GENETICS AND BREEDING

GENETIC ANALYSIS OF SEED YIELD RELATED TRAITS UNDER OPTIMUM AND LIMITED IRRIGATION IN SUNFLOWER

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ABSTRACT

In order to estimation of genetic components of variance for agronomic traits of sunflower, 16 single cross hybrids obtained by crossing between four restorer line and four CMS line as tester were evaluated as Randomized Block Design with three replications in two separate optimum and water limited conditions. The resulted data were analyzed as Line × Tester mating design fashion. According to the results under optimum irrigation condition days to maturity, seed weight and oil content were under control of additive effects while plant height was under control of none-additive effects. Flowering time, head diameter and seed and oil yield were under control of both additive and none-additive effects. Under water limited condition days to maturity was under control of additive while plant height, and seed and oil yield were under control of none-additive effects. Flowering time was under control of both additive and none-additive effects. There was a high (82%) general heritability for flowering time and an intermediate for plant height and oil yield (61-62%) under optimum irrigation. Under water limited condition the highest general heritability was obtained for flowering time, seed weight and oil yield. The values for heritability were lower under limited irrigation compared to the optimum condition which it could be resulted because of more environment effect under drought stress. The results of this study implies that selection under stressed condition is complicated and may bring illusory results, so controlling of the environmental condition is very critical in proper estimating of genetic components of variance and it is dependent to homogeneity of genetic materials and environmental condition. With environmental control, selection based methods may be efficient for production of early mature sunflower hybrids under drought condition while there is a necessity of hybridization for improvement of plant height and seed and oil yield.

Key words: Additive effect, Dominance, Heritability, Line × tester

INTRODUCTION

Sunflower whit an annual production of about 41M tones (fao.org) is the third major supplier of edible oil in the world following soybean and rapeseed. Development of hybrids with high genetic potential for seed yield and optimum plant architecture capable of adapting to the specific area of cultivation is the main objective of sunflower breeding programs (Hladni *et a*l., 2011). Breeding for desirable plant characteristics requires information about the nature of gene action and the mode of inheritance of quantitative traits as well as general and specific combining abilities of parental inbred lines. Generally different traits of any plant are under control of additive or none-additive gene action. The relative importance of these components has been reported by many authors in sunflower.

El-Hity (1992) indicated the importance of both additive and non-additive effects in controlling of 1000 seed weight and oil content. Putt (1996) reported that non-additive component was more important than the additive component in governing seed yield in sunflower. Bajaj *et al.* (1997) reported the significance of additive genetic effects in the inheritance of days to maturity, plant height, and 100 achene weight and oil contents. Kandalkar (1997) reported that seed yield was governed by both additive and non-additive genetic effects. Over dominance gene action is reported for plant height, head diameter, oil content, 100 seed weight and seed and oil yield (Gangappa *et al*, 1997). Singh *et al.* (1999) reported the predominance of non-additive genetic effects for achene yield, oil content. Ashok *et al.* (2000) found

additive gene effects for seed yield. Sharma *et al.* (2003) reported the importance of additive genetic effects in the inheritance of head diameter, achene yield per plant and oil contents. Farrokhi (2003) reported that plant height, growth duration, head diameter, 1000 achene weight, achene yield and oil contents were under control of both additive and non additive effects. Parameshwari *et al.* (2004) reported the dominance of non-additve genetic effects for days flowering, plant height, head diameter, 100 achene weight and oil contents. Devi *et al*. (2005) reported that achene yield and its components were predominantly governed by non additive genetic effects. Jan *et al*. (2005) showed dominance of non additive genetic effects for achene yield. Mijic *et al*. (2006) reported that both additive and dominant variances were involved in inheritance of 1000 achene weight.

Skoric *et al.* (2007) stressed the non-additive gene effects on oil percentage. Karasu *et al.* (2010) reported significant general combining ability for plant height, 1000 seed weight and seed number per head. They found that the non-additive effects were the most effective than other type of polygenetic effects. The gene action was changed across the years, for example additive gene action was significant for number of achenes per head and 1000-achene weight in one year but not in the second. Ghaffari *et al.* (2011) reported that days to maturity, 100 achene weight, number of achenes per head and achene yield were under the control of both additive and dominant effects, however plant height and oil contents were controlled predominantly by additive effects and life cycle duration and achene yield were controlled by dominant effects. Nooryazdan *et al*. (2011) reported additive genetic effects for days to 50 percent flowering, branching and plant height. Machikowa *et al*. (2011) reported that the additive genetic effect for these traits was more important than non additive effect for 1000 achene weight and plant height, achene yield, head diameter and oil content. According to the results of Tabrizi et al. (2012) plant height, head diameter, empty seeds per head, days to beginning of flowering, days to maturity, stem diameter and 1000 seed weight were found to be controlled mainly by additive gene effects and over-dominance effect was important for days to end of flowering. Oil yield, oil percent, head dry weight, seed weight per head, seed yield and hulled seed yield were under the control of both additive and non-additive effects.

MATERIALS AND METHODS

The experiment was carried out in Khoy Agricultural and Natural Resources Research Station in Iran. The station located in 38° 32' north latitude and 44° 58' east altitudes. The 16 single cross hybrids obtained by crossing between four restorer lines by four CMS lines as tester were evaluated as Randomized Block Design with three replications under optimum and water limited conditions. Each experimental plot consisted of 3 rows of 4 m length with 60 x 25 cm spacing between and within rows. Fertilizers were applied at the rate of 100:70:90 kg/ha for N: P: K. Drought stress was imposed by water withholding in R4-R6 growth stage. During the growth season agronomic traits as days to flowering and maturity, plant height, head diameter and seed and oil yield and the related components were measured. The resulted data subjected to line x tester analysis (Kemptorne, 1957) to estimate respective genetic variance components.

RESULTS AND DISCUSSIONS

According to the results the restorer lines explained the most part of genetic variance for flowering time, plant height and seed weight, while testers (CMS lines) had the main role in explaining of the genetic variance of days to maturity, head diameter, oil content and oil yield under optimum irrigation condition. Line × tester interaction effects were important for seed number per head and seed yield (Fig. 1A). The lines had also explained the most of variability for days to flowering, plant height and 1000 seed weight under water limited condition, however except days to maturity the variability of other traits were explained by line × tester interaction effect (Fig. 1B). This indicate that different line tester combinations can provide more variability under drought condition which itself could be aroused from activation of drought responsive genes (Oncel et al. 2000; Shao et al. 2008; Skoric, 2009). These changes may act as a protective and adaptability factor against drought condition; however the response of genotypes could be different under drought condition which affects the line × tester interaction component.

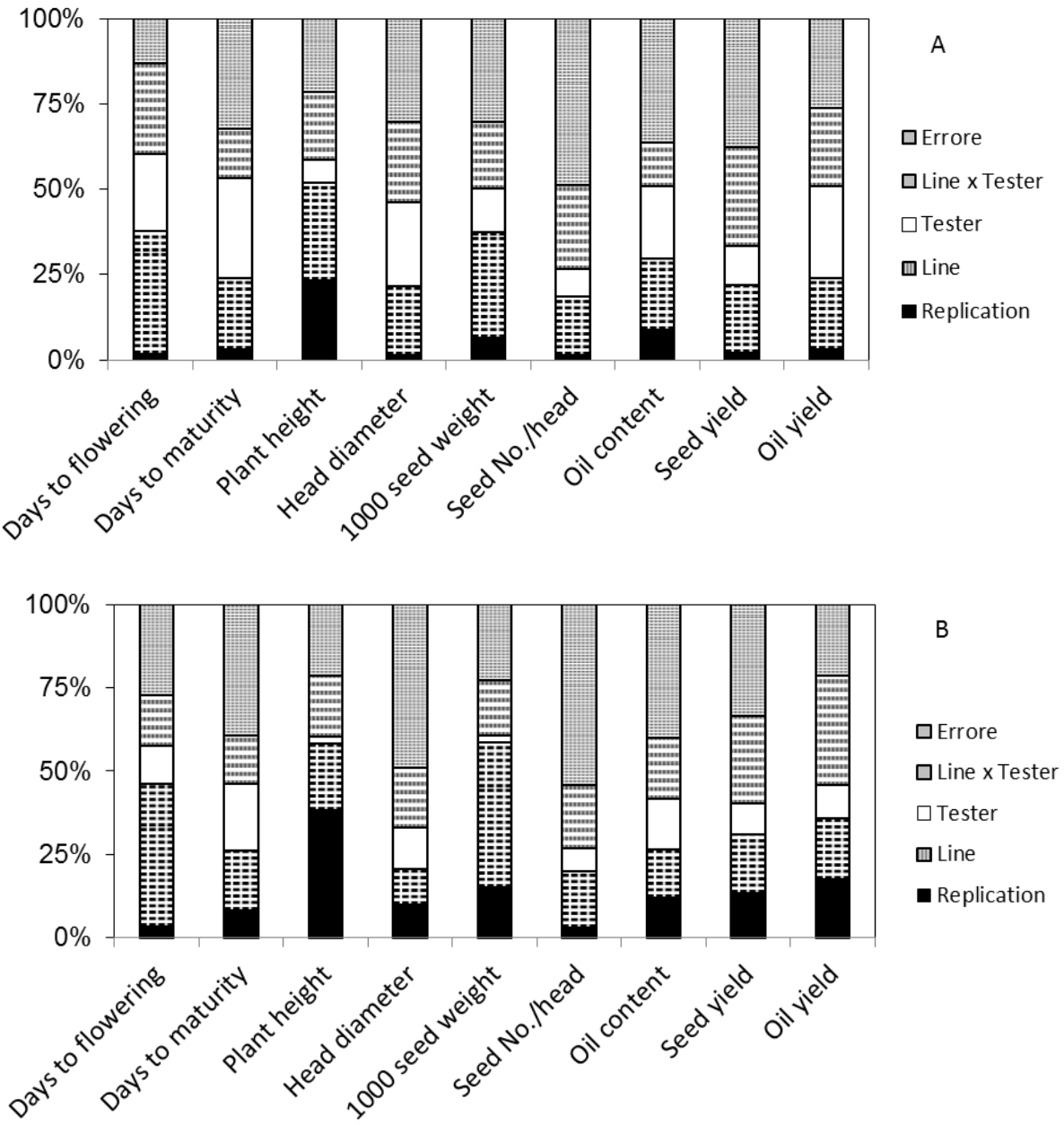

Fig.1. Relative contribution of different components on genetic variance components of agronomic traits under A) Optimum irrigation and B) water limited conditions.

The results indicated that under optimum irrigation condition growth period, seed weight and oil content were under control of additive effects, while plant height was under control of none-additive effects (Fig.2). Flowering time, head diameter and seed and oil yield were under control of both additive and none-additive gene effect. It is concluded from literature that generally the qualitative traits are predominantly under additive gene action (Singh *et al*. 1999; Sharma *et al.* 2003; Ghaffari *et al.* 2011; Nooryazdan *et al*. 2011), while quantitative characteristics as seed/oil yield are under control of both additive and non-additive gene actions (Putt, 1996; Gangappa *et al*, 1997; Devi *et al*. 2005; Jan *et al*. 2005a; Ghaffari *et al.* 2011; Tabrizi et al. 2012). Because of controversy reports it is impossible to determine absolute effect of gene action for a given trait.

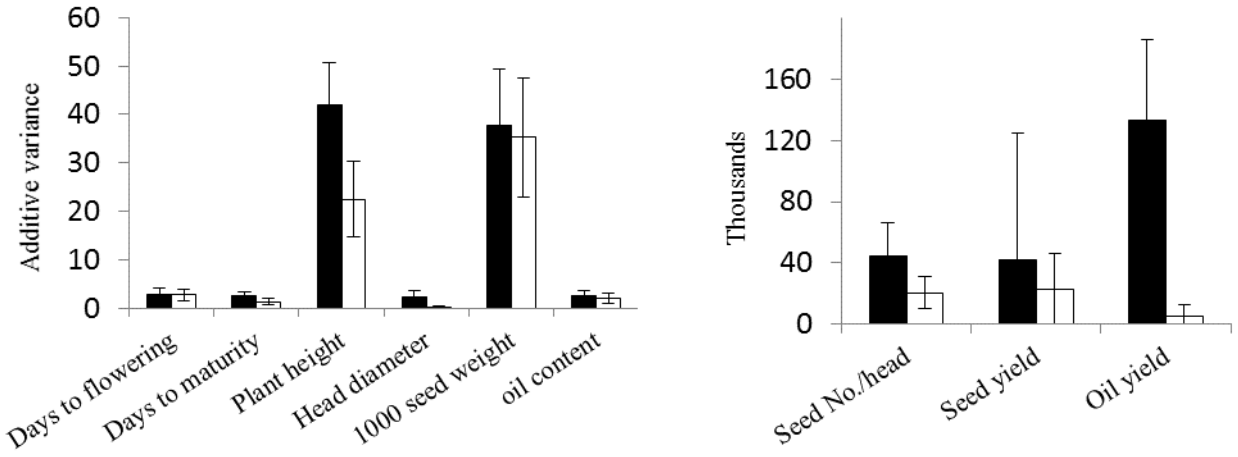

Fig. 2. Additive variance for agronomic traits under well watered (dark bars) and drought stressed condition (white bars)

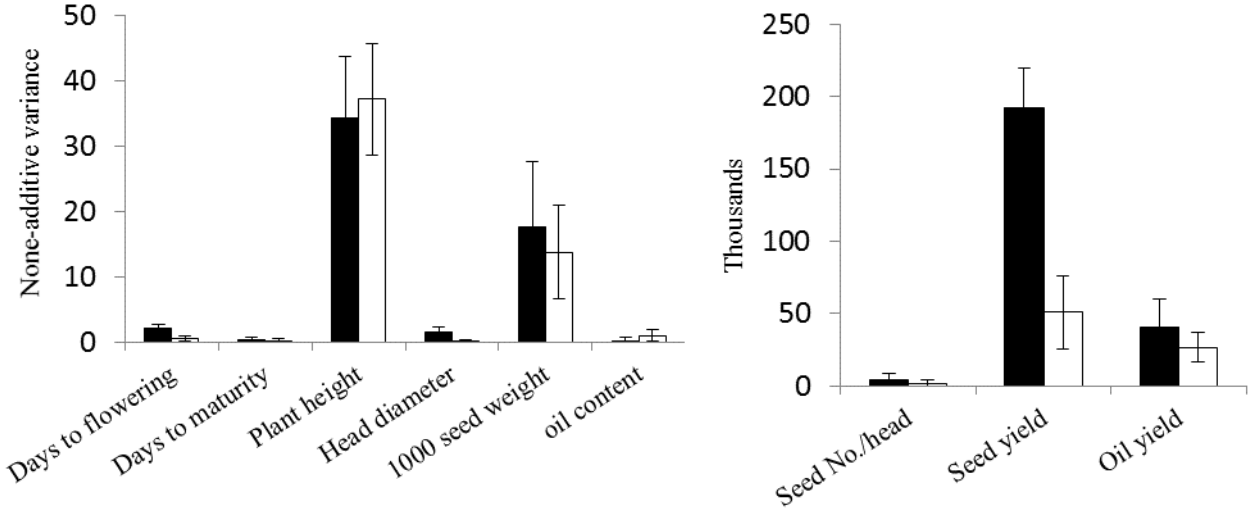

Fig.3. None- additive variance for agronomic traits under well watered (dark bars) and drought stressed condition (white bars)

Under water limited condition days to maturity was under control of additive while plant height, and seed and oil yield were under control of none-additive effects (Fig.3). Flowering time was under control of both additive and none-additive effects. These results is in accordance with other reports under non-stressed condition (putt, 1996; Bajaj et al. 1997; Gangappa et al. 1997; Devi et al. 2005; Parameshwari *et al.* 2004 and Nooryazdan *et al*. (2011). Comparison of genetic components showed that additive effects for plant height, head diameter and oil yield are significantly higher in optimum condition compared with stressed condition. There were also significant differences for days to flowering, head diameter and seed and oil yield. To the best of author's knowledge there is no information about genetic control of sunflower traits under drought condition. The results of this study indicated that additive gene action is restricted under drought condition which it could be resulted because of higher environmental effects on estimated variances. Karasu *et al*. (2010) indicated that the gene action was changed across the years, for example additive gene action was significant for number of achenes per head and 1000-achene weight in one year but not in the second.

There was high (82%) broad sense heritability for flowering time and intermediate for plant height and oil yield (61-62%) under optimum irrigation (Table 1). There was an intermediate heritability for days to flowering and maturity and oil content. Narrow sense heritability Estimates were low for all traits except seed number per head. Under water limited condition the highest broad sense heritability was obtained for

flowering time, seed weight and oil yield (Table 2). The values for heritability were lower under limited irrigation compared to the optimum condition which it could be resulted because of more environment effect under drought stress. These results indicate that the effect of environment is higher than genotype under stressed condition which could reduce the efficiency of selection under drought condition. Alza and Fernandez (1997) reported higher narrow sense heritability estimates for various sunflower traits as seed yield, number of seeds per head, seed weight, head diameter; oil content and days to bloom. On the contrary, Sayed *et al*. (2013) reported low narrow sense heritability estimates for seed and oil yields. The lower narrow sense heritability estimates in this study indicated the importance of non-additive gene effects for the agronomic traits in the sunflower genetic materials which were used in this experiment.

Table 2. Heritability estimates for agronomic traits under well watered condition

	Days to flowering	Days to maturity	Plant height	Head diameter	Seed weight
h^2_B	0.82	0.55	0.61	0.57	0.51
h^2_N	0.47	0.47	0.33	0.34	0.32

	Seed number	Oil content	Seed yield	Oil yield
h^2_B	0.70	0.45	0.39	0.73
h^2_N	0.64	0.42	0.07	0.56

Table 2. Heritability estimates for agronomic traits under drought stressed condition

	Days to flowering	Days to maturity	Plant height	Head diameter	Seed weight
h^2_B	0.62	0.40	0.50	0.21	0.64
h^2_N	0.51	0.35	0.19	0.15	0.46

	Seed number	Oil content	Seed yield	Oil yield
h^2_B	0.49	0.35	0.12	0.31
h^2_N	0.46	0.24	0.09	0.07

CONCLUSIONS

The results of this study implies that selection under stressed condition is complicated and may bring illusory results, so controlling of the environmental condition is very critical in proper estimating of genetic components of variance and it is dependent to homogeneity of genetic materials and environmental condition. With environmental control, selection based methods may be efficient for production of early mature sunflower hybrids under drought condition while there is a necessity of hybridization for improvement of plant height and seed and oil yield.

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A UNIQUE CYTOPLASMIC-NUCLEAR INTERACTION CAUSING SUNFLOWER PLANTS WITH REDUCED VIGOR AND THE GENETICS OF VIGOR RESTORATION

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ABSTRACT

Plants with pale yellow leaves and reduced vigor were observed in backcross progenies of inbred line HA 89 or HA 410 in the cytoplasms of 12 perennial *Helianthus* species, but not in the cytoplasms of annual *H. niveus*, *H. praecox*, *H. anomalus***,** and *H. neglectus*. Segregation ratios of normal (N) to reduced-vigor (RV) plants in testcrosses and self-pollination of heterozygous normal plants, respectively, suggested that single dominant gene (*V*) controls vigor restoration. A high frequency of vigor restoration genes was found in 11 cultivated sunflower lines, with the exception of HA 89, HA 410, RHA 801, and Seneca. Testcross progenies of the half-diallel crossed F1s among HA 271, HA 234, VNIIMK, Armavir, Issanka, and HA 821 onto the RV cmsRIG1 were all normal, suggesting that all these lines possess the same *V* gene. Extensive use of *H. tuberosus* in early sunflower breeding programs might explain the presence of *H. tuberosus V* gene in many cultivated sunflowers, and the possible selective advantage of the *V* gene. A new *V* gene derived from *H. giganteus* was identified, which differed from the *V* gene commonly existing in cultivated lines. Other *V* genes derived from *H. hirsutus* and *H. salicifolius* will be compared among all the *V* genes. The *V* gene commonly existing in cultivated lines has been mapped to the linkage group 7 of the sunflower genome using SSR markers. The tightly linked markers will help select for normal vigor progenies when using perennial *Helianthus* cytoplasms in a breeding program

Key Words : sunflower, cytoplasmic-nuclear interaction, reduced vigor, wild perennial *Helianthus*

CORRELATION STUDIES OF SSR MARKER BASED GENETIC DISTANCE AND HETEROSIS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) one of the most important oilseed crops in India known for its quality oil. However, in recent years the area under cultivation is decreasing owing to crop being affected by biotic and abiotic stress. This situation necessiated development of higher heterotic hybrids involving diverse germplasm to break the yield plateau. Anexperiment was conducted at Main Agricultural Research Station, UAS - Raichur to evaluate 49 sunflower hybrids along with parents to determine the correlation between SSR based genetic distance (GD) and heterosis for nine quantitative traits. The 49 hybrids were derived by crossing seven CMS lines and seven restorers in line x tester design. . Significant heterosis was recorded in hybrids for all nine traits studied. Genetics distance between pairs of tested CMS lines and testers ranged from 0.18 to 0.68. The correlation between genetic distance and heterosis was not significant for the most of characters studied. A highly significant positive correlation was observed between genetic distance and head diameter both at mid-parent ($r=0.48$; $p<0.01$) and better parent ($r=0.475$; $p<0.01$) heterosis level. However, significant negative heterosis was recorded between genetic distance and mid-parent heterosis for number of seeds per head ($r=-0.348$; $p<0.05$) and oil content ($r=-0.391$; $p<0.01$). The SSR markers included in the study are solely for their high PIC values. The poor correlation of GD with hetersis accept for head diameter indicates the need to include the markers linked to yield contributing traits to help in to rely on marker based GD to predict hybrid performance. .

Key words: Sunflower, heterosis, genetic distance, correlation

INTRODUCTION

The cultivation of sunflower at commercial scale as an oilseed crop is worldwide. The largest traditional producer is Russia and other sunflower producing countries include Argentina, the Eruopean Union, USA, China, India, Turkey and South Africa. The world sunflower production is around 30 million tones and is being cultivated over an area of 20 million hectares. . In India, sunflower is being grown over an area of 0.69 million hectares with a production of 0.54 million tones with the productivity of 791kg per ha (Anon., 2015).

Sunflower being a highly cross pollinated is an ideal crop for exploitation of heterosis. The discovery of Cytoplasmic Male Sterility by Leclercq (1969) followed by fertility restoration system by Kinman (1970) provided the required breakthrough in the development of hybrids. Hybrids are also highly self fertile and resistant to diseases, thus resulting in enhanced seed set and seed filling (Seetharam, 1981). After sunflower being introduced to India as oil seed crop in early 1970's, the first sunflower hybrid BSH-1 was released during 1980 and thereafter several hybrids have been released. The exploitation of heterosis through hybrid breeding is one of the landmark achievements in plant breeding (Duvick, 2001) and particularly in sunflower (Seetharam, 1984). In last decade (2001-2010), 6 varieties and 11 hybrids have been released for commercial cultivation (Anon., 2014) in India.

In heterosis breeding programmes, a large number of experimental hybrids need to be and are routinely produced and tested to identify hybrid vigour. This requires huge resources and manpower. In

general, heterosis is considered as an expression of the genetic divergence among inbreds/parents used for crossing. Reliable prediction of single-cross performance is crucial in hybrid breeding as the evaluation of large number inbred lines in numerous cross combinations is difficult. . Several prediction approaches have been suggested using phenotypic data with co-ancestry coefficients calculated from pedigree records or marker data (Schrag et al., 2009). The information on the genetic diversity and distance among the breeding lines and correlation between genetic distance and hybrid performance are important in determining breeding strategies, classifying the heterotic groups and predicting the hybrid performance.

Studies of genetic diversity in relation to hybrid performance have been undertaken in several crops. Investigations in corn, *Zea mays* L., have shown that the genetic diversity of parents was significantly correlated with hybrid performance and that yield heterosis could be predicted using molecular markers (Schrag et al.,2006). Genetic diversity of different sunflower gene pools has been studied with enzymes (Tersac et al., 1993), RFLP markers (Hongtrakul, 1997) and SSR markers (Solodenko et al., 2005). However, the literature data on the predication of sunflower heterosis and hybrid performance by marker based genetic distance of the parental lines is scarce (Tersac *et al*.1994, Cheres et al. 2000). The objective of the study wasto identify the reliability of SSR markers to determine the genetic diversity and association between SSR based genetic diversity and heterosis for yield component traits in sunflower.

MATERIAL AND METHODS

Seven CMS lines (CMS-2A, CMS-821A, CMS-850A, R-10-46-2A, CMS-4A, CMS-6A, CMS-10A) and seven R-lines (R-GM-39, R-GM-41, R-GM-49, R-GM-69, 83-Br, R-393 , R2F01120B) were crossed in L x T fashion during kharif-2013-14. The resultant 49 hybrids along with parents were evaluated for nine yield and yield contributing characters in RCBD design with three replications. Heterosis, expressed as per cent increase or decrease of derived F_1 over mid parent (average heterosis) and better parent (heterobeltiosis) was calculated for each character as per the method of Turner (1953) and Hayes *et al.* (1956).

Table 1: list of sunflower SSR primers used for the study

1. ORS-287	16. ORS-324	31. ORS-677
2. ORS -290	17. ORS-332	32. ORS-769
3. ORS-296	18. ORS-333	33. ORS-780
4. ORS-300	19. ORS-339	34. ORS- 807
5. ORS-301	20. ORS-337	35. ORS- 811
6. ORS-309	21. ORS-358	36. ORS- 852
7. ORS-310	22. ORS-378	37. ORS- 930
8. ORS-311	23. ORS-388	38. ORS- 938
9. ORS-315	24. ORS-407	39. ORS- 959
10 ORS-316	25. ORS- 484	40.ORS- 1068
11.ORS-318	26. ORS-546	41.ORS- 1088
12.ORS-319	27. ORS-552	42. ORS- 1159
13 ORS-321	28. ORS-578	43. ORS- 1220
14.ORS-322	29. ORS- 628	44. ORS- 1245
15.ORS-323	30. ORS- 671	

The genomic DNA of 14 parental lines was extracted by following modified CTAB method. Fourty four sunflower SSR primers were used for PCR amplification using gradient thermocycler. The amplified products were separated using 3.5% agarose gel electrophoresis. DNA polymorphism between two inbreds was estimated by comparison of amplified fragments. Jaccard similarity coeffiecient (j) was calculated according to Staub et al., (2000). Genetic distance (GD) among all parental lines was estimated as per formula GD=1-j given by Spooner et al., (1996).

The values of genetic distances as measured by SSR markers were correlated with mid-parent heterosis and better parent heterosis to estimate their relationship using Pearson's coeefficient of correlation. Correlations were done for hybrid combinations from each tester and lines separetely. Significance of correlation was determined using the table os Snedecor (1959).

RESULTS AND DISCUSSION

The 49 sunflower hybrids derived by crossing seven CMS and seven restorers in LxT fashion were evaluated for yield and yield component traits along with parents. High degree of variation was observed for all characters studied in both parents and hybrids. The mean values of the hybrids were significantly higher than the parental lines for plant height, head diameter, 100 seed weight and seed yield per plant (Table 2).

Table 2: Mean values and coefficient of variation (V) for the sunflower parental lines and their hybrids.

Character	Female parent		F1 Hybrid		Restorer lines	
	Mean	V (%)	Mean	V (%)	Mean	V (%)
Plant height (cm)	102.38	29.9	144.90	9.98	107.5	15.07
Days to 50% flowering	64.00	2.77	65.50	1.94	63.00	5.59
Head Dia (cm)	14.76	1.85	17.50	1.49	11.04	1.20
No. of leaves	20.46	3.78	27.32	2.59	21.34	2.23
100 seed wt (g)	3.27	0.71	3.94	0.48	2.39	0.33
No. of seeds/head	1206.6	127.7	1179	117.0	1349	148.9
Volume wt (g/100ml)	36.36	2.06	40.06	1.74	37.66	2.83
Seed yield/pl (g)	30.13	2.59	35.42	2.98	27.30	4.06
Oil content (%)	34.65	3.98	37.81	1.38	34.70	2.95

The heterosis level for most of the traits studied was signicantly superior viz., plant height, head diameter, 100 seed weight, seed yield per plant (Table 3). The highest level of mid-parent heterosis observed for 100 seed weight(39.28) followed by plant height (38.70) and head diameter (35.78). Whereas the highest level of better parent heterosis observed forplant height (34.65) followed by 100 seed weight(28.04) and head diameter (24.60).

Table 3: Mean and range of heterosis for nine quantitative traits in 49 sunflower hybrids

Trait	Mid-parent Heterosis		Better Parent Heterosis	
	Mean	Range	Mean	Range
Plant height (cm)	38.70**	6.8 – 88	34.65**	-5 – 61
Days to 50% flowering	3.25	-10.41 – 15.40	2.45	- 11.90 – 7.90
Head Dia (cm)	35.78**	8.35 – 79.03	24.60**	-2.73 – 46.51
No. of leaves	31.00	-2.75 – 37.43	28.20	-14.51 – 30.51
100 seed wt (g)	39.28*	11 – 45.40	28.04*	6.01 – 36.38
No. of seeds/head	-3.13	-11.77 – 11.37	-10.60	-23.20 – 11.54
Volume wt (g/100ml)	8.80	6.56 – 20.53	6.44	4.41 – 12.51
Seed yield/pl (g)	24.40*	6.96 – 58.16	19.50*	1.42 – 55.78
Oil content (%)	6.70	-4.51 – 18.80	5.81	-11.39 – 12.36

Forty four sunflower SSR primers were used to study genetic diversity among fourteen parental lines. Out of 44 primers used three primers failed to amplify and ten primers showed monomorphic amplification bands. The remaining 31 primers showed polymorphism with an avarage polymorphism of 39.65 % (PIC=39.65%). The number of amplified products ranged from 1 to 3 with an average of 1.21 bands per primer and 1.13 bands per primer were polymorphic.

The frequency of SSR polymorphism was calculated based on presence (taken as 1) or absence (taken as 0) of common bands. The binary data was used to compute pair wise similarity coefficient (Jaccard, 1908). The genetic similarity computed considering data of SSR markers showed a wide range from 0.32 to 0.82 indicating the presence of high variability among 14 sunflower genotypes.

The highest similarity was observed between the parental genotype CMS 821A and CMS 2A (0.82) while the lowest similarity was observed between the parental genotypes *viz*,. R-GM-41 and R-10-46-2A (0.32), R-GM-49 and CMS A6 (0.32).

Genetic diversity is the extent to which the heritable material differs within a group of plants, which is a result of evolution, including domestication and plant breeding. Assessing genetic diversity of cultivated crop plants is important to select proper genotype for hybridization programme. The sunflower genetic diversity and co-ancestry analysis have been carried out using RAPD (Arias *et al*., 1995). The placement of individual cultivars into different accessions based on morphological attributes do not necessarily reflect the real genetic relationship.

The recent advances in molecular biology have provided the descriptors based on protein and DNA as an aid to plant breeding programme. Genetic diversity caused by sexual reproduction i.e. hybridization, selection and mutation results in genome changes from one base pair to entire chromosome. The molecular markers that are not influenced by environmental changes provide an opportunity to examine the genetic relationship between accessions more precisely. This can help in the rationalization of existing germplasm collections and allow future collection strategies towards specific objective. Molecular markers can be used as a valuable tool for identification of parental lines and varieties for protection of plant breeder's right. DNA markers also help in studying the evolutionary/phylogenetic relationship between inbreds and varieties.

The relation between SSR based genetic diversity among inbred lines and their hybrids performance dependent on the trait of interest examined. Correlation coefficient between genetic distance,

parental means and hybrid performance in terms of heterosis were not significant for most of the characters studied. However, significant correlation was observed for GD with both mid-parent (r=0.48) and better parent (r=0.475) heterosis. For both number of seeds per head and oil content, correlation between GD and mid-parent heterosis was significantly negtive (r=-0.348 & r=-0.391).

The correlation between genetic distance and heterosis levels expressed was not significant for most of the traits studied except for head diameter, number of seeds per head and oil content. The SSR markers included in the study are solely for their high PIC values. The poor correlation of GD with hetersis accept for head diameter indicates the need to include the markers linked to yield contributing traits to help in to rely on marker based GD to predict hybrid performance. (Charcosset *et al*., 1991 and Bernardo *et al*., 1992).

Tersac *et al*. (1994) described relationships between heterosis and enzymatic polymorphism of 39 sunflower populations. The correlation coefficients for all enzyme systems were too low to be used as predictors of the general combining ability, but when enzymatic systems were analyzed separately, four of them turned out to be useful markers for breeding purposes. Zeid et al. (2004) pointed out that the lack of association between heterosis and genetic dissimilarities for inter group hybrids may be explained by the absence of crosses between related parents i.e. by the absence of variation for parental relatedness: all crosses have unrelated parents.

In the present study the GD showed poor correlation with both mid-parent and better parent heterosis except for head diameter. Similar reports were done in previous studies on pepper, alfalfa, wheat and rapeseed (Diers *etal*. 1996, Geleta *et al*. 2004, Zeid *et al*. 2004, Riday *et al*. 2003).

CONCLUSION

The conclusion on the use and reliability of SSR based genetic distance to predict hybrid performance in terms of heterosis depends on use of large number of specific markers linked to yield contributing traits. A higher accuracy would also be possible by identification of molecular markers linked to combining ability.

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STABILITY OF THE LEVEL OF PARTIAL RESISTANCE TO WHITE ROT IN SUNFLOWER

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ABSTRACT

In the southeast of Buenos Aires Province, Argentina, sunflower cultivars must be moderately resistant to *Sclerotinia sclerotiorum* infections on capitula. The stability of 32 sunflower hybrids for the relative incubation period of white rot during six years in Balcarce was assessed. Two stability methods were proposed: the linear regression model (univariate method) and the additive main effects and multiplicative interaction model (AMMI) (multivariate method). Combined analysis of variance detected highly significant effects of hybrids, years and hybrid-year interaction (GxE) effect. The linear regression model explained only 24% of the GxE sum of squares, while the two first principal components of the AMMI model retained the 66.8% of that sum of squares. Consequently, the AMMI model seemed to be more effective for characterizing the GxE effect. Two statistics based on AMMI models were considered afterwards: the AMMI Stability Value (ASV) and the Genotype Selection Index (GSI). The hybrids Paraiso 20, Paraiso 22, ACA 863, MG 60, Tehuelche CL, CF31 and Pampero DM had stability since the ASV index. According to GSI, Paraiso 22, Paraiso 20, Tehuelche CL and MG 60 showed high level of resistance to white rot besides of general adaptability across six years in Balcarce.

Key words: hybrids, partial resistance, *Sclerotinia sclerotiorum*, genotype-environment interaction, AMMI, linear regression.

INTRODUCTION

Sunflower *(Helianthus annuus*) has traditionally been an important crop in Argentina given the Premium quality of its seed-oil. In the period 2013/14, our country contributed about 5% of worldwide grain production (USDA, 2014). The Southeast of the Buenos Aires Province (SEB) produces around the third of the Argentinean production and, consequently it became the main sunflower grown area (BCBA, 2014).

Sclerotinia sclerotiorum on sunflower capitula produces white rot, a disease that may cause direct and/or indirect damages in all regions where sunflower is grown (Pereyra and Escande, 1994; Gulya *et al.*, 1997). An efficient control of white rot involves the use of sunflower hybrids with a moderate level of resistance that allows reducing the epiphyte risk and the variation of seed-productivity given the pathogen infection.

In sunflower, the resistance to white rot is of horizontal type and its inheritance mainly depends on additive gene effects (Castaño *et al.*, 2001; Godoy *et al.*, 2012). Studies conducted by the Sunflower Breeding Group in the "Unidad Integrada Balcarce" suggested that white rot can be considered as a series of phases starting in the flowering period, when the *S. sclerotiorum* infection and the mycelium invasion into capitulum are made, and ending at maturity (Castaño, 2007). These phases are assessed through components of partial resistance and among them we found the relative incubation period (RIP), which measures the relative period of time of inoculated capitulum to show white rot symptoms (Castaño and Giussani, 2009).

In conventional breeding programs, sunflower selection is carried out in the field. However, the level of hybrids resistance to white rot is affected by the genotype-environment (GxE) interaction effect (Godoy *et al.*, 2005). Recently, Delgado *et al.* (2013) found significantly GxE interaction effect when white rot partial resistance was evaluated in hybrids sown in the SEB. Therefore, in sunflower breeding for white rot resistance it will be important to determine the GxE interaction effect in order to make easier the selection of hybrids and do not overestimate the expected genetic progress (Kang and Gorman, 1989).

The genotype stability is the ability of certain genotypes to have consistently relative performance across environments. Becker and León (1988) distinguished two concepts of stability: 1) biological or static, where the stable genotype has minimum variance across environments, and 2) dynamic or agronomic, where stable cultivars are those that show a constant gain in performance with increasing potential environment. Last definition is that one preferred by agronomists and plant breeders (Alwala *et al.*, 2010).

Methods to analyze the GxE interaction effect and to detect the most stable genotypes are numerous. Among univariate methods, the join linear regression analysis (Finlay and Wilkinson, 1963) has been mostly used because its mathematical simplicity and its easy biological inferences that could be made. However, it has some disadvantages when the linearity fails and/or when there are not several genotypes and environments involved in the analysis. Another weakness is related to that the regression coefficient tends to simplify the genotype response in a single plane, when it may be described in a multidimensional space (Crossa, 1990; Flores *et al.*, 1998; Cubero and Flores, 2003).

Regarding to multivariate methods, the analysis of additive main and multiplicative interaction effects (AMMI) (Gollob, 1968; Mandel, 1971) has been used to evaluate genotype stability. Given that the AMMI method considers the sources of variation genotype and environment as additive effects and the GxE interaction effect has multiplicative effect, analyses of variance and of principal components must be done (Cubero and Flores, 2003; Farshadfar and Sutka, 2003; Alwala *et al.*, 2010; Williams Alanís *et al.*, 2010). Based on this method, the nonparametric statistics AMMI Stability Value (ASV) (Purchase *et al.*, 2000) and the Genotype Selection Index (GSI) (Farshadfar, 2008) were also proposed.

In sunflower, genotype stability has been determined for seed-yield (Lorenzo and Lorenzo, 1987; Aguirrezábal *et al.*, 2002), but there are not many studies made for other quantitative traits like horizontal disease resistances. Therefore, the objective of this study was to evaluate the stability of sunflower cultivars released in the SEB, when the white rot resistance is measured, by uni- and multivariate methods.

MATERIALS AND METHODS

Plant material and experimental design

Field experiments were at the 'Unidad Integrada Balcarce', Argentina (37 ° 45'S, 58° 18'W, 130m ASL), during six consecutive years (e.g. 2010-15). Thirty two sunflower cultivars were grown following a randomized complete block design, with three replications (Table 1). The hybrids ACA 884 and Paraíso 20 were also sown next to the experiments to be used as flowering checks.

Inoculum, inoculation protocol and measured variable

Assisted infections were carried out following the protocol suggested by Vear and Tourvieille (1984). The capitula of 12 plants/plot in the R5.3 stage (Schneiter and Miller, 1981), or its homologous F3.2 (Cetiom, 1992) were inoculated with 5ml of an aqueous suspension containing about 5000 ascospores/ml of *Sclerotinia sclerotiorum*. Given the heterogeneity of flowering period (i.e. within and among cultivars) there were four to six inoculation dates in the field experiments. Inoculated capitula were immediately covered with Kraft paper bags and trials received irrigations by micro-sprinklers once or twice a week until white rot data were scored. White rot partial resistance was evaluated by capitulum

through one of its components: the relative incubation period (RIP). The RIP is the ratio of the number of days between inoculation and first detected symptoms for each inoculated capitulum and that for the checks inoculated on the same day. A plot mean RIP value was calculated by hybrid. Hybrids with RIP higher than unity had a favorable level of resistance because their symptoms appeared after that the checks (Castaño *et al.*, 1993; Godoy *et al.*, 2005).

Statistical analyses

Analysis of variance

There was a set of six consecutive years of RIP responses for each evaluated hybrid. The assumptions required for the combined analysis of variance were evaluated by checking the residuals and using Hartley's F test of homogeneity of variances. The following mathematical model was proposed, considering both hybrids and years as fixed effects:

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \gamma_{k(j)} + e_{ijk}$$

where: i=1,…,32; j=1,…,6; k=1,…,3;

y_{ijk}: RIP of *i*th hybrid, at *k*th block within *j*th year;
μ: RIP general mean;
τ_i: main effect of hybrid *i*;
β_j: main effect of year *j*;
$(\tau\beta)_{ij}$: *i* hybrid-by-*j* year interaction effect;
$\gamma_{k(j)}$: effect of block *k* within year *j*;
e_{ijk}: residual associated with *ijk*th observation of RIP.

The joint linear regression (Finlay and Wilkinson, 1963) as well as the AMMI (Gollob, 1968; Mandel, 1971) methods were used to characterize the GxE interaction. Both methods are based on the partition of the GxE sum of squares of the analysis of variance (ANOVA).

Joint-regression analysis

The slopes of regressions of individual hybrid's RIP against annual mean RIPs, based on all hybrids in the trial (i.e. environmental index) were determined. The GxE interaction effect was partitioned into the heterogeneity of regression coefficients and the deviations from the regression model.

Additive Main Effects and Multiplicative Interaction (AMMI) model

In the AMMI model, the sum of squares of the GxE interaction was decomposed by a Principal Component Analysis (PCA). Also, two analytical indices derived from AMMI model were calculated: the AMMI Stability Value (ASV) (Purchase et al., 2000), and the Genotype Selection Index (GSI) (Farshadfar, 2008).

The ASV, that weights the contributions of each hybrid to the GxE sum of squares through the two main components selected from the PCA, was calculated by hybrid using the following formula:

$$ASV_i = \sqrt{\left[\frac{SS_{PC1}}{SS_{PC2}}(PCA1score)\right]^2 + (PCA2score)^2}$$

where:

$\frac{SS_{PC1}}{SS_{PC2}}$: is the weight given to the PCA1-score by dividing the PCA1 sum of squares by the PCA2 sum of squares;

PCA1score: is the value obtained by the 1st main component for each hybrid;

PCA2score: is the value obtained by the 2nd main component for each hybrid.

Hybrids with lower ASV values showed high stability level because of their low contributions to the GxE interaction. The GSI is an index that allows simultaneous selection of those hybrids with high level of partial resistance as well as stability. For each hybrid, it was calculated as:

$$GSI_i = rASV_i + rRIP_i$$

Where: $rASV_i$: is the ranking of hybrids according to the ASV index (value 1 corresponds to the highest stability level); $rRIP_i$: is the ranking of hybrids according to the average of RIP across evaluated environments (value 1 corresponds to the highest RIP values). According to this index, the hybrid with the least GSI is considered as the most stable with high RIP. All statistical analyses were performed by the R program (R Core Team, 2014). Also, the software 'agricolae' (De Mendiburu, 2014) was used to adjust some specific functions in the AMMI model.

RESULTS AND DISCUSSION

The RIP means of hybrids across years ranged from 0.8196 (ACA 885) to 1.2674 (SPS 3109) and the environmental indices (i.e. mean of all hybrids in a year) ranged from 0.9111 (2012) to 1.0868 (2011) (Table 1).

Table 1. Means of RIP obtained from the 32 sunflower hybrids evaluated, differentiated by year, and the average over the six years. Values obtained by the ASV (AMMI stability value) and GSI (Genotype selection index) for each hybrid. RIP averages for each year (Environmental Index) are also indicated.

HYBRIDS	YEARS						AVERAGE 6 YEARS	STABILITY INDEX	
	2010	2011	2012	2013	2014	2015		ASV	GSI
64A89	0.9063	1.2707	0.9431	0.9396	0.9902	1.0155	1.0109	0.3202	45
65A25	0.9141	0.8555	0.8593	1.0093	0.8995	0.9068	0.9074	0.2122	45
ACA 863	0.7343	0.9242	0.7565	0.8789	0.8914	0.8266	0.8353	0.0491	34
ACA 884	1.0831	1.0671	0.9958	0.9709	1.0125	1.0635	1.0322	0.129	25
ACA 885	0.8027	0.7095	0.6904	0.9951	0.8277	0.8920	0.8196	0.3502	63
ACA 886 DM	0.9356	1.0055	0.6407	0.8989	0.9029	0.9561	0.8899	0.2372	51
Agrobel 963	0.9827	1.2467	0.8277	1.0317	0.9460	0.9303	0.9942	0.2275	38
Albisol 2	1.2039	1.1105	0.9973	1.0178	1.0715	1.0630	1.0773	0.1575	22
Albisol 20	0.8240	1.0447	0.8482	0.9896	0.8843	0.9599	0.9251	0.1012	33
BuckSurcoflor	0.9161	1.1607	1.1032	0.9933	1.0021	1.0053	1.0301	0.2857	41
Cauquen	1.0453	1.0856	1.0843	0.9803	1.0363	1.0280	1.0433	0.2172	28
CF 31	1.0099	1.0162	0.8751	0.9554	0.9338	0.8790	0.9449	0.0938	29
Dekasol 3820	1.0704	1.1145	1.0003	1.0096	1.0185	1.0028	1.0360	0.114	23
Dksol OP3845	0.9731	1.0867	0.8552	1.0248	0.9205	1.0965	0.9928	0.1139	29
DM 230	0.8851	1.1183	0.9052	0.9299	0.9255	1.1432	0.9845	0.1463	33
GS 3190 RDM	1.2201	1.0384	0.9991	1.1316	1.1853	1.0960	1.1118	0.3083	32
HS-03	0.7780	1.1300	0.8228	0.8935	0.9206	0.8662	0.9019	0.2642	54
KWSBaqueano	1.0723	0.9770	0.9881	1.0016	1.0142	1.0077	1.0102	0.2217	36
Macon	1.0229	1.2322	1.0366	1.0221	0.9816	0.9700	1.0442	0.2395	32
MG 2	0.9652	1.0300	0.9138	0.9544	0.9267	0.8975	0.9479	0.1058	31
MG 60	1.0631	1.1768	0.8658	1.0121	0.9918	0.9779	1.0146	0.0758	18
NK 70	0.9645	0.9588	0.7853	0.9940	0.9510	1.0011	0.9425	0.1919	41
Pampero DM	0.8956	1.0083	0.7762	0.8960	0.9170	1.0030	0.9160	0.0973	33
Pan 7031	0.9868	1.1478	0.8725	0.9120	1.0074	0.9455	0.9787	0.1127	30

Paraíso 20	1.0374	1.1421	0.9644	1.0579	0.9966	1.0572	1.0426	0.0245	11
Paraíso 22	1.1230	1.1857	0.9816	1.2048	0.9850	1.0209	1.0835	0.0263	7
Paraíso 27	1.0747	1.2638	1.1036	1.1350	0.9824	0.9796	1.0898	0.2383	27
Paraíso 75	1.2030	1.2083	1.2238	1.1463	1.0910	1.0910	1.1606	0.2684	29
SPS 3109	1.3043	1.3165	0.9399	1.4329	1.3344	1.2763	1.2674	0.4273	33
Tehuelche CL	0.9675	1.1810	1.0380	1.1734	0.9887	1.1061	1.0758	0.0869	12
Tobsol 3004	0.8047	0.9507	0.7107	0.9471	0.8808	0.9133	0.8679	0.1546	45
VDH 487	0.9894	1.0133	0.7502	1.0089	0.9464	1.0702	0.9631	0.2434	46
ENVIROMENTAL INDEX	0.9925	1.0868	0.9111	1.0171	0.9801	1.0015			

Combined analyses of variance

Although Hartley's F test detected heterogeneity of variances among years, experimental residuals across environments did not show strong anomalies. According to Annicchiarico (2002), combined analysis of variance was made with the original data (e.g. RIP values) without transforming them.

Results of the analysis of variance are in Table 2. All evaluated effects were significant ($p<0.01$). Total sum of squares was decomposed into constituents. This showed that 13% of that total could be attributed to environmental effects (i.e. years), 40% to genotype effects (i.e. hybrids) and 21.8% to the GxE interaction effect.

Table 2. Combined analysis of variance for RIP responses obtained from 32 sunflower hybrids evaluated at Balcarce during six years.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	p-value
Year	5	1.5997	0.3199	12.69	$1.91\ 10^{-4}$
Blocks within years	12	0.3026	0.0252	3.36	
Hybrid	31	4.9123	0.1585	21.03	$1.25\ 10^{-63}$
Hybrid xYear (GxE)	155	2.6718	0.0172	2.29	$6.90\ 10^{-11}$
Error	371	2.7957	0.0075		

Joint-regression analysis

The two components of the GxE interaction effect, that is the linear regression and the deviation from regression, were both significant ($p <0.01$) and contributing with 24% and 76% to the GxE sum of squares, respectively (Table 3).

Table 3. Partition of the sum of squares of the GxE interaction according to the joint-regression method.

Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean squares**	**F ratio**	**p-value**
Hybrid x Year (GxE)	155	2.6718	0.0172	2.29	$6.90\ 10^{-11}$
Linear regression	31	0.6411	0.0207	2.76	$3.39\ 10^{-6}$
Residual	124	2.0307	0.0164	2.19	$7.16\ 10^{-9}$

According to Romagosa and Fox (1993), the contribution of the linear regression effect to the GxE sum of squares (24%) could be considered relatively low value. In addition, if we compare the linear regression component with its residual ($F= 1.26$, $p= 0.1878$) it would indicate that the variability explained by linear regression does not differ from the unexplained variability. These results suggest that

the joint linear regression would not be a suitable model to evaluate, in our case, the stability of sunflower hybrids by their level of resistance to white rot.

Additive Main Effects and Multiplicative Interaction (AMMI) model

The use of the multivariate method allowed decomposing the GxE sum of squares in five main axes (i.e. principal components, PC) (Table 4). Whereas the first three PC's showed significant effects ($p < 0.01$), the analysis stopped at PC2 given that with these two components more than two-thirds (i.e. 66.8%) of the sum of squares of GxE interaction effect were considered.

Table 4. Decomposition of the sum of squares of the GxE interaction by the main components obtained, absolute and cumulative percentages of the GxE sum of squares explained by each, and p-values associated.

Principal component (PC)	Absolute percentage	Cumulative percentage	Degrees of freedom	Sum of squares	Mean squares	p-value
PC1	39.7	39.7	35	1.0710	0.0306	$3.35\ 10^{-12}$
PC2	27.1	66.8	33	0.7323	0.0222	$3.23\ 10^{-7}$
PC3	15.2	82	31	0.4105	0.0132	$8.52\ 10^{-3}$
PC4	12.4	94.3	29	0.3347	0.0115	0.04
PC5	5.7	100	27	0.1528	0.0057	0.80

AMMI Stability Value (ASV) and Genotype Selection Index (GSI)

Estimated ASV index values ranged from 0.0245 (Paraíso 20) to 0.4273 (SPS 3109) (Table 1). In their paper, Purchase *et al.* (2000) did not suggest the most extreme ASV value from which genotypes with or without stability were determined. Therefore, in our work we considered stability in sunflower hybrids to those ones who have weighted contribution to the GxE interaction effect at a rate lower than 0.1. Consequently, there were seven hybrids accomplishing this requirement: Paraiso 20, Paraiso 22, ACA 863, MG 60, Tehuelche CL, CF 31 and Pampero DM.

The calculated GSI index values varied from 7 (Paraiso 22) to 63 (ACA 885) (Table 1). To determine the sunflower genotypes with low ASV value as well as moderate level of resistance we considered those ones whose GSI values were lower or equal than the first decile (18.4). So, the hybrids Paraiso 22, Paraiso 20, Tehuelche CL and MG 60, with RIP means across years higher than one, were selected.

CONCLUSIONS

The cumulative contribution of principal components 1 and 2 to the sum of squares of GxE interaction effect (66.8%) was 2.78 times that one explained by the linear regression (24%). Then, the AMMI model seemed to be more effective than the joint-regression analysis to evaluate the stability of sunflower hybrids by the RIP to white rot.

Further trials would allow describing better the sunflower hybrid capacity to do not change their relative RIP across different years in Balcarce. Under our experimental conditions, the selection index (GSI), derived from AMMI analysis, detected the hybrids Paraiso 22, Paraiso 20, Tehuelche CL and MG 60 having stability and moderately resistance to white rot.

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COLLECTION OF WILD *HELIANTHUS ANOMALUS* AND *DESERTICOLA* SUNFLOWER FROM THE DESERT SOUTHWEST USA

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ABSTRACT

Genetic resources are the biological basis of global food security. Collection and preservation of wild relatives of important crop species such as sunflower provide the basic foundation to promote and sustain the crop. Acquisition through exploration is the initial step in the germplasm conservation process. There are 53 species of wild *Helianthus* (39 perennial and 14 annual) native to North America. An exploration covering 3700 km to the desert southwest US in June of 2015 led to the collection of five populations of *H. deserticola* (desert sunflower) and eight *H. anomalus* (sand sunflower) accessions. All populations were collected throughout the broad distributional range of the species. Based on sand sunflower's occurrence in desert sand dune habitats of Utah and Arizona, it frequently has been recognized as drought tolerant, with the largest achenes of any wild species and high oil concentration potential, and thus is a candidate for improving cultivated sunflower. Desert sunflower is a xerophytic annual species found in sandy soils on the floor of the Great Basin Desert in small populations in western Nevada, west central Utah, and along the border of Utah and Arizona. Population size, habitat, soil type, seed set, the presence of diseases and insects, and other wild sunflower species located near the collection sites were recorded for each population. This germplasm will be important now and in the future as a genetic resource to combat emerging pests and environmental challenges, helping maintain sunflower as a viable global crop and to preserve it for future generations.

Key words: Sunflower, Crop wild relatives, Wild species, Germplasm resources, Exploration

INTRODUCTION

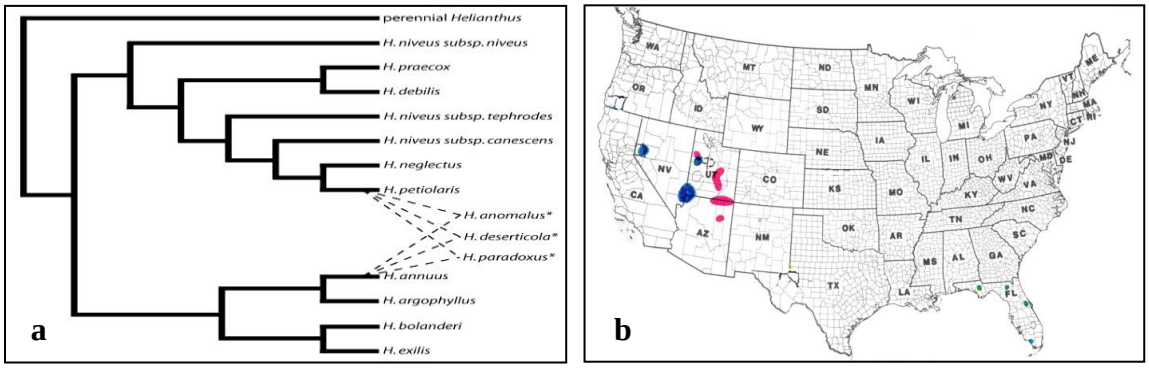
Collection and preservation of wild relatives of important crop species such as sunflower provide the basic foundation to promote and sustain the crop. Genetic resources are the biological basis of global food security, and acquisition through exploration is the initial step in the germplasm conservation process. There are 53 species of wild *Helianthus* (39 perennial and 14 annual) native to North America (Heiser et al., 1969; Schilling, 2006). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continuous source of agronomic and economic traits for cultivated sunflower (Seiler and Rieseberg, 1997; Seiler and Marek, 2011; Kane et al., 2013; Seiler and Jan, 2014). In a survey of the use of wild relatives in crop improvement over a 20 year period, among 13 crops of international importance, sunflower ranked fifth with seven traits incorporated (Hajjar and Hodgkin, 2007).

Helianthus anomalus (sand sunflower) is a rare endemic species adapted to sand dune and swale habitats in Utah and northern Arizona (Heiser, 1958, Heiser et al., 1969, Thompson et al., 1981; Nabhan and Reichhardt, 1983). It is a confirmed homoploid diploid hybrid species based on comparison of isozyme, nuclear ribosomal DNA, and cpDNA with its parental species, *H. annuus* and *H. petiolaris* that occupies an extreme environment relative to its parental species (Rieseberg, 1991; Gross et al., 2004; Ludwig et al., 2004)(Fig. 1a). *Helianthus annuus* is distributed throughout the central and western United States and typically inhabits heavy, clay-based soils. *Helianthus petiolaris*, the smaller of the two parental species, is distributed mainly through the central United States and inhabits sandier soils than *H. annuus*. The two parental species co-occur and often hybridize throughout their range. The species are all annual,

outcrossing, and have a haploid chromosome number of 17 (Heiser, 1947; Heiser et al., 1969; Rogers et al., 1982). *Helianthus anomalus* has been frequently recognized as drought tolerant, with the largest achenes of any wild species and high oil concentration potential (Seiler, 2007), and thus is a candidate for improving cultivated sunflower (Nabhan and Reichhardt, 1983; Seiler and Marek, 2006). It also appears to be more tolerant of nutrient stress than its ancestral parents based on a lower relative growth rate and higher nutrient-use efficiency (Brouillette and Donovan, 2011).

Helianthus deserticola (desert sunflower) is a xerophytic species found in sandy soils on the floor of the Great Basin Desert and distributed in small populations located in western Nevada, west central Utah, and along the border of Utah and Arizona, USA (Heiser et al., 1969) (Fig. 1b). It is also a homoploid diploid annual hybrid between two annual parental diploid species, *H. annuus* and *H. petiolaris* (Rieseberg, 1991, Gross et al., 2004). This species inhabits the desert floor, an extreme environment relative to its parental species (Gross et al., 2004) (Fig. 1a). Based on desert sunflower's occurrence in sand dune desert habitats, it frequently has been recognized as drought tolerant with high oil concentration potential, and thus a candidate for improving cultivated sunflower germplasm (Seiler, 1992; Seiler, 2007). Both species are excellent candidates for diversifying the genetic base of cultivated sunflower by enhancing oil concentration and quality improvement, as well as drought tolerance.

Figure 1a. Evolutionary relationships among annual *Helianthus* species. Homoploid hybrid species *H. anomalus* and *H. deserticola* are indicated with asterisks. Figure is redrawn from Gross et al., 2005, and based on combined nuclear ribosomal and chloroplast DNA data reported by Rieseberg, 1991; and, **1b.** Distribution of *Helianthus anomalus* (sand sunflower) in Utah and Arizona, and *H. deserticola* (desert sunflower) in Nevada, Utah and Arizona in the desert southwest US.



Unfortunately, very few populations of *H. anomalus* and *H. deserticola* have been collected and only a few are available for research purposes from the USDA-Agricultural Research Service, National Plant Germplasm System (NPGS) wild sunflower germplasm collection. Also, it is very difficult to regenerate the limited number of original seed from some of the earlier collected accessions. The objective of the study was to undertake an exploration to the desert southwest USA in Utah and Arizona in June to collect the winter-spring populations instead of the summer-fall populations previously collected in September-October of the two desert species, *H. anomalus* and *H. deserticola*, and preserve them for future generations to combat emerging pests and environmental challenges, helping to maintain sunflower as a viable and competitive global crop. The exploration was supported with funding from the Plant Exchange Office, National Germplasm Resources Laboratory, USDA-ARS, Beltsville, MD.

MATERIALS AND METHODS

The sunflower exploration for *H. anomalus* and *H. deserticola* took place from June 14 to June 22, 2015. Some populations were revisited in late July-early August 2015 to collect additional seed, and two additional populations without mature seed in June were collected. The exploration covered 3700 km in two states, Utah and Arizona. Seed heads were collected from 20 to 250 plants within each population

and bulked into a single sample. Herbarium specimens were deposited in the USDA-ARS wild *Helianthus* herbarium at Fargo, ND. The achene samples were deposited at the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, where they are maintained and distributed.

All populations were collected from the restricted distributional range of the species, Utah and Arizona for *H. anomalus*, and Utah, Arizona and Nevada for *H. deserticola* (Fig. 1b). Prior locations, generalized distribution maps, and herbaria voucher records were used to locate populations in cooperation with local natural resource officials and botanists who provided valuable information about the current year population distributions and status of the two species. Landownership was determined and all necessary permits were obtained for seed collection and inclusion of the seed in the NPGS genebank. Population size (number and extent), habitat, soil type, seed set per head, and the presence of diseases, insects, and other wild sunflower species were recorded for each population.

RESULTS AND DISCUSSION

The exploration was successful in collecting 10 representative populations of *H. anomalus* from its distributional range in Utah and Arizona (Table 1). A single population of *H. anomalus* was located in Arizona, but the plants were just flowering and no seeds were collected. It had been 15 years since this species was last collected for the NPGS (Seiler and Brothers, 2003). Attempts to recollect this endemic species over the last quarter century have met with mixed results. In September of 2000, none of 12 populations collected in the October of 1980 could be relocated in the fragile sandy habitats (Seiler and Brothers, 2003). The species appears to be very sensitive to the prevailing fall-winter and spring-summer moisture conditions. The current exploration during June located numerous populations of sand sunflower probably due to the excessive spring rains in several parts of the species' distributional range.

Figure 2 shows the typical habitat of the only population of *H. anomalus* we located in Arizona. Unfortunately, only a few plants were observed with no mature seeds to collect. Figure 3 shows one of the diverse habitats in Utah where a typical *H. anomalus* plant with multiple branches and heads, light shiny green leaves, and whitish stems grows on top of sandy hummocks with the wind causing the sand to shift and appear as waves in the sand. Figure 4 shows the unique tap root that develops to help plants survive the constant shifting sand on the dunes. Figure 5 shows a unique habitat for *H. anomalus* in a draw on the steep slope of a shifting sand dune. Figure 6 shows dried white plant stalks from the previous season(s) confirming a persistent and thriving population.

The exploration was also successful in collecting five representative populations of *H. deserticola* from its distributional range in Utah and Arizona (Table 1). *Helianthus deserticola* was not collected in Nevada because of restricted access to the areas where the species occurs. In September of 2000, an attempt to recollect several populations previously collected in October of 1980 was not successful in the fragile sandy sagebrush habitat probably due to the extremely dry 2000 growing season, although one new population was discovered (Seiler and Brothers, 2003). As with *H. anomalus*, the species appears to be very sensitive to the prevailing fall-winter and spring-summer moisture conditions. The current exploration during June 2015 located several populations of desert sunflower mainly due to the excessive spring rains in several parts of the species' distributional range.

Table 1. *Helianthus anomalus* and *H. deserticola* identification number, elevation, location, habitat, and population size collected in June 2015.

Identification Number	Elevation (m)	Location	Habitat	Population Size
ANO-2810	1310	**Utah;** San Juan Co., SE of Cal Black Memorial Airport	Shifting sand dunes, roadside	200
ANO-2811	1450	**Utah;** San Juan Co., Nokai Dome Rd, SE of Halls Crossing	Shifting sand dunes, roadside	750
ANO-2813	1147	**Utah;** Garfield Co., Notum-Bullfrog Rd	Shifting sand dunes, roadside	1,000
ANO-2815	1769	**Utah;** Kane Co., Hole-in-the-Rock Rd, Grand Staircase Escalante National Monument	Shifting sand dunes, roadside	200
ANO-2817	1394	**Utah;** Garfield Co., unnamed draw into North Wash, west side of Hwy 95	Shifting sand dunes, steep slope	250
ANO-2818	1425	**Utah;** Wayne Co., Near Hanksville	Shifting sand dunes, roadside	200
ANO-2819	1661	**Utah;** Wayne Co., Lower San Rafael Rd	Shifting sand dunes	100
ANO-2820	1565	**Utah;** Emery Co., Hans Flat Rd	Shifting sand dunes	1,000
ANO-2821	1231	**Utah;** Grand Co, White Wash Dunes	Shifting sand dunes	1,000
ANO-2822	1532	**Utah;** Emery Co, west side of Hwy 24	Shifting sand dunes	1,000s
DES-2802	1214	**Utah;** Kane Co., Beside High Desert Lodge, Big Water	Sandy desert shrub pasture	750
DES-2803	1290	**Utah;** Kane Co., End of Church Wells Rd, Grand Staircase Escalante Natl. Monument	Sand dunes, near pasture	500
DES-2805	1261	**Utah;** Kane Co., Jacobs Tanks Rd, west of Grand Staircase Escalante National Monument visitor center	Sandy swale	500
DES-2806	1294	**Arizona;** Coconino Co., Vermilion Cliffs Natl. Monument; Ferry Swale Wash	Undulating swale wash, sandy soil	1,000
DES-2807	1295	**Arizona;** Coconino Co., Southeast of Page	Sandy roadside ditch	1,000

Figure 7 shows the typical habitat of a population of *H. deserticola* located in southern Utah. While this species shares some of the habitat types of *H. anomalus*, the main difference is that it is found on the floor of the Great Basin desert in sandy soils interspersed mainly with sagebrush and other desert shrubs. Figure 8 shows one of the diverse habitats where *H. deserticola* grows in sandy soils and hummock type of topography near desert shrubs. Figure 9 shows typical plants with multiple branches and heads, dull green leaves and darker greenish-red pubescent lower stems. Figure 10 shows the unique habitat in an undulating swale wash in sandy soil among the desert shrubs. Figure 11 shows a unique habitat of *H. deserticola* scattered in a sandy pocket on an undulating swale wash underlain by shale rock.

Figure 2. Gerald Seiler standing next to the largest *Helianthus anomalus* plant in a very small population found near Dennehosto, AZ in a shifting sand dune. Only found a few plants were found with no mature seed to collect.

Figure 3. *Helianthus anomalus* (ANO-2810) on hummock sand dunes in San Juan County, UT, SE of Cal Black Memorial Airport. Notice the wave pattern in the sand from the wind shifting the sand in the dunes. Typical plants with multiple branches, light shiny green leaves, and whitish stems.

Figure 4. Population ANO-2813 in Garfield County, UT along Notum-Bullfrog Rd in shifting sand dunes near roadside. Notice the distorted exposed roots that developed to anchor the plant in the actively shifting sand dunes.

Figure 5. Population ANO-2817 in Garfield County, UT, North Wash, west side of Hwy 95, with sunflowers growing in a draw of a steep slope of a shifting sand dune.

Figure 6. Laura Marek collecting seed in population ANO-2813 in Garfield County, UT. Along Notum-Bullfrog Rd, in shifting sand dunes. Note the dead white plant stalks from previous season(s).

Figure 7. *Helianthus deserticola* (DES 2802) in Kane Co., UT near Big Water UT in a typical desert shrub habitat interspersed among the shrubs in open sandy areas.

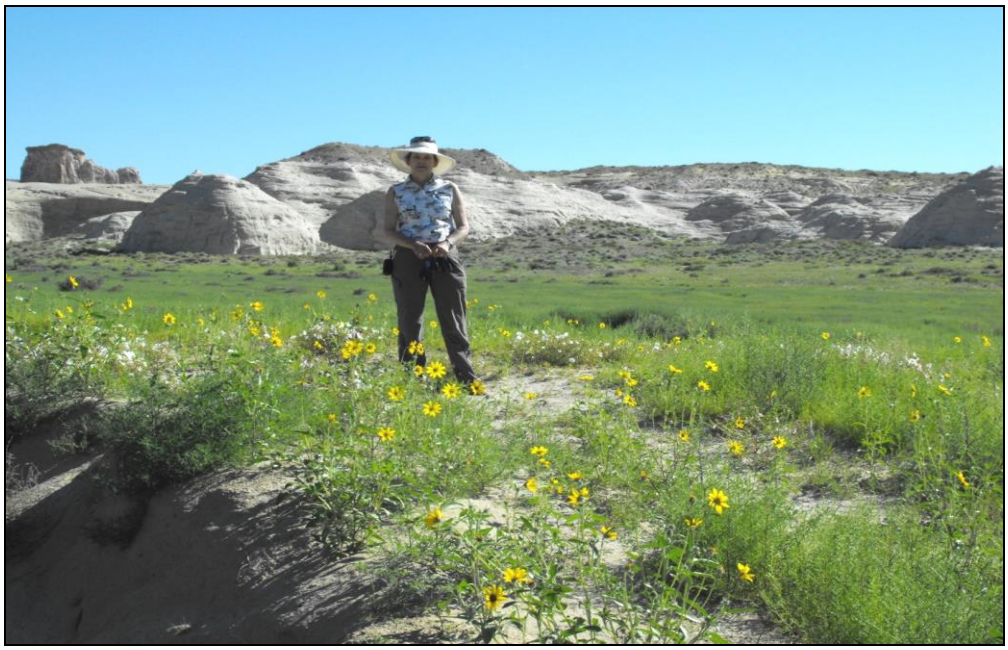


Figure 8. Laura Marek collecting seed of population DES 2803 in Kane Co., at the end of Church Wells road, west of Big Water, UT. Note the different habitat with more grayish sandy soils and hummock type topography in the background near desert shrubs.

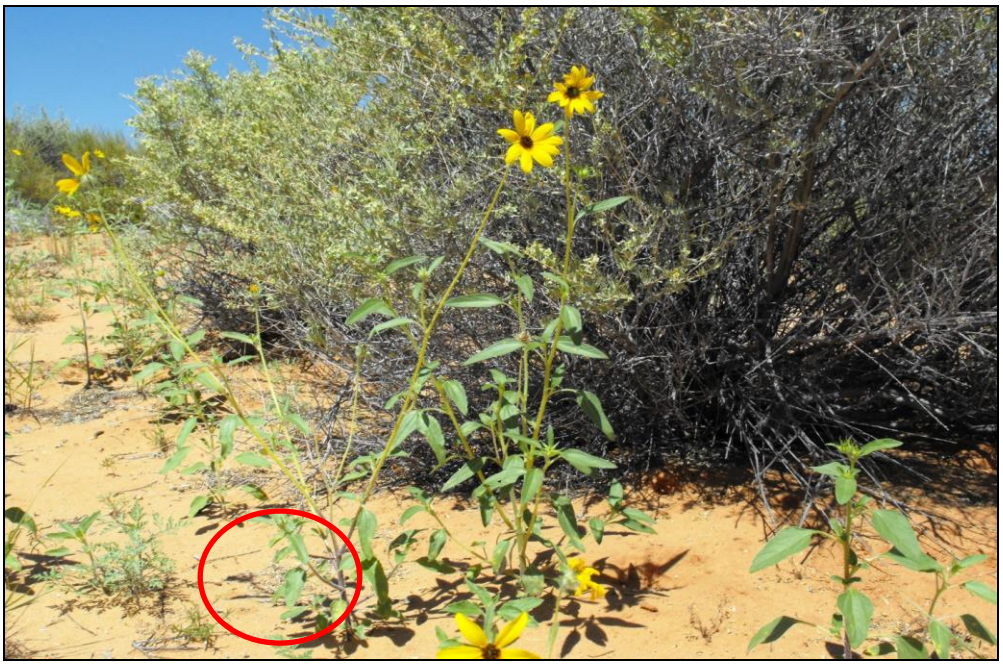


Figure 9. Population DES-2805 in Kane Co., UT, along Jacobs Tank Rd, west of the Grand Staircase Escalante National Monument (BLM), sandy pocket among the desert shrubs. Typical plant with multiple branches, dull green leaves, and darker greenish-red pubescent lower stems.

Figure 10. Population DES-2806 in Coconino Co., AZ, Ferry Swale Canyon, Vermillion Cliffs National Monument (BLM) in an undulating swale wash in sandy soil among the desert shrubs.

Figure 11. Gerald Seiler collecting seed of DES-2806 in Coconino Co., AZ, Vermillion Cliffs National Monument (BLM) in sandy soil in the undulating swale wash among the desert shrubs. Note the white shale outcropping in the background that underlies this area.

CONCLUSION

The addition of 10 *Helianthus anomalus* and five *H. deserticola* populations to the NPGS wild sunflower germplasm collection represents the first germplasm of these species collected Utah and Arizona in almost 15 years. The added populations are important as a genetic resource to combat emerging pests and environmental challenges, helping to maintain sunflower as a viable and competitive global crop and to preserve it for the future generations.

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF 400 NEW SUNFLOWER PRE-BRED LINES

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ABSTRACT

Crop wild relatives are key genetic resource for the continued improvement and diversification of sunflower. Here we describe the development of new pre-bred lines for sunflower, as well as the phenotypic and genotypic characterization of these lines. We created circa 400 pre-bred lines, each of which contain introgressions from a wild *Helianthus* genotype. The wild samples were selected to encompass as much genetic diversity as possible, and the rounds of backcrossing and selfing did not include any intentional selection. This approach was taken to maximize the wild diversity introduced into the crop gene pool for evaluation. All lines are freely available to the sunflower community under the standard material transfer agreement. Collaborators at the Uganda National Agriculture Research Organization (NARO) and SOLTIS (France) evaluated these lines, and analyses show that they contain a great deal of promising variation for valuable traits such as drought tolerance, disease resistance and flowering time. Additionally, 55 previously developed pre-bred lines were evaluated at UBC. The multi-locus genotypes of each line were assessed with genotyping by sequencing, and the number, size and locations of the wild introgressions were identified. Patterns of introgression across the genome are discussed.

Key Words : wild sunflowers, introgression, germplasm, genotyping by sequencing, phenotypic characterization

THE EVALUATION OF ANNUAL WILD *HELIANTHUS* SPECIES FOR THEIR MORPHOLOGICAL, PHENOLOGICAL AND SEED CHEMICAL CHARACTERISTICS IN FIELD CONDITIONS

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ABSTRACT

Twenty-seven wild annual *Helianthus* species and subspecies, from the USDA-ARS North Central Plant Introduction Station sunflower collection were evaluated in field trials for several morphological and phenological characteristics, concentrations of seed protein and oil, and fatty acid profile during 2012 and 2013. These were *H. agrestis*, *H. annuus* Ames 4114, *H. annuus* Ames 7111, *H. annuus* Ames 29273, *H. annuus* Ames 29348, *H. anomalus*, *H. argophyllus*, *H. bolanderi*, *H. debilis* ssp. *cucumerifolius*, *H. debilis* ssp. *debilis*, *H. debilis* ssp. *silvestris*, *H. debilis* ssp. *tardiflorus*, *H. debilis* ssp. *vestitus*, *H. deserticola*, *H. exilis*, *H. neglectus*, *H. niveus*, *H. niveus* ssp. *canescens*, *H. niveus* ssp. *tephrodes*, *H. petiolaris*, *H. petiolaris* ssp. *fallax*, *H. petiolaris* ssp. *petiolaris*, *H. porteri*, *H. praecox*, *H. praecox* ssp. *hirtus*, *H. praecox* ssp. *praecox*, *H. praecox* ssp. *runyanii*. The flowering dates of *H. argophyllus* in 2012 and 2013 were the latest with 202.00 and 202.33 days, respectively. The earliest flowering dates were observed in *H. debilis ssp. cucumerifolius* (91 days), *H. praecox* (91.00 days) and *H. praecox* ssp. *hirtus* (91.67 days) in 2012, and *H. annuus* Ames 4114 (105.33 days) in 2013. *H. argophyllus* had also the highest plant heights in both years with 325.67 and 303.0 cm. Oil concentrations of annual wild sunflower species changed between 8.02 and 31.16%. The highest linoleic acids were observed in *H. debilis* ssp. *tardiflorus* (66.77%) in 2012 and *H. praecox* ssp. *praecox* (69.13 %) in 2013. Seed protein contents changed from 6.41 to 37.05 % depend on genotypes and year.

Key words: *Helianthus* ssp., oil fatty acids, seed protein, yield components, flowering date

INTRODUCTION

Sunflower is one of the world's main sources of plant-based oil. It is also remarkably widely grown crop. Sunflowers were grown on 25 million hectares of worldwide in 2013 (FAO, 2014). Its oil ranks among the best quality edible vegetable oils and its essential fatty acids are an important part of the human diet (Skoric et. al. 2008). Common sunflower is also an important crop grown globally for production of cut flowers, fuel, commercial fiber, and seeds for snacks and bird food. Originally it was domesticated from the self incompatible common sunflower approximately 4,000 years ago. Sunflowers have historically been used by humans as a nutritious food source, a medicinal treatment for many ailments, and as a dye for body paint and coloring basketry. (Mandel et al., 2011; Anonymous, 2014).

Major goals in *sunflower breeding* remain high seed and oil yield, improved oil quality, as well as resistance to different stresses (Kaya et al., 2012). The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continued source of agronomic traits for crop improvement. The main reason for establishing a collection of wild sunflower species is the reduced genetic variability of the domesticated sunflower for a number of agronomic characteristics. The transfer of useful characters from *Helianthus* species to the cultivated sunflower started at the beginning of the 20th century and continues nowadays purposefully (Christov, 2008). In recent years, interspecific hybridization has been extensively applied in sunflower breeding. Wild sunflower species have been used as sources of desirable genes for a number of characteristics. Many

traits dealing with morphology, architecture, and disease resistances have been transferred from *Helianthus* species to sunflower. The genetic researches on the developing of new CMS - restorers of fertility have contribute to enrich diversity and to increase heterosis in sunflower (Atlagic et al., 2006; Seiler, 2007; Nooryazdan et al., 2010; Whitney, et al., 2010). Wild species are a potentially important source of abiotic tolerance; therefore, it may be desirable to introgress drought and salinity tolerant genes from wild relatives. They also contains considerable variability for biotic stress such as disease, insect pest resistance. Similarly, fatty acid composition and protein quality can also be modified by introgression from wild species. The increase in sunflower production and seed quality has been largely connected to the inclusion of wild *Helianthus* species into the improvement work on sunflower (Fernandez-Martinez, 1991; Perez et al., 2007; Christov, 2008; Nooryazdan et al., 2010)

Although, interest in using wild species in breeding programs has increased, the limited genetic variability in cultivated sunflower has slowed the future improvement of the crop, and has placed the crop in a vulnerable position should any major shifts of disease races or pests occur. In order to be competitive with other premium oils in the market, the reduction of saturated fatty acids in sunflower oil will play a key role in maintaining our continuing success. Evaluations of wild species have provided information about useful genes for future sunflower improvement. However, there are still numerous genes in wild sunflower species yet to be identified and introgressed into cultivated sunflower. The understanding of wild *Helianthus* species will increase the number of useful genes available from wild *Helianthus* species, making it possible to transfer cultivated sunflower (Jan et al., 2008, Baute et al., 2015).

In the present study, we focus on the evaluation of annual wild *Helianthus* species for their morphological, phenological and seed chemical characteristics in field conditions to provide useful features for future sunflower improvement.

MATERIALS AND METHODS

In this research, twenty-seven wild annual *Helianthus* species and subspecies getting from the USDA-ARS North Central Regional Plant Introduction Station-Iowa State University were used as materials. Their names and origins are given in Table 1. In this research, annual wild *Helianthus* species for were evaluated their morphological, phenological and seed chemical characteristics in field conditions of 2012 and 2013.

In the first year, seeds of wild sunflower were sown into multiple pots in the glasshouse on March 13, 2012, and their seedling were planted into fields on April 25, 2012. The second year, sowing time of seeds into multiple pots and planting time of seedlings on field were March 12, 2013 and May 17, 2013, respectively. The distance of plants on the rows and between rows were one meter. Irrigation was not applied into fields in both years except planting time of seedlings, and weeds were cleaned by hoeing.

The experiments were carried out on the field of the Faculty of Agriculture at Namık Kemal University in Tekirdağ, Turkey (40º59´N, 27º33´E, elevation 3 m), on soil with clay loam and low organic matter content in 2012 and 2013.

Table 1. *Helianthus* species and subspecies in this research, and their origin's

	Helianthus species and subspecies	Origin's
1	*H. agrestis*	Florida, USA
2	*H. annuus* Ames 4114	North Dakota, USA
3	*H. annuus* Ames 7111	California, USA
4	*H. annuus* Ames 29273	Texas, USA
5	*H. annuus* Ames 29348	South Australia, Australia
6	*H. anomalus*	Utah, USA
7	*H. argophyllus*	Texas, USA
8	*H. bolanderi*	California, USA
9	*H. debilis* ssp. *cucumerifolius*	Texas, USA

10	*H. debilis* ssp. *debilis*	Florida, USA
11	*H. debilis* ssp. *silvestris*	Texas, USA
12	*H. debilis* ssp. *tardiflorus*	Florida, USA
13	*H. debilis* ssp. *vestitus*	Florida, USA
14	*H. deserticola*	Nevada, USA
15	*H. exilis*	California, USA
16	*H. neglectus*	New Mexico, USA
17	*H. niveus*	Arizona, USA
18	*H. niveus* ssp. *canescens*	Utah, USA
19	*H. niveus* ssp. *tephrodes*	Mexico
20	*H. petiolaris*	South Dakota, USA
21	*H. petiolaris* ssp. *fallax*	New Mexico, USA
22	*H. petiolaris* ssp. *petiolaris*	Oklohama, USA
23	*H. porteri*	Georgia, USA
24	*H. praecox*	Texas, USA
25	*H. praecox* ssp. *hirtus*	Texas, USA
26	*H. praecox* ssp. *praecox*	Texas, USA
27	*H. praecox* ssp. *runyanii*.	Texas, USA

The main chemical characteristics of soils are shown in Table 2. Organic matter in the research area was very low, and changed between 0.92 and 1.37 % according to different depths. Phosphorus (P), potassium (K) and pH of soils ranged from 55.9 to 108.3 kg/ha, 124.5 to 209.6 ppm and 7.78 to 7.85, respectively.

Table 2. Some chemical properties of the experimental field soil

Depth (cm)	SOM (%)	WS (%)	EC µS cm^{-1}	PH	Lime (%)	P_2O_5 kg/ha	K ppm	Ca ppm	Cu ppm	Fe ppm	Mn ppm	Mg ppm	Zn ppm
0-30	1.37	42	866	7.78	1.82	108.3	209.6	6076	0.75	3.81	8.83	240.6	0.15
30-60	1.18	43	720	7.82	3.71	72.6	151.3	6055	0.67	3.62	6.60	246.9	0.10
60-90	0.92	43	631	7.85	8.06	55.9	124.5	5911	0.62	3.62	7.08	263.2	0.09

SOM = soil organic matter, WS = soil water saturation

Climatic data during growing periods of *Helianthus* ssp. in 2012 and 2013 are given in Table 3. Generally, the values of rainfall, relative humidity and temperature in the vegetative growth period and flowering duration of wild sunflower genotypes in the first year field conditions were higher than in 2013 except June rainfall and May temperature.

Table 3. Climatic data during growing periods of *Helianthus* ssp. in 2012 and 2013

Month	Rainfall (mm)		Relative humidity (%)		Temperature (°C)		The highest temperature (°C)		The lowest temperature (°C)		Sunshine duration (hour/day)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
March	18.0	52.8	81.8	98.5	7.9	9.6	12.3	13.5	3.6	5.9	6.3	4.5
April	61.4	16.0	82.4	84.8	14.1	13.5	19.3	17.7	9.6	9.4	7.4	6.7
May	62.4	8.0	91.2	69.7	18.1	19.5	22.5	23.8	14.2	15.1	7.1	9.4
June	0.2	35.0	78.2	68.7	24.1	22.4	28.4	26.7	18.9	18.1	10.9	8.4
July	6.0	0	68.7	61.4	27.0	24.7	31.5	28.8	22.1	20.0	10.6	10.5
August	7.8	0.2	62.7	62.3	26.0	25.9	31.1	30.1	20.9	21.7	10.3	9.6
September	8.4	10.2	73.6	61.4	22.2	21.6	26.6	25.6	18.1	16.9	8.1	8.4
October	54.0	96.4	87.3	76.2	19.2	14.3	23.5	17.9	15.1	10.4	6.5	6.5
November	24.8	36.6	97.0	79.0	13.7	12.9	16.9	15.9	10.7	9.6	3.4	3.6
December	184.6	2.4	97.3	74.1	6.4	6.2	9.7	9.7	3.1	3.0	2.6	2.7

Statistical analysis was conducted according to Standard procedures for a randomized complete block design with three replications. The SAS System was used to generate the analysis of variance (ANOVA) for determining treatment effects on the dependent variables (SAS Institute, 1997). Treatment mean comparisons were based on F-Protected Least Significance Differences (LSD) comparisons at $P \leq 0.05$.

RESULTS AND DISCUSSION

In this research, twenty-seven wild annual *Helianthus* species and subspecies were grown under field conditions in 2012 and 2013. But some of them such as *H. deserticola* dried during vegetative growth period under field conditions depend on ecological conditions of 2012 or 2013. Table 4 shows the results of variance analyses on fifteen morphological, physiological, yield and quality components of wild sunflower species.

Analysis of data identified that genotype and genotype x year interactions were highly significant ($p < 0.01$) for all characters. Year effects on the characters except plant height. were also significant. Therefore, data of all character were analyzed separately for each year in the study.

Statistical significant groups by the LSD test at $P < 0.05$ for plant height, canopy cover diameter of single plant and head number per plant are given in Table 5. Although genotypes displayed variable plant height between 2012 and 2013, *H. argophyllus* had the highest plant height with 325,7 cm in 2012 and 303 cm in 2013. The first year; *H. debilis* ssp. *vestitus*, *H. debilis* ssp. debilis, H. *praecox* ssp. *praecox* and *H. debilis* ssp. *tardiflorus* were the same latest group for plant height. For canopy cover diameter of single plant, the highest value was measured in *H. annuus Ames* 29273 although *H. debilis* ssp. *cucumerifolius*, *H.* neglectus, H. *bolanderi*, *H. annuus* Ames 29273, *H. praecox* ssp. *runyanii*, *H. debilis*

ssp. *tardiflorus* and *H. debilis* ssp. *vestitus* had the highest values in 2013. Head number per plant in 2013 was changed from 5 to 800. The highest head number per plant was counted on *H. debilis* ssp. *cucumerifolius. H. neglectus*, *H. praecox* ssp. *praecox*, *H. praecox, H. praecox* ssp. *runyanii* and *H. debilis* ssp. *tardiflorus* were also the same LSD groups.

Table 4. Analyses of variance of some morphological, physiological, yield and quality components of wild sunflower species under the field conditions in 2012 and 2013

	Genotype	Year	GenotypexYear	CV
Plant height (cm)	26543.64**	978.98ns	1920.42**	16.53
Canopy cover diameter of single plant (cm)	5910.17**	17368.25**	3166.62**	17.81
Head number per plant+	127995.46**			
Head diameter (cm)	45.43**	33.67**	13.57**	37.13
1000 seed weight (g.)	2409.99**	116.48**	92.71**	36.52
The first flowering day number	3275.72**	10360.63**	125.56**	4.77
50 % flowering day number	4879.93**	40480.63**	645.04**	4.71
Flowering period day	5612.44**	1400.82**	1060.68**	9.22
Seed yield per plant (g.) +	9305.93**			
Seed protein content (%)+	40.04**			10.25
Seed oil content (%)	96.16**	446.57**	70.72**	0.94
Oleic acid content of seed oil (%)	350.66**	481.78**	57.90**	0.87
Linoleic acid content of seed oil (%)	235.73**	446.55**	44.58**	0.54
Palmitic acid content of seed oil (%)	6.45**	0.07**	1.07**	0.71
Stearic acid content of seed oil (%)	2.87**	0.12**	0.54**	0.78

**: Significant differences are shown at $P < 0.01$ based on ANOVA. Statistical analysis was made only in 2013

Head diameter of wild sunflower species was changed from 1.1 to 18.3 cm in 2012 and 1.1 to 6.4 cm in 2013 (Table 6). *H. annuus* Ames 4114 had the biggest heads in both years. Table 6 also shows 1000 seed weights of genotypes. 1000 seed weight had the highest variable among wild annual sunflower species changing from 1.10 to 101.20 g. Generally *H. debilis* subspecies gave the lowest seed weights while *H. annuus* genotypes had the highest weights. Previous researchers for morphological characters, in annual wild sunflower species had similar results in this research (Seiler, 1997; Seiler and Rieseberg, 1997; Skoric, 2009, Onemli and Gucer, 2010).

Table 5. Plant height, canopy cover diameter of single plant and head number per plant of wild sunflower species

Helianthus species and subspecies	Plant height (cm)		Canopy cover diameter of single plant (cm)		Head number/plant
	2012	2013	2012	2013	2013
H. annuus Ames 4114	138.3ef	79.3j	100.7gh	37.7g	5.0e
H. annuus Ames 7111	187.7cd	165.3efg	210.0bc	143.7bcde	117.0cde
H. annuus Ames 29273	251.0b	234.7b	274.0a	157.3abc	203.7bcde
H. annuus Ames 29348	166.3cde	228.0bc	123.3efgh	129.0cdef	223.70bcde
H. argophyllus	325.7a	303.0a	156.0de	125.7cdef	228.0bcde
H. bolanderi	194.3c	198.7cd	175.0cd	159.7abc	479.0abcd
H. debilis ssp. *cucumerifolius*	118.7fgh	192.7de	152.7def	192.3a	800.7a
H. debilis ssp. *debilis*	80.3hi	61.3j	147.0def	95.7f	83.7de
H. debilis ssp. *silvestris*	145.0def	89.7ij	155.0de	112.3def	221.3bcde
H. debilis ssp. *tardiflorus*	108.0fghi	136.7gh	150.0def	156.0abcd	417.0abcde
H. debilis ssp. *vestitus*	71.0i	78.3j	143.3defg	151.7abcde	217.70bcde
H. exilis	105.7fghi	[redacted]	93.7h	[redacted]	
H. neglectus	188.0cd	171.7def	150.0def	183.0ab	593.3ab
H. petiolaris	197.7c	155.7fg	220.0b	112.0ef	245.0bcde
H. praecox	102.3fghi	91.0ij	176.0bcd	126.3cdef	501.7abc
H. praecox ssp. *hirtus*	84.0ghi	89.0ij	110.0fgh	135.3cdef	297.7bcde
H. praecox ssp. *praecox*	81.7hi	62.7j	139.0defgh	133.3cdef	536.7ab
H. praecox ssp. *runyanii*	130.7efg	121.0hi	170.0cd	157.3abc	418.3abcde
LSD	46.88	32.18	44.54	43.69	413.35

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$

Table 6. Head diameter and seed weight of wild sunflower species

Helianthus species and subspecies	Head diameter (cm)		1000 seed weight (g.)	
	2012	2013	2012	2013
H. annuus Ames 4114	18.3a	6.4a	101.20a	69.20a
H. annuus Ames 7111	6.5b	3.2c	12.50b	10.13b
H. annuus Ames 29273	5.8b	4.1b	4.40d	6.50bcd
H. annuus Ames 29348	7.6b	4.4b	12.80b	9.20bc
H. argophyllus	2.3c	3.2b	9.00c	6.53bcd
H. bolanderi	2.2c	2.3d	3.20e	5.60bcd
H. debilis ssp. *cucumerifolius*	1.9c	2.2e	1.30ij	2.86bcd
H. debilis ssp. *debilis*	1.1c	1.1h	1.40hij	0.73d
H. debilis ssp. *silvestris*	1.7c	1.8efg	1.60ghi	1.03d
H. debilis ssp. *tardiflorus*	1.4c	1.6fgh	1.10j	1.33d
H. debilis ssp. *vestitus*	1.4c	1.3gh	1.20ij	0.90d
H. exilis	1.8c	[redacted]	1.90fg	[redacted]
H. neglectus	2.2c	2.1def	1.40hij	2.93bcd
H. petiolaris	2.5c	2.3de	4.60d	3.77bcd
H. praecox	2.3c	1.8defg	1.80gh	1.90cd
H. praecox ssp. *hirtus*	1.6c	1.9def	1.40hij	1.37d
H. praecox ssp. *praecox*	1.9c	1.6fgh	1.80gh	1.43d
H. praecox ssp. *runyanii*	2.2c	1.9def	2.30f	1.23d
LSD	2.56	0.54	0.47	7.32

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$

H. argophyllus showed the latest flowering in both years (Table 7). This genotype flowered 202.0 and 202.3 day later from emerging in 2012 and 2013, respectively. Generally *H. praecox* subspecies had earlier flowering than the others. The longest flowering period was determined on *H. debilis* ssp. *vestitus* in 2012 while all *H. praecox* subspecies and *H. debilis* ssp. *vestitus* had the longest flowering in 2013. There were a few study on flowering with annual species. Generally they were on *H. annuus* and not similar detailed.

Seed yield per plant, seed protein content and seed oil content of wild sunflower species are given in Table 8. Generally *H. annuus* genotypes had higher seed yields per plant in 2013. Seed protein content of wild annual sunflower species was changed from 6.41 to 22.09 % in 2012, and from 14.01 to 44.13%

in 2013. In the first year, *H. praecox* ssp. *hirtus* gave the highest seed protein content while *H. exilis, H. petiolaris* ssp. *fallax, H. neglectus, H. debilis* ssp. *debilis and H. argophyllus* had the highest protein content in 2013.

Table 7. The first flowering day number, 50 % flowering day number and flowering period day of wild sunflower species

Helianthus species and subspecies	The first flowering day number		50 % flowering day number		Flowering period day	
	2012	2013	2012	2013	2012	2013
H. annuus Ames 4114	95.0h	105.3g	107.0j	122.0f	78.3m	40.7g
H. annuus Ames 7111	109.3cd	129.7cd	113.3gh	160.7cde	121.7i	120.3ef
H. annuus Ames 29273	108.0d	138.0bc	112.3h	161.7cde	128.3gh	126.0ef
H. annuus Ames 29348	103.0e	126.0cd	114.0g	164.7cd	127.7h	111.7f
H. argophyllus	202.0a	202.3a	221.0a	223.0a	50.0o	51.7g
H. bolanderi	102.0ef	122.7de	107.0j	162.7cde	130.3g	133.3def
H. debilis ssp. *cucumerifolius*	91.0i	125.7cd	97.0l	158.0cde	150.7c	132.7def
H. debilis ssp. *debilis*	108.3d	144.0b	158.0c	216.3a	156.7b	134.3def
H. debilis ssp. *silvestris*	111.0c	128.0cd	151.0d	169.7bc	91.0l	137.7cdef
H. debilis ssp. *tardiflorus*	101.7ef	130.0cd	141.7f	150.3de	101.3k	130.7def
H. debilis ssp. *vestitus*	103.0e	121.0def	144.0e	216.3a	162.7a	157.7abcd
H. exilis	129.0b	[redacted]	159.3c	[redacted]	73.0n	[redacted]
H. neglectus	98.0g	122.7de	143.3e	165.0cd	104.0j	142.7bcde
H. petiolaris	108.0d	126.3cd	164.7b	185.7b	134.7f	113.0f
H. praecox	91.0i	112.0efg	97.0l	149.3de	142.7d	164.3abc
H. praecox ssp. *hirtus*	91.7i	110.0efg	97.3l	146.7e	138.0e	168.3ab
H. praecox ssp. *praecox*	94.0h	108.0fg	101.7k	151.7de	141.3d	171.0a
H. praecox ssp. *runyanii*	101.0f	109.3fg	110.0i	154.0cde	120.0i	169.3ab
LSD	1.93	13.09	1.52	16.06	2.63	27.09

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.

Seed oil content of wild sunflower species in 2012 and 2013 were changed from 16.74 to 27.45 % and 8.02 to 31.16%, respectively. *H. argophyllus* in 2012 and *H. annuus* Ames 4114 in 2013 had the highest seed oil contents.

Table 8. Seed yield per plant, seed protein content and seed oil content of wild sunflower species

Helianthus species and subspecies	Seed yield per plant (g.)		Seed protein content (%)		Seed oil content (%)
	2013	2012	2013	2012	2013
H. agrestis			27.79+		
H. annuus Ames 4114	16.07c	10.69+	18.53def	24.86c	31.16a
H. annuus Ames 7111	51.67bc	19.24	17.34def	24.74c	21.53c
H. annuus Ames 29273	104.84b	13.54	14.01g	16.74h	16.68e
H. annuus Ames 29348	233.20a	14.25	22.33bc	25.76b	23.54b
H. anomalus			23.51		
H. argophyllus	15.50c	17.10	24.70ab	27.45a	8.02k
H. bolanderi	56.99bc	6.41	18.77de	21.37f	16.70e
H. debilis ssp. *cucumerifolius*	102.38b	17.10	17.34def	23.21d	14.52f
H. debilis ssp. *debilis*	1.57c	18.53	26.13a	23.14d	
H. debilis ssp. *silvestris*	15.41c	14.25	17.72def		13.82g
H. debilis ssp. *tardiflorus*	12.85c	7.84	18.53def	20.09g	9.29j
H. debilis ssp. *vestitus*	16.25c	13.57	17.10defg		10.68i
H. exilis		7.84	44.13	20.24g	
H. neglectus	56.30bc	6.41	26.84a		10.44i
H. niveus			19.95		
H. petiolaris	32.84c	14.96	18.29def	22.18e	18.02d
H. petiolaris ssp. *fallax*			37.05		
H. petiolaris ssp. *petiolaris*			17.81		
H. porteri			29.93		
H. praecox	23.75c	17.10	17.34def	22.26e	14.64f
H. praecox ssp. *hirtus*	28.93c	22.09	16.62efg		13.91g
H. praecox ssp. *praecox*	32.00c	17.10	19.95cd		16.92e
H. praecox ssp. *runyanii*	41.18bc	6.41	15.44fg		12.53h
LSD	64.97	3.28	3.29	0.26	0.33

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P < 0.05$.. + Values were not analyzed statistically.

Table 9 shows fatty acid compositions of *Helianthus* ssp. Oleic acid content of annual wild sunflower seed oil in 2012 and 2013 varied from 17.35 to 48.69% and 16.89 to 37.02%, respectively. Both of years, *H. annuus* Ames 7111 and *H. annuus* Ames 4114 had the highest oleic acid contents. Generally, oleic acid content of wild annual *H. annuus* species were higher than the other species. For high oleic acid content, this species was followed by *H. bolanderi*, *H. argophyllus* and *H. petiolaris*.

Table 9. Important oil fatty acids of the *Helianthus* ssp

Helianthus species and subspecies	Oleic acid (C18:1) content of seed oil (%)		Linoleic acid (C18:2) content of seed oil (%)		Palmitic acid (C16:0) content of seed oil (%)		Stearic acid (C18:0) content of seed oil (%)	
	2012	2013	2012	2013	2012	2013	2012	2013
H. annuus Ames 4114	42.46b	37.02a	46.38i	53.02l	6.01f	5.57j	3.94e	2.76o
H. annuus Ames 7111	48.69a	37.00a	41.39j	53.27l	5.17i	5.36k	2.67k	2.94n
H. annuus Ames 29273	38.65c	23.95f	51.39h	65.57e	5.11j	5.26l	3.33g	3.63i
H. annuus Ames 29348	36.31f	36.92a	53.99f	54.21k	4.82k	4.87m	2.79i	2.43p
H. argophyllus	37.12e	23.94f	52.92g	61.68i	5.66g	7.22e	2.72j	3.68h
H. bolanderi	38.13d	31.25b	51.38h	57.56j	5.23i	5.58j	3.13h	3.59j
H.debilis ssp. *cucumerifolius*	23.38i	27.10c	63.62c	62.55h	7.03d	5.81i	4.16d	3.94g
H. debilis ssp. *debilis*	17.35k		66.09b		9.88a		5.90a	
H. debilis ssp. *silvestris*		20.92h		66.93d		6.70g		4.33e
H. debilis ssp. *tardiflorus*	18.62j	17.91j	66.77a	68.63b	8.71b	7.67c	3.75f	3.53k
H. debilis ssp. *vestitus*		16.89l		68.12c		8.63a		5.12b
H. exilis	25.92h		59.41d		7.89c		4.92b	
H. neglectus		24.89e		65.07f		6.06h		3.09l
H. petiolaris	33.40g	26.45d	55.66e	63.20g	5.51h	5.58j	2.64k	3.05m
H. praecox	23.50i	22.06g	63.66c	62.05i	6.65e	7.69c	4.74c	5.22a
H. praecox ssp. *hirtus*		20.28i		66.59d		7.39d		4.56c
H. praecox ssp. *praecox*		17.51k		69.13a		7.92b		4.50d
H. praecox ssp. *runyanii*		20.98h		65.74e		7.13f		4.26f
LSD	0.48	0.34	0.44	0.49	0.07	0.07	0.04	0.04

*:Within each column, means followed by same small letters are not significantly different by the LSD test at $P< 0.05$.

Linoleic acid changed between 41.39 and 66.77% in 2012. *H. debilis* ssp. *tardiflorus* gave the highest linoleic acid content while *H. annuus* Ames 7111 had the lowest value. In the second year *H. praecox* ssp. *praecox* with 69.13% had the highest linoleic acid content while *H. debilis* ssp. *tardiflorus* was second wild sunflower genotypes. In contrast to oleic acid *H. annuus, H. bolanderi, H. argophyllus* and *H. petiolaris* gave the lower oil linoleic acid contents than the other species..

H. debilis ssp. *debilis* in 2012 and *H. debilis* ssp. *vestitus* in 2013 had the highest palmitic acid contents with 9.88% and 8.63%, respectively. *H. annuus* Ames 29348 in both of years gave the lowest palmitic acid contents.

Stearic acid content changed from 2.64 to 5.90% in 2012 and from 2.43 to 5.22% in 2013. *H. debilis* ssp. *debilis* in 2012 and *H. praecox* in 2013 had the highest stearic acid contents. Wild *H. annuus* genotypes except *H. annuus* Ames 29348 gave lower stearic contents than the other annual wild sunflower species in this research.

Although there were differences between 2012 and 2013 for observed characters in this research, really significant differences were found among annual species. Seiler (1998), Turhan et. al., (2010) and Onemli (2012a and 2012b) found effects of climate change on oil content and especially fatty acid composition of sunflower. Seiler (1998) also determined the lowest palmitic acid contents in *H. annuus* as in this study. In order to be competitive with other premium oils in the market, the reduction of saturated fatty acids in sunflower oil will play a key role in maintaining success

In previous studies, *Helianthus argophyllus* for drought tolerance breeding has been extensively used by the sunflower breeders (Rauf, 2008). Perez et al.(2007), studied to characterize and examine the use of wild sunflower species (*Helianthus petiolaris* Nutt), some physical properties and morphological characteristics of seeds from different locations in Argentina were determined. A collection of 168 accessions belonging to 62 species and subspecies was evaluated for fatty acid composition of the seed oil by De Haro and Fernandez-Martinez (1991) in Spain. Seventy-seven wild sunflower accessions in France for 13 quantitative characters were evaluated to assess the patterns of morphological and climatic variation (Nooryazdan et al., 2010). The wild *Helianthus* species were determined rich sources for genes determining resistance to different diseases, parasites, pests, drought and other important traits. (Christov, 2008)

In spite of these past successes, there are still numerous genes in wild sunflower species yet to be identified for cultivated sunflower breeding The understanding of wild *Helianthus* species will increase the number of useful genes available from wild *Helianthus* species, making it possible to transfer cultivated sunflower (Jan et al., 2008, Baute et al., 2015).

Generally previous studies in wild sunflower included limited number species with limited characters. In this study, almost all annual wild sunflower species were determined for their so many morphological, physiological, yield and quality components. Although the results were similar previously studies, there were a lot of new findings with especially flowering and seed quality characters in this research on a large wild annual sunflower species.

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PRINCIPAL COMPONENT ANALYSIS FOR CARBON ISOTOPE DISCRIMINATION-RELATED TRAITS IN RECOMBINANT INBRED LINES OF SUNFLOWER

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ABSTRACT

We used principal component analysis (PCA) as statistical method to analyze and grouping genetic diversity in sunflower. To utilize PCA, carbon isotope discrimination (CID) and its related traits were measured on a population of 148 F8 recombinant inbred lines (RILs) of sunflower. The RILs were treated in water-stressed as a randomized block design with two replicates. The result of Bartlett's sphericity test showed the significant value was less than alpha level. Correlations among CID-related traits were determined. The CID was negatively correlated with water use efficiency (WUE), biomass (BM) and cumulative water transpired (CWT) expressed in the biplot diagram. The first two components showed 69.28% of the cumulative variability. Based on the biplot diagram, three distinct groups could be differentiated including high WUE genotypes, high BM genotypes and high WUE-BM genotypes. The correlation between CID and the related traits and distribution of the RILs groups among the traits allow in understanding the genetic diversity of the RILs which could be used as a basic consideration before applying selection program in plant breeding.

Key Words : PCA, CID, RILs, Sunflower

NEW VIRULENCES OF *OROBANCHE CUMANA* APPEAR IN ROMANIA

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ABSTRACT

Broomrape (*Orobanche cumana*) is one of the must dangerous pathogen of sunflower in Romania. Breeding for resistance has been crucial for protecting the crops against this pathogen. In many countries area with sunflower was so much increaset beeing more and more difficult to respect a well crop technology, that beeing the must important reason which caused appearence of new and more virulent races. Since all sources up to now are attacked, new investigations should be done to discover new genes which can protect sunflower against parasite attack.

Key Words : broomrape, virulence, pathogen

THE CULTIVATED SUNFLOWER PAN GENOME PROVIDES INSIGHTS ON THE WILD SOURCES OF INTROGRESSIONS AND THEIR ROLE IN BREEDING.

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ABSTRACT

Since domestication, cultivated sunflower has accumulated gene introgreessions from its direct ancestor and closely related species. Wild relatives of sunflower are known for their enhanced tolerance for both biotic and abiotic stress and therefore are a promising genetic resource for breeding robust verieties. As the wild source used for crossing in breeding programs varies, different introgressed segments are obtained in different lines. The outcome of these introgressions is variation among lines in the genomic composition and presence/absence of specific genes. Therefore, some genes found in the cultivated gene pool are absent from the sunflower reference genome corresponding to a specific line, HA412. Identifying the overall repertoire of genes across lines, referred as the species pan genome, is of great interest for both evolutionary biologists and breeders. Here we present the cultivated sunflower pan genome based on the public association mapping population, which is comprised of 288 lines that capture most of the diversity in the cultivated gene pool. Sequences from each accession were aligned to the reference genome and badly mapped reads were extracted and used for *de-novo* assembly of sequences not found in the reference genome. Assembled sequences were further annotated to reach a unified non-redundant set of 21,081 sequences corresponding to the dispensable fraction of the cultivated sunflower pan genome. To identify the potential introgressions, low coverage whole genome sequences from 192 accessions of different wild *Helianthus* taxa were aligned to the dispensable portion of the pan genome, and 831 genes of wild parentage were identified. Thus, wild introgressions have contributed both new alleles and new genes to the cultivated sunflower gene pool. We further explore the wild origin of each introgressed gene and identify their underlying contribution to phenotypic variation in the cultivated sunflower genepool.

Key Words : Pan genome, Introgressions

STABILITY PERFORMANCE OF NEW INTRODUCED SUNFLOWER HYBRIDS FOR SEED YIELD AND ITS COMPONENTS UNDER SUDAN CONDITIONS

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ABSTRACT

Four introduced sunflower hybrids (SY-4200, SY-4045, NK Kondi and Neoma, from Syngenta Seed Company) were testing under Sudan conditions against three checks during the period of 2011-2013. The hybrids were tested in five locations (irrigated and rainfed) for their uniformity, adaptability and yield potential. The hybrids were arranged in a RCBD with three replications overall environments. Results of combined analysis of variance for seed yield showed significant effects of hybrids, environments and hybrids x environments interaction. Mean seed yield ranged from 1646 kg ha^{-1} to 2041 kg ha^{-1}. The hybrid SY-4045 out-yielded Hysun-33 by 19 % and Bohooth-1 by 23 %. While, the hybrid Kondi out-yielded the two checks by 14 % and 17 %, respectively. The results of yield stability showed that, the two hybrids (SY-4045 and Kondi) were leading, according to their means seed yield across environments. The hybrid SY-4045 had a slope of (1.16) and Kondi of (1.09) and considered as more stable hybrids. Mean oil content of Kondi (47 %) was leading; while of SY-4045 (43 %) was similar to Hysun-33 (42 %) and Bohooth-1 (44 %). Mean oil yield of SY-4045 (884 kg ha^{-1}) and Kondi (917 kg ha^{-1}) was higher than that for Hysun-33 (714 kg ha^{-1}) and Bohooth-1 (731 kg ha^{-1}). Also, both hybrids (SY-4045 and Kondi) are a single cross hybrid, in addition to their credits of earliness, medium plant height and high self-fertility for good seedset compared to checks. The introduced hybrids SY-4045 and Kondi were released in May 2015 for commercialization under Sudan conditions.

Keywords: Sunflower, Helianthus annuus, G x E interaction, Stability performance, Seed Yield,

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a member of the family Asteraceae. In Sudan, sunflower recently become an important cash crop, to strengthen the economy and fill the gap in vegetable oil production and provides a high value animal feed. The crop is grown both as a summer and winter crop under irrigated system and as a summer crop under rainfed system. Sunflower as a non-traditional crop provides an excellent alternative to cover large areas in the production of oil crops beside the major oil crops. The sunflower cultivated area in Sudan had shown an increasing trend in the last ten years. This is because sunflower is one of the crops which attracted the interest of both farmers and private companies. In addition to its wide adaptability, suitability to mechanization, low labor needs, short duration, high yield potential and good quality as a major reasons for increasing sunflower areas.

Sunflower producers in the country depended almost exclusively on imported seeds. Therefore, virtually 98% of oilseed sunflower production is with hybrid cultivars, which necessitate the need for the development and release of more hybrids that can meet farmers' standards (Mohamed *et al,* 2014). Also, this situation necessitates considerable research efforts by the Agricultural Research Corporation (ARC) to cope with increasing demand for seeds of high yielding well adapted hybrids. Hence, attempts have been made to improve hybrid seed supply through testing and releasing more sunflower hybrids as collaboration between private and public institutes. The collaboration program between ARC with some

international sunflower seed companies (Pannar, Advanta, Syngenta, May, Nuseed …etc) was started during 2006-2007 as main step for improving stability/sustainability of seed supply and probably lowering seed prices through creating free competition among more seed companies and ensures the seed supply at the optimum time. For the last five years, this program resulted in the releasing of new introduced sunflower hybrids such as Pan-7049, Pan-7033 and Aguara-4 (Mohamed *et al*, 2011), Opera and Sirena (Mohamed *et al*, 2012) Nugold Dowana and Nugold Darya (Mohamed *et al*, 2013).

Moreover, in a plant breeding program, potential genotypes are usually evaluated in different environments before selecting desirable ones that show stability across environments. For stabilizing yield it is necessary to identify the stable genotypes suitable for wide range of environments. The most widely used way to biometrically assess stability is the regression method, which is based on regression of the mean value of each genotype on the environmental index. The technique to measure stability was previously proposed by Finlay and Wilkinson (1963) and was later improved by Eberhart and Russell (1966). Therefore, the objectives of the present study were to determine the yield performance and stability of four Syngenta sunflower hybrids a cross five locations; and release of the most promising hybrids adapted to the sunflower growing areas of the Sudan.

MATERIALS AND METHODS

The performance of four Syngenta sunflower hybrids (SY-4200, SY-4045, NK Kondi, and Neoma) along with three checks; Hysun-33, Bohooth-1, and Sirena were evaluated during the period of 2011-2013 at five locations (Table 1). The seven sunflower hybrids were tested over 21 environments (seventeen environments at irrigated sites and four environments at rainfed sites). At each environment, sunflower hybrids were arranged in a randomized complete block design with three replications. The plot size was 4 rows and 8 m long ridges spaced 0.80 m apart. The effective sowing dates were during second week of July for Kharif plantings and second to third week of November for winter plantings. Seeds were sown in hills spaced 0.30 m apart within ridges and thinned to one plant per hill two weeks after planting. Irrigation was at intervals of 12-14 days depending on weather conditions. Plots were kept weed-free through frequent hand weeding. Nitrogen was applied only at irrigated sites at 80 kg urea (46% N) per hectare. All recommended agronomic practices were followed throughout the season. Data were collected from the middle two rows on days to 50% flowering, plant height (cm), number of seeds per head, percentage of empty seeds, 1000-seed weight (g), seed yield (kg ha^{-1}), seed oil content and oil yield (kg ha^{-1}). For easy reference the location/year/season combination was considered as an environment and given a number (Table 1). Analysis of variance was performed on individual trials (each environment) and the F-test was made. Combined analysis was performed separately on irrigated and rainfed environments and pooled over 21 environments, assuming random environment and fixed hybrid (Gomez and Gomez, 1984). Stability performance of each hybrid over twenty one environments was determined following the model of Eberhart and Russell (1966). IRRISTAT statistical analysis package for windows (2006) was used for the data analysis.

RESULTS AND DISCUSSION

The pooled analysis of variance showed highly significant differences ($p>0.01$) among the hybrids (G) and environments (E) for seed yield (Table 2), indicating the presence of variability among the hybrids (G) as well as environments under study. The hybrid x environment (G x E) interaction was also highly significant for seed yield, this shows that hybrids react differently at different environments for seed yield. The genotype x environment (G x E) interaction was further partitioned into linear and non-linear components (Table 2). Both hybrid-environment (linear) and pooled deviation (non-linear) were highly significant, indicating involvement of linear as well as non-linear components of variation shared G x E interaction. The significant hybrid x environment (linear) indicated that linear response of genotypic stability to change in environment was not the same for all hybrids evaluated. However the significant deviation from regression revealed the importance of linear regression component in

determining the interaction between hybrids with environments. Different models have been proposed to evaluate the yield stability of the genotypes. Finlay and Wilkinson (1963) proposed linearity of regression as a measure of stability, however, Eberhart and Russell (1966) emphasized that both linear (b_i) and non-linear components of G x E interaction should be considered in judging the phenotypic stability of a particular genotype. Further, Samuel *et al*. (1970) suggested that the linear regression could simply be regarded as a measure of response of a particular genotype that depend largely upon a number of environments whereas the deviation from regression line was considered as measure of stability, genotypes with the lowest or non-significant standard deviation being the most stable and vice versa.

Therefore, a hybrid must not only yield well in the area of its initial development or evaluation, but preferably maintains a high yielding and quality capacities in a wide range of environments intended for commercial production. Stability parameters were calculated according to procedure described by Eberhart and Russell (1966). The stability analysis results are presented in Table 3. The results showed clear differences in values of regression coefficients (b_i) greater than or around unity and relative minimal deviation from regression. This means these introduced sunflower hybrids are more responsive to environmental changes, which give the breeder an advantage to select hybrids for both adverse and favorable environments. Therefore, the resultant regression coefficient (b_i) and deviation from regression (S^2_{di}) and mean yield for each hybrid are parameters for estimating the stability of yield over the environments. The two Syngenta hybrids SY-4045 (2041 kg ha^{-1}) and Kondi (1947 kg ha^{-1}) gave the highest seed yield over the grand mean with the regression coefficients of 1.16 and 1.09, respectively; these results were very close to unity indicating their adaptability to a range of environments. They also had lower deviation from regression (S^2_{di}), indicating that these hybrids had stable seed yield over a wide range of environments.

Hybrid vigor has been the main driving force for acceptance of this oilseed crop in both the world and Sudan. The overall means seed yield for hybrids across the 21 environments ranged between 1646 kg ha^{-1} (Sirena) to 2041 kg ha^{-1} (SY-4045) with grand mean of 1772 kg ha^{-1}(Table 4). The highest means seed yield were recorded by the Syngenta hybrid SY-4045 followed by Kondi. Under irrigated sites (environments) seed yield ranged between 1183 kg ha^{-1} (E9) to 2951 kg ha^{-1} (E7) and for rainfed sites varied from 361 kg ha^{-1} (E18) to 2272 kg ha^{-1} (E20). The maximum seed yield of 2951 kg ha^{-1} was recorded by the hybrid Kondi followed by Sirena (2769 kg ha^{-1}) and SY-4045 (2749 kg ha^{-1}) at irrigated environment E7. The minimum means of seed yield were recorded by all hybrids under rainfed environments (E18 and E21). These results indicated wide variability for seed yield among Syngenta sunflower hybrids over different environments (i.e., irrigated and rainfed environments). The hybrid SY-4045 out-yielded the three checks (Hysun-33, Bohooth-1 and Sirena) across the twenty one environments. Also, Kondi (across the 21 environments) out-yielded both Hysun-33 and Sirena in 18 environments, and out-yielded the local Sudanese hybrid (Bohooth-1) in across the 21 environments. The mean seed yield across the twenty one environments for SY-4045 was 19% more than that of Hysun-33 (1709 kg ha^{-1}) and 23% more than Bohooth-1 (1664 kg ha^{-1}). Also, Kondi showed about 14 % and 17% more seed yield than Hysun-33 and Bohooth-1 respectively (Table 4). Therefore, the mean seed yield rank of the Syngenta hybrids (SY-4045 and Kondi) across the 21 environments was first for SY-4045 and second for Kondi.

Results for days to 50% flowering are shown in Table (5). There was significant difference among the hybrids for flowering time that ranged from 64 to 70 days with a mean of 68 days. Kondi was earlier than Hysun-33 by four days and earlier than Bohooth-1 by two days. Hence, this hybrid can be planted late under rainfed areas and/or under irrigation to make use of available water and to reduce irrigation cost. Across all environments the overall mean of hybrids plant height was 139 cm (Table 5). Plant heights for the selected hybrids were 141 cm for SY-4045, and 138 cm for Kondi compared to 149 cm for Hysun-33 and 141 cm for Bohooth-1. However, development of dwarf or semi-dwarf plant height is the recent trend in breeding work to avoid lodging of sunflower hybrids during storm or heavy rains. Hence, these hybrids had reasonable height, suitable for mechanical harvest and had good resistance to lodging. The mean numbers of seeds per head of individual hybrid are presented in Table (5) only for irrigated sites. Overall the irrigated environments, the hybrids; SY-4045, Kondi and Neoma recorded a higher number of seeds as compared to the three checks. This indicated that irrigated environments were the

most favorable conditions and the selected hybrids (SY-4045 and Kondi) had the capacity to exploit such environments by attaining the highest number of disc flowers formed seedset and hence high number of seeds per head. On the other hand, the lower percentage of empty is an indicator of higher seed setting and consequently high self-fertility and increased seed yield. Also, the results in Table (5) showed that the Kondi had the lowest mean percentage of empty seeds per head (6.2 %) compared to 7.7 % for Hysun-33 and 7.8 % for Bohooth-1. These results confirmed that the higher number of seeds and lower number of empty seeds per head were greatly influence by the genetic and the level of self-compatibility of hybrid and the environmental stress during the reproductive phase. Seed mass or weight is one of the most important yield components. The overall mean of thousand seed weight was 55 g (Table 5). SY-4045 (61 g) showed a mean of thousand seed weight different from that of Hysun-33 and Bohooth-1 (57 g for both), while Kondi (55 g) had a mean 1000-seed weight to some extended not different from that of checks across the 21 environments. The oil content was determined by Soxhelt method only for Medani irrigated site during kharif and winter season of 2012-2013 (Table 5). Oil content is an important component of oil yield per unit area. Results showed that the mean of oil content of SY-4045 (43%), Hysun-33 (41%), Bohooth-1 (39%) and Sirena (44.6%) were very similar in oil content percentage. While, Kondi (47%) showed higher mean oil content compared to the three checks. Regarding the mean of oil yield in Table (5), SY-4045 out-yielded Hysun-33, Bohooth-1 and Sirena by 24%, 21% and 20%, respectively. Also, Kondi out-yielded Hysun-33 by 28%, Bohooth-1 by 26% and Sirena by 25% under the environments of the study.

CONCLUSION

Consistent high mean seed and oil yields demonstrated by two Syngenta sunflower hybrids (SY-4045 and NK Kondi) and their adaptability and stability make them suitable hybrids for cultivation over a wide range of environments. In addition to credits of both hybrids for earliness, medium plant height and high self-fertility for good seedset compared to checks. Hence, the both hybrids were released last May 2015 for commercial production under both irrigated and rainfed (500-800 mm) systems in the central clay plains of the Sudan. Therefore, introduction and release of a large number of hybrids with high yielding from different countries is expected to ensure timely delivery of seeds and will probably result in lower seed prices due to free competition between suppliers.

Table 1: Locations and environments under which Syngenta sunflower hybrids were evaluated during 2011-2013

Location	**Medani**	**Suki**	**Rahad**	**Damazin**	**Gedarif**
Latitude	14° 23ˉ N	13° 25ˉ N	13° 28ˉ N	11° 49ˉ N	14° 20ˉ N
Longitude	33° 29ˉ E	33° 51ˉ E	33° 31ˉ E	34° 24ˉ E	35° 21ˉ E
Elevation (m.a.s.l.)	405 m	430 m	421 m	470 m	592 m
Environment (Season) code	E1,E2 (2011) E3,E4(2012) E5,E6(2013)	E7 (2011) E8,E9(2012) E10,E11(2013)	E12,E13(2011) E14,E15(2012) E16,E17(2013)	E18(2012) E19(2013)	E20(2012) E21(2013)
Soil type (%)	Clay 54 %	Clay 68 %	Clay 60 %	Clay 63%	Clay 75 %
pH	8.0	7.8	8.1	7.2	
Available P	3.0 mg kg^{-1}	3.0 mg kg^{-1}	1.8 mg kg^{-1}	1.8 mg kg^{-1}	3.3 mg kg^{-1}
O.C. %	0.36	0.60	0.60	0.63	0.60
N %	0.03	0.03	0.05	0.03	0.03

Source: Soil Survey Department, ARC-Sudan

Table 2: Pooled analysis of variance of seed and yields (kg ha^{-1}) in Syngenta sunflower hybrids evaluated in twenty one environments

Source of variation	d.f	Mean square
Hybrid (G)	6	603661.95**
Environment (E)	20	1592153.12**
Hybrid (G) x Environment (E)	120	61649.74**
Environment + (Hybrid x Environment)	140	280293.08**
Environment (Linear)	1	7035229.11**
Hybrid x Environment (Linear)	6	60456.53**
Pooled deviation	133	52896.46**
Pooled error	294	14092.19

** Denote significant at 0.01 probability level

Table 3: Estimates of stability parameters for seed yield of Syngenta sunflower hybrids evaluated at 21 environments.

Hybrid	Seed yield (kg ha^{-1})	Slope (b_i)	SE ±	S^2_{di}
SY-4200	1708	0.94	0.104	35138
SY-4045	2041	1.16	0.089	21870
NK Kondi	1947	1.09	0.119	49272
Neoma	1692	1.08	0.065	5086
Hysun-33	1709	0.87	0.066	5512
Bohooth-1	1664	0.87	0.070	7871
Pan-7351/Sirena	1642	0.99	0.099	30090
Mean	1772			

Where; b_i = regression coefficient, S^2_{di} = deviation from regression

Table 4: Means of seed yield (kg ha^{-1}) of sunflower hybrids evaluated over 21 environments.

Location	**Medani**						**Suki**				
Hybrid/Envi.	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11
SY-4200	1775	1917	2049	1435	1533	1765	2026	2024	1205	1795	2012
SY-4045	2356	2405	2243	1740	1731	1896	2749	2263	1611	2373	2571
NK Kondi	2129	2178	2209	1633	1629	1807	2951	2107	1547	2753	1538
Neoma	1781	1864	1715	1201	1407	1579	2311	1739	1183	2167	2004
Hysun-33	1564	1746	1828	1457	1468	1737	2302	1828	1401	2051	2346
Bohooth-1	1311	1782	1996	1495	1536	1761	1903	1816	1536	2251	1758
Sirena	1430	1479	1802	1610	1597	1742	2769	1816	1240	2104	1538
Mean	*1764*	*1910*	*1977*	*1510*	*1557*	*1755*	*2430*	*1942*	*1389*	*2213*	*1967*
SE ±	*196*	*154*	*73.5*	*45.2*	*18.4*	*32.6*	*156*	*77.9*	*99.5*	*184*	*139*
Level of sig.	**	*	**	**	**	**	**	**	*	*	**
C.V.%	*19.2*	*14.0*	*6.4*	*5.2*	*2.0*	*3.2*	*11.1*	*6.9*	*12.4*	*14.4*	*12.2*
Mean loca.	*1746*						*1988*				
Location	**Rahad**						**Damazin**		**Gedarif**		**Overall**
Hybrid/Envi.	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	**mean**
SY-4200	2362	2397	2154	1459	1216	2000	465	1340	2111	823	**1708**

SY-4045	2674	2553	2153	1945	2570	2250	382	1635	1816	949	**2041**
NK Kondi	2362	2188	2188	1598	2049	2333	677	2133	2272	603	**1947**
Neoma	2431	2397	1945	1494	2188	2000	361	1451	1578	735	**1692**
Hysun-33	2119	2345	1945	1598	1563	1958	616	1590	1571	856	**1709**
Bohooth-1	2084	2292	1841	1424	1841	1917	469	1601	1603	722	**1664**
Sirena	1911	2501	1910	1355	1841	1792	528	1132	-	702	**1646**
Mean	*2277*	*2382*	*2019*	*1553*	*1896*	*2036*	*500*	*1555*	*1825*	*770*	***1772***
SE +	*130*	*80.0*	*96.8*	*88.2*	*124*	*79.8*	*44.3*	*18.0*	*227*	*43.3*	***26.04***
Level of sig.	*	*	*ns*	*	**	**	**	**	*ns*	*Ns*	**
C.V.%	*9.9*	*5.8*	*8.3*	*9.8*	*11.4*	*6.8*	*15.4*	*2.0*	*21.5*	*21.0*	***11.7***
Mean loca.	*2027*						*1028*		*1298*		***1617***

Table 5: Means of some traits of SYNGENTA sunflower hybrids evaluated over 21 environments.

Hybrid/trait	***DF**	**PH**	**NSH**	**ES**	**SW**	**OC**	**OY**
SY-4200	70	136	1092	10.4	54	41.53	709.33
SY-4045	68	141	1115	8.2	61	43.32	884.16
NK Kondi	66	138	1152	6.2	55	47.10	917.04
Neoma	64	133	1178	7.3	48	45.53	770.37
Hysun-33	70	149	1094	7.7	57	41.76	713.68
Bohooth-1	68	141	1047	7.8	57	43.90	730.50
Sirena	68	139	1045	9.0	56	44.69	733.81
Mean	***68***	***139***	***1103***	***8.1***	***55***	***43.97***	***779.15***
SE +	*0.18*	*0.85*	*17.1*	*0.47*	*0.58*		
Sign. Level	**	**	**	**	**		
C.V.%	*2.1*	*4.8*	*12.3*	*46.6*	*8.3*		

**DF= days to 50% flowering, PH = plant height (cm), NSH = number of seeds per head, ES = percentage of empty seeds (%), SW = 1000- seed weight (g), OC = oil content (%) & OY = oil yield (kg ha^{-1}).*

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ADVANCEMENTS IN CLEARFIELD® PLUS SUNFLOWER HYBRID VARIETY DEVELOPMENT

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ABSTRACT

Clearfield® Plus sunflowers, the next generation herbicide tolerance trait based on a single mutation in the acetohydroxyacid synthase gene, *Ahas1-3,* or *CLHA-Plus,* was first launched in Argentina in 2010. Since then, Clearfield Plus hybrids have been introduced to the market in the USA, Romania, Bulgaria, and South Africa with additional countries anticipated in 2016. To speed the introduction of Clearfield Plus hybrids to the market, first generation hybrids combine the *CLHA-Plus* mutation, homozygous, on one parent with the *Ahas1-2* (*ImiSun*) mutation, homozygous, on the second parent (hetero combo). In this manner breeding companies optimize resources and inbreds from existing Clearfield breeding programs in combination with converted or new *CLHA-Plus* inbreds. Clearfield breeding programs, though, have been hampered by the necessity to spray select tolerant individuals containing both the *ImiSun* mutation plus enhancing (E)-factor(s), required for full commercial tolerance. To date, no reliable molecular markers have been developed to detect the presence and zygosity of the E-factor(s), making marker assisted selection unfeasible. This paper investigates the learnings from the past 5 years comparing Clearfield and Clearfield Plus hybrid systems both from the breeding perspective and from the grower perspective. The next generation Clearfield Plus hybrids coming to the market include CLHA-Plus (*Ahas1-3/Ahas1-3*) homozygous hybrids which benefit from more efficient breeding improvements and, like their hetero combo counterparts, demonstrate improved herbicide tolerance leading to more reliable tolerance in diverse environments. All Clearfield Plus hybrids benefit from improved herbicide products and increased weed control spectrum in South America as well as in Europe and Eastern Europe.

Key Words : Clearfield, Clearfield Plus, breeding, E factor, CLHA-Plus

GRAIN, KERNEL AND HULL CHARACTERIZATION OF OILSEED AND OILSEED X CONFECTIONARY GENOTYPES

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ABSTRACT

Sunflower breeding programs developed, through the years, an increase in grain oil concentration by increasing the kernel proportion in the grain or the oil concentration in the kernel without affecting potential grain size. Bigger grain sizes (globular form indeed) were reported as less affected by dove consumption. Although quantitative data is not available, birds appear to prefer oilseed hybrids of sunflower over confectionary types. Agronomics characteristics, hull and kernel proportion and oil content were investigated in several commercial hybrids (oilseed genotypes), two novel oilseed x confectionary (O*C) cultivar and three inbreed lines (progenitors of O*C genotypes). Fields experiments were conducted in Reconquista (Argentina) during 2014-2015 in two sowing dates (optimal and late). The measured traits were phenology, grain weight, hull and kernel percentage, whole, hull and kernel grain oil percentage, length and width grain. Days after flowering (DAF) of commercial and O*C hybrids ranged among 71 and 80, while days after maturity (DAM) varied from 99 to 123. No differences were found between oilseed and O*C genotypes in phenology. Oil percentages of commercial hybrids were 49.5±2.6 (59.7±1.4 and 19.9±1.3 kernel and hull oil percentage, respectively) while O*C genotypes were 41.7±1.9 (55.8±1.5 and 18.4±1.4 kernel and hull oil percentage, respectively). Seeds of O*C genotypes were 41 and 42% wider and longer, respectively, as well as 82% heavier than commercial hybrids. In ABSTRACT, we have assessed the reduction in oil percentage on O*C genotypes in comparison with commercial hybrids. Sunflower breeding programs may incorporate these genotypes for dove tolerance improvement.

Keywords: kernel oil, hull oil, oilseed, confectionary hybrids

INTRODUCTION

Sunflower (*Helianthus annuus* L.) seed is considered to be an important oilseed crop because it contains highly nutritious oil in large quantity (Shukla *et al.*, 1992). Sunflower kernel has between 57 to 67% oil content (Aguirrezabal & Pereyra; 1998). The hull comprises 20–30% of the seed, depends on the variety, and contains mostly crude fiber and non-significant quantity of fat (Tranchino *et al*., 1984).
Changes in environmental conditions during the grain filling period could potentially affect oil content of kernel, hull and whole seeds. For sunflower crop management, hybrids selection and his interaction with environment could also affect oil content of kernel, hull and whole seeds.
In Argentina, some species of dove (Columbidae) and parakeet (Psitticidae) can cause significant damage to sunflower (Bucher, 1990). The eared doves (*Zenaida auriculata* Des Murs) is the most numerous granivorous bird in Argentina and Uruguay and cause serious sunflower damage (Bucher, 1992; Canavelli, 2010).
In most bird-resistance studies, two main strategies were recognized: chemical repellents (Crase y Dehaven, 1976; Rodriguez *et al*, 1995) and morphological characteristics such as head angle and down-facing head (Zuil & Colombo, 2012). More uniform crops are apparently less susceptible to damage by

cockatoos (Allen, 1986). Canavelli (2010) recommended using hybrids of sunflower with bigger head angle and/or down facing head as part of crop management practices. Larger grain sizes (globular form indeed) were reported as less affected by dove consumption (Linz & Hanzel, 1997). Oilseed cultivars appear to be more attractive to doves than confectionary ones (Bernardos & Farrell, 2012). Novel hybrids were obtained from ACA breeding programs to increase grain size using an oilseed by confectionary lines.

The aim of this work was to evaluate the agronomic characteristics, hull and kernel proportion and oil content in fifteen commercial hybrids (oilseed genotypes), two novel oilseed x confectionary (O*C) cultivar and three inbreed lines (progenitors of O*C genotypes)

MATERIALS & METHODS

This research was carried out during the 2014-2015 growing season in Reconquista (29° 11' S; 59° 52' W), Santa Fe, Argentina in optimum and late sowing dates, RQTA1: 14/08 and RQTA2: 15/10, respectively. The experiment was conducted with fifteen commercial hybrids (oilseed genotypes), two novel oilseed x confectionary (Oil*Con) cultivar and three inbreed lines (progenitors of O*C genotypes). Genotypes were arranged in a randomized complete block design with four replications in each environment. Each plot was 4 rows and 5 m long, consisting of four rows of a single genotype. Mean plant densities were 4.5 pl m^{-2}. The inter-rows spacing was 52 cm and inter-plant spacing was 30 cm. The soil analysis showed the following result: 1.83 % of organic matter, pH 6.2, 14.3 $mg.kg^{-1}$ of available phosphorus and 19.9 $mg.kg^{-1}$ of $N\text{-}NO_3$. Pest and diseases were effectively controlled.

The meteorological conditions (daily temperature, precipitation and solar radiation) were measured in a weather station located 500 m approximately from the experiment. Daily incident radiation corresponding to the photosynthetically active range of the spectrum was calculated as 0.48 × global daily incident radiation (PAR incident).

Phenology (Schneiter & Miller, 1981) and plant height were recorded. The length, width and hull thickness of seeds samples were measured with calipers. Seed coats were removed with tweezers and their percentage to total seed weight was calculated. The oil percentage of whole seed, kernel and hull were measured with NMR equipment (Nuclear Magnetic Resonance Spinlok, Córdoba, Argentina).

The broad-sense heritability was calculated as the ratio of total genetic variance to total phenotypic variance (Visscher *et al.*, 2008). Results are presented as mean ± standard error. Analysis of variance was performed with the statistics program Infostat (Di Rienzo *et al.*, 2015). Data were subjected to Tukey's multiple-range tests to compare mean values at 5% level of significance. Sigmaplot software (Sigmaplot 8.0, SPSS Inc., Chicago, IL) was used to establish the relationships.

RESULTS AND DISCUSSIONS

In RQTA 1, maximum, minimum and mean air temperature averages were 27,6, 15,7 and 21,6 ºC respectively, whereas in RQTA 2 were much higher (30,2, 19,1 and 24,6 ºC). Cumulative photosynthetically active radiation incident was 1363 and 1218 $Mj.m^{-2}$ in RQTA 1 and RQTA 2, respectively. Furthermore, cumulative rainfall was 738 and 1042 mm in RQTA 1 and RQTA 2, respectively. Daily mean temperature, PAR incident and rainfall are illustrated in Figure 1.

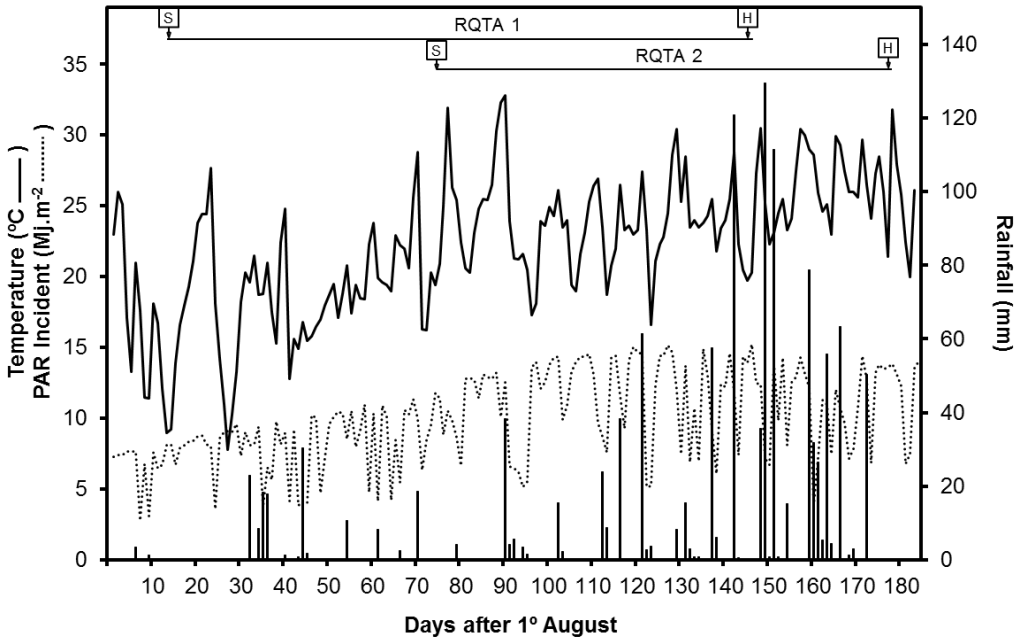

Figure 1. Daily mean temperature (ºC), photosynthetically active radiation incident (PAR incident $Mj.m^{-2}$) and rainfall (mm) since August 1º. Horizontal lines at the top of the figure represent the date of sowing (S) and harvest (H) for RQTA 1 and RQTA 2.

Days after flowering (DAF) of commercial and O*C hybrids ranged among 71 and 80 DAF, while days after maturity (DAM) varied from 99 to 123. In RQTA 1, the average days from sowing to flowering among commercial hybrids were 80 (ranged among 75 and 86), while the mean days from sowing to physiological maturity was 123 (varied among 114 and 129 days). In RQTA 2, the average days from sowing to flowering between hybrids was 71 (ranged among 65 and 79), while the mean days from sowing to physiological maturity was 99 (varied among 90 and 109 days). Furthermore, the height of commercial and Oil*Con hybrids were 137 ± 18 and 148 ± 8 cm, respectively. The Oil*Con hybrids showed a similar behavior than commercial ones in phenology and height.

Sunflower genotypes differed from each other in length ($p<0.0001$). Regarding width and hull thickness, statistically significant differences were found between genotypes and environment ($p\leq0.0009$, Table 1). Significant interaction G*E was found in width seed. The confectionary parental line "Male 2" had the highest length and width seed, compared to the commercial hybrids. Seeds of Oil*Con genotypes were 41 and 42% longer and wider, respectively, as well as 82% heavier than commercial hybrids. However, the hull was 48% thicker than commercial hybrids and similar to the confectionary males lines.

Table 1. Length, Width and Hull Thickness (in mm) of seeds for different environments (RQTA 1 and 2) of 15 commercial genotypes of sunflower, 3 parental lines and two confectionary*oilseed hybrids.

Hybrids	Type	Length		Width		Hull Thickness	
		RQTA 1	RQTA 2	RQTA 1	RQTA 2	RQTA 1	RQTA 2
		mm					
ACA 861	Oilseed	10,5 ± 0.1*	10,6 ± 0,6	4,8 ± 0,2	5,9 ± 0,1	0,5 ± 0,048	0,5 ± 0,025
ACA 885	Oilseed	10,9 ± 0,1	10,6 ± 0,2	5,3 ± 0,3	5 ± 0,2	0,4 ± 0,001	0,3 ± 0,021
ACA 887	Oilseed	11,0 ± 0,0	11,4 ± 0,3	4,7 ± 0,4	5,4 ± 0,2	0,4 ± 0,048	0,4 ± 0,036
AD66 CL	Oilseed	9,6 ± 0,2	9,8 ± 0,1	4,9 ± 0,1	5,4 ± 0,2	0,3 ± 0,046	0,5 ± 0,01
Cacique CL	Oilseed	10,4 ± 0,2	10,3 ± 0,3	4,6 ± 0,3	5,4 ± 0,2	0,3 ± 0,031	0,4 ± 0,044
Diagora	Oilseed	10,4 ± 0,2	10,4 ± 0,4	4,8 ± 0,3	4,8 ± 0,1	0,4 ± 0,043	0,5 ± 0,01
DK 3970 CL	Oilseed	10,3 ± 0,3	9,8 ± 0,3	4,7 ± 0,1	5,3 ± 0,3	0,3 ± 0,038	0,4 ± 0,041
DK 4045	Oilseed	10,4 ± 0,1	10,8 ± 0,3	4,8 ± 0,3	5,3 ± 0,1	0,5 ± 0,001	0,5 ± 0.001
DK 4065	Oilseed	8,8 ± 0,5	9,1 ± 0,3	4,8 ± 0,1	5,0 ± 0,0	0,3 ± 0,027	0,3 ± 0,0087
NEON	Oilseed	10,8 ± 0,1	10 ± 0,4	5,3 ± 0,1	5,3 ± 0,1	0,5 ± 0,025	0,5 ± 0,025
P 1100 CLP	Oilseed	10,1 ± 0,3	9,5 ± 0,5	4,6 ± 0,2	4,9 ± 0,2	0,3 ± 0,001	0,4 ± 0,044
P102CL	Oilseed	8,9 ± 0,1	9,1 ± 0,1	4,4 ± 0,1	5,3 ± 0,3	0,5 ± 0,043	0,4 ± 0,041
PAN 7031	Oilseed	10,8 ± 0,3	10,4 ± 0,3	4,8 ± 0,3	5,2 ± 0,1	0,4 ± 0,04	0,4 ± 0,027
PAN 7076	Oilseed	10,9 ± 0,3	10,1 ± 0,5	4,5 ± 0,3	5,3 ± 0,3	0,4 ± 0,001	0,4 ± 0,01
PROTON 290	Oilseed	10,1 ± 0,1	10,6 ± 0,2	4,8 ± 0,3	5,9 ± 0,1	0,3 ± 0,047	0,4 ± 0,042
Female (F)	Oilseed	12,0 ± 0,0	11,5 ± 0,3	5,5 ± 0,0	5,3 ± 0,1	0,2 ± 0.01	0,3 ± 0,029
Male 1 (M1)	Con	15,5 ± 0,3	14,5 ± 0,3	6,0 ± 0,0	6,5 ± 0,3	0,6 ± 0,029	0,6 ± 0,029
Male 2 (M2)	Con	17,0 ± 0,0	16,5 ± 0,3	7,5 ± 0,0	8,0 ± 0,6	0,5 ± 0,029	0,6 ± 0,029
Hybrid 1 (F*M1)	Oil*Con	14,5 ± 0,0	15,0 ± 0,0	7,5 ± 0,0	7,0 ± 0,0	0,6 ± 0,029	0,6 ± 0,029
Hybrid 2 (F*M2)	Oil*Con	13,8 ± 0,1	14,3 ± 0,4	6,8 ± 0,1	7,3 ± 0,4	0,5 ± 0,029	0,7 ± 0,029

	Significance		
Genotype	<0,0001	<0,0001	<0,0001
Environment	0,2341	<0,0001	0.0009
G*E	0,0915	0,0211	0,0539
DMS (Tukey p<0,05)	1,6	1,3	0,23

*mean ± standard error

Sunflower genotypes were statistically different in term of hull/kernel ratio and hull proportion among genotypes, environment and the interaction G*E ($p \leq 0.0311$, Table 2). Although the difference found in hull thickness was 48% between commercial and oil*con genotypes, the proportion of hull between them was 6%. The hull/kernel ratio were higher in RQTA 2 (47.6 and 50.5 for commercial and oil*con genotypes, respectively) than RQTA 1 (41.2 and 45.5 for commercial and oil*con genotypes, respectively).

Table 2. Hull/Kernel ratio and Hull proportion of seeds for different environments (RQTA 1 and 2) of 15 commercial genotypes of sunflower, 3 parental lines and two confectionary*oilseed hybrids.

Hybrids	Type of hybrid	Hull/Kernel Ratio		Hull Proportion	
		RQTA 1	RQTA 2	RQTA 1	RQTA 2
		……%.....			
ACA 861	Oilseed	42 ± 1,8	48 ± 4	30 ± 0,9	32 ± 1,8
ACA 885	Oilseed	44 ± 1,7	49 ± 1,1	31 ± 0,8	33 ± 0,5
ACA 887	Oilseed	46 ± 2,7	62 ± 4,9	31 ± 1,3	38 ± 1,8
AD66 CL	Oilseed	32 ± 0,9	33 ± 3,6	24 ± 0,5	24 ± 1,8
Cacique CL	Oilseed	36 ± 1,8	37 ± 2,1	26 ± 1,0	27 ± 1,1
Diagora	Oilseed	31 ± 2,5	41 ± 2,2	24 ± 1,4	29 ± 1,1
DK 3970 CL	Oilseed	29 ± 1,1	39 ± 1,7	22 ± 0,6	28 ± 0,9
DK 4045	Oilseed	55 ± 4,6	56 ± 1,5	35 ± 1,9	36 ± 0,6
DK 4065	Oilseed	36 ± 2,8	46 ± 7,3	26 ± 1,6	31 ± 3,4
NEON	Oilseed	60 ± 6,7	57 ± 5,0	37 ± 2,4	36 ± 2,1
P 1100 CLP	Oilseed	34 ± 1,0	40 ± 0,6	25 ± 0,6	28 ± 0,3
P102CL	Oilseed	41 ± 1,1	48 ± 3,7	29 ± 0,6	32 ± 1,6
PAN 7031	Oilseed	44 ± 1,7	51 ± 3,3	30 ± 0,8	34 ± 1,4
PAN 7076	Oilseed	45 ± 1,4	57 ± 5,2	31 ± 0,7	36 ± 2,1
PROTON 290	Oilseed	44 ± 2,7	51 ± 5,9	30 ± 1,3	34 ± 2,4
Female (F)	Oilseed	24 ± 1,4	28 ± 0,4	19 ± 0,9	22 ± 0,3
Male 1 (M1)	Con	73 ± 2,1	63 ± 1,2	42 ± 0,7	39 ± 0,5
Male 2 (M2)	Con	65 ± 0,3	69 ± 1,6	39 ± 0,1	41 ± 0,5
Hybrid (F*M1)	Oil*Con	41 ± 2,2	45 ± 0,1	29 ± 1,1	31 ± 0,0
Hybrid 2 (F*M2)	Oil*Con	50 ± 0,5	56 ± 0,4	33 ± 0,2	36 ± 0,2
		Significance			
Genotype		<0,0001		<0,0001	
Environment		<0,0001		<0,0001	
G*E		0,0311		0,0305	
DMS (Tukey p<0,05)		17,1		7,5	

*mean ± standard error

Whole seed oil, kernel oil and hull oil percentage showed statistically differences among genotypes, environment and the interaction G*E ($p \leq 0.0001$, Table 2). Oil percentages of commercial hybrids were 49.5±2.6 (59.7±1.4 and 19.9±1.3 kernel and hull oil percentage, respectively) while O*C genotypes were 41.7±1.9 (55.8±1.5 and 18.4±1.4 kernel and hull oil percentage, respectively). The whole seed oil percentage was higher in RQTA 1 (50.2 and 45.5 for commercial and oil*con genotypes, respectively) than RQTA 2 (48.8 and 39 for commercial and oil*con genotypes, respectively). The kernel oil percentage was higher in RQTA 1 (63 and 56.5 for commercial and oil*con genotypes, respectively) than RQTA 2 (56.6 and 55 for commercial and oil*con genotypes, respectively), while hull oil percentage was 18.7 and 19.5 for commercial and oil*con genotypes, respectively in RQTA 1 and 21.2 ad 17.5 for commercial and oil*con genotypes respectively in RQTA 2. The confectionary males lines resulted in less oil percentage in kernel and hull.

Table 2. Percentage of Whole Seed Oil, Kernel Oil and Hull Oil for different environments (RQTA 1 and 2) of 15 commercial genotypes of sunflower, 3 parental lines and two confectionary*oilseed hybrids.

Hybrids	Type	Whole Seed Oil		Kernel Oil		Hull Oil	
		RQTA 1	RQTA 2	RQTA 1	RQTA 2	RQTA 1	RQTA 2
		……%……					
ACA 861	Oilseed	47 ± 1,0*	49 ± 0,4	62 ± 1,3	55 ± 1,1	18 ± 0,6	22 ± 0,7
ACA 885	Oilseed	49 ± 1,4	48 ± 0,7	64 ± 1,4	55 ± 0,9	18 ± 1,0	21 ± 0,3
ACA 887	Oilseed	49 ± 0,5	47 ± 1,7	66 ± 0,7	58 ± 1,8	17 ± 0,5	20 ± 0,4
AD66 CL	Oilseed	51 ± 1,6	52 ± 1,1	61 ± 1,2	55 ± 0,7	20 ± 0,6	24 ± 0,5
Cacique CL	Oilseed	51 ± 1,4	50 ± 1,1	62 ± 1,3	54 ± 0,5	20 ± 0,3	23 ± 0,4
Diagora	Oilseed	55 ± 0,8	49 ± 1,3	64 ± 0,7	54 ± 1,1	20 ± 0,2	23 ± 0,3
DK 3970 CL	Oilseed	54 ± 0,8	57 ± 0,4	60 ± 2,1	60 ± 0,5	21 ± 1,3	23 ± 0,3
DK 4045	Oilseed	46 ± 0,9	49 ± 3,0	63 ± 1,4	57 ± 1,3	17 ± 0,6	21 ± 1,5
DK 4065	Oilseed	57 ± 0,9	47 ± 4,4	64 ± 1,8	55 ± 2,3	21 ± 1,1	21 ± 1,0
NEON	Oilseed	43 ± 0,7	46 ± 0,7	62 ± 0,9	57 ± 1,7	16 ± 0,4	20 ± 0,6
P 1100 CLP	Oilseed	51 ± 1,2	49 ± 1,6	63 ± 1,6	57 ± 1,7	19 ± 0,7	21 ± 0,3
P102CL	Oilseed	48 ± 1,1	45 ± 2,5	63 ± 0,4	56 ± 0,8	18 ± 0,3	20 ± 1,4
PAN 7031	Oilseed	50 ± 1,9	48 ± 0,8	64 ± 1,2	58 ± 1,7	18 ± 0,5	20 ± 0,8
PAN 7076	Oilseed	51 ± 0,4	46 ± 1,2	65 ± 1,8	60 ± 3,2	18 ± 0,9	18 ± 1,6
PROTON 290	Oilseed	51 ± 2,2	50 ± 1,5	63 ± 1,3	58 ± 1,5	19 ± 0,7	21 ± 0,7
Female (F)	Oilseed	55 ± 0,1	47 ± 0,1	54 ± 0,6	50 ± 0,7	25 ± 0,3	23 ± 0,3
Male 1 (M1)	Con	35 ± 0,1	27 ± 1,8	50 ± 1,3	51 ± 0,5	17 ± 0,4	13 ± 0,8
Male 2 (M2)	Con	26 ± 0,6	23 ± 0,6	45 ± 0,5	48 ± 0,2	14 ± 0,2	12 ± 0,3
Hybrid 1 (F*M1)	Oil*Con	45 ± 0,2	41 ± 0,0	55 ± 0,1	54 ± 0,0	20 ± 0,0	19 ± 0,0
Hybrid 2 (F*M2)	Oil*Con	44 ± 0,6	37 ± 0,2	58 ± 0.0	56 ± 0,8	19 ± 0,2	16 ± 0,4
				Significance			
Genotype			<0,0001	<0,0001		<0,0001	
Environment			<0,0001	<0,0001		<0,0001	
G*E			<0,0001	<0,0001		<0,0001	
DMS (Tukey p<0,05)			7,8	3,9		7,4	

*mean ± standard error

Hull/kernel ratio was curvilinear and negatively in relation to whole seed oil percentage (Fig 2). Hull/kernel ratio accounted for the variation in whole seed oil percentage of all genotypes ($p < 0.0001$, $r^2=0.74$, Fig. 2 a) where lower hull/kernel ratios showed the highest seeds oil percentages (commercial hybrids and female line). The oil*con genotypes had mean values of hull/kernel ratio and seed oil percentages. Whole seed oil percentage was curvilinear and positively related to kernel oil ($p<0.0001$, $r^2=0.58$ Fig 2 b) and hull oil ($p<0.0001$, $r^2=0.67$, Fig. 2 C). Dedio (1982) reported that each of the two components of the seed (kernel and hull oil) have a significant effect on the oil content of the whole seed. Regarding both variables, oil*con genotypes has shown an intermediate performance between confectionary and oilseed genotypes.

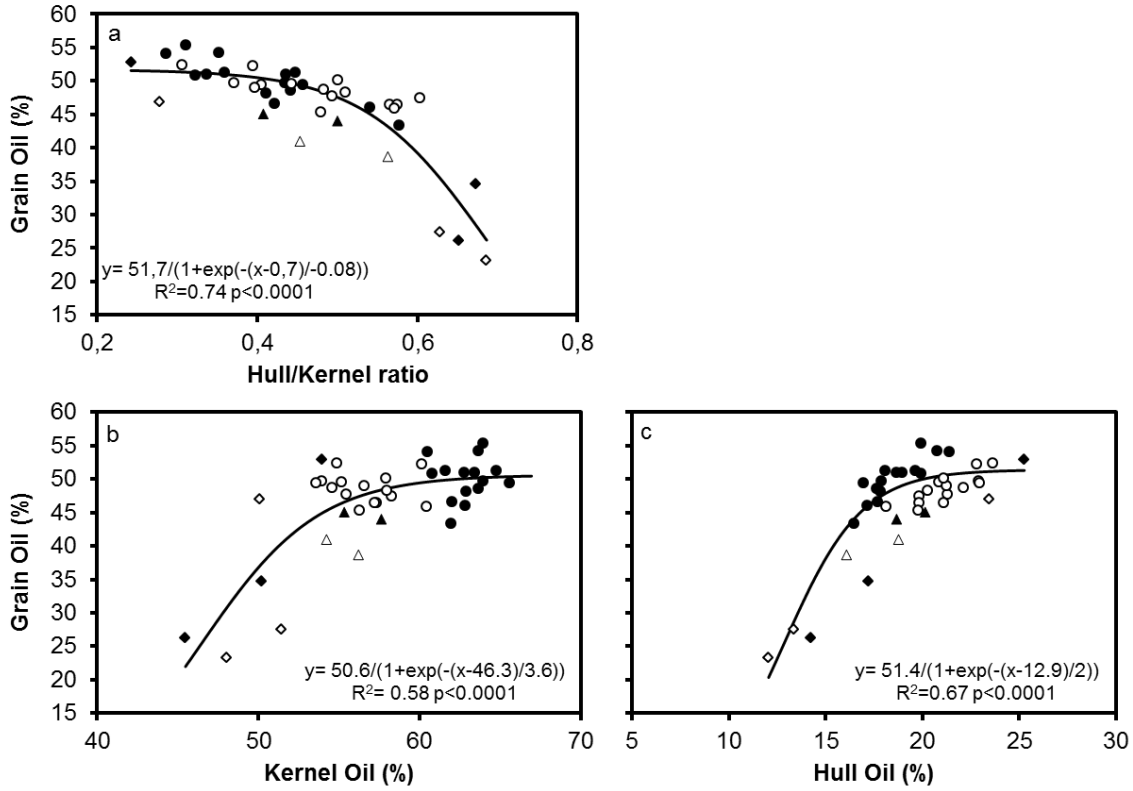

Figure 2. Relationship between grain oil and Hull/Kernel ratio (a), Kernel Oil (b) and Hull oil (c) of sunflower hybrids. Data correspond circle, diamond and triangle to commercial hybrids, parental lines and oil*con genotypes, respectively. Full and empty icons represent RQTA1 and RQTA 2, respectively.

For whole seed oil, hull and kernel weight and seed length, the higher component of variance was genetic and the heritability (broad sense) estimated ranged between 61 and 98 % (Table 4). High heritability estimates for oil content has been reported by Shrinivasa (1982) and Khair *et al.* (1992). Dedio (1982) reported that due to the lack of genetic linkage between kernel and hull oil, a breeder could screen for both component while striving to improve oil content. Further investigations should be developed to understand the effect of the environmental conditions over the oil parameters measured in this work. Furthermore, it would be interesting to develop studies involving a wider range of genetics background of confectionary, oilseed and their crossing genotypes.

In ABSTRACT, oil*con genotypes referred in this work has a good performance in oil percentage compared to confectionary and commercial oilseed. Thus, the grain size (length and width) of Oil*Con genotypes was higher than commercial hybrids and could be used in bioassays against eared doves to confirm the reduction of birds consumption. We have assessed the reduction in oil percentage on O*C genotypes in comparison with commercial hybrids.

Table 4. Genotypic variance, environment variance, interaction G*E variance and heritability in wide sense for Seed Oil percentage, hull weight, kernel weight, seed length, seed width, kernel oil (%), hull oil (%), Hull/Kernel Ratio and Hull.

	σ^2_g	σ^2_{ga}	σ^2_a	H^2
Whole Seed Oil	432	32,2	242,4	0,61
Hull Weight	599	30,8	37,1	0,90
Kernel Weight	966	81,8	170,2	0,79
Seed Lenght	36	0,5	0,4	0,98
Seed Width	6	0,4	6,8	0,47
Kernel Oil percentage	127	23,4	945,9	0,12
Hull Oil percentage	47	12,7	71,3	0,36
Hull/Kernel Ratio	0,1	0,01	0,11	0,45
Hull percentage	0,02	0,0013	0,02	0,48

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DEVELOPING WELL ADAPTED HYBRIDS IN EUROPE BY USING A G*E APPROACH

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ABSTRACT

The expression of sunflower yield is determined by biotic and abiotic factors. Breeding work allows, most of the time, to find efficient answers for pests or diseases damages, but the interaction between varieties and environmental criteria is more difficult to analyze and therefore to select. First studies carried out in France by INRA and TERRES INOVIA demonstrated the possibility to structure the Genotype *Environment interaction using pedo-climatic parameters, at different stages of the sunflower cycle, and highlighted differences of behavior between hybrids. On that basis, we developed our own research program using data collected for the last 10 years on our European sunflower testing network, in order to find original agro-climatic indicators that explain the Genotype *Environment interaction. Using strong statistical methods and computer tools, we were able to identify a limited number of different "climates" that summarize most frequent agro-climatic conditions present in the European sunflower area (10 different countries), and to find specific combinations of indicators for each "climate". Moreover, a statistical model established with these indicators significantly explains part of the Genotype * Environment interaction and highlights the capacity of some varieties to obtain stable and high enough yield in all the identified "climates". The model is a powerful tool for the breeding or the marketing teams to characterize *a posteriori* testing locations and better analyze how relevant their network is. The use of that method in the breeding process will also be helpful in creating new hybrids broadly adapted to abiotic stresses.

Key Words : abiotic stress, Europe, G*E interactions

OPTIMIZATION OF AGROBACTERIUM-MEDIATED GENE TRANSFER SYSTEMS IN TURKISH SUNFLOWER (HELIANTHUS ANNUUS L.) VARIETIES

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ABSTRACT

This study aimed to establish the plant tissue culture and gene transfer systems in some elite Turkish sunflower (*Helianthus annuus* L.) varieties. Plant tissue culture systems were established on Murashige and Skoog (MS) media supplemented with various plant-growth regulators using cotyledonary nodes and meristematic shoots as explants. After surface sterilization, seeds were germinated in MS media for 12 days in growth chamber under 16/8 photoperiods, %60 humidity and 24oC temperature. Following germination, explants were isolated and cultured in MS media containing 0.25 mg/l NAA (1-Naphthaleneacetic acid) and 1 mg/l BAP (6-Benzylaminopurine). Isolated shoots were inoculated with an upper-virulent strain of *Agrobacterium tumefaciens*, which included a kanamycin resistance gene as selective marker, a cauliflower-mosaic-virus-derived 35S promoter, a GFP coding sequence and an antibiotic resistance gene (BAR) for selection of transformed plants. All regenerated shoots were rooted on MS media supplemented with 1 mg/l IBA. GFP protein expression was detected on gel as well as visualized using Fluorescent microscope. *Agrobacterium*-mediated gene delivery system in the meristematic tissues is regarded as an efficient method in production of transgenic sunflowers as well as it forms a baseline for the effective delivery of agronomically valuable gene/s in some Turkish elite sunflower varieties. Many commercial sunflower varieties are seriously affected by various biotic and abiotic stress factors, and also require the chemical control while maintaining the product quality. Traditional breeding strategies do not have ability to address all these limitations but biotechnology does. Thus, present study emphasized the application of molecular and biotechnological methods to improve the some elite sunflower varieties in Turkey.

Key Words : Sunflower, tissue culture, gene transfer, regeneration, organogenesis

INCLUSION OF DOMINANCE EFFECT IN GENOMIC SELECTION MODEL TO IMPROVE PREDICTIVE ABILITY FOR SUNFLOWER HYBRID PERFORMANCE

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ABSTRACT

Hybrids dominate the world sunflower production, mainly because of the important heterosis in this crop. Therefore, predicting hybrid performance is of high importance to improve the efficiency of sunflower breeding programmes. Rather than seeking to identify individual loci significantly associated with a trait, genomic selection (GS) uses all marker data as predictors and consequently delivers more statistically accurate predictions. Dominance, a major factor of heterosis together with epistasis, is often neglected in GS model, adding to the residual error. So the inclusion of dominance effect in the GS model may improve the predictive ability. An incomplete factorial design of 36 males and 36 females was used in this study. We used genotypic data on 635,155 SNPs and phenotypic data (flowering time and leaf senescence) on 452 hybrids in six environments. We used a mixed model to correct spatial variation in each environment and we performed genomic prediction of the genetic hybrid value with a genomic best linear unbiased prediction (GBLUP) method, based on relationship matrices. Two models were compared: an additive model with the male and female relationship matrices and a dominance model taking into account the male, the female and the dominance, the latter defined as the interaction between male and female. Prediction accuracies of these models were estimated as the correlation between genetic hybrid values and observed phenotypes. Comparison of these models and prediction gain of the inclusion of the dominance in GS will be discussed for the two traits chosen for their contrasting heritability

Key Words : genomic selection, parents effect, dominance

ASSESSMENT OF SUNFLOWER GERMPLASM SELECTED UNDER AUTUMN PLANTING CONDITIONS

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ABSTRACT

Agronomic potential of traditional sunflower spring varieties is low because its flowering and grain filling are often exposed to mid and end-season drought. To overcome this, new breeding strategy consisted of selecting varieties tolerant to winter cold in order to shift to autumn or early winter planting. Nowadays, 'Ichraq' is the only one registered autumn variety. The objective of this research is to evaluate various genotypes having been selected in different environments under autumn planting conditions. This germplasm was planted early at winter during two years (2013 and 2014) at 'Annoceur', a mountain site known for its pronounced winter cold. Morphological, physiological, agronomic and technological parameters were considered for the germplasm assessment. Analysis of variance showed significant differences between genotypes for most of these parameters. Plantlet initial vigor average was 3.5 varying from 1 for genotype M32 to 5 for AN8. Leaf area average was 162 cm² varying from 25 to 375 cm² for genotypes M17 and AN34, respectively. Total chlorophyll content average was 43 mg/g, varying from 28 to 79 mg/g for genotypes K7 and M29, respectively. Number of days from sowing to flowering varied from 162 for genotype AN21 to 180 for genotypes M27 and M29. Mean seed yield per plant was 49 g, with a large variation from 8 to 110 g for M18 and K8, respectively. Mean seed oil content was 36%, ranging from 22% for M8 to 47% for K4. Genotypes having exhibited more performance than 'Ichraq' were selected to develop new sunflower germplasm suitable for autumn or early winter sowing.

INTRODUCTION

Agricultural sector continues to dominate Morocco's economic activity. The rural population accounts for 40% of the total population. The Agriculture thus proved an effective engine of economic growth and guaranteed food security. To upgrade and boost domestic agriculture, different strategies have been implemented during the Moroccan contemporary history. The latter being the Green Morocco Plan implemented since 2008. Owing to its importance in the cropping system and the food security challenge in vegetable oils, the oilseed is considered among the priority sectors. Since 2001, the year of oilseed sector reform, and until 2013, the year of signature of a sector program contract, sunflower was the major annual oilseed crop grown in Morocco, with an average area about 50000 ha and an average seed yield below 1 t/ha. Indeed, national seed oil production covers barely 2-3% of the overall needs of the edible oil in the country estimated at over 410.000 t. The gap is covered by importation which has negative repercussion on the economy and food security of our country (Nabloussi et al., 2015).

In Morocco, sunflower traditionally sown in spring has limited productivity as it does not benefit the fall and/or winter precipitation, and it is often exposed to drought and high temperatures of mid and late cropping cycle. Such constraints coincide with periods of flowering and seed filling that are critical for determining seed productivity and seed oil content (Ouattar et al., 1992). Its cultivation is often secondary and is considered as catch crop, following early droughts or floods that affect growing of autumn crops, mainly cereals. However, several studies have shown the benefits of early planting (autumn or early winter) in improving the seed yield and oil content in Morocco (Boujghagh, 1993 ; Gosset and Vear, 1995 ; Aboudrare et al., 2000), Spain (Gimeno et al., 1989) and France (Allinne et al., 2009). Early

sowing, two to three months earlier than conventional sowing, induced a significant drop in temperature at planting and during early stages of vegetative growth (Allinne et al., 2009). The characterization and evaluation of sunflower genotypes adapted to low temperature conditions, during early vegetative growth stages, requires analyzing the impact of such conditions on the physiological processes associated with initial seedling vigor and plant cold tolerance. Agronomic, morphological, physiological, technological and biochemical attributes could be taken as valuable criteria to identify and select genotypes adapted to winter cold conditions. Nowadays, "Ichraq" is the only autumn variety registered in the Moroccan Official Catalogue (Nabloussi et al. 2008). It is a late maturing, winter cold tolerant and combines good seed yield and high seed oil content. Current research continues to develop new sunflower populations, resistant (or tolerant) to winter cold and agronomically performant, which would be the basis for selection of new improved varieties better than the variety "Ichraq". Thus, the present work aimed to evaluate new sunflower genetic materials for agro-morphological, physiological and technological traits under early winter planting conditions.

MATERIALS AND METHODS

Plant materials

The plant material used in this study consisted of 46 sunflower genotypes including 'Ichraq', the first and only one autumn variety, considered as check, and 45 individual selected plants derived from 'Ichraq'. As this latter is a population variety (Nabloussi et al., 2008), there was opportunity to select individual plants (PS) in order to release new germplasm that will be more performant than 'Ichraq'. The 45 PS were selected in various environments for their vigor, habit and agro-morphological performances.

Methods

The 46 genotypes were planted on 2 January 2014 at the INRA experimental station located at 'Annoceur', mountainous area known for its rough winter cold. It is located 50 km from Fez city, in the north of Morocco, at an altitude of 1350 meters. During the cropping cycle, the minimum temperature was -5°C, registered in January and February whilst the maximum temperature was 37°C, recorded in May.

Trial was conducted under rainfed conditions following a randomized complete blocks with two replications. Each genotype was sown in two 5 m rows spaced by 60 cm. In each row, plants were spaced by 30 cm. Initial N-P-K fertilization was 80-80-30 units, respectively, followed by cover N fertilization with two inputs of 40 units, one at stem elongation stage and the other at flowering stage. No phytosanitary treatment was applied.

Morphological, phenological, physiological, agronomic and technological parameters were studied. During plant vegetative growth, plant height (cm), growth rate (cm/d), collar diameter (mm), initial vigor of young seedlings (following a grading scale of 1 to 5), number of leaves per plant, leaf area (cm^2) and number of branches per plant were measured. Flowering time of each genotype was determined by counting the number of days between planting date and the date when 50% of plants of this genotype have flowered. Chlorophyll content (mg/g) was calculated according to the method of Billore and Mall (1975). The optical density (OD) of all the supernatant obtained was measured in a spectrophotometer at 645 nm and 663 nm. The concentrations of chlorophyll pigments are given by the following formulas:

CHL A = 12.7 (OD 663) – 2.69 (DO645)

CHL B = 22.9 (OD 645) – 4.56 (DO663)

At maturity, head diameter and head aborted diameter were measured (cm). After harvest, total seed yield (q/ha), seed yield per plant (g) and its components (number of seeds per propeller and 1000 seeds weight) are determined. Also, seed oil content was determined using RMN method (Oxford 4000).

Descriptive analysis of gathered data, analysis of variance and analysis of correlation were performed using different procedures of SAS program. Duncan's new multiple range test was applied to compare genotypes means.

RESULTS AND DISCUSSION

Morphological parameters

Analysis of variance showed there were significant differences ($P < 0.001$) between the 46 genotypes for all studied parameters (Table 1). Initial vigor of young seedlings varied from 1 for genotype M32 to 5 for genotype AN8, with an average of about 3.5, higher than the check vigor (3). In many studies, seedling and plantlet initial vigor was found as a good selection criterion correlated with the adaptation and the performance of genotypes under environmental abiotic stresses (Foolad and Lin, 2001). In the present work, all genotypes having an initial vigor of 4 or 5 will be selected for further evaluation and germplasm improvement. For growth rate, the overall mean was 2.31 cm/d, with a minimum of 0.53 cm/d, registered for genotype AN21 and a maximum of 3.92 cm/d for genotype M34, slightly higher than that of the check, which was 3.15 cm/d (Table 1). Genotypes having growth rate higher than that of the check will be selected. The average plant height was 147 cm, with a variation from 75 to 200 cm for M18 and K20, respectively. Plant height of the check was about 167 cm. Higher is a plant more it is susceptible to lodging and late drought (Sposaro et al., 2008). Plants with a height less than the observed average (< 145 cm) could be interesting for selection. Number of leaves per plant varied from 17 for M4 to 38 for K3, with an average of 27.5 leaves per plant. The check had 25 leaves per plant. The average leaf area was 162 cm², which is equal to the check value. The genotypes M17 and AN34 exhibited the extreme values: 24.5 and 374.85 cm², respectively. Elevated number of leaves per plant and high leaf area are correlated with high plant transpiration (Romero-Aranda et al., 2001). Thus we aimed to select those plants having less than 25 leaves and a leaf area less than 162 cm². Regarding collar diameter, genotype M7 exhibited the strongest value which was about 31 mm, whilst genotype M18 showed the lowest value which was 11 mm. The overall mean value was 20 mm and the check value was 23 mm. Like initial vigor, collar diameter in an indicator of good adaptation under stressed environments (Liua et al., 2012). Therefore, all the genotypes exhibiting a collar diameter more than the observed average (20 mm) could be selected for further evaluation. Among the 46 studied genotypes, 27 ones, including 'Ichraq' the check variety, had no branching, whilst 19 ones were branched, with a number of branches per plant varying from one to six. Genotype M30 was the most branched, having six branches per plant. The overall average was 0.93. Sunflower branching is an indicator of plants susceptibility to cold conditions (Alba et al., 2010). The plants selected for further evaluation and new germplasm constitution should have no branching.

Physiological parameters

Analysis of variance revealed significant effect of genotype on flowering earliness, chlorophyll a content and chlorophyll b content ($P < 0.001$), and non-significant effect on total chlorophyll content (Table 1). However, a large variation was observed, ranging from 28 mg/g for genotype K7 to 79 mg/g for genotype M29. The average total chlorophyll content was 43.21 mg/g, while the content concerning the check variety was 54.6 mg/g (Table 1). Genotypes maintaining high chlorophyll content under abiotic stresses, like as drought or cold, exhibit tolerance to such stresses (Yang et al 2015). All genotypes having total chlorophyll content higher than that of the check will be selected. Regarding chlorophyll a and chlorophyll b content, the genotype K4 exhibited the highest values for both types, 11.3 and 19.8 mg/g, respectively. The lowest contents were 0.86 mg/g, registered in genotype K9, and 1.74 mg/g, registered in genotype K8, for chlorophyll a and chlorophyll b, respectively. The check variety had 1.86 and 2.77 mg/g for these parameters, respectively. Vegetative period before flowering was too long, with an average duration exceeding 170 days from sowing date to flowering date. It ranged from 162 days for genotype AN21 to 180 days for genotypes M27 and M29. The check variety has bloomed in 170 days after sowing.

Flowering earliness is a desired character in environments under terminal drought stress (Ribot et al., 2012). Thus, genotypes having a sowing-flowering period shorter than that of the check will be selected.

Table 1. Analysis of variance (Mean square and significance level of differences) for agromorphological, physiological and technological traits of 45 sunflower accessions evaluated under early winter planting conditions in Annoceur 2014.

Parameter	Genotype	Average	Minimum		Maximum		value from Control Ichraq	Threshold for selection
			Value	Genotype	Value	Genotype		
IV(1)	*** (2)	3.49	1	M32	5	AN8	3	4-5
GR	***	2.31	0.53	AN21	3.92	M34	3.15	>3.15
PH	***	146.77	75	M18	200	K20	166.66	<145
NLP	***	27.46	17	M4	38	K3	25.33	<25
LA	**	162.23	24.5	M17	374.85	AN34	161.7	<162
CD	***	20.08	11.11	M18	30.82	M7	23.11	>20
CHLT	ns	43.21	28.2	K7	79.1	M29	54.6	>54
CHLA	***	4.04	0.86	K9	11.31	K4	1.86	>1.86
CHLB	***	6.77	1.74	K8	19.89	K4	2.77	>2.77
DSF	***	170.48	162	AN21	180	M29 M27	170	<170
NBP	***	0.93	0		6	M30	0	0
THD	***	12.79	6	M22 M18	23	K9	16.33	>16
AHD	ns	2.48	0		7	K3 K9	2	<2
PAD	ns	21.2	0		62.5	M32	13.96	<13.96
NSP	***	18.39	8	M22	27	K20 K8	20	>20
HSY	***	49.36	8.184	M18	110.3	K8	62.14	>62
TSW	***	47.52	12.4	M18	83.6	K8	56.86	>56
SOC	***	36.43	21.83	M8	46.85	K4	38.52	>38
TSY	***	27.42	4.54	M18	61.3	K8	34.52	>34

(1) IV: initial vigor, GR: growth rate, PH: plant height, NLP: number of leaves per plant, LA: leaf area, CD: collar diameter, CHLT: total chlorophyll content, CHLA: chlorophyll a content, CHLB: chlorophyll b content, DSF: days from sowing to flowering, NBP: Number of branches per plant, THD: total head diameter, AHD: aborted head diameter, PAD: percentage of aborted diameter, NSP: number of seeds per propeller, HSY: head seed yield, TSW: 1000 seeds weight, SOC: seed oil content, TSY: total seed yield (per hectare).

(2) *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively. ns not significant.

Agronomic and technological parameters

Analysis of variance showed there were significant differences ($p < 0.001$) between the studied genotypes for all agronomic and technological parameters, excepted aborted head diameter (AHD) and percentage of aborted diameter (PAD) (Table 1). However, one could observe some variation between genotypes for AHD and PAD (Table 1). Most of the evaluated genotypes had no AHD, and among the few ones having

AHD, genotypes K3 and K9 exhibited the largest AHD, 7 cm. The overall average AHD was about 2.5 cm. The overall average PAD was about 21%, ranging from 0%, for most of the genotypes, to more than 62%, for genotype M32. All genotypes exhibiting some AHD should be discarded from the selected population as aborted sunflower head is an indicator of plant susceptibility to cold (Hladni et al., 2010). Average total head dimeter (THD) was 12.8 cm, ranging from 6 cm, for genotypes M22 and M18, to 23 cm, for genotype K9. The check variety 'Ichraq' had a THD of 16 cm, an AHD of 2 cm and a PAD of 14%. A large range was observed for number of seeds per propeller, from 8 in genotype M22 to 27 in genotypes K8 and K20. The check variety had a number of 20 seeds per propeller. Regarding seed yield per head, the overall mean was slightly higher than 49 g, and a large range was found, from 8 g in genotype M18 to 110 g in genotype K8, which is much higher than head seed yield of the check (62 g). Thousand seed weight (TSW) ranged from 12.4 g in genotype M18 to 83.6 g in genotype K8, and the average was 47.52 g. TSW of the check was about 57 g. The average total seed yield (TSY) was around 27 q/ha and there was a large variation from 4.54 q/ha in genotype M18 to 61.30 q/ha in K8. TSY of the check was slightly higher than 34 q/ha. Total head diameter, number of seeds per propeller, single head seed yield and TSW are components of TSY which are correlated with this latter, and thus could be considered as selection criteria for seed yield breeding (Yasin and Singh, 2010). In our study, we will select all those genotypes showing values higher than those of the check. Finally, seed oil content (SOC) fluctuated from 21.80% in genotype M8 to 46.85% in genotype K4, and had a mean value of 36.43%. The check 'Ichraq' had a SOC of 38.52%, which was slightly higher than the overall average. Genotypes with SOC exceeding that of the check will be selected. Table 2 shows the pools of genotypes selected, according to described threshold, for each of the studied parameters.

Pearce 1999 subdivided the plants into three categories according to their tolerance to cold and ability to adapt to low temperatures. Susceptible plants to low temperatures that suffer damage as early as 12 °C, tolerant plants to low positive temperatures and plants capable to acclimate to survive under temperatures below zero degree. Xin and Browse 2000 showed that they are a large number of physiological mechanisms that allow plants to better withstand severe stress (temperatures below zero) after a long time at low temperature (acclimatization). Many studies have shown low temperature direct effects on cells (Pearce, 1999), on seed germination (Durr et al., 2001), on photochemical reactions of photosynthesis and carbon fixation (Liua et al., 2012). Likewise, cold causes reduction of cell water content (Kacperska, 2004).

Our findings have shown there was a genetic diversity among the sunflower genotypes evaluated for most of the studied parameters. In all cases, these genotypes were compared with the check variety 'Ichraq'. This study allowed us to identify and select genotypes more interesting than the check for morphological, physiological, agronomic and technological parameters under winter early planting conditions. Globally, taking into account all these parameters, the genotypes AN8, AN3, AN34, AN33, AN27, AN23, AN21, AN24, K30, K20, K10, K3, K8, K7, K4 seemed to be performant and promising. After confirming their performance in further seasons, they could be useful for intercrossing to develop a new variety more performant and more tolerant to winter cold than 'Ichraq', the only one autumn variety registered to day in Morocco.

Table 2. Pools of sunflower genotypes selected for their performance on basis of morphological, physiological, agronomic and technological parameters under early winter planting conditions.

Parameters	Sunflower genotype pools
IV	AN8;AN6;AN3;M45;M43;M30;M26;M17;K8;K7;K6;K5;K4; A35;A34;A33; A32;A27;A23;A21;A13;A11
RG	M34;M32;M30;K30;K20;K10;K9;K8;K5;
PH	AN8;AN3;M43;M41;M37;M34;M32;M29;M26;M27;M22;M19;M18;M17;M13;M8;M7;M6; M5;M4;AN21
NLP	AN8;AN3;M41;M37;M34;M32;M29;M27;M22;M19;M18;M17;M8;M6;M4;K8;AN33;AN27;AN21;
LSA	AN6;AN3;M45;M37;M34;M32;M29;M26;M22;M19;M18;M17;M8;M7;M6;M4;K30;K20;K9; K8;K4;K3;AN32;AN31;AN27;AN24;AN23;AN21;AN13;AN11;AN9;
CD	AN8;AN3;M45;M41;M37;M34;M30;M26;M22;M13;M7;M5;K30;K20;K10;K9;K8;K7;K6; K5; K4;K3;AN35;AN34;AN32;AN31;AN27;AN24;AN23;AN21;AN13;AN11;
CHLT	M29;M27;M22;M8;M6;K30;K3;AN31;
CHLA	All genotypes except: M45;M34;M30;K9;K8;AN35;AN31;AN23;AN9
CHLB	All genotypes except: M30;K8;AN35;AN34;AN31;AN23;
DSF	AN8;AN6;M22;M18;M7;M4;K30;K7;K4;AN35;AN34;AN33;AN27;AN31;AN24;AN23; AN21;AN13;AN9
NGB	AN8;AN6;AN3;AN45;M43;M41;M37;M13;M7;K30;K20;K10;K9;K8; K7;K3;K4;K5;K6;AN35;AN32;AN27;AN24;AN9;AN11;AN13
DTC	AN8;M45;M37;K20;K10;K9;K8;K7;K5;K4;K3;AN31;AN24;AN27;
DFA	AN3;M43;M34;M32;M27;M29;M30;M18;M7;K20;K10;K9;K8;K7;K5 ;K3;AN34;AN32;AN27;AN24;AN23;AN13;AN9
PDA	AN3;M43;M37;M34;M32;M27;M29;M30;M18;M7;K20;K10;K9;K8;K7; K5;K3;AN34;AN32;AN31;AN27;AN24;AN23;AN13;AN9
NGP	AN8;AN3;M45;M43;M41;M37;M34;M32;M30;M13;M8;M7;M6;M5;K30;K20; K10;K9;K8;K7;K6;K5;K4;K3;AN34;AN31;AN27;AN24; AN23;AN11;AN9
SYC	AN8;AN6;AN3;M45;M41;M37;M32;M30;M8;M7;K30;K20;K10;K8;K7;K4;K3; AN35;AN34;AN27;AN24;AN23;AN21;AN11;
TSW	AN8;AN6;AN3;M45;M43;M41;M37;M34;M32;M30;M18;M7;K30;K20;K10;K8;K7;K5; K4;K3;AN35;AN34;AN33;AN27;AN24;AN23;AN21;AN13;
SOC	AN8;AN6;AN3;M41;M22;M19;M17;M6;M5;M4;K30;K20;K10;K9;K8;K7; K6;K5;K4;K3; AN34;AN33;AN32;AN31;AN27;AN24;AN23;AN21;AN13; AN11;AN9
SYP	AN8;AN6;AN3;M45;M41;M37;M32;M30;M8;M7;K30;K20;K10;K8;K7;K4;K3 ;AN35;AN34;AN33;AN27;AN24;AN23;AN21;AN11;

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TESTING ANNUAL WILD SUNFLOWER SPECIES FOR RESISTANCE TO *OROBANCHE CUMANA* WALLR.

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is a holoparasitic weed that attacks the roots of sunflower (*Helianthus annuus* L.) causing yield losses in excess of 30%. It affects mostly warm and dry regions. Development of resistant cultivars and optimization of agricultural practices are the most important tasks for broomrape control in affected countries. In Serbia, first severe infestations were recorded in the early 90s. IFVCNS breeding program for transfer of *O. cumana* resistance from wild *Helianthus* species first pointed to *H. petiolaris* ssp. *petiolaris* as an excellent donor of *Or* genes. Resistance of annual wild species to *O. cumana* has been evaluated in a long term characterization program. Starting from 1996, multiple tests were performed in the greenhouse and in the field with broomrape presence. Total of 7 annual *Helianthus* species and 182 accessions were screened for resistance. The highest percentage of accessions with no segregation for resistance was found in *H. petiolaris* (81%) followed by *H. niveus* and *H. argophyllus*. If resistance is expressed per plant, *H. petiolaris* and *H. niveus* had more than 90% of plants with no infection. *H. argophyllus, H. debilis, H. praecox* and *H. neglectus* were in the range of 77-86%, while *H. annuus* had only 37% of resistant plants and proved to be the most susceptible of the tested annual species. The obtained results pinpoint the most useful species and accessions for further work on keeping cultivated sunflower resistant to broomrape.

Key Words : wild annual Helianthus, resistance, Orobanche cumana

STUDY OF THE CHARACTERISTICS OF CULTIVATED VARIETIES OF SUNFLOWER, REGARDING THE PRODUCTION OF HIGH QUALITY SUNFLOWER MEAL WITH DEHULLING PROCESS

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ABSTRACT

Dehulling sunflower seeds, before crushing, increases the protein content in the meal up to 36%, whereas a cake obtained without dehulling contains 27-29% protein. The quality of sunflower seeds directly impacts the possibility of obtaining a high protein meal. The purpose of this study was to assess the varietal effect on the protein content and the hullability of sunflower seeds. Genetic effect was studied with seed samples from a network of variety evaluation trials in France during the two years. The protein content in seeds was expressed as a percentage of Defatted Dry Matter. Hullability was obtained by measuring the initial weight of the seeds and the weight of extracted hulls, removed by a laboratory dehulling equipment. Other measured characteristics were oil content, seed size, crude fibre content. Significant differences between varieties for protein content were observed within the medium early/medium late group in 2013 (from 33.2% to 41.3%), as for hullability (from 3.7% to 14.7%). As a consequence, the potential protein content of their dehulled meals also ranged widely (34-44%). Crude fibre content was closely correlated to hullability. An equation was established to estimate the protein content of dehulled sunflower meal as a function of protein content and crude fibre content in seeds. The protein content of sunflower seeds proved to be the key characteristic determining the quality of sunflower meal. Genetic selection, which allowed great improvements in the oil content and fatty acid composition, should therefore also help to improve the quality of sunflower meal.

Key words: Sunflower, Hullability, Protein, Variety

INTRODUCTION

Sunflower seed processing produces 2 principal co-products: oil, mainly for human consumption, and meal, for animal feed. Two main variants exist in the crushing industry: oil extraction from whole seeds and oil extraction from partially dehulled seeds. In the first process, the resulting meal is of low protein content (27-29%). In the second process, the resulting meal has a higher protein content (36% protein content is a standard quality for this type of meal) and reduced fibre. The second process is highly developed in Eastern Europe and Argentina. In France, until recently, dehulling was carried out in only one oil mill which has a limited dehulling capacity offering only a modest improvement in meal protein content. Capacity has begun to develop since 2013, with a larger factory now partially dehulling prior to crushing.

Dehulling offers 2 advantages:

- The higher protein and lower fibre content meal has an increased economic value on the animal feed market. Peyronnet *et al.* (2012) demonstrated that the interest price of a 36% protein content

meal (i.e., that maximum price at which it remained competitive) was 70% of the soybean meal price, whereas for a 29% protein meal it was only 43%.

- The hulls removed can be used as an energy source for steam production in a high-performance biomass boiler. Rising energy costs and environmental concerns have led to a growing interest within the crushing industry for using hulls as energy source instead of fossil fuels (Tostain *et al*., 2012).

In the 1980s, energy prices were very high; towards the end of the decade and the early 1990s, research was undertaken in France to prepare the crushing industry for greater use of the dehulling process. Genetic studies were carried out concerning the ease with which hulls could be removed from sunflower seeds (hullability). These studies showed that this characteristic could be introduced through breeding programmes. Cultivars producing seeds with a smaller hull mass, higher oil content but a good hullability offered the most promise for improving the quality of sunflower meal. Such genotypes were rare, but a recurrent selection programme could be used to increase the frequency of favourable genes (Denis and Vear, 1996). Although all this work constituted a favourable basis for the growth of sunflower dehulling in the French crushing industry, the technology was not developed. In France, the oil mill that only recently reintroduced dehulling actually abandoned the technique in the early 1990s, the context being one where the oil content of the new cultivars was improving but hullability was decreasing, resulting in considerable losses of oil from the hull fraction. At that time moreover, boiler technology for burning hulls had not achieved an adequate level of efficiency. So, the economics were against dehulling. As a consequence, sunflower breeding has ignored the characteristic of hullability, and likewise protein content.

The supply of vegetable protein to livestock is now a matter of political concern. Oilseed meals are an attractive source of proteins. Moreover, sunflowers have the advantage of containing no anti-nutrients or toxic components. Increasing the protein content in sunflower meal would therefore be advantageous. The quality of seeds, notably the protein content expressed as a percentage of Defatted Dry Matter (DDM) and their hullability, determines the potential protein content of the resulting meal. It has been shown that the most profitable way to reach a set requirement of protein content in the meal is to produce seeds with high protein content as a percentage of DDM, in order to require extraction of only a minimum amount of hulls (Dauguet *et al.*, 2015).

The economic focus remains on sunflower oil (about 700-800€/t in 2015, as compared with approximately 180-200€/t for non-dehulled meal and 250-280€/t for 36% protein meal). Hence, breeding has always been centred on obtaining varieties combining high yield and high oil content. These are the 2 criteria that are currently taken into account in the registration of new sunflower varieties; protein content is not a criterion in the registration of new sunflower varieties and nor is it measured in the official trials. Studies have shown that soil and climatic conditions exert a greater influence on protein content than genetics (Nel, 2001; Oraki *et al.*, 2011; Dauguet *et al.*, 2015). This can be explained by the fact that breeding programmes have not searched for variability in protein content. No relationship has been observed between oil content and protein content as a percentage of DDM (Dauguet *et al.*, 2015). So, the independence of these 2 features would suggest that there is considerable scope for improving the protein content of the defatted fraction without penalizing oil content.

Hullability increases with the size of seed and decreases with their oil content; these are varietal characteristics and so genetic improvements of hullability might be considered (Baldini *et al.*, 1994; Denis *et al.*, 1994; Evrard *et al.*, 1996; Nel, 2001; Sharma *et al.*, 2009; Dauguet *et al.*, 2015).

In a previous study (Dauguet *et al.*, 2015), we examined seed samples taken from a wide network of farmers' fields in South West France, looking at 3 varieties, over 2 years (2 varieties each year). Both protein content and hullability were found to be influenced by the environment, with water stress having a substantial effect. Some differences between cultivars could be identified, affecting protein content and

hullability. In contrast, the influence of agricultural practices such as nitrogen fertilization could not be established. In order to improve meal quality, and the competitiveness of sunflowers in the food chain, boosting the protein content of sunflower seeds through breeding would be very beneficial, so long as there was no negative effect on oil content and hullability remained adequate.

The objective of the present study, designed in close collaboration with Terres Univia, the French oil and protein crops inter-branch organization, was to improve knowledge of the sunflower cultivars traded on the French market, in particular with regard to their characteristics that impact the possibility of producing good quality meal: seed protein content as a percentage of DDM and hullability. We studied genetic and climatic effects on these characteristics, with samples from a network of varietal evaluation trials, during 2 consecutive years to evaluate the variability in of marketed sunflower varieties for these or other characteristics not taken into account in breeding programmes. We also measured crude fibre content in order to study its correlation with hullability, and the possibility of evaluating hullability using this more simple analytical result rather than employing laboratory dehulling equipment.

MATERIALS AND METHODS

Samples: Seed samples were collected from the Terres Inovia experimental network. This is constructed each year to evaluate the performance of varieties marketed in France (agronomic performance such as yield and diseases resistance; quality traits of the seeds such as oil content and Thousand Seed Weight). For the purposes of this study, additional analyses were performed on the seed samples to measure their protein content, hullability and crude fibre content. The varieties studied were oleic and linoleic types in 2 maturity groups (early or medium early/medium late). Each year, the Terres Inovia experimental network includes about 30 variety trials for each maturity group.
The seed samples collected for this study came from 2012 and 2013. Each year, we collected samples from several experimental locations, from regions where sunflowers are commonly cultivated: South-West, West and Central France (see Figure 1).

Each year, we studied the protein content of 5 to 7 different varieties in each maturity group, in the 8 different trial locations. For hullability and crude fibre analyses, the number of varieties studied was reduced to 2 per maturity group, as the measurements were time-consuming and costly.

In order to investigate the variability of profiles for protein content and hullability, we also studied each year a larger number of varieties (12 and 16) in only 2 or 3 trials (see Table 1).

Overall, the data set included 275 samples of sunflower seeds, with 40 different sunflower varieties, at 23 different locations (see Table 1).

Table 1: Cultivar distribution according to year and locations

Year	Maturity group	Trial locations (postal code of French department)	Cultivars
2012	Early	Antoigné (79), Frozes (86), Levroux (36), Saint Branchs (37), Rhodon (41), Maslacq (64)	ES Biba, Vellox, Extrasol, ES Balistic, ES Ethic
		Saint-Martial (16), Vibrac (17)	ES Biba, Vellox, Extrasol, ES Balistic, ES Ethic, Ullys, Fydgi, Volltage, ES Violetta, P64LL41, ES Atletic, SY Valeo
	ME/ML	Virson (17), Loudun (86), Vicq / Nahon (36), Lévignac (31), Tané (32), Le Saumont (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO
		Vibrac (17), Duras (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Breha, Sherllok, Dougllas, Mobill, SY Edenis, ES Akustic, NK Adagio, LG5625, ES Tektonic, ES Unic
2013	Early	Vibrac (17), Levroux (36), Meung sur Loire (45), Ivoy le pré (18), Maslacq (64)	ES Biba, Vellox, Extrasol, SY Valeo, ES Violetta, Fydgi
		Antoigné (79), Triaize (85), Trouy (18)	ES Biba, Vellox, Extrasol, SY Valeo, ES Violetta, Fydgi, SY Sanbala, P63LL78, LG5377, Bering, MAS83R, ES Lumina, ES Columbella, SY Revelio, ES Atletic, ES Balistic
	ME/ML	Antoigné (79), Benet (85), Lévignac (31), L'Isle Jourdain (32), Montagnac Auvignon (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Dougllas, ES Tektonic
		Pompertuzat (31), Tané (32), Duras (47)	NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO, Dougllas, ES Tektonic, Clloser, Meddia CS, LG5687HO, SY Explorer, LG5528, SY Edenis, ES Akustic, LG5625

ME/ML=Medium early/medium late

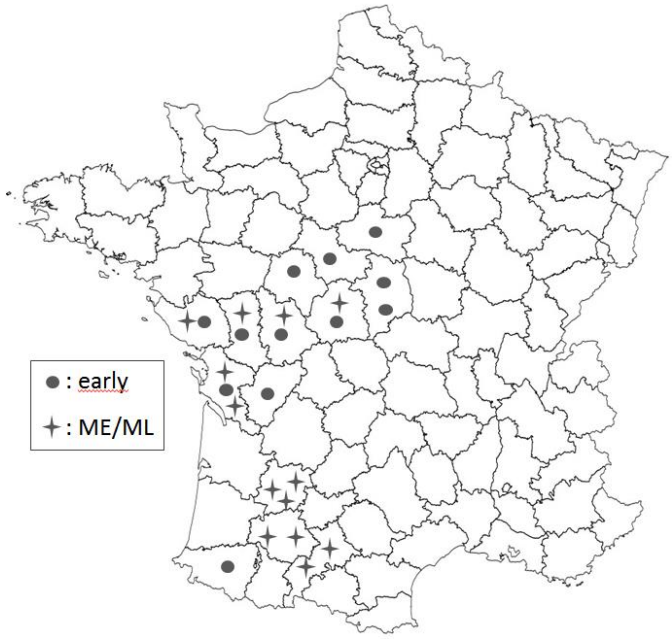


Figure 1. Location in France of the selected sunflower variety trials in 2012 and 2013, by maturity group.

Chemical analyses: For each seed sample collected, the oil content was assessed by Nuclear Magnetic Resonance (NF EN ISO 10565) and expressed as a percentage at marketing standard (9% moisture content and 2% impurities level), as commonly used in varietal trials. Protein content was assessed by the Dumas method (NF EN ISO 16634-1) and expressed as a percentage of Defatted Dry Matter (DDM) or Dry Matter (DM). The crude fibre content was measured by the Weende method (NF V03-040 with previous oil extraction by hexane), and was expressed as a percentage of Defatted Dry Matter (DDM) or Dry Matter (DM). The expression of results as a percentage of DDM, for protein or crude fibre content, has an obvious interest from an end-user perspective, as it gives information on the content that would be obtained in the meal, after oil extraction.
An indicator of the seed size, the Thousand Seeds Weight (TSW), was measured on clean dry grain (0% moisture).
While each sample was analysed for it oil and protein content and TSW, the crude fibre content was assessed in only 2 varieties in each maturity group each year.
All of these analyses were carried out at Terres Inovia's Analysis Laboratory in Ardon.
Hullability determination: What we refer to as "hullability" was obtained by measuring the initial weight of the seeds and the weight of extracted hulls, removed by a standard procedure: Hullability (%) = (mass of extracted hulls (g))/ (mass of initial seeds (g)).
Seed hullability is affected by water content (Sharma et al, 2009). Since the seeds had been stored at various levels of humidity, they were taken out of cold storage and placed in Petri dishes that were then left open for 48 hours, to facilitate equilibration of water content prior to dehulling. The water content of the seeds was low, as they had previously been dried slightly to favour long-term storage: about 5.5-6.0% (mean moisture 5.7%) and sufficiently uniform (standard deviation 0.7%) to permit a comparison of hullability.

A conical divider was used to produce 4 identical subsamples of approximately 15g from the primary sample. Three replicates were used in the dehulling test; the 4th was used to measure water content. Employing a method determined by a previous study (see Dauguet et al., 2015), the weighed samples were passed 3 times through the laboratory dehulling equipment, a Techmachine, at 2 000 revolutions per minute (rpm). This is equivalent to limited or moderate dehulling in an industrial dehulling process which would result in 10% hull extraction, whereas 15% hull removal is current practice in industry.

After sorting using laboratory sorting equipment, the various fractions (kernels, whole seeds, fines and hulls) were weighed (to the nearest 0.01 g). The percentage of extracted hulls was taken from the average of 3 replicates. Water content was assessed from the difference in seed weight before and after 15 hours in an oven at 103°C (NF V03-909).

Hullability also was measured only for 2 varieties in each trial, except for the trials with a wider range of varieties studied (2 or 3 trials each year in each maturity group) where hullability was measured for each cultivar.

Statistical analyses: Data were analysed using analysis of variance (ANOVA). F-test and differences were evaluated via the Student-Newman-Keuls Test (software SAS 9.4). The coefficients of determination, and associated probability (Student) were also established using SAS software. Shapiro-Wilk tests were performed to check the normality of the residuals; homoscedasticity was verified visually. *Calculation of the protein content in sunflower meal post-dehulling:* Given the defined quality characteristics of sunflower seeds (protein content, hullability), we developed a formula to estimate for each sample the protein content of the dehulled meal. In this way, we were able to assess the potential of a particular variety to produce meal of the required quality. This formula is based on measured values: initial seed protein content and the degree of hullability (percentage of extracted hulls), as well as assumptions regarding oil and moisture content of the meal, and the protein, oil and moisture content of the sunflower hulls. These assumptions were based on a yearly study of meal quality in the French crushing industry (Terres Inovia's unpublished results from a particular factory) and from an online database on feedstuffs (Feedipedia) for parameters on hulls.
Assumptions:

A = Oil content in meal in raw matter (RM) = 1.2%

B = Moisture content in meal = 11.5%

C = Protein content in hulls in RM = 6%

Moisture content in hulls = 10%

Oil content in hulls in RM = 2%

X = mass of removed hulls (g/100g seeds)

Formulae:

D = Defatted Dry Matter (DDM) of seeds = 1 – (moisture content of seeds) – (oil content of seeds on NMR)

E = Protein content of seeds (%DDM) = protein content of seeds (%DM)/ (1 - oil content of seeds (%DM))

F = Defatted Dry matter of the hulls = 1 – (moisture content of hulls) – (oil content of hulls) = 88%

G = Protein content of non-dehulled meal (% RM) = $E * (1 - A - B) * \left(\frac{D}{1-A-B}\right)$

H = Protein content of extracted hulls (% RM) = X * C

I = Protein content of dehulled meal (%RM) = $\frac{G-H}{D-X*F} * (1 - A - B)$

RESULTS AND DISCUSSION

Analysis of variance

The results were aggregated by maturity group and by year, see Tables 2 and 3. Here, we assessed the influence of location and cultivar on the seed characteristics in 8 trials.

Table 2: Results for the Early group cultivars grown in eight trial locations (mean by cultivar, t comparison tests at 5% and levels of significance of ANOVA of seed components).

Year	Factor		Oil content (% at marketing standards)	Thousand Seeds Weight (g DM)	Protein content (% DDM)	Protein content (% DM)	Crude fibre (% DDM)	Crude fibre (% DM)	Hullability (% extracted hulls)	Calculated protein content in dehulled meal (% RM)
2012	Cultivar	ES Biba	46.1 (B)	42.4 (BC)	34.5 (A)	16.7 (B)				
		Vellox	47.9 (A)	39.6 (C)	36.2 (A)	16.8 (B)	28.3 (B)	13.5 (B)	11.0 (B)	38.9 (A)
		Extrasol	45.4 (B)	45.4 (AB)	35.5 (A)	17.4 (B)	30.3 (A)	15.0 (A)	15.5 (A)	41.5 (A)
		ES Balistic	42.9 (C)	46.3 (A)	35.9 (A)	18.6 (A)				
		ES Ethic	46.0 (B)	42.4 (BC)	34.5 (A)	16.7 (B)				
	Level of significance	Location	***	***	***	***	**	**	NS	NS
		Cultivar	***	***	NS	***	***	***	**	NS
2013	Cultivar	ES Biba	48.1 (C)	49.7 (C)	32.4 (AB)	14.9 (AB)				
		Vellox	52.3 (A)	50.2 (C)	33.3 (A)	13.8 (B)				
		Extrasol	47.7 (C)	57.9 (A)	33.5 (A)	15.6 (A)				
		SY Valeo	48.2 (C)	50.4 (C)	31.3 (AB)	14.4 (B)	35.5 (A)	16.6 (A)	10.2 (A)	33.1 (A)
		ES Violetta	47.7 (C)	55.6 (AB)	30.9 (B)	14.3 (B)				
		Fydgi	50.7 (B)	52.1 (BC)	32.9 (AB)	14.2 (B)	35.2 (A)	15.3 (B)	9.6 (A)	34.7 (A)
	Level of significance	Location	***	***	**	**	NS	NS	*	NS
		Cultivar	***	***	**	**	NS	*	NS	NS

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per year within a column followed by the same letter are not significantly different ($P < 0.05$)

Table 3: Results for the ME/ML group cultivars grown in eight trial locations (means by cultivar and levels of significance of ANOVA of seed components)

Year	Factor		Oil content (% at marketing standards)	Thousand Seeds Weight (g DM)	Protein content (% DDM)	Protein content (% DM)	Crude fibre (% DDM)	Crude fibre (% DM)	Hullability (% extracted hulls)	Calculated protein content of dehulled meal (% RM)
2012	Cultivar	NK Kondi	44.9 (A)	41.1 (B)	35.3 (A)	17.5 (C)				
		Kapllan	44.9 (A)	42.7 (AB)	36.1 (A)	17.9 (BC)				
		Extrasol	43.6 (AB)	46.0 (A)	36.7 (A)	18.7 (AB)	29.1 (A)	14.8 (B)	16.8 (A)	43.7 (A)
		DKF3333	43.2 (B)	45.8 (A)	36.1 (A)	18.6 (AB)	30.0 (A)	15.4 (A)	15.3 (A)	41.4 (A)
		LG5656HO	42.3 (B)	44.1 (AB)	36.3 (A)	19.0 (A)				
	Level of significance	Location	***	***	***	***	**	**	**	NS
		Cultivar	***	*	NS	**	NS	*	NS	NS
2013	Cultivar	NK Kondi	47.9 (A)	49.1 (D)	35.5 (C)	16.4 (C)	36.8 (A)	17.4 (A)	9.7 (B)	37.3 (B)
		Kapllan	48.2 (A)	52.4 (CD)	38.0 (AB)	17.5 (B)				
		Extrasol	46.6 (B)	56.3 (B)	37.2 (ABC)	17.8 (AB)				
		DKF3333	46.9 (B)	51.0 (CD)	37.7 (ABC)	17.9 (AB)				
		LG5656HO	44.5 (C)	49.8 (CD)	37.4 (ABC)	18.7 (A)				
		DOUGLLAS	47.6 (A)	60.1 (A)	39.0 (A)	18.0 (AB)				
		ES TEKTONIC CL	46.0 (B)	53.7 (BC)	36.2 (BC)	17.6 (B)	36.1 (A)	17.9 (A)	14.4 (A)	41.8 (A)
	Level of significance	Location	***	***	***	***	*	**	*	*
		Cultivar	***	***	**	***	NS	NS	***	**

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per year within a column followed by the same letter are not significantly different ($P < 0.05$)

ANOVA were performed on the parameters studied using location and cultivar as explicative factors (see Tables 2 and 3). The impact of location was significant, except on hullability and crude fibre content in the early group and for the calculated protein content of the dehulled meal. This impact might be attributable to different meteorological and soil conditions affecting plant growth.

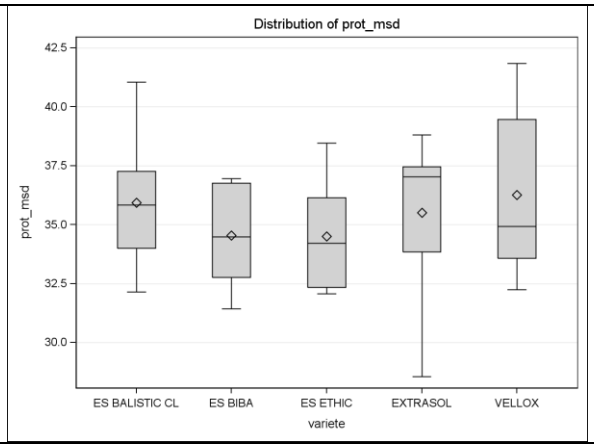
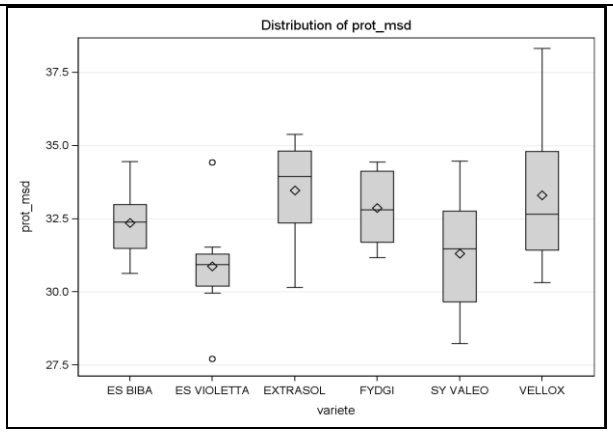
Cultivar was the principal factor affecting oil content, seed size (TSW) and percentage protein of DM. It did not however systematically affect the protein content of DDM: the variability for each variety was high (see Figure 2). Significant differences between varieties for protein content as a percentage of DDM were observed only in 2013: ES Violetta was significantly lower than Extrasol and Vellox (30.9%

versus 33.5 and 33.3%) within the early group. Within the ME/ML group, NK Kondi and ES Tektonic had significantly lower protein contents than Dougllas (35.5% and 36.2 % versus 39%). For this parameter, in 2012 the differences between locations were greater than the differences between cultivars.

For hullability, Vellox was significantly more difficult to dehull than Extrasol in 2012 (11% extracted hulls versus 15.5), and NK Kondi had also a lower hullability than Es Tektonic in 2013 (9.7% extracted hulls versus 14.4%). However, there was no significant difference between SY Valeo and Fydgi in 2013, or between Extrasol and DKF3333 in 2012, as the differences between locations were high (see Figure 3).

It is difficult to conclude from the results for crude fibre content. Crude fibre associated with higher hull content in seeds could be a favourable factor for hullability. For example, Extrasol had significantly higher crude fibre content (on DDM and on DM) than Vellox, which could be related to a better hullability, but, ES Tektonic showed better hullability than NK Kondi, although these 2 varieties had comparable crude fibre contents. This led to a conclusion that crude fibre content was not the unique factor affecting hullability. Seed size, hull structure and the phenomenon of adherence were probably important also.

The final aspect, the right hand column in Tables 2 and 3, a calculation of the potential protein content in meal that would be obtained after dehulling, based on protein content of the seeds and hullability, did not show significant genetic differences, except between NK Kondi and ES Tektonic, as the second had a better hullability and gave a richer meal. This parameter suggests that the protein content of meal could be quite high, above the standard level in high-protein meal (36%), since for some cultivars in some years it exceeded 40%. It was only in the early group in 2013 that the protein content was low for all the varieties, and hullability was moderate; so the calculated protein richness in the meal was less than the standard 36%. This highlighted the importance of initial protein content in seeds in the production of good quality meal.

Group	2012	2013
Early		

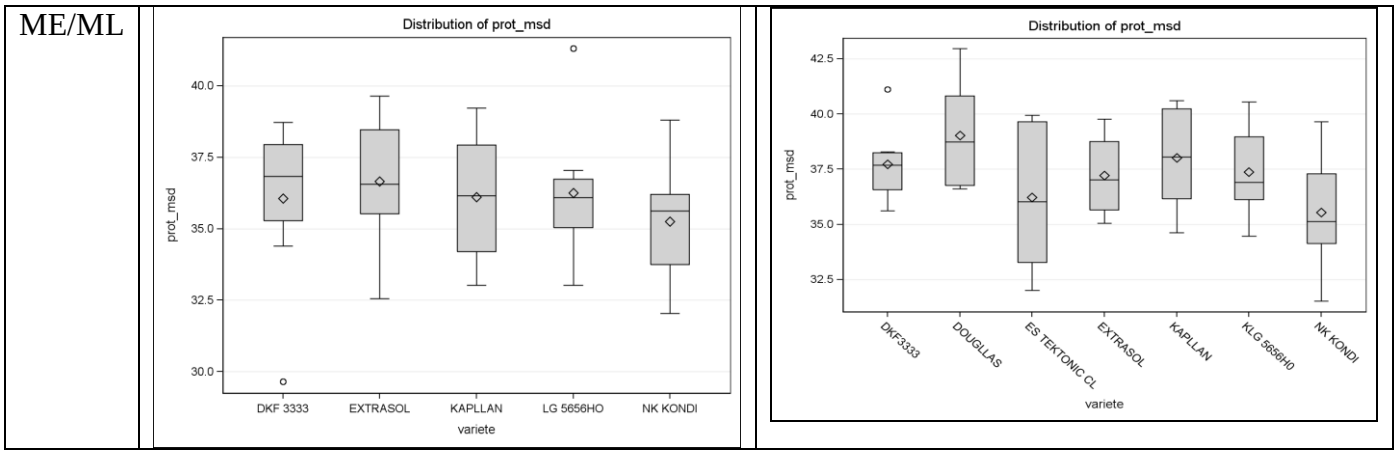


Figure 2: Boxplots of cultivar effect on protein content (%DDM) showing the median (line in the middle), mean (diamond), interquartile range (box) and total range (whiskers) not including atypical values (circle symbols, where they exist)

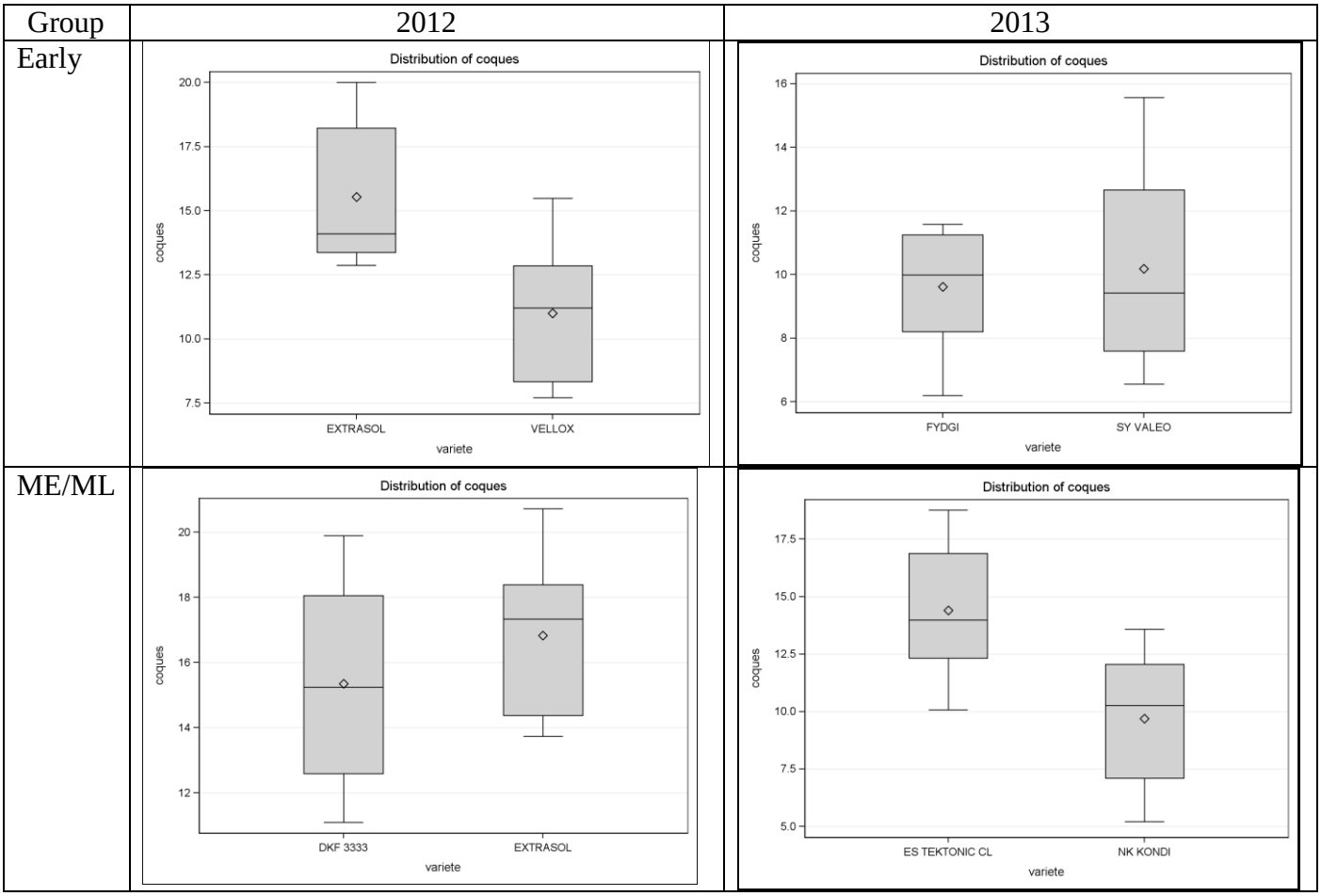


Figure 3: Boxplots of cultivar effect on hullability (% of extracted hulls) showing the median (line in the middle), mean (diamond), interquartile range (box) and total range (whiskers)

3.2. Year effect

Some trial locations and cultivars were constant in both years, which enabled the evaluation of the effect of year (including the climatic effect) (see Table 4):

- for the early group, 4 locations (Antoigné, Levroux, Vibrac and Maslacq) and 3 varieties (ES Biba, Vellox, Extrasol),

- for the ME/ML group, 3 locations (Duras, Tané, Lévignac) and 5 varieties (NK Kondi, Kapllan, Extrasol, DKF3333, LG5656HO).

Table 4: Analysis of variance for year, location and cultivar effects on seed characteristics (means, t comparison tests at 5% and levels of significance)

Factors		N	Oil content (% at marketing standards)	TSW (g DM)	Yield (t/ha at marketing standards)	Protein content (% DDM)	Protein content (% DM)
Early	2012	12	47.5 (B)	45.4 (B)	3.80 (A)	35.8 (A)	16.7 (A)
	2013	12	49.3 (A)	55.3 (A)	3.57 (A)	33.3 (B)	14.9 (B)
	Year		*	***	NS	*	**
	Location		NS	NS	NS	**	**
	Cultivar		**	*	NS	NS	NS
ME/ML	2012	15	44.3 (B)	41.6 (B)	3.42 (B)	37.4 (A)	18.8 (A)
	2013	15	48.0 (A)	47.5 (A)	3.66 (A)	37.2 (A)	17.2 (B)
	Year		***	**	*	NS	***
	Location		**	NS	*	NS	NS
	Cultivar		**	NS	***	NS	**

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means within a column followed by the same letter are not significantly different ($P < 0.05$) with t test

It was rainier and less sunny in 2012 than in 2013. During 2013, water stress occurred after flowering, which led to lower yields in the South and West of France and in the country as a whole (respectively 2.38 t/ha in 2012, and 2.14 t/ha in 2013, according to public statistics). In the studied, this trend was observed for the early cultivar group, but it was not substantial (yield 2012 3.8 t/ha and yield 2013 3.57 t/ha); while the situation was the opposite for the ME/ML cultivar group with better yields in 2012 (3.66 t/ha) than in 2013 (3.42 t/ha). This is due to the fact that the varietal evaluation trials were grown in more optimal conditions than normal farmers' fields, and therefore were not representative of national sunflower production. The climatic effect influenced the oil content and seed size (TSW), higher in 2013 than in 2012, and percentage protein content of DM, higher in 2012, which was a consequence of lower oil content in 2012. For the percentage protein content of DDM, a significant difference was observed only in the early cultivar group, with higher levels observed in 2012 than in 2013.

The wider range of varieties

Each year, 15 cultivars were sampled and analysed in 2 (2012) or 3 (2013) locations for each maturity group, to obtain some idea of the diversity of cultivar profiles for protein content as a percentage of DDM and their hullability, and for their potential to produce good quality meal.

This larger panel of cultivars made it possible to assess the potential variability of the protein content in dehulled meals. The range for the calculated protein content of dehulled meal (see Table 5) is large, with 10 percentage points between the poorest and the best cultivars within ME/ML group in 2013 (for other maturity groups and years, the range was 7 to 9 points; the results are not presented here).

Table 5: Analysis of variance for seed components from 15 ME/ML group cultivars, grown in 3 locations in 2013 (means and level of significance of ANOVA on seed components)

Cultivar	Oil content (% at marketing standards)	TSW (g DM)	Protein content (% DDM)	Hullability (% extracted hulls)	Calculated protein content of dehulled meal (% RM)
CLLOSER	49.7 (A)	53.2 (ABC)	39.0 (AB)	7.3 (CD)	39.1 (ABCD)
MEDDIA CS	49.0 (AB)	47.1 (C)	41.3 (A)	3.7 (E)	38.6 (BCD)
KAPLLAN	48.5 (BC)	53.1 (ABC)	38.9 (AB)	10.3 (BC)	41.5 (ABC)
DOUGLLAS	47.6 (CD)	61.3 (A)	39.4 (AB)	11.0 (B)	42.5 (AB)
NK KONDI	47.4 (CD)	51.3 (BC)	36.1 (BC)	9.5 (BCD)	37.4 (BCD)
LG5687HO	47.2 (DE)	48.0 (C)	35.6 (BC)	8.6 (BCD)	36.2 (CD)
SY EXPLORER	46.9 (DEF)	51.4 (BC)	36.9 (ABC)	9.8 (BCD)	38.5 (BCD)
DKF3333	46.8 (DEF)	51.8 (BC)	38.3 (AB)	7.0 (D)	37.8 (BCD)
LG5528	46.8 (DEF)	51.5 (BC)	38.4 (AB)	8.7 (BCD)	39.3 (ABCD)
SY EDENIS	46.6 (DEF)	50.8 (BC)	33.2 (C)	10.0 (BCD)	34.7 (D)
EXTRASOL	46.5 (DEF)	57.9 (AB)	38.5 (AB)	9.7 (BCD)	40.2 (ABC)
ES AKUSTIC	46.2 (DEF)	57.0 (AB)	39.2 (AB)	10.0 (BCD)	41.1 (ABC)
LG5625	45.7 (EF)	55.4 (ABC)	35.0 (BC)	14.3 (A)	39.9 (ABCD)
ES TEKTONIC CL	45.6 (F)	54.6 (ABC)	37.0 (ABC)	13.5 (A)	41.5 (ABC)
LG5656HO	44.4 (G)	50.8 (BC)	38.8 (AB)	14.7 (A)	44.4 (A)
Cultivar effect	***	***	***	***	***
Location effect	***	***	***	***	***

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

Means per cultivar within a column followed by the same letter are not significantly different ($P < 0.05$) with Student-Newman-Keuls comparison test

Location and cultivar effects were significant for all parameters in Table 5: oil content, seed size (TSW), percentage protein content of DDM, hullability and the calculated protein content of the dehulled meal. We were able, therefore, to distinguish varieties with contrasting characteristics, not only for oil content, but also concerning parameters that affect the possibility of obtaining meal with high protein content.

Cultivars LG5656HO and Dougllas had significantly higher calculated protein content in their dehulled meal (44.4% and 42.5%) than cultivars LG5687HA and SY EDENIS (34.8% and 34.9%). However, for all other varieties, we could not draw a firm conclusion, as the differences concerning this parameter were not significant. Thus, with this wider number of varieties, various combinations of seed characteristics were identified:

- Varieties with low or medium oil content, but high protein content (as a percentage of DDM) and good or medium hullability, giving a high protein meal (LG5656HO, ES TEKTONIC, Extrasol, ES Akustic)
- Some varieties with high oil content, high protein content (as a percentage of DDM) and medium hullability, giving a high protein meal (Dougllas, Kapllan)
- Some varieties with medium oil content, poor protein content (as a percentage of DDM) and medium hullability, giving a lower protein meal compared to other varieties (SY Edenis, LG5687HO)
- Some varieties with very high oil content, high protein content (as a percentage of DDM) but low or very low hullability, giving a medium protein meal (Clloser, Meddia CS).

Turning to the economic aspect, some varieties would be more profitable than others. The outlines of an economic approach can be suggested, but would require further development if sunflower ideotypes

are to be determined. Using 2015 market data (oil price of 750€/t, 36% protein meal at 260€/t and hulls at 80€/t), and by calculating the rate of hull removal necessary to produce a 36% protein-content meal (based on the formula presented in section 2.5), we calculated the likely achievable income of some cultivars. Oil content was the main factor affecting income, with percentage protein content of DDM as the second factor (using high protein content seeds, a lower percentage of hulls can be removed to produce 36% protein meal, the quantity of which is therefore greater). The cultivars that would produce the highest expected incomes belonged to the varietal groups combining high or very high oil contents and high protein contents: Meddia CS, Clloser, Kapplan, Douglllas (494 to 505 €/ton of processed seeds). The lowest incomes were obtained for cultivars displaying low/medium oil contents: LG5625, SY Edenis, LG5656HO and ES Tektonic (472 to 477 €/ton of processed seeds).

Crude fibre content analysis could replace hullability tests?

In 2013, hullability (percentage of extracted hulls) was significantly correlated with: oil content, Thousand Seeds Weight, percentage protein content of DDM, percentage crude fibre content of DDM and percentage crude fibre content of DM (Table 6). Only percentage protein content of DM was not correlated. The closest correlation was with crude fibre content in DM (see Figure 4). The results of the 2012 correlation matrix gave the same conclusions.

Table 6: Pearson correlation matrix concerning 2013 trials (first line Pearson correlation coefficient (R), second line number of samples)

	TSW	**Protein content (%DDM)**	**Protein content (%DM)**	**Crude fibre (%DDM)**	**Crude fibre (%DM)**	**% extracted hulls**	**Calculated protein content of dehulled meal (% RM)**
Oil content	-0.38452*** 157	-0.20423* 157	-0.59629*** 157	-0.17711 *NS* 32	-0.78159*** 32	-0.59706*** 107	-0.51507*** 107
TSW		0.08842 *NS* 157	0.23748** 157	0.33684 *NS* 32	0.31760 *NS* 32	0.40733*** 107	0.39198*** 107
Protein content (%DDM)			0.90667*** 157	0.08418 *NS* 32	0.43585* 32	-0.28675** 107	0.76529*** 107
Protein content (%DM)				0.13256 *NS* 32	0.63598*** 32	-0.00416 *NS* 107	0.85291*** 107
Crude fibre (%DDM)					0.72126*** 32	0.35681* 31	0.25643 *NS* 31
Crude fibre (%DM)						0.70678*** 31	0.64457*** 31
% extracted hulls							0.38716*** 107

NS: non-significant; ∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001

This could be explained by the fact that the percentage cellulose content of dry matter was both highly correlated with the percentage cellulose content of DDM (the richer is the whole seed in fibre, the

richer also in fibre is the defatted fraction of the seed) and oil content (the richer is the seed in oil, the greater is the reduction of the defatted fraction, which lowers the proportion of cellulose). Previous studies had shown on the one hand that hullability is significantly and negatively correlated with the seed oil content (Denis *et al.*, 1994; Dauguet *et al.*, 2015); and on the other, since the crude fibre is concentrated mainly in the hulls, that hullability is strongly and positively correlated with the seed hull content (not assessed in this present study, but demonstrated by Denis *et al.*, 1994; Baldini *et al.*, 1994; Nel, 2001). Thus, the crude fibre content as a percentage of DM incorporates both the effect of fibre content as a percentage of DDM, and the effect of oil content on hullability.

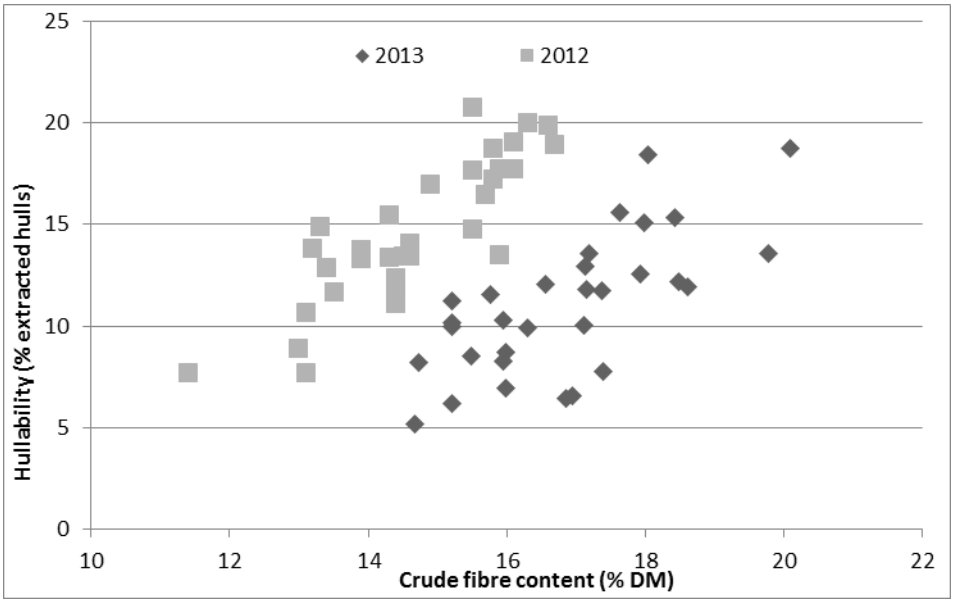


Figure 4. Relationship between on hullability (% of extracted hulls) and crude fibre content of sunflower seeds

An analysis of covariance was conducted, testing the effects of crude fibre content expressed as a percentage of DM, year and the interaction "Crude fibre DM * Year" on the extracted hull rates (Table 7). It showed a year effect, i.e. a different intercept but, no interaction effect (similar slopes).

Table 7: Analysis of covariance results for the percentage of extracted hulls variable (2012 and 2013 data)

Parameter	**Estimated value**	**Standard error**	**Pr > |t|**
Intercept	-22.85	3.57	<0.0001
Crude fibre content	2.01	0.21	<0.0001
Year 2012	8.04	0.72	<0.0001
Year 2013 (reference)	0		
R^2 model = 0.69			

The crude fibre content analyses were performed on: DKF3333, Vellox and Extrasol in 2012 and NK Kondi, ES Tektonic, Fydgi and SY Valeo in 2013. However, the climatic context was probably the most impacting parameter since hullability was lower overall in 2013 compared to 2012 (see Tables 2 and 3), which may be linked to a higher oil content in 2013 compared to 2012 (see Table 4).

Thus, if significant advances are made in the near future in the development of rapid non-destructive analysis methods, determining the crude fibre content as a percentage of DM would be an appropriate way to assess the hullability of varieties in sunflower breeding programmes. An annual calibration does, however, appear necessary.

Predicting the potential of a variety for producing a meal with high protein content?

It appears that protein content of dehulled sunflower meal (calculated data for each sample from the seed protein content and rate of extracted hulls by the method outlined in section 2.5) was most closely correlated with seed protein content as a percentage of DDM ($p < 0.0001$ and $R^2 = 0.59$, see Table 6 for 2013 data) and much less related to the rate of hulls extracted ($p < 0.0001$, $R^2 = 0.15$, see Table 6 for 2013 data). From this, it may be concluded that the initial seed protein content is of paramount importance for obtaining high protein meals.

Taking all the data for 2012 and 2013 together, we obtained Equation 1.

Equation 1. Relationship between protein content of dehulled meal and protein content as a % of DM (2012 and 2013 data, Figure 5)

Prot_dehulled_meal = 18,198 + 0,6391 * (protein content DDM) [$R^2 = 0.43$]

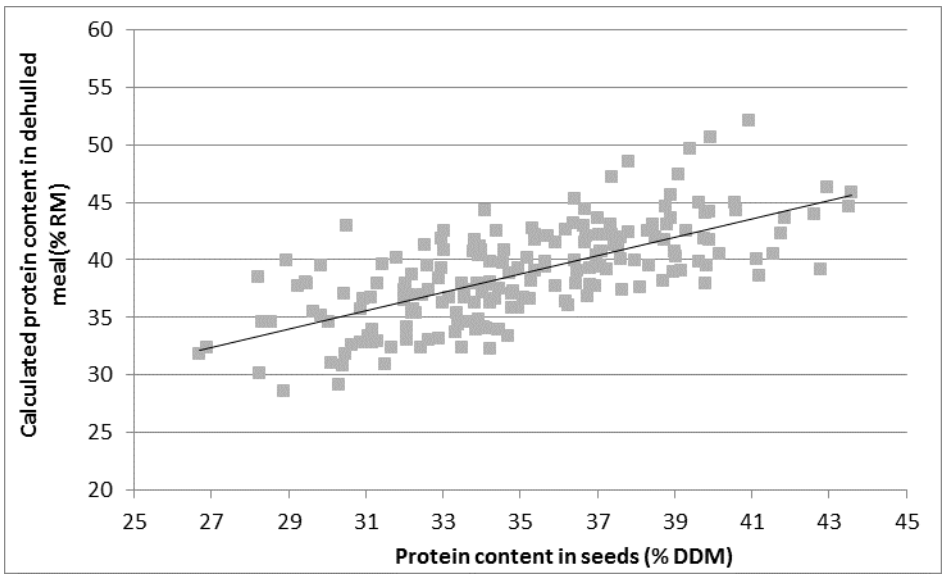


Figure 5. Relationship between calculated protein content in dehulled meal (% RM) and protein content of sunflower seeds (% DDM)

Adding crude fibre content to the model could improve the equation, as this parameter was correlated with hullability. Taking into account the year effect, as shown in section 3.4, could further improve the predictive model.

Equation 2. Protein content of dehulled meal (% RM) as a function of protein content DM and crude fibre content DM, and including year effect (2012 and 2013 data)

2013: Prot_dehulled_meal = -26.22 + 1.12*(protein content DDM) + 1.48*(Crude Fibre content DM)

2012: Prot_dehulled_meal = -20.73 + 1.12*(protein content DDM) + 1.48*(Crude Fibre content DM) Significance: General model $p<0.0001$; Intercept $p<0.0001$; protein content DDM $p<0,0001$; Crude Fibre content DM $p<0.0001$; Year effect $p<0.0001$

R^2 (model)=0.86 ; partial R^2 (protein content DDM)=0.66 ; partial R^2 (Crude Fibre content DM)=0.16 ; partial R^2 (year effect)=0.05

A calibration of this Equation 2 according year results in a more accurate estimate, and enables classification of varieties according to their capacity to produce meal with improved protein content after dehulling.

4. CONCLUSION

At present, sunflower breeding programmes do not take into account the characters of protein content and hullability. So if the crushing industry wishes to produce a high protein meal it would have to review the dehulling process. In this study, we identified sunflower varieties that combine both high oil and high protein content. The protein content of sunflower seeds proved to be the key characteristic determining the quality of sunflower meal; improvement by breeding would help to improve both meal quality and the profitability of the crushing process. Selection of varieties with particularly high oil contents could have a negative impact on hullability; it may be worthwhile checking this in order to avoid difficulties at crushing plants.

Results observed in this study proved that for selection of cultivars producing sunflower meal with more than 40% protein content is perfectly feasible without having to remove more than 13% of seed mass in hulls. This study also highlighted important environmental effects (year and location) on protein content and hullability; this indicates that cultivar selection alone is not sufficient to ensure the production of a precise quality target for the seeds, although it should reduce the risk of failing to deliver meal of a commercial standard and/or losing too much oil in the hulls extracted. The question remains open as to whether the stakeholders in sunflower oil mills would benefit from negotiating specifications with their suppliers to segregate crops that have strong potential for producing high protein meal. A framework for sharing the earnings attributable to seed protein content could be set up for farmers. Further technical and economic assessments are needed to comprehensively address these possibilities. Action on them could lead to the adoption of new breeding strategies and significant improvements in the quality of sunflower meal.

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THE B1 LOCUS THAT CONTROLS APICAL SHOOT BRANCHING IN *HELIANTHUS ANNUUS* EXHIBITS A MOLECULAR DIVERSITY LINKED TO THE BREEDING HISTORY OF HYBRIDS

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ABSTRACT

During domestication, shoot branching has been discarded for selection in sunflower. However, when selection entered the F1 hybrid era approximately 50 years ago, a recessive shoot branching gene was widely deployed in male lines in numerous breeding programs, mainly because multi-head architecture allows a more extended time window for pollen availability, thus securing hybrid seeds production. The vast majority of male lines used in breeding programs for sunflower have the branched phenotype. The *b1* locus controlling apical shoot branching mapped on LG10 in a segregating population (recombinant inbred lines) obtained from the cross between XRQ (an unbranched line) and PSC8 (a branched line). We developed two near isogenic lines (NILs) that only differed one from the other by the genomic region of the chromosome 10 containing the *b1* gene. A large F2 population (approximately 6500 individuals), derived from the two NILs, was used to reduce the genetic window containing the *b1* locus. The entire population was genotyped with two markers surrounding the *b1* locus. All recombinant plants were phenotyped and the locus was mapped in a 0.3cM window. BAC clones located in the b1 region were identified *in silico* and sequenced. The genomic region didn't fully cover the genetic interval but candidate genes were identified. Re-sequencing experiments inside and around the *b1* locus, on a set of 192 lines, allowed us to analyze the molecular diversity. We performed diversity analysis (HKA test, Tajima's D, π) in order to describe the history of the branching in sunflower. Our results suggest that the *b1* locus, including the branching gene, was under selection during domestication and modern breeding. We also developed universal molecular markers to follow this trait in breeding programs.

Key Words : shoot branching, map-based cloning, molecular diversity, breeding

EFFECTS OF OSMOTIC STRESS WITH DIFFERENT HORMON COMBINATIONS ON CALLUS INDUCTION IN SUNFLOWER ANTHERS

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ABSTRACT

Sunflower is one of the most important oil crop in the world. Recently, genetic and breeding programs involve to improve new hybrids which have better properities. Anther culture is an alternative technique to obtain desired properities in a short time. Effective callus induction is critical for successful anther culture. Callus induction can be effected by different treatments. The purpose of this research is to identify the best callus induction treatment using different hormon combinations with the pretreatment of Mannitol for osmotic stress in sunflower anthers. An oil seed variety, 08TR003, of *Helianthus annuus* L. was used for this study. Sunflowers were grown in greenhouse under semi controlled conditions (16 h photoperiod). Buds collected from mid to late uninucleate stage of microspores. Anthers were pretreated 0.5 M Mannitol solution in the dark for 5 days after transferred to B5 media supplemented with 2 mg/L 2-4D, 0.5mg/L Kin; 1mg/L 2-4D, 0.5mg/L Kin; 2mg/L NAA, 0.5mg/L BAP; 1mg/L NAA, 0.5mg/L BAP. The best result was observed using 1mg/L NAA, 0.5mg/L BAP with compared the other applications and control group.

Key Words : sunflower, callus, mannitol, anther culture

CONFECTIONERY SUNFLOWER HYBRID BREEDING IN VNIIMK (RUSSIA)

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ABSTRACT

Experiments were conducted at the Central Station (Krasnodar) of All-Russia Research Institute of Oil Crops (VNIIMK). Released, prospective and experimental sunflower hybrids and inbred lines of VNIIMK breeding were used as a material. Randomized block design was used to test the obtained hybrids. Plant density was 50 000 plants per ha. Plots were 25.2 m^2 in size and had four rows; two central rows were harvested to evaluate seed yield, oil and husk content, 1000-seed weight. Field resistance to all pathogens was registered under the natural conditions. The aim of our breeding efforts was to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid. No such commercial hybrids were available at this time in Russia. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight. Results show that the best hybrid (VK-905 A × VK-944) have significantly higher seed yield in comparison with the check. Seed yield level was rather high in the trial. Check OP variety Oreshek gave 3.23 t/ha. Oil content in the absolutely dry seeds was 454 g/kg for the check variety, and varied from 431 to 480 g/kg in the seeds of studied hybrid combinations. As a result tested hybrids could be used in two different ways (for oil production and for confectionery use) along with released confectionery OP varieties (Oreshek, SPK and Lakomka). Significantly less oil content is typical for the confectionery sunflower produced outside the Russia. To evaluate general combining ability (GCA) of our new confectionery lines we crossed two CMS-lines with four restorer lines. As a conclusion line with the best GCA value for the seed yield was VK-944 (0.40) and VK-905 A was the best line among the testers. Combination of two the most important traits (seed yield and seed size) allowed us to define the most prominent hybrids and lines. It was proved that to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid – is quite possible. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight. Three-year trial allows us to define the most prominent hybrid combination (VK-905 A × VK-944).

Key words: Hybrid – sunflower – confectionery – broomrape resistance

INTRODUCTION

Sustainable market demand for confectionery sunflower seeds made VNIIMK initiate a special breeding program with the aim to develop modern confectionery open-pollinated varieties. Dr. S. Borodin with his colleagues released three OP varieties – SPK, Lakomka and Oreshek (Gontcharov and Beresneva, 2009). Their seeds are close to the oil-type one by structure but larger in size and 1000-seed weight, has bigger husk content and less oil content (450-490 g/kg). Husk is black or black with grey stripes in color. This type of seeds has special Russian name “mezheumok” and means intermediate. People in Russia and Ukraine prefer such types of sunflower seeds for the direct consumption. The seeds also could be easily dehulled by the machinery for confectionery use. Now these four OP varieties covered more than 700 thousands hectares in Russia. Commercial success of confectionery OP varieties encouraged us to start confectionery hybrid breeding program also. This program started in 1999. Russian market demands for sunflower with 1000-seed weight 80 g or more, oil content on the level 450-490 g/kg and seeds should be easily dehulled.

As a new initial breeding material we used non-oil samples of sunflower from Iran and Syria, Russian modern confectionery OP varieties and high-oil inbred lines of our breeding with relatively big seed size.

As a result of crossing this material and self-pollination we developed a number of inbred lines for confectionery hybrid breeding. Lines were crossed with CMS-lines to test their ability to restore pollen fertility. So we found some restorer lines and some maintainer lines. Several of such lines were converted to CMS-lines by back-crossing.

The aim of our breeding efforts was to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight.

MATERIALS AND METHODS

Experiments were conducted at the Central Station (Krasnodar) of All-Russia Research Institute of Oil Crops (VNIIMK). Krasnodar region is situated in the Southern part of Russia near the Black Sea. Climatic conditions are very favorable here for sunflower production. Sunflower usually covers about 0.5 million ha in this region.

Released, prospective and experimental sunflower hybrids of VNIIMK breeding were used as a material. To produce confectionery hybrids we used two CMS-lines of our own breeding (VK-905 A and VK-934 A). Restorer lines were developed from crosses of our elite lines with dolichocarpous sunflower. The most interesting sample was bought in the local Iranian market. It was very specific dolichocarpous sunflower *Helianthus annuus var. armeniacus Wenzl.& Anashcz* (Anaschenko, 1971). This botanical variety of cultivated sunflower considered to be the most genetically distant from usually used sunflower cultivars. Main traits for individual selection were early flowering time (Iranian sample was very late in our conditions), short stem (initial material population was very tall – up to 3 m and more), bigger seed and kernel size, resistance to diseases.

Randomized block design was used to test the obtained hybrids. Plant density was 50 000 plants per ha. Plots were 25.2 m2 in size and had four rows; two central rows were harvested to evaluate seed yield, oil and husk content, 1000-seed weight. Field resistance to all pathogens was registered under the natural conditions. Shneiter and Miller's method (1981) was used for phenological observations. Oil content was evaluated by NMR-analyzer.

RESULTS AND DISCUSSION

New breeding program with the aim to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid – started at VNIIMK in 1999. As a first result the most prominent hybrid combination Katyusha (VK-905 A × VK-944) was put in the State trial (table 1).

Oil content in the absolutely dry seeds was 454 g/kg for the check variety, and 477 g/kg in the seeds of studied hybrid combination. As a result seeds could be used in two different ways (for oil production and for confectionery use) along with released confectionery OP varieties (Oreshek, SPK and Lakomka). Significantly less oil content is typical for the confectionery sunflower produced outside the Russia. But such material had no commercial success here.

1000-seeds weight of all tested hybrid combinations was higher 80 g, thought superiority of check variety was obvious. Comparison of 1000-seeds weight of all tested combinations showed big variation for this trait. 1000-seed weight varied from 79.1 g (VD-354 × K-3) to 109.9 g (VK-905 × K-3).

To evaluate general combining ability (GCA) of our new confectionery lines we crossed two CMS-lines (VD-354 A and VK-905 A) with four restorer lines (K-1, K-3, K-4 and K-5). CMS-lines were used as testers. Obtained hybrids were tested for the seed yield. Analysis of results allowed us to calculate GCA values (Table 2).

Table 1. Trial results of new confectionery sunflower hybrid Katyusha (Krasnodar, 2009-2011)

Hybrid or OP variety	Seed yield		Oil content, %	Oil yield		1000-seed weight, g
	t/ha	± to check		t/ha	± to check	
Oreshek (check)	2.29	-	45.4	0.94	-	117.2
Katyusha	2.66	+0.37	47.7	1.14	+0.20	106.8

After three years of State trial this hybrid was released.

Table 2. General combining ability evaluation of confectionery sunflower lines for seed yield (Gontcharov and Beresneva, 2011)

Line	Type	GCA value
K-1	Paternal (pollinator) line	-0.35
K-3	Paternal (pollinator) line	0.13
K-4 (VK-944)	Paternal (pollinator) line	0.40
K-5	Paternal (pollinator) line	-0.18
VK-905 A	Mother line (tester)	0.17
VD-354 A	Mother line (tester)	-0.17

As a conclusion line with the best GCA value for the seed yield was K-4 (0.40), average value was demonstrated by K-3 line (0.13). Other two lines showed poor results. VK-905 A was the best line among the testers. Next breeding effort allowed us to develop new CMS-line 934 A (confectionery type) and to identify restorer line VK-930 (oil-type) with high combining ability. New hybrids were tested in 2015 (Table 3).

Table 3. Trial results of new confectionery sunflower hybrids (Krasnodar, 2015)

Hybrid or OP variety	Seed yield		Oil content, %	Oil yield		1000-seed weight, g
	t/ha	± to check		t/ha	± to check	
Oreshek (check)	3.49	-	44.5	1.40	-	104.1
VK-934 A×VK-930	4.12	+0.63	45.6	1.69	+0.29	85.2
VK-934 A×VK-944	3.92	+0.43	39.4	1.39	-0.01	106.6
VK-905 A×VK-930	3.61	+0.12	47.2	1.53	+0.13	75.6
LSD_{05}	0.26					

It was proved that to develop sunflower hybrids suitable for double-using – as a confectionery and oil-type hybrid – is quite possible. Such hybrids should perform high seed yield, rather high oil content and high 1000-seeds weight. The most prominent hybrid combinations (VK-934 A×VK-930, VK-934 A×VK-944 and VK-905 A×VK-930) are recommended for the future trials. Their parental forms (CMS-lines VK-905 A and VK-934 A and restorer lines VK-944 and VK-930) will be used for the future breeding work.

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POPULATION STRUCTURE, LINKAGE DISEQUILIBRIUM AND ASSOCIATION MAPPING FOR MORPHOLOGICAL TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower is one of the most important oil crops worldwide. Ago-morphological traits are important for sunflower breeders in selection of genotypes with high performance and other traits. The objectives of this study were to determine the population structure and linkage disequilibrium among 106 dispersed sunflower genotypes and to identify the genomic regions associated with agro-morphological traits using the association mapping approach. High genetic variability was observed among the sunflower genotypes for the studied agro-morphological traits. In molecular experiments, the genetic variability among the genotypes was assessed by using simple sequence repeat (SSR, or microsatellite), inter-retrotransposon-amplified polymorphism (IRAP) and retrotransposon-microsatellite amplified polymorphism (REMAP) markers. In this study, 248 loci were detected using 28 IRAP and REMAP primers and also a total number of 67 alleles were detected using 30 SSR loci. The studied sunflower lines were divided into two subpopulations using IRAP+REMAP data and into five subpopulations using SSR data. By using a mixed linear model procedure, 224 loci showed significant association with quantitative trait loci (QTL) controlling the investigated traits. The identified and associated markers are expected to be useful in marker-aided selection in sunflower breeding programs

Key Words : Association Mapping, Linkage Disequilibrium, Microsatellite, Mixed Linear Model, Retrotransposon-Based Molecular Markers, Sunflower

MAPPING QTL CONTROLLING SALT TOLERANCE INDICES IN SUNFLOWER (*HELIANTHUS ANNUS* L.)

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ABSTRACT

Salt stress is an important limiting factor for plant growth. Using selection indices, it is possible to identify cultivars with high yield in normal and stressed conditions. So, the genetic analysis of salt tolerance indices can play important role in plant breeding programs. In order to identify molecular markers associated with salt tolerance indices in sunflower, recombinant inbred lines produced from a cross between RHA266 and PAC2, were studied in a factorial experiment with a completely randomized design with three replications under normal and salinity stress conditions. Stress tolerance indices such as mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress tolerance index (STI), yield stability index (YSI) and tolerance index (TOL) was calculated based on yield data under normal and salinity conditions. High correlation was observed between yield under normal and salinity conditions with geometric mean, geometric mean productivity and harmonic mean. So, these indices are introduced as most appropriate measures to identify sunflower lines tolerant to salinity stress. Based on three dimensional plots constructed by yield in normal and salt stress conditions and each one of appropriate indicators (GMP, MP and HM), lines such as C86, C61, C142, C134a, C62, C70a, LR1, C153, C108, C6, C106, C98b and C148 are considered tolerant lines. Using composite interval mapping, a total of 9 QTL were identified for salt tolerance indices. The results indicate co-localization of the identified QTL for GMP, MP and HM in linkage group 14 with QTL identified for grain yield under salt stress conditions.

Key Words : Biplot, Molecular Markers, Stress Tolerance Indices, Sunflower

GENETIC DIVERSITY OF SUNFLOWER (*HELIANTHUS ANNUS* L.) LINES UNDER NORMAL AND SALT STRESS CONDITIONS USING MULTIVARIATE STATISTICAL ANALYSIS

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ABSTRACT

To study genetic diversity of several agronomic and physiologic traits and the effect of salt stress on these characters in 100 inbred lines of sunflower, an experiment was conducted as a split-plot based on randomized complete block design with three replications outside the greenhouse in an open air area under natural environmental conditions with 2 salinity stress levels (0 and 8 dS/m) in Research Station of University of Urmia in 2014. Analysis of variance revealed significant differences among genotypes for all studied traits, indicating the existence of genetic variation among population. The highest coefficient of genetic variation was observed for head dry weight, plant grain yield and the lowest for date of flowering time in both stressed and non-stressed conditions. The results of correlation analysis showed that there is significant and positive correlation between seed yield per plant with most of the studied traits in both stress conditions. Stepwise regression analysis revealed that under salt stress condition 40.3 percent of seed yield per plant variation was determined by head diameter, one hundred seed weight, bottom leaf length, leaf number, bottom petiole length, upper leaf width, chlorophyll concentration and in normal condition 30.3 yield grain per plant variation explained by head diameter, one hundred seed weight and plant height. Cluster analysis grouped lines into 3 clusters in each one of normal and salt stress conditions but the disruption of lines within groups were different depending to stress environment that present the genetic variability for salt tolerance in sunflower lines.

Key Words : Cluster Analysis, Genetic Correlation, Phenotype Correlation, Salt Stress, Split-Plot, Sunflower

FOUR DECADES OF SUNFLOWER GENETIC RESOURCES ACTIVITIES IN INDIA

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ABSTRACT

Sunflower crop is introduced in India during early 1970's with commercial hybrids, parental lines and germplasm accessions received from USA and erstwhile USSR. The work on collection, evaluation and maintenance of sunflower germplasm was carried out at Germplasm Management Unit (GMU) located at the Project Coordinating Unit (PC, Unit Sunflower), Bangaluru from 1983 till 2001. During 2001 PC unit sunflower was transferred to Indian Institute of Oilseeds Research (IIOR). IIOR gene bank maintains 3273 sunflower accessions under the Germplasm Management Unit. The collection includes germplasm **(**GMU 1200**)** exotic collection (350), genetic stocks (97), inbreds (360), populations (5), gene pool (GP) for high oil, yield and autogamy (390), back cross converted lines (15) and wild species (42) including their derivatives (154). Screening of wild *Helianthus species*lead to the identification of both annual and perennial species confirming resistance to major biotic stresses *viz*., powdery mildew, *Alterneria helianthi*, rust and *Spodoptera litura*. Appropriate strategies are being developed for utilization of the resistance sources in introgression breeding progrmamme. Characterization of the germplasm resulted in identification of 37 trait specific germplasm *viz*., high yield (7), high to medium yield coupled with medium to high oil% (8), high oil (6), early maturity (3), dwarfness (4), late maturity (2), powdery mildew tolerance (2), white pollen(1), high 'p' acquisition(1), high oleic acid content (2) and ornamental type (1). Recently a total of 660 germplasm accessions including core germplasm are augmented in the gene bank from European countries. Wide variability among the available accessions exists for key quantitative traits like seed yield/plant (3 to 55 g), oil content (14 to 42 %), 100-seed weight (2 to 16.0 g) and plant height (50 to 360 cm). Utilization of germplasm resulted in identification of two sunflower varieties from Solapur centre i.e. Phule Bhaskar (SS 0808) from germplasm selection (GP-688-1) and Bhanu from gene pool selection (GP-775). Till date 21 varieties; populations and 35 hybrids were released in India. The present focus is an augmentation of trait specific germplasm and utilization of promising cultivar germplasm and wild *Helianthus species* in inbred development, maintainer/restorer gene pool development, parental lines improvement and resistance breeding programme.

Key Words : sunflower , gene bank, genetic resources , utilization, India

QTL MAPPING FOR BROOMRAPE (*OROBANCHE CUMANA* WALLR.) RESISTANCE IN SUNFLOWER

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is one of the most important biotic factors *causes* reduction in sunflower *yield. Although breeding for* broomrape resistance is most effective method to control the disease, development of cultivars resistant to broomrape is not easy due to quantitative nature of the resistance. Although few QTLs were identified for broomrape race F resistance, none of the QTLs is suitable for marker assisted selection (MAS) due to small effect. In the present study three major QTLs were identified on LG7, LG11 and LG12 for broomrape race F resistance in sunflower by using high density SNP map constructed by *genotyping by sequencing (GBS) approach. Total* phenotypic variation (PVE) explained by the identified QTLs was 82%. This is the first report of *QTL mapping for* broomrape race F resistance *using a high density SNP map.* QTLs identified in this study will be valuable molecular genetics tools for broomrape resistance.

Key Words : *Helianthus annuus*, molecular breeding, genotyping by sequencing (GBS), broomrape race F

PERSPECTIVE AND CHALLENGES TO DEVELOP HIGH YIELDING, DISEASE RESISTANT AND OIL QUALITY SUNFLOWER HYBRIDS IN INDIA

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the third important oilseed crop in the world after soybean and groundnut. It is grown over an area of 25.6 m ha with a production of 44.8 m. tons and average productivity of1749 kg/ha in the world. Due to its wide adaptability, it is grown in all the continents. Important sunflower growing countries are Argentina, CIS countries, France, Spain, USA, China and India. China, France, Turkey are the highest yielding countries with an average yield of >2 tonnes/ha as against the lowest yielding countries like Kazakistan, Myanmar and India with <1 tonne per ha. Russia and Ukraine have largest share of about 50% in total sunflower production in the world. The crop has become an important oilseed crop in India covering an area of 0.55 m ha with a production of 0.41 m tons with the average yield of 752 kg/ha.(Annon.2014). In India the cultivation of sunflower is confined to southern states of Karnataka, Tamil Nadu, Maharashtra and Andhra Pradesh. During the last 20 years, the crop has made a dent in nontraditional areas in the northern states of Punjab, Haryana and Uttar Pradesh, in Spring/zaid season. The productivity of sunflower in these states is highest (2111 Kg/ha) in the country.

HISTORICAL DEVELOPMENT

Breeding in sunflower began around 1912 in the former Soviet Union and the most successful early breeding programme was that of V.S.Pustovoit. The concerted efforts for four decades resulted in increasing oil content from about 30 to 52%. High oil sunflower varieties, such as Peredovik, Armavirskiy 3497, Mayak,VNIIMK 8931, VNIIMK 6540 and Smena developed by V.S.Pustovoit and his associates enabled the spread of sunflower crop not only in Soviet Union but also to other continents. In the 1940s, Putt in Canada developed shorter, early maturing cultivars (Miller,1992). Resistance to rust was incorporated from wild species. Breeding programs were started about the same time in Argentina and several other countries. In the USA, Kinman began breeding programme around 1950, intensive breeding programmes were pursued in several countries around the world, as a result of which sunflower is now grown over a large area in many countries.

Development of hybrids in maize and other crops stimulated sunflower breeders to work towards developing hybrids in sunflower. Kinman in USA, Putt in Canada and several other workers started a hybrid production scheme taking advantage of self-incompatibility system in the crop (Fick, 1978). Although a high per cent of hybrid seed could be obtained on the female line, the % seed set varied with the lines and environmental conditions. Evolving hybrids using genetic male-sterility was developed by Vranceanu (1974) in Romania. Male-fertility (MS ms) was linked with red anthocyanin pigmentation enabling rouging of fertile plants in the female line in the seed production plots. Hybrid seed production cost was high in view of the labour requirement to remove fertile plants.

The landmark in the development of commercial sunflower hybrids was the discovery of cytoplasmic male-sterility by Leclercq (1969) in the progeny of a cross between Helianthus petiolaris Nutt and cultivated sunflower. The system was stable as evidenced by sterility obtained in the progeny of male-sterile plants crossed with fertile cultivated sunflower plants. Genes for genetic restoration of

fertility were found in the wild species by Kinman (1970). Subsequently, Leclercq (1971), Enns et al. (1970) and Vranceanu and Stoenescu (1971) also reported fertility restoring genes. First commercial hybrids based on cytaoplasmic male-sterility were made available in1972 in the USA. Subsequently, the cultivation of sunflower hybrids spread to all parts of the world.

Development of Hybrid Sunflower in India

The value of hybrids and heterosis breeding was recognized with the inception of AICRP on sunflower in 1972-73. Experimental hybrids were developed at Bangalore in 1974-75 using 4 CMS lines(CMS 2, CMS 124, CMS 204 and CMS 234) and 2 restorer lines (RHA 266 and RHA 274) introduced from the USA. All the hybrids were distinctly superior to the check variety EC 68415 both in seed and oil yield .Thus, the first sunflower hybrid BSH-1 (CMS 234A X RHA 274) was released for commercial cultivation in 1980(Seetharam et.al. 1980).Since then the hybrid base has been further widened in the country through extension of heterosis breeding work to other research centres. Many hybrids have been developed by different Public and private Institutes/universities (Table 1).

HETEROSIS STUDIES

Heterosis studies carried out in sunflower have been presented for inter-line, inter-varietal and top cross hybrids involving genetic and cytoplasmic male sterility. Kovacic (1960) in a study of inter varietal crosses observed superior response with an increase in seed yield. But only few lines exceeded the parents in oil content. However, F_1s were more vigorous and flowered earlier than the parents. Popov and Lazarav (1963) developed inbred lines from high yielding varieties and reported that single cross and top cross hybrids surpassed their parents in oil content and seed yield. Some inter-varietal hybrids exceeding their parents in seed yield were also obtained. Schuster (1964) observed heterosis for seed yield to the extent of 70%, heterosis for plant height to an extent of 47% and heterosis for head diameter was upto 60 percent.

Leclercq (1971) observed heterosis to an extent of 12-40% over standard variety Peredovik. Shuravina (1972) found that 16 out of 24 hybrids exhibited heterosis over tester parent to an extent of 39% and 20% for seed weight and seed yield, respectively. In another study, 14 out of 18 hybrids showed heterosis upto 90% for seed weight and 40% for seed yield. However, ten hybrids had reduced hull per cent and three exhibited heterosis upto 4.8% for oil content. Kloczowskii (1972) observed heterosis upto 90 to 160% for seed yield in the F_1, but in F_2 achene yields and oil content were dropped by 20 and 4%, respectively. Fick and Zimmer (1976) reported an increase in yield upto 31% over Peredovik in hybrids. Hybrids were also found to have higher oil content. Kloczowskii (1975) reported heterotic effect upto 43% for achene yield in inerline hybrids while in line x variety hybrids it was18%. In a diallel crosses between short and tall varieties.. The highest yielders were obtained by crossing short lines, families and varieties with variety Cernyanka-66. Skoric (1977) observed that four single cross hybrids yielded 25 to 30% higher seed yield, earlier, shorter and more resistant to diseases than Peredovik and Vniimk-8931. The hybrids had approximately three % higher oil content.

Seetharam *et al*. (1977) while studying the performance of hybrids produced by four cytoplasmic male sterile and two fertility restorer lines observed a significant positive heterosis for days to flower, plant height, head size, test weight, oil content and seed yield. But heterosis was not significant for stem girth and number of leaves. BSH-1 and BSH-2 were considered as best hybrids which surpassed the check variety EC-68415 by 30% in seed yield. In a study of single, double and three way cross hybrids. Shrinivasa (1982) observed a significant heterosis for plant height, stem girth, head diameter and yield per plant in all nine crosses, heterosis for oil content was significant only over the mid parental valve and was negative for 100 achene weight.While evaluating100 F1 involving 20 inbred lines and 5 pollen parents, Choudhary and Anand (1984)observed 62.3% heterosis for 1000-seed weight, 62.8% for seed yield,

64.6% for head diameter, 23.2% for oil content and negative heterosis of 7.7% for days to flowering over better parent.

Singh *et al.* (1984) in a study on performance of variety x inbred crosses observed heterosis for yield to an extent of 47-206%. The studies of Shivaraju (1984) on ten F_1 hybrids indicated an average heterosis to an extent of 175% for seed yield, 129% for number of filled seeds, 39%for head diameter, 22% for stem girth and 7% for oil content. Majority of the hybrids showed negative heterosis for days to 50% flowering and days to maturity.

Among the 49 hybrids studied, Reddy *et al*. (1985) recorded heterobeltiosis for achene yield and oil percentage in 46 and 41 hybrids, respectively. In eight hybrids, heterobeltiosis for achene yield exceeded 100% while in ten, heterobeltiosis for oilyieldwas10%. Giriraj *et al*. (1986) by crossing five CMS lines with two restorers observed average heterosis of -8% for days to flowering and 192% for achene yield per plant. Low heterosis was exhibited for oil content and number of leaves. In a study of 18 hybrids. Wali (1987) observed that heterosis varied considerably for yield and its component characters. The highest heterosis of 259% and 363% over mid-parent was observed for seed yield per plant in summer and kharif seasons, respectively. Heterosis was high and positive for leaf area index,100 seed weight, head diameter and number of filled seeds per plant while it was negative for days to flowering over both mid-parental and better parental value.

Fernandez et al. (1989) crossed lines breeding true for oil with high oleic acid (at least 85%) with standard lines having an oleic acid content of 30%. They analyzed the oil of F_1 seed and showed that high oleic acid content was a dominant trait and had maternal influences. Dedio (1993) observed heterosis for kernel oil content as well as achene oil content. In a comparative study of single cross and three-way cross hybrids, Naresh (1993) indicated that more number of three-way cross hybrids have manifested significant positive average heterosis for all the characters studied except days to 50% flowering and seed filling. The average heterosis registered for seed yield ranged upto 128%.

Disease resistance:

Sunflower (*Helianthus annuus* L.), is prone to attack by several pests and diseases (Mayee, 1997).
Several diseases are known to cause yield loss in sunflower. In India the important diseases are: alternaria leaf spot caused by *Alternaria helianthi*; rust caused by *Puccinia helianthi*; downy mildew caused by *Plasmopora helstedii* and various root and stem rots caused by *Sclerotium sp.* and *Rhizoctonia sp*. But little information is available about genetic control of disease resistance. Wild species of sunflower are known to harbor genes for resistance against diseases.

Sunflower Necrosis Disease:

Sunflower cultivation was seriously affected in India by an unusual necrosis disease caused by Sunflower necrosis virus (SNV). It was first observed in Karnataka state in 1997 and in the subsequent years it spread to other states viz., Tamil Nadu, Andhra Pradesh and Maharashtra with the average disease incidence up to 50%.Necrosis appears on the part of leaf lamina near the mid rib resulting in twisting of leaf and then extends through one side of the lamina to the petiole and stem and finally terminate to the shoot of the plant leading to partial paralytic symptoms . Necrosis at bud formation stage leads to partial twisting of the capitulum .Thrips suspected to act as a vector in transmission of this disease. No resistance source has been reported so far. However, through management this disease can be controlled Sunflower necrosis virus disease (SNVD) became a major threat to the successful cultivation of all the sunflower hybrids and varieties and devastating the crop since 1998. Significant reductions in terms of total crop loss up to 90% were reported due to early infection in the farmers' fields (Bhat et al., 2001; Ramaiah et al., 2001a; Lavanya et al., 2005). This has resulted in the substantial loss of sunflower production to 0.733 M tones in 2000–2001 in comparison to 2.0 M tones in 1998–1999 (Bhat et al., 2002a; Jain et al., 2003). According to Ravi et al. (2001) SNV belongs to the ilarvirus sub group I and is related to tobacco streak virus (TSV) as the former shared 90% amino acid sequence identity with the latter. It has been

reported that the SNV is a single stranded circular RNA virus with isometric virions; the sunflower ilarvirus was related to TSV on the basis of coat protein gene sequence (Prasada Rao et al., 2000; Bhat et al., 2002b). Initially, Jain et al. (2000) reported that the SNV was associated with tospovirus, but later it was confirmed that the ilarvirus, antigenically related to TSV was associated with SNVD (Jain et al., 2003). A disease similar in nature to SNVD has been reported in the Netherlands (Dijkstra, 1983) and Australia (Brunt et al., 1996). Thrips mediated SNV transmission has already been reported in sunflower (Jain et al., 2003; Lokesh et al., 2005). Notably, a groundnut (Arachis hypogaea L.) isolate of TSV was transmitted by a thrip, Frankliniella schultzei Trybom (Reddy et al., 2002). Although ilarvirus is transmitted through seeds (van Regenmortel et al., 2000), there is no report confirming the transmission of SNV through seeds in sunflower. Limited attempts were made for the management of SNVD using border crops and insecticides mainly to control the SNV carrier, thrips (Jain et al., 2000, 2003; Ramaiah et al., 2001b). Apart from vector control, no effective control measures are available for the management of SNVD. Therefore, the majority of the farmers depend only on chemical control of the insect to minimize the virus spread, despite the fact pesticides can be hazardous to the environment and public health. In this scenario, biological control, an eco-friendly disease control. strategy is worth testing as a supplement or an alternative to chemical control. Application of biocontrol agents (BCAs) is an important strategy in crop protection against plant pathogens. The most important control to this disease is to develop sunflower hybrids which show resistance to this virus.

Alternaria leaf spot:

It is a common disease in many countries but causes more damage in India. Only field resistance has been reported in cultivated sunflowers and no information is available on the inheritance of resistance to this disease.

Rust:

Rust is one of the most destructive diseases of sunflower in the world and can appear throughout the plant growth. Racial differentiation exists in this fungus. So far, four races and the corresponding resistant genes have been identified. Resistance to rust found commonly among wild species of *Helianthus* has been successfully incorporated into commercial cultivars. In India, although rust is of a common occurrence, the race pattern is not known.However, resistant source for local races have been identified and incorporated in the hybrids under cultivation.

Downy mildew:

At present, downy mildew is more serious under temperate conditions than in tropics. Its occurrence in India was reported in Maharashtra in 1984 (Mayee and Patil, 1986). The pathogen shows wide variation with many races and corresponding resistant genes in the host. Resistance to each race was found to be controlled by a single dominant gene. Apart from major genes several modifiers are also known to influence resistance. The resistance to the disease appears to be rare in cultivated annuals but more common in wild perennials. One of the popular restorer lines, RHA 27A, has the P12 gene for resistance to races 2and 3.

Wilt: Resistance to wilt is present in both cultivated and wild sunflowers. The
available information indicates that resistance is both simple and complex depending upon the material involved in the study.

This disease was reported first time in India during 1997 in Bagepally area of Kolar distt. in Karnataka and also in Rangareddy distt. of A.P.This disease was also reported in Parts of Maharastra and TamilNadu.

High Oil Quality hybrids:

Oil percentage and fatty acid profile is an important trait to develop high quality hybrids of sunflower. Several environmental factors also influence oil percentage as well as fatty acid composition. Low temperature at seed development stage increases the linoleic component and decreases the other fatty acids whereas it is reverse under high temperature conditions (Sheoran *et. al*, 2014.) For this purpose breeder must go for selection of inbred lines which shows high oil content and fatty acid profile and

emphasis should be laid on the stable lines so that the hybrids with high yield and high oil quality trait could be developed.

Perspective

Sunflower has become a crop of major economic importance worldwide. It is cultivated mainly as an edible oil seed crop. As a source of edible vegetable oil, it is one of the important oil seed crops in the world. Sunflower has made a significant dent in a number of tropical and temperate countries because of the following desirable features including wide adaptability of the crop enabling its cultivation in different agro-climatic regions and soil types. Being day neutral, the crop can be grown in different seasons. Being a short duration crop, it can fit into various multiple cropping systems. Ideal crop for contingency cropping plans. The versatile nature of crop and its increasing contribution to oilseeds production calls for concerted efforts to evolve hybrids with higher productivity. To achieve quantum jumps in the productivity levels among the large areas of Asia, Africa and other countries, production of quality hybrid seed is required. Further to keep pace of the new challenges, broadening the genetic base of male sterile as well as restorer lines, development of superior hybrids and supply of genetically pure hybrid seeds to commercially exploit maximum heterosis assumes greater importance. The gains in productivity of sunflower crops have been achieved primarily through exploitation of available genetic variability Conventional breeding coupled with modern tools such as biotechnology should now be the primary focus in crop improvement programs. Heterosis breeding should be the major focus in this crop. To facilitate better exploitation of the available gene pools and overcome the production constraints, research emphasis needs to be on (i) augmentation/ identification of trait specific germplasm; (ii) prebreeding and genetic enhancement; (iii) allele mining, (iv) functional genomics, proteomics, metabolomics, and interactomics; (v) marker assisted breeding and gene pyramiding; and (vi) trait improvement through genetic engineering.

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MOLECULAR AND GENETIC ASPECTS OF SUNFLOWER DEFENSIVE RESPONSE TO DOWNY MILDEW

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ABSTRACT

Sunflower represents one of the most important oilseed crops worldwide. In Republic of Moldova it is placed third after wheat and maize according to cultivated area. An excessive extension of cultivated areas and high susceptibility of this crop to wide number of diseases determine necessity of obtaining of resistant hybrids. One of the most devastating pathogens of sunflower is downy mildew (DM) *Plasmopara halstedii*, which causes significant yield losses in rainy years. In this context, the aim of this study was to determine resistance potential among sunflower genotypes from RM and some key processes within molecular mechanisms of genetic control of sunflower downy mildew resistance. Performed study included genotyping of sunflower lines using SSR markers, screening of *Pl1* and *Pl6* downy mildew resistance genes and expression studies of 22 genes involved in ROS metabolism and Systemic Acquired Resistance (SAR). The resistance potential of sunflower genotypes cultivated in RM was estimated. *Pl1* gene was identified in 36 genotypes, *Pl6* gene – in 37 and both genes were identified in 24 genotypes. Investigations related to gene expression revealed new insights of sunflower DM resistance mechanism such as differential expression of genes involved in maintenance of oxido-reduction homeostasis in function of infection degree; involvement of transcription factor *Why1* from Whirly family in insurance of sunflower response to *P. halstedii* attack.

Key Words : sunflower, downy mildew, resistance genes, defensive response

COMPARATIVE ASSESSMENT OF ANDROGENIC RESPONSE IN SUNFLOWER (HELIANTHUS ANNUS)

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ABSTRACT

A number of factors include genotype, donor plant growing conditions, developmental stage of pollen, pretreatments and media constituents influence anther culture. The androgenetic ability of four hybrid sunflower genotype using two different growth environments (field and growth chamber), two pretreatment (cold and heat) and also different media compositions were tested and compared. Flower buds (capitulum) containing anthers were collected when the microspores were at the late uninucleate stage. Capitulums from field grown plants were reaching to this stage after 50-60 days after planting but this time is prolonged up to 70 days in the growth chamber. The capitulum diameter of growth chamber grown plants (1-2 cm) was smaller than field grown plants (2-5 cm). The capitulum obtained from growth chamber grown plants includes anthers at optimal developmental stages more than growth chamber grown plants as well. Anthers were excised from capitula of field and growth chamber grown plants and pretreated with cold for 24, 48 or 72 hours at 4°C in the dark. Cold pretreatment for 24 hours produced the highest frequency of embryonic calli induction (34,4 %) on the medium that was supplemented with NAA (0,5 mg/l) and heat pretreatment at 35 0C for 0, 2, 4, 8 or 12 days were applied to anthers and 2 days pretreatment produced the highest frequency of embryogenic calli induction (41%) in growth chamber grown capitulum. The studies on capitulum collected from field have been currently under investigation. This research has been supported by TUBITAK KBAG (Project No: 214O274).

Key Words : Sunflower, anther culture, growth environment.

APPLYING THE TOOLS OF GENOMICS TO SUNFLOWER BREEDING ISSUES

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ABSTRACT

The last four years have been an illuminating time in the history of sunflower genomics. We have seen the release of the first draft of a public sunflower genome. This reference has been wrought with complexity that is still being investigated, but has presented opportunities to understand our favorite species in ways that we have only dreamed of before. From the perspective of the evolutionary biologist, we can obtain greater understanding of how selection has changed this crop, in both expected and unexpected ways. We are beginning to understand how our inbred lines and hybrids are different in terms of genome size and organization, and not simply on their phenotypic trait structure. In this plenary, I will discuss some of the practical tools that arise out of the sequenced genome. At the USDA in Fargo, together with collaborators at University of Colorado, we have used our historical breeding records complete with phenotypic data to develop a Genomic Selection system. Genomic Selection takes sequence information from parents and progeny in the breeding program, trains a model that assigns breeding values to each polymorphic site in the genomic dataset, and uses the model to make informed selection decisions, fully utilizing the historical phenotypic data that is pertinent to the environments of interest. This should improve accuracy and balance in selection in early generations of progeny, resulting in optimized genetic gain compared to previous inefficient, unbalanced methods. A more familiar model is the GWAS or association mapping model. Community resources have been developed around this system, and can result in greater understanding of quantitative traits of large importance (e.g. fatty acid variation in oilseed sunflower). Genomics has also allowed us to better understand the importance of mutation in breeding programs, allowing us to see for the first time the variety in mutations found in mutagenesis experiments. These advances allow the sunflower community to enter a new age of informed breeding, which could lead to acceleration of genetic gain in the future.

Key Words : genomics, breeding, quantitative genetics, genomic selection, GWAS

DETERMINATION OF SUPERIOR HYBRID COMBINATIONS IN SUNFLOWER AND TESTING OF THEIR RESISTANCE TO BROOMRAPE (OROBANCHE CUMANA WALLR.) IN INFESTED AREAS

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ABSTRACT

This research was conducted during 2004-07 in order to study the F_1's hybrid vigor and genetic structure of a hybrid sunflower population in terms of phenological characters, agronomical traits, yield and quality characters and to identify suitable parents and promising hybrid combinations showing superior general and specific combining abilities and resistant to Broomrape. Twenty five experimental hybrids were created using 5 cytoplasmic male sterile (CMS) and 5 pollen tester (restorer) lines having different levels of resistance to Broomrape in sunflower. Field trials of the research were made at three different locations (Center, Ferhadanli and Banarli districts) in Tekirdag province. The experiments were designed in a randomized complete block with three replications. According to the results, the general and specific combining ability (sca) variances were highly significant for all traits investigated except days to 50% flowering. According to the general combining ability effects obtained from the all locations, A_3 (TTAE 4156A) for oil content, seed yield and oil yield were determined as the most suitable parents. The significant SCA effect and high mean values of hybrids combinations showed that the crosses A_4 x R_7 A_3 x R_7, A_4 x R_8, A_5 x R_6, A_3 x R_9 and A_3 x R_8 were promising hybrid combinations for seed and oil yields. It was found that A_3 x R_6, A_3 x R_7, A_3 x R_{10} and A_4 x R_7 hybrids produced 20-25 % more oil yield compared with the mean of checks varieties in some locations. The results of Broomrape test indicated that only R_{10} male line was resistant to broomrape population in all locations. But instead of that male line's hybrids, A_3 x R_6 and A_3 x R_7 experimental hybrids were found highly tolerant to broomrape in all locations. As a result, genotypes A_3 (TTAE 4156A), A_4 (TTAE BAH8 A), R_6 (RHA14) and R_7 (RHA 20) were the parents involved in the best-yielding crosses. Among these parents, A_3 and B_7, which possesses a considerable positive gca effect, might be utilized as a good parent in hybrid sunflower breeding programs. On the other hand, A_3 x R_6, and A_3 x R_7 might be considered as promising hybrid combinations for higher yield based on their sca effects and resistance to Broomrape.

Key Words: Broomrape, Combining ability, *Helianthus annuus* L., heterosis, line x tester, sunflower, yield and quality.

INTRODUCTION

In general, hybrid varieties in open-pollinated crops provide heterosis in terms of yield performance, some agronomic traits such as plant height, earliness and product quality. Therefore, most of the sunflower growers in many countries prefer to use hybrid varieties. Hybrid sunflowers are more stable, highly self fertile and more uniform at maturity (Dedio and Enns, 1976, Seetharam, 1979). Resistance to diseases and broomrape has also increased the importance of hybrid varieties. The heterotic performance of a hybrid combination depends upon to combining abilities of its parents (Kadkol et al.,1984; Allard 1999). Due to high heterosis occurring generally in hybrids between genetically unrelated inbred lines, all plant breeders use as a selection criterion the heterosis to find good combiners. Breeding programs can take advantage from such information on combining abilities to find best selection strategy for developing high yielding lines and hybrids. In that reason, general and specific combining abilities are becoming very important information in plant breeding. (Skoric, 1992). Heterosis and combining ability

studies are frequently used by breeders to improve the superior synthetics and hybrids in sunflower as well as in the other open-pollinated crops (Goksoy et al., 2000).

Regarding combining ability analysis, SCA variance higher than GCA variance means that non-additive genes have higher effects than additive genes in determining the studied characters. Conversely, higher GCA variance indicates that additive gene effects play a more important role in determining these traits. If neither variance is significant, it implies the existence of epistatic gene effects (Marinkovic *et al*., 2000; Joksimovic *et al*., 2000). Various researchers have studied on the general and specific combining ability variances for certain characters in sunflower. Some researchers found that additive gene effects had more important roles in certain yield traits such as plant height (Mruthunjaya *et al*., 1995; Mihaljcevic, 1988; Goksoy *et al*., 1999; Joksimovic *et al*., 2000), 1000-seed weight (Tyagi, 1988; Mruthunjaya *et al*., 1995; Mihaljcevic, 1988; Goksoy *et al*., 1999; Khan, 2001), flowering time (Mihaljcevic, 1988), physiological maturity date (Mihaljcevic, 1988; Tyagi, 1988), etc. Others observed that non-additive genes affected dominantly some yield components such as head diameter (Mruthunjaya *et al*., 1995; Mihaljcevic, 1988) and physiological maturity date (Mihaljcevic, 1988).

The environmental conditions of course influence the evaluation of combining abilities (Petakov, 1996) but, most of the breeders obtained their superior hybrids by crossing inbred (*cms)* female and male (restorer) lines having high GCA and SCA values.

In this study, it was aimed to estimate the genetic structure in 25 hybrids obtained from five CMS and five restorer sunflower lines and to identify parental lines having good combining abilities and superior sunflower hybrids the resistant to broomrape.

MATERIAL AND METHODS

Broomrape observations were evaluated as frequency (F), intensity (I) and attacking rate (AR) according to Pustovoit's method. The plants having 0 – 10 % frequency and 0-1 AR values were considered as resistant (Vranceanu *et al.*, 1980 and Pacureanu-Joita *et al.*, 1998).

Test of the resistance to broomrape was made on natıral infected fields at Karaevli, Tekirdag in 2004. The results of orobanche test showed that except P4223 (Pioneer Seed Co.) all other commercial checks and released inbred lines from UUAF (Uludag University Agricultural Faculty) and TARI (Thrace Agricultural Research Institute) were susceptible to the new broomrape race. Five female and five male were selected from broad-sense sunflower germplasm according to their plant vigor and yield abilities and resistance to broomrape (Table 1).

Five cytoplasmic male sterile lines and five fertility restorer lines illustrated in Table 1 were crossed in all possible combinations in 2004 and 2005. All resultant 25 experimental hybrids, 10 parents and three commercial hybrids (as check) were planted in randomized complete block design with three replications at Center in 2005 and Ferhadanli and Banarli locations of Tekirdag in 2006. In the experiments, parent lines, experimental hybrids and check varieties were planted by hand in mid-April in a well prepared soil. Plot size was 12.6 m^2 (6.0 x 2.10 m) ; row spacing was 0.70 m; plant-plant spacing was 0.30 m. Sixty kilogram of nitrogen per hectare as ammonium nitrate was applied prior to sowing and a further 60 kg N ha^{-1} was added when the plants were 25-30 cm in height. Hand hoeing was done when necessary.

The data were recorded on ten randomly selected plants from middle row of each plot for plant height, stem diameter and head diameter. Additionally, days to 50 % flowering and days to physiological maturity were also observed and recorded to field book. Yield components such as hectoliter, plot yield, 1000 seed weight and number of seeds per head were measured based on plot harvest at the Ministry of Agriculture, Tekirdag Province Control Laboratory. Oil content was measured by NMR at Thrace Oil Seeds Union Laboratory.

All data were subjected to analysis of variance for each character using MINITAB (University of Texas, Austin, version 14) software. Analysis of variance for combining ability was done according to the Line x Tester method in which estimates gca variances and sca variances were obtained as suggested by Singh an Chaudhary (1977). Analysis of combining ability was made using TARPOPGEN (Ege University, Izmir, Turkey) software as outlined by Ozcan and Acikgoz (1999).

Table 1: Selected male and female lines

Parents		**Female/Male**	**Type**	**Source**
Code	**Pedigree**			
A_1	CMS 16 X N 42	Female	CMS	UUAF
A_2	CMS 10 X N 11	Female	CMS	UUAF
A_3	4156 A	Female	CMS	TARI
A_4	BAH 8 A	Female	CMS	TARI
A_5	H1 CMS 88 X N Record (109)	Female	CMS	UUAF
R_6	RHA 14	Male	Restorer	UUAF
R_7	RHA 20	Male	Restorer	UUAF
R_8	RHA 22	Male	Restorer	UUAF
R_9	RHA 03	Male	Restorer	UUAF
R_{10}	RHA 09	Male	Restorer	UUAF

UUAF: Uludag University Agriculture Faculty

TARI : Thrace Agriculture Research Institue

RESULTS AND DISCUSSION

Broomrape screening results are illustrated in Table 6 (at the final page). Commercial varieties used as check in the study, Sanbro (Sygenta Seed Co.) and C70165 (Advanta Seed Co.) are known resistant to 5 races (A to E) of Broomrape. None of them were found even tolerant to broomrape populations in trial areas. Third check variety, hybrid P4223 (Pioneer Seed Co.) known as resistant to new race was found resistant to broomrape in all trial areas.

The results showed that obviously trial areas were infested by new race or races. In Ferhadanli (2006) in spite of high frequency level of the genotypes, all hybrid combinations and parent lines except check variety Sanbro had low attack degree. The results of Broomrape test indicated that only R_{10} (Rha 09) was found resistant in all locations. But instead of hybrid combinations including R_{10} (Rha 09) male line, A_3 x R_6 and A_3 x R_7 experimental hybrids were found highly tolerant to broomrape in all locations. The commercial checks Sanbro and C70165 were susceptible to the new races.

Broomrape races A to E are controlled by the single dominant gene (Sunko et al. 1999). These results confirmed by the earlier researchers revealed that the new race resistant gene actions is mainly determined by dominant - recessive epistatic gene effects. In that reason, depends on its broomrape

sensitivity would be variable one hybrid combination obtained from the cross between broomrape resistant line and non resistant line. (Martinez et al. 2005).

Some of the experimental hybrids had less attacking rate than critical limit level but none of them had less frequency level than 10 %. Therefore none of these experimental hybrids were found resistant to the new races. However, some of experimental hybrids had lower frequency level and attacking rate than commercial checks Sanbro and C70165. Therefore these hybrids can be described as tolerant to the new races. Shindrova and Encheva (1994) reported that orobanche parasite reduced mainly seed yield and some yield components such as 1000 seed weight, plant height, head diameter, oil and protein content but fatty acid and quality composition of kernel were not affected by broomrape. Therefore, using of varieties with resistant to broomrape is very important in infested areas.

In the research, commercial checks reached to the flowering stage at 75 – 78 days and also to the physiological maturity stage at 111 – 112 days while the experimental hybrids required 69 – 86 days to reaching flowering stage and 100 – 120 days for the physiological maturity. Our findings on this subject are in agreement with those of Kaya (1998 and 2001) and Ergen and Saglam (2005) who reported that the days to 50 % flowering and days to physiological maturity ranged between 63 and 81 days and 94 and 110 days, respectively. As seen from general combining ability effects for days to 50% flowering in Table 3, gca effects were negative for A_1 (CMS 16 x N42), A_3 (TTAE 4156 A) and A_4 (TTAE BAH 8 A) whereas that effect was positive for A_5 (H1 CMS 88 X N Record 109) in all locations but gca effects were only statistically significant for A_1 and A_5 parental lines at Banarli location. For the male lines, gca effects were only found positively significant in R_8 (Rha 22) line at Ferhadanli location. Specific combining ability (sca) effects were no significant for days to 50 % flowering (Table 4).

For the days to physiological maturity, only A_1 (CMS 16 x N42) line had significantly negative effect whereas the restorer lines' gca effects were not significant. Sca effects of experimental hybrids were not significant in Tekirdag and Banarli locations. The experimental hybrids A2 x R_{10} and A_3 x R_{10} had significant sca effects in Ferhadanli. In observed p*henological* traits, gca variance was lower than sca variance. Due to σ^2_{GCA} / σ^2_{SCA} variance ratio indicates dominant or epistatic genes' actions more effective than additive genes. Our results do not correspond to those of Mihaljcevic (1998) and Tyagi (1988) who reported that additive gene actions were more effective than non-additive gene actions. The proportion of genetic factors (H) into phenotypic variance was 5.6 % for the days to flowering and 17.4 % for days to physiological maturity. The proportion of additive genetic variance (h^2) into phenotypic variance for both traits was 1.71 % and 2.06 %, respectively.

In the research, plant height for check varieties varied between 147 and 180 cm. That agronomic traits for experimental hybrids were among 138.3 to 194.4 cm. Significant gca effects for plant height were found positive for A_1 (CMS 16 x N42), A_2 (CMS 10 x N11), R_7 (Rha 20) and R_8 (Rha 22) lines, but it was negative for A_5 (H1 CMS 88 X N Record 109) line in all locations (Table 3). Parental lines having positive and significant gca effects for plant height have been reported earlier by Dua and Yadava (1983) and Goksoy et al (2000). In the present study, plant height sca effects were positively significant for A_5 x R_7 and A_5 x R_9 at Tekirdag and Banarli locations whereas it was negatively significant for A_2 x R_8 experimental hybrid at Ferhadanli location (Table 4a).

For the plant height, σ^2_{GCA} / σ^2_{SCA} ratio was higher than 1 at Banarli while it was lower than 1 at the other locations. This case indicated that additive genes were more effective than non-additive genes for this hybrid population at Banarli but non-additive gene actions were found more effective in Ferhandanli and Tekirdag. Previous researchers reported that additive gene actions were more effective than non-additive gene actions for plant height Muruthunjava, 1995, Mihaljcevic, 1988, Goksoy et al, 1999 Joksimovic et al. 2000). The proportion of genetic factors (H) and additive genes (h^2) into phenotypic variance for plant height was 54.4 % and 7.81 %, respectively (Table 5).

The gca effects for 1000 seed weight were found positively significant in A_3 (TTAE 4156) at Banarli and R_8 (Rha 22) line at Ferhadanli, while this effect was negatively significant in R_{10} (Rha 09) at Ferhadanli location. Sca effects for 1000 seed weight were not significant in all locations.

Neither general nor specific combining ability variances were significant for 1000 seed weight. Most of the total genetic variation for this trait was caused by epistatic gene action, since the sca variance was higher than gca variance. On the other hand, negative sca variance for 1000 seed weight indicated that epistatic genes affected 1000 seed weight in decreasing direction (Table 4a). The results of earlier Goksoy and Turan (2004) reported similar findings. Broad-sense (H) and narrow-sense (h^2) heritabilities were estimated as zero because of the very low and negative genotypic variance. Contrary, Pathak (1974) reported that broad-sense heritability ranged between 20 % and (0 % for 1000 seed weight. Also Alza and Fernandez-Martinez (1997) found that heritability for seed weight in sunflower was 84 %.

The gca effects for number of seeds per head were significant on some female lines at Ferhadanli and Banarli locations. A_2 (CMS 10 X N11) had negative gca effect in all locations while A_3 (TTAE 4156) was positive gca effect (Table 3). The sca effects were highly significant for all of hybrid combinations in all locations (Table 4b).

The ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ were found lower at Tekirdag (Center) and Ferhadanli locations except one the other. The ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ indicated that the gca variance was significant at Banarli location but sca variance at Tekirdag. However, both gca and sca variances were no significant at Ferhadanli location (Table 5). These results revealed that additive gene actions were more effective at Banarli but dominant gene actions at Tekirdag for number of seeds per head. On the other hand, epistatic gene actions were more effective at Ferhadanli. Opposite results were obtained in different studies on combining ability in number of seeds per head. Goksoy and Turan (2004) reported that the number of seeds per head was influenced by dominant gene action, as the variances due to sca were highly significant for this character. On the other hand, Goksoy et al. (2000) detected that additive gene action was significant for the number of seeds per head.

Heredity values for the number of seeds per head were estimated 43.2 % for broad-sense and 4.77 % for narrow sense heritability. Fick (1978) noted that heritability for the number of seeds per head was higher than that for seed yield.

Hectoliter weight was measured ranging from 26.3 to 37.8 kg in female lines, and 32.9 to 39.8 kg in male lines. Hectoliter weight of commercial hybrids ranged between 33.7 and 37.6 kg while experimental hybrids had 29.3 kg to 41.3 kg hectoliter weight. Previous researchers reported similar results (Kaya and Atakisi 2004, Marinkovic et al. 2006).

As the ratios of $\sigma^2_{GCA} / \sigma^2_{SCA}$ were lower than 1 for test weight, non-additive gene actions were more effective than additive gene actions on this trait. Our findings do not correspond to those of Marinkovic et al. (2006) who reported that epistatic gene action was more effective than other gene actions on the heredity of hectoliter weight. The sca effects were found significant for all hybrids except A_1 x R_7 hybrid combination in Tekirdag location. The gca effects in parent lines were significant positively for B_{10} (RHA 09) and negatively for B_8 (RHA 22) at Ferhadanli location. However, at Banarli location, gca effects were significant positively for A_2 (CMS 10 X N11) and negatively for A_4 (TTAE BAH 8 A), also.

Environmental and genotype x environment interaction variances were found higher than genotypic variance for hectoliter weight. Therefore, heritabilities for the broad-sense and narrow sense were estimated as 11.80 % and 2.30 %, respectively. The biggest proportion of phenotypic variance was affected from environmental factors. Similar results were also reported earlier by Marinkovic et al. (2006).

In the present study, oil content ranged from 40.5 percent to 42.79 percent for female lines, 37.4 percent to 42.8 percent for male lines, 37 percent to 46 percent for commercial checks and 38.8 percent to 43.6 percent for experimental hybrids (Table 3 and Table 4b).

The gca effects for oil content were only significant in Ferhadanli location while sca effects were no significant for all location. The ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ was found lower than 1 and sca variance was negative and both gca and sca variances were not statistically significant. Therefore, epistatic gene action was more effective than the other gene actions for this trait. Our results support the previous work of Hladni et al. (2007) who reported that non-additive gene actions were more effective than additive gene actions on the heredity of oil percentage, as the ratio of gca:sca variance was lower than 1 for this character.

Heredity values of oil content were estimated 15.30 % for broad-sense and 3.43 % for narrow-sense heritability. Fick (1975) reported that broad-sense heritability for oil content ranged between 52 % and 61 %. On the other hand, Cespedes Torres et al. (1984) found that broad-sense heritability was 26.8 % for oil percentage. In this study, seed yields ranged between 3221 and 3953 kg ha^{-1} for commercial checks and 1971 and 3571 kg ha^{-1} for experimental hybrids over all locations.

The gca effects were found significant in A_2, A_3, A_5 female lines at Ferhadanli and also A_1 and A_4 female lines at Banarli. The gca effects were no significant in the male lines at Tekirdag and Ferhadanli locations while these effects were significant in R_6, R_7 and R_9 male lines at Banarli. The sca effects were only found significant in A_5 x R_7 experimental hybrid at Tekirdag location. Additive gene action was described more effective than non-additive gene action for seed yield at Ferhadanli whereas dominant gene action was more effective than additive gene action, as the ratio of $\sigma^2_{GCA} / \sigma^2_{SCA}$ was lower than 1 at Tekirdag (Center). In addition, epistatic gene action was more active at Banarli. In the most of previous studies, significant non-additive gene action for seed yield has been reported earlier by Dua and Yadava (1983), Castiglioni et al. (1999), Goksoy and Turan (2004), Jan et al. (2005) and Ortis et al. (2005).

Heredity values for seed yield were estimated as 53 % for broad-sense heritability and 6.54 % narrow-sense heritability. In previous studies, degree of broad-sense heritability was found as 57 % (Pathak 1974), 18 % (Kloczowski 1975), 48.4 % (Cespedes Torres et al. 1984) while degree of narrow-sense heritability was obtained 50.5 % (Mogali and Virupakshappa 1994) and 65 % (Alza and Fernandez-Martinez 1997).

In the present study, oil yields were found between 1259 and 1445 kg ha^{-1} for commercial checks and 693 and 1386 kg ha^{-1} for experimental hybrids over all locations. The gca variance was significant for oil yield at Ferhadanli and Banarli locations whereas sca variance played an important role Tekirdag (Center). These results revealed that additive gene actions were more effective than non-additive genes at Ferhadanli and Banarli whereas dominant gene effects were more active than other gene actions at Tekirdag (Table 5). In earlier researches, Jan et al. (2005) found that non-additive gene actions were significant for oil yield. Contrary to that, Del Gatto et al. (2005) reported that additive gene actions were more effective than non-additive gene actions.

Heredity values were estimated 52.9 % for broad-sense and 7.84 % for narrow-sense. In close agreement with our findings, Mogali and Virupakshappa (1994) reported. Degrees of broad and narrow-sense heritability for oil yield were 64.1 % and 50.7 %, respectively.

The present study showed that A_3 (TTAE 4156) and R_7 (Rha 20) lines were good combiners and they could be evaluated for further cross combinations by present breeding program. Cross combinations A_3 x R_6, A_3 x R_7, A_3 x R_8 and A_4 x R_7 were determined as promising hybrid combinations in terms of higher yield and resistance to broomrape for pre-commercial trials (Table 6).

CONCLUSION

As a results, parental lines A_3 (TTAE 4156A), A_4 (TTAE BAH8 A), R_6 (RHA14) and R_7 (RHA 20) were found as the best-combiners because of their high gca effects for yield and the other characters. It was found that the crosses A_4 x R_7 A_3 x R_7, A_4 x R_8, A_5 x R_6, A_3 x R_9 and A_3 x R_8 were promising hybrid combinations for seed and oil yields. Especially, A_3 x R_6, A_3 x R_7, A_3 x R_{10} and A_4 x R_7 hybrids produced 20-25 % more oil yield compared with the mean of checks varieties in some locations. Among of these experimental hybrids, A_3 x R_6, and A_3 x R_7 might be considered as promising hybrid combinations for higher yield based on their sca effects and resistance to Broomrape.

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Table 3: General Combining Abilities of Parents

Parents		# Days Of Flowering (50 %)						# Days Of Physiological Maturity						Plant Height (Cm)					
		2005		2006				2005		2006				2005		2006			
		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
		Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Female Lines																			
A_1	CMS 16 X N 42	85.3	-0.96	73.0	-0.44	75.0	**-1.16**	114.0	-2.31	101.7	-0.83	103.7	**-2.63**	126.7	**-7.35**	146.6	**-8.80**	126.7	**-11.91**
A_2	CMS 10 X N11	86.0	1.11	76.3	1.29	76.7	0.97	120.7	1.03	111.0	2.17	111.3	0.77	138.3	**-5.68**	150.5	**-12.33**	133.7	-5.51
A_3	TTAE 4156 A	80.0	-0.23	73.0	-0.71	76.0	-0.56	115.0	0.76	108.7	-3.56	109.3	0.91	136.7	**7.72**	153.8	**2.53**	158.0	-0.51
A_4	TTAE BAH 8 A	90.7	-0.89	69.3	-0.51	76.0	-0.29	125.3	1.16	109.0	2.51	105.0	1.64	181.7	**-9.68**	145.7	**5.00**	148.7	5.83
A_5	H1 CMS 88 X N Record (109)	83.3	0.97	73.7	0.36	76.0	**1.04**	114.7	-0.64	105.0	-0.29	107.3	-0.69	176.7	**14.99**	175.5	**13.60**	170.6	**12.09**
Male Lines																			
R_6	RHA 14	79.0	-1.09	74.0	-0.84	74.0	-0.43	117.7	-0.51	112.7	0.71	112.7	0.04	121.7	-2.68	131.1	**1.80**	111.1	-1.44
R_7	RHA 20	83.7	0.97	73.0	0.96	75.3	-0.29	113.0	-0.11	102.3	0.84	104.7	-1.49	133.3	3.32	135.0	**2.20**	117.2	5.49
R_8	RHA 22	81.3	0.77	75.3	**1.29**	76.0	1.11	114.7	0.83	108.7	2.31	109.3	1.04	121.7	4.32	140.0	**6.20**	139.5	**8.83**
R_9	RHA 03	81.3	0.31	73.7	0.63	76.0	0.71	110.7	-0.84	103.0	1.84	105.3	-0.49	113.3	-5.28	129.4	**-2.73**	110.6	-0.44
R_{10}	RHA 09	79.0	0.96	70.7	-2.04	69.7	-1.09	109.3	0.63	101.0	-5.69	100.0	0.91	98.3	0.32	126.1	**-7.47**	111.1	**-12.44**

Parents		1000 Seed Weight (gr)						# Seeds Per Head						Hectoliter Weight (Kg)					
		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
		Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Female Lines																			
A_1	CMS 16 X N 42	55.7	-0.77	50.5	-0.47	47.2	-1.51	564.7	-127.98	883.7	11.88	1085.2	**-138.78**	34.0	10.97	37.8	8.25	35.6	11.05
A_2	CMS 10 X N11	53.9	-2.45	52.6	0.17	57.7	-1.83	1021.9	-28.88	812.1	**-417.67**	836.2	**-221.39**	37.2	11.37	34.0	-3.81	32.3	**18.59**
A_3	TTAE 4156 A	58.3	2.95	55.4	0.25	74.3	**3.63**	711.7	48.33	622.3	**143.82**	603.8	**208.62**	34.5	-4.69	29.0	-2.75	26.3	-12.55
A_4	TTAE BAH 8 A	56.8	1.18	53.7	0.05	48.6	0.06	795.8	73.35	849.7	27.43	1131.2	**160.37**	36.5	-0.96	32.0	-1.15	34.4	**-20.68**
A_5	H1 CMS 88 X N Record (109)	51.5	-0.91	51.1	-0.01	53.0	-0.35	912.3	35.19	1033.3	**234.54**	662.6	-8.82	35.5	-16.69	36.6	-0.55	30.8	3.59
Male Lines																			
R_6	RHA 14	23.5	-0.64	25.0	-0.13	23.3	1.27	542.5	9.76	417.1	107.32	372.7	96.57	38.4	0.24	38.9	1.72	36.2	-4.61
R_7	RHA 20	24.3	-1.12	25.7	0.35	23.9	1.48	273.7	-72.11	384.7	-14.32	358.8	96.76	33.0	-11.63	36.0	-6.88	32.9	-5.81
R_8	RHA 22	27.7	1.44	25.7	**0.67**	22.5	-1.01	355.6	5.75	335.7	21.29	384.8	-54.07	35.0	-4.23	36.3	**-13.15**	35.6	4.25
R_9	RHA 03	24.0	-1.31	25.7	0.19	23.6	-1.22	494.5	-10.45	173.9	-187.56	289.7	-76.83	37.1	9.17	36.1	-1.55	35.7	-2.75
R_{10}	RHA 09	23.6	1.63	24.7	**-1.07**	19.8	-0.52	342.5	67.06	346.1	73.27	329.3	-62.43	37.9	6.44	39.8	**19.85**	38.9	8.92

Parents		Oil Level (%)						Grain Yield (Kg/ha)						Oil Yield (Kg/ha)					
		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
		Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Female Lines																			
A_1	CMS 16 X N 42	41.3	0.78	45.9	0.45	39.3	0.33	1486	-41.25	2125	2.85	2431	**-37.28**	558	-14.60	877	-0.70	855	**-15.34**
A_2	CMS 10 X N11	41.5	-1.48	42.3	**-2.28**	37.9	-1.40	2630	-20.45	2030	**-96.15**	2352	**-59.15**	998	-11.14	767	**-45.61**	776	**-25.89**

A_3	TTAE 4156 A	43.5	2.02	42.7	0.65	39.1	1.61	1978	33.41	1637	**37.92**	1775	**58.05**	776	20.42	978	**22.44**	622	**32.55**
A_4	TTAE BAH 8 A	41.4	0.32	43.8	**1.22**	41.8	-0.04	2155	22.08	2172	-3.35	2619	**39.65**	805	8.20	629	3.82	625	**12.27**
A_5	H1 CMS 88 X N Record (109)	41.5	-1.64	43.9	-0.05	42.7	-0.50	2153	6.21	2488	**58.72**	1594	-1.28	802	-2.88	859	**20.05**	622	-3.60
Male Lines																			
R_6	RHA 14	43.3	-0.15	44.0	0.49	41.1	0.25	1201	-1.52	993	14.32	827	**35.65**	473	-1.72	392	10.05	306	**11.79**
R_7	RHA 20	39.5	0.04	37.1	**-0.88**	37.8	0.37	617	-24.25	940	1.59	795	**33.85**	220	-9.64	292	-4.30	270	**11.86**
R_8	RHA 22	35.9	0.34	42.2	**0.80**	39.0	-0.34	939	8.81	821	12.72	805	-15.41	303	3.44	312	4.65	283	-8.44
R_9	RHA 03	38.5	0.18	37.6	-0.10	35.9	-0.28	1064	-9.12	425	-42.01	653	**-36.68**	369	0.46	142	-12.32	210	-6.84
R_{10}	RHA 09	39.8	-0.40	44.4	-0.31	40.5	0.00	730	26.08	818	13.39	632	-17.41	261	7.46	326	1.92	231	-8.38

Significant at 5 %, Significant at 1 %

Table 4a: Spesific Combining Abilities of Hybrids

Hybrids	# Days Of Flowering (50 %)						# Days Of Physiological Maturity						Plant Height (Cm)						1000 Seed Weight (gr)					
	2005		2006				2005		2006				2005		2006				2005		2006			
	Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
Cross	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**	**Mean**	**SCA**
A_1 x R_6	81.3	1.89	70.3	-4.30	72.0	0.40	116.3	2.11	105.3	**-1.24**	107.0	0.7	146.7	3.01	158.9	-6.67	144.5	-2.36	52.4	-2.56	49.7	0.05	48.9	-0.95
A_1 x R_7	80.0	-1.51	73.0	0.00	71.3	-0.40	113.0	-1.63	106.0	-0.70	104.3	-0.4	151.7	2.01	162.2	-3.73	151.1	-2.96	56.3	1.73	49.9	-0.22	47.9	-2.22
A_1 x R_8	80.0	-1.31	74.7	0.70	73.7	0.50	115.0	-0.56	109.7	**1.49**	108.7	1.4	145.0	-5.65	172.2	1.93	160.5	3.04	62.1	4.99	50.9	0.36	49.9	2.3
A_1 x R_9	81.3	0.49	71.7	-2.30	72.7	-0.10	117.3	1.97	107.7	0.00	108.7	1.5	140.0	-1.05	168.9	7.87	148.9	0.97	51.0	-3.35	49.9	-0.13	47.4	-0.04
A_1 x R_{10}	80.0	0.43	68.7	-4.40	70.7	-0.30	112.0	-1.89	100.7	0.50	102.7	-3.1	148.3	1.68	156.7	0.6	137.2	1.31	56.4	-0.82	48.7	-0.07	49.0	0.92
A_2 x R_6	81.0	-0.51	73.7	-2.00	73.7	-0.10	115.3	-2.23	108.0	**-1.57**	108.0	-1.7	143.3	-1.99	168.9	6.87	148.9	-4.43	54.8	1.46	50.5	0.21	47.4	-2.18
A_2 x R_7	85.7	2.09	74.0	-0.90	74.0	0.10	118.3	0.37	106.7	**-3.04**	106.7	-1.5	153.3	2.01	160.6	-2.2	161.1	0.64	52.0	-0.84	50.9	0.11	49.7	-0.08
A_2 x R_8	82.7	-0.71	72.3	-4.60	75.7	0.40	121.0	2.11	110.7	-0.50	114.0	3.3	146.7	-5.65	157.8	**-8.53**	161.7	-2.03	54.6	-0.81	50.3	-0.81	48.6	1.33

A_2 x R_9	82.0	-0.91	73.3	-2.20	74.0	-0.90	117.7	-1.03	109.0	**-1.71**	109.7	-0.9	151.7	8.95	162.8	5.07	154.4	-0.09	55.2	2.53	50.3	-0.37	49.2	2.14
A_2 x R_{10}	81.7	0.03	73.7	0.20	73.7	0.60	118.0	0.77	110.0	**6.83**	110.0	0.8	145.0	-3.32	151.7	-1.2	148.3	5.91	53.3	-2.33	50.2	0.86	46.6	-1.22
A_3 x R_6	77.7	-2.51	70.3	-4.30	71.0	-1.20	115.7	-1.63	108.3	**4.49**	109.0	-0.8	151.7	-7.05	176.7	-0.33	160.0	1.57	63.2	4.49	50.7	0.27	55.0	-0.03
A_3 x R_7	82.7	0.43	72.3	-0.90	72.3	0.00	120.7	2.97	110.3	**6.36**	110.3	2.0	168.3	3.61	184.4	6.93	170.0	4.64	57.5	-0.76	50.0	-0.87	54.7	-0.58
A_3 x R_8	82.3	0.29	72.3	-2.50	73.3	-0.40	117.0	-1.63	107.0	**1.56**	108.0	-2.8	171.7	5.95	181.1	-0.07	165.0	-4.03	56.7	-4.13	51.6	0.44	53.3	0.49
A_3 x R_9	81.3	0.09	70.8	-3.50	73.9	0.60	117.7	-0.76	107.5	**3.69**	109.8	-0.7	148.7	-7.45	177.4	-3.13	160.0	0.57	61.2	-6.04	50.4	0.29	53.7	1.28
A_3 x R_{10}	82.0	1.69	69.0	-3.90	72.7	1.10	118.0	1.04	107.3	**-16.11**	105.0	2.4	166.7	4.95	164.4	-3.4	144.5	-2.76	67.4	6.44	49.3	-0.12	52.1	-1.17
A_4 x R_6	79.3	-0.17	69.3	-3.30	75.0	2.50	115.3	-2.36	105.3	**-4.57**	111.0	0.4	146.7	5.35	179.4	0.2	163.3	-1.43	52.5	-4.43	50.1	-0.13	54.4	2.87
A_4 x R_7	83.0	1.43	72.3	1.60	73.3	0.70	119.7	1.57	109.0	**-1.04**	110.0	1.0	150.0	2.68	173.9	-6.2	161.1	10.36	56.4	-0.05	50.5	-0.17	54.5	2.87
A_4 x R_8	80.3	-1.04	73.7	1.80	72.0	-2.00	120.0	0.97	113.3	**1.83**	111.7	0.1	146.7	-1.65	188.3	4.47	181.7	6.64	58.3	-0.75	51.6	0.65	48.4	-0.82
A_4 x R_9	81.3	0.43	74.0	3.50	73.3	-0.30	118.3	-0.49	111.0	0.00	110.3	-1.1	138.3	-0.39	171.1	-3.93	178.9	12.91	66.8	10.55	50.6	0.09	46.5	-2.48
A_4 x R_{10}	79.0	-0.64	68.7	-1.90	71.0	-0.80	117.7	0.31	107.3	**3.83**	109.7	-0.4	138.3	-5.99	175.5	5.47	146.1	-7.76	53.9	-5.32	48.8	-0.45	47.3	-2.43
A_5 x R_6	82.7	1.29	72.7	-1.60	72.3	-1.50	120.0	4.11	110.0	**2.89**	109.7	1.4	166.7	0.68	188.4	-0.07	177.8	6.64	55.9	1.04	49.7	-0.41	51.4	0.29
A_5 x R_7	81.0	-2.44	73.7	0.50	73.7	-0.30	113.0	-3.29	105.7	**-1.57**	105.7	-1.0	161.7	**10.32**	193.3	5.2	186.1	8.04	54.3	-0.08	51.8	1.15	51.3	0.02
A_5 x R_8	86.0	2.76	74.0	-0.70	77.0	1.60	116.3	-0.89	104.3	**-4.37**	107.3	-1.9	180.0	7.01	194.4	2.2	177.8	-3.63	57.6	0.7	50.3	-0.63	45.5	-3.3
A_5 x R_9	82.7	-0.11	71.7	-2.70	75.7	0.70	117.3	0.31	106.3	**-1.91**	110.3	1.2	163.3	-0.05	175.5	-5.87	157.8	**14.36**	50.5	-3.69	50.6	0.11	47.7	-0.9
A_5 x R_{10}	80.0	-1.51	70.3	-2.50	72.7	-0.50	115.3	-0.23	105.7	**4.96**	108.0	0.3	171.7	2.68	177.2	-1.47	163.3	3.31	59.2	2.03	49.0	-0.23	53.2	3.9

Significant at 5 %, Significant at 1 %

Table 4b: Spesific Combining Abilities of Hybrids

Hybrids	# Seeds Per Head						Hectoliter Weight (Kg)						Oil Content (%)						Grain Yield (Kg/da)						Oil Yield (Kg/da)					
	2005		2006				2005		2006				2005		2006				2005		2006				2005		2006			
	Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli		Tekirdag		Ferhadanli		Banarli	
Cross	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
A_1 x R_6	1070.1	**151.41**	1241.6	**-152.82**	1010.3	**-60.44**	36.3	**-6.04**	39.3	-0.19	34.1	6.88	41.1	0.03	44.3	-0.81	40.3	-0.21	261.8	3.01	294	-6.67	234	-2.36	96.7	**10.56**	117.8	-14.72	84.8	-8.33
A_1 x R_7	557.1	**-279.72**	1450.2	**177.39**	1171.9	**101.01**	36.0	2.16	38.9	4.75	35.0	17.41	41.6	0.38	44.7	0.9	40.4	-0.29	147.8	2.01	345	-3.73	268	-2.96	55.2	**-23.05**	138.7	20.49	97.1	3.86
A_1 x R_8	1004.5	**89.98**	1069.5	**-238.92**	1026.8	**106.70**	37.5	**10.09**	37.1	-6.99	33.3	-9.65	40.5	-0.99	45.2	-0.25	40.5	0.58	289.4	-5.65	260	1.93	240	3.04	105.8	**14.48**	104.5	-22.59	87.0	14.09
A_1 x R_9	1164.9	**266.55**	1189.1	**89.62**	824.8	**-72.55**	38.7	**9.03**	39.0	0.41	34.0	4.35	43.9	2.47	43.6	-0.99	39.3	-0.75	282.6	-1.05	283	7.87	187	0.97	111	**22.69**	110.6	0.45	65.8	-8.75
A_1 x R_{10}	747.6	**-228.22**	1485.1	**124.73**	837	**-74.72**	36.0	**-15.24**	41.3	2.01	32.8	-18.99	38.9	-1.89	45.5	1.16	41.1	0.68	201.4	1.68	344	0.6	198	1.31	70.7	**-24.68**	140.8	16.38	72.1	-0.87
A_2 x R_6	834.6	**-183.09**	1126.1	**161.27**	1003.8	**15.67**	34.4	**-25.44**	37.7	-3.45	34.2	1.01	37.8	-1.04	42.6	0.15	38.3	-0.55	216.1	-1.99	270	6.87	228	-4.43	73.6	**-16.07**	103.9	16.3	77.8	-4.81
A_2 x R_7	849.1	**-86.72**	863.3	**20.11**	754.8	**-233.48**	36.1	**3.43**	37.1	-1.19	33.4	-6.45	38.6	-0.42	39.8	-1.21	37.9	-1.03	204.1	2.01	209	-2.2	178	0.64	72.1	**-9.58**	74.9	1.6	60.8	-21.79
A_2 x R_8	955.7	**-57.88**	817.1	**-61.87**	671.5	**-165.89**	34.8	**-16.97**	38.2	15.75	34.3	-6.85	40.4	1.11	43.0	0.31	39.0	0.81	246.3	-5.65	196	-8.53	154	-2.03	91	**-3.72**	75.8	-6.38	54.1	-8.29
A_2 x R_9	1305.1	**307.65**	712.3	**42.31**	853.1	**38.40**	40.8	**6.96**	38.2	4.81	32.9	-14.52	37.6	-1.5	42.5	0.6	37.8	-0.51	342.8	8.95	170	5.07	200	-0.09	118.4	**26.56**	63.9	-1.41	68.0	4.01
A_2 x R_{10}	1095.1	**20.04**	769.1	**-161.82**	1174.4	**345.30**	35.6	**32.03**	38.3	-15.92	38.2	26.81	40.4	1.84	41.7	0.15	39.8	1.28	278.6	-3.32	186	-1.2	257	5.91	101.6	**2.82**	69.4	-10.11	93.3	30.88
A_3 x R_6	1045.7	**-49.14**	1310.2	**-216.18**	1477.9	**59.83**	33.4	**2.63**	37.5	-7.19	29.0	-19.85	42.4	0.06	45.0	-0.38	43.6	1.75	313.4	-7.05	316	-0.33	387	1.57	121.5	0.27	128	-27.62	151.5	10.52
A_3 x R_7	1014.5	1.50	1469.8	**65.06**	1514.8	**96.51**	36.7	**-7.51**	38.8	14.41	31.9	10.35	44.4	1.91	44.0	0.06	41.5	-0.47	278.7	3.61	350	6.93	386	4.64	111.5	-1.81	138.7	-2.55	144.3	3.22
A_3 x R_8	1136.7	**45.80**	1772.5	**332.14**	1288.9	**21.40**	34.3	**17.43**	35.7	-10.32	30.8	-11.05	42.7	-0.12	45.7	-0.02	40.1	-1.17	308.3	5.95	436	-0.07	327	-4.03	118.4	**-7.92**	179	28.78	118.3	-2.49
A_3 x R_9	1106.4	**-74.66**	1498.8	**-131.48**	1356.9	**-64.72**	36.7	**4.69**	38.3	3.08	32.5	12.61	42.5	-0.13	44.9	0.14	41.6	0.32	268.8	-7.45	379	-3.13	226	0.57	122.9	-0.47	145.6	12.32	129.2	6.81
A_3 x R_{10}	1228.7	**76.50**	1442.8	**-49.54**	1146.1	**-113.02**	35.5	**-17.24**	40.0	0.01	33.2	7.95	40.3	-1.72	44.7	0.19	41.1	-0.43	387.4	4.95	339	-3.4	278	-2.76	140.3	**9.93**	136.6	-10.93	102.8	-18.05

A_4 x R_6	1146.8	**26.91**	1634.9	**224.94**	1315.4	**-54.42**	36.0	**-2.44**	38.6	2.55	29.3	-8.72	37.9	-2.68	45.6	-0.28	36.7	-3.48	285.3	5.35	390	0.2	338	-1.43	97.1	**-11.9**	159.9	22.84	112.7	-8.04
A_4 x R_7	1445.7	**407.68**	1229.5	**-58.76**	1362.6	**-7.44**	34.8	**14.43**	37.8	3.48	29.5	-5.85	41.3	0.5	45.2	0.62	42.4	2.11	390.6	2.68	297	-6.2	349	10.36	144.7	**43.62**	119.1	-3.63	133.4	12.62
A_4 x R_8	1116.4	0.48	1306.8	**-17.14**	1325.9	**106.65**	34.5	**-5.31**	35.7	-11.25	31.7	6.08	43.4	2.3	46.3	0.08	39.4	-0.26	313.5	-1.65	321	4.47	305	6.64	123.8	**9.71**	133.4	1.73	107.9	7.38
A_4 x R_9	756.2	**-343.52**	1010.1	**-105.06**	1246.1	**49.64**	34.5	**-21.04**	37.6	-3.85	29.4	-10.25	39.1	-1.84	46.0	0.74	41.0	1.36	232.2	-0.39	243	-3.93	274	12.91	82.6	**-28.61**	100.3	-14.33	101.0	-1.05
A_4 x R_{10}	1085.6	**-91.56**	1332.1	**-43.98**	1116.4	**-94.43**	37.8	**14.36**	41.1	9.08	33.4	18.75	42.1	1.7	43.9	-1.15	40.2	0.28	278.7	-5.99	309	5.47	247	-7.76	105.3	**-12.81**	122.3	-6.61	89.7	-10.91
A_5 x R_6	1135.6	**53.90**	1599.9	**-17.20**	1240	**39.37**	37.3	**31.29**	39.2	8.28	34.7	20.68	42.2	3.62	46.0	1.32	42.2	2.49	305.8	0.68	379	-0.07	305	6.64	115	**17.14**	156.5	3.21	115.5	10.66
A_5 x R_7	957.1	**-42.73**	1291.6	**-203.80**	1244.3	**43.41**	31.7	**-12.51**	35.4	-21.45	31.0	-15.45	36.5	-2.37	42.9	-0.37	39.5	-0.33	246.9	**10.32**	319	5.2	302	8.04	80.8	**-9.17**	123	-15.92	107.0	2.09
A_5 x R_8	999.3	**-78.39**	1516.8	**-14.21**	981.2	**-68.86**	33.2	**-5.24**	38.2	12.81	35.7	21.48	36.8	-2.3	44.8	-0.12	39.1	0.04	276.4	7.01	363	2.2	210	-3.63	90.5	**-12.54**	146.3	-1.53	73.9	-10.69
A_5 x R_9	905.5	**-156.02**	1426.8	**104.60**	1076.5	**49.23**	35.1	0.36	37.6	-4.45	33.6	7.81	40	0.99	43.6	-0.49	38.8	-0.41	217.5	-0.05	344	-5.87	244	14.36	79.9	**-20.16**	133.9	2.98	85.2	-1.02
A_5 x R_{10}	1362.3	**223.24**	1713.7	**130.61**	978.6	**-63.14**	33.4	**-13.91**	40.7	4.81	30.5	-34.52	38.5	0.06	43.5	-0.34	37.7	-1.79	374.2	2.68	400	-1.47	247	3.31	131.8	**24.74**	156.4	11.27	83.7	-1.05

Significant at 5 %, Significant at 1 %

Table 5: Variance Components and Heredity Degrees

Variance Components	# Days Of Flowering (50 %)			Plant Height (Cm)			1000 Seed Weight (gr)			# Seeds Per Head		
	2005	2006		2005	2006		2005	2006		2005	2006	
	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli
σ^2 GCA	0.051	0.121	0.073	7.334	7.873	8.319	-0.157	0.024	0.266	-633	3946	2156.7
σ^2 SCA	0.322	0.599	0.105	14.976	16.076	3.104	1.135	0.152	-11.813	29929.2	10353.8	-278.7
σ^2 GCA / σ^2 SCA	0.158	0.202	0.695	0.49	0.49	2.68	0.138	0.158	0.022	0.021	0.4	7.7

σ^2_A	0.102	0.242	0.146	14.668	15.746	16.638	-0.314	0.048	0.532	-1266	7892	4313.4
σ^2_D	0.322	0.599	0.105	14.976	16.076	3.104	1.135	0.152	-11.813	29929.2	10353.8	-278.7
$\sqrt{(\sigma^2_D / \sigma^2_A)}$	1.777	1.573	0.848	1.01	1.01	0.432	1.901	1.779	4.712	4.8	1.1	0.3
$H = \sigma^2_G / \sigma^2_F$	5.64 %			54.40 %			0.0 %			43.20 %		
$h2 = \sigma^2_A / \sigma^2_F$	1.71 %			7.81 %			0.84 %			4.77 %		

Variance Components	Hectoliter Weight (Kg)			Oil Level (%)			Grain Yield (Kg/da)			Oil Yield (Kg/da)		
	2005	2006		2005	2006		2005	2006		2005	2006	
	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli	Tekirdag	Ferhadanli	Banarli
σ^2_{GCA}	4.5	8.04	10.29	0.047	0.125	0.023	-5.21	218.864	185.099	3.188	43.882	5.824
σ^2_{SCA}	185.2	53.02	67.82	1.892	-0.708	0.826	2416.02	106.13	586.153	291.67	128.33	70.01
$\sigma2_{GCA} / \sigma2_{SCA}$	0.02	0.15	0.15	0.024	0.176	0.028	0.002	2.06	0.316	0.01	0.34	0.08
σ^2_A	9	16.08	20.58	0.094	0.25	0.046	-10.42	437.728	370.198	6.376	87.76	11.65
σ^2_D	185.2	53.02	67.82	1.892	-0.708	0.826	2416.02	106.13	586.153	291.67	128.33	70.01
$\sqrt{(\sigma^2_D / \sigma^2_A)}$	4.53	1.81	3.29	4.486	1.683	4.237	15.23	0.492	1.258	6.76	1.21	2.45
$H = \sigma^2_G / \sigma^2_F$	11.80 %			15.30 %			53.30 %			52.90 %		
$h2 = \sigma^2_A / \sigma^2_F$	2.30 %			3.43 %			6.54 %			7.84 %		

Table 6: Combined Results Comparison Of Hybrid And Commercial Checks For All Traits.

Genotypes			Days of flowering (50%)	Days of physiological maturity	Plant height (cm)	Tem girth (mm)	Head diameter (Cm)	1000 seed weight (gr)	# seed per head	Hectoliter weight (kg)	Oil level (%)	Seed yield (Kg/da)	Oil yield (Kg/da)	Frequency (%)	Intensity	Degree of Attack
1	C70165	C70165	**77**	112	159	23	19	**56**	1233	36	**44**	322	143	68	**4**	**2.7**
2	Sanbro	Sanbro	**71**	113	170	25	21	51	**1407**	**37**	40	340	138	64	3	1.9
3	P4223	P4223	73	112	160	25	22	53	**1566**	35	41	**395**	**162**	**2**	**1**	**0.0**
4	A_1 x R_6	CMS 16 X N 42 / RHA 14	72	110	150	24	17	50	1107	**37**	42	263	111	46	**2**	**0.9**
5	A_1 x R_7	CMS 16 X N 42 / RHA 20	**71**	108	155	24	21	51	1060	**37**	42	254	108	47	**2**	**0.9**
6	A_1 x R_8	CMS 16 X N 42 / RHA 22	74	111	159	25	21	54	1034	36	42	263	111	51	**2**	**1.0**
7	A_1 x R_9	CMS 16 X N 42 / RHA 03	73	111	153	23	20	**49**	1060	**37**	42	251	107	52	**2**	**1.0**
8	A_1 x R_{10}	CMS 16 X N 42 / RHA 09	**71**	**105**	**147**	24	21	51	1023	**37**	42	248	105	49	3	1.5
9	A_2 x R_6	CMS 10 X N 11 / RHA 14	74	110	154	23	20	51	988	35	40	238	95	58	**2**	1.2
10	A_2 x R_7	CMS 10 X N 11 / RHA 20	74	111	158	25	21	51	822	36	**39**	**197**	**77**	47	3	1.4
11	A_2 x R_8	CMS 10 X N 11 / RHA 22	76	**115**	155	24	21	51	**815**	36	41	**199**	**81**	51	3	1.5
12	A_2 x R_9	CMS 10 X N 11 / RHA 03	74	112	156	25	**24**	52	957	**37**	**39**	238	92	58	**4**	2.3
13	A_2 x R_{10}	CMS 10 X N 11 / RHA 09	74	113	148	23	20	50	1013	**37**	41	240	97	57	3	1.7
14	**A_3 x R_6**	**TTAE 4156 A / RHA 14**	**71**	111	163	24	19	**56**	1278	33	**44**	339	**148**	**29**	**2**	**0.6**
15	**A_3 x R_7**	**TTAE 4156 A / RHA 20**	72	114	174	**26**	21	54	1333	36	**43**	338	**146**	**26**	**2**	**0.5**
16	**A_3 x R_8**	**TTAE 4156 A / RHA 22**	73	111	173	**26**	19	54	**1399**	34	**43**	**357**	**154**	43	**2**	**0.9**
17	A_3 x R_9	TTAE 4156 A / RHA 03	74	112	162	24	20	55	1321	36	**43**	291	126	60	3	1.8
18	A_3 x R_{10}	TTAE 4156 A / RHA 09	73	110	159	24	21	**56**	1273	36	42	335	141	47	3	1.4
19	A_4 x R_6	TTAE BAH 8 A / RHA 14	75	111	163	**26**	22	52	**1366**	35	40	338	137	50	**4**	2.0
20	**A_4 x R_7**	**TTAE BAH 8 A / RHA 20**	73	113	162	**26**	22	54	**1346**	34	**43**	**345**	**148**	49	**2**	**1.0**
21	A_4 x R_8	TTAE BAH 8 A / RHA 22	72	**115**	172	**26**	22	53	1250	34	**43**	313	135	69	**2**	1.4
22	A_4 x R_9	TTAE BAH 8 A / RHA 03	73	113	163	**26**	**17**	55	1004	34	42	250	105	63	**4**	2.5
23	A_4 x R_{10}	TTAE BAH 8 A / RHA 09	**71**	112	153	25	21	50	1004	**37**	42	278	117	61	**2**	1.2
24	A_5 x R_6	H1 CMS 88 X N Record (109) / RHA 14	72	113	178	23	**23**	52	1178	**37**	**43**	330	144	64	3	1.9
25	A_5 x R_7	H1 CMS 88 X N Record (109) / RHA 20	74	108	180	24	20	52	1325	**33**	40	289	115	63	**2**	1.3

26	A_5 x R_8	H1 CMS 88 X N Record (109) / RHA 22	**77**	109	**184**	25	19	51	1164	36	40	283	116	**78**	**4**	**3,1**
27	A_5 x R_9	H1 CMS 88 X N Record (109) / RHA 03	76	111	166	**21**	18	50	1166	35	41	268	110	69	3	2,1
28	A_5 x R_{10}	H1 CMS 88 X N Record (109) / RHA 09	73	110	171	24	22	54	1136	35	40	340	137	61	**2**	1,2

RECENT MOLECULAR STUDIES ON DOWNY MILDEW DISEASE

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ABSTRACT

Downy mildew is a common sunflower disease and appears as yellow to white patches on the upper surfaces of older leaves. *Plasmopara halstedii*, which is a fungal parasite, causes that disease. Downy mildew has been spread to the whole world from North America and this disease causes more yield lost in temperate climate than subtropical climate. Antifungal chemicals are used for struggling with the disease. As a choice, the resistance hybrid cultivars are also preferred. In some cases these solutions cannot be enough due to development of fungicide or resistant gene resistance pathogens. Thus, molecular studies on downy mildew disease have been conducted. In this presentation, recent development in molecular studies on downy mildew disease will be introduced.

Key words: Downy mildew, sunflower, disease

INTRODUCTION

Sunflower, the forth wildly produced oil crop in the world, right after soybean, rapeseed, cotton seed with 41.3 million tonnes production in 2013/14, is a valuable oil crop (FAO, 2014). The market value of sunflower is provided with its oil content rather than the meal. The oil concentration of the sunflower seeds might range from 260 g/kg to 720 g/kg among the genotypes (Hu et al., 2010). Sunflower oil is exceptional having high unsaturated linoleic fatty acid and low linoleic acid concentration (Dorrell and Vick, 1997).

Downy mildew (*Peronospora halstedii*) disease was spread from North America, where is the gene center of sunflower, to Europe (Anonymous, 1984). The first determination of the disease was in 1922 in North America, later on the disease was spread to Europe in 1966 starting from France (Sakr, 2014). The disease could cause yield loss until 80%, thus struggling with the disease is very important for sunflower breeders (Molinero-Ruiz et al., 2003). The symptom of the disease could be in two ways: systematic and secondary symptoms. The systematic symptoms are usually observed at rooting stage of the seedling and this situation causes killing of the seedlings. Hence, these will be some blank spots in the field, where seedlings are supposed to be. However, if seedlings survive, the systematic symptoms of the disease occur on the cotyledons or first true leaves with thickness and yellow parts. Another symptom is white cottony appearance underside of the leaves, which is caused by fungal mycelium and spores. Besides, the infected plants are dwarfed and have very less amount of seeds when they reach maturity stage. Secondary symptom of the disease is small angular lesions on the upper surface of leaves. Usually, secondary symptoms do not cause systemic symptoms or yield loss (Friskop et al., 2009; Gascuel et. al., 2015).

The management of the disease has been obtained by two methods; fungicide application and production of hybrid plants. Metalaxyl and mefenoxam were used until end of 1990s. However, by the finding resistant *P. halstedii* isolates, azoxystrobin and fenamidone have been started to use for downy mildew disease. Development of resistant *P. halstedii* lines is still possible. Thus, using

fungicide is not the most effective method for management of downy mildew disease. The other method is production of hybrid sunflower lines. It is, therefore, resistance genes, which are called *PI* genes, are used. Until 1980s resistant sunflower lines carried Pl_1 and Pl_2 genes, after 1980s these genes were replaced with Pl_6 and Pl_7 genes (Tourvieille de Labrouhe et al., 2010).

History of *Plasmopara halstedii*

The pathogen was discovered by Farlow in 1882 as *Peronospora halstedii.* After modification of Peronospora genus, the fungus was named *Plasmopara halstedii* in 1888 (Sackston, 1981). Over the years, many fungus, which caused downy mildew disease in sunflowers, were detected and classified as *Plasmopara halstedii* due to their sporangiophores and spoangia morphology (Stevens, 1913). According to Novotelnova's observation, the pathogen in Europe was different form the North America and she renamed the fungus as *Plasmopora helianthi;* however, the observations of Novotelnova were just based of morphological. Thus, this name is not accepted today.

Geographical distribution of *Plasmopara halstedii*

The first downy mildew syndrome in *Helianthus annuus* were detected in 1890s and it became a serious problem for sunflower production in 1920s in North America (Henry and Gilbert, 1924; Young and Morris, 1927). In the middle of the 20th century the disease was spread to the Europe (Novotelnova, 1966). The distribution map of *P. halstedii* is shown in Figure 1. As it seen in the figure, the pathogen has been distributed all over the world.

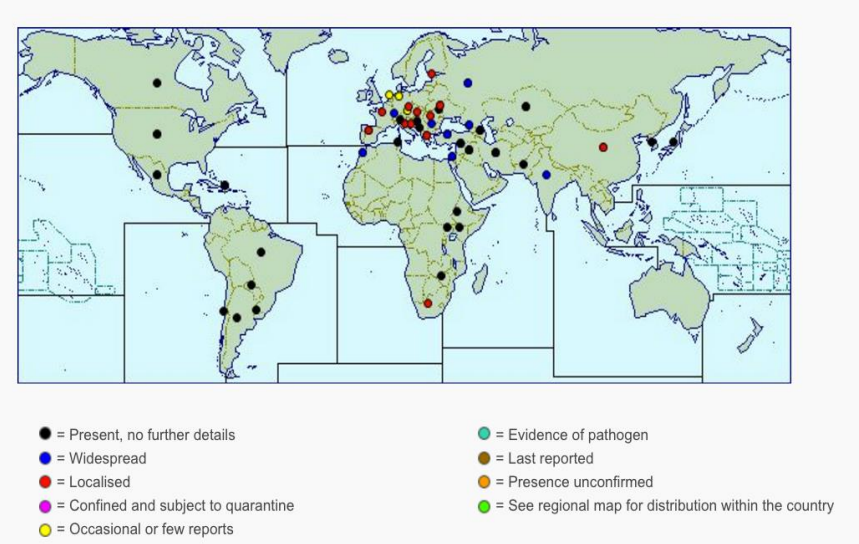

Figure 7: Geographical distribution of *Plasmopara halstedii*

Apart from the figure, the distribution map is given in Table 1 in detailed.

Table 1: Geographical distribution of *Plasmopara halstedii*

Region	**Countries**
EPPO (European and Mediterranean Plant Protection organization)	Albania, Austria, Bulgaria, Czech Republic, Egypt, Estonia, France, Germany, Hungary, Italy, Moldova, Morocco, Poland, Romania, Slovakia, Spain, Switzerland, Turkey, Russia, Ukraine, Yugoslavia
Asia	Azerbaijan, China, Georgia, India, Iran, Iraq, Israel, Japan, Kazakhstan, Pakistan, Russia, Turkey
Africa	Egypt, Ethiopia, Kenya, Morocco, Zimbabwe, Uganda
North America	Canada, USA (California, Kansas, Minnesota, North Dakota, South Dakota)

Region	Countries
Central America and Caribbean	Dominican Republic
South America	Argentina, Brazil, Chile, Uruguay, Paraguay

Evolution of *Plasmopara halstedii* in sunflower cultivated zones

Plasmopara halstedii is native to North America, then it spread to Europe. According to its physiological properties, the organism is spread into two races; race 300 in North America and race 100 in Europe. Later on, race 710 was introduced into sunflower zones in Europe from USA in 1980s (Tourvieille de Labrouhe et al. 2000, Delmotte et. al. 2008, Ahmed et. al. 2012). Recombination between races was occurred and new races were appeared and 14 races in Europe and 35 races in the world have been identified (Delmotte et. al. 2008, Ahmed et. al. 2012, Gulya 2007).

New generation races are able to beaten *Pl* loci, were discovered over the years all over the world as listed in Table 2. Although hybrid sunflowers having Pl_6-Pl_7 gene are grown in all Europe, 304, 307, 314, 334, 704 and 714 races have never been recorded out of France.

Table 2: Virulence of 15 *Plasmopara halstedii* races on sunflower differential lines

Race	Differential lines								
	D1 Ha-304[a]	D2 Rha-265[a]	D3 Rha-274[a]	D4 PMI3[b]	D5 PM-17	D6 803-1[c]	D7 HAR-4[a]	D8 QHP1[b]	D9 Ha-335[a]
100	S	R	R	R	R	R	R	R	R
300	S	S	R	R	R	R	R	R	R
304	S	S	R	R	R	R	R	R	S
307	S	S	R	R	R	R	S	S	S
314	S	S	R	S	R	R	R	R	S
334	S	S	R	S	S	R	R	R	S
700	S	S	S	R	R	R	R	R	R
710	S	S	S	S	R	R	R	R	R
703	S	S	S	R	R	R	S	S	R
704	S	S	S	R	R	R	R	R	S
707	S	S	S	R	R	R	S	S	S
714	S	S	S	S	R	R	R	R	S
717	S	S	S	S	R	R	S	S	S

Race	Differential lines								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
	Ha-304[a]	Rha-265[a]	Rha-274[a]	PMI3[b]	PM-17	803-1[c]	HAR-4[a]	QHP1[b]	Ha-335[a]
730	S	S	S	S	S	R	R	R	R
770	S	S	S	S	S	S	R	R	R

[a] USDA genotypes (USA), [b] INRA genotypes (France), [c] IFVC genotypes (Yugoslavia), * S: susceptible, R: resistant (Tourvieille de Labrouhe et. al. 2000)

Pl gene has vital importance on sunflower production. Unfortunately, the gene has a short life period, which is not enough to obstruct virulence emerge of *P. halstedii* (Sakr 2014).

Apart from morphological observation, the development in biotechnology has given an opportunity for classification of *P. halstedii* in detailed by molecular techniques. First of all, 21 RAPD markers were used to differentiate 77 samples from 12 different countries at low levels in 2003 by Roeckel-Drevet et. al. 2003. In a study, conducted by Spring et. al. (2006), ITS regions were partially sequenced by molecular markers. By this study, the polymorphism between 100, 310 and 330 was detected, along with populations, which are exemplified by 700, 701, 703, 710 and 730. Giress and colleagues (2007) identified high genetic variability between isolates from French and Russia by SNP markers. Ahmet et. al. (2012) defined that recombination facilitated by multiple introductions are reasons for appearing new races of *P. halstedii*.

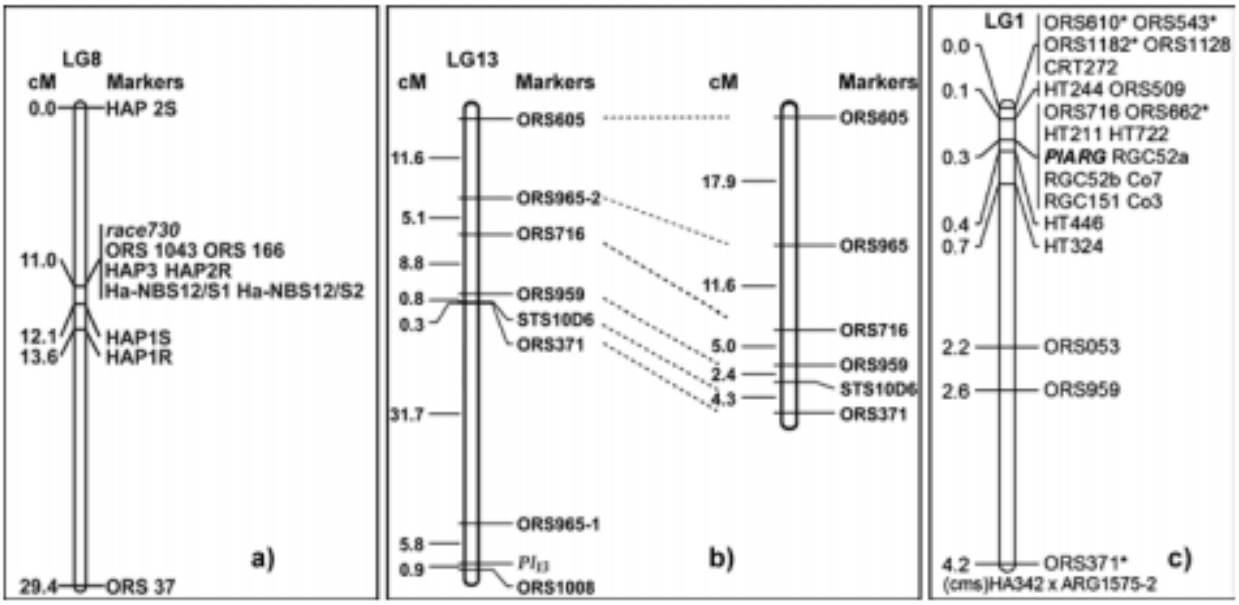

Figure 8: Mapping of *PI* genes (Jocic et. al., 2012)

Genetic background of sunflower (*Helianthus annus* L.)

Cultivated sunflower is diploid crop (2n=2x=34) (Rieseberg and Seiler 1990). On the point of having 14 annual ad 39 perennial, there are 53 wild species of genus *Helianthus* (Moyers and Roisberg 2013, Marek et al. 2014). Wild annual *Helianthus* species are all diploid (2n=2x=34), just

like s cultivated sunflower. On the other hand, perennial varieties could be diploid (2n=2x=34), tetraploid (2n=4x=68) or hexaploid (2n=6x=102) (Qi et. al. 2016).

Introducing new genes to cultivated sunflowers is one of the struggling methods to downy mildew disease. Having gain resistance of pathogens to resistance genes is the reason of the introducing different genes at different times. Inbred lines HA 335, HA336 (*Pl*$_6$), HA 337, HA 338, HA 339 (*Pl*$_7$) and RHA 340 (*Pl*$_8$) have been broadly used all over the world as a defense to downy mildew disease (Miller and Gulya 1988,1991). However, at least eight races , resistant to *Pl*$_6$ and *Pl*$_7$, were identified at France by Gulya et. al. (2011). e. Up to now, 20 downy mildew resistance genes (*Pl*) have been discovered (*Pl*$_1$-*Pl*$_{18}$, *Pl*$_{21}$ and *Pl*$_{Arg}$) (Table 3). Fourteen of these genes (*Pl*$_1$, *Pl*$_2$, *Pl*$_5$-*Pl*$_8$, *Pl*$_{13}$-*Pl*$_{18}$, *Pl*$_{21}$ and *Pl*$_{Arg}$) have been introduced to specific linkage groups (LGs) of cultivated sunflowers (Figure 2) (Mouzeyar et al. 1995; Roeckel-Drevet et al. 1996; Vear et al. 1997; Molinero-Ruiz et al. 2003; Yu et al. 2003, Mulpuri et al. 2009, Romano et al 2010, Bachlava et al. 2011; Liu et al. 2012; Qi et. al. 2015; Qi et. al. 2016).

Table 3: *PI* genes that are used for Downy mildew disease resistance

Name of Gene	Gene source	Inbred line	LG region	Introducing Year	Referance
Pl$_1$	-	RHA265, RHA266	LG8	1970	Kinman
Pl$_2$	-	RHA274	LG8	1975	Fick et. al.
Pl$_5$	-	DM-2	LG13	1984	Miller and Gulya
Pl$_6$	*H. annuus*	HA335, HA336	LG8	1991	Miller and Gulya
Pl$_7$	*H. praecox*	HA337, HA338, HA339	LG8	1991	Miller and Gulya
Pl$_8$	*H. argophyllus*	RHA340	LG13	1991	Miller and Gulya
Pl$_{13}$	*H. annuus*	HA-R5	LG1	2009	Mulpuri
Pl$_{14}$	-	-	LG1	2011	Bachlava et. al.
Pl$_{15}$	RNID	RNID	LG8	2010	Romano et. al.
Pl$_{16}$	*H. annuus*	HA-R4	LG1	1996	Roeckel-Drevet
Pl$_{17}$	*H. annuus*	HA 458	LG4	2015	Qi et. al.
Pl$_{18}$	*H. argophyllus*	HA-DM1	LG2	2016	Qi et. al.
Pl$_{21}$	-	PAZ2	LG13	2012	Vincourt
Pl$_{Arg}$	*H. argophyllus*	Arg1575-2	LG1	1991	Seiler

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MOLECULAR STUDIES OF SUNFLOWER RESPONSES TO ABIOTIC STRESSES

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the third major crop for vegetable oil production worldwide among *Asteraceae* species. Mutant resources or routine protocols to transform genes to sunflower are not available as sunflower genome has not been completed yet. Abiotic stress conditions like drought, extreme temperatures, and high salt causes series of biochemical, physiological and morphological changes. These conditions lead to the production of excess reactive oxygen species (ROS) and osmotic imbalances that limit the productivity and growth of plants. In a scientific literature search, it was found that several genes were characterized in abiotic stress tolerance of sunflower. For instance, sunflower *HaWRKY6* shows functional response in temperature stress, and it is regulated by a miRNA. Sunflower HD-Zip I members *HaHB4*, *HaHB1* and *HaHB11* have critical functions in drought, freezing, and submergence tolerance, respectively. The functions of many sunflower regulatory genes and transcription factors in abiotic stresses are still unclear due to divergent genes encoding for transcription factors. For further studies, outstanding experimental strategies can be applied to overcome difficulties of studying divergent genes encoding for transcription factors in sunflower in abiotic stress tolerance. Understanding of plant responses to abiotic stresses is essential for structural and functional characterization of environmental stress-induced genes. Here we present the current molecular studies of sunflower responses to abiotic stresses.

Keywords: sunflower, abiotic stress, drought, salinity, heat, low temperature

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the significant oil crops in the world and is North America's native crop. It is used in medicine ad as food and is first domesticated by Indians (Kaya, Jocić, & Miladinović, 2012). Sunflower is also used as an ornamental plant and grown commercially. High oil and protein containing commercial sunflower hybrids are used for oil crop breeding (Cvejić, 2016). It is cultivated area is over 22 million ha and annual production of sunflower is over 9 million tonnes in the world (Fernández-Martínez et al., 2009). Sunflower production is mainly concentrated in Ukraine, Russia, Argentina and India (Gulya 2014). According to Turkish Statistical Institute, sunflower production is generated as 2.6% and became 1.7 million tonnes in Turkey (Tuik, 2015). Development of varieties including high oil content became a milestone in oil seed sunflower breeding in the world. Another milestone in the development of sunflower was the discovery of cytoplasmic male sterility. This highly reliable method paved the road to the production of commercial hybrid seeds with inherent advantages (Leclercq, 1969). In early efforts, breeders tried to cope with parasitic weeds (broomrape, *Orabanche cumana*) and insects (Homeosome electellum, sunflower moth) (Fick, 1997) by genetic control. In 1910-1912, Krasnodar by Vasilii Stepanovich Pustovoit started a scientific sunflower breeding program from locally developed varieties. Sunflower is more tolerant to abiotic stresses compared to other field crops because its main organs such as stem, leaves, head and roots have developed specific

structures able to grow under negative conditions or in marginal soils in semiarid zones. To increase the genetic tolerance of cultivated sunflower against abiotic stresses, diversity of the wild *Helianthus* species has been used with good reactions (Škorić, 2009). *H. argophyllus* and *H. paradoxus* showed the best results as wild sunflower species in sunflower breeding against drought and salinity, respectively. Integration of molecular breeding techniques is essential to provide the genetic tolerance mechanisms of wild Helianthus species towards enhancing the abiotic stress tolerance in sunflower breeding program. More progress has been carried out about heat tolerance compared to cold tolerance in sunflower breeding. On the other hand, special breeding programs are needed to be develop in sunflower to deal with mineral toxicities and deficiencies.

Under abiotic stress conditions, transcription factors (TFs) (bZIP, MYC, MYB and DREB), protein kinases and proteases are essential for the regulation of transcriptional changes under adverse environments such as abiotic stress conditions (Pradeep et.al. 2006). Transcription factors are induced by abiotic stress conditions to activate transcription machinery (Figure 1). Cold stress, salinity and drought cause production of reactive oxygen species (ROS) in photosynthesis pathway, limit the availability of CO_2 for the dark reaction and this, in turn, leaves oxygen as the main reductive product of photosynthesis (Mitter, 2002). Abiotic stresses such as drought, salt and cold lead to the accumulation of hydroxyl radicals, hydrogen peroxide, and superoxide in the cells (Hasegawa et.al. 2000). Because of the accumulation of these products along the oxidative stress, many expressed sequence tags (ESTs) from leaf and stem cDNA libraries express catalases, thioredoxins, oxygen-evolving enhancer proteins and peroxidases (Kawasaki et. al., 2001). Due to oxidative stress and accumulation of ROS, most of those proteins are up-regulated in stress conditions (Kawasaki et al., 2001).

In literature, several TFs of *Asteraceae* species are defined as essential in abiotic stress tolerance. During the last five years, characterized *Asteraceae* TFs include sunflower *HaWRKY6* that is regulated by a miRNA in temperature response; chrysanthemum *DgWRKY3* that is involved in salt tolerance; sunflower HD-Zip I members *HaHB4*, *HaHB1* and *HaHB11* that are functional in drought, freezing and submergence tolerances, respectively; chrysanthemum DREB subfamily member of the AP2/ERF family *CgDREBa* and the bHLH member *CdICE1* that are essential in freezing, salt and drought stress tolerance; chrysanthemum MYB TF, *CmMYB2* that is involved in salinity and drought stress; chrysanthemum NAC *DgNAC1* that confers salt tolerance; chrysanthemum zinc finger protein *DgZFP* bringing about salt tolerance (Table 1).

Table 1. *Asteraceae* genes encoding transcription factors under abiotic stress conditions.

Gene name and source	**Function**	**Reference**
Sunflower *HaWRKY6*	High temperature tolerance	Giacomelli et al., 2012
Chrysanthemum *DgWRKY3*	Salt tolerance	Liu et al., 2013
Sunflower *HaHB4*	Drought tolerance	Dezar et al., 2005
Chrysanthemum *CgDREBa*	Freezing, salinity and drought tolerance	Chen et al., 2012
Sunflower *HaHB1*	Freezing and drought tolerance	Cabello et al., 2012
Chrysanthemum *CdICE1*	Freezing, salt and drought tolerance	Song et al., 2014
Chrysanthemum *CmMYB2*	Salinity and drought tolerance	Shan et al., 2012
Chrysanthemum *DgNAC1*	Salinity and drought tolerance	Liu et al., 2011

From drought and salinity stress samples, microsatellites located within ESTs in *H. annuus* are analyzed from populations from arid desert and salty areas. Test statistics of lnRV and lnRH were used to select candidate genes that have a wide variety of functions. 17 significant loci of included genes were analyzed based on BLAST hits with homology search. According to the results, genes were categorized as five transcription factors, three cellular components, four genes with catalytic or metabolic functions, four genes of unknown homology or function and one DNA-repair gene (Kane et al. 2007).

A large quantity of ESTs from *Helianthus* spp. are available in public databases, but they are not studied well (Giacomelli, 2010). Giacomelli et al. (2010) estimated 97 sunflower WRKY members derived from EST databases. They report that *Asteraceae* WRKY family can be the source of specific new functions with a particular diversification. Additionally, they suggest that the sunflower *WRKY*s can be used as markers of tolerance to necrotrophic pathogens because they could have a significant function in biotic stress response. Furthermore, specific features of the sunflower WRKY family are identified. For instance, they suggest that *HaWRKY4* may function in senescence (Giacomelli, 2010).

Flooding is one of the environmental abiotic stress conditions that affect food production negatively (Boyer, 1982). Cabello et al. (2016) studied on sunflower transcription factor *HaHB11*, which is a member of the sunflower homeodomain-leucine zipper I subfamily of transcription factors. According to their results, overexpression of HaHB11 in transgenic Arabidopsis plants led to larger rosettes, wider stems and significantly increased biomass compared to wild type plants. Transgenic Arabidopsis plants expressing *HaHB11* showed enhanced tolerance to flooding stress. Additionally, transgenic plants produced twice the amount of seeds that the wild type plants produced (Cabello, 2016).

DROUGHT TOLERANCE IN SUNFLOWER BREEDING

Quartacci and Navari-Izzo (1992) indicated that sunflower seedlings exposed to water deficiency accumulated lower levels of soluble proteins, chlorophyll, and total and polar lipids compared to control plants. Under water stress, root growth extension is observed into moist soil regions. To escape from desiccation tolerance, there are available mechanisms, pathways and reactions, including the accumulation of intracellular proteins such as late embryogenesis-abundant (LEA) proteins. They stabilize other proteins and membranes against drying. Dehydrins are among drought stress induced proteins in D-11 subgroup of LEA family (Giordani, 1999).

Mayrose et al. (2011) analyzed protein phosphatase 2C and the HD-Zip transcription factor ATHB8 under drought stress conditions. Protein phosphatase 2C gene is from a group of serine /threonine protein phosphatases. These proteins are negative regulators in plant responses under abiotic stress conditions such as drought (Schroeder et al. 2001; Tahtiharju&Palva, 2001). HD-Zip transcription factor ATHB8 induces developmental reactions to the environmental conditions. *ATHB8* expression decreased under drought stress such that 1.3 fold repression in native plants, 2.6 fold repression in weeds were observed, while the highest repression was found in crops as 3.2 (Mayrose et al., 2011). Interestingly, they showed that no control plant has the expression of the *ATHB8* gene. Additionally, *ATHB8* transcription factor is available in reduced growth and weedy plants under drought conditions.

Members of the sunflower (or other *Asteraceae* species) WRKY family are not clear completely so far. HaWRKY76 is a sunflower transcription factor whose biological role is not found yet because the WRKY family is highly diversified in the *Asteraceae* (Giacomelli et al. 2010). Raineri et al. (2015) indicated that HaWRKY76 is a divergent sunflower WRKY transcription factor. It enhances the dehydration and submergence tolerance in Arabidopsis when expressed in transgenic plants. It is suggested that HaWRKY76 could be potential tool to make drought tolerant plants (Raineri, 2015).

SALINITY TOLERANCE IN SUNFLOWER BREEDING

Mineral salt accumulation in global arable lands leads to abiotic stresses. After moisture stress, salinity is in the second rank in causing agricultural problems. Accumulation of excess amount of soluble salts, mineral toxicity or deficiency may cause this stress (Singh, 2006). Salinity stress limits plant growth and productivity (Khan et al., 2014). Khan&Asim (1998) evaluated that limited cell division resulted from salt stress causes cell volume reduction. Salt stress negatively affects biochemical and physiological changes, placement of solute dissolved proteins, nutrient uptake, ion-uptake and carbon assimilation (Schroeder et al., 2013; Naz&Bano, 2015). Selectivity of root membranes is impaired by excess amount of Na^+ and Cl^- that are predominant ions causing high ionic imbalances (Bohra&Dörffling, 1993). To examine the comparative differences of salinity effect, different physiological characters such as compartmentation of Na^+ and Cl^- ions, osmotic adjustment, selectivity for K^+ ahould be taken into consideration regarding to the salt tolerance in crops (Wyn Jones&Storey, 1981). Ahmed et al. (2005) explained that sunflower cultivars grown in saline environment show crucial reductions in height, leaf area and stem girth. These growth limitations cause oil percentage reductions. In salinity conditions, plant cell turgor pressure is reduced and then this causes stomatal closure, which limits carbon fixation and photo-assimilation rate (Gale & Zeroni 1984).

Fernandez et.al (2008) studied eighty genes isolated from organ-specific cDNA libraries under salinity (NaCl) and low temperature conditions. They looked at microarray profiling of chilling and NaCl-treated sunflower leaves, and indicated significant changes in transcription factors, defense/stress related proteins, transcript abundance and effectors of homeostasis under both stresses. They categorized results of differentially expressed genes according to their functions (Table 2). In Table 2, down-regulated and up-regulated number of genes in categorized metabolism are given under salinity stress.

Table 2. Number of genes involved in different functional categories (Fernandez, 2008).

	Cold			**Salinity**		
Functionally classified proteins	**NC**	**Up**	**Down**	**NC**	**Up**	**Down**
Central metabolism/Photosynthesis	1	2	7	2	2	6
Translation machinery	2	3	1	1	3	2
Transcriptional machinery	2	2	-	2	2	-
Signaling machinery	-	1	1	-	1	1
Protein turnover/folding/interactions		3	2	2	1	2
Transport	-	3	-	-	2	1
Secondary metabolism	1	-	2	-	1	2
ROS machinery	-	5	2	2	3	2
Total	6	19	15	9	15	16

NC: No change.

First genetic map of sunflower was constructed by the help of quantitative traits controlling physiological characters regarding to the oil yield and the adaptive responses of sunflower to abiotic stresses (Tang, 2003). This type of genomics-based approach allows the development of low-cost

procedures that will be used further by researchers in breeding programs whose goals are enhancing sustainability and yield stability under abiotic stress conditions.

Fernandez et al. (2008) analyzed that EST T411, similar to a plastidic aldolase is up-regulated under salinity stress. Plastidic aldolase genes are indicated in *Nicotiana* plants and are grouped as AldP1 and AldP2. Yamada et.al. (2000) firstly reported that AldP2 was up-regulated under salt stress while AldP1 was suppressed in salt stress conditions. EST H136 (similar to a chloroplast drought-induced stress protein) is down-regulated under chilling and salinity stresses (Fernandez et al., 2008). CDSP (CHLOROPLAST DROUGHT-INDUCED STRESS PROTEIN) is a type of thioredoxins, which play role in oxidative stress (Broin et al., 2000)

It is found that salinity induces transcription of the *MIPS* (*MYO-INOSITOL-1-PHOSPHATE SYNTHASE*) during biosynthesis pathway of myo-inostol and its derivatives (Nelson, 1998; 1999). Myo-inositol-1l-phosphate synthase (MIPS) is functional in *de novo* inositol biosynthesis pathway (Loweus and Loweus, 1983). In *M. crystallinum*, salinity stress induces higher expression of *MIPS* mRNA as 5-folds, resulting in free inositol accumulation of 10-folds (Ishitani et al., 1996).

Understanding of genetic mechanism to salty environment will improve plant responses to changing conditions and develop insights to long-standing questions. Edelist et al. (2009) reported constitutively under- or over-expressed genes regarding to potassium and calcium transport (homologues of *KT1, KT2, ECA1*) in hybrid species of *H. paradoxus.* They found that salinity treatment induced over-expression of homologues of the potassium transporter *HAK8* and its transcriptional regulator.

In sunflower, a small family of three genes (*HAS1*, *HAS1.1* and *HAS2*) encodes asparagine synthetase (AS; EC 6.3.5.4) (Herrera-Rodríguez et al., 2007). They are regulated differentially by nitrogen, carbon and light availability. Gene specific probes are used in Northern analysis under osmotic stress, heavy metal stress and salt stress. They reported that stress treatments did not induce any changes in the expression of *HAS2*. Osmotic and salt stresses decreased the expression of *HAS1* and *HAS1.1* genes in light conditions (Herrera-Rodríguez et al., 2007).

SALT OVERLY SENSITIVE2 (SOS2) and PLASMA MEMBRANE PROTEIN3-1 (PMP3-1) are functional in homeostasis. They were analyzed in two salinity-contrasting sunflower lines, Hysun-38 (salt tolerant) and S-278 (moderately salt tolerant) (Saadia, 2013). In sunflower root tissues from both tolerant and moderately tolerant lines, *SOS2* expression showed gradual increase under salt stress. A gradual increase of *SOS2* expression was observed in leaf tissues of tolerant variety compared to moderately tolerant one. They observed highest level of *PMP3-1* expression in the roots of tolerant sunflower line in the post-salinity level (6 and 12 h of stress treatment). Higher expression of *PMP3-1* was observed in moderately tolerant line at 12 and 24 h of salt treatment (Saadia, 2013).

NAC family transcription factors in plants are functional in abiotic stress responses (Jeong, 2010). In tolerance to abiotic stresses, only a few stress-responsive NAC proteins are characterized (Nakashima, 2011). Manjunath et al. (2013) developed a simple and effective screening methodology to identify transformants under salt tolerance. They created leaf discs of *EcNAC1* gene transformants. They analyzed *EcNAC1* gene with *HPT II* specific primers and *Sac I* restriction enzyme is used to digest the amplified *EcNAC1* gene product. They suggest that initial identification of promising transformants result from *in vitro* screening strategy at plant level based on the target gene (Manjunath, 2013).

HEME OXYGENASE1 (HO1) is functional in protecting mechanisms against environmental stress responses (Zhu, 2014). It is a stress responsive antioxidant enzyme that cleaves heme to biliverdin IXα (BV). BV functions in concominant release of carbon monoxide (CO) and production of free iron (Fe^{2+}) (Shekhawat 2010). Zhu et al. (2014) cloned sunflower *HaHO1* gene, which is required for sunflower salinity acclimation. They showed the induction of *HaHO1* was closely associated with the sunflower salinity acclimation.

HEAT TOLERANCE IN SUNFLOWER BREEDING

Heat stress is defined as high temperature lasting in enough duration that cause important yield reduction compared to control plants (Singh, 2004). Emissions of heat stress in environment resulting from automobiles, industry and urbanization cause temperature increase that endangers diversity of fauna and flora (Singh et al. 2006). High temperature causes heat stress in plants that affects physiological, morphological and physiological traits negatively (Table 3).

High temperatures may cause stomatal closure, a rise in respiration rate, leaf, or canopy temperature, cell membrane injuries, disruption of the photosynthetic apparatus, and the induction of stress-specific growth regulators, which shorten the total growth period due to changes in crop phenology, biomass, fruiting sites, gamete sterility, seed fruit, seed size, and seed quality (Moriondo & Bindi 2006; Moriondo et al. 2011).

In the growing environment, plants are more vulnerable to heat stress in their flowering stages. Under such conditions, large quantities of pollens are selected by breeders. To obtain the best pollination and seed formation, it is necessary to maintain pistil, stigma or disk flowers that are tolerant to high temperature (Škorić, 2012).

Table 3. Effects of heat stress on sunflower.

Traits	**Effects**	**LITERATURE**
Leaf growth period (d)	Decreased by 1.04 days per °C above 36°C	Rawson and Hindmarsh (1982)
Grain weight /yield (g)	Grain weight was reduced up to 21% and final grain yield reduced by 10% at 38°C	Ploschuk and Hall (1995)
Grain-filling duration (d)	Reduced by 2–6 days at 38°C	Ploschuk and Hall (1995)
Grain weight (g)	40% decrease when temperature is >35°C during early grain development	Rondanini et al. (2003, 2006)
Respiration rate (mmol m^{-2} s^{-1})	Increased 19% when night temperature 5°C higher than control	Manunta and Kirkham (1996)
Oleic acid (%)	Increased oleic acid production at the expense of linoleic acid	Harris et al. (1978); Fernández-Moya et al. (2002)
Leaf temperature	1–2°C higher than ambient air temperature (42°C) in susceptible lines	Kalyar et al. (Forthcoming 2013)
Heat stress injury (%)	Decreased 10–65% in sunflower germplasm with variable resistance evaluated at 40°C	Kalyar (2013)

WRKY transcription factors are functional in plant stress responses. The sunflower *HaWRKY6* contain a target site for the binding of miR396. Giacomelli et al. (2012) analyzed the possible post-transcriptional regulation of *HaWRKY6* by miR396 in the *Asteraceae*. They found that the silencing of *HaWRKY6* due to miR396 accumulation is responsible for high-temperature protection in sunflower (Giacomelli, 2012).

LOW TEMPERATURE TOLERANCE IN SUNFLOWER BREEDING

Low temperature limits crop productivity in many environments. When the temperature is above freezing level (> 0 °C), it is called as chilling; if it is below 0 °C, it is called as freezing. Kalaydzhyan et al. (2009) developed sunflower genotypes that are tolerant to cold after mutagenizing the plants by dimethyl sulfate (DMS) as chemical mutagen. 44.000 seeds of about 2.000 mutagenic progenies were screened under low temperatures by planting them in early and late winter. 499 plants from 72 mutagenic progenies were able to grow under harsh winter and low temperature conditions (down to −20°C).

HaF455 involved in ribosomal activity is induced by cold and salinity stresses in sunflower (Fernandez et al. 2008). Fernandez et.al. (2008) showed that the expression of EST H123 [GenBank: BU672069] having high identity with myo-inositol phosphate synthase (MIPS protein, isomerase involved in inositol metabolism) was decreased by chilling and salinity stresses.

CONCLUSION

There are many reports on molecular mechanism of sunflower abiotic stress tolerance. However, molecular attempts to sunflower abiotic stress tolerance have not been enough as compared to the molecular studies performed with other crops. Especially, the use of molecular techniques such as QTL identification and associating mapping will enable a faster and more efficient breeding program in sunflower abiotic stress tolerance. For further studies, the application of different molecular methods such as transcriptomics will help development of new sunflower cultivars that are more tolerant to abiotic stress conditions. In further studies, array based cDNAs will contribute to the understanding of functions of more sets of genes functioning in sunflower abiotic stress tolerance.

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MOLECULAR STUDIES INVOLVED IN SUNFLOWER RESPONSES IN DROUGHT STRESS

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ABSTRACT

Sunflower is a major oil seed crop worldwide that shows adaptations to diverse environmental conditions, such as water and salt stresses. It is adapted to grow in semi-arid conditions. To provide expansion of cultivated area, it is crucial to determine and create drought tolerant sunflower genotypes. There are diverse physiological and biochemical responses against water stress by up- or down-regulating stress tolerance genes and other mechanisms, including hormones and secondary metabolite accumulations. For instance, an increase in the expression of sunflower aquaporin gene *HaTIP7* was observed in guard cells and root phloem under drought conditions. Overexpression of a drought-responsive dehydrin gene, *HaDHN1*, makes the sunflower line tolerant to water-limited conditions. For further studies, outstanding transcriptomic strategies can be applied to examine the genes encoding for transcription factors in sunflower in drought tolerance. Here, we present recent molecular studies associated with drought stress tolerance in sunflower genotypes.

Keywords: sunflower, drought stress, molecular studies, tolerance

INTRODUCTION

Sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.) oil is the fourth most significant vegetable oil after palm, canola and soy in the world. Planted all over the world, sunflower is used for a wide range of purposes including oil production from oil seed sunflower, consumption of seed directly from confectionary sunflower and cosmetics production from ornamental sunflower. Sunflower production is mainly concentrated in Ukraine, Russia, Argentina and India (Gulya 2014). According to Turkish Statistical Institute data, sunflower production is increased 2.6% and became 1.7 million tonnes in Turkey (Tuik, 2015). It is cultivated in over 22 million ha land, and annual production of sunflower is over 9 million tonnes in the world (Fernández-Martínez et. al., 2009). Geographical, archeological, morphological and molecular evidences suggest that sunflower was first domesticated in eastern North America (Rieseberg, 2001). Sunflower breeding programs date back to early 20th century. In 1910-1912, Krasnodar by Vasilii Stepanovich Pustovoit started a scientific sunflower breeding from locally developed varieties. In first efforts, breeders tried to cope with parasitic weeds (broomrape, *Orabanche cumana*) and insects (*Homeosome electellum*, sunflower moth) (Fick, 1997) by genetic controlling. Development of varieties including high oil content became a milestone in oil seed sunflower breeding in the world. Another milestone in the development of sunflower was the discovery of cytoplasmic male sterility. This highly reliable method paved the road to the production of commercial hybrid seeds with inherent advantages (Leclercq, 1969). In recent years, sunflower breeding programs have started to concentrate on the development of varieties tolerant to environmental stresses.

Drought stress is the most significant abiotic stress factor that affects the plant production because around one third of the soils are affected by drought worldwide. All plant organs show reactions to drought stress with morphological, physiological, and metabolic alterations. To

minimize yield reduction due to drought stress, there are various mechanisms that are categorized in three groups of (1) dehydration avoidance, (2) drought escape, and (3) dehydration tolerance (Singh, 2000). In the slowly dehydrated attached leaves of drought-stressed plants, dehydration avoidance by osmotic adjustment is improved (Levitt, 1985). The activation of drought escape mechanism in plants is a significant process in early maturation that provides suitable environment for late-season drought stress conditions (Singh, 2000). Water uptake and consumption is minimized by soil drought. In order to withstand drought conditions, plants decrease the transpiration rate by stomatal closure, which in turn leads to overheating in the leaves due to local high temperature. Because of water loss, photosynthesis rate also decreases gradually (Žolkevič, 1968). Increasing the water retention capacity is a protection mechanism in plants to escape from water loss. Reduction in transpiration rate and enhancement of water uptake from soil are together referred to as water or turgor potential under drought conditions. In drought stress, dehydration tolerance increases with leaf number. (Levitt, 1985)

Several morphological and physiological parameters are used to examine the drought tolerance levels in sunflower (Škorić, 2009). They include leaf water potential, yield stability, root xylem diameter, root growth, stomatal conductance, osmotic adjustment, canopy temperature, abscisic acid (ABA) accumulation, seedling recovery after stress, growth under stress, proline accumulation and leaf rolling. These parameters were used to evaluate the differences in drought tolerance levels among sunflower varieties in attempts to develop drought tolerant sunflower genotypes. In an early attempt, Škorić (1992) evaluated over 30 different parameters to study the drought tolerance in sunflowers. In another attempt, Petrović et al. (1992) evaluated the free-proline accumulation rate and nitrate reductase activity under conditions of water stress, and found large amounts of differences between sunflower varieties. Therefore, proline accumulation and nitrate reductase activity levels were proposed to be used as indicators for the estimation of drought stress in sunflower varieties. Unlike other plant species such as Arabidopsis, wheat, rice and soybean, water stress tolerance mechanism is still not completely comprehended in molecular basis in sunflower even though most of drought-related genes are cleared in other plant species (Cellier et al., 1998).

MOLECULAR STUDIES OF DROUGHT TOLERANCE IN SUNFLOWER

Morphological, physiological, and metabolic modifications respond to drought stress in whole plants. At the cellular level, water deficit causes cell damage in all plant organs. In adaptive processes, other responses occur (Ingram and Bartels, 1996). In sunflower, there are several management mechanism to coper with drought stress. Water deficit reduces root proliferation, leaf size and stem extension by disturbing water relations. A variety of physiological and biochemical responses are controlled at cellular and whole-organism levels start to manage drought stress (Farood, 2009). CO_2 assimilation in the leaves is reduced by membrane damage, stomatal closure and disturbed activity of various enzymes that function in adenosine triphosphate synthesis and CO_2 fixation. Oxidative load generating reactive oxygen species (ROS) is increased by enhanced metabolite flux through the photorespiratory pathway. ROS cause injury to biological macromolecules under drought stress (Farood, 2009). This is among the major obstacles for growth. Plants display some mechanisms to cope with the drought stress. The major mechanisms that curtail the effects of water loss include the diffusive resistance, enhanced water uptake by deep and prolific root systems, and succulent and smaller leaves to limit the transpirational loss. Moreover, potassium ions provide osmotic adjustment while silicon develops root endodermal silicification and enhances the cell water balance. To sustain cellular functions under drought conditions, low-molecular weight osmolytes including proline, glycine-betaine and other amino acids, polyols and organic acids have significant emerging roles. Plant growth substances such as auxins, gibberrellins, salicylic acid, ABA and cytokinins regulate the plant responses toward drought stress. To reduce the adverse effects of drought stress, citrulline polyamines and several enzymes act as antioxidants (Farood, 2009).

In breeding programs, development of tolerant cultivars and selection of drought tolerance is achieved by the help of physiological trait improvements or genetic modifications. More recently, molecular markers were effectively applied to select for enhanced drought tolerance among sunflower varieties (Škorić, 2009). At molecular level, several drought responsive genes and transcription factors have been characterized in sunflower. However, the studies to identify drought responsive genes, such as the genes encoding for late embryogenesis abundant (LEA) proteins, dehydration-responsive element-binding proteins and aquaporins in sunflower have been limited under drought stress conditions. Although a lot of physiological studies have been carried out in sunflower, molecular studies and genetic modifications over drought stress tolerance are limited.

Expressions of many genes are up- and down-regulated by water deficit (Table 1). Liu et al., (2003) analyzed the structural and functional characterization of environmental stress-induced genes under drought and salinity stresses in sunflower. Differential display was used to compare overall differences in gene expressions between drought- or salinity-stressed and unstressed (control) plants of sunflower. Guanylate kinase (signal transduction), *lytB* (antibiotic/drug resistance), selenium-binding protein (heavy metal stress), polyprotein (reverse transcriptase), and AC-like transposable element were identified from sequence analysis of used clones under drought and salinity stress conditions. To regulate water fluxes in plants, aquaporins are one of the major functional transporters in plants. Under water deficit conditions, sunflower aquaporin gene *HaTIP7* accumulated in the roots inducing stomatal closure (Aguado, 2014). In drought and exogenous ABA conditions, sunflower *HaABRC5* of ABI5-Interacting Proteins (AFP family) was up-regulated in roots, seedling shoots and leaves (Liu et al. 2004). This gene is predicted to be an ABA-responsive nuclear protein playing a role in plant stress responses in sunflower. In drought conditions, hydrophilin and LEA proteins are essential as soluble proteins to provide maintenance of cellular integrity. Drought induced transcripts of *HaELIP1*, *HaDHN1*, and *HaDHN2* accumulated in leaves of tolerant sunflower variety under progressive drought (Cellier, 1998). Water stress induced *HaELIP1* gene expression and accumulation of dehydrins in sunflower leaves (Ouvrard et al. 1996). Dehydrins are from D-11 subgroup of LEA proteins that are functional as water stress-induced proteins. Dehydrins accumulate in desiccation tolerant seed embryos during water stress conditions (Close, 1997). Actually, the function of dehydrins in drought stress tolerance mechanism is not clear, but their accumulation in drought stress was shown to be increased in previous studies (Giordani et al., 1999; Hundertmark and Hincha, 2008). Interestingly, the expression of the *HaDHN1A* dehydrin gene was under the control of two possible mechanisms of ABA-dependent or ABA-independent pathways. The expression level of *HaDHN1A* transcript was lower in ABA-deficient sunflower mutant compared to ABA-sufficient non-mutant under water limited conditions, suggesting the involvement of ABA-dependent tolerance mechanism in sunflower responses under drought stress (Giordani, 1999). In ethylene synthesis, ACCO (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE) is a main regulatory enzyme. *HaACCO2* transcript expression increased in sunflower leaves (Liu et al. 1997). Drought and exogenous ABA application induced the expression of this gene as well (Ouvrard et al. 1996).

Gago et.al. (2002) reported that the transcript abundance of a homeodomain-leucine zipper protein, *Hahb-4*, *was* controlled by water stress conditions in sunflower whole seedlings, roots, stems and leaves. Additionally, ABA was proved to function as a component in signal transduction pathway that regulates *Hahb-4* expression under water stress conditions. ABA is involved in various regulations in plant physiological and developmental stages, and in pathways to cope with drought and salinity stresses (Skriver & Mundy 1990; Leung & Giraudat 1998). The functional analysis of the promoter sequences of genes involved in sunflower drought tolerance identified *ABRE* (ABA responsive elements) consensus sequences indicating the involvement of ABA signaling in drought tolerance in sunflower.

Table 1. Genes involved in sunflower drought tolerance.

Genes	Results	Reference
HaELIP1, HaDHN1,* and *HaDHN2	drought-induced genes	Ouvrard et al. 1996
HaTIP7	transcript accumulation by water deficits	Sarda et.al. 1997
HaACCO2	induced by drought and exogenous ABA application	Ouvrard et al. 1996
ABI5-Interacting Proteins (AFPs)	involved in ABA response	Garcia et al. 2008
LTP genes	induced by water deficit and ABA application	Colmenero-Flores et al, 1997

Water deficit stress and ABA application induce *LTP* genes encoding for Lipid Transfer Proteins. These proteins are functional as epidermal cell wall proteins that are essential for the secretion of extracellular lipophilic substances (Martin and Brewbaker, 1971). Ouvrard et. al. (1996) showed that *HaLTP* gene expression increased in ABA treatment and drought stress conditions in sunflower. Mitotic activity and DNA synthesis activity is constrained by drought stress and ABA treatment (Robertson et al., 1990). Liu et.al (2003) showed that *HaRPS28* expression in different organs of sunflower decreased under drought and salinity stresses. Additionally, they realized that *ABRE* repeats in 3' UTR of *HaRPS28* mRNA are expressed in low levels, suggesting a new comprehension and study about ARE-mediated decay pathway under drought stress conditions in sunflower (Liu, 2003).

Giordani et al. (2011) analyzed 8 genes, namely *NAC1*, *DREB*, *ABA-C5*, *ABP1*, *DHN*, *HSP*, *LTP* and *DES*, which are functional in drought responses in eight sunflower inbred lines with phenotypic characters. Gene expression analyses proved that these genes are putatively essential in drought stress responses. *NAC1* gene belongs to the NAC family of transcription factors functioning in morphogenesis and stress responses (Ooka et al. 2003). Drought-responsive-element-binding (DREB) protein encoding genes are transcription factors, which bind DRE cis-elements of drought-responsive genes (Shinozaki and Yamaguchi-Shinozaki 2007). ABA-responsive-C5 (ABAC5) encoding gene was involved in ABA-mediated drought response, and there are two copies in the sunflower genome (Liu and Baird 2004). A sunflower specific gene named *AUXIN-BINDING PROTEIN* (*ABP1*) was shown to be involved in the auxin transport within the cell and was predicted to be the auxin receptor (David et al. 2007). Induction of *ABP1* under drought stress in sunflower suggests the involvement of auxin signaling in drought tolerance mechanism. In addition to these genes, genes encoding for heat shock proteins (HSP) and desaturase enzyme are also induced under drought conditions in sunflower. The nucleotide diversity values of four genes (*NAC*, *ABA-C5*, *DREB*, *ABP1*) were shown to be lower than the other four, although they were highly functional genes encoding for proteins involved in the regulation of transcription or signaling cascades under stress conditions (Giordani, 2011).

Drought tolerance of sunflower has not been studied in details although different plant characters have been analyzed and numerous attempts have been carried out to understand the mechanisms involved in drought tolerance in sunflower. Wild sunflower species provide high level of drought tolerance by controlling various sets of genes to create new drought tolerant sunflower lines (Škorić, 2009). Appropriate screening techniques, controlling genetic backgrounds and analyzing physiological mechanisms of drought tolerance can be developed by the help of selection methods and breeding programs (Škorić, 2009).

Molecular Markers in Sunflower Breeding Against Drought

In crop breeding programs, molecular markers play a crucial role in detection of characters. Molecular marker tools can be applied successfully to oil seed crops such as sunflower, soybean and groundnut to control the seed quality or other characters affected by abiotic and biotic stresses (Sujatha, 2009). Ali et al., (2009) analyzed physico-chemical attributes of sunflower seeds under drought stress at different growth stages, i.e. vegetative and reproductive stages. From results of their comprehensive studies, it was concluded that drought affected some constituents of sunflower seed oil in different cultivars. Distinctive parameters such as fatty acid composition, oil yield, iodine value and oil tocophenol content are significant factors that are the most vulnerable to water deficit (Ali et al., 2009). Studies conducted in unfavorable conditions, especially drought showed that it affects the seed composition and seed numbers (Nel, 2001; Anwar et al., 2006). In quantitative trait loci (QTL) identification studies, several physiological traits were associated with genomic locations (Hervé 2001). Hervé et.al. (2001) analyzed traits related to photosynthesis including internal CO_2 concentration, net photosynthesis rate, leaf chlorophyll content, and water status traits including transpiration, stomatal conductance, relative water potential and leaf water potential in recombinant inbred sunflower lines. Analyzed traits showed a correlation between water potential and transpiration, and between transpiration and photosynthesis rates. This study was the first one about the identification of genetic characters involved in water status and photosynthesis in sunflower under drought conditions. Genetic markers associated with these physiological characters were also identified in sunflower inbreed lines, and their utilization in future breeding programs was evaluated.

Haddadi et.al. (2011) detected genomic regions associated with leaf related traits and yield components in recombinant inbred sunflower lines under water stress. This study is a suggestive work for the development of future marker-based approaches in sunflower. This study can develop improved understanding of positional cloning of related genes in development, and improvement of near-isogenic lines in sunflower varieties. Kiani et al. (2007) detected QTLs related to water status and osmotic adjustment in sunflower with two water treatments in greenhouse conditions.

In a recent study, Abdi et.al. (2013) compared the relative water content and chlorophyll concentration in 70 recombinant sunflower inbred lines under drought and control conditions. By using 210 simple sequence repeats (SSRs), 11 genes were placed in 17 linkage groups. A total of 10 and 8 QTLs were identified for chlorophyll levels and relative water content, respectively. Utilization of SSR markers to develop an association mapping gives a greater precision in decoding the genetic map. Identification of genomic regions related to drought tolerance phenotypes will develop a future understanding of marker-based approaches in drought stress conditions in sunflower. These molecular studies give new insights to the development of drought tolerant sunflower varieties in molecular ways.

CONCLUSION

Although a large number of drought-induced genes have been characterized in other plant species, molecular basis of sunflower tolerance to water deficit has not been completely understood. There are many reports on molecular mechanism of sunflower drought stress tolerance. Sunflower drought tolerance can be managed by the use of molecular techniques such as marker-assisted selection, QTL identification and associating mapping. At the molecular level, several drought responsive genes and transcription factors have been characterized in sunflower drought stress tolerance. However, the application of different molecular methods such as transcriptomics will help the development of new sunflower cultivars that are more tolerant to drought stress conditions. Applications such as mass-screening and breeding, exogenous application of hormones and osmoprotectants to growing plants are also ongoing studies in sunflower. Additionally, array based

cDNAs will contribute to the understanding of functions of more sets of genes functioning in sunflower drought stress tolerance.

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DETERMINATION THE GENETIC CHARACTERIZATION OF DIFFERENT LINES OF SUNFLOWER (HELIANTHUS ANNUUS L.)BY USING GENETIC RESOURCES BASED ON SSRS (SIMPLE SEQUENCE REPEAT)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the most important sources of oil crops in the world and Turkey. The oil of sunflower seed is known as high qualified oil due to having unsaturated acid like oleic and linoleic acid. Using conventional plant breeding methods for determine a high oleic acid sunflower lines are laborious and time consuming but the process of new breeding system is now being accelerated and carried out with more precision and fast-track manner than the classical breeding techniques: also utilization of DNA markers especially by SSR markers has many advantages for example recently, in many plant species marker system has successfully undertaken for identification the genetic resources of different plant species: it also has an important place in sunflower.These markers are used to measure the differences in DNA level. İn Turkey it can be found infrequent researches regarding DNA identification, therefore in this research it was decided to use SSR molecular markers method to provide more genetic levels information about sunflower for further studies. In this research, 10 SSR primers and 41 oleic sunflower lines were used as a material in order to estimation of genetic diversity among high oleic sunflower lines. Sunflower lines were obtained from Trakya Agricultural Research Institute, Turkey. As a result maximum genetic similarity among 41 high oleic asid lines was obtained betweenG3 K6 ASN and K7 RSN 7/13; YDAH SN 2/13 and K7 RSN 7/13; YDAH SN 3/13 and K7 RSN 7/13.A minimum genetic similarity was observed between (K7 RSN 7/13) and (YDAH SN 1 /13). It was identified a total number of 79 alleles. t he number of alleles per locus ranged from 2 (ORS598) to 12 (ha4136). Based on physiological analysis among sunflower lines G3K6 7/13 ASN, ASN G3K8 YDAH SN 3/13 and 1/13, ASN G5K8 YDAH SN 5/13 and 4/13 YDAH SN 2/13 and G3K6 7/13 ASN was performed the best.

Key words: Sunflower (*Helianthus annuus* L.), DNA, SSR**,** oleic acid

INTRODUCTION

Sunflower is one of the most important oil crops in the world due to higher adaptation capability. (Kaya et al., 2012, Skoric, 2012; Kaya, 2014b). The sunflower oil quality is determined by the saturated and unsaturated fatty acid ratio. The sunflower oil is a high qualitative one, due to very high percentage of poly-unsaturated fatty acids which can reach 90% from the total (Kinman and Earle, 1964; Vrânceanu, 1974, 2000; Skoric, 1989; Schuster, 1993). Among unsaturated fatty acids, the linoleic one is dominant in classical sunflower. There is an important genetic variation regarding the fatty acid composition of the sunflower oil (Cummins et al., 1967; Simpson and George, 1985).
Using conventional plant breeding methods for determine a high oleic acid sunflower lines are laborious and time consuming but the process of new breeding system is now being accelerated and carried out with more precision and fast-track manner than the classical breeding techniques: using of DNA technology in agricultural research has progressed rapidly over the last two decades. The

procedure of DNA extraction should also be quick, simple and cheap. Molecular markers are powerful tools to study genetic variation and relate them to phenotypic variation (Varshney et al., 2005). SSRs (Simple Sequence Repeats) show high reproducibility and genomic covering, co-dominance, neutrality and they are highly polymorphic (Spooner et al., 2005). In sunflower (Helianthus annuus L.), microsatellites and SSR method have been particularly useful in the studies of phylogenic relationships, genotype identification and calculation of genetic relationship between inbred lines(Tang et al. 2002; Poormohammad Kiani et al 2007; Darvishzadeh 2012; Grandon et. al, 2012; Singchai, et. al., 2013). SSR is a powerful technique for assessment of genetic diversity at molecular level. This method aleso (SSR) takes less time, reliable and gives good quality of DNA even without using RNas.

MATERYAL AND METHOD

This research was conducted in Laboratory of The Institute of Biotechnology at Ankara University. In this research, 41 sunflower lines (19 female and 22 male) i.e. G3K6, G5K8, YDAH, K6, K7 were used as a plant material. The seeds were obtained from National Sunflower Breeding Project conducted by Trakya Agricultural Research Institute.

DNA Extraction:

Leaves were harvested from sunflower lines in the field conditions, freeze-dried and ground to powder. DNA extraction was performed according to the cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). The extracted DNA content was measured using DNA standards in agarose gel (0.8 % w/v).

The PCR reaction contained 20 ng DNA, 1X reaction buffer, 1.5 mM Mgcl2, 0.2 mM of each of dNTP, 0.5 uM of each forward and reverse primer, 0.3 IU Taq DNA polymerase. DNA amplification was performed in a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems Inc., Foster city, CA) with 10 uL reaction volume. DNA samples were denatured initially at 94 °C for 3 min, then subjected to the following 20 cycles: 94 °C for 30 s, 63 °C for 30 s with a decrement of 0.5 °C per cycle, and 70 °C for 1 min. This was followed by another 20 cycles of 94 °C for 15 s, 55 °C for 30 s, and 70°C for 1 min. A 10 min extension was performed at 72 °C as the last step. Amplified products were analyzed using 1.5 % agarose gel. Electrophoresis was performed at 120 volts DC for 2.5 hrs in a submarine electrophoresis system (Maxi sub XL). After electrophoresis, remove the gel from the tank and view the gel under UV illumination and photograph using gel documentation system (Table 1).

Table 1. Sequences of the SSR primers, Fluorescent Dye and references used in the investigation

NO	SSR loci	Forward primer 5'-3'	Revers primer 5'-3'	Fluorescent Dye	References
1	ORS 149	Gctctctatctcccttgactcg	tgctctaagatctcaggcgtgc	D3	Darvishzadeh. 2012
2	ORS 154	Gcacctttggtgaggagata	tgcatcagtagctattgtctat	D3	Darvishzadeh. 2012
3	ORS 1068	Aatttgtcgacggtgacgatag	ttttgtcatttcattacccaagg	D3	Tang et al. 2002
4	ORS 488	Cccattcactcctgtttcca	ctccggtgaggatttggatt	D4	Tang et al. 2002
5	ORS 598	Ccaaatgtgaggtgggagaa	atagtccctgacgtggatgg	D4	Tang et al. 2002
6	ha4136	Cctattcctgataattcactaagc	ggtagcatgcttacattaagatg	D4	Poormohammad Kiani et al 2007
7	ha3513	Tgacccattcaacttcttaa	tcatggttcctgatgagaat	D4	Poormohammad Kiani et al 2007
8	ha1604	Gcaaatgcactaaaggcccc	ccctactcaaaccttacctc	D3	Poormohammad Kiani et al 2007

The quality and quantity of the extracted samples was estimated by using Spectrophotometer (NanoDrop ND-1000) and by 0.8% Agarose Gel Electrophoresis. Polymerase chain reaction (PCR) amplifications were performed as described by Şelli et al. (2007). Forward primers of each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green), and D4 (blue) (Proligo, Paris, France).

The PCR products were first separated on a 2% (w/v) agarose gel and visualized under UV light. For further determination of polymorphisms, the PCR products were run on CEQTM 8800 XL capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA). Allele sizes were determined for each Sunflower SSR loci using the Beckman CEQ Fragment Analysis software.

Genetic Analysis

Identical cultivars, number of alleles, allele frequency, expected (HE) and observed (HO) heterozygosities, estimated frequency of null alleles (r), and probability of identity (PI) were calculated for each loci using the IDENTITY 1.0 program (Wagner and Sefc, 1999) according to Paetkau et al. (1995). Similarity matrix and Dendrogram was constructed with the unweight pair-group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973), using the Numerical Taxonomy and Multiware Analysis System software (NTSYS-pc) (version 2.0) (Rohlf, 1988).

RESULT AND DISCUSSION

SSR Analysis

10 SSR primers were successfuly amplified and electrophoresed by CEQTM 8800 XL capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA). The size of the markers are varied among 300 bp to 400 bp in 1% agarose gels (Figures 1).

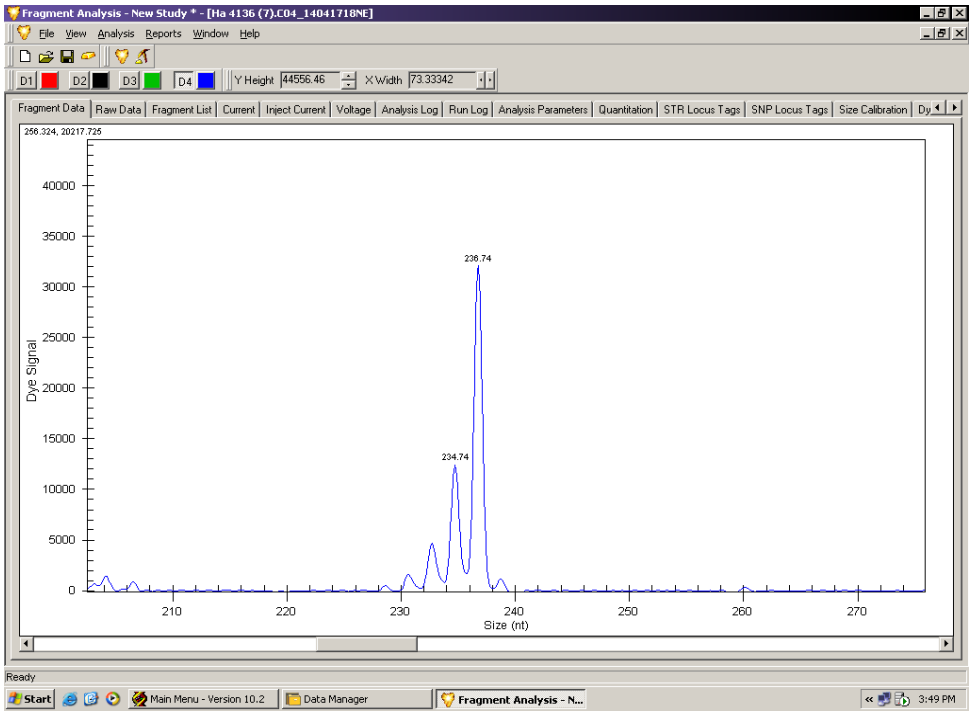


Figure. 1. Homozygote pic profile (allele) at Ha4136 SSR loci

The image of PCR products in agrose jel was observed (Figure 2).

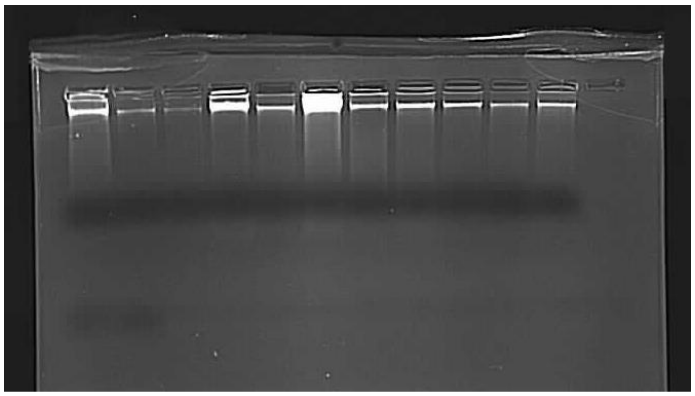


Figure 2. High resolution agarose gel images of SSR markers in Sunflower line.

It was observed that a total number of 57 alleles. the number of alleles per locus ranged from 2 (ORS598) to 12 (ha4136) Table 3. In this research the maximum allel frequency was observed on 3513 lokusuna (536) aittir. Daha sonra OR598 (378), ORS 1068 (364), Ha 4136 (288), ORS 546 (228), ORS 154 (223), ORS 488 (177), ORS 78 (162), ORS 149 (142) and Ha 1604 (121) primers.

Table 3. Number of alleles (*N*A), expected heterozygosis (*H*E), observed heterozygosis (*H*O), probability of identity (PI), and the frequency of null alleles (r)

Loci	Number of alleles (n)	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Probability of identity (PI)	Null alleles (r)
ha1604	3	0.392	0.048	0.549	0.246
ha4136	12	0.740	0.634	0.129	0.060
ha3513	7	0.713	0.926	0.221	-0.124
ORS546	5	0.674	0.975	0.243	-0.179
ORS598	2	0.195	0.170	0.704	0.020
ORS488	4	0.320	0.024	0.507	0.224
ORS78	4	0.433	0.000	0.424	0.302
ORS1068	8	0.762	0.951	0.171	-0.106
ORS154	7	0.660	0.585	0.209	0.044
ORS149	5	0.617	1.000	0.355	-0.236
Total	57	5.506	5.313	-	-
Mean	5.7	0.550	0.531	-	-

Genetic Similarity

Overalls, the values for genetic distances ranged from % 40 - % 90 (Figure 3). The average of genetic similarity was % 48, depicting a high level of genetic variation among studied sunflower genotypes. Among the sunflower genotypes "34 (K7 RSN 7/13)" showed significant distinction, being grouped in a different branch than the other genotypes.

The results of similarity revealed a low genetic similarity was observed as follows:

G3 K6 ASN & K7 RSN 7/13 : YDAH SN 2/13 & K7 RSN 7/13 : YDAH SN 3/13 & K7 RSN 7/13 : G5K8 ASN 6/13 & K7 RSN 7/13 : G5K8 ASN 2/13 & K7 RSN 7/13 with % 16 genetic similarity. The high similarity among sunflowers lines were observed among G5K8 ASN 5/13 & YDAH SN 3/13: G5K8 ASN 5/13 & YDAH SN 1 /13 lines with % 94 (0.94).

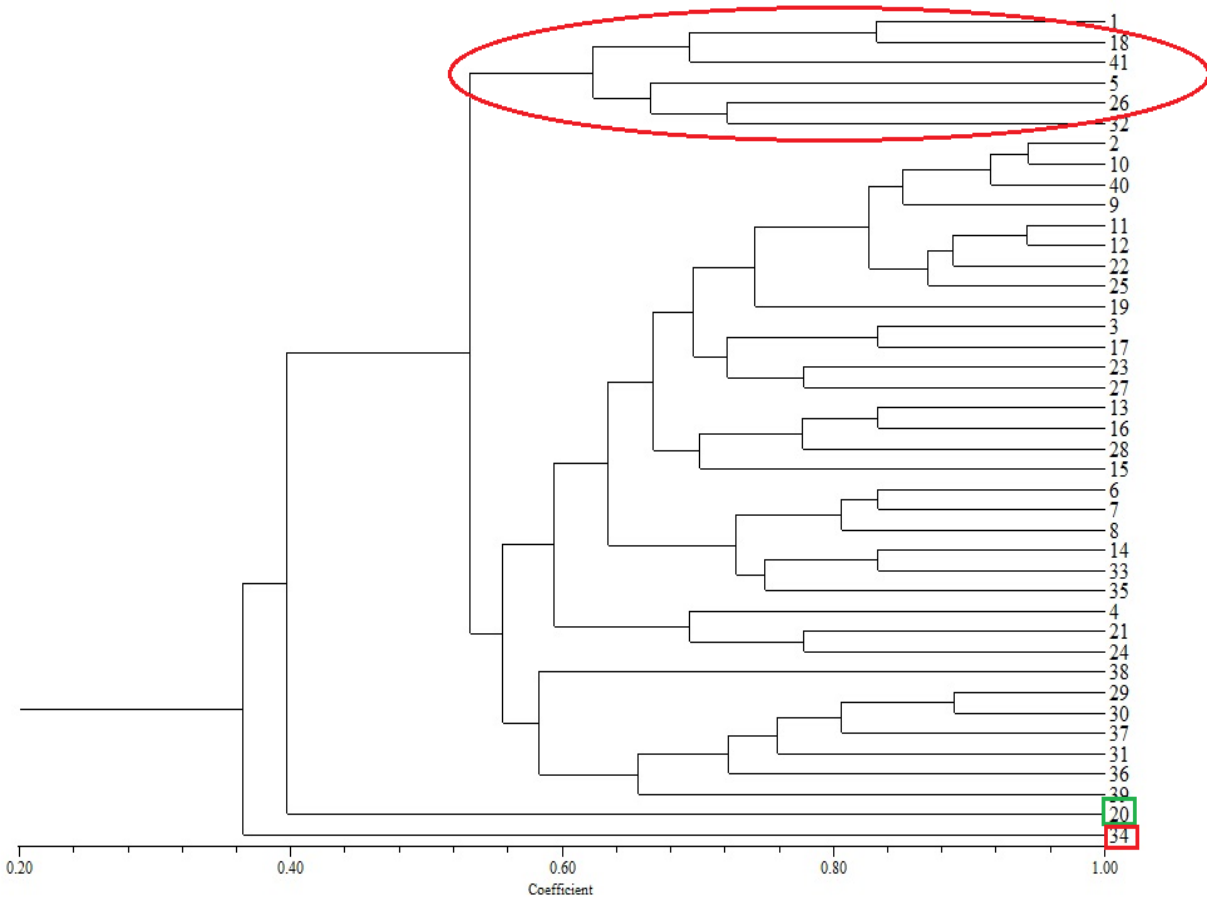


Figure 3: Dendrogram showing the genetic relationship among 41 Sunflower Lines

As sunflower is a highly cross pollinated crop therefore, a high number of alleles per locus could be a result of the natural out crossing among the parental material and also due to having a broad genetic base (Zia et. al, 2014). The average number of alleles found in our study is comparable to those reported in other studies on sunflowers. For instance, Darvishzadeh 2012, used 38 markers for15 sunflower genotypes; the average allele frequency in his study was 2.32. but in our study we detected an average allele frequency 5.7. İt shows that the markers selected in this present study have high polimorphic content.

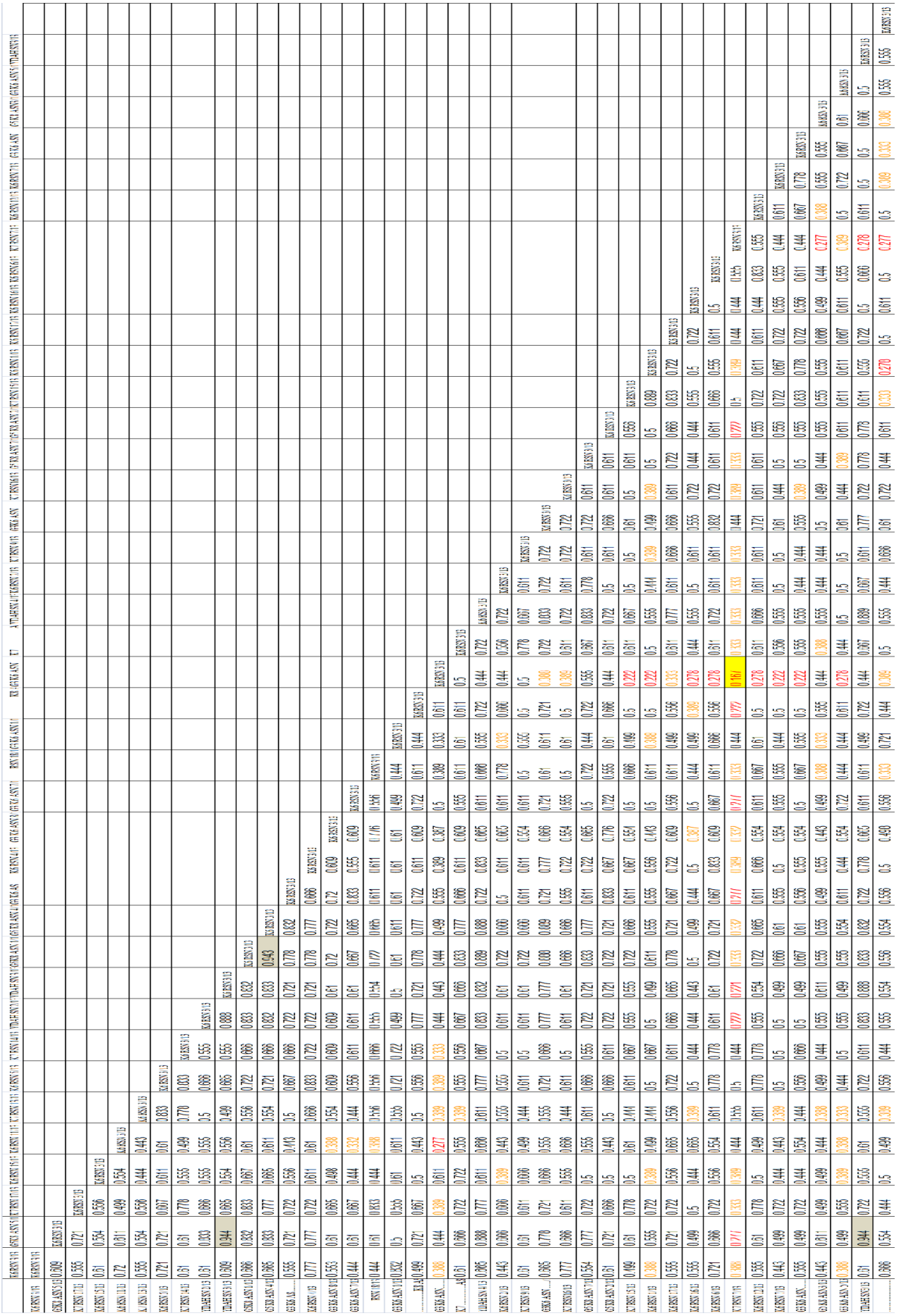
In the other study which was carried out by Darvishzadeh and his colleagues in 2010, 38 SSR locus was used for the characterization of 28 sunflower cultivars. It was reported that the loci ORS 598 gave high PI but surprisingly in our study it was observed that the ORS598 loci gave the lowest number of alleles. Duca and his colleagues (2013) used 13 pairs of SSR primers were used for genotyping of sunflower lines, in their study ORS78 SSR primer were excluded from the analysis due to unclear profiles with stutter bands. This outcome was different in our research means ORS78 SSR primer gave 4 alelle and showed 0.433 expected heterozygosity (H_e).

The present study, using molecular markers and morphological traits, investigated the genetic relationships of 41 sunflower lines from Turkey. Among the sunflower cultivars analyzed in this study, no identity, synonym and homonym genotypes were found.

The SSR technique used here was found to be quite effective in determining the genetic relationships among sunflower lines.

it is expected that the results of this study will assist current sunflower breeding efforts in Turkey as well as maintain the genetic integrity of the genetic resources.

Table 4: Genetic distances (GD) between different lines of sunflower



LITERATURE

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GENETIC DIVERGENCE IN SUNFLOWER ACCESSIONS

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Importance of sunflower-

Sunflower (*Helianthus annuus* L.), belonging to the family '*Asteraceae*' (*Compositae*) and genus '*Helianthus*', is a diploid species (2n = 2x = 34) and native to southern parts of USA and Mexico. Sunflower is an important oilseed crop and is the preferred source of oil for domestic consumption and cooking worldwide (Hu *et al*., 2010). Sunflower was introduced to India during 1969 and gained popularity during 1980's with the development of first sunflower hybrid BSH-1 (Seetharam, 1980). In the oilseed scenario, sunflower competes with other three major oilseeds, *i.e.* soyabean, groundnut and rapeseed mustard at global level. Sunflower has a great potential in bridging the gap between the demand and supply of edible oil in future. Sunflower holds great promise because of its short duration, wider adaptability, photo-insensitivity, drought tolerance and higher amount of superior quality oil. The oil and protein content in sunflower ranges from 35-45 per cent and 18-20 per cent, respectively. Higher oil yield is an ultimate objective of sunflower researchers as this oil is considered as a good quality oil from health point of view, due to presence of polyunsaturated fatty acids which are known to reduce the risk of cardiac related problems (Monotti, 2004). Additionally, due to the possibility of using its oil as raw material for manufacturing biodiesel, it is arousing the interest of farmers, agriculture professionals and companies in the world.

Importance of genetic divergence in crop improvement-

In plant breeding, genetic diversity in parental lines is a pre-requisite for developing genetically superior hybrids. More diverse the parents, greater are the chances of obtaining heterotic expression in F1 with possibility of broad spectrum of variability in segregating generations. Yield is the combination of numerous components which are influenced by environmental instabilities. It is highly recommended to explore configuration of yield via breeding approaches. Sunflower being a highly cross-pollinated crop has a great scope for increasing productivity by diversifying hybrid base. In downsizing the breeding lines to be maintained, assessment of genetic divergence also helps a lot. The concept of D^2 statistics, based on measurements of morphological characters is frequently used as a tool for estimating genetic divergence by the plant breeders (Mahalanobis, 1936; Rao, 1952). Tracing D^2 as a generalized statistical distance, genotypes are grouped on the basis of minimum genetic distance using Tocher's method as described by Rao (1952). Varieties from different localities are generally included in the hybridization programmes assuming genetic diversity and greater likelihood of recovering promising segregants. However, Murthy and Anand (1966) noted that there is no parallelism between geographical and genetical diversity. Genetic diversity existing within and between groups of germplasm is important, and particularly, useful in proper choice of parents for realising higher heterosis and obtaining useful recombinants. D^2 statistic is a useful tool for estimating the genetic divergence in plant breeding experiments.

The knowledge about the magnitude and nature of variability present in a population due to the genetic and non-genetic causes is an important prerequisite for a breeding programme to improve the yield potential of genotypes, as greater variability among the genotypes leads to better chance for further improvement in the crop.

Methods to measure genetic divergence-

Evaluation of genetic divergence is performed through methods based on agronomic, morphology and molecular characteristics. Mahalanobis (1936) outlined a statistical procedure 'D^2 statistic' to measure the genetic divergence among the test genotypes involving quantitative characters in a given population. This concept is based on the technique of utilising measurement in respect of an aggregate of characters. Clustering methods objectify to separate a group of original observations on different subgroups in order to obtain homogeneity within and heterogeneity between subgroups. Among these methods, optimization and hierarchical ones are employed on a large scale by plant breeders. Visualization and interpretation of distances may be facilitated by the use of a clustering method and/or graphical dispersion. Multivariate analysis has been considered as an important tool in quantifying the degree of genetic divergence in different crops (Rao, 1952).

Genetic divergence in sunflower-

Genetic divergence is a process of one species diverging over time into more than one species, *i.e*., passing small random changes over time, from one generation to next generation. Varieties from different localities are generally included in the hybridization programmes assuming genetic diversity and greater likelihood of recovering promising segregants. However, Murthy and Anand (1966) noted that there is no parallelism between geographical and genetical diversity. Several investigations that evaluated the genetic divergence in sunflower crop were conducted by using morphoagronomic characters (Arshad *et al*., 2007). Genetic divergence estimation between different sunflower genotypes has been studied, aiming to develop parents for hybrids constitution or even the formation of new segregating populations, from the intercross of divergent genotypes with complementary agronomic characteristics.

Reddy and Devasenamma (2004) studied 61 genotypes of sunflower and grouped them into 19 clusters based on their genetic diversity. Based on inter cluster distance value and *per se* performance of genotypes, the genotypes namely, EC-376211, EC-399318, RHA-344 and BLC-P6 were selected which could be intercrossed to obtain high heterotic expression and also to recover desirable transgressive segregants.

Reddy *et al*. (2005) assessed genetic divergence among 102 genotypes and grouped them into 12 clusters. Based on the inter cluster distances and *per se* performance, the genotypes namely, GMU-4, GMU-11, GMU-14, GMU-16, GMU-25, GMU-40 and GMU-70 were selected which could be intercrossed to obtain high heterosis and also to recover desirable transgressive segregants. Seed yield/plant contributed maximum to divergence (40.2%) which was followed by number of leaves/ plant (25.8%) and 100-seed weight (17.0%).

Loganathan *et al*. (2006) conducted multivariate analysis of divergence among 50 genotypes of sunflower which led to their grouping into 14 clusters. Seed yield contributed maximum towards genetic divergence, followed by 100-seed weight and plant height.

Mahalakshmi *et al*. (2006) studied 29 genotypes of sunflower for their genetic divergence by D^2 analysis. The genotypes were grouped into 7 clusters. The character, days to first flowering contributed more towards genetic divergence.

Sridhar *et al*. (2006) assessed genetic divergence using D^2 statistics among 44 sunflower genotypes and grouped them into 9 clusters. Plant height and oil content contributed more towards genetic divergence.

Binodh *et al*. (2007) studied genetic divergence of 24 breeding lines for 8 traits in sunflower. The genotypes were grouped into 10 clusters where cluster I was the largest containing 13 genotypes, followed by cluster IV with 3 genotypes. The inter-cluster distance was the maximum between cluster VI and cluster VIII, followed by cluster IV and cluster VI and cluster VI and cluster IX. The study revealed that plant height contributed maximum towards divergence (45.29%),

followed by seed yield per plant (25.72%) and oil content (15.94%). Based on the inter-cluster distance and *per se* performance, the genotypes *viz.*, 17A, 47A, CSFI 5325, CSFI 5415, CSFI 5436 and CSFI 5013 were identified as suitable parents which could be intercrossed to obtain high heterosis.

Camarano *et al*. (2010) investigated genotypic divergence among 10 sunflower populations using Mahalanobis' D^2 statistics and canonic variables to identify more similar and/or divergent groups. The results of the individual variance analyses pointed out significant differences for the initial flowering, final flowering, plant height, oil content, moisture content and yield in all the experiments. Very high genetic variability was noted among the populations for these traits. The traits, stem diameter, head diameter, 1000-seed weight and number of seeds per head presented differences, which were sometimes significant and sometimes not, indicating that these traits show genotype-environment interaction.

Punitha *et al*. (2010) assessed genetic diversity among 17 sunflower genotypes using 9 agronomic characters and indicated the presence of substantial genetic diversity. The genotypes were grouped into 4 clusters. Among the investigated characters, seed yield, plant height, oil content and oil yield exhibited high contribution towards genetic divergence. It was observed that the inclusion of CSFI 5076, CSFI 5162, CMS 47A, CSFI 5005, CMS 17A, CMS 47A, CSFI 5069, CSFI 5422, CSFI 5109, CSFI 5155, CSFI 5002, COSF 1A , CSFI 5161 and CSFI 5015 in future breeding programs could result in the development of superior sunflower cultivars.

Mandel *et al*. (2011) conducted population genetic analysis of the primary gene pool of sunflower based on a broad sampling of 433 cultivated accessions and 24 wild sunflower populations. Gene diversity across the cultivars was 0.47, as compared with 0.70 in the wilds, indicating that cultivated sunflower harbours roughly two-thirds of the total genetic diversity present in wild sunflower.

Kumari and Sheoran (2012) evaluated 80 sunflower genotypes for genetic divergence using D^2 analysis. The genotypes were grouped into 10 clusters. Cluster I was the largest one with 22 genotypes, followed by cluster II (18), IV (17), VI (11), III (7) and V, VII, VIII, IX and X with one genotype each. The genotypes, DRSF-120 R, P70R, Nandyal-1 and RHA-586 were identified as divergent and superior performers. Likewise, genotypes from different sources were grouped in the same cluster, thus, suggesting that geographical diversity does not necessarily represent genetic diversity.

Reddy *et al*. (2012) studied genetic divergence in 64 genotypes of sunflower and grouped the genotypes into 9 clusters. The pattern of distribution of genotypes into various clusters was random and indicated that the geographical and genetic diversity were not related. Plant height contributed maximum towards genetic divergence, followed by stem diameter and head diameter.

Ayaz *et al*. (2014) evaluated seventeen sunflower hybrids and fifteen inbred lines including ten Cytoplasmic male sterile lines and five restorer lines for flower initiation days, full flowering days, full developmental days, height of plant, disk diameter, stem thickness, leaves per plant, hundred achenes weight, achenes yield and oil content percentage. The maximum achenes yield was contributed by Hysun-33 2119 kg/h followed by SMH-0924 and SMH-0925. SMH-1028 and SMH-0926 were suggested as potential significant hybrids for future breeding plans to incorporate maximum achenes yield and oil content percentage. The CMS-11, CMS-25 and CMS-10 were long statured with vigorous stem and all the restorers were early maturing recommended for including in hybridization program to generate high heterotic factions.

Chandirakala *et al*. (2014) assessed genetic divergence of 38 sunflower genotypes using Mahalanobis D2 statistics. These genotypes were grouped into 13 clusters, among which the cluster IX with 9 genotypes was the largest. Maximum inter cluster distance was recorded between cluster XII and XIII (39.58) followed by clusters II and XII (38.18). Hence hybridizing between these

divergent groups may lead to higher variation in segregating population. In this study, the genotypes *viz.*, GMU 322, COSF3B and COSF4B in the cluster II, the genotypes *viz.*, GMU 503, GMU 1074, GMU 1108 in the cluster XII and the genotype COSF1B in the cluster XIII are widely divergent and crosses may be effected among the genotypes of these clusters to get more heterosis among the hybrids.

Pandya *et al.* (2014) evaluated forty genotypes of sunflower [*Helianthus annuus* (L.)] for seed yield and its components and grouped them in 5 clusters. The clustering pattern of genotypes was independent of their geographical distribution. Taking into account cluster mean for important seed yield components, the various clusters which can provide the desired parents like GMU-1033, GMU-411 for hybridization for improvement of characters.

Masvodza *et al*. (2015) used 16 cytoplasmic male sterile (CMS) lines and 10 male restorer (R) lines and characterised them for ten morphological variables namely, days to 50% flowering, head diameter, leaf length, leaf width, petiole length, nodding, lodging, number of leaves, plant height, stem diameter and uniformity. The genetic base of the collection was observed as narrow and would need more diversification.

Sunflower improvement in relation to genetic divergence-

Genetic diversity existing within and between groups of germplasm is important, and particularly, useful in proper choice of parents for realising higher heterosis and obtaining useful recombinants. D^2 statistic is a useful tool for estimating the genetic divergence in plant breeding experiments. To get more heterotic F_1's and large number of desirable transgressive segergants, selection of parents for hybridization should be properly based on genetic diversity rather than geographic diversity. The mating systems in any field crop determine the gene flow and hence the propensity with which reference population can be improved through genetic amelioration. Sunflower is predominately a cross- pollinated crop and the pollination is by and large insect-mediated, though some degree of self pollination cannot be ruled out in some genotypes for the reasons of hermophroditism and some homogamy. This necessitates that heterozygosity *per se* be maintained in sunflower populations.

Sunflower improvement strategies include; development of heterotic hybrids, elite composites and/ or improved open-pollinated populations developed through random mating (hand pollination or male sterility mediated), followed by selection (various recurrent selection procedures). All these methods would necessarily entail in their objectives for accumulation of gene constellations for intra and inter allelic interactions in genotypic background(s) of agronomic significance. In hybrids, dominance deviation of alleles, and in improved populations, accumulation of additive genes with greater complimentary effects are harnessed for better trait expression and hence higher economic yield.

The success of any chosen breeding programme would depend upon the extent of heritable genetic variation, response of selection pressure exerted, the magnitude and direction of associations among various yield contributing traits and selection indices used in reference population(s). Hence , analysis of genetic diversity among inbred accessions is of vital importance in Sunflower breeding.

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COMBINING ABILITY AND GENETIC COMPONENTS FOR SEED YIELD IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Being one of the most important oil crops in the world main goals in sunflower breeding are increased seed and oil yield per hectare. Bearing in mind breeding direction and global importance of this oil crop objective of this study was to evaluate general combining ability (GCA) of six sunflower genotypes and specific combining ability (SCA) of their crosses as well as to estimate components of genetic variability for seed yield/plant. Genotypes were crossed according to incomplete diallel method (without reciprocals) and fifteen F1 progenies were derived. Both, additive and non-additive, genetic components were significant in seed yield expression but according to GCA/SCA ratio additive component was more important. The highest GCA value was recorded in G1 genotype, while the highest SCA value was recorded in combination G2xG3, for seed yield. Analysis of components of genetic variability revealed that dominant gene effects (H1) were more important than additive (D) and frequency of dominant genes was greater than recessive ones. Dominant and recessive genes were not equally distributed among parents as presented by the $H_2/4H_1$ ratio which was different than 0.25 (0.17). According to average degree of dominance (1.14) superdominance was the case in seed yield expression.

Key words: seed yield, combining ability, dominant and recessive genes

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the World and main crop for production of edible oil in Serbia. Consequently, main goals in sunflower breeding are increased seed yield and thus oil yield per hectare. Sunflower seed yield, as a complex trait, requires the most effort in breeding process because all other goals in breeding through improving individual properties are in a function of increasing seed yield and thus oil yield. The success in creating new, superior, genotypes largely depends on the possession of adequate genetic variability in the parental material because the greater are the chances for obtaining superior F1 plants. Furthermore, for sucessful breeding it is necessary to dispose information about mode of inheritance and combining ability. Obtained information helps in breeding proces through selection of perspective parental lines with the aim of creating genotypes that will improve production. There are many papers that deal with this topic which differ in the results. Previous results have found that both, additive and non-additive, components are important in the inheritance of seed yield (Škorić et al., 2007). Some authors emphasize the larger importance of additive component of genetic variance for seed yield (Putt, 1966; Sindagi et al., 1979; Petakov, 1992 and Karasu et al., 2010). Contrary to them, larger role of non-additive component on the inheritance of seed yield was determined in previous research by many authors (Marinković, 1984; Mihaljčević, 1989; Joksimović, 1992; Lande et al., 1997; Rather et al., 1998; Goksoy et al., 2000; Cecconi et al., 2000; Jocić, 2002, 2012; Sakthivel, 2003; Farrokhi et al., 2008; Gvozdenović et al., 2008 and Hladni et al., 2010). Combining abilities are divided into general (GCA), which represent the value of the parent used in crosses, and specific (SCA), representing the value of certain crossing. The most reliable method for testing combining abilities of genotypes is diallel (Živanović et al., 2006). Diallel crossing was proposed by the zoologist-geneticist Dr. Schmidt (1919) and firstly applied in plants by Sprague and Tatum (1942). Masood et al. (2005) used 8x8 diallel to test combining ability in sunflower and

found greater importance of non-additive effects in controlling seed yield. Vice versa Mijić et al. (2008) in 6x6 diallel of sunflower inbred lines found greater significance of additive component. Investigating the GCA/SCA ratio in inbred lines of sunflower using the method line x tester Andarkhor et al. (2013) have found greater importance of non-additive genetic components in controlling seed yield, considering that GCA/SCA ratio was less then 1 (<1).

Objective of this study was to evaluate combining abilities of sunflower genotypes through their crossings and to obtain information about components of genetic variance.

MATERIAL AND METHODS

Six sunflower genotypes were crossed according to incomplete diallel (without reciprocals). F1 progeny and parents were sown in three replicates in a randomized block design at Rimski šančevi experimental field of the Institute of Field and Vegetable Crops from Novi Sad. Experimental plot size was 10 m^2 with four, 3.6 m long rows and 70x30 cm plant spacing. The data were recorded on 10 plants in each replicate from middle rows. Harvest was done at the stage of physiological maturity and seed yield/plant was recorded in laboratory on a technical scale with an accuracy of 0.01 g. General combining abilities (GCA) of parents and specific combining abilities (SCA) of F1 were tested according to diallel method 2 by Griffing (1956). The assumption of this method is that there are no differences in reciprocal crosses.

Mathematical model for analysis of combining abilities as follows:

$Y_{ij}=m+g_i+ g_j+s_{ij}+1/bc\sum\sum e_{ijkl}$

Analysis of components of genetic variance was performed according to the method suggested by Mather and Jinks (1971).

RESULTS AND DISCUSSION

Analysis of variance of combining ability for seed yield/plant showed statistically highly significant differences in the general (GCA) and specific (SCA) combining abilities between parents used in this experiment (Tab. 1). As GCA and SCA provide information for additive and non-additive gene actions considering that GCA/SCA ratio was higher than 1 it can be concluded that additive gene action played greater importance in the inheritance of seed yield/plant. In earlier studies Putt (1966) and Sindagi et al. (1979) found that general combining ability for seed yield in sunflower is more important than specific combining ability, indicating that additive component is more important than non-additive. In contrast, a significant impact of non-additive component of genetic variance in the inheritance of seed yield/plant in sunflower was observed by many authors (Marinković, 1993; Bajaj et al., 1997; Kumar et al., 1998; Chandra et al., 2011 and Andarkhor et al., 2012). The highest and statistically significant and positive GCA value was calculated for the G1 genotype so it can be concluded that this genotype represents the best general combiner for improving this trait (Tab. 2). Parental genotypes G4 and G5 also demonstrated positive GCA values but without statistical significance. In other parent genotypes were established negative GCA effects. Significant and positive value of SCA effect was recorded only in crossing combination G2xG3. In other crossing combinations SCA values were not statistically significant, while in five crossings were found negative SCA values.

Table 1. Analysis of variance of combining abilities for seed yield/plant in sunflower.

Source	Df	SS	MS	F-value
GCA	5	2718.59	543.72	11.31**
SCA	15	2664.86	177.66	3.70**
Error	40	1922.97	48.07	

Table 2. GCA (diagonal) and SCA (above diagonal) effects for seed yield/plant in sunflower

Genotypes	G1	G2	G3	G4	G5	G6
G1	13.62**	-21.39	-0.04	-1.83	-9.65	10.48
G2		-5.83	17.59*	-10.48	3.22	1.92
G3			-3.78	13.45	11.96	1.26
G4				3.81	14.29	-14.33
G5					1.61	5.52
G6						-9.43

$LSD_{0.05}$ GCA= 7.00 $LSD_{0.05}$ SCA= 17.16
$LSD_{0.01}$ GCA= 9.37 $LSD_{0.01}$ SCA= 22.96

The analysis of components of genetic variance revealed that the dominant component (H1) was greater than the additive (D), which shows that most of the genetic variation in the inheritance of seed yield/plant makes the non-additive component (Tab. 3). According to the calculated F value dominant genes prevailed in relation to recessive ones, as confirmed by the calculated frequency of dominant (u) and recessive (v) genes. Furthermore, calculated value of the H2/4H1 ratio indicated the unequal representation of dominant and recessive genes in parents. From the KD/KR ratio, which is greater than one, is also evident that the dominant genes prevailed in respect to the recessive ones, in the inheritance of seed yield/plant. Average degree of dominance $\sqrt{H_1}/D$ (1.14) indicated that superdominance was the case in expression of seed yield/plant, considering all crossings.

Table 3. Components of genetic variance for seed yield in sunflower.

Components	Value
D	535.98
H_1	698.17
H_2	473.63
F	544.60
E	48.07
u	0.78
v	0.22
$H_2/4H_1$	0.17
$\sqrt{H_1}/D$	1.14
K_D/K_R	2.60

CONCLUSIONS

Main objective in breeding programe is development of superior synthetics or hybrids. To achieve this aim it requiers estimation of gene action in various traits in order to design an efficient breeding plan for further genetic improvement of the initial material. Sunflower seed yield is one of the most important traits considering this crop and information about combining abilities and components of genetic variance are necessary for improving this valuable trait. In this research we found that both, additive and non-additive, genetic components were important in expression of seed yield/plant but additive genetic component prevailed. According to GCA value genotype G1 is the best general combiner for improving this trait and that genotype will be used in further hybrid combinations in order to obtain highly productive hybrids of sunflower. Crossing combination G2xG3 had the highest and significant SCA value and that combination will be tested with other

perspective hybrid combinations. Analysis of components of genetic variance revealed that dominant genes were prevalent in expression of seed yield/plant, as confirmed by the frequency of dominant genes. Dominant and recessive genes were not equally distributed among parents as presented by the $H_2/4H_1$ ratio which was different than 0.25 (0.17). According to average degree of dominance (1.14) superdominance was the case in seed yield expression.

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RECOMBINATION AND SELECTION IN SUNFLOWER POPULATIONS FROM EEA PERGAMINO INTA

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ABSTRACT

The achievement of new sources of genetic variability is central for the development of breeding programs. At Pergamino EEA (33º 53'S, 60º 35'), in the 2014-15 , the following populations were evaluated: P7xBulkVert C3/Bulk AO C2(C1), Bulk AO / Bulk Vert(C1),P7xBVert C3/Bulk AOC2(C2),Bulk AO/ Bulk Vert (C2) ,Bulk A.O(C2), Bulk AO(C3) and the hybrid Paraíso 20. The characters were flowering days, seed yield and *Verticillun* resistance. In the populations Bulk AO / Bulk Vert (C1); Bulk AO/ Bulk Vert (C2) , P7BulkVert(C1) , P7BulkVert(C2), P7BulkVert(C3), Bulk AO(C2), Bulk AO(C3), P7xBulkVert C3/Bulk AO C2 (C1), P7xBulkVert C3/Bulk AO C2(C2), PGRK (C0), PGRK(C2), Bulk AO(C1), BulkVert(C3), BulkVert (C4) and P7 (C0) were also evaluated percentage of oil content, plant height, head diameter, weight of 100 seeds, number of seeds/ head and percentage of kernel. The objective was to describe the variability obtained in germplasm of different origin and the advance achieved in the improvement of characters from recombination and selection cycles. The combination of different origin germplasm allowed to achieve yields similar to the hybrid. In P7xBulkVert C3/Bulk AO C2(C2), the cycles of selection and recombination improved *Verticillium* resistance. The highest variability was obtained in number of seeds /head and the slowest was observed in weight of 100 seeds. The variability of germoplasm in different characters would allow to obtain improved genotypes

Key words*: Helianthus annuus; breeding , variability*

INTRODUCTION

The achievement of new sources of genetic variability is central for the development of breeding programs .The first step in a cultivars development program me is to form a population with genetic variability for the characters of interest.

The potential advantages of a complex population (involving hundreds of parents) are that the number of alleles for each locus increases with the number of parents used and the probability of heterozygote in multiple loci is greater. Mass selection has been effective in sunflower breeding for precocity oil and diseases resistance (Pustovoit 1964). Open pollination sunflower populations would be a good source for the development of inbred lines (Eberhart 1967)

Verticillium wilt, caused by pathogen *Verticillium dahliae* (Kleb), is one of the main diseases of sunflower (*Helianthus annuus* L.) in Argentine; thus obtaining of cultivars of good performance against the pathogen is a priority in breeding programs. Pereyra et al, 1999, reported that this disease causes up to 73% yield losses. The search of germplasm with diseases resistance, industrial quality, yield and oil content led to improvement to obtain populations that meet these characters. The sunflower Group of INTA Pergamino Experimental Station (EEA) develops as part of its work methodology the formation of populations of different origin with traits related to the quality, disease resistance and yield to be used as a source of new cultivars.

The objective was to describe the variability obtained in germplasm of different origin and the advance achieved in the improvement of characters from recombination and selection cycles.

MATERIALS AND METHODS

The populations studied were the following:**P7**: Recombination and selection of lines with outstanding agronomic characteristics and good seed yield. **Bulk Vert :** Obtained by recurrent selection of germplasm of good performance in seed yield and resistance to *Verticillium dahliae.* **Bulk AO**: It is obtained from recombination lines of high oleic acid content. **P7xBulkVert**./ **Bulk AO.** It is obtained from the crossing and recombination of populations (P7xBulk Vert) x Bulk AO. **PGRK:** Obtained by recurrent selection from Ruso x Klein. **Bulk AO/ Bulk Vert:** It is obtained from the crossing and recombination of populations Bulk AO xBulk Vert

At EEA Pergamino INTA (33º 57' S, 60º 34' W) were planted a trial with the statistical design of a randomized complete block with 3 replications and plots of 3 rows of 6.0 m, row spacing of 0.7 m. The germplams evaluated were the populations (P7xBVert C3/Bulk AO C2)C1, Bulk AO / Bulk Vert C1,(P7xBVert C3/Bulk AO C2)C2,Bulk AO/ Bulk Vert C2 ,Bulk A.OC3 y Bulk A.Oc2 and the commercial hybrid Paraíso 20. The analyzed characters were: days to flowering, yield (kg ha-1) and *Verticillium* resistance. Seedling inoculation method was applied to evaluate *Verticillium* resistance. The scale was R (resistant), MR (moderately resistant), MS (moderately susceptible), AS (very susceptible)

In Bulk AO / Bulk Vert C1; Bulk AO/ Bulk Vert C2 , P7BVc1 , P7BVc2, P7BVc3, Bulk A.Oc2, Bulk A.OC3, (P7xBVert C3/Bulk AO C2)C1,(P7xBVert C3/Bulk AO C2)C2PGRK Cycle 0, PGRK Cycle 2, Bulk A.OC1, BVert C3, BVert C4, P7 Co, Paraíso 20, Olisun 4 and ACA 885 were analyzed oil content, plant height, head diameter, 100-Seed weight (g); Seed nº/head and kernel content

RESULTS AND DISCUSSION

Table 1 Yield, days to flowering and *Verticillium* resitance in populations of Pergamino INTA

Population	Days to flowering	Yield (kg/ha)	Verticillium(*)
Paraiso 20(commercial hybrid)	66	1938	1,4
(P7xBVert C3/Bulk AO C2)C1	65	1619	2,03
Bulk AO / Bulk Vert C1	68	1555	2,4
(P7xBVert C3/Bulk AO C2)C2	68	1450	1,8
Bulk AO/ Bulk Vert C2	69	1183	2,17
Bulk A.OC3	64	947	--
Bulk A.Oc2	67	706	--
Mean	67	1342	
C.V.%	2,25	16,03	
LSD 5%	3,00	444	

(*) (1-1.9) moderately susceptible , (2-2.9): susceptible

Table 1 analyzes days to flowering, yield adjusted by oil content and Verticillum resistance in populations of the Pergamino EEA. We observed that P7xBVert C3/Bulk AO C2)C1 y Bulk AO / Bulk Vert C1 didn't have significant yield differences with Paraíso 20. The longest cycles was found in Bulk AO / Bulk Vert and the shortest was found in Bulk A.OC3. The most advanced selection cycles improved *Verticillium* resistance.

The different origin germplasm combinations allowed to reach similar yields to the commercial hybrids. Concerning *Verticcillum* resistance, it was observed that even though (P7xBVert C3/Bulk AO C2) C2 performance was inferior to the commercial hybrids, selection and recombination allowed to improve that character.

Table 2 Characters in different origins populations of Pergamino INTA

Population	Plant height (cm)	Head diameter (cm)	Seed nº/head	100-Seed weight (g)	Oil content (%)	Kernel content (%)
Bulk AO / Bulk Vert C1	156	19	1295	5,7	37,4	72,9
Bulk AO/ Bulk Vert C2	157	15	996	5,1	36,1	73,1
P7BVc1 (w 3153)	147	18	1228	5,1	35,7	70,8
P7BVc2	164	20	1521	5,1	39,5	73,8
P7BVc3	162	18	1288	4,9	39,6	75,8
Bulk A.Oc2	106	13	-	4,3	36,9	75,4
Bulk A.OC3	118	16	-	5,2	40,4	78,3
(P7xBVert C3/Bulk AO C2)C1	128	16	801	4,6	39,5	77,2
(P7xBVert C3/Bulk AO C2)C2	145	15	689	4,5	37,5	73,2
PGRK Ciclo 0	179	15	-	4,5	31,8	63,1
PGRK Ciclo 2	205	16	996	5,4	34,3	64,3
Bulk A.OC1	144	18	-	5,7	46,1	73,5
Paraiso 20(commercial hybrid)	140	16	1705	4,6	42,1	79,0
Olisun 4 (commercial hybrid)	141	16	1125	5,5	45,8	75,1
ACA 885(commercial hybrid)	173	17	1263	5,7	42,7	72,7
BVert C3	173	15	1081	5,2	40,6	75,0
BVert C4	172	16	-	4,6	36,0	74,5
P7 Co	147	12	573	3,3	34,1	71,2
Promedio	153,0	16,2	1120	4,9	38,7	73,3
C.V.(%)	12,2	24,1	36,0	8,6	12,9	8,6
LSD 5%	12,6	2,7	480	1,1	4,3	5,5

Table2 shows the analyzed characters of populations from Pergamino INTA. Highest plant height was found in PGRK and the smallest in Bulk A.Oc2 and Bulk A.Oc3. Highest head diameter was found in in P7BulkVertC2 and the smallest in P7 C0. Paraiso 20 had the highest seed nº/head., P7BVc2, Bulk AO / Bulk Vert C1 and P7BVc3 had also high values. Highest **100**-seed weight values were found in Bulk AO / Bulk Vert and ACA 885 , and the smallest was found in P7C0. Bulk A.OC1, Olisun 4, ACA 885 and Paraíso 20 had the highest oil content and PGRK C0 had the smallest. Highest kernel content was found in Paraíso 20, Bulk A.OC3 and (P7xBVert C3/Bulk AO C2)C1 and the smallest was found in PGRK.

Table 3 Characters in populations composed and original

Population	Plant height (cm)	Head diameter (cm)	Seed nº/head	100-Seed weight (g)	Oil content (%)	Kernel content (%)
Bulk AO / Bulk Vert	156	17	1145	5,4	36,7	73
Bulk AO	123	16	sd	5,1	41,2	73,5
Bvert	173	16	1081	4,9	38,3	74,8
P7BVert	158	19	1345	5,0	38,3	73,5
P7 Co	147	12	573	3,3	34,1	71,2
Bvert	173	16	1081	4,9	38,3	74,8

Table 3 compares the average values of composed populations with values of original populations. Bulk AO / Bulk Vert showed medium plant height value and an increase in the head diameter, 100 seed weight and seed nº/head, on the other hand there was a diminution of oil and kernel content values with those of the original populations. P7BVert showed medium plant height value and an increase in the head diameter , 100 seed weight and seed number/head, on the other hand the oil content remained the same for the original population with the value more high (BVert).

Table 4 Characters in recombination cycles Bulk AO/Bulk Vert population

	Bulk AO / Bulk Vert C1				Bulk AO/ Bulk Vert C2			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	156	110	192	21,8	157	125	182	13,8
Head diameter(cm)	18,9	12,5	24,7	3,9	15,1	9,3	18,5	2,8
Seed nº/head	1287	649	1612	552,6	993	725	1388	255,9
100 seed weight (gr)	5,7	2,5	8,4	1,7	5,2	3,5	8,7	1,4
Kernel content(%)	72,8	52,4	82,9	8,6	73,0	67,9	78,3	3,6
Oil content (%)	37,2	27,6	42,6	4,4	36,1	28,8	43,7	3,4

Table 4 compares two recombination cycles of the Bulk AO/Bulk Vert population. In cycle 2 there was a diminution in the averages of all the characters, except for plant height and kernel content.

Table 5 Characters in recombination cycles of P7xB Vert population

	P7BVc1				P7BVc2				P7BVc3			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	147	125	170	15,5	164	125	192	15,0	162	145	178	7,8
Head diameter(cm)	18,0	12,5	23,4	3,4	20,3	10,5	27,4	4,6	17,9	12,5	23,5	3,5
Seed nº/head	1207	884	1555	279,7	1529	1125	1761	350,9	1287	766	1610	362,4
100 seed weight (gr)	5,0	2,6	6,2	1,2	5,1	2,8	6,5	1,3	4,82	3,5	6,5	0,9
Kernel content(%)	71,0	61,7	81,0	6,4	73,8	64,4	85,0	6,8	75,9	69,3	83,2	4,4
Oil content (%)	35,0	24,9	44,9	6,2	39,8	33,1	50,3	5,7	39,4	33,4	48,4	4,6

Table 5 shows the values of the three recombination cycles of the P7xB Vert population. The highest values of plant height, head diameter, seed number/head, 100 seed weight and oil content were obtained in cycle 2. Cycle 3 had the highest kernel content.

Table 6 Characters in cycles of recombination of the population Bulk AO

	Bulk A.OC1				Bulk A.Oc2				Bulk A.OC3			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	146	140	160	7,2	106	100	120	5,4	118	90	170	16,0
Head diameter(cm)	18,3	13,5	26,2	4,8	13,4	9,2	20,1	3,0	15,6	7,3	20,0	3,9
100 seed weight (gr)	5,4	4,4	6,5	0,8	4,2	2,4	6,0	1,3	5,1	4,0	7,1	1,1
Kernel content(%)	74,1	70,1	78,9	3,8	75,6	65,0	81,7	5,1	78,5	77,6	79,5	0,7
Oil content (%)	45,6	41,3	48,0	3,0	36,4	31,4	42,9	3,8	40,1	35,8	45,0	3,1

Table 6 analyzes three recombination cycles of the Bulk AO population. The highest 100 seed weight and oil content values were obtained in cycle 1. The highest kernel content was obtained in cycle 3.

Table 7 Characters in recombination cycles of the P7xBVert C3/Bulk AO C2 population

	(P7xBVert C3/Bulk AO C2)C1				(P7xBVert C3/Bulk AO C2)C2			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	128	100	165	15,8	145	110	190	21,1
Head diameter(cm)	16,5	9,2	28	4,6	15,4	10,5	21,0	2,9
Seed nº/head	789	532	1420	368,6	697	401	1545	360,2
Peso 100 Aq. (gr)	4,7	2,4	6,9	1,5	4,5	2,4	7,0	1,4
Kernel content(%)	77,1	65,0	94,0	9,0	73,2	67,3	79,3	4,3
Oil content (%)	39,4	34,4	50,8	5,3	37,5	27,4	45,7	5,5

Table 7 compares two recombination cycles of the P7xBVert C3/Bulk AO C2. Cycle 1 showed the highest head diameter, seed number/head, 100 seed weigh, kernel content and oil content . Cycle 2 reached the highest plant height.

Table 8 Characters in recombination cycles of the PGRK population

	PGRK Ciclo 0				PGRK Ciclo 2			
	Mean	Mín	Máx	S.D.	Mean	Mín	Máx	S.D.
Plant height(cm)	179	160	202	12,7	205	160	245	19,2
Head diameter(cm)	15,1	8,5	27,2	5,0	16,7	8,5	22,5	3,7
Seed nº/head	-	-	-	-	1010	449	1617	349,6
Peso 100 Aq. (gr)	4,5	2,9	5,8	1,0	5,5	3,2	7,2	1,1
Kernel content(%)	63,2	59,5	67,1	2,6	64,2	57,8	72,4	4,9
Oil content (%)	32,2	25,8	39	5,4	34,4	26,7	41,5	4,5

Table 8 compares the cycles of the PGRK population. In all characters , cycle 2 reached the highest values. The method applied was the recurrent selection and it was possible to improve the values of all characters.

Table 9 Characters in recombination cycles of BVert the population

	BVert C3				BVert C4			
	Media	Mín	Máx	D.E.	Media	Mín	Máx	D.E.
Plant height(cm)	173	130	203	19,8	172	150	190	10,8
Head diameter(cm)	14,9	6,0	22,1	5,1	16,3	9,0	23,8	4,1
Seed nº/head	1058	742	1585	367,3	953	865	1040	123,7
Peso 100 Aq. (gr)	5,2	3,6	7,7	1,3	4,5	3,2	6,4	1,0
Kernel content(%)	75,1	70,0	80,1	3,5	74,6	64,8	88,5	7,4
Oil content (%)	40,9	34,5	44,1	3,2	38,4	26,2	46,4	7,0

Table 9 shows two recombination cycles of the BVert. population. Plant height values were similar in both cycles. The highest seed number/ head, 100 seed weight, kernel and oil content values were achieved in cycle 3 . The average of head diameter was higher in cycle 4.

In all the analyzed populations the character that showed most variability was seed number/head. The character that showed least variability was 100 seed weight.

In general, composed populations allowed to improve yield components and, in some cases, to reach similar yields to commercial hybrid.

The existent variability in most of the studied germplasm would allow to keep and obtaining improved genotypes

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AN EMS MUTATION ALTERING OIL QUALITY IN SUNFLOWER INBRED LINE

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ABSTRACT

The main objective of this research was to increase genetic variability of sunflower in terms of oil quality and productivity using induced mutations. A preliminary sensitivity test was performed to establish optimal ethyl-methane-sulphonate (EMS) doses for seed treatement. The results showed that high EMS concentrations (0.5-2.5%) caused low survival rates, therefore lower EMS doses were used. Thousand seeds of the sunflower high-oleic inbred line L31 were treated with 0.1% solution of EMS to induce mutations. In the M_2 generation, seeds were screened for fatty acid composition and alterations occured in individual plants. In the next generation a putative mutant line, ML31-1, was isolated with significantly lower oleic acid content compared to the wild type L31 grown in the same year. We assumed that heterozygous mutation occurred, manifested by changing a dominant allele *Ol* to the recessive *ol*. After self-polination in the next generation the segeregation of oleic acid was from 346.6 to 949.1 g/kg and of linoleic acid from 39.9 to 339.3 g/kg. In subsequent generations, individual selection and evaluation of progenies continued in several directions depending on the content of oleic acid: low, increased or high. The stable progenies were evaluated in micro-plot tests for seed yield and other agronomic traits in comparison with their respective wild type.

Key words: sunflower, induced mutation, ethyl-methane-sulphonate, fatty acids, oleic acid

INTRODUCTION

Sunflower oil has been traditionally appreciated as a high-quality commodity in the world oil market (Fernandez-Martinez et al., 2009). Standard sunflower oil is liquid at room temperature due to high content of unsaturated fatty acids. The most abundant is polyunsaturated linoleic acid (C 18:2), about 550-700 g/kg, followed by monounsaturated oleic acid (C 18:1) with 200-250 g/kg. Keeping up with the trends of the food and other industries, sunflower breeders have been able to significantly change the quality of the oil (Cvejić et al., 2014). The high-oleic sunflower hibrids have increased content of oleic acid 800 g/kg and more, compared to a standard type of sunflower. Oil of the high-oleic hybrids has excellent nutritional properties, is a suitable raw material for many derivatives of the chemical industry and for the production of high quality biodiesel, is more favorable because of higher oxidative stability, more resistance to heating and heart-healthy properties (Haddadi et al., 2011). Sunflower breeders have developed a large number of high-oleic hybrids because of the rapidly increasing interest of oil industry (Škorić et al., 2008). However, selection pressure to one particular trait can influence variability of other traits.

Sunflower genetic variability is often limited, as its genetic base of available inbred lines is narrowed. Genetic variability can be broadened by interspecies hybridization with wild species and mutation breeding (Cvejić et al., 2015). The great variability arising after mutagen treatment offers breeders unique challenge for the development of new genetic combinations (Velasco et al. 1999). Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics that significantly increase seed yield and quality (Cvejić and Bado, 2009). The first high-oleic sunflower variety Pervenec was obtained by induced mutagenesis by

seed treatment of the variety VNIIMK 8931 with the solution of dimethyl sulfate (DMS) and selection for increased content of oleic acid over 840g/kg (Soldatov 1976). Worldwide, Pervenec is used as a high-oleic trait donor in breeding programs. There are publications about other sources of high-oleic mutants with 800g/kg of oleic acid (Ivanov et al. 1992) and with 900g/kg of oleic acid (Andrich et al., 1992). Recently, the new high oleic sunflower mutant was obtained which ultra-high oleic content was not affected by temperature during grain filling, representing an advantage over the high oleic Pervenets and traditional genotypes (Leone et al. 2013, Alberio et al. 2016). The mode of inheritance of oleic acid content proved to be complex and has been studied by numerous authors, but there is no unanimity among scientists over the number of genes which control this trait (Fick, 1984; Urie, 1984; 1985; Miller et al., 1987; Fernandez-Martinez, 1989; Fernandez et al., 1999; Demurin and Škorić, 1996; Velasco et al., 2000; Lacombe and Berville, 2001; Lacombe et al., 2002; Perez-Vich et al., 2002; Vares et al., 2002; Schuppert et al., 2006). The common conclusion of all studies is that the presence of gene *Ol* is crucial for creating high oleic sunflower genotypes, while number and function of genes controlling this inheritance of this trait remain to be determined.

The main objective of this research was to increase genetic variability of sunflower inbred line in terms of oil quality and productivity. The first step was to assess the efficiency of ethyl-methane-sulphonate (EMS) mutagenic treatments, while the second is to detect mutant lines with different (changed) oil quality; this would provide new genetic variability and better crop productivity and stability.

MATERIAL AND METHODS

Plant material: Sunflower inbred line L31 (wild type) was used for mutagenesis. Line was developed in Institute of Field and Vegetable Crops in Novi Sad, Serbia. This line has over 800 g/kg oleic acid and has potential for further improvement of productivity and stability.

Mutagenic treatment: Ethyl-methane-sulphonate (EMS) mutagenesis of seeds from line L31 was performed in the Joint FAO/IAEA Laboratories in Seibersdorf, Austria. In order to determine the survival rate, fifty seeds were treated with 5 concentrations of EMS solution, 0.5, 1.0, 1.5, 2.0 and 2.5% (v/v), respectively; treatment concentrations were based on studies of other species (Kodym and Afza, 2003). Before the treatment, seeds were transferred to nylon meshes and pre-soaked in distilled water for 24 hours at room temperature. Seeds were then incubated in 200 ml of sodium phosphate buffer (0.1 M, pH 7.4) with gentle shaking (100 rpm) and different EMS concentrations were added. Incubation lasted 4 h. After the EMS treatment, the seeds were washed in distillated water several times. The control, non-mutagenized seeds were treated similarly, except for exposure to the mutagen. All treated seeds and the controls were sown in boxes using the flat method (Gaul, 1963) in a glasshouse under controlled environmental conditions (22-35ºC, lighting of 12h photoperiod). The parameter used to assess the dose response was the survival rate. The number of viable seedlings were calculated after a week of sowing and survival rate was determined by calculating number or survived seedlings per total number of planted seeds. Based on these results, batches of seeds were treated with two concentration of EMS, 0.1% (v/v) and 0.25% (v/v), respectively, and planted in the field.

Selection method: After the mutagenesis, M_1 seeds were planted in the nursery field of the Institute of Field and Vegetable Crops in Rimski Šancevi, Novi Sad, Serbia and after self-pollination of M_1 surviving plants, M_2 seeds were harvested. Seeds from each head were screened for fatty acid composition. Seeds of the wild type were grown and screened at the same time/. Mutants with altered fatty acid content were selected by screening. Seed from selected plants were planted next year in the field and after self-pollination, the M_3 seeds were collected. In next

generations plants were selected by pedigree method and seeds were screened for fatty acid composition. Fatty acid composition was measured by gas chromatography.

Agronomic evaluation: Selected mutants (M_6) and wild type were planted in comparative trial. The trials were organized in randomized block design with three replicates. Following traits were analyzed: days to flowering (from plant emergence to full flowering - UPOV - stage F3.2), plant height (10 plants per replication), seed yield per plant, thousand seed weight, oil content (NMR) and fatty acid composition by gas chromatography (AOCS Official Method Ce 1-62, 1993).

Statistical analysis: The statistical data analysis of mutant generation was performed using Statistica 12 (StatSoft, DEL, USA). The selection progress in successive generations is illustrated in table and figures. Statistically significant differences between examined traits was determined by of t-test? In order to compare distributions of oleic and linoleic acid among mutant generations it was necessary to make corrections for their fluctuation over the years (Spasibionek, 2006).

RESULTS AND DISCUSSION

In order to obtain optimal concentration of EMS solution, seeds were treated with five different doses. The effect of treatment was evaluated by calculating the survival rate. The survival rate varied from 25% (2.5% EMS solution) to 32% (0.5% EMS solution) in the glasshouse (Table 1). This drastical reduction of survival rate showed that all five doses were too high for mutagenic treatment. For that reason further bulk treatments were adjusted with 0.1 and 0.25% of EMS. Depending on the concentration of EMS treatment, the survival rate was 86% (by use of 0.1% EMS solution) and 31% (by use of 0.25% EMS solution) of the M_1 seedlings growing in the field (Table 1). Since the plants treated with 0.25% EMS solution had poor seed set (19.2%), further analysis were based on plants treated with 0.1% EMS solution. In general, results of the sensitivity test showed high frequency of lethality leading to the conclusion that less drastic EMS concentrations should be used for sunflower inbred line L31 seed mutation induction. Generally, optimal EMS concentration for mutation induction differs not only between plant species, but also between different genotype of the same crop. Osorio et al. (1995) reported that EMS concentration of 70m*M* (0.87% EMS) was used to obtain mutagenic sunflower line CAS-3. In *Arabidopsis thaliana*, the LD50 rate determined for Ler and Cor-0 seeds was 0.2% EMS for 16h and 0.13-0.25% for 12.5h, respectively (Jander et al., 2003). The LD50 rates for sugar beet seed balls were 1% EMS for 12h (Hohmann et al., 2005).

Table 1. Results from EMS treatment.

EMS Treatments	No of treated seeds	M_1 seedlings - Survival (%)	Sterility (%)	Seed set (%)
Glasshouse				
0.5%	50	32		
1%	50	30		
1.5%	50	28		
2%	50	27		
2.5%	50	25		
Total	250			
Field				
0.1%	500	86.0	0.0	75.6
0.25%	500	31.0	0.4	19.2
Total	1000	58.5	0.2	47.4

Since no mutant selection is recommended in M_1, as mutation may remain masked or undetectable due to chimerism presence (Bado et al. 2015), M_2 generation of 0,1% EMS-mutagenized population was developed. To isolate the mutants, 378 individually harvested M_2 seed stocks were screened for fatty acid composition and alterations occured in individual plants. These individual plants were planted in the next generation and the mean content of oleic acid in the seed oil decreased from 867 to 603 g/kg while mean linoleic content increased from 40 to 305 g/kg. Mutant line, designated ML31-1, was identified (Table 2). Mutant line had significantly changed oleic acid content compared to the wild type, L31, grown in the same year. We assumed recessive mutation occurred, manifested by changing a dominant allele *Ol* to the recessive *ol*, especially due to the fact that the effect of recessive gene is manifested in the later generations (Knowles, 1989). For that reason, seeds were collected from each ML31-1 plant and used as a source of segregating mutant plants.

Table 2. Oleic and linoleic acid concentrations (g/kg) in the seed oils of mutants (ML31-1, ML-31-11, ML31-12, ML31-13) and the wild type (L31) of sunflower in five M generations.

Gen eration	No of plants	Fatty acid (g/kg)	Mutants				Check	CV			
			ML31-1	ML31-11	ML31-12	ML31-13	L31	ML31-1	ML31-11	ML31-12	ML31-13
M_2	378	Oleic	790.0** (590.0[a])				821.0	22.32			
		Linoleic	144.0** (325.0[a])				94.0	25.01			
M_3	45	Oleic	603.0**				867.0	40.82			
		Linoleic	305.0**				40.0	45.84			
M_4	163	Oleic		519.9**	649.9**	863.6	850.8		12.25	22.55	8.24
		Linoleic		339.3**	231.5**	39.9	40.1		19.88	20.01	12.32
M_5	275	Oleic		514.7**	624.0**	855.0	832.5		12.26	11.22	4.86
		Linoleic		359.3**	269.0**	57.0	50.9		15.48	12.83	8.38
M_6	308	Oleic		482.0**	613.0**	887.0	867.0		9.62	10.21	8.66
		Linoleic		391.3**	270.0**	13.0	40.0		8.29	10.11	8.73

*,**significant at P=0.05 and P=0.01, respectively

[a]meaen value of individual plants

In the next generation (M_3), it was convenient to maintain mutant selection as in segregating population. After harvesting seeds were screened for fatty acid composition. The content of oleic and linoleic acid was significantly changed comparing to the wild type (Table 2). The segregation of oleic acid ranged from 346.6 to 949.1 g/kg and of linoleic acid from 39.9 to 339.3 g/kg (Fig. 1 and 2). In subsequent generation (M_4), individual selection and evaluation of progenies continued in several directions depending on the content of oleic acid: low, increased or high. We identified three subsequent mutants, designated ML31-11, ML31-12 and ML31-13.

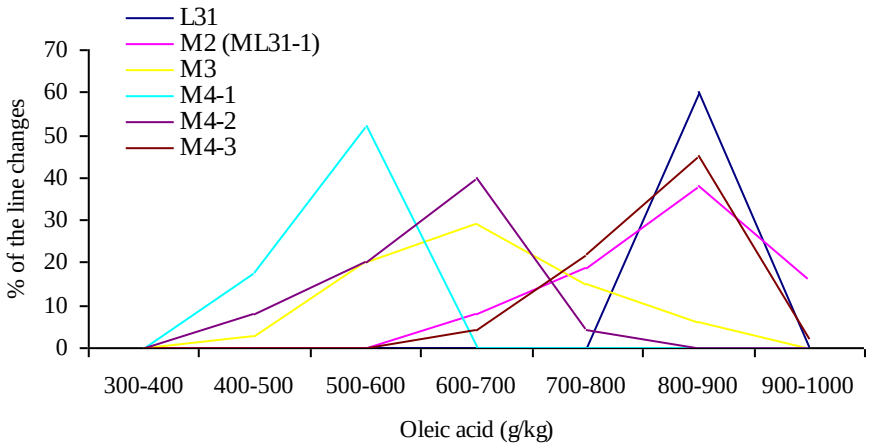

Fig.1. Distribution of oleic acid content (g/kg) in four M generations of sunflower mutant ML31-1 and subsequent mutants ML31-11 (M4-1), ML31-12 (M4-2) and ML31-13 (M4-3) compare to wild type L31

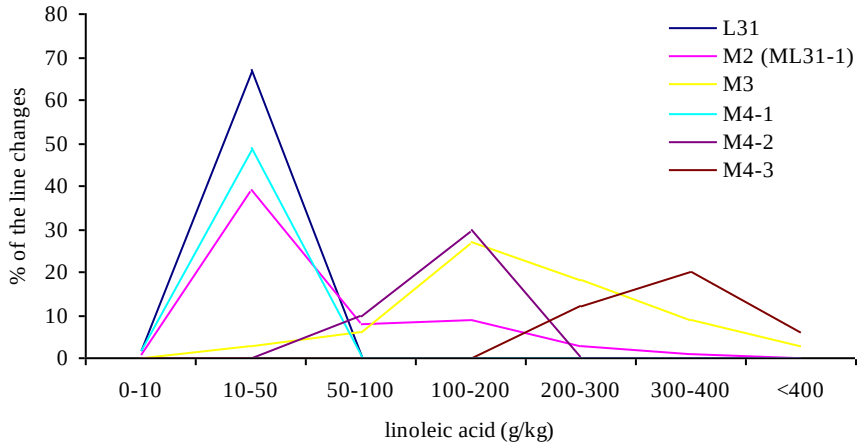

Fig.2. Distribution of linoleic acid content (g/kg) in four M generations of sunflower mutant ML31-1 and subsequent mutants ML31-11 (M4-1), ML31-12 (M4-2) and ML31-13 (M4-3) compare to wild type L31

In M_6 generation subsequent mutants ML31-11, ML31-12 and ML31-13 were evaluated and showed significant differences in one or more characteristics in regards to wild type (Table 3). Due to fatty acid content, mutant lines ML31-11 and ML31-12 had significantly lower concentration of oleic acid and significantly higher concentration of linoleic acid compared to the wild type. The content of oleic acid was higher in ML31-12 mutant line than in ML31-11. The thousand-seed-weight of these mutant lines was significantly higher than of the wild type. With respect to oleic acid content, values obtained were similar between the wild type and the mutant ML31-13, however, other examined traits such as oil content, thousand-seed-weight and seed yield were significantly higher in mutant line than the wild type (Table 3). This improvement represents the progress of wild type (line L31) thought mutation breeding since the seed yield and its components are the most important traits in sunflower production. Two mutant lines (ML31-12, ML31-12) exhibited highly significant increase in seed yield compared to the wild type. The oil content in the seed is closely linked to seed yield, which is the main purpose of sunflower growing (Škorić, 2012). Significant increase in oil content was observed in the mutant line ML31-13. This obtained increase is a very notable result, since no drastic mutation has been reported for seed oil content in sunflower (Vranceanu and Iuoras, 1991, Cvejić et al., 2015).

Table 3. Comparison between mutants ML31-11, ML31-12, ML31-13 and wild type L31 for some agronomic traits and fatty acid composition investigated in the field trials.

Traits	Mutants			Wild type
	ML31-11	ML31-12	ML31-13	L31
Full flowering (days)	58.0(±0.33)	57.0(±0.33)	58.0(±0.67)	57.0(±0.01)
Plant height (cm)	134.8(±2.10)	126.6(±0.62)	133.2(±1.21)	133.6(±0.15)
Seed yield (g/plant)	25.9(±0.13)	29.5**(±0.08)	30.1**(±0.32)	24.7(±0.05)
Thousand-seed-weight (g)	63.61**(±0.13)	63.13**(±0.11)	64.79**(±0.15)	59.5(±0.12)
Oil content (%)	50.56(±0.13)	50.09(±0.14)	54.1**(±0.13)	50.4(±0.10)
Palmitic acid (g/kg)	54.3(±0.20)	48.2(±0.08)	34.8(±0.01)	39.2(±0.01)
Stearic acid (g/kg)	59.5(±0.41)	57.6(±0.02)	50.8(±0.10)	49.6(±0.08)
Oleic acid (g/kg)	482.0**(±1.13)	613.0**(±2.23)	887.0(±2.40)	867.0(±3.08)
Linoleic acid (g/kg)	391.3**(±2.14)	270.0**(±0.21)	13.0**(±0.01)	40.0(±0.70)
Linolenic acid (g/kg)	1.1(±0.00)	1.0(±0.00)	1.2(±0.01)	1.0(±0.00)

*,**significant at P=0.05 and P=0.01, respectively

Induced mutagenesis lead to genetically inherited variability of sunflower inbred lines in terms of oleic and linoleic acid content, which will be more suitable for use in breeding programmes. Further studies will include identification of molecular changes that led to changes in oleic acid content in new mutant lines.

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SUNFLOWER GENETIC GAIN IN ARGETINA

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ABSTRACT

Genetic gain studies help breeders to refine and/or change their breeding programs in desired directions and to make estimates of future progress. There are two methods to estimate genetic gains: comparing an historic set of cultivars with uniform management or from the trial data collected by breeding programs. Using the first approach, a set of historical hybrids released to the Argentinian market by a private breeding program between 1984 and 2015 were evaluated in a four location experiment during 2014/2015 season. We included hybrids of four segments: conventional (CONV), herbicide tolerant (HT), high oleic (HO) and herbicide tolerant high oleic (HTHO). Genetic gain for oil yield in CONV hybrids was 25.5 $kgha^{-1}year^{-1}$. For the HT hybrids, the genetic gain was 63.6 $kgha^{-1}year^{-1}$. In the case of the HO, genetic gain was 49.6 $kgha^{-1}year^{-1}$. For the HTHO hybrids genetic gain was 29.1 $kgha^{-1}year^{-1}$. When the HT hybrid was launched to the market in 2003, the oil yield compared with the best CONV hybrid was 25.6% lower. The HO hybrid was launched to the market in 2004; the oil yield compared with the best CONV hybrid was 19.5% lower. In the case of the HTHO, the oil yield compared with the best CONV hybrid was 26.3% lower. The gaps have been closed for HT and HO and reduced for HTHO. Once the gaps are closed the genetic gain will depend on the level of resources dedicated to each segment.

Key words: *Helianthus annuus* L., Genetic Gain, Breeding, Sunflower, Yield drag

INTRODUCTION

Maximize the genetic gain is one of the main objective of most of the commercial breeding programs. The genetic gain can be defined as the yield increase divided by the time consumed to develop the higher yielding cultivars.

There are two different ways to evaluate the genetic gain. One is to analyze a dataset of different hybrids during a given period of time using linear mixed models (de la Vega et al., 2007). The other approach is to produce an historic set of cultivars and evaluate them in a trial network (López Pereira et al., 1999; Sadras et al., 2000; Vear et al., 2003). However, this approach has a couple of sources of inaccuracies related with the weather, the diseases and the weather by management interactions (Bell et al., 1995).

Sunflower (*Helianthus annuus* L.) crop is one of the main edible oil crops in Argentina. Cultivation of Sunflower varieties began in the early 30s by immigrants who brought seeds from Europe. In the 60s, new varieties were obtained by official breeding programs coming from the combination wild species that improved disease tolerance (Bertero and Vazquez, 2003). In 1969, Leclercq discovered the cytoplasmic male sterility and several restorer genes were discovered after, allowing a rapid spread of hybrid production by public and private companies.

The germplasm evaluated in this study is coming from the same breeding program and will be restricted to hybrids developed by Dekalb from 1984 to 1999, by Monsanto from 2000 up to 2009 and from that year to the present by Syngenta. The breeding activities started in the mid-70s in Ines Indarht, then it continues in Bragado and now it is based in Camet, all in Buenos Aires province, Argentina.

The main objectives of this breeding program have been grain yield and oil content. Also some diseases like Verticillium wilt (*Verticillium dahliae* Klebahn) and Downy mildew (*Plasmopara halstedii (Farl.) Berl. & De Toni*) have been very important breeding traits for selection.

From the 90s, new type of hybrids appeared in the market and today the Argentinean market of oil type sunflower can be separated in four segments: conventional hybrids (CONV), herbicide tolerant hybrids (HT), high oleic hybrids (HO), and herbicide tolerant- high oleic hybrids (HTHO).

After the discovery of Imazethaphyr resistance in wild sunflower in 1998 by Al-Khatib et al.; seed companies incorporated this source of tolerance to create the IMISUN hybrids with resistance to different imidazolinone herbicides. This technology that allows better weed control was rapidly adopted by farmers in Argentina. It was reported that some linkage drag around IMISUN gene coming from wild sunflower produced a decrease in oil content in the seed (Sala et al., 2012).

As a request from the industry to get better quality of sunflower oil, the high oleic market was developed. Breeding programs incorporated the Pervenets mutation to increase the percentage of oleic acid content in the seed. The hybrids containing this mutation produce a different profile of fatty acids with high percentage of oleic acid and industry pays a premium price to the farmers for high oleic content above 80%. Depending of the donor of this mutation and the quality of the conversion, more or less yield drag has been observed in this type of hybrids compared with the CONV hybrids.

The newest market segment in Argentina is the HTHO that combine herbicide tolerance and high oleic acid content. Hybrids from this segment presented the largest yield drag.

The objectives of this work were: To quantify the genetic gain of this breeding program considering hybrids developed from 1984 up to 2015 and to calculate yield drags between market segments and the evolution they had.

MATERIAL AND METHODS

SITES AND CROP MANAGEMENT

Rainfed trials were conducted in five locations: Quemú Quemú, América, Olavarría, Camet and Necochea. All of them were located within the sunflower production area and the planting dates where the same as farmers (Table 1). Rainfalls during growing season were adequate for crop development. A final plant density of 50000 plants ha-1 was achieved planting higher densities and then doing manual thinning at the stage of V2 (Schneitter and Miller, 1981). Herbicides were applied after planting and remaining weeds were controlled manually. Insecticides were applied for insect control. Fungicides were applied in R1, no pressure of disease was observed.

Table 2. Location, planting dates and coordinates.

Location	planting date	GPS coordinates
America	10-Oct-2014	35°30'S 63°30'W
Quemú Quemú	14-Oct-2014	36°30'S 63°35'W
Olavarría	2-Oct-2014	36°41'S 60°22'W
Camet	16-Nov-2014	37°46'S 57°53'W
Necochea	22-Nov-2014	38°33'S 58°53'W

PLANT MATERIAL

Hybrids were produced during summer 2013-2014 in Syngenta sunflower breeding station in Camet. Most of the materials were chosen by the year of registration, performance and farmer adoption level. Table 2 summarizes hybrids, year of registration and market segment.

Table 3. Hybrids included in the trials, year of release to the market and market segment.

Hybrid name	year of release	market segment	Hybrid name	year of release	market segment
DK G100	1984	CONV	DK 3880CL	2003	HT
DK G105	1990	CONV	DK 4000CL	2003	HT
DK 3881	1993	CONV	DK 3910CL	2008	HT
DK 4100	1994	CONV	DK 3948CL	2008	HT
DK 3878	1997	CONV	SYN 3970CL	2012	HT
DK 4040	1997	CONV	SYN 4070CL	2012	HT
DK 3915	1997	CONV	DK_OILPLUS3845	2004	HO
DK 4050	1999	CONV	DK_OILPLUS3945	2004	HO
DK 3920	2002	CONV	SYN 3950HO	2011	HO
DK 3820	2003	CONV	SX132397HODM	2015	HO
SPS3150RDM	2004	CONV	DK 3955CLHO	2009	HTHO
DK 3810	2004	CONV	SYN 3965CLHO	2013	HTHO
DK 4045	2005	CONV			
DK 3940	2006	CONV			
DK 4065	2009	CONV			
SYN 3825	2013	CONV			

EXPERIMENTAL DESIGN AND MEASUREMENTS

A randomized complete block design with two repetitions was used for trials. Plot size was 7 meters by 4 rows. Interrow distance was 0.7 meters. Only the 2 central rows were harvested with a combined harvester machine. Samples were collected to measure oil content with nuclear magnetic resonance equipment. Days from planting to R 5.5 were measured in Quemú Quemú and Camet.

CALCULATIONS

Genetic gain was calculated as the slope of the regression between the trait and year of release. To make it comparable with other studies the genetic gain was also expressed as the % of the average yield for the considered period. Oil yield was calculated as the product of grain yield and percentage of oil content. Oil yield gap between market segments was calculated by the difference among best yielding CONV hybrid and traited hybrid expressed as the percentage of the CONV.

RESULTS AND DISCUSSION

YIELD PERFORMANCE OF CONV MARKET SEGMENT

When genetic gain of oil yield in each location was analyzed, a similar slope in Camet, America and Necochea and Olavarría was observed (Table 3). Yield data from Quemú Quemú was excluded from the analysis due to poor quality.

Table 4. Slope and determination coefficient for the linear regression analysis between oil yield and year of release for trial locations.

Location	Slope	r^2
América	21.14	0.391
Camet	25.58	0.398
Necochea	24.20	0.505
Olavarría	31.08	0.638

The genetic gain derived from the combined analysis of 4 locations was 25.51 kg year^{-1} for oil yield (Fig. 1). That rate represents 1.77 % of the average oil yield of the period considered. De la Vega et al. (2007) also found a positive trend in oil yield using a linear mixed model to calculate the best linear unbiased predictor. The reported genetic gain was up to 14.4 kg year^{-1} for oil yield in a dataset from central Argentina from 1983 to 2005.

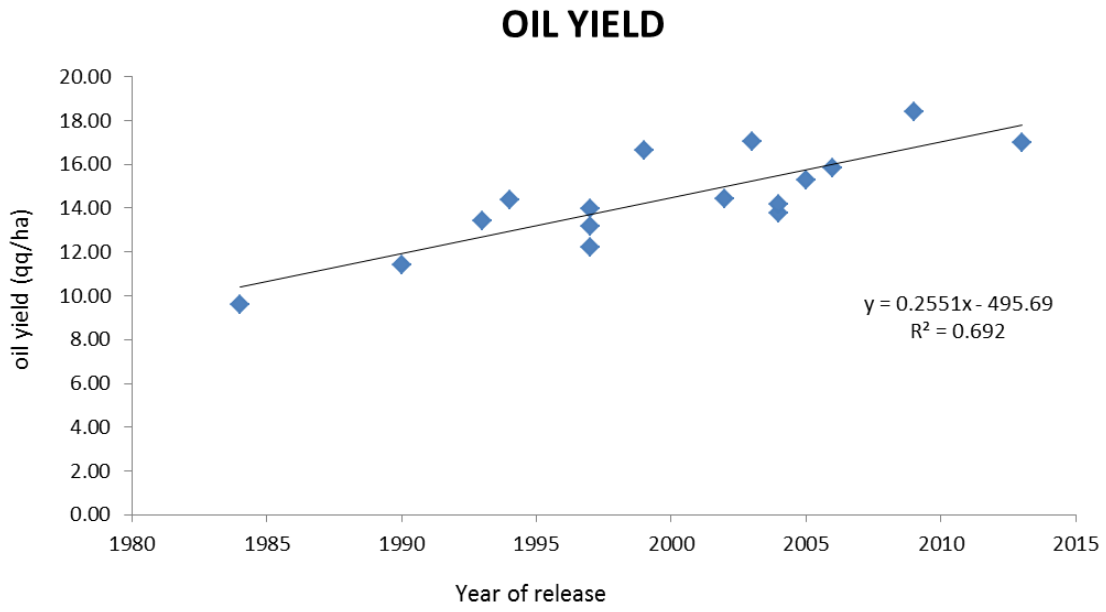

Figure 9. Linear regression between oil yield and year of release combining four locations.

Sadras et al. (2000) found a positive correlation between oil yield and year of release when cultivars from 1963 to 1998 were evaluated. However, no genetic gain was calculated. López Pereira et al. (1999) studied set of historical cultivars released between 1930 and 1995 and found positive association between grain and oil yield and year of release. This association has not been constant, a clear turning point was observed during the seventies with the introduction of hybrid cultivars. No significant improvement has been found after that point. These authors postulate that the lack of improvement might be related with "defiency" breeding (Richards et al., 1997). This means that breeding for disease tolerance, grain quality and a narrow genetic base might have restrained genetic gain for yield.

In France, a genetic gain study using a similar approach of this one but using cultivars released from 1960 up to 2000 found an improvement that represents 1.3% for grain yield per year (Vear et al., 2003). In a more recent study in the United States of America, Hulke and Kleingartner (2014) found a genetic gain of 0,698% for cultivars released from 1975 up to 2013. The main reasons for this level of genetic gain were related with the focus on defensive breeding. In this study, a clear progress in oil yield was found. The difference in the results of this study and the others found in literature might be explained by the fact that other assessment of the genetic gain for sunflower used different set of hybrids, different periods and different methodologies.

The genetic gain in oil yield for this particular study was only explained by grain yield and not by oil content. When components of oil yield were analyzed separately, the grain yield genetic gain was 46.92 kg year^{-1} but no clear tendency for oil content was observed (Fig. 2). The lack of association between oil content and year of release in this germplasm could be because the first released hybrids already have similar levels of oil content to the recently released hybrids. An example of this is the hybrid G100 registered in 1984 with an average oil content of 52.5%.

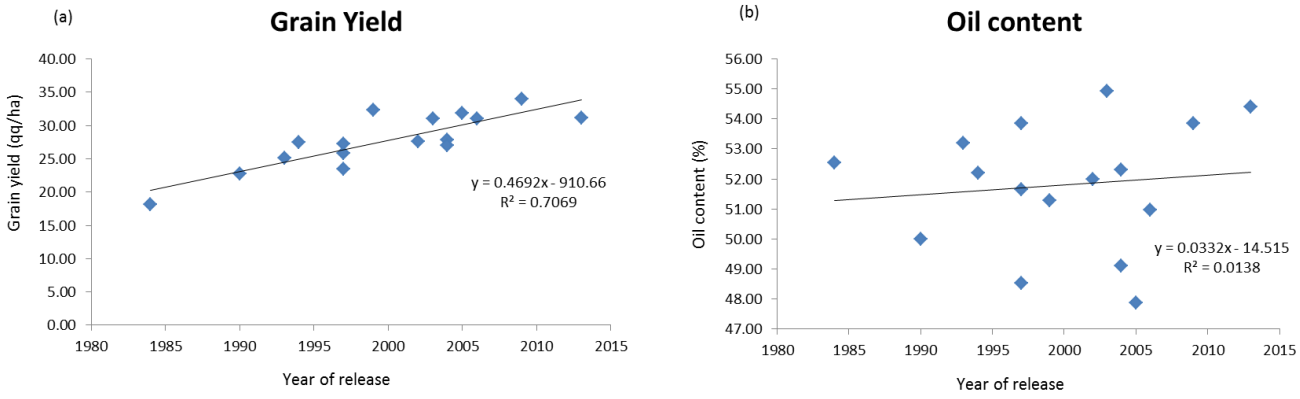

Figure 10. (a) Grain yield and (b) oil content linear regression with year of release.

De la Vega et al. (2007) found a bi linear function for the description of the relation of grain yield and year of release with a clear improvement from 1983 until 1995 and no improvements after that. Two reasons are postulated to explain this plateau. First, that in Argentina, during that period, a change in breeding process resulted in an increase of oil yield mainly due to the higher grain oil concentration rather than grain yield. Second, from 1991 to 2005 the explosive growth of soybean in central Argentina pushed the sunflower industry toward more marginal, lower rainfall western environment giving as result the declining of grain yield genetic gain.

COMPARISON BETWEEN MARKET SEGMENTS

Genetic gain for different market segments are presented in Fig. 3. Trendlines for each market segment are shown. Genetic gain for oil yield was 25.5 kg year^{-1}, 63.6 kg year^{-1}, 49.6 kg year^{-1} and 29.1 kg year^{-1} for CONV, HT, HO and HTHO respectively. It is important to point out that only few hybrids were used to calculate genetic gain for segments HT, HO and HTHO.

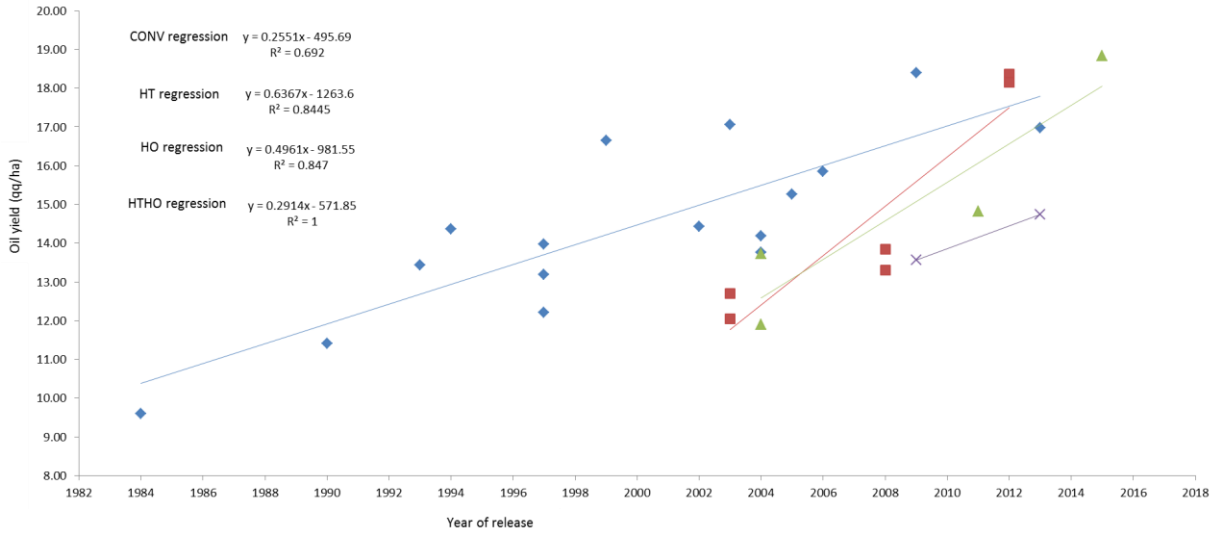

Figure 11. Linear regression between oil yield and year of release for four market segments. Symbols: diamond = CONV market, squares = HT market, triangles = HO market, cross = HTHO market.

As expected, HT, HO and HTHO have higher genetic gain than CONV segment mainly due to the fact that the first hybrids registered including the new traits presented a yield gap compared with the best yielding CONV hybrids. The reason of that gap might be related with the introduction of undesired alleles coming from the donor lines used as source of the new traits. The other reason for that gap could be related with the conversion process that had few backcrosses and the lack of tools like molecular markers for trait detection and background recovery.

First HT hybrids were released in 2003 with an oil yield gap of 25.52%. By 2012 this gap was closed reaching similar levels of performance with the CONV segment. A total of 10 years were needed to close this gap. The genetic gain in oil yield in HT market segment was explained by both, an increase in grain yield and oil content (Fig. 4). This situation differs from CONV segment where genetic gain in oil yield was explained only because of grain yield. This is an indicator of oil content linkage drag with of the introduction of IMISUN trait, as IMISUN gene comes from wild sunflower.

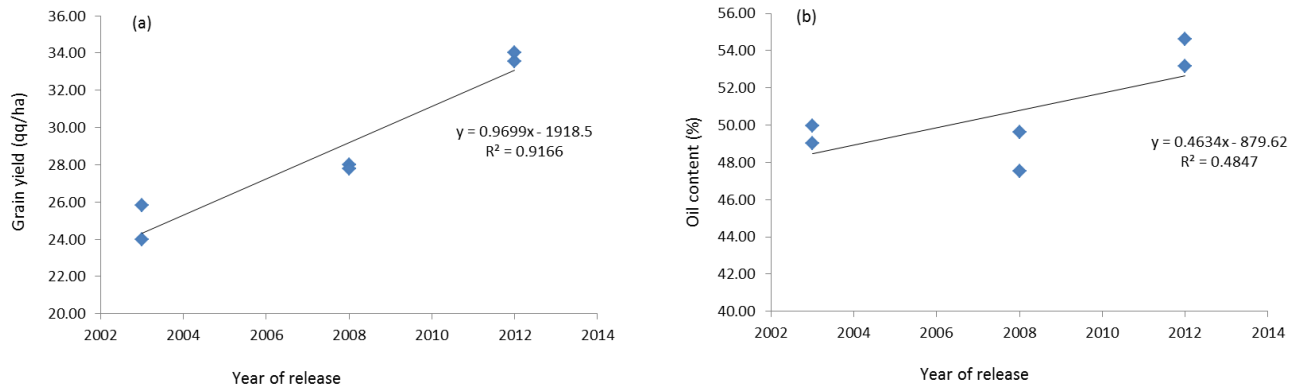

Figure 12. (a) Grain yield and (b) oil content linear regression with year of release for HT market segment.

First HO hybrids were released in 2004 with an oil yield gap of 19.5% and by 2015, after 12 years of breeding, this gap was closed and now HO hybrids have similar performance to CONV hybrids. The genetic gain in oil yield in HO segment was explained because of grain yield, as there is no tendency in oil content (Fig. 5).

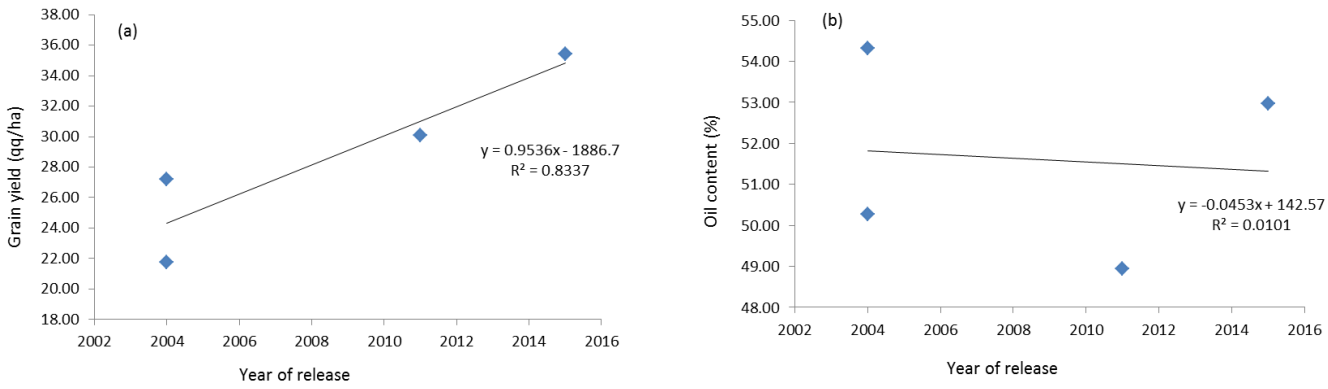

Figure 13. (a) Grain yield and (b) oil content linear regression with year of release for HO segment.

In the HTHO segment only two hybrids were included as this is the newest market segment. First HTHO hybrid was released in 2009 with an oil yield gap of 26.3% compared with the best CONV at that time. The second release in 2013 reduced the oil yield gap to 19.9%.

CYCLE LENGTH.

Cycle length defined as days from planting to R 5.5 showed a positive correlation with the year of release in all market segments (Fig. 6 a-b-c). Considering harvest moisture as indirect measurement of cycle length also presented positive correlation with year of release (Fig. 6 c-d-e). The same positive correlation was found comparing cycle length and oil yield (Fig. 7).

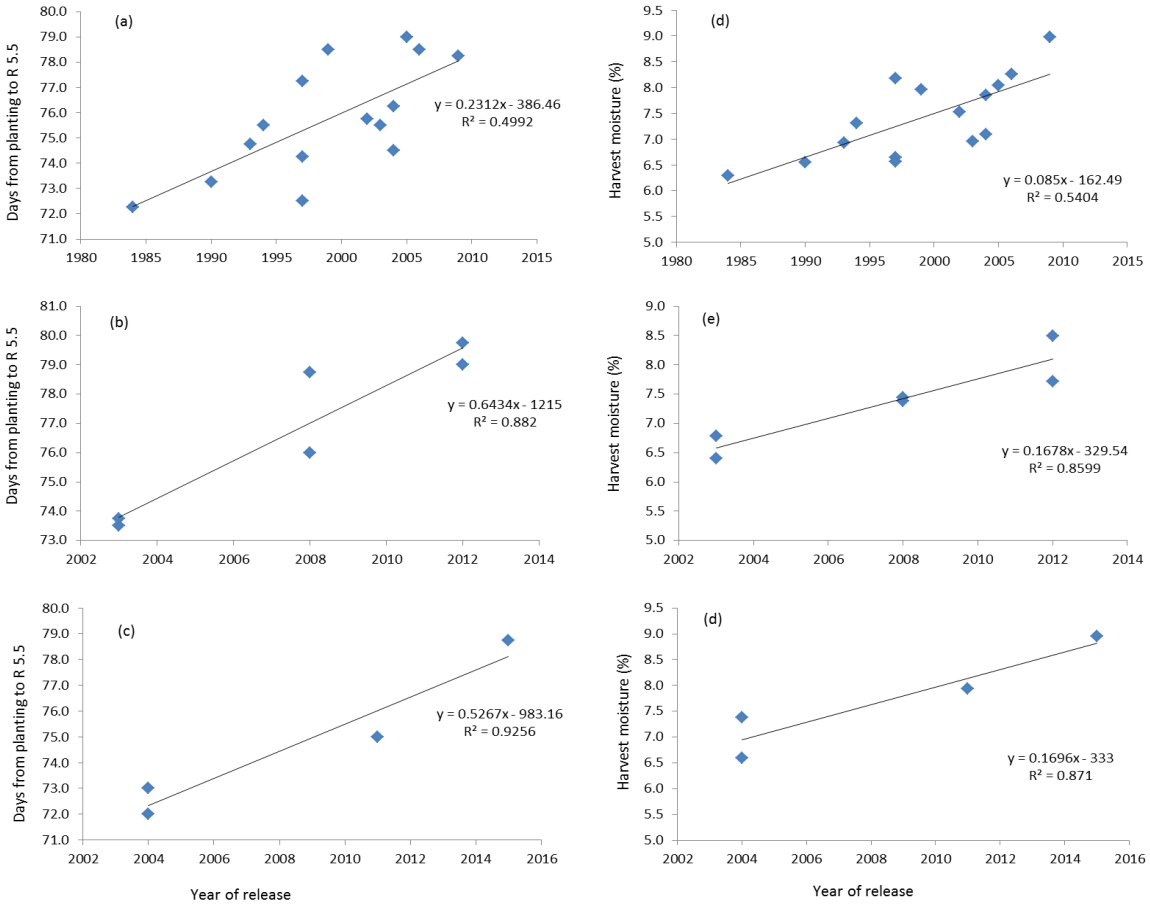

Figure 14. Relationship between cycle length and year of release. Cycle length as days from planting to R 5.5: (a) CONV market segment, (b) HT market and (c) HO market. Cycle length as harvest moisture: (d) CONV market segment, (e) HT market segment and (f) HO market.

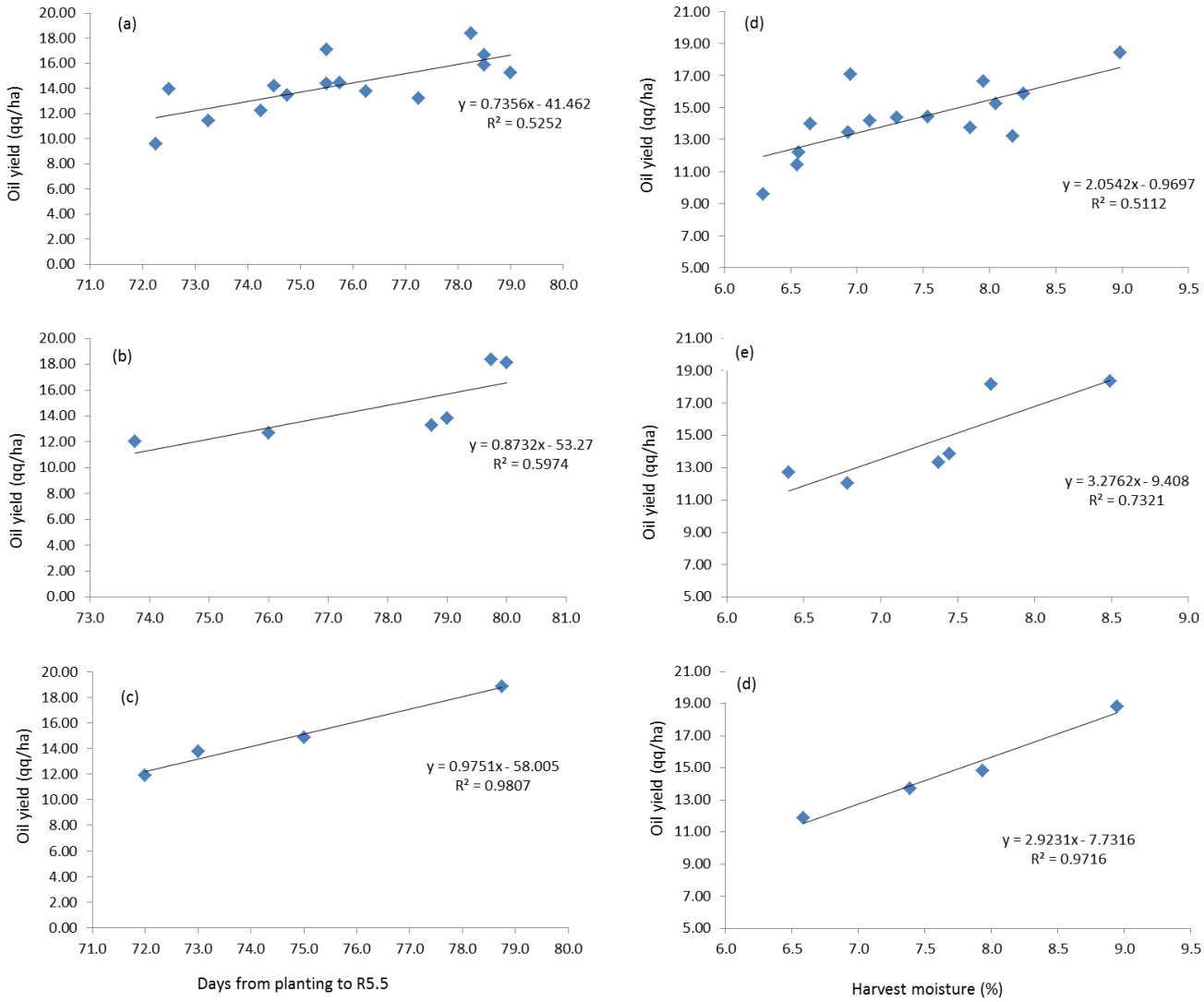

Figure 15. Relationship between oil yield and cycle length. Cycle length as days from planting to R 5.5: (a) CONV market segment, (b) HT market segment and (c) HO market segment. Cycle length as harvest moisture: (d) CONV market segment, (e) HT market segment and (d) HO market segment.

Earlier studies indicated no relation between days to anthesis and year of release (De la Vega et al., 2007; Sadras et al., 2000). Even more, Lopez Pereira et al., 1999 reported that breeding and selection shortened cycle length during the period 1930 and 1995 and that most of this reduction in cycle length was accounted for a reduction in time to anthesis.

CONCLUSIONS

Oil type sunflower breeding programs objective is to increase oil yield but strategies to achieve this could be different. In this study, and for the hybrids included, we reported different tendency in grain yield, oil content and time to anthesis compared with other studies that included a different set of Argentinean or foreign cultivars.

As breeding strategies could be different among breeding programs, studies that mix many pools of germplasm could be undercovering specific effects or tendency for different traits in each germplasm. Genetic gain studies done by specific germplasm might allow detecting these tendencies that each breeding program improved to get the final objective of increasing oil yield.

This particular germplasm is in active growth as no plateau was detected either for grain or oil yield. The increase in cycle length has reached the maximum value for suitable season growth in Argentina. The challenges for this germplasm will be to maintain the same rate of genetic gain in oil yield.

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PRODUCTION POTENTIAL OF NEW SUNFLOWER HYBRIDS DEVELOPED AT DOBRUDZHA AGRICULTURAL INSTITUTE – GENERAL TOSHEVO

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ABSTRACT

Sunflower breeding at Dobrudzha Agricultural Institute – General Toshevo (DAI) is traditionally carried out at a high level showing very good results. The working collection includes over 6 000 inbred lines. In the past decade, many new materials with very good combining ability and valuable properties were developed here. The new released hybrids possess high production potential. Over 1400 hybrid combinations are annually being tested in Bulgaria and abroad. Work is primarily focused on two-linear simple hybrids with full fertility restoration. A large part of the new genotypes were included in official varietal testing networks in Bulgaria and abroad; having demonstrated high performance, they were registered in the varietal lists of EU and other countries. These are hybrids Alpin, Veleka, Vokil, Velko, Gabi, Mihaela, Dea, Sevar. A number of foreign companies included our hybrids in their catalogs and are now promoting them. The testing of already released hybrids is ongoing with a view of their distribution on larger territories under variable environments. The developed sunflower lines and hybrids are the result from the efforts of the entire research team of the Sunflower Breeding Department at DAI who are working on several important projects aimed at higher productivity and oil content, variable chemical composition of oil, early maturity, drought tolerance and resistance to economically important diseases and the parasite *Orobanche*. The aim of this investigation was to present a brief characterization of the new DAI hybrids, demonstrating their productivity and adaptability potential.

Key words: Sunflower, New hybrids and lines, Production potential, Official varietal testing

INTRODUCTION

Sunflower is an oil seed crop of primary importance in Bulgaria. The country is one of the greatest exporters of sunflower worldwide (Christov et al., 2009). In 2013, the export reached a peak – over 1 000 000 tons, which was 17.8 % of the global export. Almost two thirds of the production from this crop are being annually exported.

During the last decade the volume of the produce increased twice, from 788 000 tons in 2003 to 1 697 000 tons in 2015. This is a result from both the larger areas sown with sunflower and the higher mean yield, which was about 1200 kg/ha in 2003, and 2120 kg/ha in 2015. Annually 700 000 – 800 000 ha of sunflower are sown in Bulgaria. It has been noted that during the recent years the normal crop rotation is not being observed and sunflower is sown at an interval of 2-3 years, some times even only one year. Although profitable, this practice should be limited because it can cause the distribution of a number of diseases and pests, which, on its turn, can compromise irreversibly the sunflower crops.

Under the contemporary conditions characterized with certain variations of the abiotic and biotic environmental factors, adequate response is needed; such a response requires development of new

breeding materials with enhanced productivity and resistance to stress influences of various nature (Ivanova and Mihova, 2012, Marinković et al, 2011; Mihova, 2011; Gonzáles et al, 2013; Khan et al., 2013). Specific methods for evaluation of the genetic variability and selection of genotypes with high adaptability potential are applied (Mihova, 2013). The year 1917 can be considered the beginning of sunflower breeding in Bulgaria (Stoyanova et al., 1977). In 1963, the breeding and improvement work on development of hybrids started, using the method of inter linear hybridization (Petrov et al., 1994). The practical application of the methods for heterosis breeding became possible after the discovery of a stable CMS source by Leclercq (1969) and fertility restorer genes for this type of CMS (Enns et al.; Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenesko, 1971). There are several schemes for hybrid seed production developed at DAI (Velkov and Stoyanova, 1974), but only one of them is used in practice – a simple hybrid with full restoration of fertility.

In 1979, the first Bulgarian hybrid Start was released and distributed on the territory of the entire country (Gotsov et al., 1981; Ivanov et al., 1988). A new page of Bulgarian sunflower breeding was opened in 1988, when the new hybrid Albena was registered in France, followed by registration in Bulgaria as well on the next year. During the years to come it became the most widely distributed hybrid in Bulgaria, while in France it occupied as much as 42 % of the sunflower production areas. The hybrid was grown very successfully in other European countries as well. The high results of hybrid Albena in Bulgaria and abroad made it a world standard. More than 20 own and joint hybrids involving the mother line of Albena have been registered. Up to now mainly early hybrids have been developed at DAI. The best known among them are Super Start, Dobrich, Mussala, Maritsa, Rada, Merkuryi, Perfekt, Diamant, and the large-seeded variety Favorit.

During the 1990's, hybrid San Luka was released, which occupied about 90 % of the sunflower areas in Bulgaria till 2008. For more than a decade now, the invasion of foreign hybrids in Bulgaria has increased considerably (Georgiev et al., 2009). Almost all major sunflower seed companies introduced new products in the domestic market and aided by their enormous financial potential logically displaced our own hybrids from the sunflower production areas. We were unprepared economically for this situation. However, efforts were made and opportunities were found for distribution of our products abroad. Now our hybrids are successfully produced and traded on the Ukrainian, Russian, Romanian, Moldovian and other markets. The testing of our hybrids with the aim of their registration is ongoing.

At DAI, the breeding of sunflower is carried out in three main directions:

Higher productivity;

Higher resistance to the economically important diseases, the parasite *Orobanche*, some herbicides, soil and air drought; Higher oil percent in seed и and higher variability of its chemical composition.

The work in these directions is in accordance with the present reality of sunflower production, the scientific achievements in this field, the conventional and bio technology methods applied in breeding, the human resources and last, but not least, the financial means available in our system. Our work is focused on the use of heterosis by developing and investigating inbred lines, testing of experimental hybrids and production of seeds from parental lines of already developed and registered hybrids (Petrov et al., 1994).

The aim of this investigation was to present a brief characterization of the new developed and registered hybrids of DAI – General Toshevo, revealing their production and adaptability potential.

MATERIAL AND METHODS

The main investigations related to this study were carried out at DAI – General Toshevo. They are the result from the implementation of a long-term program which encompasses several 4-year periods. The main purpose of this program is to develop sunflower hybrids with enhanced production potential, resistant to economically important diseases and the parasite *Orobanche* by combining conventional and biotechnology methods.

Breeding material was used, which includes Bulgarian and foreign direct and hybrid varieties, landraces and foreign populations, our old sterility maintainer lines, their sterile analogues and fertility restorers, wild species of genus *Helianthus*, and species from other genera of *Compositae* family. To obtain new forms, lines and hybrids, the following methods are applied: hybridization (interspecific, interlinear, intraspecific and intergeneric), experimental mutagenesis, selection, gamma-induced parthenogenesis, embryo culture, somaclonal variation, combined use of *in vitro* methods and physical mutagenesis.

Eight new male fertile two-linear sunflower hybrids were developed through the method of interlinear hybridization. These are Alpin, Veleka, Vokil, Velko, Gabi, Mihaela, Dea and Sevar. The mother components of these hybrids are lines 2607, 217, 3607 and 807. They possess very good general and specific combining ability and resistance to economically important diseases; with the exception of line 2607, they also possess resistance to the parasite *Orobanche*, races A-F.

Using these mother lines, other new hybrids registered abroad have also been developed, as well as hybrids which are now in the process of official testing. The fertility restorers involved in the new hybrids are 10681R, 166R, 340R, 105R, 127R, 10671R, 509R and 626R. They are all resistant to the above diseases and the parasite *Orobanche* and have excellent combining ability. All are branched and rich in pollen.

Each new sunflower hybrid goes through three-year testing in the trial fields of DAI according to a growing technology approved for this crop (Georgiev et al., 1997). The standards used in this testing were the most widely distributed hybrids in Bulgaria – San Luka and Maritsa, as well as the most highly productive and most marketed foreign hybrids Brio, Diabolo, Meldimi, Clarica, PR64F50, LG5665, PRLE19, PRLE25, etc.

Having demonstrated very good results, the new hybrids were provided to our partners from Saaten Union – Romania to produce and distribute them. Following one more year of testing in their experimental fields, they were subjected to a three-year official testing at 10 locations within the system of the State Institute for Variety Testing and Registration. Having once again demonstrated very good results, the new sunflower hybrids were officially registered. They are enlisted in the European catalog of field and vegetable crops. The trait seed yield kg/ha was read. The observations and the evaluation of the morphological characters were made in accordance with the UPOV protocol (2002). The phytopathological characterization of the hybrids was made at DAI – General Toshevo. The resistance to downy mildew (*Plasmopara halstedii*) was determined according to the standard methodology (Vear and Tourvieille, 1987) adapted to the working conditions of the institute. The response of the hybrid to races 700 and 731 of the pathogen was presented as percent of resistance.

The resistance to grey spots on sunflower (*Phomopsis helianthi*) was done according to the method of Encheva & Kiryakov (2002) under field conditions against artificial infection background. The type of attack was read one week after full flowering and at stage milk maturity according to the following scale: 0 – no symptoms; 1 – necrotic spot up to 5 cm in diameter; 2 – necrotic spot with diameter more than 5 cm; 3 – several merged necrotic spots on stem; 4 – stem broken at the place of infection.

The testing for black spots on sunflower (*Phoma macdonaldii*) was carried out under field conditions in an artificial infection field. Inoculation was done at stage budding – beginning of flowering according to the method of Maric et al. (1981). The response of the plants was read at stage yellow-brown maturity according to a 4-degree scale: 0 – no symptoms; 1 – necrotic spot localized around the petiole; 2 – several merged necrotic spots on stem; 3 – entire stem covered with necrotic spots or broken.

The resistance to the parasite broomrape (*Orobanche cumana*) was determined by the method of Panchenko (1975). The evaluation was made under greenhouse conditions using the index percent of resistance. The experimental data were analyzed by ANOVA 3. The applied statistical model was:

$$Yijk = Y.. + Gi + Yj + Rk + (GY)ij + (GR)ik + (YR)jk + (GYR)ijk + Eijk$$

where Gi is the factor genotype , Yj is the factor climatical conditions, and Rk - the factor location.

The Additive Main Effects and Multiplicative Interaction (AMMI) model has developed a new statistical method for analyzing the genotype by environment interaction. In the AMMI method, first the main additive effects of genotype and environment are considered by variance analysis, then are analyzed by principal characteristics of remain value from variance analysis model (Gauch et al, 1996; Dias et al., 2003, Lee, 2004). Totally, AMMI follows three basic purposes: first, this is an appropriate method for primary analysis of performance tests. Second, it explains the effect of the genotype × environment interaction. Third, performance estimate is done with greater accuracy. This method is applied to estimate the ecological stability and plasticity of the hybrids. Data were analyzed with the help of the software SPSS, version 19.0.

RESULTS AND DISCUSSION

Following the official testing, all new hybrids underwent a two-year test for distinctness, uniformity and stability at the State Institute for Variety Testing and Registration – Romania. They were acknowledged as distinct, uniform and stable. Their morphological descriptions were done using the methodology of UPOV (2002).

BIOLOGICAL AND ECONOMIC PROPERTIES

The eight new Bulgaria hybrids presented here are from the group of the medium early varieties, with growth season 110 – 120 days; however, the earliest among them are Alpin, Mihaela and Sevar. They all have linoleic type of oil, with oil percent within the range 46-50 %. The number of seeds per plant can reach up to 1200 – 1300, which makes their production potential very high, exceeding sometimes 4300 kg/ha. The stems are medium high and resistant to lodging. All hybrids possess very high plasticity with regard to the growing conditions. Hybrids Velko and Dea demonstrated highest drought resistance.

The possibility of sowing both parental forms simultaneously is a great advantage of all presented hybrids. The father lines are strongly branched and very rich in pollen, which, on its part, allows planting design of 10:2 female to male lines. This design should naturally be provided with at least 3-4 good bee hives per ha.

PRODUCTIVITY

All eight new hybrids were subjected to three-year official testing at the State Institute for Variety Testing and Registration – Romania. The testing was carried out at 10 locations representative for regions with various soil and climatic conditions from all over the country. The first three, Alpin, Veleka and Vokil were tested during 2009 – 2011 (Table 1).

The three years of investigation were with comparatively similar climatic conditions as evident from the given mean yields, which were with similar values and low variation. Averaged for the investigated period, all new hybrids exceeded the standard. Hybrid Alpin demonstrated highest mean results with an average yield for the entire period of testing 3572 kg/ha, followed by Vokil and Veleka.

Table 1. Mean results from 10 locations of official testing of hybrids Alpin, Veleka and Vokil

Hybrids	Yield kg/ha	% from standard	Yield kg/ha	% from standard	Yield kg/ha	% from standard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2009		2010		2011			
Standard	3108	100	3240	100	3659	100	3336	100
Alpin	3489	112	3619	112	3609	99	3572	108
Veleka	3272	105	3368	104	3485	95	3375	101
Vokil	3259	105	3356	104	3635	99	3417	103

The group of hybrids including Velko, Mihaela and Gabi were tested during 2011 – 2013 (Table 2). In this case, again, the mean results of the three hybrids were above the mean standard of the entire investigated period. The exceeding was within the range 4 – 9 %, hybrid Velko being the highest yielding. It was also the hybrid with highest mean results out of all eight according to the index seed yield during the entire official testing - 3578 kg/ha. Hybrid Velko also gave best results during the less favorable year 2012.

Table 2. Mean results from 10 locations of official testing of hybrids Velko, Mihaela and Gabi

Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Averaged for 3 years kg/ha	Relative yield according to the standard, averaged for 3 years
	2011		2012		2013			
Standard	3659	100	2648	100	3642	100	3316	100
Velko	3694	101	3072	116	3969	109	3578	109
Mihaela	3618	99	2934	111	3757	103	3436	104
Gabi	3689	101	2879	109	3947	108	3505	106

The last two hybrids, Sevar and Dea, were officially tested during 2012 – 2014 (Table 3). They also exceeded the standard, averaged for 3 years. Dea showed better results, especially in 2012, the year with lowest precipitation, which defines this hybrid as drought resistant.

Table 3. Mean results from 10 locations of official testing of hybrids Sevar and Dea

Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Averaged for 3 years кг/ха	Relative yield according to the standard, averaged for 3 years
	2012		2013		2014			
Standard	2648	100	3642	100	3234	100	3175	100
Sevar	2650	100	3959	109	3210	99	3273	103
Dea	2954	112	3783	104	3424	106	3387	107

The results from the dispersion analysis are presented in Table 4.

Table 4. Dispersion analysis for the index ***productivity***

	Mean of square	df
G	11089,8 [c]	8
Y	15793 [c]	2
R	7726,6 [c]	7
G x Y	1416,1 [c]	16
G x R	438,1 [a]	56
Y x R	4061,7 [c]	14
G x Y x R	474,3	112
Error	221,4	278

The applied dispersion analysis proved with a high degree of significance (p=0.001) the differences in the genetic potential of the selected hybrids according to the index ***productivity***. The effects of the factors climatic ***conditions*** and ***location*** were with highest significance.

The interaction between the indices ***genotype x climatic conditions*** and ***genotype x location*** was also determined statistically. The lower significance of /G x R/ showed that the predominant part of the genotypes responded in a similar way to the various locations of testing. Their response to the year conditions /G x Y/ was specific. This fact allowed applying AMMI (1, 2) models for evaluation of the ecological plasticity and stability of the investigated hybrids with regard to the index productivity (Table 5).

Five of the investigated Bulgarian hybrids had ASV < 0.200 indicative of high ecological stability and plasticity. Hybrids Alpin, Veleka and Vokil showed lower response to the changeable environments with regard to the index productivity. Hybrids Sevar, Dea and Gaby had more susceptible reaction.

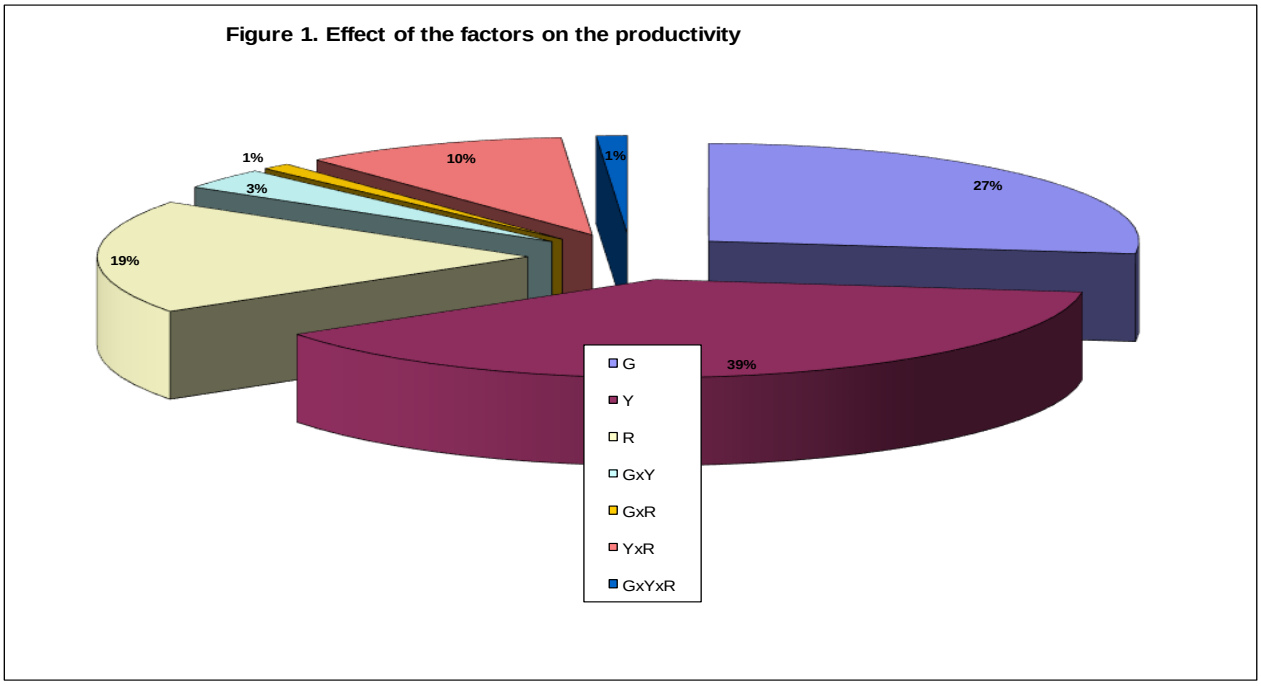

Table 5. Ranking of investigated hybrids by the index ***productivity*** according to their ecological plasticity and stability

Hybrid	**Parameters**		
	Rank	**ASV**	**I**
Alpin	1	0,022	2,432
Veleka	2	0,084	2,213
Vokil	3	0,111	1,945
Velko	4	0,165	1,929
Standard	**5**	0,180	1,896
Mihaela	6	0,187	1,854
Gabi	7	0,208	1,805
Dea	8	0,233	1,725
Sevar	9	0,276	1,664

All eight Bulgarian sunflower hybrids were officially registered and included in the European catalog of field and vegetable crops. Because of the demonstrated very good productivity potential, they were included also in the systems of official varietal testing of non-EU countries such as Ukraine, Serbia, Kazakhstan, Russia, etc

In 2013 the testing of our hybrids continued in Hungary as well, at three locations in the central and southern parts of the country (Table 6). Seed yield varied from 3150 до 5304 kg/ha, most of the results being within the range of the higher values and demonstrated a very good level of the index ***productivity***. Only hybrids Velko and Dea exceeded the standard at all three locations, and the mean value of hybrid Mihaela also exceeded it. Hybrid Dea demonstrated highest mean results - 4441 kg/da, showing once again that it is hybrid with high plasticity and productivity.

Table 6. Results from testing in Hungary

	Bekecsaba		Lanycsok		Cegled		Average	
Hybrids	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from strd
STANDART	4381	100	3892	100	3621	100	3965	100
ALPIN	3679	84	3301	85	3438	95	3473	88
VELEKA	3714	85	3411	88	3435	95	3520	89
VOKIL	3559	81	3458	89	3617	100	3545	89
MIHAELA	4926	112	3800	98	3924	108	4217	106
VELKO	4628	106	3969	102	3772	104	4123	104
GABI	3403	78	3219	83	3503	97	3375	85
SEVAR	4118	94	3554	91	3150	87	3607	91
DEA	5304	121	3922	101	4098	113	4441	112

Table 7. Results from the testing in Ukraine

Hybrids	KHARKIV		ODESSA		ZAPOROGYE		KIROVOGRAD		AVERAGE	
	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std	Yield kg/ha	% from std
STANDARD	3445	100	817	100	1823	100	3075	100	2290	100
Neoma CL	3376	98	670	82	1422	78	3290	107	2190	96
Adagio CL	3796	110	1006	123	1977	108	2970	97	2437	106
ALPIN	3397	98	930	114	1792	98	3670	119	2447	107
VELEKA	3108	90	980	120	1802	99	2520	82	2103	92
VOKIL	3498	101	1007	123	2226	122	3210	104	2485	109
MIHAELA	3671	106	630	77	2029	111	3360	109	2423	106
VELKO	3985	116	500	61	2482	136	3600	117	2642	115
GABI	3434	99	840	103	2039	112	2910	95	2306	101

In 2014, at four locations in the central, southern and eastern part of Ukraine, some of the most recent hybrids of DAI – General Toshevo were tested and compared to the most widely distributed

and highly productive commercial hybrids (Table 7). In the regions of Odessa and Zaporogye, where the climate is dryer, the results were naturally lower, while in Kharkiv and Kirovograd the hybrids revealed their true potential. The maximum yields obtained were 3985 kg/ha from Velko in Kharkiv region and 3670 kg/ha from Alpin in Kirovograd. In general, all tested Bulgarian hybrids, with the exception of Veleka, exceeded the standard at the four locations, and Alpin, Vokil, Mihaela and Velko were at the productivity level of the foreign hybrids currently dominant on the market.

PHYTOPATHOLOGICAL CHARACTERISTICS

The phytopathological characterization was done under laboratory conditions and in the infection field of DAI where all newly developed materials of the Sunflower breeding department are subjected to testing for the economically important diseases and the parasite *Orobanche*. The results from these investigations are given in Table 8.

Table 8. Phytopathological evaluation of sunflower hybrids in artificial infection field at DAI – General Toshevo.

Hybrid	*Phomopsis helianthi*		*Phoma macdonaldi*		*Plasmopara helianthi*		*Orobanche cumana*
	Attacking rate	#	Attacking rate	#	Resistance to race 700, %	Resistance to race 731, %	Resistance to races A-F, %
San Luka	3/3(3)	3	1/3(1)	1	100.0	92.9	100.0
Perfekt	1/3(1)	1	1/3(1)	1	84.5	-	100.0
Diabolo	2/3(2)	2	1/3(1)	1	100.0	90.5	100.0
Brio	1/3(1)	1	0	0	100.0	100.0	100.0
Meldimi	2/3(2)	2	1/3(1)	1	100.0	90.0	100.0
PR64F50	1/3(1)	1	0	0	100.0	100.0	100.0
Valin	2/3(2)	2	1/3(1)	1	100.0	95.0	100.0
Alpin	2/3(2)	2	1/3(1)	1	100.0	100.0	100.0
Veleka	1/3(1)	1	0	0	100.0	100.0	100.0
Vokil	1/3(1)	1	0	0	100.0	90.0	100.0
Mihaela	2/3(2)	2	1/3(1)	1	100.0	100.0	100.0
Gabi	1/3(1)	1	0	0	100.0	100.0	100.0
Velko	1/3(1)	1	0	0	100.0	100.0	100.0
Dea	1/3(1)	1	0	0	100.0	70.0	100.0
Sevar	1/3(1)	1	0	0	100.0	100.0	100.0

Attacking rate - what part of the plant stem was covered with spots of the pathogen (1/3, 2/3, 3/3). In brackets – number of spots. Rank: 0 – immune; 1 – resistant; 2 – moderately resistant; 3 – moderately susceptible; 4 – susceptible.

Hybrids Veleka, Vokil, Gabi, Velko, Dea and Sevar were resistant to the fungal pathogen *Phomopsis helianthi*, similar to other foreign high-yielding hybrids widely distributed in Bulgaria, such as Brio and PR64F50. In comparison to San Luka and the other hybrids presented in this study, they performed as more tolerant to this disease. With regard to the other important leaf pathogen *Phoma macdonaldi*, these six hybrids demonstrated immune reaction. Although with one degree only, the other hybrids were below their resistance to this disease.

With the exception of Perfekt, the other hybrids demonstrated 100 % resistance to downy mildew (*Plasmopara helianthi*) race 700. To the most recent race 731, full resistance was demonstrated by Brio, PR64F50, Alpin, Veleka, Mihaela, Gabi, Velko and Sevar. In the greater number of Bulgarian hybrids, this resistance came from the male parent. The resistance to the parasite *Orobanche cumana* was 100 % due to the contribution of both parental components.

CONCLUSION

The new Bulgarian sunflower hybrids were officially registered in Romania and were enlisted in the European catalog of the field and vegetable crop varieties. They are distinct, uniform and stable. They demonstrated high and stable yields under variable environments in Bulgaria and abroad, which makes them hybrids with high adaptability potential. They also possess high field resistance to the economically important diseases and the parasite *Orobanche*.

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HYBRIDIZATION BETWEEN CULTIVATED SUNFLOWER AND WILD ANNUAL SPECIES HELIANTHUS NEGLECTUS HEISER

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ABSTRACT

Interspecific hybridization was carried out between sterile analogues of cultivated sunflower lines with normal cytoplasm and wild annual *Helianthus neglectus* accession E-017 from collection of DAI-General Toshevo. The obtained F1 progenies were characterized from morphological, biochemical and phytopathological point of view. They were distinguished with diversity of seed oil content. The most variable phenological phases of hybrid plants from all crosses were duration of flowering period and germination. The hybrid plants from crosses 325 A x E-017, 818 A x E-017 and 3482 A x E-017 were characterized with higher seed oil content than the other studied crosses. Hybrid forms distinguished with resistance to stem canker, downy mildew and the parasite broomrape were obtained. The hybrid plants, carriers of Rf genes, could be used in sunflower breeding programs for developing restorer lines.

Key words: Hybridization, *Helianthus neglectus,* Resistance

INTRODUCTION

Helianthus species were not only the material, the sunflower varieties originated from, but also they continue to be the source of useful characters in sunflower improving work. (Thompson et al., 1981; Atlagic, 2004). Wild species are adapted to a wide range of habitats and possess a considerable amount of genetic diversity that might be a rich source of alleles for continued improvement of the cultivated sunflower (Seiler and Rieseberg, 1997; Burke et al., 2002; Škorić, 2009). The narrow genetic base of domesticated sunflower is a main concern of breeders worldwide. Wild *Helianthus* species offer a significant amount of genetic diversity, including important traits, such as disease resistance, fertility restoration, cytoplasmic male sterility, various seed-oil characteristics, protein content, fatty acid composition, tolerance to abiotic stress factors (Seiler and Rieseberg, 1997). They have been included in the breeding process as sources of valuable characters for the cultivated sunflower, but their use was attended with some difficulties, such as poor crossability and frequent F_1 sterility in interspecific hybrids, abortion of the hybrid embryo and long period of dormancy. This fact limited the usefulness of many wild Helianthus species. The wild annual *Helianthus neglectus* was the subject of research investigations, carried out by Atlagic (2004), Christov (2008), Škorić (2009), etc. They found that this wild sunflower species provided valuable genetic diversity for the improvement of cultivated sunflower. According to Christov (1993) in the annual species, differentiation of a genotype is possible, because of self-pollination of a single plant in natural or artificial conditions. In that way, a different number of stabilized genotypes, which, in total, expressed the diversity of the given species, represented each species. It was known, that within each *Helianthus* species, there is a great diversity in form and the value of individual traits.

The aim of this investigation was to obtain hybrid plants, carriers of Rf genes, resistant to some diseases (*Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii*) and to the parasite broomrape, with varied seed oil and protein content, suitable for including in sunflower breeding programs for developing diverse restorer lines.

MATERIAL AND METHODS

The investigation was carried out in Dobrudzha Agricultural Institute, General Toshevo. The accession E-017 of wild annual species *H. neglectus* was included in this study. The cultivated sunflower was represented by five CMS lines– 325 A, 818 A, 3482 A, 828 A and 846 A. The classical methods of interspecific hybridization were applied for obtaining of hybrid seeds. Interspecific crosses *cultivated sunflower x wild species* were performed and the obtained hybrid plants were grown in field conditions. As paternal component in the realized crosses was the accession of wild *Helianthus neglectus*. The sterile analogues of fertile sunflower lines with normal cytoplasm were used as maternal parents.

Phytopathological evaluations of F1 hybrid progenies were carried out in laboratory conditions and in artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berlese et de Toni) was carried out on the method of Vear and Tourvieille (1987). Evaluation for resistance to grey spots on sunflower (*Phomopsis /Diaporthe helianthi* Munt.-Cvet. et all.) was carried out on the method of Encheva and Kiryakov (2002) in field conditions on artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema / *Phoma oleracea var. helianthi-tuberosi* Sacc) was carried out on the method of Fayralla i Maric (1981) in field conditions on artificial infection plot. The seed oil and protein content was determined on the method of Rushkovskii (1957). The weight of 1000 seeds was measured on three samples, where each sample consists of 25 or 50 seeds. Ten plants from the accession, grown in field conditions, were used for this investigation in aim to collect a sufficient pollen quantity. The follow phenological characters, conformed to UPOV characteristics, were determined: germination (days), beginning of button formation (days from germination), beginning of flowering (days from germination), period of flowering (days), beginning of maturity of central inflorescence (days from germination), vegetation period (days). Germination was reviewed at cotyledons emergence of 75% of the sown seeds. Beginning of button formation was reviewed at inflorescence formation of the central stem, and beginning of flowering – beginning of flowering of central head for 25% of the plants. For the end of vegetation period was accepted the withering of stems and leaves of all plants. The seed set (%) was calculated as a correlation between a number of inseminated disk florets to the total number of disk florets in one inflorescence. The analysis of experimental data was done by the statistical package BIOSTAT 6.0. For analyzing the obtained results, consistent to the aims of investigation, the follow quantities were analyzed: arithmetic mean ($\bar{x}$) and the coefficient of variation (VC), which showed the relative uniformity or variability of the studied characters.

RESULTS AND DISCUSSION

Plants from one population of annual species *H. neglectus* was crossed with sterile analogues of cultivated sunflower lines. All obtained F1 interspecific hybrid plants were fully branched with or without central head. Cultivated lines were not branched. The branching was typical character for wild species. Anthocyanin coloration was observed on stems, leaves and rarely on the petioles of some hybrid plants. The presence of anthocyanin pigmentation and branching on F1 plants were the suitable markers for early establishment of the hybrid type of obtained plants. The obtained hybrid plants were 120 cm to 160 cm tall. Some differences in plant height, color of disk florets and leaves, length of branches were observed among plants from the same cross and between different crosses. This was due to the fact, that the paternal parent was a population. Leaves were mostly alternate, ovate and slightly serrate on the margins, truncate at the base. The crossability between *H. neglectus,* accession E-017, and cultivated sunflower was 53,3% (Table 1). This was because both species were close from the taxonomic point of view (Seiler G.J., 1992). The lower crossability was determined for the crosses 828 A x E-017 and 846 A x E-017. Seeds were obtained from all pollinated inflorescences. Similar results were obtained by Hristov M. (1990) and Nikolova L.

(1998). The difficulties, connected to the low crossability of this species, resulted by obtaining of seeds with immature endosperm and sterility of the hybrid plants.

The results from hybridization showed that the seed set was low and varied from 2,9 % for the hybrid 828 A x E-017 to 11,7 % for the hybrid 325 A x E-017. Some differences in viability of hybrid seeds were established. The percentage of viable F1 plants varied from 20 % for the hybrid 846 A x E-017 to 58,3 % for hybrid 325 A x E-017, which was distinguished also with the biggest number of seeds per head. The crosses 325 A x E-017, 818 A x E-017 and 3482 A x E-017 were characterized with better crossability and the biggest number of obtained hybrid seeds originated from them.

Table 1. Crossability between wild species *H. neglectus* (E-017) and cultivated sunflower lines (*H. annuus*).

Hybrid combination	Pollinated inflorescences			Obtained seeds			Obtained hybrid plants	
	Total number	With seeds		Average per head	Total number	Seed set %	Total number	Average per seeds, %
		number	%					
325 A x E-017	3	2		12	24	11,7	14	58,3
818 A x E-017	3	2		11	22	10,8	12	54,5
828 A x E-017	3	1		3	3	2,9	1	33,3
846 A x E-017	3	1		5	5	4,9	1	20
3482 A x E-017	3	2		12	24	11,6	10	41,7
***H.annuus* x E-017**	**15**	**8**	**53,3**	**8,6**	**78**	**8,4**	**54**	**78,46**

The phenological observations were done and the main phenological phases were studied. The mean values of the studied phenological phases and their variation for the parents and the obtained hybrid F1 progenies were presented on table 2. Hybrid plants were distinguished with higher variation than their parents regarding all studied phenological phases were. Differences were observed both between plants from the different crosses and among plants from the same cross. This was because the accession E-017 was maintained as population. Vegetation period of hybrids was shorter than that of wild species *Helianthus neglectus* and varied from 85 to 110 days for the earlier to 120-130 days for the other progenies. The duration of flowering period of hybrid plants was determined as the most variable phenological phase for all studied progenies, followed by variation in germination and vegetation.

Variation was also observed in some biochemical characters connected to seed oil and protein content of parental forms and the obtained progenies. The average values of the studied biochemical characters were presented on figure 1. Seeds of wild *H. neglectus* were characterized with low 1000 seeds weight and seed oil content, and high content of hull. The seeds of sunflower hybrid plants were with higher content of oil in seeds and kernels. The oil content of hybrids in kernel varied from 64,5% to 71% and in seeds – from 35,8% to 49%. The highest seed oil content (49%) was determined for the crosses 325 A x E-017, 818 A x E-142 and 3482 A x E-017.

Table 2. Mean values and variation coefficients of the studied phenological phases of the parents and their F_1 hybrids.

Phenological phases	P_1		P_2		F_1	
	cultivated sunflower (*H. annuus* L.)		*Helianthus neglectus* Heiser		*H. annuus x H. neglectus*	
	$\bar{x}$	VC	$\bar{x}$	VC	$\bar{x}$	VC
Germination, days	9,6	3,4	13,7	7,32	12,5	14,9
Beginning of button formation, days from germination	41,9	3,3	49,5	3,9	46,5	7,7
Beginning of flowering, days from germination	53,9	5,2	72,2	2,9	58,6	10,3
Flowering, days	6,3	13,6	48,2	4,5	12,8	18,7
Beginning of maturity of central inflorescence, days from germination	94,5	6,16	104	5,9	94,5	11,9
Vegetation period, days	112,5	10,3	155,5	4,8	120,5	12,9

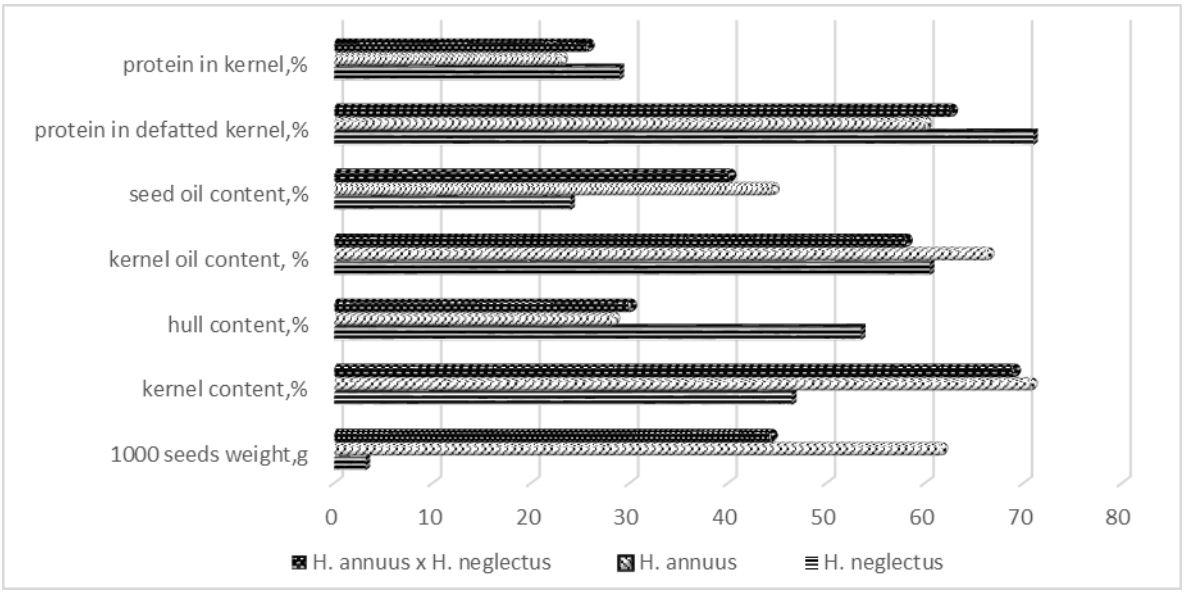

Fig. 1. Average values of characters, connected to seed oil and protein content of hybrid plants and their parents.

The hybrid seeds, originated from wild sunflower species have lower oil content compare to the cultivated sunflower, but it could be changed quickly to acceptable level by backcrossing with cultivated sunflower lines (Seiler and Rieseberg, 1997). The kernel content (%) was of importance for the total oil yield per hectare. Increasing of kernel content gave the opportunity for accumulation of bigger oil quantity in it and lead to increasing the seeds oil content, respectively, to increasing the seed yield. Seed oil content was a character, which reflected simultaneously on the kernel content and the oil content in it. Therefore, the sunflower seed oil content of hybrid plants was set up by the relative portion of kernel to the whole seeds, and the content of oil in it. The seed oil content of the new registered hybrids and cultivars was increased due to decreasing of hull content predominantly than increasing the other characters (Nikolova V., 1987). According to this dependence, hybrid plants with the lowest hull content and highest oil content were selected.

The reaction of hybrid materials to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the sources for resistance to these pathogens (Tables 3-4).
The hybrid combinations 3482 A x E-017 and 818 A x E-017 were resistant (100%) to downy mildew and the parasite broomrape. They were characterized with immune type of reaction to the pathogens caused grey and black spots on sunflower. Their vegetation period was 115-118 days. The other crosses also demonstrated a certain resistance to *Pl. helianthi* and *Orobanche cumana*. They could be successfully included in the sunflower breeding programs for developing new resistant lines.

Table 3. Phytopathological evaluation of F_1 hybrid progenies for resistance to *Pl. helianthi* and *Orobanche cumana.*

Resistance, %	Hybrid combination	
Resistance 100 % to *Pl. helianthi* Novot. and 76-99% to *Orobanche cumana* Wallr.	3482 A x E-017	818 A x E-017
Resistance 76-99% to *Pl. helianthi Novot*. and *Orobanche cumana* Wallr.	828 A x E-017 846 A x E-017	325 A x E-017

Resistant type of reaction to the pathogens caused grey and black spot on sunflower showed three hybrid forms -828 A x E-017, 846 A x E-017 and 325 A x E-017.

Table 4. Phytopathological evaluation of F_1 hybrid progenies for resistance to *Phomopsis helianthi Munt.-Cvet. et all.* and *Phoma macdonaldii Boerema.*

Type of reaction	Hybrid combination	
Immune to *Phomopsis helianthi* and *Phoma macdonaldii*	3482 A x E-017	818 A x E-017
Resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	828 A x E-017 846 A x E-017	325 A x E-017

CONCLUSION

Wild *Helianthus* species have been included in sunflower breeding programs mainly as donors for resistance to diseases and to the parasite broomrape. Transfer of genes, controlling the resistance, into cultivated sunflower lines gave the opportunity for diversification of cultivated sunflower. Resistance 100% to downy mildew and immune type of reaction to the pathogens *Phomopsis helianthi* and *Phoma macdonaldii* was established for some crosses, obtained with participation of E-017 accession.
Plants from the hybrid combinations 325 A x E-017, 818 A x E-017, 828 A x E-017, 846 A x E-017 and 3428 A x E-017 could be used as donors for resistance. They also carried Rf genes and distinguished with varied seed oil content.
The obtained hybrid materials were useful initial materials for application in sunflower breeding programs for producing restorer lines with high seed oil content and resistance to the main diseases with economic importance.

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COMPARATIVE INVESTIGATION OF IMMATURE EMBRYOS GROWING OF INTERSPECIFIC SUNFLOWER HYBRIDS

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ABSTRACT

Comparative investigation of immature embryos growing of interspecific sunflower hybrids was carried out in vitro conditions. Four hybrid combinations, in which wild annual and perennial sunflower species participated, were used. These were the hybrid combinations 807A x E-131 (*Helianthus argophyllus*), 3A x E-130 (*H. argophyllus*), 807A x M-129 (*H.divaricatus*) and 3A x M-146 (*H.tuberosus*). In in vivo conditions the embryos necrotized and died because of incompatibility between species. In in vitro conditions, the method of embryo rescue was applied. On the base of this method, different number of hybrid embryos was isolated. The tissue culture of Azpiroz et al. (1987) was applied, when some modifications of the tissue were used. The results of the investigation showed that using embryo rescue the hybrid plants could be grown. The seed set of hybrids, originated from perennials was too low than that of hybrids, originated from annual species. The obtained hybrid plants were cultivated in greenhouse conditions and sufficient quantity of seeds was obtained. Next generations were grown in field conditions. They were used as initial material for developing sunflower lines with valuable agricultural characters, resistant to biotic and abiotic stress factors.

INTRODUCTION

For the breeding of most crops was necessary to accelerate or shorten the process repeatedly. That was due to the need of fast developing of hybrids and uniformed lines, fast transfer of new genetic material, valuable for sunflower breeding programs. Shortened vegetation period, improving the quality of seed production, overcoming of new pathogens races and overcoming of incompatibility of cultivated and wild forms were the subjects of the main research work. The method of embryo culture, used in this connection, allowed faster creation of lines. This method led to reducing the duration of lines obtaining. Six generations could be produced in one year as contrasted with classical methods (Alissa et al., 1986; Aspiroz et al., 1987). Applying the embryo rescue method was a precondition for developing the effective method for decreasing the sterility and obtaining of hybrid forms in *Helianthus* genus (Chandler & Bear, 1983; Bohorova *et al*., 1985; Krauter & Fried, 1991 and etc.). Paul & Barthou (1994) suggested method for cultivation of embryos from commercial hybrids in unsterile conditions. Special equipment, preliminary treatment of embryos and different chemical substances were necessary for their cultivation in "in vitro" conditions. In this study we presented the data of successful obtained crosses between cultivated sunflower and annual and perennial species of genus *Helianthus* using embryo rescue method and classical breeding methods.

MATERIAL AND METHODS

Plant material

The investigation was carried out in Dobrudzha agricultural institute (DAI), General Toshevo. The embryos were isolated from preliminary crosses, obtained using classical methods in sunflower breeding – isolation, pollen collecting, pollination. Cultivated sunflower lines 807A and 3A, developed in DAI were used as maternal parent. Some accessions of wild sunflower species were used as paternal component. The annual species *Helianthus argophyllus*, (accession *E-131*)

and perennial species *H. divaricatus* (accession *M-129*) and *H.tuberosus* (accession *M-146*) were used as pollinators.

Explants and sterilization

The immature embryos are isolated from the inflorescence from the 3rd to the 14th day after crossing. The day of performing embryo isolation is different for each cross. Depending on the used genotype, the embryo size is from 2 to 7 mm. The formed young embryos are removed from the inflorescence and placed in a lint bag. Sterilization is done with commercial bleaching solution (without diluting it) for 20 min, then the bags are transferred to a laminar box and washed with sterile distilled water. Using scalpel and pincers, the husk is removed from the not well formed seed, and the embryo is separated from the endosperm and immediately placed on a nutrition medium.

Nutrition media, sterilization and cultivation

For the separated 2-3 mm large embryos the nutrition media suggested by Chander and Beard (1983) are used. The initial medium on which the embryos are cultivated is B5 with added amino acids as follows: L-alanine – 100 mg/l, L-glutamine – 800 mg/l, L-serine – 160 mg/l, L-tryptophane – 5- mg/l, L-cysteine – 10 mg/l and NOK – 0.05 mg/l. Sucrose is 120 g/l, and the agar is 7g/l; pH of the medium is 5.7. The nutrition medium is distributed in 10 cm Petri dishes after autoclaving, and 10 embryos are plated on each dish. Five to seven days after chlorophyllization of the cotyledons, the embryos are transferred to solid agar nutrition medium. The nutrition medium contains only B5 – salts and 10 g/l sucrose.

For cultivation of embryos bigger than 3-4 mm, the methodology of Azpiroz et al (1987) is applied, which is simpler and with a shorter cycle. The medium for cultivation of the isolated embryos is MS, the macrosalts being reduced in half, the vitamins are of medium B5, the sucrose is 20 g/l, with 100 mg/l of inositol, pH being 5.7.This medium is distributed in 5 Petri dishes each with diameter 10 cm. Ten embryos are placed in each dish.

The cultures are placed in a phytosanitary room at temperature 24±2^{o} C and illumination 2500 – 3000 lux, with photoperiod 18/8 h. The plants are grown in the cultivation constructions till beginning of budding stage at temperature 18-20^{o} C, and the next stages occur at temperature 22-26^{o} C. When roots reach length 2-3-4 cm, the plants are transferred to a soil under non-sterile conditions, covering them with glass cover for about 4-6 days to ensure successful rooting and acclimatization.

RESULTS AND DISCUSSION

The process of creating of sunflower lines, parental lines of a hybrid, was long, difficult, and needed at least 10-12 years. For acceleration of that process, some alternative ways in the field of plant biotechnology were searched. Different theoretical opportunities and in vitro technics existed for intensification of the breeding process, but not all of them could be applied, because of their low effectiveness in sunflower. One of the methods, which could be successfully used, was embryo cultivation. This method was used in sunflower for quick obtaining of lines, restorers of fertility, as well as sterile analogues (Plotnicov, 1983). In perfect conditions this method allowed vastly shortening the breeding process and obtaining 6 generation in one year, which was impossible to be done by classical methods (Alissa *et al*., 1986; Azpiroz *et al.,* 1987). This method was easy and cultivated sunflower embryos could be grown on simple synthetic tissue with small quantity of hormone supplements. The cultivation of interspecific embryo rescue was disparate. Our investigations showed that they had small size and aborted prematurely. In this case the tissues were more complicated and at least one preliminary investigation had to be done and determined when

exactly the embryos died. It depended on type of interspecific crosses – crosses with annuals and perennials (Drumeva, M. & Nenova, N., 2012; N. Nenova *et al.*,2014; Valkova D. *et al.*,2014) described in the previous chapter. описани в предишния раздел.

Based on our previous investigations, in this study we included interspecific hybrids obtained with participation of annuals and perennials. Hybrid combinations, seed set and number of obtained plants, grown in the soil were presented on table 1.

Table 1. Number of isolated embryos and obtained plants grown in the soil from F_1 interspecific sunflower hybrids.

№	Hybrid combination	Seed set, %	Number of embryos	Number of plants
1	*807 A x H.argophyllus E- 131*	53	62	57
2	*3A x H.argophyllus E -131*	71	117	66
3	*3A x H.tuberosus M-146*	0.2	5	1
4	*807 A x H.divaricatus M-129*	0.2	5	2

The results showed, that the highest seed set was determined for the hybrid combination *807 A x E-* 131 (*H. argophyllus*). The species *H.argophyllus* (acc. E- 131) was included in other cross too but the difference in seed set was 18%, which showed that the maternal component also had an effect for the successful crossability. In this combination there were 55 more isolated embryos, which was precondition for surviving of bigger number of plants after sowing in the soil. The embryos from both crosses with participation of *H.argophyllus* (E- 131) were with different size. The embryo size of combination *807 A x E-131* (*H. argophyllus*) was 4-7 mm, and for the other hybrid combination *807 A x E-131* (*H. argophyllus*) - 3-4 mm. It was suggested that larger embryos possessed more endosperm than the others. They contained more nutrients and their survival mechanisms were better.

The testing results of hybrids, obtained with participation of perennials were quite different. The seed set was too low 0.2%, and five embryos were isolated from both hybrids - *3A x M-146 (H. tuberosus)* and *807A x M-129* (*H. divaricatus*). Obviously, the incompatibility of wild annuals and cultivated sunflower was lower than that of perennial species.

These three species, *H. argophyllus*, *H. divaricatus* and *H. tuberosus*, included in the investigation possessed valuable economic characters and could be used as sources of new genetic material to be transferred to the genome of cultivated sunflower. The barriers of incompatibility between most of wild species and cultivated sunflower were hardly overcome using conventional methods. This enforced using of rescue embryo method. All F_1 plants were planted and grown in nursery conditions and after that in the field. Each plant was isolated separately. Selection on morphological characters, evaluation for resistance to downy mildew, phoma, phomopsis and broomrape were carried out. The obtained plants from the cross *cultivated sunflower x perennial species* died during their vegetation. The presented results concerned the plants from hybrid combination *cultivated sunflower x annual species*.

The results of evaluation for resistance to diseases and parasite broomrape were presented on table 2.

Table 2. Resistance of interspecific hybrids to diseases and parasite broomrape.

Resistance, %	Hybrid combination
Resistance 100% to *Pl.helianthi* Novot. and 76-99% to *Orobanche cumana* Wallr.	807 A X E-131
Resistance 76-99% to *Pl.helianthi* Novot. and *Orobanche cumana* Wallr.	3A X E-131
Type of reaction	Hybrid combination
Immune to *Phomopsis helianthi* and *Phoma macdonaldi*	807 A X E-131
Resistant to Phomopsis helianthi and *Phoma macdonaldi*	3 A X E-131

One of the main purposes of interspecific hybridization was directed to transfer of genetic material from wild *Helianthus* species into the genome of cultivated sunflower. The obtained materials with participation of wild annual species *H. argophyllus* (accession E-131) possessed resistance to *Pl. helianthi* from 76% до 100%. The resistance to broomrape varied from 76% до 99%. The type of reaction to the leaves pathogens (*Phomopsis helianthi* and *Phoma macdonaldii*) varied from immune to resistant. The present resistance was transferred from wild accessions because the cultivated sunflower was susceptible regarding the studied pathogens.

Seed oil content was an important character in developing new sunflower forms for including in breeding programs. Wild sunflower species were characterized with low seed oil content. Hybrid combinations were distinguished with higher seeds oil content. The highest seed oil content 41.7% was determined for the cross 3 A x E-131, followed by 39.5% for hybrid combination 807 A x E-131. The pointed values oil content in hybrid seeds were comparatively low, but after backcrossing and self-pollination it could be increased.

CONCLUSIONS

On the base of the pointed results in this investigation, some conclusions could be made:

- Wild annual species were characterized with lower incompatibility with cultivated sunflower than wild perennial species. The improved by us embryo rescue method was suitable for overcoming the incompatibility of wild annual *Helianthus* species and cultivated sunflower.

- The species *H.argophyllus*, accession E-131 was a source of Rf genes and genes for resistance to economically important diseases and parasite broomrape. Transfer of these genes was determined by evaluation of hybrid material.

- Seed oil content was low, but in next generations after backcrossing and self-pollination it could be increased.

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DEVELOPMENT OF SUNFLOWER HYBRIDS RESISTANT TO HERBISIDES

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ABSTRACT

Broomrape (*Helianthus annuus*) and weed control are the main problems in sunflower seed production in Bulgaria. Surmount these problems conducted to development and spreading of hybrids, tolerant to herbicides. CL Plus source was used for creation of hybrids, resistant to herbicide from the imidazolinone group. During the period of study, 112 IMI resistant hybrids were tested. Seed yield, oil yield and seed oil content were tested. Seed yield above the mean standard of 51 hybrids possessed gene for resistance to herbicides Pulsar 40 + Stomp 330 EC at variety testing trails-1 and 2 was reported. Best results at variety testing trails-3 for seed yield were reported for combinations 1111 A x 185 (128.1%) and 1111 A x 437 R (124.8%). Exceeding the mean standard for the character oil yield was reported for 50 hybrid combinations at variety testing trails-1 and 2. Hybrid combination 1111A x 51/2/2 was relieved with the highest oil content 51.4% in air-dry seeds. Best results at variety testing trails-3 for oil yield were reported for combinations 1111 A x 185 R (132.7%) and 1111 A x 437 R (128.5%).

Key words: *Helianthus annuus*, Sunflower, Hybrids, IMI herbicides resistance

INTRODUCTION

Cultivated sunflower is a main crop for oil production in Bulgaria. Significant problems for its cultivation in Bulgaria were *Xanthium strumarium* L., creeping thistle (*Cirsium arvense* L.), wild hemp (*Cannabis sativa* L.), black nightshade (*Solanum nigrum* L.) and others. There were not so many available herbicides for sunflower, as for example in cereals. Some of registered herbicides were not effective enough in the aforementioned weeds (weed mention above). An additional problem in climate change becomes more significant is the lack of effect of soil herbicides under conditions of prolonged drought.

In recent years, they created conditions for widespread parasite broomrape in Bulgaria. There was a rapid change in the population of the parasite, namely the appearance of new more virulent races (Shindrova, 1994; Shindrova, P., 2006). A similar process was observed in other countries such as Turkey, Spain, Romania and others. (Pacurenu-Joita *et al*. 1998, Kaya *et al*, 2014, Fernandez-Martinez *et al*, 2000). This situation requires the search for means to control and reduce losses that broomrape causes. At this stage, control is carried out by sowing of broomrape resistant hybrids. IMI herbicides used with imidazolinone (IMI) tolerant hybrids on the other hand control of both broomrape and key weeds in sunflower.

Al-Khalib *et al.*, 1998 reported for transferring of resistance to imidazolinone into cultivated sunflower and developing "IMISUN" line. Meanwhile, Alonso (1998) first reported for 100% chemical control of broomrape in sunflower resistant to imazethapyr. New source for IMI (Imazethapir) resistance (CLPlus) created by induced mutagenesis (Ethyl methane sulfonate) in wild type *H. annuus* was published by Sala *et al*., 2008. Biochemical studies in various field conditions indicate that CLPlus delivers a higher level of tolerance to IMI compared to the IMISUN (Sala *et al*., 2012).

MATERIAL AND METHODS

In 2008 started a work on transfer of genes for resistance to herbicides from the group of imidazolines in restorer lines of DZI- Toshevo. They were characterized by morphological uniformity, good economic performance and very good combining ability. Some of them are resistant to *Plasmopara helianthi*. To create sustainable materials a source of resistance - CL Plus was used. The best time for testing sunflower plants for resistance is in phase 3-5 pair of true leaves. The dose of the herbicides from the group of imidazolinone was 120 ml/da (Pulsar 40) and 230 ml/da (Stomp 330 EU). After 15 days of treatment, the hybrids were characterized phenotypically in terms of herbicide activity and selectivity according to 9-point system of EWRS- (European Weed Research Society). Three trials were performed: Variety testing trials-1 included 31 hybrid combinations tested for second year; Variety testing trials-2 included 65 hybrid combinations, tested for first year; Variety testing trials-3 - 16 hybrids combinations.

Hybrids were tested in 2015 at the experimental breeding fields of DZI-General Toshevo in a randomized block method in three repetitions, as the area of each repetition is 10 m2 (Barov and Shanin, 1965). Aim of the experiment: testing of experimental imidazolinone (IMI) tolerant hybrids

RESULTS AND DISCUTIONS

Sunflower is the main crop for oil production in Bulgaria. The sunflower production fields have been increased during the recent years and spread on more than 700 000 ha. It needed to work on reducing the impact of poor environmental condition. Creation and implementation in practice of herbicide-tolerant hybrids would enable farmers to cope with the problems that cause weeds and parasite broomrape.

Since 2008 the scientists at the department on sunflower breeding in G. Toshevo were working on the task of creating hybrids tolerant to herbicides from the group of imidazolinone (IMI). There were created 112 hybrid combinations in which allele called *Ahasl1-3* is in the homozygous state. This new traits present better stability of the herbicide tolerance in different environmental condition, permit developing new herbicide formulations providing more flexible and reliable weed control, higher oil content, etc. than previous IMISUN trait (Sala *et al.,* 2008 a, b; Weston *et al*., 2012).

After 15 days of treatment the hybrids were characterized phenotypically (Fig 1) in terms of herbicide activity and selectivity according the 9 point system of EWRS- (European Weed Research Society). The character seed yield was evaluated**.** The standards for comparison of the new hybrids in terms of seed yield are Neoma and LG 5661 CL. Thirty-one hybrid combinations were tested in variety testing trials 1 for second year. Fourteen of the hybrids exceeded the mean standard by seed yield. In table 1 were presented 8 hybrids with the highest values. Two hybrids possessed seed yield over 4,000 kg/ha. These are 1111 A x 67/1 R - 4390 kg/ha and 1111 A x 102/2/3 R - 4270 kg/ha.

Table 1. Seed yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC from the class of imidazolinones, increasing adjacent mean standard. Variety testing trials 1. Tested for 2th year

№	Cross	Seed yield kg/ha	% of mean St
82	1111 A x 14/1/1 R	3670	113.6
83	1111 A x 14/1/2 R	3560	110.2
84	1111 A x 14/1/2 R	3570	110.5
85	1111 A x 14/1/3 R	3490	108.0
92	1111 A x 16/2/2 R	3550	109.9
93	1111 A x 16/2/3 R	3400	105.3
St	***NEOMA***	***3400***	***105.3***
St	***LG 5661 CL***	***3060***	***94.7***
	Mean standard	***3230***	***100.0***
108	1111 A x 67/1 R	**4390**	115.1
115	1111 A x 102/2/3 R	**4270**	111.9
St	***NEOMA***	***3970***	***104.1***
St	***LG 5661 CL***	***3660***	***95.9***
	Mean standard	***3815***	***100.0***

In 2015, 65 IMI tolerant hybrid combinations in variety testing trials-2 were tested for the first year. The best results were presented in table 2. The commercial hybrids Adagio, Alego, Neoma and LG 5661 CL were used as standards. Thirty-six hybrids exceeded the mean standard on seed yield. For eight of them the exceeding was more than 10 %. The best results were obtained for the crosses 1111 A x 53/3 R (126.8%) and 1111 A x 77/1/1 R (124.5%). Two hybrids had seed yield more than 4000 kg/ha. These were 1111 A x 77/1/1 – 4110 kg/ha and 1111 A x 40/2/2 – 4070 kg/ha.

The results of Variety testing trials-3 were presented on table 3. All hybrids, included in table 3, exceeded the mean standard of the used Neoma and LG 5661 CL. Ten combinations, from the tested 16 (62.5%), exceeded the mean standard from 0.1 to 28.1 %. The highest seeds yield was obtained from hybrids 1111 A x 185 R (128.1%), 1111 A x 437 R (124.8 %) and 1111 A x 410 R (119.0 %).

Table 2. Seed yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC from the class of imidazolinones, increasing adjacent mean standard. Variety testing trials 2. Harvest year 2015

№	Cross	Seed yield kg/ha	% of mean St
124	1111 A x 25/1 R	3990	111.8
128	1111 A x 40/2/2 R	**4070**	114.0
St	***ADAGIO***	***2790***	***78.2***
St	***ALEGO***	***3570***	***100.0***
St	***NEOMA***	***3960***	***110.9***
St	***LG 5661 CL***	***3960***	***110.9***
	Mean standard	***3570***	***100.0***
147	1111 A x 51/2/2 R	3600	115.8
150	1111 A x 53/3 R	3940	**126.8**
155	1111 A x 57/1 R	3700	119.0
156	1111 A x 57/2 R	3630	116.8
St	***ADAGIO***	***3170***	***102.0***
St	***ALEGO***	***3260***	***104.9***
St	***NEOMA***	***3330***	***107.1***
St	***LG 5661 CL***	***2670***	***85.9***
	Mean standard	***3108***	***100.0***
162	1111 A x 77/1/1 R	**4110**	**124.5**
181	1111 A x 20/2 R	3670	111.2
St	***ADAGIO***	***3250***	***98.5***
St	***ALEGO***	***3340***	***101.2***
St	***NEOMA***	***3730***	***113.0***
St	***LG 5661 CL***	***2880***	***87.3***
	Mean standard	***3300***	***100.0***

Table 3. Seed yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC from the class of imidazolinones, increasing adjacent mean standard. Variety testing trials 3. Harvest year 2015

№	Cross	Seed yield	
		kg/ha	% of Mean St
3	1111 A x 100/2/3 R	2995	103.7
5	1111 A x 100/3/3 R	3272	113.3
6	1111 A x 175 R	3197	110.7
7	1111 A x 414 R	2944	102.0
8	**1111 A x 185 R**	**3698**	**128.1**
9	1111 A x C 61	2891	100.1
10	1111 A x 514 R	2960	102.5
13	1111 A x 462 R	2917	101.0
14	1111 A x 410 R	2997	103.8
15	**1111 A x 437 R**	**3603**	**124.8**
St	***Neoma***	***3051***	***105.7***
St	***LG 5661 CL***	***2723***	***94.3***
	Mean standard	***2887***	***100.0***

Biochemical study of sunflower hybrids, tolerant to herbicides Pulsar 40+ Stomp 330 EC

The testing results of 31 imidazolinone (IMI) tolerant hybrid combinations in Variety testing trials-1 in respect of the trait oil yield per hectare are presented in Table 4. The oil content in air-dry seeds of new hybrids ranged from 44.1% to 46.8%. At table 4 were presented hybrid combinations with the greatest increasing in compared to the adjacent mean standard. These are 1111 A x 67/1 R - 122.8%, 1111 A x 16/2/2 R - 120.4% and 1111 A x 102/2 / 3 R - 119.4%.

Our results confirmed the conclusion of Weston *et al*., (2012) that absence of genes from a wild source around *Ahasl1-3* determines that the oil content in the hybrids carrying the CLPlus trait show the same oil yield per hectare as those of their conventional counterparts (Weston *et al*., 2012).

Table 5 presented the hybrid combinations at Variety testing trials-2 with the highest increase compared to the adjacent mean standard in terms of oil yield. In 30 hybrid combinations (46%) oil content in the seed is higher than the mean standard. The oil content of the new hybrids ranged from 42.0% to 51.4%. Hybrid combination 1111 A x 51/2/2 R stand out with 7.8% excess oil content in the seeds compared to the mean standard. Very good results in terms of oil yield were reported in combinations 1111 A x 53/3 R- 141.7%, 1111 A x 51/2/2 R - 139.8% and 1111 A x 77/1/1 - 136.7%.

Table 4. Oil yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC, increasing adjacent mean standard. Variety testing trials 1**.** Tested for 2th year

№	Cross	Oil content %	Oil yield	
			kg/ha	% of mean St
82	1111 A x 14/1/1 R	44.1	1618	117.2
83	1111 A x 14/1/2 R	46.1	1641	118.9
85	1111 A x 14/1/3 R	45.5	1588	115.1
92	1111 A x 16/2/2 R	46.8	1661	**120.4**
93	1111 A x 16/2/3 R	46.8	1591	115.3
St	***NEOMA***	***45.8***	***1557***	***112.8***
St	***LG 5661 CL***	***39.3***	***1203***	***87.2***
	Mean standard	**42.6**	**1380**	**100.0**
108	1111 A x 67/1 R	46.8	2055	**122.8**
112	1111 A x 99/1 R	46.6	1831	109.4
114	1111 A x 102/2/1 R	46.3	1810	108.2
115	1111 A x 102/2/3 R	46.8	1998	**119.4**
St	***NEOMA***	***47.3***	***1878***	***112.3***
St	***LG 5661 CL***	***40.1***	***1468***	***87.7***
	Mean standard	***43.7***	***1673***	***100.0***

Table 5. Oil yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC, increasing adjacent mean standard. Variety testing trials 1. Harvest year 2015

№	Cross	Oil content in seeds %	Oil yield	
			kg/ha	% of mean St
147	1111 A x 51/2/2 R	**51.4**	1850	**139.8**
150	1111 A x 53/3 R	47.6	1875	**141.7**
155	1111 A x 57/1 R	47.8	1769	133.7
156	1111 A x 57/2 R	**49.3**	1790	135.3
St	***NEOMA***	***48.1***	***1602***	***121.1***

St	***LG 5661 CL***	***39.1***	***1044***	***79.0***
	Mean standard	***43.6***	***1323***	***100.0***
162	1111 A x 77/1/1 R	47.4	1948	**136.7**
St	***NEOMA***	***45.6***	***1693***	***118.8***
St	***LG 5661 CL***	***38.3***	***1157***	***81.2***
	Mean standard	**41.9**	**1425**	**100.0**

Table 6. Oil yield of hybrid combinations tolerant to herbicides Pulsar 40 + Stomp 330 EC, increasing adjacent mean standard. Variety testing trials 3. Harvest year 2015

№	Cross	Oil content in seeds,%	Oil yield	
			kg/ha	% of mean st
3	1111 A x 100/2/3 R	43.1	1291	108.7
5	**1111 A x 100/3/3 R**	**42.7**	**1398**	**117.7**
6	1111 A x 175 R	40.8	1209	101.8
7	1111 A x 414 R	44.0	1296	109.1
8	**1111 A x 185 R**	43.1	**1527**	**128.5**
9	1111 A x C 61	42.5	1228	103.4
10	1111 A x 514 R	43.0	1275	107.3
13	1111 A x 462 R	43.7	1275	107.3
14	**1111 A x 410 R**	**44.8**	**1342**	**113.0**
15	**1111 A x 437 R**	43.7	**1576**	**132.7**
St	***Neoma***	***44.6***	***1361***	***114.6***
St	***LG 5661 CL***	***37.2***	***1014***	***85.4***
	Mean standard	***40.9***	***1188***	***100.0***

The results for the oil content in the seed and oil yield per hectare for imidazolinone (IMI) tolerant hybrids, included in the Variety testing trials-3 are presented in Table 6. Ten hybrid combinations (62.5%) demonstrated excess over adjacent mean standard. Values above 105% were reported for 9 hybrids. The highest values were reported for crosses 1111 A x 437 R (132.7%), 1111 A x 185 R (128.5%), 1111 A x 100/3/3 R (117.7%) and 1111 A x 410 R (113.0%).

CONCLUSIONS

During the period of study, 112 imidazolinone (IMI) tolerant hybrid combinations were tested. All hybrid combination possessed allele *Ahasl1-3* in the homozygous state.

Seed yield, seed oil yield and seed oil content were determined. Seed yield above the mean standard at Variety testing trials 1 and 2 were observed in 51 hybrids.

Best results for seed yield at Variety testing trials 3 are reported for combinations 1111 A x 185 R (128.1%) and 1111 A x 437 R (124.8%). Hybrids exceeded the mean standard by oil yield per hectare were demonstrated in 50 hybrid combinations at Variety testing trials 1 and 2. Hybrid combination 1111 A x 51/2/2 R was stand out with the highest oil content- 51.4% in

air dry seeds. Best results for oil yield at Variety testing trials 3 are demonstrated in combinations 1111 A x 185 R (132.7%) and 1111 A x 437 R (128.5%).

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RESPONSE TO WATER STRESS INDUCED BY PEG 6000 ON GROWTH OF PLANTLETS IN SOME SUNFLOWER GENOTYPES RESULTED FROM INTERSPECIFIC HYBRIDISATION

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ABSTRACT

In 2008 at NARDI Fundulea a breeding program was initiated to improve sunflower for drought resistance by genes introgression from the *H. argophyllus* species into cultivated sunflower. Test of new genetic material was represented by hybrid combinations resulting from a selection process that took place over a period of 7 years including all stages of the breeding (hybridization: *H. annuus* x *H. argophyllus*, backcross, self pollination and selection). For this study, the testing of genotypes was performed under laboratory conditions to water stress induced by the presence of PEG 6000 in 5 concentrations and 3 replications/variant. In contrast with plantlets from backcross 7, at higher concentrations of PEG (15% and 20%) many plantlets from maternal lines did not survive until the end of the experience. The 10% PEG 6000 concentration permitted the best analysis of the tested material and it will be maintained in our further studies. For most studied lines were pointed out backcross descendents that exceed their maternal lines and combinations that are bellow the initial levels especially at low stress levels. A number of eight backcross 7 descendents were selected for the final stage of improving for drought resistance, in order to obtain new hybrids.

Key Words : Helianthus argophyllus, interspecific hybridization, self pollination, backcross, water stress, PEG 6000, growth

A NEW BULGARIAN SUNFLOWER HYBRID DEA

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ABSTRACT

Sunflower Dea was developed at Dobrudzha Agricultural Institute – General Toshevo (DAI). It is a male sterile two-linear hybrid derived through interlinear hybridization. The mother is line 217 possessing cytoplasmic male sterility, and the father is line 626 R, a branched fertility restorer. Both parental forms have excellent general and specific combining ability. Hybrid Dea is of medium maturity, growth period 116 – 119 days, plant height 160-165 cm and head diameter 20-23 cm. Absolute seed weight is 58-62 g and oil content is 48-49%. Flowering is 12-14 days. The new hybrid successfully underwent 3-year testing at DAI according to the standard testing practice. The maximum seed yield was 5313 kg/ha, while oil content reached 50 %. It is resistant to economically important diseases and to the parasite *Orobanche*. In 2012 hybrid Dea was submitted for official testing within the structures of the Romanian Varietal Commission at 10 locations. In the first three years it exceeded the Romanian standard with averagely 7,3 % by seed yield. The mean seed yield per ha for the three years of testing was 3387 kg. The hybrid was officially registered in Romania and was enlisted in the European Catalog of Field and Vegetable Crop Varieties.

Key words: Hybrid, Seed yield, Oil yield, Growth period

INTRODUCTION

The main task and priority of contemporary sunflower breeding are the development of high-yielding hybrids resistant to the economically important diseases and parasites. This tendency imposes the necessity to introduce new hybrids meeting the demands of the market. The good hybrid, apart from being high-yielding, should also be adaptive to the changeable biotic and abiotic environmental factors thus revealing its maximum potential under specific conditions. The wide use of sunflower justifies the necessity of increasing the improvement work on this crop, determining the various directions and specificity of tasks in its breeding of (Encheva and Georgiev, 2009; Encheva et al., 2014; Encheva V. *et al.*2014; Valkova D. *et al.* 2014). The breeding of a new hybrid is a long process which involves collecting and developing of initial material, selection and choice of parental components, testing of the new forms, their registration and distribution. The successful outcome of each breeding project is highly productive genotypes which not only possess valuable properties from a research point of view, but which are also able to find good realization in production (Chamurliyski and Tsenov, 2013; Chamurliyski et at., 2011).

The aim of this investigation was to present a detailed morphological, biological and economic characterization of a modern Bulgarian hybrid (Dea) which meets the requirements for high productivity, resistance and adaptability. It was registered and enlisted in the European Varietal List of 2015. The hybrid is medium early, high-yielding and possesses very good drought resistance.

MATERIAL AND METHODS

Hybrid Dea was developed by the method of inter linear hybridization. It is a male fertile two-linear hybrid. The mother component is line 217 which possesses cytoplasmic male sterility and which has been developed through hybridization between the Bulgarian candidate variety No 72 and line 246 originating from Russian cultivars. By using the methods of selfing and selection, the line was developed as morphologically and genetically homogenous. It is characterized with very good general and specific combining ability.

Its successful use as the mother component in the most recent Bulgarian sunflower hybrids Veleka, Vokil, Divna, Vyara and Gabi confirmed its excellent properties. Its phytopathological evaluation determines it as resistant to the parasite *Orobanche* up to race F, moderately resistant to phoma and alternaria and moderately susceptible to phomopsis.

The father component is line 626 possessing Rf fertility restorer genes, which was developed through hybridization of lines 654 R and 620 R, selfing and selection. The line is strongly branched, with specific lemon yellow coloration of the ray flowers and rich in pollen. It has very good general and specific combining ability. It is resistant to downy mildew up to race 731 and to the parasite *Orobanche* up to race F, moderately resistant to phoma, phomopsis and alternaria. The hybrid cross was developed in 2009 and was tested for two years in a competitive varietal trial and in a unified varietal trial, where it exceeded the standard with over 6 %. The testing was carried out after predecessor wheat applying agronomy practices suitable for growing of this crop. The trial plots were each 12 m with standard block design, in three replications of two rows. The plant density was 61220 plants/ha. Three standards were used – San Luka, Brio and PR 64F50. During the growth season all morphological and phenological characters of the hybrid cross were determined according to the UPOV Protocol (2002). The main elements of yield were taken into account: seed yield kg/ha, oil content in seed, %, and oil yield, kg/ha. Phytopathological evaluation of the hybrid and the parental lines was done at DAI – General Toshevo. The resistance to downy mildew (*Plasmopara halstedii*) was determined according to a standard methodology (Vear F., Tourvieille D., 1987) adapted to the working conditions of DAI. The percent of resistance was expressed in the response of the hybrid to races 700 and 731.

The resistance to the parasite broomrape (*Orobanche cumana*) was determined by the method of Panchenko (1975). The evaluation was done under greenhouse conditions using the index percent of resistance. The resistance to gray spots on sunflower (*Phomopsis helianthi*) was determined by the method of Encheva and Kiryakov (2002) under field conditions against artificial infection background. The type of attack was determined one week prior to flowering at stage milk maturity. The following scale was used: 0 – no symptoms; 1 – necrotic spot with diameter up to 5 cm; 2 – necrotic spot with diameter more than 5 cm; 3 – several merged necrotic spots on the stem; 4 – stem broken at the place of infection.

The testing for black spots on sunflower (*Phoma macdonaldii*) was carried out under field conditions against artificial infection background. The inoculation was done at stage budding – beginning of flowering by the method of Maric et al. (1981). The reaction of the plants was read at stage yellow-brown maturity according to a 4-degree scale as follows: 0 – no symptoms; 1 – necrotic spot localized around the petiole; 2 – several necrotic spots on stem; 3 – entire stem covered with necrotic spots or broken.

RESULTS

In 2012 the hybrid cross 217 A x 626 R was provided to Saaten Union – Romania as a candidate hybrid for registration in the European Catalog of Field and Vegetable Crops and for testing within the system of the State Institute for Variety Testing and Registration – ISTIS Romania. Following a three-year testing during 2012 – 2014, it was officially released with certificate No 4935/09.06.2015 under the trade name Dea.

Morphological description

The morphological description was done according to the methodology of UPOV (2002) and is presented in (Table 1).

Table 1. Morphological characteristics of sunflower hybrid DEA

№	Traits	Expression	Degree
1.	Hypocotyl:anthocianin coloration	Absent	1
2.	Hypocotyl:anthocianin coloration	Absent	1
3.	Leaf: size	Large	7
4.	Leaf: green color	Medium green	5
5.	Leaf: blistering	Weak	3
6.	Leaf: serration	Medium	5
7.	Leaf: shape of cross section	Concave	1
8.	Leaf: shape of distal part	Acuminate	8
9.	Leaf: auricules	Large	7
10.	Leaf: wings	Absent	1
11.	Leaf: angle of lowest lateral veins	Right or nearly right angle	2
12.	Leaf: height of the tip of the blade compared to insertion of petiole (at 2/3 height of plant)	High	7
13.	Stem: intensity of hairiness at the top	Very strong	9
14.	Time of flowering	Medium	5
15.	Ray flower: density	Dense	7
16.	Ray flower: shape	Narrow ovate	2
17.	Ray flower: disposition	Flat	1
18.	Ray flower: length	Medium	5
19.	Ray flower: color	Oringe yellow	4
20.	Disk flower color	Orange	2
21.	Disk flower: anthocyanin coloration of stigma	Absent	1
22.	Disk flower: intensity of anthocyanin coloration of stigma	-	-
23.	Disk flower: presence of pollen	Present	9
24.	Bract shape	Rounded	3
25.	Bract: length of the tip	Long	7
26.	Bract: green color of the external part	Medium	5
27.	Bract: attitude in relation to head	Not embracing or very slightly embracing	1
28.	Plant: natural height	Medium to tall	6
29.	Plant: branching	Absent	1

30.	Plant: type of branching	-	-
31.	Plant: natural position of closest lateral head to the central head	-	-
32.	Head: attitude	Half-turned down with straight stem	4
33.	Head: size	Medium	5
34.	Head: shape of grain side	Weakly concave	2
35.	Seed: size	Medium	5
36.	Seed: shape	Narrow ovoid	2
37.	Seed: thickness relative to width	Thin	3
38.	Seed: main color	Dark brown	6
39.	Seed: stripes on margin	Strongly expressed	3
40.	Seed: stripes between margin	Strongly expressed	3
41.	Seed: color of stripes	Brown	3

Biological and economic properties

The sunflower hybrid Dea is medium early, with duration of the growth season 116-119 days. Plant height is within the range 160-165 cm, with head diameter 20-23 cm. The absolute weight of seeds is 58-62 g and oil content is 49-50 %. The oil is of linoleic type. The percent of kernel in seed reaches up to 74-75 %, and the protein in seed is 19-20 %. Seed weight per plant is 78-84 g, and seed number is 1150-1300. The duration of flowering is 12-14 days. The maximum yield obtained in the experimental fields of DAI was 4545 kg/ha, and in neighboring Romania – 5313 kg/ha.

The seed production of the new hybrid allows simultaneous sowing of the two parental lines because their flowering coincides. This is a great advantage with a view of the necessary agronomy practices. The father line 626 R is strongly branched and rich in pollen. The most suitable seed production scheme is 10:2 (mother to father lines), with at least 3-4 well developed bee colonies available per ha.

Preliminary testing at DAI – General Toshevo

Hybrid Dea was subjected to three-year testing in the trial fields of DAI, involving two-year testing in a competitive varietal trial and one-year testing in a unified competitive trial (Table 2).

Table 2. Testing of hybrid DEA at DAI - General Toshevo

Hybrids	Seed yield, kg/ha	% from mean standard	Oil percent, %	Oil yield, kg/ha	% from mean standard
2009 – competitive varietal trial					
Dea	4039	115,5	48,2	1947	121,4
San Luka (st.)	3031	86,7	44,6	1352	84,3
Klarisa (st.)	3317	94,9	49,3	1635	101,9
Brio (st.)	4139	118,4	44,1	1825	113,8
Mean standard	3496	100,0	46,0	1604	100,0
2010 – competitive varietal trial					
Dea	4097	114,5	50,8	2081	118,6
San Luka (st.)	3528	98,6	46,8	1651	94,1
Klarisa (st.)	5319	92,8	53,6	1779	101,4
Brio (st.)	3886	108,6	47,2	1834	104,5
Mean standard	3578	100,0	49,2	1755	100,0
2011 – unified varietal trial					
Dea	3767	106,1	49,1	1850	106,6
San Luka (st.)	3189	89,9	47,1	1502	86,5
Klarisa (st.)	3553	100,1	52,3	1858	107,0
Brio (st.)	3906	110,0	47,3	1848	106,5
Mean standard	3549	100,0	48,9	1736	100,0

During the period of testing, hybrid Dea exceeded the mean standard by seed yield with 6.1 – 15.5 %. The exceeding was highest in 2009 both by seed yield (15.5 %) and oil yield (21.4 %). The exceeding by oil yield for the three years of testing was within 6.6 – 21.4 %.

Both yields were highest in 2010: 4097 kg/ha seed yield and 2081 kg/ha oil yield.
In the unified varietal trial, hybrid Dea was compared to the most promising and most productive hybrids of DAI, showing the following results: 6.1 % above the mean standard by seed yield and 6.6 % above the mean standard by oil yield.

The oil content of this hybrid reached 50.8 % and was higher than the standards San Luka and Brio.

Official testing

In 2012 hybrid Dea was provided to Saaten Union – Romania for official three-year testing on the territory of Romania and for registration. The results are given in (Table 3).

Table 3. Results from the official testing of hybrid “DEA”

Region	Hybrids	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Yield kg/ha	% from stan dard	Relative yield according to the standard, averaged for 3 years
		2012		2013		2014		
1.Troian	Standard	1999	100	3821	100	1868	100	
	Dea	2030	102	3683	96	2366	127	108
2.Tecuci	standard	3154	100	4316	100	3393	100	
	Dea	3125	99	3511	81	3227	95	92
3.Rm.Sarat	standard	2124	100	4531	100	3292	100	
	Dea	2906	137	5313	117	3292	112	122
4.Portaresti	standard	2492	100	3487	100	3763	100	
	Dea	3437	138	4564	131	4614	123	131
5.Peciu Nou	standard	2873	100	3363	100	3230	100	
	Dea	3567	110	3470	103	3421	119	111
6.Negresti	standard	3057	100	4408	100	4201	100	
	Dea	2553	84	4043	92	3882	92	89
7.Mircea Voda	standard	2204	100	2698	100	4580	100	
	Dea	3123	142	3172	118	4958	108	123
8.Inand	standard	2441	100	3507	100	2495	100	
	Dea	2529	104	3578	102	2658	107	104
9.Dalga	standard	3801	100	4055	100	3518	100	
	Dea	3777	99	4367	108	3767	107	105
10.Cogealac	standard	1981	100	2240	100	2716	100	
	Dea	2496	126	2135	95	2059	76	99
Средно от 10 пункта	standard	2648	100	3642	100	3234	100	
Averaged from 10 locations	Dea	2954	112	3783	104	3424	106	109

The official testing of new hybrids in Romania is carried out at ten locations representative for almost all soil-and-climate regions suitable for growing of field crops.

Two Romanian hybrids were involved as standards. During the second and third year hybrid Daniel was used as a standard, and during the first year – hybrid Alex.

During the first year of official testing the new hybrid Dea gave a mean yield from all locations 2954 kg/ha, which was a 12 %-exceeding, the highest during the three-year testing. The low yield of kg per da was due to the unfavorable conditions in 2012 related to very high air temperatures for a long period of time and to the long-lasting drought during almost the entire growth season. Nevertheless, hybrid Dea demonstrated the best results among all tested hybrids in that year and performed as resistant to drought and high air temperatures.

During the second year the exceeding of the standard was with 4 % at seed yield 3783 kg/ha, which was the highest result from the official three-year testing. During the third year the exceeding was 6 %, the seed yield being 3424 kg/ha.

The highest seed yield was 5313 kg/ha from location Rm.Sarat in 2013, and the lowest - 2030 kg/ha from Troian in 2012. The highest exceeding of the standard with 12 % was at location Mircea Voda in 2012.

In seven out of ten locations hybrid Dea showed results exceeding the standard with up to 31 %. For the three years of testing, Dea exceeded the standard with 9 % and this was the main reason for its official registration and enlisting in the European catalog of field and vegetable crops.

Phytopathological characterization

The evaluation of the resistance of the hybrid to economically important diseases and the parasite *Orobanche* were carried out in the infection fields of DAI. The results from them are presented in (Table 4).

Table 4. Phytopathological evaluation of sunflower hybrids in artificial infection field at DAI – General Toshevo

Hybrid	Phomopsis helianthi		Phoma macdonaldi		Plasmopara helianthi		Orobanche cumana
	Attacking rate	Rank	Attacking rate	Rank	Resistance to race 700, %	Resistance to race 731, %	Resistance to races A-F, %
San Luka	3/3(3)	3	1/3(1)	1	100.0	92.9	100.0
Diabolo	2/3(2)	2	1/3(1)	1	100.0	90.5	100.0
Brio	1/3(1)	1	0	0	100.0	100.0	100.0
PR64F50	1/3(1)	1	0	0	100.0	100.0	100.0
Dea	1/3(1)	1	0	0	100.0	60.0	100.0

Hybrid Dea was resistant to the fungal pathogen *Phomopsis helianthi.*
To the other important leaf pathogen *Phoma macdonaldi* the hybrid demonstrated immune reaction.

The resistance of hybrid Dea to downy mildew on sunflower *Plasmopara helianthi*, race 700 was 100 %, and to the most recent race 731 its resistance was moderate.

To the parasite *Orobanche cumana* the resistance was 100%.

CONCLUSIONS

- Hybrid Dea is clearly distinct, uniform and stable.
- It was officially registered in Romania and was enlisted in the European catalog of the field and vegetable crop varieties.
- It possesses very good adaptability and realizes its high potential under variable soil-and-climatic conditions.
- The hybrid is resistant to drought and high temperatures.
- It is also resistant to the economically important diseases and the parasite *Orobanche*.

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INVESTIGATION ON SUNFLOWER LINES AND HYBRIDS (*HELIANTHUS ANNUUS* L.) FOR EXPRESSION OF HETEROSIS AND DOMINANCE RATE OF IMPORTANT ECONOMIC TRAITS IN F_1 UNDER THE CONDITIONS OF NORT-EAST BULGARIA

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ABSTRACT

The investigation wascarried out during 2013–2014 in the trial field of Dobrudzha Agricultural Institute (DAI). Nine hybrid combination of oil seed sunflower were investigated, which were obtained from the crossing of three sterile lines to three fertility restorers. The following traits were studied: plant height, head diameter, and 1000 kernel weight. Hypothetical and actual heterosis and heritability rate were determined in all hybrid combinations. For statistical processing of the results, two factor dispersion analysis and variation and correlation analyses were applied using software XLSTAT Pro. ver 7.0.1.

It was demonstrated that 1000 kernel weight was affected most by the head diameter in the tested lines and hybrid combinations. Lower was the effect on 1000 kernel weigh by plant height according to the performed correlation analysis. The variation analysis showed highest variation of the traits 1000 kernel weight and head diameter, while plant height was with the lowest value of variation among the investigated traits. Highest values of hypothetical and actual heterosis of the trait plant height were found in the cross 89R x 217A. For the trait head diameter, highest values of hypothetical and actual heterosis and heritability rate were determined in the cross 89R x 813A.

Key words: sunflower, heterosis, heritability rate, hybrid,

MATERIAL AND METHODS

The investigation was carried out during 2013 – 2014 in the trial field of Dobrudzha Agricultural Institute – General Toshevo (DAI). Nine hybrid combinations of oil seed sunflower were investigated, which were derived from the crossing of three sterile lines to three fertility restorers. The hybrid combinations were tested in a trial of three replications designed by the Latin square method. The size of the plot was 7.35 m^2. The standards used were the Bulgarian hybrid San Luka and some of the most productive and well established in Bulgaria foreign hybrids Meldimi, Clarissa and P64LE19.

The traits plant height, head diameter and 1000 kernel weight were studied. Hypothetical heterosis, real heterosis and heritability rate were determined for all hybrid combinations. Two-factor dispersion, variation and correlation analyses were applied for statistical processing of results using the software XLSTAT Proversion 7.0.1.

The data on the investigated traits were subjected to genetic analysis to determine the theoretical (the mean value of the two parents) and the real (the value of the better parent)

heterosis in F_1, and the percent of heritability of the investigated traits (according to Omarov, 1975). The dominance rates in the heritability of the traits in F_1 were determined using the methodology of Romero and Frey, 1973.

RESULTS AND DISCUSSION

Highest value of hypothetical heterosis in (Table 1) for the trait plant height was determined in the cross 2003A x 89R (58.22%) while real heterosis with highest index was determined in the hybrid combination 217A x 85R. Highest heritability rate of this trait, averaged for two years, was found in the cross 217A x 85R.

The results presented in (Table 2) characterize the expressions of heterosis in F_1 and the rate of dominance in F_1 generation for head diameter. In cross 813A x 89R, a clearly expressed positive heterosis was observed in the heritability of this trait both with regard to the mean value of the two parents and with regard to the parent with higher value regardless of the genotype and the year of growing. The mean values from the two years of investigation on the hypothetical heterosis in generation F_1 were different and varied from 27.67 % in cross 217A x 85R to 52.34 % in cross 813A x 89R. In cross 2003A x 89R a high heritability rate was determined for this trait – 1.75 %, averaged for the two years.

Highest hypothetical heterosis for the trait 1000 kernel weight (Table 3) was determined in cross 2003A x 84R - 85.40 %. Positive real heterosis according to the better parent was observed in hybrid combination 813A x 84R – 71.58 %, averaged for two years. High mean value was obtained in this combination for dominance rate in F_1, averaged for two years. The dominance rates in generation F_1 for the three traits had positive values higher than 1 implying super dominance. In (Table 4) it was proved that 1000 kernel weight was influenced most by the head diameter of the tested lines and hybrid combinations. Lower was the effect of 1000 kernel weight in comparison to plant height according to the correlation analysis carried out. Head diameter was influenced by plant height.

It becomes clear from the variation analysis in (Table 5) that highest was the variation of the traits 1000 kernel weight and head diameter, while plant height had lowest value of variation among the investigated traits.

CONCLUSIONS

Highest value for hypothetical heterosis of the trait plant height was determined in the cross 2003A x 89R (58.22%) while real heterosis with highest index was found in the hybrid combination 217A x 85R. Highest was the heritability rate of this trait in the cross 217A x 85R, averaged for two years.

Highest hypothetical heterosis of the trait 1000 kernel weight was determined in the cross 2003A x 84R - 85.40%. Positive real heterosis and heritability rate of the trait 1000 kernel weight according to the better parent was observed in hybrid combination 813A x 84R – 71.58 %, averaged for two years.

In cross 813A x 89R, a clear positive heterosis was observed in the heritability of the trait with regard to both the mean value of the two parent and to the parent with higher value regardless of the genotype and the year of growing. In cross 2003A x 89R, high heritability rate of this trait was determined – 1.75 %, averaged for two years.

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Table 1. Heterosis (%) and dominance rate in F_1 of the trait plant height

		Heterosis %		Dominance rate in F_1
Hybrid	Year	Hypothetical	Real	
217A x 84R	2012	24.35	8.64	1.74
	2013	22.74	6.44	1.43
	mean	**23.55**	**7.54**	**1.59**
217A x 85R	2012	46.43	48.45	36.23
	2013	34.81	37.29	27.33
	mean	**40.62**	**42.87**	**31.78**
1017A x 89R	2012.00	50.72	45.72	17.32
	2013.00	27.29	23.15	9.39
	mean	**39.01**	**34.44**	**13.36**
813A x 84R	2012	12.76	8.27	18.3
	2013	7.39	0.72	10.61
	mean	**10.08**	**4.50**	**14.46**
813A x 85R	2012	15.49	3.79	1.36
	2013	23.75	14.16	2.82
	mean	**19.62**	**8.98**	**2.09**
813A x 89R	2012	18.84	8.01	1.87
	2013	26.17	11.66	1.77
	mean	**22.51**	**9.84**	**1.82**
2003A x 84R	2012	36.68	30.07	8.27
	2013	31.72	19.70	5.70
	mean	**34.20**	**24.89**	**6.99**
2003A x 85R	2012	57.40	39.34	4.42
	2013	43.39	36.60	8.72
	mean	**50.40**	**37.97**	**6.57**
2003A x 89R	2012	58.90	42.62	5.16
	2013	57.53	37.60	6.00
	mean	**58.22**	**40.11**	**5.58**

Table 2. Heterosis (%) and rate of dominance in F_1 of the trait head diameter

		Heterosis %		Dominance rate in F_1
Hybrids	Year	Hypothetical	Real	
217A x 84R	2012	31.73	4.76	1.23
	2013	42.76	3.33	1.12
	mean	**37.25**	**4.05**	**1.18**
217A x 85R	2012	25.74	0	1
	2013	29.6	1.19	0.77
	mean	**27.67**	**0.59**	**0.88**
1017A x 89R	2012	40.12	4.76	1.18
	2013	48.27	2.38	1.07
	mean	**44.20**	**3.57**	**1.13**
813A x 84R	2012	27.27	2.38	1.11
	2013	34.86	0	0.89
	mean	**31.06**	**1.19**	**1**
813A x 85R	2012	34.73	4.76	1.09
	2013	44.73	4.76	1.17
	mean	**39.73**	**4.76**	**1.13**
813A x 89R	2012	43.31	8.12	1.3
	2013	61.37	11.42	1.36
	mean	**52.34**	**9.77**	**1.33**
2003A x 84R	2012	27.48	2.5	1.13
	2013	53.28	16.66	1.69
	mean	**40.38**	**9.58**	**1.41**
2003A x 85R	2012	29.62	5	1.26
	2013	50.36	14.44	1.6
	mean	**39.99**	**9.72**	**1.43**
2003A x 89R	2012	31.57	2.5	2
	2013	57.69	7.89	1.5
	mean	**44.63**	**5.20**	**1.75**

Table 3. Heterosis (%) and dominance rate in F_1 for the trait 1000 kernel weight

		Heterosis %		Dominance rate in F_1
Hybrids	Year	hypothetical	real	
217A x 84R	2012	50.8	0.62	1
	2013	78.38	64.68	4.71
	mean	**64.59**	**32.65**	**2.86**
217A x 85R	2012	30.52	2.2	1.1
	2013	39.95	9.59	1.44
	mean	**35.23**	**5.89**	**1.27**
1017A x 89R	2012	39.54	7.58	1.33
	2013	109.4	61.61	3.69
	mean	**74.47**	**34.60**	**2.51**
813A x 84R	2012	39.4	43.6	2.65
	2013	104.8	99.56	19.3
	mean	**72.1**	**71.58**	**10.97**
813A x 85R	2012	38.4	30.8	6.25
	2013	54.47	26.5	2.46
	mean	**46.44**	**28.65**	**4.36**
813A x 89R	2012	37.6	41.1	6.25
	2013	66.9	50	5.93
	mean	**52.25**	**45.55**	**6.09**
2003A x 84R	2012	38.1	32.9	12.7
	2013	132.7	82.08	4.77
	mean	**85.40**	**57.49**	**8.74**
2003A x 85R	2012	37.1	37.6	14.09
	2013	57.6	42.74	4.59
	mean	**47.35**	**40.17**	**9.34**
2003A x 89R	2012	36.3	44.5	10
	2013	68.9	50.25	4.75
	mean	**52.60**	**47.38**	**7.38**

Table 4**.** Correlation analysis

	Plant height	*Head diameter*	*1000 kernel weight*
Plant height	1		
Head diameter	0.44	1	
1000 kernel weight	0,58**	0,76***	1

Table 5. Variation analysis

	Plant height	*Head diameter*	*1000 kernel weight*
Mean value	122.2	17.3	48.9
Standard Error	4.4	1.0	2.8
VC % Variation	177.8	249.5	242.5

CORRELATIONS AND PATH COEFFICIENT ANALYSIS OF CONFECTONERY SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

The most important criterion for introducing new confectionary hybrids into production is high protein yield. Breeding for increase of kernel protein content results in increased protein yield. Path coefficient analysis was performed to separate direct and indirect effects of studied traits on seed kernel protein content, and to identify traits which could be used as selection criteria in sunflower breeding. The research was conducted during three vegetation seasons on 22 NS high-protein two-line confectionary sunflower hybrids produced within the breeding program at IFVCNS, Novi Sad, Serbia. Strong and very strong correlations were found among the largest number of examined traits. Based on the analysis of simple correlation coefficients, strong negative correlation was determined between kernel protein content and kernel ratio (-0.516*). A weak negative interdependence was determined between head diameter, seed protein content, and kernel protein content. Positive but weak correlation was determined between kernel protein content and thickness of seed, length of seed, width of seed, and 1000 seed weight. Path coefficient analysis for kernel protein content at phenotypic level showed that the thickness of seed had a strong positive direct effect on kernel protein content (DE=382*). Kernel ratio and width of seed had a very strong direct negative effect on kernel protein content (DE=-0.990**; DE=0.600**). A weak direct positive effect of head diameter, seed protein content and length of seed was established, whereas 1000 seed weight had a weak direct negative effect on kernel protein content. This indicates that thickness of seed has high influence on kernel protein content.

Key Words : confectionary sunflower, correlations, kernel protein content, path coefficient analysis, quantitative traits

MORPHOLOGICAL CHARACTERIZATION OF UGA-SAM1 SUNFLOWER ASSOCIATION MAPPING POPULATION

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ABSTRACT

In order to use germplasm collections more efficiently and effectively, it is important to characterize the diversity of the germplasm. The objective of this study was to assess morphological diversity of a sunflower association mapping population UGA-SAM1 composed of 286 accessions and obtained from the USDA sunflower collection. Accessions were characterized for 10 traits to determine available morphological variability. The Shannon-Weaver diversity index (*H'*) was used to determine allele richness according to the frequency of genotypes in each nominal class. Phenotypic variation was found for all evaluated traits with *H'* values ranging from 0,45 to 0,90. The highest diversity was found for leaf lateral veins angle and height of the tip of the leaf blade compared to insertion of petiole, while least diversity was found for seed color and leaf shape. Homogeneity analysis by means of alternating least squares (HOMALS) grouped accessions to three major clusters: 1) RHA-Oil, 2) RHA-Oil and RHA-non Oil and 3) a mix of remaining accessions including Oil and non Oil accessions. The presented results confirm usefulness of UGA-SAM1 as a rich source of variability and as such a valuable resource for sunflower research.

Key Words : Characterization, diversity index, Germplasm, morphological, UGA-SAM1

HIGH OLEIC SUNFLOWER HYBRID OXY WITH CHANGED SEED TOCOPHEROL CONTENT

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ABSTRACT

Single cross sunflower hybrid Oxy was developed in VNIIMK, Krasnodar and registered by Russian State Commission on testing and protection of breeding achievements in 2014. Hybrid Oxy is considered an innovation from biochemical genetics to breeding for oil quality. All of the hybrid, female and male parent lines are homozygotes for a dominant high oleic mutation *Ol*, as well as recessive tocopherol mutation *tph1*, *tph2* and *tph3*. The main breeding character of the hybrid Oxy is increased oil oxidative stability up to 14 times due to high content of oleic content, gamma- and delta-tocopherols. Tocopherol mutations in sunflower seeds were originated from spontaneous mutagenesis within a gene pool of cultivated plants of *Helianthus annuus* L. All *tph1*, *tph2* and *tph3* mutations are monogenic recessive and non-lethal. The recombination of these mutations allows producing nearly any types of tocopherol profiles with four known alpha-, beta-, gamma- and delta-homologues. Mid-ripening hybrid Oxy possesses acceptable seed yield potential about 3 t/ha, broomrape resistance to race A-E, phomopsis tolerance and stay-green stem. Seed oil content averages 48%, hull content – 23%. Hybrid Oxy is not a GMO. The oil of the hybrid is intended to be used in the area of native oil with maximum level of oxidative stability i.e. for frying purposes.

Key words: Oil, Stability, Breeding, Oleic Acid, Tocopherol, Mutation

INTRODUCTION

Genetic research has led to an entirely new level in the global plant breeding for oil quality associated with overcoming interspecific barriers in genetic variation of seed lipid composition between different oil crops. For example, sunflower can produce oil similar to olive in fatty acid composition and flax - to sunflower. Plant varieties are developed with industrial-commodity targeting.

Sunflower breeding for oil quality based on the current trends to transfer the individual steps of industrial technology in the cells of living organisms. This process allows obtaining the desired substances of natural origin with ecological clean biosynthetic approach. Breeding strategy in this case is to create varieties with new types of oil determined by its use. There is selection of genotypes both on the extreme manifestation of the trait, i.e. minimum or maximum and the optimum content of desired substances. It is obvious that each type of oil has individual quality parameters.

Unlike breeding for yield increase, when traits of productivity, resistance to diseases and abiotic stresses are formed and implemented in the field during harvesting, breeding for improved quality deals with so-called “cross-cutting” characters of chemical composition of the seeds. These traits are formed in the plant in the field conditions and pass to the raw

materials and products of technological processing, i.e. they are realized in the industrial or consumer sectors.

The quality of the oil, i.e. its nutritive, biological and technological properties, depends on composition of fatty acids of a triacylglycerol molecule, and presence of related compounds. One of the important problems in improving of oil quality is to increase its resistance to oxidation for preventing toxic products of rancidity during storage and use.

The degree of unsaturation of fatty acids which correlated positively with the ability to oxidation and the presence of natural antioxidants, especially tocopherols, protecting against the free radical accumulation are the main factors of oil oxidation (Velasco *et al*., 2003, 2004; Warner *et al*., 2008).

HYBRID OXY DEVELOPMENT

The first major achievement in sunflower breeding for oil oxidative stability was held with high oleic variety of Pervenets in VNIIMK, Krasnodar (Soldatov, 1976). This variety has become a unique donor of the high oleic mutation in breeding programs worldwide.

Found in further studies the effect of synergism in the joint action of fatty acids and tocopherols on the stability of oil to oxidation has opened up opportunities in sunflower breeding by combining the desired genes (Demurin, 1993; Demurin *et al*., 1996). As a result of this work sunflower commercial hybrid, named Oxy, was developed in VNIIMK. It has both traits combined of high oleic acid and the high content of powerful antioxidants such as gamma- and delta-tocopherols (Table 1).

Table 1. Composition of fatty acids and tocopherols in the oil of sunflower hybrid Oxy

Hybrid	Fatty acid composition, %				Tocopherol composition, %			
	palmitic	stearic	oleic	linoleic	α	β	γ	δ
Standard	5,1	3,5	31,2	60,2	100	<1	<1	0
Oxy	4,3	3,8	86,2	5,7	<1	<1	60	40

Single-cross sunflower hybrid Oxy was obtained in the framework of breeding and genetic program to improve the quality of oil by the crossing inbred lines VK876 A × VK195. All parent forms, including female CMS analogue and maintainer, as well as a male fertility restorer are homozygous on four genes controlling the trait of high oleic acid content (dominant mutation *Ol*) and high content of powerful antioxidants such as gamma- and delta-tocopherol (triple homozygote for recessive mutations of *tph1*, *tph2* and *tph3*). The main valuable character of the hybrid Oxy is increased to 14-fold oxidative stability of the oil compared to the normal genotype due to the simultaneous change in the composition of fatty acids and tocopherols, which gives this hybrid world priority (Table 2).

Table 2. Breeding characteristics of the sunflower hybrid Oxy

Trait	Standard	Oxy
Vegetation period, days	94	94
Seed yield, t/ha	3,3	3,1
Seed oil content, %	51,8	47,8
Oil yield, t/ha	1,5	1,3
Oil type	linoleic, α-tocopherol	high oleic, γ- and δ-tocopherols
Oxidative stability, hours (Rancimat-test, 120 °C)	3,1	44,3

Hybrid Oxy belongs to the middle ripening group. The seed yield does not differ from the standard. The hybrid is resistant to broomrape (A-E), downy mildew, tolerant to phomopsis (stay-green). The vegetation period from germination to biological ripeness is 94 days, the achenes oil content of 48% and hull content of 23%. It is obvious that this hybrid is designed to produce special oil, i.e. for long shelf-life or frying. Hybrid Oxy has been included in the Russian state register of admitted and protected varieties since 2014.

CONCLUSIONS

Thus, sunflower hybrid Oxy was developed with common breeding methods without the use of transgenic techniques. The seed oil possesses the highest level of oxidative stability by combining high oleic acid content with the increase concentration of gamma- and delta-tocopherols asstrong endogenous antioxidants. This natural oil without any chemical modification and addition of exogenous ingredients can be useful in the industries with high demands for resistance to oxidation.

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CYTOGENETIC STUDY OF HELIANTHUS STRUMOSUS AND ITS F_1 AND BC_1F_1 HYBRIDS WITH CULTIVATED SUNFLOWER

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ABSTRACT

Helianthus strumosus L. is represented in Novi Sad collection of wild sunflower species with large number of accessions (14 with seed reserves and 20 in the field collection). It is often used as a source of resistance to disease-causing agents in the breeding of cultivated sunflower. Interspecific crosses with cultivated sunflower lines were performed using 17 accessions of this species. Six F1 hybrid combinations were obtained using two*H.strumosus* accessions with a total of 48 plants, while in backcrossing 51 BC1F1 plant was obtained. Nine originated from crossing F1 and 42 from crossing F1OP with cultivated sunflower. Cytogenetic analysis showed 3 levels of ploidy in the examined accessions of *H.strumosus* (n = 17, 34 and 51) and high pollen viability ranging from 83.13- 98.93%. F1 hybrids exhibited reduced pollen viability (26.83 - 55.34%), and there were occurrences of male sterility. Analysis of chromosomal association of F1 hybrids showed that chromosome number was 68, and that most commonly observed associations were 25-34 bivalents with the occurrence of quadrivalents, hexavalents and univalents. BC1F1 hybrids also had male sterile plants, while pollen viability ranged from 5.66 - 80.85%. Analysis of chromosomal associations in diakinesis showed a varying number of chromosomes (55 - 70), while the number of bivalents was 15-27, trivalents 0-3, quadrivalents 0-4, hexavalents 0-1 and univalents 1-5. In addition to irregular patterns of chromosome pairing in diakinesis, F1 and BC1F1 hybrids also exhibited irregularities like fast, lagging chromosomes and chromosome bridges in other stages of meiosis. Cytogenetic analyses show the difficulties in obtaining progenies of interspecific hybrids that will contain the desirable genes from *H.strumosus*.

Key Words : Sunflower, *Helianthus strumosus* L., Interspecific crosses, Cytogenetic analyses

VALIDATION OF SCAR-MARKER FOR RESTORATION FERTILITY GENE IN UKRANIAN INITIAL MATERIAL OF SUNFLOWER

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ABSTRACT

The breeding lines, lines of mutant origins, samples of interspecific hybrids and varieties of sunflower (in general 105 sample) for the presence of HRG01 locus, which linked with the Rf_1 gene have been analyzed. The HRG01 locus has always identified when this gene is present in the plant material. In the result of amplification band 426 bp has been synthesized. In the absence of the Rf_1 gene the specific band in the samples of sunflower was not found. Therefore, amplified product in male sterility restoration lines was not synthesized, while its synthesis was in male restoration lines. Percentage of samples with HRG01 locus in samples obtained from interspecific hybrids was 62. It was found that the allele of HRG01 locus occurred in 30 % of sunflower varieties. Its frequency was varied from 0.053 to 0.263.

Key words: Sunflower, SCAR marker, *Rf* gene

INTRODUCTION

The different types of DNA markers widely used in genetic and breeding research of sunflower. A significant amount of information about variability of markers is accumulated by RAPD, AFLP, SSR and SNP analyses. It has been constructed detailed genetic maps of sunflower including resistance genes to pathogens, morphological and biochemical traits, which are relative to a particular type of molecular markers (Jan et al., 1998; Lai et al., 2005; Heesacker et al., 2008; Tang et al., 2002). It was possible not only with the development of molecular methods, but detailed studies of genetics of resistance, morphological and biochemical traits of sunflower that studying the effects of most genes (Sharypina et al., 2008). Therefore, information on linkage marker and gene can be used in marker-assisted selection, which is widely used in many countries to intensify the breeding process (Popov & Kirichenko, 2010).

Restoration of pollen fertility in sunflower is controlled by Rf_1 gene with the possible interaction between at least two or three *Rf* genes. Features of genetic control of pollen fertility restoration are summarized in review articles and monographs (Gavrilova & Anisimova, 2003; Vedmedeva & Tolmachev, 2006; Popov & Kirichenko, 2010). The main step of creation of the sunflower restorer lines is determination of the ability of these lines to fully restore fertility of pollen in their crosses with male sterile lines. This selection process is laborious and time consuming (Popov & Kirichenko, 2010). Therefore, the creation of inbred sunflower lines should involve different DNA markers for screening the presence of *Rf* genes in various initial material that optimizes the breeding process.

Currently, the details of mapping of restoring fertility pollen gene Rf_1 has been gathered (Jan et al., 1998; Horn et al., 2003; Kusterer et al., 2005; Schnabel et al., 2008). Thus, based on polymorphic of RAPD fragments two SCAR markers – HRG01 and HRG02 were developed (Horn et al., 2003). One of the closely linked markers TRAP was converted to STS marker (Yue et al. 2010). Distance between STS and Rf_1 was 0.4 cM. Construction of a genetic map based on SSR-markers revealed that the *Rf1* gene is located in 13 linkage group (Tang et al., 2002). Also the molecular mechanisms of interaction between mitochondrial and nuclear genes were clarified (Moneger et al., 1994; Horn et al., 1999).

For efficient use of molecular markers in the breeding process their initial material of various origins should be validated. The purpose of this study was to establish the presence of SCAR-marker (HRG01) linkage with the gene Rf_1 in various breeding material of sunflower.

MATERIAL AND METHOD

Thirty seven inbred lines of sunflower created in the laboratory of breeding and genetics of sunflower of the Plant Production Institute named after V. Ya. Yuriev of NAAS (Kharkiv, Ukraine) were involved, including 11 male sterile lines, 19 male sterility restoration lines and 7 mutant lines. In addition, the study used 29 sunflower samples that obtained from interspecific hybrids. Also we involved 39 sunflower varieties of different origins.

SCAR-marker identification was performed by PCR with a pair of primers that flank certain areas of genomic sunflower DNA. The nucleotide sequences of primers to locus HRG01 were as follow: F: TATGCATAATTAGTTATACCC and R: ACATAAGGATTATGTACGGG (Horn et al., 2003).

PCR was performed using reagent kit GenePak PCR Core of LLC "Laboratory Izogene" (Russia). The final volume of the reaction mixture was 20 μl and contained 20 ng of genomic DNA with the addition of 0.2 mM of each primer. In test tubes of reaction mixture 20 μl of mineral oil was added. PCR was performed in thermocycle "Tertsyk" (Russia) using program according (Kusterer et al., 2005).

PCR products were run on 2 % agarose gel with high resolution and the addition of ethidium bromide in the low-molarity buffer. The amplified products were visualized using photography in UV light with photosystem NikonD50. For determination of the lengths of PCR products DNA ladders 50 bp and Mcombi (LLC " Laboratory Izogene", Russia) were used.

RESULT AND DISCUSSION

Cytoplasmic male sterility (CMS) always is in use for creation of high yields sunflower hybrids. On the base of CMS the inbred lines of three types – male-sterile lines ($cyt^S rf_1 rf_1$), sterility fixing lines ($cyt^N rf_1 rf_1$) and male sterility restoration lines ($cyt^N Rf_1 Rf_1$) create. The creation of inbred lines of sunflower is based on the interaction between classical cytoplasm PET1 with gene *Rf*.

At the first stage of the testing of marker HRG01 the inbred lines of sunflower (male-sterile and male sterility restoration lines) from collection of initial material of the laboratory of sunflower breeding and genetics of Plant Production Institute named after V. Ya. Yuriev of NAAS were involved. These lines are used to create single cross and three-way cross hybrids of sunflower in Plant Production Institute (Kirichenko et al., 2014). According to literature PCR product with size 426 bp indicates the presence of HRG01 locus and as a consequence of *Rf1* gene presence in sunflower genotypes. Involvement of inbred lines of Kharkiv breeding in the research allowed us to conduct the validation of HRG01 markers. Thus, absence of amplicon has been observed in all male-sterile lines, while in male sterility restoration lines

were identified PCR product with size of 426 bp. These results confirm the diagnostic ability of the marker to identify gene *Rf1* in the plant genotypes (fig.1).

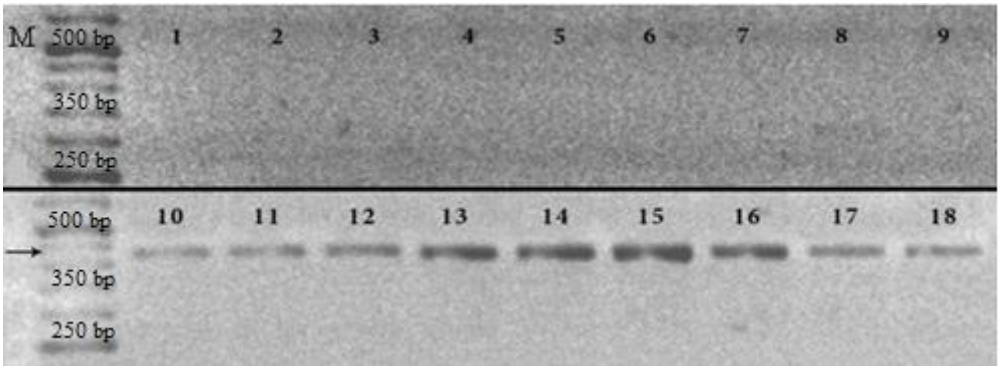

Fig.1 Electrophoregram of separation of amplified products of SCAR-marker HRG 01. 1–9 – male-sterile lines; 10–18 – male sterility restoration lines. The arrow shows the PCR product of the size of 426 bp.; M – DNA ladder «Mcombi».

Six lines of mutant origin were also tested with a pair of primers to HRG01. In the three lines of sunflower – Mkh1829, Mkh4 and Mkh42 the amplified product with size of 426 bp was found. It was absent in the lines Mkh2122, Mkh108, Mkh1091.

For the molecular genetic analysis samples obtained from interspecific hybrids of different origin have been involved. They were created with using annual wild species of sunflower *H. annuus*, *H. argophyllus* and *H. debilis*. It should be noted, that these samples are not analyzed for the presence of *Rf1* genes using classical plant breeding methods. As a result, molecular analysis revealed that 17 samples had specific PCR product. The size of this product was 426 bp, which corresponds with male sterility restoration lines, in which the presence of *Rf1* gene has been clearly identified by hybridization with male-sterile lines. The frequency of such lines was 0.586. In 12 samples obtained from interspecies hybrids PCR product with size of 426 bp (frequency 0.414) was not observed. The results allow to differentiate experimental material into two groups – the samples male-sterile lines type (absence of amplicon 426 bp) and the samples of male sterility restoration lines type (presence of amplicon size 426 bp).

Sunflower varieties were further involved to test the marker HRG01. The varieties of sunflower are the source of initial material for a complex of traits, including genotypes with genes *Rf1*. However based on the genetic structure the most varieties are fixers of sterility. This means that the populations consists mainly of genotypes $cyt^{N}rf_1rf_1$. Therefore, for intensification of creation of male fertility restorer lines from sunflower varieties DNA markers linked to the gene *Rf1* are need to use. Using pairs of primers to HRG01 locus amplified product with size of 426 bp was obtained. Results of separation of amplified products are shown on figure 2. It should be noted that amplified product 426 bp was not identified in all varieties-populations because most genotypes of these varieties are fixers of sterility.

In the studies of the molecular genetic structure of Ukrainian varieties the 426 bp allele of HRG01 locus were identified only in four varieties – Zaporiz'kii konditerskii, Mistsevii 1, Mistsevii 2, Mistsevii 15, ChaS. The frequency of allele 426 bp for these varieties was 0.105, 0.263, 0.579, 0.684 and 0.316, respectively. In other varieties this band was not detected.

However 426 bp allele of HRG01 locus was not identified in a sample of sunflower varieties of Russian breeding. In the varieties of sunflower breeding of Greece only variety Rodopi detected allele of 426 bp size with frequency 0.053. In other varieties allele of this size is not identified.

In French variety Nain noir and varieties Slovenska siva and Bucianska olejna from Czechoslovakia we also not found band 426 bp.

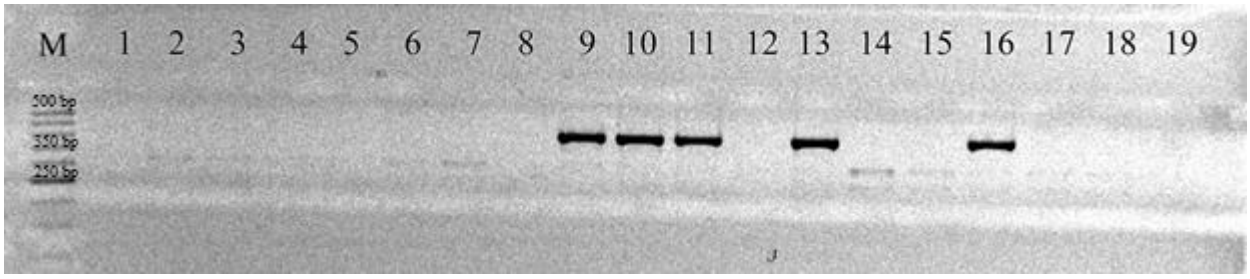

Fig. 2. Electrophoregram of separation of amplified product of SCAR-marker HRG 01 in sunflower variety Mennonite (Canada). 1–19 – genotypes of variety Mennonite; M – DNA ladder 50 bp.

Analysis of the distribution of frequency of allele 426 bp of HRG01 locus for the Hungarian varieties revealed that allele was present only in varieties Mezoeheguesi and Lovaszpatonai. The allele frequency was 0.053 in both varieties. In two other varieties of Hungarian breeding allele was not detected.

According to the analysis of HRG01 locus allele 426 bp was identified in three out of four USA varieties. These are the varieties Untitled (PI 432515), Arrowhead, Ghray Mammoth. The frequencies of allele in these varieties were 0.105, 0.158 and 0.211 respectively. In variety Mingren allele 426 bp is not detected.

In varieties of Canadian breeding the allele of size 426 bp of HRG01 locus was identified only in variety Mennonite. Its frequency was 0.263. In other varieties allele is not detected.

In general, it should be mentioned that the 426 bp allele of HRG01 locus in 12 (30 %) of sunflower varieties is distributed and in 27 varieties (70 %) is not identified.

CONCLUSION

SCAR-marker (locus HRG01) clearly identified in breeding lines, which are shown of the presence or absence of a gene Rf_1. Using samples of sunflower, created with the involvement of annual wild species and varieties has proved the presence in their genotypes of PCR products with size of 426 bp, which indicates also the presence of the Rf_1 gene. The results make it possible to conduct targeted selection of male lines from interspecific hybrids and varieties.

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THE PUBLIC SUNFLOWER ASSOCIATION MAPPING POPULATION

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ABSTRACT

In recent years, major steps have been made toward generating genetic and genomic resources in sunflower; however, the tools for associating phenotype with genotype have remained largely undeveloped. To help fill this gap, we developed a permanent, publicly-available association mapping resource for sunflower. The Sunflower Association Mapping (SAM) population consists of 271 diverse inbred lines and captures nearly 90% of the genotypic diversity in cultivated sunflower. We have grown the SAM population in replicate at three locations and phenotyped it for a number of agronomically important traits including days to flower (DTF) and plant architecture. All individuals within the SAM population were sequenced to a minimum of 8-10x depth with the Illumina platform. Using custom bioinformatics pipelines developed in collaboration with the software company SAP, we extracted 613,011 high confidence SNPs to be employed for genome-wide association study (GWAS) analyses. To identify the alleles associated with phenotypes characterized in the SAM population, we have completed development of a GWAS pipeline which includes imputation, population structure calculation, kinship, mixed linear model, p-values adjustment, meta-analysis across replicates and environments, tests for GxE, and calculation of variance components and effect sizes at each SNP position. We implemented this custom pipeline on the SAM phenotypic data for DTF and total branching which was previously analyzed using genotypes from a 10K Illumina SNP array. We present details of the SAM population including phenotypic and genetic diversity, results from the newly developed custom GWAS pipeline, and comparisons to previous association results in sunflower employing SNP arrays.

Key Words : association mapping, whole genome sequencing, GWAS, flowering time, genetic diversity

FH-586- A SHORT DURATION HIGH YIELDING SUNFLOWER HYBRID UNDER SEMIARID CONDITIONS

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ABSTRACT

Oilseeds Research Institute, Faisalabad Pakistan is working on the development of high yielding sunflower hybrids, resistant to insect pest and diseased suitable in existing cropping pattern of variable agro climatological condition of Pakistan.

17 hybrids viz., FH- 533, FH-557, FH-558, FH-572, FH-583, FH-585, FH-586, FH-587, FH-592, FH-593, FH-594, FH-595, FH-596, FH-598, FH-600 and two checks FH-331 and Hysun-33 were evaluated at this Institute for their performance under semi-arid conditions of Pakistan during autumn (August to November) 2014. The data depicted highest seed yield of 1950 kg per ha for the hybrid FH-586 and followed closely by Hysun-33 the check the seed yield of which was 1930 kg per ha. The 2nd check FH-331 yielded 1825 kg per ha. The important aspect shown by FH-586 was its early physiological maturity (14 days) than imported hybrid Hysun-33. The former matured in 77 days as compared to later (91) days. Early maturity helps farmers to prepare land for wheat crop, the best sowing time of which in Pakistan is 1st fortnight of November. It is also supportive in mitigating the import bill incurred on edible oil on one hand and maximizing wheat seed yield on the other hand. The entire advantage reaped due to its sowing during autumn is not only for Pakistan but for the entire humanity as the wheat grain produced in the process may be helpful for eradicating hunger in the world.

Key Words : Short duration, Physiological maturity, Import bill, High yield, Hunger eradication.

CYTOMORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF INTERSPECIFIC HYBRIDS BETWEEN *HELIANTHUS ANNUUS* AND H. *ARGOPHYLLUS* T. & G.

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ABSTRACT

Successful interspecific hybrids were obtained through sexual hybridization between cultivated *Helianthus annuus* (ARM-243B; 2n=34) and a wild *Helianthus* species [*H. argophyllus;* 2n=34*;* PI-468649], using the later as pollen parent for transferring desirable traits like downy mildew resistance, oil content and hopper resistant from wild species into cultivated background. Morphological, cytological and simple sequence repeats (SSR) based molecular analyses were carried out to confirm the hybrid nature of F1 plants. The hybrids exhibited morphological features intermediate to both the parents for few attributes and more related to wild *Helianthus* species like, leaf and stem hairiness, flower colour, stem size, branching, disc floret pigmentation, plant height, seed size and seed shape, etc. A reduction (89.9%) in pollen fertility was recorded in F1 plants as compared to both the parents. Meiotic analysis revealed a mixture of univalents, bivalents, trivalents and quadrivalents in all the pollen mother cells (PMCs) analysed. In addition to bivalents and univalents, a trivalent was also observed in few PMCs, indicating segmental homology between chromosomes. Higher level of chromosome configurations like quadrivalents was also observed in 42 out of 50 PMCs. Frequently observed chromosome configurations in diakinensis were 15 II + 1 IV and 13 II + 2 IV. The results suggested that the species *H. argophyllus* and *H. annuus* differ by 1-2 translocations and 1-2 inversions. Hybridity of interspecific hybrids was confirmed through sunflower specific molecular markers. Primers ORS-05, ORS-896 and ORS-908 were found to reveal highly polymorphic bands in the parents and were used for confirmation of hybridity of the F1s. The informative SSR markers screened in the study will be useful marker resources for tracking the flow of *H. argophyllus* genetic material among the progenies that may be produced by future backcrosses to *H. annuus*. Results show that the classical method of crossing is applicable in sunflower breeding programs for obtaining interspecies hybrids.

Key Words : sunflower, wild species, prebreeding, inter-specific hybridization

BROADENING THE GENETIC BASE OF CULTIVATED SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN INDIA THROUGH PREBREEDING

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ABSTRACT

In India, sunflower production is severely constrained by heavy yield losses due to diseases like *Alternaria* ster leaf spot (*Alternaria ster helianthi*), downy mildew (*Plasmopara halstedii* (Farl.), powdery mildew (*Golovinomyces cichoracaerum*), rust (*Puccinia helianthi* Schw.) and sunflower necrosis disease. Further, susceptibility to water stress in rainfed cultivation results in low yields. Hence, recent years have witnessed a decline in the acreage under the crop mostly in the traditional sunflower growing regions. Among the various approaches to manage these stresses, host plant resistance is the most reliable and economical to the end users. With its large potential for export as confectionary or non-oilseed, there is a need to develop genotypes with specific quality characteristics. However, plant breeding efforts to develop varieties/hybrids with the desired economic characteristics are constrained by the narrow genetic base of the cultivated sunflower. Concerted efforts are required to incorporate additional genetic variability from reliable sources by integrating modern biotechnological tools and conventional breeding methods. Wild *Helianthus* species are rich sources of genetic variability in terms of resistance to biotic and abiotic factors, altered plant architecture, high yield, oil content, maturity duration, oil quality and continue to serve as sources of cytoplasm and fertility restorer genes. Prebreeding is required to broaden the genetic material potential for increased heterosis and to integrate useful genes such as resistance to biotic and abiotic stresses, better oil quality and higher yield performance into developed inbred lines. Successful introgression of desirable genes from the distantly related wild *Helianthus* into cultivated sunflower requires a clear understanding of the genome relationships of the wild *Helianthus* species and the cultivated sunflower through extensive genetic, cytogenetic, and molecular investigations. This review is mainly focused on the current status of the ongoing prebreeding work and the utility of the prebreeding materials developed in India for sunflower improvement.

Key Words : Sunflower, prebreeding, Helainthus species

MOLECULAR BREEDING FOR MAJOR DISEASES OF SUNFLOWER IN INDIA: PRESENT STATUS AND FUTURE NEEDS

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ABSTRACT

Sunflower is an important sources of vegetable oil in the world. The adaptability and versatility of the crop is being demonstrated by its cultivation from subtropical to sub arctic areas. Asia accounts for nearly 20–22% of the global sunflower and contributes to about 18% of the production. The productivity of sunflower in Asia is about 1.0 t/ha which is lower than the world average. India is the second largest grower of sunflower in the Asian continent. The major problems confronting sunflower productivity in India is the vulnerability of the crop to various biotic stresses. Climate change has lead to the evolution of minor diseases into epidemic status in sunflower. The major diseases of sunflower in India include *Alternaria* leaf spot (*Alternaria helianthi*), downy mildew (*Plasmopara halstedii* (Farl.), powdery mildew *(Golovinomyces cichoracaerum*), Sclerotinia rot (*Sclerotinia sclerotiorum*) sunflower necrosis disease (SND) caused by *Tobacco necrosis virus*, rust (*Puccinia helianthi* Schw.) and dry head rot (*Rhizopus* spp). Polygenic inheritance of resistance is reported in case of *Alternaria* leaf spot and powdery mildew. The resistance to *P. halstedii* is known to be controlled by dominant *Pl* genes, grouped in clusters (*Pl*1-*Pl*13 & *Pl*Arg) each conferring resistance to different races. Resistance for *P. helianthi* is reported to be controlled by seven genes *R*1-*R*5, *R*Adv and *Pu*6. Nature of resistance to Sclerotinia is described as partial, quantitative and mostly additive. Promising resistance sources for these fungal diseases have been found in wild sunflowers and exotic germplasm derived through interspecific hybridisation. Fine mapping of these diseases will aid in precision breeding. SND being a virus disease is transmitted by aphid *Myzus persicae* and*Capitphorus elaegni*, breeding for resistance against these vectors is known to reduce incidents of SND. In this review, emphasis is mainly focused on the current status of knowledge related to 'R' genes controlling resistance against major diseases of sunflower in India and their sources as well as markers associated with these genes.

Key Words : sunflower, diseases, breeding, markers

GENE EFFECTS AND COMBINING ABILITIES OF SUNFLOWER YIELD AND MORPHOLOGICAL TRAITS BY LINE X TESTER MATING DESIGN

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ABSTRACT

Recent changes in vegetable oil production in Uganda has realized that sunflower is now the main oilseed crop for producing vegetable oil. However the oil production is not enough for domestic needs and as such, much of the seed in production is basically from the imported sunflower hybrids. The development of sunflower hybrids with high genetic potentials for seed yield and other seed yield components requires information on the GCA and SCA for agronomically important traits. Understanding the genetic basis and mode of gene action for grain yield and important agronomic traits of sunflower would facilitate the improvement of sunflower production in Uganda. Choosing suitable lines for breeding as a parental component of a hybrid variety is of great importance. Seven CMS inbred lines used as females and six restorers used as males were crossed in a line x tester mating design to produce 42 single cross hybrids. Planting was done at the National Semi-Arid Resources Research Institute (NaSARRI) in 2013. The design was alpha-lattice (7 x 8) with three replications. One experimental hybrid (Belmonte) was used as a check. The traits recorded were days to 50% flowering, days to maturity, head diameter, plant height, number of seeds per head, and weight of seeds per head. The objective was to investigate the GCA and SCA effects of the F1 hybrids to the expression of the mentioned morphological characters.

Key words: Combining ability, gene effect, Line x tester design, Sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) has become the main oilseed crop in Uganda especially in the eastern and northern districts of the country. It is a source of livelihood for a number of resource poor farmers in these areas as the main immediate source of income. It is still the only main source of edible oil in the country followed by palm oil which was introduced into the country recently. By late 1980, Uganda was importing 98% of the total edible oil in the country. Considering the high oil content compared to other oilseeds, sunflower shows the greatest potential in reducing Uganda's dependence on imported edible oil. Its oil is used mainly as cooking oil and for soap making while the seed cake is being used as livestock feed. Most rural poor farmers and commercial producers in the sunflower growing areas obtain much lower yields than the expected potential yields due to lack of improved varieties and poor agronomic practices. There is severe lack of sufficient seed of acceptable varieties to meet the demand for the required plantings. Most of the released hybrids that have been released so far are being imported from South Africa. Their prices are expensive and not affordable by the poor resource farmers in Uganda.

Production of sunflower in a country needs genotypes that are widely adaptable so that it can be grown in a wider area and becomes of economic importance to the country. Breeders usually attempt to identify superior varieties for most of the characters of economic interest. A variety might be high yielding in a geographic region for which the breeder is evolving varieties but when employed as a parent in crosses, this variety may emerge as a poor combiner. In other words, this line does not appear to transmit desirable genes for the better

performance to its progeny. Such a behavior could result from intra- and/or inter-allelic interaction of genes concerned with the character. Thus superior performance of a variety is not always reflected in its combining ability.

The concept of combining ability is a measure of gene action. Combining ability analysis is used in the breeding programme for testing the performance of lines in hybrid combinations and also for characterizing the nature and magnitude of gene action involved in the expression of quantitative traits. General combining ability (GCA) of a line/variety refers to the average value of that line/variety estimated on the basis of its performance when crossed with other lines (Falconer, 1989). General combining ability is largely due to additive genetic effects and additive x additive epistasis. Meanwhile specific combining ability (SCA) is used to designate in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines crossed. Specific combining ability is largely a function of non additive dominance and other types of epistasis. The concept of general and specific combining ability is of practical importance to the breeders. It is therefore, in the interest of breeders to know how the two combining abilities are related to various components of heritable variations.

A successful breeding programme depends on the variability present among the different genotypes and in-depth understanding of the underlying gene action and genetic architecture of traits related to yield. Selection of parents based on their performance *per se* alone may not always be a sound procedure, since phenotypically superior genotypes may yield inferior hybrids and/or poor recombinants in the segregating generations. It is very important to identify parents with high general combining ability (GCA) value for the trait to be improved (Banerjee and Kole, 2009).

Information on gene action and combining ability helps in the choice of suitable parents for hybridization programmes for developing superior F_1 hybrids so as to exploit hybrid vigour and building genotypes to be used in the breeding programme. The objectives of the present study were to assess the nature and magnitude of gene action controlling the inheritance of seed yield and yield characters in selected sunflower genotypes.

MATERIALS AND METHODS

The sunflower lines were introduced from USA, Canada and Australia. Seven CMS inbred lines were used as females and six restorers used as males in a line x tester mating design to produce 42 single cross hybrids. Planting was done at the National Semi-Arid Resources Research Institute (NaSARRI), Serere in 2013. The design was alpha-lattice (7 x 8) with three replications. One experimental hybrid (Belmonte) was used as a check. Each plot had four rows and two middle rows were used for data recording. The spacing was 75 x 30 cm at a length of 4 m long. No fertilizer was applied since most farmers do not use fertilizer in their fields. Yield data was obtained from the two middle rows during harvest. The traits recorded were days to 50% flowering, days to maturity, head diameter, plant height, number of seeds per head, and weight of seeds per head.

RESULTS AND DISCUSSIONS

Analysis of variance is presented in Table 1. There was highly significant difference among the female lines for days to 50% flowering, days to maturity and plant height. No significant

difference was recorded for head diameter, number of seeds per head and seed weight per head. Similar observations were recorded for males whereby high significant differences were observed for days to 50% flowering, days to maturity and plant height. Female x male interaction only showed significant difference for days to 50% flowering and days to maturity.

Table 1: Mean square variances for the different traits studied

SoV	d.f	Days to 50% flowering	Days to maturity	Head diameter (cm)	No of seeds per head	Plant height (cm)	Seed weight per head (gm)
Rep	2	11.90***	6.16	10.89	127586	185	553
Female	6	9.47**	5.76***	9.34	73216	772***	352
Male	5	25.48***	4.32***	6.78	39936	987***	165
F x M	30	3.67***	0.74***	8.51	49920	231	226
Residual	60	1.20	3.20	8.05	83735	155	
Total	124	3.61		8.22		240	

*, **, *** Significant at 5%, 1% and 0.1% Probability level.

Table 2 shows the mean performance of the single cross hybrids evaluated. Days to 50% flowering ranged from 57 to 63 days while days to maturity ranged from 88 days in Cms850 x RHA346 to 99 days in Cms850 x RHA374-1, Cms850 x RHA373 and Cms412 x RHA374-1. The highest number of seeds per head was recorded in Cms383 x RHA447 with 1291 mean number of seeds per head followed by Cms404 x RHA447 and Cms383 x RHA271. The tallest hybrid was cms403 x RHA374-1 which recorded 153 cm in height followed by Cms404 x RHA373. For seed weight per plant, Cms383 x RHA271 and Cms383 x CM632 had the highest seed weight per plant with 79 gms.

Table 2. Mean performance of the single crosses evaluated at Serere, 2013

Hybrids	Days to 50% flowering	Days to maturity	Head diameter (cm)	No of seeds per head	Plant height (cm)	Seed weight per head (gm)
Cms383x CM632	58	91	19	1112	129	79
Cms 402x CM632	61	95	14	704	109	46
Cms 403x CM632	60	96	13	662	112	44
Cms 404x CM632	60	98	16	959	111	67
Cms 412x CM632	61	95	20	905	133	72
Cms 433x CM632	60	91	15	870	119	53
Cms 850x CM632	58	91	16	1057	144	66
Cms383XRHA271	60	95	18	1208	136	79
Cms 402x RHA271	61	98	15	990	113	63
Cms 403x RHA271	61	98	15	691	116	45
Cms 404x RHA271	61	98	15	707	128	51
Cms 412x RHA271	60	96	18	941	141	66
Cms 433x RHA271	62	95	16	685	119	57
Cms 850x RHA271	58	89	16	1174	150	68
Cms383x RHA346	61	92	12	690	114	41
Cms 402x RHA346	60	96	15	880	130	50

Cms 403x RHA346	60	92	16	889	117	77
Cms 404x RHA346	60	97	17	1196	134	77
Cms 412x RHA346	62	96	14	703	129	37
Cms 433x RHA346	61	91	15	966	122	57
Cms 850x RHA346	59	88	14	615	121	47
Cms383x RHA373	61	98	15	719	142	57
Cms 402x RHA373	61	98	15	621	122	45
Cms 403x RHA373	63	98	14	612	115	37
Cms 404x RHA373	61	98	15	955	148	64
Cms 412x RHA373	61	95	16	778	139	48
Cms 433x RHA373	61	91	14	795	116	47
Cms 850x RHA373	63	99	18	1107	141	72
Cms383X RHA374-1	65	98	17	794	139	49
Cms 402x RHA374-1	63	98	14	750	133	44
Cms 403x RHA374-1	63	99	16	852	153	51
Cms 404x RHA374-1	62	98	16	939	145	70
Cms 412x RHA374-1	63	99	16	925	146	64
Cms 433x RHA374-1	63	98	14	643	137	43
Cms 850x RHA374-1	60	93	14	760	147	44
Cms383x RHA447	60	92	16	1291	141	70
Cms 402x RHA447	61	97	13	705	104	37
Cms 403x RHA447	60	97	16	951	138	61
Cms 404x RHA447	62	97	17	1284	136	72
Cms 412x RHA447	61	98	14	840	134	44
Cms 433x RHA447	60	91	16	969	128	65
Cms 850x RHA447	57	91	13	610	122	33

The GCA effects for females and males are presented in Table 3. For days to 50% flowering, Cms850 was the only female line that recorded significant negative GCA effect. In order to reduce the time for days to 50% flowering, HA850 could be useful. No male showed any significant difference in days to 50% flowering however, RHA374-1 could be used for increasing days to 50% flowering as days to 50% flowering is positively correlated to yield. All the female lines recorded highly significant difference ($P<0.001$) for days to maturity. Since days to maturity is positively correlated to yield, the females with positive GCA effects such as Cms402, Cms403, Cms404 and Cms 412 would be useful. In areas with less rainfall, Cms433 and Cms850 could be useful in the breeding programme. Among the males, highly significant difference ($P<0.001$) was also recorded. RHA373 and RHA374-1 had high positive GCA effects which could also improve yield. CM632 and RHA346 had high negative GCA effects which would be good for rainfall areas. No significant GCA effects were recorded for head diameter for the females and the males. However, Cms383, Cms404 and Cms412 among the females and CM632 and RHA271 among the males could be useful in improving the head diameter in the breeding programme. For number of seeds per head, only Cms404 had a significant positive GCA effect. For plant height, Cms402 was the only female line with a significant negative effect GCA effect. It could be useful in decreasing the plant height against lodging. Meanwhile, among the males, RHA374-1 had high significant positive ($P<0.001$) GCA effect. No any genotype among both the female and male lines had any positive significant GCA effect for seed weight per head. However, among the female lines, Cms383, Cms404 and among the male lines, CM632 and RHA271 could be useful in improving the yield performance of the sunflower lines.

Table 3. General combining ability effects for various yield components in sunflower at Serere

	Days to 50% flowering	Days to maturity	Head diameter (cm)	Number of seeds per head	Plant height (cm)	Seed weight per head (gm)
Females						
Cms383	0.096	-0.92***	0.68	99.82	3.5	6.4
Cms402	0.270	1.61***	-0.93	-94.13	-11.3**	-8.6
Cms403	0.253	1.59***	-0.48	-93.12	-4.7	-3.6
Cms404	0.123	2.50***	0.52	137.7*	3.8	10.4
Cms412	0.430	1.10***	0.96	-20.4	7.2	-1.0
Cms433	0.443	-2.46***	-0.54	-47.93	-6.2	-2.4
Cms850	-1.615*	-3.42***	-0.21	18.07	7.6	-1.2
SE	0.365	0.153	0.945	67.98	4.2	5.0
Males						
Cm632	-0.993	-1.59***	0.76	26.36	-7.4	5.0
RHA271	-0.487	0.37*	0.65	44.52	-0.6	5.1
RHA346	-0.733	-2.17***	-0.67	-20.81	-6.1	-0.9
RHA373	0.828	1.53***	-0.15	-70.90	1.8	-3.5
RHA374-1	2.041	2.39***	-0.20	-60.07	13.1***	-4.0
RHA447	-0.656	-0.53***	-0.39	80.90	-0.8	-0.5
SE	0.337	0.14	0.88	62.94	3.8	4.6

The SCA effects are presented in Table 4. SCA effects are indicators of dominance gene effect. For days to 50% flowering, positive SCA effects were observed in the crosses Cms433 x RHA271, Cms850 x RHA373, Cms383 x RHA374-1 and Cms404 x RHA447. For negative SCA effect, this was recorded in Cms383 x CM632, Cms403 x RHA374-1 and Cms850 x RHA447. This results in early flowering hybrids. For days to maturity, Cms850 x RHA373 had the highest positive SCA indicating that it had the highest maturity period. Meanwhile, Cms403 x RHA374-1 had the highest negative SCA effect indicating that it was the earliest hybrid in maturity. For number of seeds per head, Cms850 x RHA373 had significant ($P<0.05$) positive SCA effect followed by Cms850 x RHA271 and Cms383 x RHA 447. For plant height, negative significant SCA effect were recorded in Cms404 x CM632, Cms403 x RHA 374 and Cms850 x RHA 447 while positive significant SCA effect for seed weight per head was recorded in Cms403 x RHA346 and Cms850 x RHA373.

Conclusion

A number of genotypes have shown variability with desirable GCA and SCA effects that can be used in the sunflower breeding programme in Uganda. High variability is recorded especially in days to maturity.

Table 4: Estimates of specific combining ability effects for yield and yield components in sesame at Serere

Hybrids	Days to 50% flower	Days to maturity	Head diameter (cm)	No of seeds per head	Plant height (cm)	Seed weight per head (gm)
Cms383x CM632	-1.47*	-1.87***	2.41	116.2	2.8	11.7
Cms 402x CM632	0.50	-0.73**	-1.31	-97.5	-1.9	-6.7
Cms 403x CM632	-0.48	0.89**	-2.75	-140.4	-5.5	-13.2
Cms 404x CM632	0.53	1.65***	-0.42	-74.0	-15.5*	-4.6
Cms 412x CM632	0.64	-0.24	3.13	29.8	3.7	12.0
Cms 433x CM632	0.15	-0.31	-1.04	22.3	3.2	-5.6
Cms 850x CM632	0.13	0.62*	-0.02	143.7	13.9	6.3
Cms383XRHA271	-0.67	-0.17	1.19	194.6	3.4	11.8
Cms 402x RHA271	0.08	0.92***	-0.20	170.8	-4.7	10.4
Cms 403x RHA271	0.12	1.07***	-0.99	-130.0	-8.4	-13.2
Cms 404x RHA271	0.69	0.01	-1.65	-344.3**	-4.9	-20.8*
Cms 412x RHA271	-1.13	-0.54*	0.57	47.9	5.0	5.8
Cms 433x RHA271	1.48*	1.74***	0.74	-181.2	-3.7	-1.6
Cms 850x RHA271	-0.58	-3.03***	0.34	242.1*	13.3	7.6
Cms383x RHA346	0.54	-0.03	-3.49*	-258.5*	-13.6	-20.6**
Cms 402x RHA346	0.19	1.12***	1.45	125.5	17.6*	3.6
Cms 403x RHA346	-0.61	-2.45***	2.00	133.3	-2.4	25.6*
Cms 404x RHA346	-1.18	1.46***	1.68	210.4	6.5	11.0
Cms 412x RHA346	1.08	1.40***	-1.44	-124.7	-2.2	-17.6*
Cms 433x RHA346	0.56	0.24	0.39	165.5	4.7	4.5
Cms 850x RHA346	-0.58	-1.73***	-0.59	-251.5*	-10.8	-6.6
Cms383x RHA373	-0.59	2.36***	-0.68	-178.9	6.5	-2.5
Cms 402x RHA373	-1.14	-0.32	0.93	-82.7	1.7	0.8
Cms 403x RHA373	0.98	-0.13	-0.85	-92.8	-12.5	-11.8
Cms 404x RHA373	-0.62	-1.07***	-0.52	19.5	12.5	0.5
Cms 412x RHA373	-1.23	-2.64***	-0.63	0.4	0.2	-3.5
Cms 433x RHA373	-0.77	-3.42***	-1.13	44.0	-9.9	-3.6
Cms 850x RHA373	3.37*	5.22***	2.88	290.3*	1.5	20.1*
Cms383X RHA374-1	2.49***	1.35***	0.69	-115.0	-7.8	-9.6
Cms 402x RHA374-1	-0.24	-1.11***	-0.03	35.2	1.2	0.5
Cms 403x RHA374-1	-60.42***	-95.74***	-14.62***	-733.0***	-115.0***	-53.9***
Cms 404x RHA374-1	-1.05	-1.98***	-0.14	-8.0	-1.3	7.1
Cms 412x RHA374-1	0.06	-0.36	0.09	136.4	-4.3	12.6
Cms 433x RHA374-1	-0.52	3.06***	-0.74	-118.0	0.4	-6.5
Cms 850x RHA374-1	-1.07	-0.62*	-0.74	-66.7	-3.1	-6.5
Cms383x RHA447	-0.30	-1.63***	-0.12	241.6*	8.6	9.2
Cms 402x RHA447	0.61	0.12	-0.84	-151.4	-13.9	-8.7
Cms 403x RHA447	-0.35	0.96***	1.72	93.7	14.0	10.2
Cms 404x RHA447	1.63*	-0.08	1.05	196.4	2.6	6.8
Cms 412x RHA447	0.58	2.38***	-1.72	-89.8	-1.9	-9.4
Cms 433x RHA447	-0.90	-1.31***	1.77	67.3	5.2	12.7
Cms 850x RHA447	-1.27*	-0.45	-1.88	-358.0**	-14.8*	-20.8*
SE	0.63	0.26	1.64	117.8	7.2	8.7

SOURCE-SINK RATIO EFFECTS ON THE EXPRESSION OF GENES ASSOCIATED WITH GRAIN GROWTH IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Grain size is the result of the coordinated growth of the embryo, endosperm and maternal tissues. Understanding the clues of the development and growth of these tissues is essential for increasing grain weight, a key component of sunflower yield and quality. This research was aimed at evaluating the effect of pre-anthesis shading (source-sink ratio reduction) on grain growth and the expression of genes associated with grain size between R3 and physiological maturity in sunflower. Two sunflower genotypes contrasting in grain weight were sown in a split plot design with three replicates. Shading treatments (nets intercepting 80% of incident radiation) were set over the plots from R3 to R5 stage. Ovaries and grains (the last divided in pericarp and embryo) were sampled from R3 to R9 stage. RNA was extracted from ovary and grain tissues. The time-course of the expression of putative orthologous genes for sunflower of HaGW2 (RING-type E3 ubiquitin ligase-like) and HaAP2 (EREBP-like), were assessed by qPCR. Grain weight was affected ($P < 0.05$) by both genotype and shading treatments. The lower source-sink ratio decreased final grain weight. Interestingly, the expression of HaGW2 and HaAP2 genes was affected by the genotypes and the source-sink ratio in flowers and grains tissues across the developmental stages. Results presented here suggest that HaGW2 and HaAP2 genes act in the pericarp and might be involved in driving the growth of grains in this crop.

Key words: Sunflower, grain weight, grain size, genetic control, AP2, GW2.

INTRODUCTION

Grain weight in crop plants is an important agronomic trait and a key component of sunflower yield. Grain development requires a double fertilization event generating two products within the embryo sac: the embryo and the endosperm (Lopes and Larkins, 1993). The embryo is surrounded by the endosperm, which, in turn, is enclosed within the maternal seed coat. The grain size is regulated by the coordinated growth of the embryo, endosperm, and maternal tissues (Fang et al., 2012; Xia et al., 2013).

In recent years, the knowledge about grain development improved considerably, and some genetic and molecular mechanisms are now known, mainly in model plants (Sundaresan, 2005; Sun et al., 2010; Li and Li, 2015; Orozco-Arroyo et al., 2015). However, in grain crops like sunflower this knowledge is still partial. Grain size is affected by the

maternal and/or zygotic tissues. It is known that in various crops the grain weight and grain size has a polygenetic control (Zhang et al., 2012; Kesavan et al., 2013). In *Arabidopsis* APETALA 2 (AP2) encodes a member of the AP2/EREBP (ethylene responsive element binding protein) may restrict grain growth by limiting cell proliferation in the integuments (Jofuku et al., 2005; Ohto et al., 2005). Ap2 mutant grains exhibit delayed cellularization of the endosperm resulting in larger embryo sacs and bigger embryos that show increased cell number and size and this larger grain trait was passed through the maternal sporophyte and endosperm genome (Jofuku et al., 2005; Ohto et al., 2005, 2009).

The role of the ubiquitin pathway on the grain size determination has been widely investigated over the last years in *Arabidopsis* (Li and Li, 2014). Several members involved in this pathway have been identified. DA1 and DA1-related (DAR) encode for plant-specific ubiquitin receptor protein. DA1 protein might act antagonistically with native DA1 or DAR, and would be negatively regulating cell proliferation in maternal grain tissues (Li et al., 2008). DA2 and enhancer of DA1 (EOD1) encode protein with E3 ubiquitin ligase activity and are also negative regulators of grain size (Xia et al., 2013). In rice GW2 (RING-type E3 ubiquitin ligase) functions as a negative regulator of grain width and weight, the loss of function of GW2 leads to increased cell number, a widers pikelet hull and an accelerated grain milk-filling rate, which increases grain width, grain weight and yield (Song et al., 2007). In recent years, extensive studies of GW2 have been carried out in wheat (Su et al., 2011; Yang et al., 2012; Zhang et al., 2013; Qin et al., 2014; Simmonds et al., 2014, 2016; Hong et al., 2014). Most of them reported that GW2 is a negative regulator of grain size and weight, except the study accomplished by Bednarek et al. (2012), where the authors reported the positive effect of GW2.

In the last decades, many key regulators of grain size have been identified; however, it is still limited the knowledge on genetic control of grain size and weight in crops, and this knowledge is virtually lacking in sunflower. The present research was aimed at evaluating the effect of pre-anthesis shading (source-sink ratio reduction) on flower and grain growth as well as the expression of genes associated with grain size between R3 and physiological maturity in sunflower the dynamics of grain dry matter accumulation and grain dimensions were analyzed in parallel with the expression of AP2 and GW2 genes.

MATERIALS AND METHODS

Field site description, treatments and experimental conditions

A field experiment was conducted at the Agricultural Experimental Station (EEAA) of Universidad Austral de Chile in Valdivia (39°47´S, 73°14´W, 19 m ASL), Chile in the 2014-2015 growing season. Two genotypes contrasting in grain weight and with similar phenology were arranged in a split plot design with three replicates, where the source-sink manipulation treatment was assigned to main plots and genotypes to subplots. Plant density was 6 plants m^{-2} and the dimension of plots (experimental units) was 9 rows at 0,70 m apart. 20 plants were sown per row. The treatments were the outcome of combining (i) two genotypes and (ii) two source-sink rates (control without shading and shading treatment by nets intercepting 80% of incident radiation, imposed during the pre-anthesis period, from R3 to R5). Genotypes were Alybro from Panam Seeds of small size grain (oilseed) and RHA-280 of big size grain (confectionery), from NCRPIS, USDA-ARS.

Sampling and grain measurements

Phenology was recorded by using the scale proposed by Schneiter and Miller (1981). Flowers and grains (the last separated in pericarp and embryo) were sampled from R3 to R9. Samples were individually removed, and were immediately frozen in liquid nitrogen. Frozen samples were stored at -80 °C until analysis. Dry matter, water content and dimensions

(length, width, and height) of flowers and grains were evaluated every three days. Final grain weight, dimensions, and the timing of physiological maturity were estimated using a linear model as in Calderini et al. (1999). The model was fitted using the iterative optimization technique of TableCurve 2D V5.01 (Systat Software Inc., Chicago, IL, USA).

Search and isolation of candidate gene sequences

To find sequences of genes AP2 and GW2 in sunflower we conducted a search in the sunflower transcriptome database Heliagene (https://www.heliagene.org/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/), based on orthologous genes from *Arabidopsis* and rice. Using the "ExpressionPatterns" tool available in Heliagene, we chose candidate gene sequences, which had greater expression in grain tissue. Sequences were analyzed by blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Putative proteins were predicted by ExPASy (http://web.expasy.org/translate/). A protein analysis was conducted by InterPro (https://www.ebi.ac.uk/interpro/) and Conserved Domain Database (http://www.ncbi.nlm.nih.gov/cdd) to verify the structure of the protein, domains and conserved sites and ensure that the sequences corresponding to the putative genes.

Real-time (qPCR) expression analysis

Total RNA was extracted from flower ovaries, pericarps and embryos from control and shaded plants using the kit NucleoSpin® RNA Plant (Macherey-Nagel). It was treated with DNase I (Invitrogen). First strand cDNA synthesis was performed using an Affinity Script cDNA Synthesis Kit Reverse Transcription System (Agilent Technologies) following the manufacturer's instructions. Three biological replicates for each sampling date were used. Specific 5′-3′ primers for AP2, GW2 and β-tubulin (as internal control) genes from Heliagene database, were designed using Primique (Fredslund and Lange, 2007) (http://cgi-www.daimi.au.dk/cgi-chili/primique/front.py), with high stringency to avoid amplification of non-specific PCR products. All primers were synthesized by Macrogen Inc. (South Korea) (http://www.macrogen.com/). Primer pair sequences were: AP2: AGGATGGGCCAATTTTTAGG (forward), ATGGCAGCCTTATCATACGC (reverse), GW2: GAAGCCATCTGGTTGTCGTT (forward), TGGATGCTAAGAGGCGAACT (reverse), and β-tubulin: GGCGTCTACCTTCATTGGT (forward), TCCATCTCATCCATTCCTTC (reverse) (Meimoun et al., 2014). The amplicon sizes were 92 bp for the AP2 gene, and 115 bp for GW2 gene.

The amplification reactions were performed using Brilliant II SYBR® Green QPCR Master Mix (Agilent Technologies) according to the manufacturer's instructions in an AriaMx real-time PCR system (Agilent Technologies Inc., Santa Clara, CA, USA). PCR conditions were: 95°C for 10 min; 35 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 15 s. No template control (NTC) and no reverse transcriptase control (no-RT) were included for detecting gDNA contamination. A dilution series was built to estimate the amplification efficiency using a cDNA mix as template prepared from control ovaries samples (-14 to 0 days after anthesis). Each reaction was performed in triplicate, and a negative water control was included in each run. Fluorescence was measured at the end of each annealing step. The amplification efficiency was estimated through a melting curve and amplification products were visualized on agarose gels (1.5%, w/v). The relative expression levels were first normalized against the β-tubulin gene and using non-shading samples from day one as calibrator, with a nominal value of 1. The method described by Livak and Schmittgen (2001) was used to make all calculations.

Statistical analysis

The recorded data were assessed by two-way analysis of variance. LSDs were calculated using STATISTICA v7.0 (StatSoft Inc.) and used for mean separation ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The final grain weight of peripheral grain position measured at physiological maturity stage showed a wide range (58.2 – 148.8 mg) of values (Table 1). Both grain weight and dimensions (length, width and heigh) were significantly affected by genotype ($p \leq 0.001$) and the source-sink ($p \leq 0.001$) treatments (Table 1). Previous studies demonstrated that grain weight and grain number are sensitive to shading during pre-flowering in sunflower (Cantagallo and Hall, 2002; Alkio et al., 2003; Cantagallo et al., 2004; Lindström et al., 2006). Our results confirm the high sensitivity of sunflower under strong source shortage at the immediately pre-anthesis stage. These results support that grain traits are set at pre-anthesis, therefore, the size of the ovary would determine the potential weight of grains (Cantagallo et al., 2004; Rondanini et al., 2009) like in wheat (Hassan et al., 2011). The dynamic of dry weight of peripheral ovaries and grains from R3 to maturity is shown in Fig. 1 a, b.

Peripheral grain weight showed a positive relationship with grain length ($r^2 = 0.92$; $P < 0.05$), grain width ($r^2 = 0.98$; $P < 0.05$) and grain height ($r^2 = 1$; $P < .0.001$). Taking into account the close associations shown by the final grain weight and dimensions, especially the height and width, these would seem to be crucial for grain weight determination. In fact, studies by Lizana et al., 2010; Hasan et al., 2011 in wheat show that length grain is very important in final grain weight determination. Unlike wheat where length grain shows better associaciations with grain weight, in sunflower it seems the height and width would be more important in grain weight determination, probably because they are different species, and different architecture grains.

Table 1. Final grain weight, grain dimensions (length, width and height) of peripheral grain position in Alybro and RHA-280 genotypes measured at in physiological maturity.

Genotype	Treatment	Length	Width	Height	Final grain weight (mg)
RHA-280	Control	13,01 a	9,80 a	6,49 a	148,78 a
	S-S	12,30 b	8,40 b	5,21 b	106,97 b
Alybro	Control	10,96 c	6,78 c	4,35 c	79,20 c
	S-S	9,62 d	5,54 d	3,72 d	58,15d
Genotype		***	***	***	***
Treatment		***	***	***	***
Genotype x treatment		***	ns	***	ns

Different letters indicate LSD test differences ($p < 0.05$). S-S: source-sink treatment. *** Mean significant at 0,001 probability level; ns: non-significant.

The putative sequences of AP2 (accession: HaT13l014337) and GW2 (accession: HaT13l009504) were obtained from Heliagene database (https://www.heliagene.org/). Both two sequences in sunflower were named HaAP2 and HaGW2 respectively. These sequences encoding proteins similar to *Arabidopsis* and grape vine (*Vitis vinifera* L.) respectively. The complete coding sequence of HaAP2 shares 100% identity with APETALA 2 (AP2) of *Arabidopsis* (NCBI accession: NP_195410) (Mayer et al., 1999), and the analyses of the protein sequence predict an AP2-like ethylene-responsive transcription factor, DNA-binding domain and AP2/ERF domain. Regarding HaGW2, the sequence shares 61% identity with RING-type E3 ubiquitin ligase (VvGW2) from grape vine (GenBank accession: AII80417.1). The protein analyses of HaGW2 predict a Zinc finger, RING-type domain. These results

support that the sequences evaluated in this study correspond to the HaAP2 and HaGW2 putative sunflower genes.

To investigate the source-sink manipulation effect on HaAP2 and HaGW2 candidate genes and grain development, we evaluate genes expression at eight times from R3 (14 days before anthesis) to R9. (32 days after anthesis), i.e. three times in pre-anthesis, and five times in post-anthesis (Fig. 1). Our results show a differential expression of the two genes between genotypes and among grain tissues.

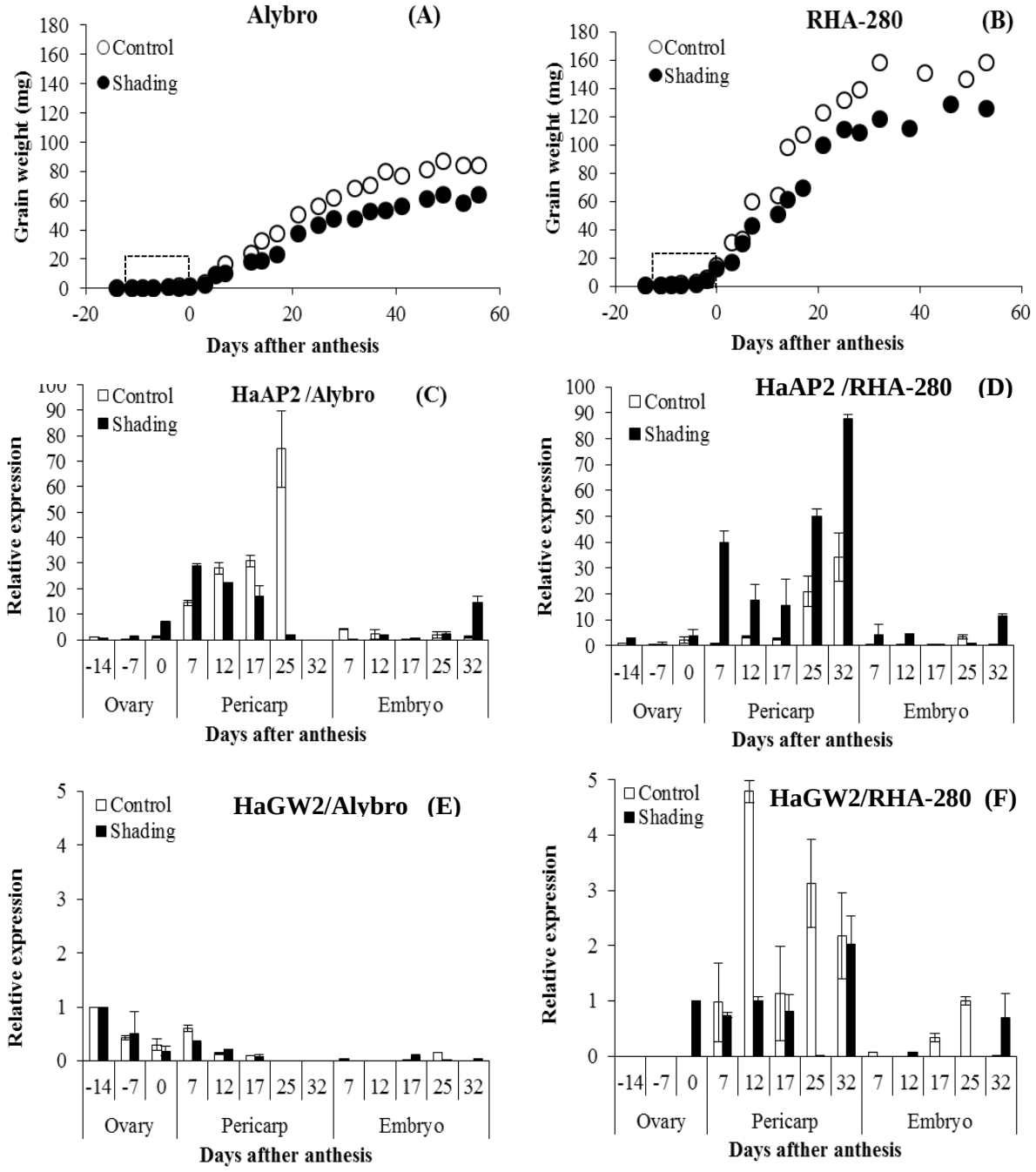

Figure 1. Relative expression of HaAP2 and HaGW2 genes in growing grains of contrasting grains weight genotypes of sunflower before and after anthesis under source-sink manipulation. Time-course of grain dry weight in Alybro (a) (small grains) and

RHA-280 (b) (large grains). Bar dashed line indicates the shading period. Relative expression of HaAP2 in Alybro (c) and RHA-280 (d). Relative expression of HaGW2 in Alybro (e) and RHA-280 (f). Bars on the graphs indicate the standard error. Open symbols indicate the control plants and filled symbols indicate shaded plants.

In control plants the HaAP2 gene showed higher expression in the genotype Alybro (small grain) than in genotype RHA-280 (large grains) (Fig. 1c, d). The expression of HaAP2 in both genotypes was mainly detected in the pericarp tissues, in Alybro from 7 to 25 days after anthesis, while in RHA-280 a longer expression was found (until 32 days after anthesis).

Shaded plants and control plants of Alybro showed similar expression pattern of HaAP2 in pericarp tissues, however, the relative expression in shade plants was lower compared to controls plants between 12 and 25 days after anthesis (Fig. 1c). Surprisingly, higher expression levels in shade plants were found in RHA-280 compared to controls, mainly in the pericarp tissues (Fig. 1d). This higher expression under shading of the large grain genotype parallels with the lower grain weight dynamic and lower final grain weight (Fig. 1d Tabla 1.). These results suggest that this gene may be acting in the pericarp maternal tissues, downregulating the growth of grains. Our results agree with previous studies of AP2, where that the authors suggest that AP2 negatively regulate the size of *Arabidopsis* grains. AP2 may restrict grain growth by limiting cell proliferation and cell expansion in the integuments (Jofuku et al., 2005; Ohto et al., 2005, 2009). In addition, AP2 is required for ovule and seed coat development (Leon-Kloosterziel et al., 1994; Modrusan et al., 1994; Jofuku, 1994).

HaGW2 gene showed low expression in control plants in Alybro genotype (small grain) (Fig. 1e), whereas in RHA-280 genotype (large grain) a higher expression was observed (Fig 1f). The expression of this gene also was mainly detected in the pericarp tissues in RHA-280 and Alybro, although with lower expression in the last genotype, suggesting that the HaGW2 gene might positively affect grain size and weight. The greatest expression of HaGW2 found in control plants of RHA-280 was observed early after anthesis (from 7 to 12 days after anthesis) in agreement with the linear growth phase of the ovule and ovary (Lindström and Hernández, 2015). The expression of GW2 in the pericarp decreased at 12 days after anthesis, when these tissues reached the final size and weight. In shaded plants of Alybro, the HaGW2 gene expression was similar to the controls (Fig. 1e), however, in RHA-280 a lower expression profile was observed in the reduced source-sink treatment compared to the controls (Fig. 1f). Similarly, the expression was tissue-specific in the pericarp. The dry weight of grains under shading was lower than controls during grain filling (Table 1, Fig. 1b), supporting upregulation of this gene in the growth of the pericarp tissue in sunflower.

In plants, the ubiquitin pathway has recently been shown to play important roles in seed size control (Li and Li, 2014), In *Arabidopsis* was reported that DA1, an ubiquitin receptor with two ubiquitin-inter- acting motifs (UIMs), and a single zinc-binding LIM domain (Li et al., 2008), acts as maternal control of seed size. DA1 regulates seed growth by limiting cell proliferation in the maternal integuments of developing ovules and seeds (Li et al., 2008; Xia et al., 2013). In the present study, we found evidence that the HaGW2 gene, a putative RING-type E3 ubiquitin ligase, would be acting in maternal tissues of sunflower, because primarily observed expression in pericarp tissue, but different to most studies that have examined the ubiquitin pathway as a negative regulator of seed size in cereals (Song et al., 2007; Li et al., 2008; Hong et al., 2014; Simmonds et al., 2016), we observe evidence of upregulation. The HaGW2 gene seems to have a positive effect on grain weight and size in sunflower, in wheat, where TaGW2 has been extensively studied in recent years, there are controversial results about the regulation of this gene on grain size (Bednarek et al., 2012). Therefore, it is likely that HaGW2 upregulates the weight and size of sunflower grain.

CONCLUSIONS

A clear association was found in this study between grain growth dynamics and the expression of HaAP2 and HaGW2 putative genes. The expression, tissue-specific pericarp suggests that these genes would be acting by maternal tissues. The present study provides clear evidences on the genetic control of grain growth and could be helpful to improve the knowledge f of grain weight and grain size determination in sunflower.

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PRODUCTIVITY AND QUALITY TRAITS OF SUNFLOWER INBRED LINE COLLECTION OF KAZAKHSTAN

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ABSTRACT

Assessment of 43 restore (R) lines, 36 male – sterile (A) lines and 44 maintainer (B) lines on seeds quality and productivity was done for the searching linkage between economic valuable traits and protein, molecular characteristics. 41 lines were selected as sources for breeding hybrids with high level of oil content, weight of 1000 seeds, total seed number per head and low percent of seeds hull. Screening purity of inbred lines on the base of seeds storage protein electrophoreses revealed 3 heterogeneous lines among restore lines collection (VKU 34RR, VKU 250R, VKU 360R), 3 heterogeneous mail-sterile lines (CMS): VKU 270 A, VKU 116 A, VKU 136 A and 5 heterogeneous maintainer lines : VKU 1B, VKU 183B, VKU 108B, VKU 286B, VKU 110B. According to helianthinin spectra in all sets of inbred lines 4 types of band composition were revealed. Ratio of 1: 2: 3: 4 types in a set of R lines was 79,1%; 4,7%; 11,6% and 9,3%, in a set of A lines 56,4%; 7,7%; 25,6% and 10,3%, in a set of B lines – 61,3%; 4,5%; 25%; 9,0%.

Key words: sunflower, inbred lines, seeds quality, productivity, line purity

THE EFFECT OF SOWING DATE AND DENSITY ON CALLUS INDUCTION AND SHOOT REGENERATION FROM SUNFLOWER ANTHERS

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ABSTRACT

The success of anther culture depends on numerous factors such as genotype, donor plant growing conditions, anther pre-treatment and development stage, as well as incubation conditions. We have investigated the effect of sowing date and sowing density of donor plants on callus induction and shoot regeneration from cultivated sunflower anthers. Anthers were collected from of three commercial sunflower hybrids that were sown in four different sowing dates, and at three different sowing densities. Anthers were surface sterilized and placed on MS-medium based solid regeneration media. The appearance of organogenesis or somatic embryogenesis was observed and obtained data statistically analysed. The experiment was set as completely randomised, with two factors. Callus, somatic embryo, shoot and root regeneration on the anthers of the tested genotypes was observed. Data were analysed by ANOVA. Statistical analysis enabled us to determine effect of sowing date and density on anther culture and shoot regeneration induction. Sowing date had a significant effect on all observed parameters, with earlier sowing dates having significant positive effect on shoot regeneration. Sowing density had no effect on either of observed parameters in all tested genotypes. The obtained results will contribute to the better understanding of the conditions needed for haploid production in sunflower and its introduction in sunflower breeding programs.

Key words: Anther culture, Dihaploid, Donor plant, Regeneration, Sunflower

INTRODUCTION

Anther culture results in sunflower (*Helianthus annuus* L.) have been rather unsatisfactory up to now (Marinković et al., 2003). As in other species, anther culture response of sunflower is strongly affected by numerous factors such as genotype, donor plant growing conditions, anther pre-treatment and development stage, as well as incubation conditions (Gurel et al, 1991; Miladinović et al, 2012). By testing a number of different parameters, that is, donor plant growing conditions and stages, as well as culture media and conditions, appropriate protocol could be worked out for the successful regeneration of shoots - at least for a number of genotypes.

Various environmental factors that the donor plants are exposed to may affect haploid plant production. Light intensity, photoperiod, and temperature have been investigated, and at least for some species, these are found to influence the number of plants produced from anther cultures (Reed, 2005). Seasonal variations have been reported to influence anther response in *Triticum aestivum* (Ouyang et al., 1987) and *Solanum tuberosum* (Tiainen, 1992), while different temperature regimes were found to affect anther response in wheat hybrid plants

(Orshinsky and Sadasivaiah, 1997). Growing season and conditions, as well as donor plant age had an effect on anther culture of *Capsicum annuum* (Ercan et al., 2006; Buyukalaca et al, 2004).

Up to our knowledge, there are no reports on effect of donor plant growing conditions on sunflower anther culture. There are only reports on influence of medium composition variation on the frequency of anther callusing and/or somatic embryogenesis and subsequent plant regeneration (Marinković et al., 2003; Miladinović et al., 2012). Different compositions of media used for establishing anther culture were extensively reviewed by Friedt et al. (1997), and variation of other culture parameters by Nichterlein and Horn (2005).

We have investigated the effect of sowing date and sowing density of donor plants on callus induction and shoot regeneration from cultivated sunflower anthers.

MATERIAL AND METHODS

Anthers were collected from three commercial sunflower hybrids (NS Oskar, NS Fantazija, and Orfej). The hybrids were sown in four different sowing dates and at three different showing densities (30,000; 50,000 and 70,000 plants per ha).

After collection, anthers were surface sterilized and placed on solid regeneration media, supplemented with basic MS macro and micro salts (Murashige and Skoog, 1962), 0.3% gelrite, pH 5.7, while composition of hormones varied (Vasić et al., 2000; Miladinović et al., 2012). Anthers were cultured in the dark at 30^{o}C.

Two experiments were set as completely randomized, with two factors. In the first experiment factors were sowing date and genotype of donor plants, while in the second experiment factors were sowing density and genotype. Callus, somatic embryo, shoot, root and plant regeneration on the anthers of the tested genotypes was observed. The data were transformed by *arc sine* transformation in order to obtain normal distribution of their frequencies, which is required for further statistical analysis. Analysis of variance and Fisher's least significant difference test were performed in statistical program STATISTICA 12.0 (StatSoft Inc., 2013) in order to establish the significance of factor effects and their interaction, and significance of difference among treatments. Based on results of ANOVA, in order to estimate the relative importance of examined sources of variance, expected variances and their contribution to the total variance were calculated.

RESULTS AN DISCUSSION

Sowing date

Regarding contribution of components of variance to the total variance, sowing date had the highest effect on root regeneration since its variance contributed to over 50% of total variance (Figure 1). It also had the strongest effect on callus formation, as well as shoot and plant regeneration, contributing to 45%, 20% and 15% of the total variance, respectively. The effect of sowing date and interaction was similar for embryo formation, as their variances contributed approximately to 25% of total variance, each.

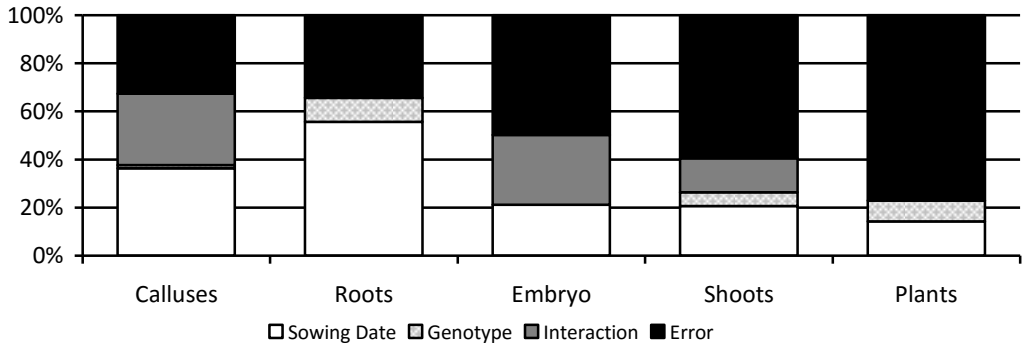

Figure 1. Contribution of expected variances of tested variation sources to the total variance (%)

Although sowing date significantly affected only plant regeneration, LSD test showed that earlier sowing dates had more positive effect on all observed parameters (Table 1). This especially stands for shoot and plant regeneration, as there were no regenerants from anthers collected at later sowing dates. Anthers collected from the plants sown at the earliest planting date had the best androgenic response, as they formed the highest number of calluses and embryos, and had the highest percentage of regeneration of roots, shoots and plants. Genotype had significant effect on shoot and plant regeneration. LSD test indicated that there was no significant difference among tested hybrids for embryo formation.

Table 1. LSD test for percentage of callusogenesis, somatic embryogenesis, root, shoot and plant regeneration at different sowing dates

		Calluses	**Roots**	**Embryos**	**Shoots**	**Plants**
		p	p	p	p	p
Date		0.000	0.000	0.000	0.009	0.071
Genotype		0.027	0.025	0.025	0.606	0.119
Date*Genotype		0.009	0.562	0.562	0.035	0.448
Variant	**Genotype**					
DATE I		86.809a	27.898a	1.612a	0.974a	0.374a
DATE II		83.351a	32.995a	0.784a	0.244ab	0.042ab
DATE III		48.322b	8.441b	1.008a	0.000b	0.000b
DATE IV		52.103b	2.950b	0.014b	0.000b	0.000b
	OSKAR	79.006a	24.313a	0.747a	0.000b	0.000b
	FANTAZIJA	70.243ab	10.526b	0.868a	0.457a	0.211a
	ORFEJ	56.924b	13.811b	0.442a	0.189ab	0.023ab

The values within the same column marked with different letters differed significantly at $\alpha_{0.05}$

Sowing density

Sowing density did not have any effect on the observed parameters (Figure 2). Genotype had the strongest effect on callus formation, as it contributed to 55% of total variance. Embryo formation was equally influenced by genotype and interaction (25% of total variance, each) while interaction had the highest effect on root regeneration (20% of total variance).

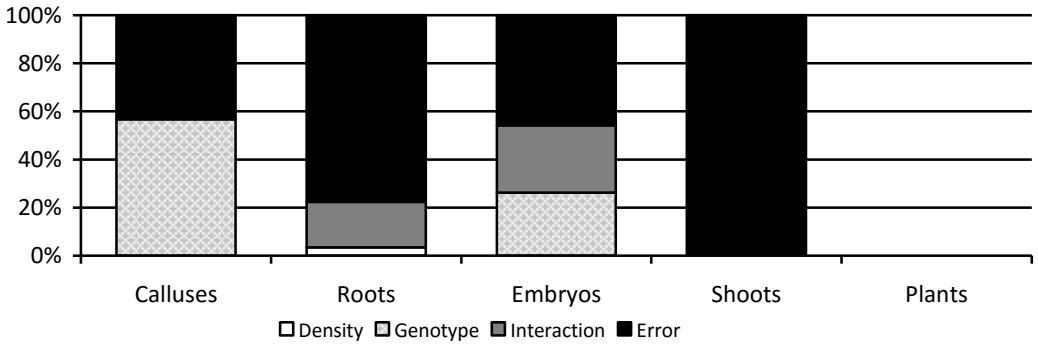

Figure 2. Contribution of expected variances of tested variation sources to the total variance (%)

Analysis of variance showed that sowing density generally had significant effect on tested parameters, but that there were no significant differences among different densities (Table 2). Interaction was significant for all tested traits, while genotype had significant effect on root and shoot formation.

Table 2. LSD test for percentage of callusogenesis, somatic embryogenesis, root, shoot and plant regeneration at different sowing densities

		Calluses	**Roots**	**Embryos**	**Shoots**	**Plants***
		p	p	p	p	p
Density		0.428	0.273	0.863	0.387	-
Genotype		0.000	0.816	0.003	0.387	-
Density*Genotype		0.961	0.186	0.055	0.433	-
Variant	**Genotype**					
30,000		78.057a	19.743a	0.510a	0.000a	-
50,000		70.928a	17.376a	0.694a	0.000a	-
70,000		78.131a	22.920a	0.420a	0.042a	-
	OSKAR	70.408b	19.419a	0.355b	0.000a	-
	FANTAZIJA	63.339b	21.206a	2.182a	0.000a	-
	ORFEJ	90.220a	19.291a	0.014b	0.042a	-

The values within the same column marked with different letters differed significantly at $\alpha_{0.05}$
*This parameter did not vary in some variants, so it was not possible to do variance analysis.

In our study, we have found that sowing date had an effect on establishment and plant regeneration from sunflower anther culture. Higher regeneration frequencies were obtained with plants from earlier sowing dates. The prevailing temperature during the growth of donor plants is reported to play a crucial role in microspore embryogenesis in Crucifers (Pratap et al., 2009). A high frequency of embryogenesis was consistently obtained in donor plants grown at low temperatures (Keller et al., 1987; Dunwell et al., 1985). This could be the reason for better regeneration frequencies in earlier sowing dates in our experiment, as low temperature is thought to increase the number of microspores suitable for embryogenesis due to slow pollen development, and it also prolongs the duration for which suitable microspores are available in a crop (Pratap et al., 2009). The opposite results were observed in wheat anther culture where embryo regeneration was usually greater when anthers were obtained from plants grown at high temperatures than plants grown at lower temperatures (Orshinsky and Sadasivaiah, 1997).

The lack of effect of sowing density on the observed parameters indicates that in our experiment irradiation and the temperature within the canopy were not important for androgenic response in tested hybrids, and that sowing date and temperature conditions during the plant growth have greater effect on this trait.

The results obtained in our study indicate that although genotype plays an important role in sunflower anther culture, the regeneration frequency could be improved by taking care of growing conditions of donor plant. Future studies should be focused on further optimisation of donor plants growing conditions in order to minimize genotype effect and sunflower androgenic potential and enable creation of the environment that will favour haploid plant regeneration.

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DEVELOPMENT OF SUNFLOWER NECROSIS VIRUS (SNV) DISEASE IN SOUTH INDIA

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ABSTRACT

Sunflower is the fourth important oilseed crop in India, with the major area concentrated in its southern states viz. Karnataka, Andhra Pradesh, Maharashtra & Tamilnadu. The crop is largely cultivated under rainfed conditions in these states. Besides abiotic stress, the biotic factors *viz.* viral and fungal diseases directly impact the yield. Sunflower Necrosis Virus Disease (SNV) is reported as the most devastating disease in south India. It was observed for the first time in year 1997 at village Bagepally, in Kolar District, Karnataka State. The disease is caused by Tobacco Streak Virus (TSV) belonging to Genus – *Ilarvirus*, Family - Bromoviridae. Natural infection of the virus on peanut, cotton, green gram, okra, soybean and marigold crop has been reported in India. The virus is naturally transmitted through pollen of weed host *viz. Parthenium hysterophorus* with the aid of *Thrips sp.* Temperature between 25-35 ^{o}C and moderate relative humidity is favourable for spread of *Thrips* vector and TSV. Disease incidence is high in wet and winter cultivation. Key field symptoms are distortion and necrosis of leaf, stem and head. Early infected plants remain stunted and develop malformed heads with chaffy or no seeds. Late infected crop has poor seed setting. Yield losses ranging from 30 to 100% have been reported due to SNV disease in South India. Considering the availability of alternate host crops and *Parthenium* weed prevalence throughout the country, there is a potential threat of spread of SNV in central and north India.

Key words: Sunflower, SNV, TSV, *Parthenium*, Thrips

INTRODUCTION

Sunflower is an important oilseed crop in India, besides groundnut, mustard & soybean. It is cultivated on 6.7 million hectares area (Annon. 2014) in the country with majority of cultivation in the Southern states viz., Karnataka & Andhra Pradesh followed by Maharashtra and Tamilnadu. In North India Sunflower is cultivated in state of Punjab and some parts of North Uttar Prasesh in spring season.

The productivity of sunflower is impacted by both abiotic and biotic stresses. Fungal diseases *Alternaria* blight and Powdery mildew occur in wet and winter season respectively. Among the viral diseases, Sunflower Necrosis Virus Disease is one of the most devastating diseases in South India and is observed in both wet and winter season. The disease was observed for the first time in 1997 in a seed production field near the village of Bagepally, Kolar District, Karanataka State, India (Singh et al., 1977). In subsequent years, outbreaks of this disease in major sunflower-growing states of India, especially Andhra, Karanataka and

Maharashtra, have virtually threatened the sunflower cultivation and yield losses ranging from 30 to 100% have been reported (Chander Rao et al., 2000).

EPIDEMIOLOGY & SYMPTOMS

The Sunflower Necrosis disease is caused by Tobacco Streak Virus (TSV) belonging to the Genus – Ilarvirus, Family - Bromoviridae.(Ravi.et.al., 2001). The virus is reported to naturally infect peanut, cotton, green gram, okra, soybean and marigold crop. Weed sp. *Parthenium hysterophorus* is the common alternate host for the virus. The major method of transmission of TSV is by infected pollen, which can spread by wind or carried by insect vector Thrips sp. (Harvir Singh 2005). Transmission of TSV to plants depends on entry of virus from the infected pollen in plant cells through the feeding injury caused by thrips. Temperature between 25-35 ^{o}C and moderate relative humidity is favourable for spread of Thrips vector and TSV. In Southern India, the SNV disease occurs in Wet and Winter season only. The summers being hot (38-45 ^{o}C) does not support effective multiplication of vector and dissemination of virus.

The SNV disease infects the sunflower crop at all growth stages. This results in severe economic loss. Early infected crop at seedling stage, results in necrosis and death of plant. Crop infected in vegetative stage show symptoms like distortion of apical shoot and necrosis of leaf & stem. Such plants remain stunted and develop malformed heads. The infection during flowering stage affects the seed setting and results in chaffy seeds or no seed set. In susceptible hybrids mosaic and yellowing of leaves is also observed.

CURRENT SCENARIO

Sunflower necrosis disease incidence has a variable trend in the southern states. The disease spread was on rise in Karnataka and Andhra Pradesh states, for a decade after its first appearance in 1997. Subsequently it spread to other states in south. With erratic monsoon pattern there has been rise and fall in area of sunflower crop in wet and early winter cultivations. It also impacts cultivation of other alternate hosts of TSV viz. peanut & cotton. Hence variability in disease incidence is noted across years. Also the availability of disease tolerant hybrids in these states has limited the disease spread and severity.

According to disease survey data in the annual report of All India Co-ordinated Research Project on Sunflower- 2014, the sunflower necrosis disease incidence was variable in different sunflower growing districts of southern states. In eastern dry zone of Karnataka *viz*. Chitradurg district the SNV incidence in wet season at vegetative stage was (20-30%) and at flowering stage (40-45%). In northern districts of the state *viz*. Raichur the disease incidence was (5-10%) at vegetative and (20-45%) at full blom stage, in Bagalkote (8-10%) in vegetative and (30-35%) at flowering and in Koppal (1-2%) at vegetative and (15-20%) at full bloom stage. In Tamil Nadu state, among the sunflower growing districts viz. Erode, Tirrupur, Tiruchi, Dindigul, Tirunelveli and Dharampuri, the SNV incidence in Wet season ranged from (1-4%). In Andhra Pradesh, in the major sunflower cultivation district Kurnool; very low disease incidence (0-4%) was recorded at 15 locations surveyed. In Maharashtra, the SNV disease incidence was recorded to be very low (4-8%) at Akola and Latur districts.

FUTURE CHALLENGES

The sunflower crop is cultivated along with other cash crops like peanut and cotton in south and north part of India. These crops are known to harbour Tobacco Streak Virus, which

is causal pathogen of Sunflower Necrosis disease. Weed host *viz. Parthenium hysterophorus* is the reservoir for TSV and acts as inoculum source. The spread and adaptability of this weed host across different geographies is of serious concern. Simultaneous availability of alternate crops and weed hosts with favourable weather conditions for *Thrips* vector can be a vulnerable combination; favouring dissemination and establishment of the virus, in other sunflower growing areas in India. Indeed there is a need to speed up resistant hybrid development program to counter the challenge of Sunflower Necrosis Virus disease in coming years.

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GENOME WIDE ASSOCIATION STUDIES ON SUNRISE GWA POPULATION

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ABSTRACT

The world oilseed production will face an increasing demand in the next years, for edible oil, biofuel or green chemistry. In the same time, the climate change will cause a water deficit: yield losses of 10 to 30 % have been predicted at 2030 horizon in Europe for sunflower crop. The SUNRISE project aims to improve the oil production from culture of sunflower hybrids in condition of water deficit, thanks to a genomic prediction approach. On the basis of genotypic and phenotypic data, GWAS (Genome Wide Association Study) allows to look for chromosomical regions linked to agronomical traits of interest. The GWA hybrid population was developped to produce these data. This population was an incomplete factorial design of 36 males and 36 females crossed to generate 452 hybrids. Parents were sequenced and SNP calling led to about 2,000,000 SNPs. An association study was performed on phenotype adjusted for field effect, using a mixed model having male, female and their interaction random effects. Results for a multi-locus association analysis will be presented on traits of interest, as flowering time.

Key Words : GWAS, mixed model, parent effects

SCREENING FOR RESISTANCE TO HIGHLY VIRULENT RACES OF SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA*)

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ABSTRACT

Sunflower broomrape (*Orobanche cumana* Wallr.) is a major constraint for sunflower production in most production areas around the world, particularly in areas of Eastern and Southern Europe and the Middle East. The yield losses caused by this parasitic weed can reach 100% for the susceptible cultivars which are heavily infested. The development of resistant cultivars as well as optimized managing strategies is a high priority in sunflower breeding programs all over the world.

Since the problem of resistance to broomrape race E is fully resolved, almost all commercial sunflower hybrids have incorporated resistance gene *Or5*, the main goal in sunflower breeding program in the terms of resistance to broomrape is to find new sources of resistance genes to the new broomrape races which are present in Europe. The objective of this research was to screen newly developed inbred lines in order to find resistance to highly virulent races overcoming race F of broomrape present in Southern and Eastern Europe

Screening procedure of testing for resistance to broomrape was combination of greenhouse and field trials during the several years. First field trials were performed in broomrape infested area in Northern Serbia in two locations where the race present was race E. The greenhouse testing was performed in the winter period under artificial inoculation using broomrape seeds collected from broomrapes attacking sunflower hybrids with incorporated gene of resistance Or5.Only the genotypes with complete resistance to broomrape are further tested in Spain, Turkey and Romania. In this way, the inbred lines resistant to virulent races of broomrape, overcoming race F were detected in NS breeding material. These lines are from the different gene pools and they were fully resistant to races which overcame race F in all tested areas in 2014 and 2015. Preliminary results of studies of the mode of inheritance of resistance to the broomrape races higher than F indicate that this trait is controlled by dominant gene(s).

Key Words : Sunflower, *Orobanche cumana*, resistance, high virulence

PREVALENCE OF SUNFLOWER DOWNY MILDEW AND PATHOGEN VIRULENCE IN THE UNITED STATES NORTH CENTRAL GREAT PLAINS

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ABSTRACT

Genetic resistance is one of the most important management tools of sunflower [*Helianthus annuus* L.] downy mildew caused by *Plasmopara halstedii* [(Farl.) Berl. and de Toni]. However, many resistance genes have been overcome by the pathogen and the incorporation of additional resistance genes into commercial hybrids is needed. Assessment of pathogen virulence is critical for determining what resistance genes should be incorporated into hybrids. The objectives of this study were to determine the prevalence of downy mildew and determine the virulence of *P. halstedii* isolates collected from United States (U.S.) north-central Great Plains states. In 2014 and 2015, 105 and 76 fields, respectively, were surveyed in North Dakota and South Dakota by visually assessing 40 plants at five locations for signs and symptoms of downy mildew. In 2014, 65% of those fields had downy mildew and ten fields (10%) had field-wide incidence levels higher than 5%. In 2015, 78% of fields had downy mildew and sixteen (21%) had field-wide incidence levels higher than 5%. To determine the virulence phenotypes of *P. halstedii*, 185 pathogen samples were evaluated on the international standard nine *P. halstedii* differentials and up to 13 supplemental lines were evaluated as additional differential candidates containing additional resistance genes. Virulence was observed on all nine differential lines and some candidate differential lines. Downy mildew remains common in the north-central U.S., and pathogen virulence on all current differential lines and additional resistance genes indicate that the inclusion of additional differentials is needed.

Key words: Downy mildew, *Plasmopara halstedii*, Races, Resistance genes, Sunflower, Virulence phenotype

INTRODUCTION

Downy mildew caused by the biotrophic, Oomycete pathogen, *P. halstedii* (Farl.) Berl. and de Toni, is an economically significant seedling disease of cultivated sunflower, *Helianthus annuus* L., grown in temperate regions. Cool temperatures around 11°C and wet soil conditions between germination and emergence favor infection of sunflower radicles by *P. halstedii* zoospores (Baldini et al. 2008). If seedlings do not damp-off, cotyledons and the first true leaves become thickened, puckered and chlorotic (Gulya et al. 1997). Later, leaves show chlorosis along the veins and across the leaves while mycelia and zoosporangia appear on the underside of the leaves below the chlorotic areas. Plants that survive are severely dwarfed with shortened internodes and horizontal heads. Yield losses due to downy mildew are dependent on the number of systemically infected plants and their distribution within the field (Friskop et al. 2009).

Qualitative genetic resistance is one of the most important management tools for sunflower downy mildew; however, many previously deployed, single, dominant resistance genes (denoted *Pl*) have been overcome by the pathogen (Tourvieille de Labrouhe et al. 2008). Therefore, the incorporation of additional resistance genes into commercial hybrids as well as the use of fungicidal seed treatments has been and continues to be necessary. A field survey was conducted in order to determine the effectiveness of sunflower downy mildew control in the main production area of the U.S., the north-central Great Plains. Assessment of pathogen virulence is critical for evaluating effectiveness of resistance genes which have been incorporated into hybrids; therefore, a set of nine internationally recognized differential lines became standard in 2000 to identify virulence phenotypes or races of sunflower downy mildew (Tourvieille de Labrouhe et al. 2000). In 2012, Institut National de la Recherche Agronomique (INRA) proposed two additional sets of three differentials to update the race nomenclature bringing the total number of digits in the virulence phenotype code to five (Tourvieille de Labrouhe et al. 2012). These differentials and up to seven supplemental lines lines containing additional sources of resistance were evaluated to determine their effectiveness as additional differential candidates containing additional resistance genes.

MATERIALS AND METHODS

From June 30 to July 10, 2014 and July 8 to 24, 2015, 105 and 76 fields, respectively, were surveyed in the states of North Dakota and South Dakota. To determine field incidence, a visual inspection was made for downy mildew symptoms of 40 plants at five points in an inverted W-shaped pattern for a total of 200 plants. Prevalence was determined based on whether the disease was present or absent in the field. Pathogen isolates were collected from each field for a total of 436 isolates. An additional 125 viable isolates from North Dakota, South Dakota, Minnesota and Nebraska were collected and sent in by personnel from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), state Extension services, and seed companies.

In order to increase *P. halstedii* samples collected, susceptible sunflower seedlings were inoculated in a zoosporangia suspension prepared from symptomatic leaves for three to six hours using methods described by Gulya (1996). Inoculated seedlings were planted in a sand-perlite mixture and grown in the greenhouse for eight to ten days. Then, seedlings were placed in a glass chamber in a cool (16-18^{o}C) room and sprayed with a fine mist of water to achieve 100% relative humidity for 16 to 48 hours to induce sporulatation. The cotyledons covered with zoosporangia were harvested, desiccated, and stored in cryotubes at -80^{o}C.

After inoculum increases were completed, one isolate from each field or research plot was arbitrarily selected for virulence phenotyping. In total, 185 isolates were evaluated on the nine international standard *P. halstedii* differential lines and up to thirteen supplemental lines containing additional resistance genes. Differential seedlings were inoculated and planted using the previously described method. After 11 to 14 days, when true leaves were easily visible, sporulation was induced. Plants were allowed to air dry before susceptibility and resistance of plants was evaluated.

The following new rating system proposed by INRA for susceptible and resistant plants was used: RI = resistant, no sporulation; RII = weak sporulation on cotyledons; SI = susceptible, sporulation on cotyledons and true leaves and SII = abundant sporulation on cotyledons only (Tourvieille de Labrouhe et al. 2012). Moderate, easily visible sporulation on the cotyledons was considered to be a RII reaction. To determine race in the triplet code system, each set of three differential lines is given a numerical value. The first three lines correspond to the first digit (Table 1), the second three lines correspond to the second digit and the third three lines correspond to the third digit. If a line is resistant, it is given a value

of 0. Otherwise, the first line is given a 1, the second line a 2 and the third line a 4. The values for all three lines in each of the three sets are then added. Each digit ranges from 0 if all three lines were resistant to 7 if all three lines were susceptible. The proposed five-digit code adds two additional sets of three differential lines.

Table 1. Standard and Proposed International Differentials.

	Digit	Differential Line	Sunflower Line	Genes
Standard	1	1	Susceptible (MYC 270)	None
		2	RHA 265	Pl_1
		3	RHA 274	Pl_2/Pl_{21}
	2	4	DM-2	Pl_5
		5	PM 17	?
		6	803	?
	3	7	HA-R4	Pl_{16}
		8	HA-R5	Pl_{13}
		9	HA 335	Pl_6
Proposed	4	10	Y7Q	Pl_{6-}
		11	PSC8	Pl_2
		12	XA	Pl_4
	5	13	PSS2RM	Pl_6/Pl_{21}
		14	VAQ	Pl_5
		15	RHA 419	Pl_{Arg}

RESULTS AND DISCUSSION

In 2014, 65% of fields surveyed had sunflower downy mildew and 10% of fields had incidence levels greater than 5% (Table 2). In 2015, 78% of fields had downy mildew and 21% of fields had incidence levels higher than 5%. These fields did not appear to be concentrated in any particular region. Prevalence was high, but yield impacting incidence was low. Yield losses start to occur between 5 and 15% depending on the distribution of systemically infected plants within a field; therefore, if scattered infection occurs, incidence below 15% should result in minimal yield loss (Bradley et al. 2007). In 2015, most infected plants appeared to be scattered throughout the fields, so other plants should have compensated in this incidence range. Over the two years of the survey, six of 181 fields would be expected to have significant yield loss due to sunflower downy mildew.

Table 2. Prevalence and Incidence of Sunflower Downy Mildew for 2014 and 2015.

	2014	2015
Prevalence	65% (68/105)	78% (56/76)
Incidence		
0	65%	55%
0.5 - 4.5%	25%	24%
5 - 14.5%	9%	14%
≥ 15	1%	7%

Virulence was observed on all nine differential lines and some supplemental differential lines (Table 3). Minimal virulence was found on lines HA-R4 and HA-R5, which were released in 1984, containing *Pl16* (1%) and *Pl13* (1%) genes, respectively, (Liu et al. 2012; Mulpuri et al. 2009; Vear et al. 2008). In 1986, the USDA released six downy mildew resistant lines: *Pl6* in HA 335 and HA336 from wild *H. annuus*, *Pl7* in HA337, HA 338 and HA 339 from *H. praecox* and *Pl8* in RHA 340 from *H. argophyllus* (Miller and Gulya 1991). *Pl6* and *Pl7* were found to be similar (Miller and Gulya 1991). Between 2009 and 2013 nine races overcame the *Pl6* gene in the United States (Gulya et al. 2014). Isolates virulent on the *Pl6* gene have been between 38 and 60% since 2011 with an average of 51% (Gulya et al. 2014). Virulence on the *Pl6* gene was found on 47% of the isolates from 2014 and 2015. Seven isolates were found over the two years in North Dakota that were virulent on RHA 340, which contains the *Pl8* gene. No isolates were virulent on both the *Pl6* and the *Pl8* genes. Resistance genes in the supplemental lines evaluated include *PlArg* in RHA 419 and RHA 420 which was released in 1999 from *H. argophyllus*, *Pl17* in HA458 released in 2006 from wild *H. annuus*, an unknown gene in RHA 468 released in 2006, *Pl18* in HA DM 1 released in 2015 from *H. argophyllus* and *Pl15* in RNID a proprietary inbred line from NIDERA in Argentina (DuBle et al. 2004; Paniego et al. 2012; Qi et al. 2015, 2016; Vear et al. 2008). No virulence was found on six supplemental lines containing *PlArg*, *Pl15*, *Pl17*, *Pl18* and two other lines with unknown resistance genes.

Table 3. Results for Standard and Supplemental Sunflower Downy Mildew Differential Lines for 2014 and 2015.

Differential Line		Sunflower Lines	Genes	2014 Isolates Virulent / Isolates Screened	2015 Isolates Virulent / Isolates Screened	Total Isolates Virulent /Isolates Screened	Percent
Standard	1	Susceptible (MYC 270)	None	105/105	80/80	185/185	100%
	2	RHA 265	*Pl1*	105/105	80/80	185/185	100%
	3	RHA 274	*Pl2/Pl21*	101/105	70/80	171/185	92%
	4	DM-2	*Pl5*	83/105	56/80	139/185	75%
	5	PM 17	?	10/105	4/80	14/185	8%
	6	803	?	9/105	3/80	12/185	6%
	7	HA-R4	*Pl16*	1/105	1/80	2/185	1%
	8	HA-R5	*Pl13*	1/105	1/80	2/185	1%
	9	HA 335	*Pl6*	53/105	34/80	87/185	47%
Supplemental		RHA 340	*Pl8*	2/105	5/80	7/185	4%
		RHA 419	*PlArg*	0/105	0/80	0/185	0%
		HA 458	*Pl17*	0/61	0/80	0/141	0%
		HA DM 1	*Pl18*	0/87	0/80	0/167	0%
		RHA 468	?	0/66	0/80	0/146	0%
		TX 16R	?	0/84	0/80	0/164	0%
		RHA 428	?	15/66	0/0	15/66	23%
		RNID	*Pl15*	0/66	0/80	0/146	0%

Based on the current standard nine *P. halstedii* differentials, twelve races were found in 2014 and 2015 in isolates from North Dakota, South Dakota, Minnesota and Nebraska (Table 4). In both years, the most common downy mildew races were 714, 710 and 700,

comprising 77% of the total. Race 774 was the 4th most frequent race in 2014, while race 314 was the 4th most frequent race in 2015. Three races, 304, 707 and 717, have been identified in France, but are new to the U.S. (Virányi et al. 2015). Seven isolates that were virulent on the *Pl8* gene are currently differentiated by the addition of a "+" following the current race type since RHA 340 has not been proposed as a differential. 58% of the 26 fields with incidence greater than 5% had races that were not virulent on the *Pl6* or the *Pl8* genes.

Table 4. ABSTRACT of Sunflower Downy Mildew Races for 2014 and 2015.

Race	2014	2015	Total
304	1	0	1
314	3	10	13
700	18	18	36
700+	1	1	2
704	1	4	5
707	1	0	1
710	32	21	53
710+	1	4	5
714	37	17	54
717	0	1	1
730	0	1	1
734	1	0	1
770	0	1	1
774	9	2	11

+ = Virulent on the *Pl8* gene

A selection of the 185 isolates collected in 2014 and 2015 were virulence phenotyped using the INRA proposed differential lines to determine how races in the main sunflower production area of the U.S. would compare to the 17 races used in the proposal (Table 5) (Gascuel et al. 2015; Tourvieille de Labrouhe et al. 2012).

Table 5. ABSTRACT of Proposed Downy Mildew Races for 2014 and 2015.

Race	2014 Isolates	Proposed Race	2015 Isolates	Proposed Race
304	1/1	30430	---	---
314	0/3	---	9/10	31430
700	7/18	70060	0/18	---
700+	1/1	70060+	1/1	70060+
704	1/1	70471	2/4	70471
707	1/1	70771	---	---
710	14/33	71060	2/20	71060
710+	1/1	71060+	3/3	71060+
710+	---	---	1/1	71070+
714	6/37	71471	2/17	71471
717	---	---	1/1	71771
730	---	---	1/1	73060
734	1/1	73471	---	---
770	---	---	1/1	77062
774	1/9	77473	2/2	77473

+ = Virulent on the *Pl8* gene

Races already evaluated by INRA were the same virulence phenotypes in the U.S., but this is the first time U.S. races 700+, 710+, 734, and 770 have been evaluated with these proposed differentials. One of the 710+ isolates from 2015 conferred virulence on multiple seed batches of Y7Q which has the postulated Pl_{6-} gene, but not on HA 335 with the Pl_6 gene.

CONCLUSIONS

Virulence was observed on all nine differential lines and some supplemental differential lines. Downy mildew remains common in the north-central U.S., and pathogen virulence on all current differential lines and additional resistance genes indicate that inclusion of additional differentials is needed. Use of resistant hybrids in combination with fungicidal seed treatments and crop rotation is currently limiting field incidence based on surveyed fields.

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OILSEED AND CONFECTIONARY (SUNFLOWER (*HELIANTHUS ANNUUS* L.) RESEARCHES IN AEGEAN AGRICULTURAL RESEARCH INSTITUTE (AARI)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops in Turkey. To increase production, sunflower with high yield capacity can be grown at both first and second crop production seasons in the Aegean Region of Turkey. The mission of the "Sunflower Research Project for Aegean Region" at Aegean Agricultural Research Institute (AARI) is to improve well adapted and high yielding varieties, to develop knowledge and technology for sunflower industry in Turkey. A number of different approaches are utilized to achieve the goal. Research is conducted to develop; a diverse germplasm base that leads to enhanced yield potential and quality characteristics; transfer useful traits from wild species, landraces and other sources into cultivated sunflower. To improve sunflower varieties with desired characters, genetic investigations and germplasm development of sunflower with improved yield, oil quality, resistance to disease [Sunflower rust (*Puccinia helianthi* Schw.) and downy mildew (*Plasmopara helianthi* Farl de Toni)], *Orobanche* sp., and adverse conditions. Oilseed and confectionary type of sunflower germplasm (A, B and Rf lines) including improved populations, hybrid and open pollinated varieties have been developed by conventional breeding techniques and biotechnological methods. High oleic types and resistance to herbicide (IMI groups) sunflower varieties are undergoing. The research program is leading to develop oilseed and confectionary type of sunflowers for both first and second crop production seasons. Sunflower landraces were characterized to utilization of development of new varieties. Sunflower germplasm have been developing from sources such as cultivars, populations created through breeding methods and tested for general and specific combining ability were undertaken with line x tester analysis. In the breeding program, lines, candidate and commercial varieties evaluated in preliminary and yield trials as regional basis under first and second crop production season at Aegean Region and other parts in Turkey since 1983. Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance, approximately 500-550 kg da^{-1} at both first and second crop production seasons in Aegean Region of Turkey. Oilseed type of open pollinated variety Ege-2001 was developed by using S 0:1 generation testing (Recurrent selection) method and hybrid variety Turay and parental lines has been registered. In addition effect of plant population, planting time, fertilizing, irrigation, honeybee pollination, on seed yield, oil percentage and other plant characteristics, and silage quality of sunflower (*Helianthus annuus* L.) were determined. Sunflower rust (*Puccinia helianthi* Schw.) race identification under field conditions was determined.

Keywords: Sunflower, *Helianthus annuus* L, *Helianthus* spp., Breeding, Genetics, Germplasm, Hybrid variety, Open polinated variety, Agronomy, Adaptation, CMS line, Restorer line, technology, Sunflower diseases, insects and weeds, Yield components.

INTRODUCTION

Vegetable oils and fats are vital component of human diet because they are an important source of energy. Sunflower is one of the major oilseed crops in Turkey. According to production data, sunflower was grown 657458 ha area with 1637900 metric ton seed production, and average seed yield of 2690 kg ha^{-1} in Turkey in 2014 (Anonymous, 2015). Because of gap for vegetable oil production in Turkey, sunflower is one of the alternative and leading oilseed crops to increase vegetable oil production. Growing sunflower as a first and second crop in Aegean Region is one of the possibility to increase the production. The Aegean Region has suitable ecological conditions for first and second crop sunflower production (Tan, 2007; Tan, 2010; Tan, 2011; Tan, 2014).

Sunflower research activities has been conducted since 1979 and breeding program initiated in 1984 at Aegean Agricultural Research Institute (AARI) in Menemen, Izmir, Turkey. The mission of the Sunflower Research Project is to develop improved germplasm by conventional and biotechnical breeding techniques for both first and second crop production areas in Turkey. New germplasm, breeding lines, hybrid varieties have been developed. To improve oilseed and confectionary sunflower varieties with desired characters, genetic investigations, and germplasm development of sunflower with improved yield, oil quality, resistance to diseaseases such as *Plasmopara helianthi* (Farl.) Berl de Toni., *Puccinia helianthi* Schw., and *Orobanche cumana* Walr. Adverse conditions are also under consideration. This studies are also incorporated with agronomic and other related researches.

RESEARCH FINDINGS

Breeding and Genetics

In the breeding programme a number of different approaches are utilized. Improved germplasm (populations and germplasm), new germplasm for hybrid development and breeding lines of oilseed and confectionary type of sunflower germplasm [A (CMS), B (maintainer) and Rf (restorer) lines] including hybrid and open pollinated varieties have been developed by conventional breeding techniques.

Sunflower germplasm have been developing from sources such as cultivars, populations created through breeding methods or crosses with wild germplasm, and tested for general and specific combining ability, oil percentage, and resistance to prevalent disease and adverse conditions to construct improved variety. In the breeding program, hybrids parental lines have been evaluated and developed according to their General (GCA) and specific combining ability (SCA).

Combining ability studies in oilseed and confectionary sunflower breeding program were undertaken with a set of line x tester including parents for the characters; seed yield, 1000-seed weight, days to flowering, days to physiologic maturity, plant height, head diameter, stem diameter, oil content, fatty acid content (oleic, linoleic, palmitic, and stearic acids), protein content, seed length, seed width, and hull percentage. General (GCA) and specific combining ability (SCA) of inbreed lines and their hybrids were estimated in a line x tester analysis at first and second crop production seasons in 1991. The variances due to GCA and SCA were highly significant for most of the characters under both environments. Based on GCA effects under first and second crop production seasons, the inbreeds (CMS and Rf lines) exhibited desirable GCA effects, and were found to be good general combiners for most of the traits; thus they can be used for developing superior genotypes and hybrids in sunflower (Tan, 1993a; Tan, 2005a, 2005b; Tan, *et. al.*, 2013a; 2015a).

More than 300 oilseed and confectionary type of CMS and restorer (Rf) lines with higher GCA and SCA effect developed since 1983 (Tan, *et. al.*, 2013a; 2015a).

Genetic and inheritance studies in sunflower to understand genetic make-up of the traits and genetic inheritance including disease resistance [Such as *Puccinia helianthi* Schw.; *Plasmopara halstedii* (Farl.) Berl de Toni.] in sunflower, and to improve drought tolerant varieties etc. in the breeding programs under consideration.

In the sunflower breeding program, Parental lines, candidate variety and commercial variety have been evaluated under first and second crop production seasons. Oilseed and confectionary type of sunflower germplasms including hybrid and open pollinated variety have been developed. Oilseed open pollinated variety EGE-2001, and hybrids TURAY, and SUN 2235 ant their parental lines have been registered (Tan, *et. al.*, 2013a; Tan, 2014; Tan, *et. al.*, 2015a).

Sunflower Genetic Resources

Evaluation and characterization of sunflower genetic resources to improve oilseed and confectionary type of sunflower varieties are under consideration. Therefore; sunflower landraces, collected, conserved, regenerated, characterized and evaluated for source of breeding for broaden the genetic base. This studies are allow us to develop new and desired sunflower varieties, breeding lines, and populations (Tan and Tan, 2010; Tan and Tan, 2011; Tan and Tan, 2012; Tan *et. al*., 2013a; Tan *et. al*., 2013b; Tan *et. al*., 2015b).

Orobanche cumana Wallr. Studies

In the breeding program, all test hybrids are tested for genetic resistance and or tolerance of *Orobanche cumana* Wallr. Imidazolinones (IMI) herbicides provide excellent broad-spectrum weed and broomrapes (*Orobanche cernua* and *O. cumana*) in sunflower. some oilseed and confectionary varieties have also been improved for imidazidol herbicide resistance (Tan, *et. al.*, 2013a; 2015a).

On-farm study of *Orobanche cumana* Wallr.

Resistance and susceptibility of sunflower (*Helianthus annuus* L.) to *Orobanche cumana* Wallr. was determined under natural infections. In this on-farm study, sunflower hybrids an control variety of Vniimk-8931 were evaluated for their rates of resistance to *O. cumana*, and evaluated for their agronomic performance. The hybrid varieties found to be consistently resistant, and Vniimk 8931 was susceptible to *Orobanche cumana* Wallr., and had very low yield losses under natural infection. (Tan and Karacaoglu, 1991b).

Rust race identification

Sunflower rust incited by *Puccinia helianthi* Schw. is considered one of the important foliar diseases of sunflower and is present wherever sunflower is grown in the world. The objective of this study was to identify the races of sunflower rust in the main sunflower production areas in Turkey. Experiments were conducted in six provinces (Aydın, Balıkesir, Bursa, Denizli, Izmir, and Edirne) of Turkey in 1992. Race identification of *P. helianthi* was determined in the field conditions. Eighteen differential genotypes were used to identify races of *P. helianthi*. Sunflower rust reaction of the differential genotypes were scored on a scale of 0 to 4, where 0 to 2 = resistant, 3 and 4 = susceptible. Race 1 of *P. helianthi*, causal agent of sunflower rust, was identified at Menemen-Izmir, Susurluk-Balıkesir, Koçarlı-Aydın, Çivril-Denizli. However, Races 1 and 3 of *P. helianthi* were found in Bursa and Edirne (Tan, 1993b; Tan, 1994a; b).

In the breeding program, all lines are selected for *Puccinia helianthi* Schw. genetic resistance under field conditions.

Yield trials

Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance (500-550 kg da^{-1}) at both first and second crop production seasons in Aegean Region of Turkey (Tan, 2010; Tan, *et. al.*, 2013a; Tan, 2014; Tan, *et. al.*, 2015a).

On-farm research and adaptation studies (genotype x environment interaction) to find out higher yielding varieties for different geographical regions and ecological conditions in both first and second crop production seasons. More than 2000 lines, candidate variety and commercial variety have been evaluated in preliminary and yield trials under first and second crop production seasons since 1979 (Tan, *et. al.*, 2013a; Tan, 2014; Tan, *et. al.*, 2015a).

Agronomic techniques have been applied for higher yield and to develop culturing techniques in sunflower.

Effect of plant population on seed yield, oil percentage and other plant characteristics in sunflower

In this study, the highest yield values, 2190 and 1920 kg ha^{-1} were obtained from the plant populations, 40820 and 47620 plants ha^{-1}, respectively. The experiment design was RCBD and plots were consisted of four row 7.70 m in length, spaced 0.7 m apart. Plants spaced 15, 20, 25, 30, 35 and 40 cm on each row of experiments plots. In this study, the most important observation was that degree of lodging effected harvestable yield greatly. Lodging is associated with plant population size, stalk diameter, plant height and wind speed. The number of lodged plants increased when plant population size increased. The maximum wind speed were 9.0 and 12.0 m s^{-1} in 1986 and 1987, respectively. The highest percent of lodged plants were obtained at 95240 plants ha^{-1} (15x70 cm plant spacing) as 69% and 19% in 1986 and 1987, respectively (Tan and Karacaoglu, 1991a).

Effect of planting date on plant characteristics in sunflower

Effect of planting date on seed yield, oil content, fatty acid composition and other plant characteristics in sunflower (*Helianthus annuus* L.) were determined at seven planting date including both first and second crop production times. The highest yields were obtained with early planting in the first and second crop planting times which are at the beginning of April and July, respectively. In general, oil content was not affected by planting times. However, the fatty acid composition was changed significantly by planting dates depending upon temperature. Oleic acid composition was 45% in the early planting time and as planting time was delayed it decreased to 25% in the late planting time. The lineloic acid proportion increased from about 45% to 65% with the delaying plantings from first to second crop planting time. The changes in fatty acid composition can be associated with temperature during seed development of the crop. The plant height, head diameter, and 1000 seed weight were also negatively affected as planting date was delayed (Tan, 1991).

The effects of honeybee pollination on some economic characters of oil type sunflower varieties

The objectives of this research were to determine the effect of honeybee pollination on sunflower varieties (Super-25, AS-503, and ETAE-Y1) in 1997 and 1998, at AARI in Menemen, Izmir. Data were collected on sunflower yield (kg ha^{-1}), 1000-seed weight (g), plant height (cm), head diameter (cm), days to flowering, days to physiological maturity, oil percentage (%), protein percentage (%), hull percentage (%), and seed sizes. The experimental design was randomized block with split plot arrangement with three replications. The main plots were assigned to the pollination treatments and the sub-plots for sunflower varieties. Plot size was 16.17 m^2 and the plant density was 4081,6 plants ha^{-1}. The plots of the treatments were 1) plots with honeybee pollination, 2) plots caged to exclude honeybee and other insect pollination, and 3) and open pollinated plots.

The results of statistical analysis indicated a significant difference among the treatments. Seed yield of honeybee pollinated plants of plots, an average, were 4440 and 4130 kg ha^{-1} in 1997 and 1998 respectively. All cultivars shoved a significant increase in yield in the presence of bees. Seed yield on honeybee pollinated plants of 3 different sunflower varieties were, on average, 95% higher than on plots caged to exclude bees in 1997 and were 124% in 1998. Yields were higher on plots pollinated by honeybees than on those caged to exclude bees and open pollinated plants; but the difference varied between cultivars, and between years. It is concluded that the use of honeybees for pollination were also effective on the seed size, 1000 seed weight, oil and protein content (Tan, 2000; Tan, *et. al*., 2002.).

The effect of irrigation at various growth stages on some economic charecters of first crop sunflower

The objectives of this research were to determine number of and optimum and more economic irrigation during specific growth stages oil type sunflower varieties (Super-25 and Trakya-129) conducted in 1996 and 1997, at AARI in Menemen, Izmir. The experimental design was randomized block with split plot arrangement with three replications. The main plots were assigned to the irrigation treatments and the sub-plots for sunflower varieties. Treatments were: 1. Non-irrigated (Control); 2. One irrigation at the begining of heading stage; 3. One irrigation at the begining of flowering (blooming) stage; 4. One irrigation at the begining of milk stage; 5. Two irrigation at the begining of heading and blooming stages; 6. Two irrigation at the begining of heading and milk stages; 7. Two irrigation at the begining of blooming and milk stages; and 8. Three irrigation at the begining of heading, flowering and milk stages. Data were collected on sunflower yield (kg da^{-1}), 1000-seed weight (g), plant height (cm), head diameter (cm), days to flowering, days to physiological maturity, oil percentage (%), protein percentage (%), hull percentage (%), seed length (mm), seed width (mm), and stem diameter (cm). The results of statistical analysis showed a significant difference among the irrigation treatments. According to the results the best yield obtained from three times irrigation with 427 kg da^{-1} and 373 kg da^{-1} in 1996 and 1997 respectively. Whereas, 347 kg da^{-1} and 265 kg da^{-1} yields were obtained from the control (non-irrigated) plots in 1996 and 1997 respectively.

Marginal analysis method was used for economic analysis. Generally, both registered varieties showed positive response to the applications in question. The 8th application seems as if it is the most profitable treatment in terms of having the highest gross revenues. According to results, three irrigation (Treatments-8) can be applied for high yield; but, one irrigation at the development of head stage is recommended because of satisfactory yield and the maximum marginal revenue (Tan *et. al.* 2000).

Silage quality of sunflowers

Sunflower (*Helianthus annuus* L.) is known as one of the drought tolerant crop. Because of this property, it can be used as an alternative silage crop at both first and second crop production seasons when irrigation is limiting factor. This study was conducted to determine the most suitable harvest stage of sunflowers for silage and evaluate silage quality of sunflowers harvested at different vegetation stage. In this study confectionary material ETAE-14 was planted by machine at populations of 40816 plants ha^{-1} in 1993 at Menemen, Izmir. Plants were harvested at 5 different growth stages (R3, R5.1, R5.5-5.9, R6, R9), and cut about 0.8 - 1.0 cm in length by silage machine then stored in plastic barrels for silage. In the study, the material was evaluated for forage yield, flieg score, sensory quality test, dry matter, crude protein, crude oil, crude fiber, N-free extract, ash, Ca, P, and pH. Research results indicated that harvesting sunflower for silage during R6 stage (complete flowering stage) was found as the most suitable stage for silage (Tan and Tumer, 1996).

Training Programs

Technical and applied training programs organized in each year for both agricultural engineers and farmers.

CONCLUSIONS

In Aegean Sunflower Research Project, desired oilseed and confectionary type of germplasm, populations, lines (CMS and Rf), test hybrids, and varieties developed in the breeding program. Agronomic studies achieved to increase sunflower yield under first and second crop production times in Turkey.

Research findings have been supported that instead of increasing total acreage in sunflower production second crop sunflower production to increase sunflower production in Turkey. Therefore, first and second crop sunflower production should be considered in Aegean Region in order to decrease vegetable oil gap in Turkey.

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PERFORMANCE OF SOME OILSEED SUNFLOWER (*HELIANTHUS ANNUUS* L.) VARIETIES IN AEGEAN REGION OF TURKEY

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ABSTRACT

Sunflower is one of the major and leading oilseed crops in Turkey. It is grown mainly Thrace Region of Turkey. Oilseed sunflower was grown 568995 ha area with 1500000 metric ton seed production, and average seed yield of 2640 kg ha^{-1} in Turkey in 2014. The main objectives of this study were to determine performance of oilseed hybrid varieties which in Aegean region. The experiments were conducted at first crop growing seasons in 2013 on the experiment field of Aegean Agricultural Research Institute in Menemen, Izmir in Aegean Region; Edirne and Luleburgaz in Thrace. The experiments were established in randomized complete block design with four replications. As a material, sunflower oilseed candidate varieties and commercial hybrids were used in this study. Adaptation study were undertaken for the characters seed yield, seed oil content (%), 1000 seed weight, plant height, head diameter, seed length, seed width, hull percentage (%), days to flowering and days to physiological maturity. The results indicated that statistically significant differences were found among the sunflower varieties for the characters in question. The highest seed yield 516 kg da^{-1} and the lowest 347 kg da^{-1} was obtained from the varieties 08 TR003 and TE-TM-2012-2 respectively in Menemen. However, in the rain fed condition, the highest seed yields were 209 and 204 kg da^{-1} were obtained from 08 TR003 in Edirne and LG 5550 in Luleburgaz locations respectively. The results indicated that TE-TM-2012-6 and TE-TM-2012-1 found to be promising candidate hybrids with the high yields over the locations. The results of this study indicated that the production for oilseed sunflower in this region ha the great potentiality. Because of gap for vegetable oil production in Turkey, Aegean Region is one of the possibilities to increase the vegetable oil production.

Keywords: Sunflower, *Helianthus annuus* L, hybrid variety, open pollinated variety, adaptation, yield, yield components.

INTRODUCTION

Because of an increasing world population it is difficult to deal with human feeding in the world. Vegetable oils are an important source of energy. To reduce oilseed production gap in Turkey, it is possible to grow sunflowers with high yield, oil percentage and oil quality; consequently, increasing oilseed production will result in increasing vegetable oil and decreasing import of vegetable oil (Gobbelen et al., 1989; Schneiter, 1997; Tan, 2007).

Turkey is one of the leading countries for sunflower production. According to production data oilseed sunflower was grown on 568995 ha area with 1500000 metric ton seed

production, and average seed yield of 2640 kg ha^{-1} in Turkey in 2014 (Anonim, 2015). However, amount of oilseed production including sunflower is not sufficient for the consumption; therefore, amount of the production should be increased. There are some other potential sunflower production areas such as Aegean Region and South East Anatolia besides main production area of Thrace of Turkey (Fırat and Tan, 1992; Tan, 2007; Tan, 2010a; b; Tan, 2014).

In Sunflower research project at AARI, oilseed and confectionary type of sunflower germplasms including hybrid and open pollinated variety have been developed, and candidate varieties are evaluated in yield trials under first and second crop production season. Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance (500-550 kg da^{-1}) at both first and second crop production seasons in Aegean Region of Turkey (Tan, 2007; Tan, 2010a: b; Tan, *et. al*., 2013a; Tan, 2014; Tan *et. al*., 2015). The Aegean Region that has suitable ecological conditions for first and second crop sunflower production should be considered for sunflower production to decrease vegetable oil gap in Turkey.

The main objectives of this study were: (1) to determine newly developed oilseed hybrids varieties which could be grown with satisfactory yield performance in Aegean region.

MATERIAL AND METHODS

This study was conducted to determine performance of oilseed hybrid for Menemen, Izmir conditions. The experiments including oilseed hybrid cultivars were conducted separately at first crop growing seasons in 2013 on the experiment field of Aegean Agricultural Research Institute (AARI) in Menemen, Izmir. They were also tested in Lüleburgaz and Edirne locations as well. Adaptation study were undertaken for the characters seed yield (kg da^{-1}), seed oil content (%), 1000 seed weight (g), plant height (cm), head diameter (cm), seed length (mm), seed width (mm), hull percentage (%), seed color (white, black, and intermediate), days to flowering and days to physiological maturity.

In this study, as a material, 6 oilseed hybrids sunflower candidate varieties developed at AARI sunflower breeding program were used in the experiments. Restorer lines of these hybrids were developed by sunflower breeding program of AARI and CMS lines of these hybrids were developed by sunflower breeding program of Trakya Agricultural Research Institute (TARI). The newly developed oilseed hybrids used in this study were; TE-TM-2012-1; TE -TM-2012-2; TE -TM-2012-3; TE -TM-2012-4; TE -TM-2012-5; TE -TM-2012-6. Oilseed commercial hybris, LG-5550 (St-4); TURAY (ST-1); LG-5580 (St-5); P64G46 (St-2); 08 TR 003 (St-3) were used as control varieties.

The oilseed hybrid and OP confectionary variety variety experiments were conducted a randomized complete block design with four replications. The experiment plots were consisted of four row 7.70 m in length, spaced 0.7 m apart. There were 22 plants, spaced 0.35 (in Menemen) and 0.30 (in Luleburgaz and Edirne) cm on each row of experiments plots. Recommended agronomic crop production practices were followed. In Menemen and Edirne experiments 50 kg da^{-1} ($N_{10}P_{10}K_0$) and 25 kg da^{-1} ($N_{10}P_{10}K_0$) composed fertilizer applied respectively during the soil preparation. Three irrigation were applied in 2013 (27/06/2013, 12/07/2013, 31.07.2013) in Menemen experiment, however, irrigation were not applied in Edirne and Luleburgaz experiments. In all experiments weed control were routinely applied. The experiments were planted in 10, 20, and 21 May 2013 in Edirne, Luleburgaz and Menemöen locations respectively.

Data were obtained on;

Seed yield (kg da^{-1}): The yield was obtained from each of the two middle rows of the four row plots in the experiment. At the harvest, 1st and 4th rows and first and last plants of the middle row are removed as edge effect in the confectionary variety experiment. The first and last plants of the rows are removed as edge effect in the experiment for evaluation. Heads were hand harvested, threshed, and evaluated at 0% moisture.

Days to physiological maturity: days from planting to R9 stage (Schneiter, and Miller, 1981).

Days to flowering maturity: days from emergence to 75% of the flowering.

1000 seed weight (g): Weight of 1000 seed (g) determined from dried seed (0% moisture) sample.

Oil content (%): Sample from harvested seed was dried to 0% moisture and percent oil was determined by nuclear magnetic resonance (NMR).

Plant height (cm): Height of ten plants were measured at R9 (Schneiter, and Miller, 1981) from ground level to the base of the head (cm).

Head diameter (cm): Head diameter of ten plants were measured at R9 (Schneiter, and Miller, 1981).

Seed size (mm): The length and width of a sample of 10 seeds were measured in mm for only confectionary yield trial.

Hull percentage (%): Sample from harvested seed was dried to 0% moisture and the husk of seed was removed and weighted.

Uniformity: At the 75% of the flowering stage plants were observed whether they were uniform or not.

Statistical analyses were performed to determine the differences among the varieties (Steel, and Torrie, 1980).

RESULTS AND DISCUSSION

Results showed that statistically significant differences were found among the sunflower varieties for the characters in question. In the experiments; the highest seed yields (516, 483, and 443 kg da^{-1}) were obtained from the varieties 08 TR003, TE -TM-2012-6, and TE -TM-2012-1 respectively and the lowest seed yield of 347 kg da^{-1} was obtained from TE -TM-2012-2 in Menemen.

While, the highest seed yields (516, 483, and 443 kg da^{-1}) were obtained from the varieties 08 TR003 (239 kg da^{-1}), TE-TM-2012-6 (237 kg da^{-1}), and TE -TM-2012-1 (221 kg da^{-1}) in Edirne location. In Luleburgaz location, the highest yields; 204 kg da^{-1}, 199 kg da^{-1}, and 190 kg da^{-1} were obtained from the varieties 08 TR003, TE -TM-2012-6 and TE -TM-2012-1 respectively (Tan, *et. al*., 2013).

The lowest flowering days (45 days) observed from ETAE TE-TM-2012-1 and the highest flowering day (50 days) observed LG-5580 (St-5). The lowest physiological maturity days (97 days) observed from 08 TR003 (St-3) and highest flowering day (104 days) observed from P64G46 (St-2). The highest plant height (201 cm) was obtained from ETAE-TM-4 and the lowest plant height (155,50 cm) was obtained from P64G46 (St-2). The highest head diameter (19,6 cm) were obtained from LG-5550 (St-4) and the lowest head diameter (15.5) was obtained from TE-TM-2012-5. The highest 1000-seed weight (82.97 g) was obtained from 08 TR003 (St-3) and the lowest 1000-seed weight (53.54 g) was obtained from TE -TM-2012-2. The highest oil content (45.88 %) was obtained from TANAY, and the lowest oil content (34.55 %) was obtained from TE-TM-2012-3. The highest hull percentage (27.27 %)

was obtained from TE-TM-2012-2, and the lowest hull percentage (20.29 %) was obtained from 08 TR 003 (St-3).

Hybrids varieties had suitable physiological maturity days for both first and second crop production seasons in Aegean and Thrace Regions. These hybrids and especially TE-TM-2012-6 and TE-TM-2012-1 showed satisfactory results of other plant characters in Izmir, Edirne, and Luleburgaz experiments (Tan, *et. al*., 2013a; b).

According to *Orobanche cumana* tests, P64G46 (K), TE-TM 2012-1, TE-TM 2012-2, TE-TM 2012-3, TE-TM 2012-5,TE-TM 2012-6 were found to be tolerant to *Orobanche cumana* (Tan, *et. al*., 2013). The oilseed hybrid variety TE-TM-2012-6 was one of the best candidate hybrid variety for registration because of high yield capacity and high tolerance to Orobanche (*Orobanche cumana* Wallr.) in the yield performance trials in Turkey (Sezgin and Yasar, 2015).

CONCLUSIONS

In the oilseed variety experiments; the highest seed yield (516 kg da^{-1}) and the lowest seed yield (347 kg da^{-1}) were obtained from the varieties. These oilseed hybrids varieties with short physiological maturity days that is suitable for both first and second crop production seasons in Aegean and Thrace Regions.

Research results indicated that Oilseed candidate hybrid TE-TM-2012-6 showed the highest yield and quality performance in Menemen, Edirne and Luleburgaz locations, and found to be highly tolerant to existing races of *Orabanche Cumana* Wallr. (Tan, et. al., 2013a; b; Sezgin and Yasar, 2015), and TE-TM-2012-6 was registered as SUN 2235 in April, 2016 (Sezgin and Yasar, 2016).

Increase in sunflower production could be possible by the expansion of acreage, giving importance to the high-yielding varieties need to be planted. Planting high yielding hybrids in Aegean Region that has suitable ecological conditions for first and second crop sunflower production may play an important role to decrease vegetable oil gap in Turkey.

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Table 1. Oilseed sunflowers yield trials yield, (kg/da), oil content and oil yield, 1000 seed weight (g), and husk percentage (%) values of the varieties. AARI, Menemen-Izmir (2013).

Variety Name	Seed yield (kg da^{-1})*	Yield groups (α=0.01)	Seed yield over St. mean (%)	Seed yield order	Oil content (%)	Oil yield (kg da^{-1}) *	Oil yield over St. mean (%)	Oil yield Order	1000 Seed weight (g)**	Husk Percentage (%)
LG-5550 (St-4)	400	CDEF	90	10	36.83	147	77	12	76.16	25.05
TE-TM-2012-1	**443**	**ABCDE**	**100**	**6**	**43.51**	**193**	**102**	**7**	**55.17**	**22.25**
TE -TM-2012-2	347	F	78	14	36.82	128	67	14	53.54	27.27
TE -TM-2012-3	382	DEF	86	11	34.55	132	70	13	56.42	25.07
TE -TM-2012-4	374	EF	84	12	41.46	155	82	11	58.74	24.10
TURAY (ST-1)	440	A BCDE	99	7	45.30	199	105	6	54.12	25.92
TE -TM-2012-5	372	EF	83	13	42.57	158	83	10	56.33	26.70
TE -TM-2012-6	**483**	**AB**	**108**	**2**	**43.58**	**210**	**111**	**3**	**57.10**	**24.35**
TANAY	478	ABC	107	3	45.88	219	115	2	56.57	20.85
ETAE-TM-4	456	ABCD	102	5	45.16	206	109	4	55.07	21.35
LG-5580 (St-5)	471	ABC	106	4	42.81	202	106	5	68.97	22.18

P64G46 (St-2)	402	CDEF	90	9	41.66	167	88	9	60.54	23.34
08 TR003 (St-3)	516	A	116	1	45.33	234	123	1	82.97	20.29
EGE 2001	422	BCDEF	95	8	44.05	186	98	8	68.09	22.97
CV (%)	9.83								9.87	5.34
LSD (0.05)	60.13								8.67	1.08
LSD (0.01)	80.50								11.60	2.42

* 10% seed moisture; ** 0% seed moisture.

Table 2. Phenological Data of Firs Crop Oilseed Sunflowers Yield Trials. AARI, Menemen-Izmir (2013).

Variety Name	Days to flowering (day)	Pphysiological maturity (day)	Plant height (cm)	Head diameter (cm)	Uniformity (1-5)**
LG-5550 (St-4)	47	103	168.5	19.6	1.5
TE-TM-2012-1	**45**	**100**	**159.1**	**17.7**	**1.0**
TE -TM-2012-2	46	101	174.9	17.3	4.0
TE -TM-2012-3	46	98	165.3	18.4	1.8
TE -TM-2012-4	45	99	165.1	18.2	1.6
TURAY (ST-1)	47	100	187.4	17.9	1.1
TE -TM-2012-5	47	103	183.6	15.5	4.0
TE -TM-2012-6	**47**	**99**	**189.5**	**18.0**	**1.4**
TANAY	47	100	195.4	19.1	3.8
ETAE-TM-4	49	100	201.0	18.5	1.1
LG-5580 (St-5)	50	102	192.4	18.6	1.8
P64G46 (St-2)	48	104	155.5	19.1	1.4
08 TR003 (St-3)	46	97	165.4	18.6	1.4
EGE 2001	47	101	174.9	19.2	2.4
CV (%)	1.22	0.54	3.72	5.56	
LSD (0.05)	0.81	0.77	9.41	1.45	
LSD (0.01)	1.09	1.04	12.60	1.94	

*: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

Table 3. Oilseed sunflowers yield trials yield, (kg/da), oil content and oil yield, and 1000 seed weight (g), values of the varieties. TARI, Edirne (2013).

Variety Name	Seed Yield (kg da^{-1})	Yield Groups (α=0.01)		Seed Yield over St. mean (%)	Seed Yield Order	Oil Content (%)	Oil Yield (kg da^{-1})	Oil Yield Groups (α=0.01)		OilYield Order	1000 Seed weight (g)*
08 TR 003 (K)	239	A		113.1	1	48.0	115	A		1	59.28
TE-TM 2012-6	237	A	B	112.4	2	46.3	110	A	B	2	40.24
TE-TM 2012-1	221	A	C	104.7	3	48.9	108	A	B	3	43.56
EGE 473 A x TT 119 R	217	A	C	102.8	4	46.3	100	B	C	5	50.40
P64G46 (K)	215	B	C	102.0	5	47.2	102	B	C	4	48.24
LG 5550 (K)	206	C	D	97.6	6	42.6	88	D	E	7	55.04
TE-TM 2012-4	204	C	D	96.6	7	46.1	94	C	D	6	40.32
TE-TM 2012-3	200	C	D	95.0	8	43.4	87	D	E	8	44.48
EGE 436 A x TT 119 R	185	D	E	87.6	9	43.4	80		E	10	39.12
TE-TM 2012-2	185	D	E	87.6	10	43.7	81		E	9	40.64
LG 5580 (K)	184	D	E	87.0	11	42.7	78		E	12	39.64
TE-TM 2012-5	169		E	80.0	12	47.6	80		E	11	41.28
CV (%)	7,65						7,60				
LSD (0.05)	22,57						10,24				

* 10% seed moisture.

Table 4. Phenological Data of Firs Crop Oilseed Sunflowers Yield Trials. TARI, Edirne (2013).

Variety Name	Days to flowering (day)	Pphysiological maturity (day)	Plant height cm)	Head diameter (cm)
P64G46 (K)	58	102	133	15
LG 5580 (K)	60	102	147	18
LG 5550 (K)	58	101	137	16
08 TR 003 (K)	57	99	145	16
EGE 436 A x TT 119 R	59	103	158	14
EGE 473 A x TT 119 R	60	107	133	19
TE-TM 2012-1	56	105	124	20
TE-TM 2012-2	58	106	145	16
TE-TM 2012-3	57	105	142	14
TE-TM 2012-4	57	105	147	16
TE-TM 2012-5	58	106	158	15
TE-TM 2012-6	58	105	169	20

Table 5. Oilseed sunflowers yield trials yield, (kg/da), oil content and oil yield, and 1000 seed weight (g), values of the varieties. TARI, Luleburgaz, Edirne (2013).

Variety Name	Seed yield (kg da^{-1})	Yield groups (α=0.01)		Seed yield over St. mean (%)	Seed yield Order	Oil content (%)	Oil yield (kg da^{-1})	(α=0.01) Oil yield groups		Oil yield Order	1000 Seed weight (g)
LG 5550 (K)	204	A		109.5	1	44.7	91	A	C	3	54.66
TE-TM 2012-6	199	A	B	106.7	2	46.9	93	A	B	2	48.12
TE-TM 2012-1	190	A	C	102.1	3	51.1	97	A		1	42.64
EGE 473 A x TT 119 R	187	A	C	100.3	4	48.2	90	A	C	5	43.80
08 TR 003 (K)	185	B	D	99.6	5	49.0	91	A	C	4	50.32
P64G46 (K)	180	C	D	96.4	6	47.3	85	B	D	6	49.48
EGE 436 A x TT 119 R	179	C	D	96.2	7	46.1	83	C	E	8	39.96
LG 5580 (K)	176	C	D	94.3	8	43.0	76		E	11	43.32
TE-TM 2012-5	174	C	D	93.7	9	48.0	84	C	E	7	41.16
TE-TM 2012-3	172	C	D	92.5	10	44.3	76		E	12	41.52
TE-TM 2012-2	168		D	90.3	11	45.7	77	D	E	10	39.56
TE-TM 2012-4	168		D	90.2	12	46.4	78	D	E	9	45.48
CV (%)	7,00						7,01				
LSD (0.05)	18,32						8,58				

* 10% seed moisture.

Table 6. Phenological Data of Firs Crop Oilseed Sunflowers Yield Trials. TARI, Luleburgaz, Edirne (2013).

Variety Name	Days to flowering (day)	Pphysiological maturity (day)	Plant height (cm)	Head diameter (cm)
P64G46 (K)	59	103	137	11
LG 5580 (K)	61	102	146	9
LG 5550 (K)	59	101	144	10
08 TR 003 (K)	57	100	139	11
EGE 436 A x TT 119 R	59	104	164	10
EGE 473 A x TT 119 R	61	106	171	12
TE-TM 2012-1	57	106	144	11
TE-TM 2012-2	59	107	148	9
TE-TM 2012-3	59	105	154	10
TE-TM 2012-4	58	106	159	9
TE-TM 2012-5	59	106	168	9
TE-TM 2012-6	59	104	160	12

Table 7. Oilseed sunflowers yield trials combined data of Edirne and Luleburgaz on yield, (kg/da), oil content and oil yield, and 1000 seed weight (g), values of the varieties.

TARI, Luleburgaz and Edirne (2013).

Variety Name	Seed yield (kg da^{-1})*	Yield groups (α=0.01)		Seed yield over St. mean (%)	Seed yield Order	Oil content (%)	Oil yield (kg da^{-1})	(α=0.01) Oil yield groups		Oil yield order
08 TR 003 (K)	212	A	B	106.8	2	48.5	102,7	A		1
TE-TM 2012-1	206	A	C	103.6	3	50.0	102,6	A		2
TE-TM 2012-6	218	A		109.8	1	46.6	101,5	A	B	3
EGE 473 A x TT 119 R	202	B	C	101.7	5	47.3	95,2	B	C	4
P64G46 (K)	197	C	D	99.4	6	47.3	93,3		C	5
LG 5550 (K)	205	A	C	103.2	4	43.7	89,4	C	D	6
TE-TM 2012-4	186	D	E	93.7	8	46.3	86,0	D	E	7
TE-TM 2012-5	172		F	86.5	12	47.8	82,1	E	F	8
TE-TM 2012-3	186	D	E	93.9	7	43.9	81,6	E	F	9
EGE 436 A x TT 119 R	182	E	F	91.7	9	44.8	81,4	E	F	10
TE-TM 2012-2	177	E	F	88.9	11	44.7	78,8		F	11
LG 5580 (K)	180	E	F	90.5	10	42.9	77,0		F	12
CV (%)	7,38						7.35			
LSD (0.05)	14,26						6.55			

* 10% seed moisture.

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PERFORMANCE OF SOME CONFECTIONARY SUNFLOWER (*HELIANTHUS ANNUUS* L.) VARIETIES IN AEGEAN REGION OF TURKEY

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ABSTRACT

Confectionary sunflower was grown 116322 ha area and 180700 metric ton seed was harvested with the average yield of 1550 kg ha^{-1} in Turkey in 2015. Aegean Region region produce a significant number of approximately 36% of confectionary seed in Denizli, İzmir, Kütahya, Usa, Afyon, Balıkesir, and Manisa provinces of Turkey. However, farmers generally use low yielding landraces for confectionary sunflowers production. The main objectives of this study were to determine confectionary varieties which could be grown with satisfactory yield performance in Aegean Region. The experiments were conducted at the experiment field of Aegean Agricultural Research Institute in Menemen, Izmir in 2009 to 2015. The experiments were established in randomized complete block design with four replications. As a material, open pollinated and hybrid confectionary sunflower candidate varieties were used in the experiments. This study were undertaken for the characters of seed yield, seed oil content (%), 1000 seed weight, plant height, head diameter, seed length, seed width, hull percentage (%), seed color, days to flowering and days to physiological maturity. Results showed that statistically significant differences were found among the varieties for the characters in question. In the experiments; the highest seed yield as 546 kg da^{-1} was obtained from the candidate open pollinated variety ETAE-D1-1-B2 in 2009 and 619 kg da^{-1} was obtained from the candidate hybrid variety ETAE-C-TM-10-2010 in 2011. The results indicated that OP and hybrid varieties could grow with their high yield performance in Aegean Region; thus, in order to increase production landraces or old cultivars need to be replaced with modern varieties.

Keywords: Sunflower, *Helianthus annuus* L, confectionary variety, adaptation, yield, yield components.

INTRODUCTION

Turkey is one of the leading countries for sunflower production. According to production data confectionary sunflower was grown on 116322 ha area and 180700 metric ton seed was harvested and the mean seed yield was 1550 kg ha^{-1} in Turkey in 2015. The Aegean Region has suitable ecological conditions for first and second crop sunflower production. Aegean Region region produce a significant number of approximately 36% of confectionary seed in Denizli, Afyon, Manisa, Uşak, Kütahya, and Izmir provinces (Anonim, 2015).

There is not enough certified seed production with desired quality. Consequently, land races are generally used for Confectionary sunflower production in Turkey.

The land races or local varieties are not suitable for combine harvest because of their ununiformity of the plant development in the field (Tan, 2010b; Tan and Tan, 2010; Tan, *et. al*., 2013b). In Sunflower research project at AARI, oilseed and confectionary type of

sunflower germplasms, open pollinated and hybrid varieties have been developed, and candidate varieties are evaluated in yield trials under first and second crop production season. Variety performance tests and yield trials indicated that sunflower can grow with satisfactory yield performance (500-550 kg da^{-1}) at both first and second crop production seasons in Aegean Region of Turkey (Tan, 2007; Tan, 2011; Tan, 2010a; b; Tan, *et. al.*, 2013a; Tan, *et. al.*, 2015). The main objectives of this study were; to determine open pollinated confectionary varieties which could be grown with satisfactory yield performance for Aegean region.

MATERIAL AND METHODS

The experiments conducted separately at first crop growing seasons in 2013 to 2015 on the experiment field of Aegean Agricultural Research Institute (AARI) in Menemen, Izmir.

The study were undertaken for the characters seed yield (kg da^{-1}), seed oil content (%), 1000 seed weight (g), plant height (cm), head diameter (cm), seed length (mm), seed width (mm), hull percentage (%), seed color, days to flowering and days to physiological maturity.

In this study, open pollinated (OP) confectionary sunflower candidate varieties developed at AARI sunflower breeding program were used in the experiments. The OP confectionary type of varieties, developed by using sunflower collections conserved National Gene Bank at AARI, Menemen, Izmir.

The varieties used in this study.

Varieties	Seed coat color
ETAE-D1-1-B1	White with a light gray stripe
ETAE-D1-1-B2	White with a light gray stripe
ETAE-D1-1-B3	White with a light gray stripe
ETAE-D1-1-B6	White with a gray stripe
D-2012-1-1	White with a light gray stripe
D-2012-1-2	White with a light gray stripe
ETAE-NGL	White
ETAE-ALA (D2)	Dark brown with a light gray stripe
ETAE-Ç-P-1-2	Black
ETAE-Ç-P-11-1	Black
ETAE-K-1	White with a few pale gray stripe
Cigdem (Check variety)	White with gray stripe
Palancı-1 (Check variety)	Gray with a white stripe

Planting times of the experiments were 21.05.2013, 02.07.2014, and 15.04.2015. The experiments were conducted a randomized block design with four replications. The

confectionary variety test experiment plots were consisted of four rows, 8.80 m in length, spaced 0.7 m apart. There were 22 plants, spaced 0.40 cm on each row.

The experiments were conducted on sandy loam soil. Recommended agronomic crop production practices were followed. Fifty kg da^{-1} ($N_{10}P_{10}K_0$) composed fertilizer applied during the soil preparation. Weed control were routinely applied. Three irrigation were applied in 2013 (27/06/2013, 12/07/2013, 31.07.2013) and 2014 (25.07.2014, 22.08.2014, 09.09.2014), and only one irrigation was applied in 2015 (10.07.2015).

Data were obtained on;

Seed yield (kg da^{-1}): The yield was obtained from each of the two middle rows of the four row plots in the experiment. At the harvest, 1st and 4th rows and first and last plants of the middle row are removed as edge effect in the experiment. Heads were hand harvested, threshed, and evaluated at 0% moisture.

Days to physiological maturity: Days from planting to R9 stage (Schneiter, and Miller, 1981).

Days to flowering maturity: Days from emergence to 75% of the flowering.

Plant height (cm): Height of ten plants were measured at R9 (Schneiter, and Miller, 1981) from ground level to the base of the head (cm).

Head diameter (cm): Head diameter of ten plants were measured at R9 (Schneiter, and Miller, 1981).

Seed size (mm): The length and width of a sample of 10 seeds were measured in mm.

Hull percentage (%): Sample from harvested seed was dried to 0% moisture and the husk of seed was removed and weighted.

1000 seed weight (g): Weight of 1000 seed (g) determined from dried seed (0% moisture) sample.

Hectoliter (g): Sample of each parcel hectoliter weight (g).

Oil content (%): Sample from harvested seed was dried to 0 g kg^{-1} moisture and percent oil was determined by nuclear magnetic resonance (NMR).

Uniformity: At the 75% of the flowering stage plants were observed whether they were uniform or not.

Statistical analysis was performed to determine the differences among the varieties (Steel, and Torrie, 1980).

RESULTS AND DISCUSSION

According to three-years results of this research in Menemen conditions, among the confectionary varieties, statistically significant (α: 0.05 and 0.01) differences were found on seed yield, flowering date, physiological maturity date, plant height, head diameter, 1000-seed weight, oil percentage (%), Seed length (mm), Seed width (mm), and hull percentage (%) (Table 1, 2, and 3).

In the experiments; the highest seed yield 537 kg da^{-1} and the lowest 216 kg da^{-1} were obtained from the varieties ETAE-D-2012-1-2 and ETAE-D1-1-B6 respectively in 2013; the highest seed yield 431 kg da^{-1} and the lowest 171 kg da^{-1} were obtained from the varieties ETAE-D-2012-1-1 and ETAE-C-P-1-2 respectively in 2014; and the highest seed yield 326 kg da^{-1} and the lowest 167 kg da^{-1} were obtained from the varieties ETAE-K1 and ETAE-C-P-1-2 respectively in 2015.

The lowest flowering days were recorded from the variety D-2012-1-2 as 50, 47, and 56 days in 2013, 2014 and 2015 growing seasons respectively. However; the highest flowering days (64 days) observed from ETAE-Ç-S-1-2 in 2015.

The lowest physiological maturity days were recorded 104 days (D1-1-B6), 95 days (ETAE-K1 and Ç-P-1-2), and 100 days (Palancı-1) in 2013, 2014 and 2015 growing seasons respectively. However; the highest physiological maturity days (109 days) observed from D-2012-1-2 in 2015. The highest plant height (228.20 cm) was obtained from D-2012-1-1 and the lowest plant height (166.40 cm) was obtained from Ç-P-1-2 in 2013 growing season.

The highest head diameter (27.83 cm) was obtained from D-2012-1-1 in 2015 growing season, and the lowest head diameter (19,40 cm) was obtained from (ETAE-D1-1-B6 and ETAE-Ç-P-11-1) in 2013 growing season. The highest 1000-seed weight 184.60 g (Palancı-1) and 182.90 g (D-2012-1-1) were obtained in 2015 growing season, however; the lowest 1000-seed weight (86,96 g) was obtained from ETAE-Ç-P-11-1 in 2013 growing season.

The highest seed length (25.41 mm) was obtained from ETAE-NGL in 2015 growing season, and the lowest seed length (18.11 mm) was obtained from ETAE-Ç-P-1-2 in 2009 growing season. The highest seed width (8.77 mm) was obtained from ETAE-NGL in 2015 growing season, and the lowest seed width (5.77 mm) was obtained from ETAE-Ç-P-1-2 in 2014 growing season. The highest hull percentage (53.62%) was obtained from ETAE-K1 in 2013, and the lowest hull percentage (35.95%) was obtained from Palancı-1 in 2015 growing season.

CONCLUSIONS

Results showed that statistically significant differences were found among the sunflower varieties for the characters in question.

In the experiments; the highest seed yield for (563 kg da^{-1}) and the lowest seed yield (202 kg da^{-1}) were obtained from the varieties ETAE-D1-2-B2 and ETAE-Ç-P-1-2 in 2009 growing season respectively, in confectionary variety experiments.

Research results indicated that both oilseed hybrids and OP confectionary varieties had suitable physiological maturity days for both first and second crop production seasons in the region.

Research results indicated that the yield performance of the oilseed varieties ranges 171 to 537 kg da^{-1}, and this results shows similarity of the long term adaptation results in Aegean Region (Tan *et. al*., 2013a; 2015).

Planting high yielding varieties in Aegean Region that has suitable ecological conditions for first and second crop sunflower production may play an important role to decrease good quality production gap in Turkey. Research results were also indicated that candidate confectionary varieties with their desired seed characters and quality will aid to decrease seed demands of the farmers.

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Table 1. First Crop confectionary sunflower variety yield trial. AARI, Menemen, Izmir (2013).

Variety name	Days to flowering (days)	Days to physiological maturity (days)	Yield* (kg da^{-1})	Yield Groups (α=0.01)	Plant heigth (cm)	Head diameter (cm)	1000 seed weight (g)	Seed length (mm)	Seed width (mm)	Oil percentage (%)	Hull percentage (%)	Uniformity (1-5) **
D1-1-B1	53	107	372	C	185.6	22.6	118.6	19.86	7.21	25.26	44.31	3.0
D1-1-B2	52	106	411	BC	203.9	21.1	113.2	19.78	7.26	28.60	43.25	2.8
D1-1-B3	52	107	462	AB	196.9	22.6	124.9	19.17	7.47	25.58	44.68	2.5
D-2012-1-1	51	109	485	AB	228.2	21.8	132.7	22.43	7.34	28.89	44.49	2.5
D-2012-1-2	50	108	537	A	218.9	22.3	143.3	22.96	7.75	28.24	44.51	2.5
D1-1-B6	52	104	216	D	171.1	19.4	116.2	21.42	6.11	26.32	41.33	2.5
ETAE-ALA (D2)	52	104	443	BC	202.3	20.2	119.0	21.64	7.55	29.16	45.53	2.3
Ç-P-1-2	58	105	238	D	166.4	21.5	100.2	18.79	5.93	21.83	45.45	2.5
Ç-P-11-1	61	105	280	D	169.6	19.4	86.96	19.79	6.12	24.30	45.90	2.8
ETAE-K-1	53	105	487	AB	189.4	21.1	135.8	19.88	7.48	20.11	53.62	2.3
CV (%)	1.58	0.47	11.59		3.78	5.28	6.42	2.57	4.85	5.16	3.39	
LSD (0.05)	1.22	0.73	66.09		10.59	1.63	11.09	0.77	0.49	1.94	2.23	
LSD (0.01)	1.65	0.98	89.25		14.30	2.19	14.97	1.04	0.67	2.61	3.01	

* Seed yield and 1000 seed weight were adjusted to 0% moisture.

**: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

Table 2. Second crop confectionary sunflower variety yield trial. AARI, Menemen, Izmir (2014).

Variety name	Yield* (kg da^{-1})	Yield Groups (α=0.01)	Days to flowering (days)	Days to physiological maturity (days)	1000 seed weigth (g)	Hull percentage (%)	Seed length (mm)	Seed width (mm)	Uniformity (1-5) **
D-2012-1-1	431	A	48	97	129.29	42.07	23.31	7.76	2.1
D-2012-1-2	353	AB	47	99	150.95	43.86	23.87	8.24	2.6
ETAE-ALA (D2)	385	A	50	93	130.35	42.05	20.55	8.23	2.0
ETAE-K-1	263	BC	49	95	132.45	49.51	20.67	7.80	1.9
ETAE-Ç-P-1-2	171	C	50	95	89.40	42.06	17.91	5.77	1.3
CV (%)	15.53		0.84	0.48	6.95	2.47	2.69	5.68	
LSD (0,05)	76.72		0.63	0.70	13.54	1.67	0.87	0.66	
LSD (0,05)	107.60		0.88	0.99	18.89	2.34	1.22	0.93	

* Seed yield and 1000 seed weight were adjusted to 0% moisture.

**: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

Table 3. First crop confectionary sunflower variety yield trial. AARI, Menemen, Izmir (2015).

Variety name	Days to flowering (days)	Days to physiological maturity (days)	Yield* (kg da^{-1})	Yield Groups (α=0.01)	Plant heigth (cm)	Head diameter (cm)	1000 seed weigth (g)	Seed length (mm)	Seed width (mm)	Hectoliter (g)	Hull percentage (%)	Oil percentage (%)	Uniformity (1-5) **
D-2012-1-1	56	104	279	A	193.9	27.83	164.61	25.05	8.35	206	43.57	24.05	2.3
ETAE-ALA (D2)	57	104	257	A	191.8	26.02	149.58	22.08	8.27	262	42.97	24.38	2.3
ETAE-K-1	59	103	293	A	181.7	25.30	159.66	21.17	7.79	306	47.83	26.58	2.1
ETAE-NGL	60	105	283	A	205.4	28.60	172.35	25.41	8.77	252	44.62	21.10	2.5
ETAE-Ç-S-1-2	64	105	150	B	187.9	22.70	108.27	18.11	5.81	314	39.36	24.20	1.6
Cigdem (St-2)	58	106	286	A	195.4	27.30	142.47	20.16	7.71	300	39.66	26.55	2.3
Palanci-1 (St-3)	56	100	271	A	186.3	24.13	166.14	18.98	8.43	323	35.95	31.90	1.5
CV (%)	1.50	1.37	12.87		3.77	3.67	3.91	2.62	2.29	2.95	3.05	4.52	
LSD (0,05)	1.30	2.12	55.22		10.75	1.42	9.79	0.84	0.27	13.99	1.91	1.71	
LSD (0,05)	1.78	2.90	75.65		14.73	1.94	13.41	1.15	0.36	19.16	2.61	2.35	

* Seed yield, 1000 seed weight, and hectoliter weight (g) were adjusted to 0% moisture.

**: 1 highly uniform, 2: uniform, 3: heterogen, 4: highly heterogen.

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OILSEED AND CONFECTIONARY SUNFLOWER (HELIANTHUS ANNUUS L.) LANDRACES OF TURKEY

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ABSTRACT

Turkey is one of the significant countries for the plant crop diversity. Turkey is also center of origin for many crop species. Flora of Turkey consists of high endemism about 3000 out of the 9500 plant species. Turkey is described as microcenters for some crops which are originated in different part of the world. The sunflower (*Helianthus annuus* L.) and its wild relatives were originated and domesticated in North America, providing an important genetic diversity for crop improvement. It is one of the important oilseed crops and their landraces have significant diversity in Turkey. The extend diversity of sunflower landraces or primitive commercial varieties are also very important source of genetic variability because they have adapted to local environments as a result of natural selection over centuries. Within the framework of National Industrial Plant Genetic Resources Project, sunflower landraces have been collected and conserved ex situ at the National Gene Bank in Izmir, Turkey. There are 389 oilseed and confectionary sunflower accessions conserved and maintained at the National Gene Bank of Turkey. In this study; eco-geographical distribution of sunflower landraces and the characterization result of agro-morphological variation of National sunflower collection will be presented. IPGRI / UPOV characters were evaluated to analyze the similarity and dissimilarity. Principle Component Analysis (PCA) was performed for diversity determination of sunflower accessions. The distribution areas of sunflower samples showed great diversity and very variable for morphological characters. The results of analysis exhibited broad morphological variation model of sunflower land races. The diversity among and between the landraces is result of adaptation of different ecologies and the farmers' selection in their pLITERATURE. The informal seed exchange mechanism among the farmers effect the some degree of similarity of the some accessions collected from different localities of different provinces.

Keywords: Sunflower, *Helianthus annuus* L., landraces, conservation, diversity, agro-morphological variation, eco-geographical variation, characterization, Multivariate analysis.

INTRODUCTION

Turkey is one of the distinctive countries for the plant diversity as being center of origin and/or center of diversity or microgene center for many crop species (Harlan; 1951; Tan, 2010a; Tan, 2010b; Karagoz *et. al*., 2010). Two of the Center of Origin is overlapped in Anatolia. Turkey is also described as microcentres for some crop species that are not originated in Turkey but they are divers in many characteristics. Turkey is the meeting place of three phytogeographical regions; Euro-Siberian, Mediterranean, and lrano-Turanian. Turkey's wealth in plants is apparent in the fact that about 3,700 out of the 11,707 plant taxa are endemic to the area (Güner *et. al*.2012).

Tanksley and McCouch (1997) emphasized that narrowing of the genetic base occurred firstly when changing the wild species into a domesticated species and secondly when landraces were

replaced by modern cultivars. Therefore the landraces, before the replacement with modern varieties should be collected, conserved and evaluated for source of breeding for broaden the genetic base. Highly organized National Plant Genetic Resources Program (NPGRP) of Turkey conducts survey, collection, conservation both *ex situ* and *in situ* (including on farm conservation of landraces), characterization and evaluation of Turkish genetic resources and genetic diversity since 1960s (Tan, 2000; Tan, 2010b).

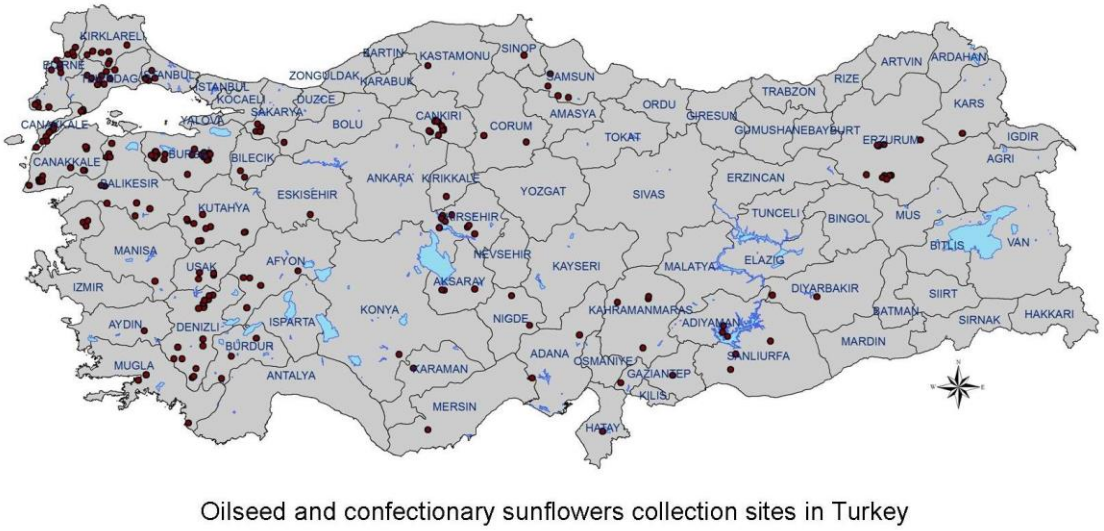
The Industrial Crops Genetic Resources Program of NPGRP is responsible for survey, collection, conservation, regeneration, evaluation, and characterization of industrial crops species (landraces and wild species (Tan and Tan, 2010; Tan and Tan, 2011; Tan and Tan, 2012; Tan et al., 2013). Environmental factors affecting the lost of wild species, the threats on landraces/local varieties are mainly the result of the replacement of landraces with modern varieties and changing the agricultural farming system. So, Industrial Crops Genetic Resources Program has yearly survey and collection program for long-term conservation of the collection at National Gene bank at Aegean Agricultural Research Institute (AARI).

Extremely variable domesticated crops as well as landraces with unique characteristics are still grown by farmers in Turkey. Fragmentation of lands lets farmers run several fields and to keep local landraces with application of traditional farming. Marginal agronomic conditions, especially steep slopes and heterogeneous soils of mountain agriculture make local landraces competitive with improved cultivars, at least in part of farming system. Economic isolation creates market limitation and minimizes to competitive advantages of improved cultivars. Local traditions and preference of diversity lead farmers to keep local landraces are the factors affect the farmers, even modern farmers, to keep their landraces or traditional crops (Tan, 2009).

From different provinces and different sources, like fields, farmer storage, threshing place and local markets of the villages, about 390 accessions confectionary and oilseed type sunflower land races were collected and stored and maintained long-term at National Gene Bank, so far. The collection and passport data, storage and characterization data are stored in National Plant Genetic Resources Data Base (Tan et al. 2015). Figure 1 shows the collection sites of sunflower land races. The collection, passport and characterization data are stored in National Plant Genetic Resources Data Base (Tan and Tan, 1998a; Tan and Tan, 1998b; Tan, 2010a; Tan, 2010b).

The Sunflower and its wild relatives were originated in North America (Heiser ve ark., 1969; Heiser, 1978; Putt, 1978; Zeven and deWet, 1982; Miller, 1987) and providing and important genetic diversity for crop improvement. Landraces are also important source of genetic variability because they have adapted to local environments as a result of natural selections over centuries. Thus, the characterization of existing collection is essential for the breeders. Characterization of genetic resources collections of confectionary and oilseed sunflower is significant to assess collection diversity for increased utilization (Tan and Tan, 2010; Tan and Tan, 2011; Tan and Tan, 2012; Tan et al., 2013; Tan et al., 2015b).

The main objectives of the this study were to analyze the degree of similarity or differences among sunflower landraces and to determine the extent of genetic diversity in sunflower landraces based on agro-morphological traits to provide information and utilization in plant breeding program.



Oilseed and confectionary sunflowers collection sites in Turkey

Figure 1. Sunflower land races collection sites in Turkey (Tan et al., 2015).

MATERIALS AND METHODS

Fifty four confectionary sunflower accessions collected from West and East Turkey, and 36 oilseed accessions collected from West Turkey maintained at National Seed Gene Bank, were characterized for assessing sustainable utilization.

The accessions were grown in two rows and fifty plants. Twenty randomly selected plants were observed from each accession. IPGRI (Anonymous, 1985) and UPOV (Anonymous, 2000) Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Sunflower" were used to observe the thirty two morphological characters of plant, head/flower and seed characteristics (Table 1). The agronomic characters, days to flowering and days to physiological maturity were also recorded (Anonymous, 1985; Anonymous, 2000).

Statistical analysis and Multivariate Analysis (Principal Component Analysis-PCA) were applied to conclude the variation among the accessions (Sneath and Sokal, 1973; Clifford and Stephenson, 1975; Tan, 1983). The statistical values of quantitative characters were calculated (Steel and Torrie, 1980).

Table 1. The observed morphological characters (Anonymous, 1985; Anonymous, 2000).

Plant characteristics: Plant height (cm), Stem width (cm), Branching, Leaf shape of cross section, Leaf shape, Leaf auricules, Leaf wings, Leaf pubescence, Leaf blistering, Leaf serration, Leaf width (cm) Leaf length (cm), Leaf distribution on stem, Stem hairiness, Stem diameter (cm), Leaf angle of lowest lateral veins, Leaf: height of the tip of the blade, compared to insertion of petiole.
Head/flower characteristics: Head diameter (cm), Head attitude, Head shape, Disk flower color, Disk flower anthocyanin coloration, Pollen fertility.
Seed characteristics: 1000 seed weight (g), Seed length (mm), Seed width (mm), Seed main color Seed type (Oilseed/confectionary), Seed hairiness, Seed stripes, Seed shape.

RESULTS

I. Characterization of Confectionary Sunflower Genetic Resources of Turkey

The morphological variation on the observed characters was found highly variable for most of the characters. All accessions have released the fertile pollen, and alternate leaf arrangements, hairy stem, absent branching, short hairy leaves, triangular leaf shape, confectionary type of kernel, dark yellow head flower. No anthocyanin coloration on the disk flower was observed. Plants were mostly vigor.

Almost all leaf characters showed variation. Leaf blistering was mainly strong and medium; Leaf serration coarse and medium; Leaf shape of cross section flat and weakly convex; Leaf auricules medium and large; Leaf wings none or very weakly expressed and weakly expressed; Leaf angle of lowest lateral veins acute and right angle or nearly right angle; Leaf height of the tip of the blade compared to insertion of petiole low and medium. Seed shape presented mainly as elongated, narrow ovoid and broad ovoid. Seed main color was white and whitish grey; Seed Stripes was observed with all types as none or very weakly expressed, weakly expressed and strongly expressed. Head attitude was variable at maturity; mainly half-turned down with straight stem and turned down with slightly curved stem were observed. Head shapes were presented as concave, flat, convex. In case of days to physiological maturity, they exhibited high range (97-104 days) and some of the accessions from Erzurum had shorter maturity period, *i.e.* 97 days, representing earliness. Similar pattern were observed in 1000 seed weight (80.60-183.50 g). The variations on quantitative characters were shown in Table 2.

Table 2. The statistical values of the agromorphological characters (Tan *et. al*., 2013).

Statistical value	Days to flowering	Days to physiological maturity	Plant height (cm)	Head diameter (cm)	1000 seed weight (g)
Mean	50.87	100.69	206.69	21.47	152.44
Min.	46.00	97.00	162.90	16.70	80.60
Max.	60.00	104.00	226.30	25.70	183.50
S^2 (Variance)	6.61	3.43	173.22	3.19	385.82
S (Standard error)	2.57	1.85	13.16	1.79	19.64
SE $\bar{x}$ (Standard error of the mean)	0.35	0.25	1.79	0.24	2.67
CV (%)	5.05	1.84	6.37	8.32	12.89

Statistical value	Seed width (mm)	Seed length (mm)	Leaf width (cm)	Leaf length (cm)	Stem diameter (cm)
Mean	7.56	21.85	12.90	28.24	17.90
Min.	5.60	16.68	2.57	24.00	2.83
Max.	9.16	26.18	33.20	33.30	31.50
S^2 (Variance)	0.47	3.94	161.28	6.17	141.90
S (Standard error)	0.68	1.99	12.70	2.48	11.91
SE $\bar{x}$ (Standard error of the mean)	0.09	0.27	1.73	0.34	1.62
CV (%)	9.05	9.09	98.43	8.79	66.56

Principal Component Analysis showed that the first eight principal components (PRINs) was accounted for 73.721 % of the total variation. The detailed result of PCA with latent roots (Eigen values), percentage variance and cumulative variance values were given in Table 3. First two Principal Components (PRIN1 and PRIN2) accounted with 33.272 % of total variance. Head diameter, leaf shape of cross section, leaf distribution on stem were effective variables on PRIN1 while seed length, 1000 seed weight, head shape, and seed width were effective variables on PRIN2 to form the groups and the scattering the accessions. Highly variable one group was formed and some of the Erzurum accessions and Denizli accessions were separated from the outside of this group (Figure 2).

Table 3. Result of Principal Component Analysis (Tan *et. al*., 2013).

PRINs	Latent Roots (Eigen values)	Percentage variance	Cumulative variance
PRIN 1	4.212	19.147	19.147
PRIN 2	3.107	14.124	33.272
PRIN 3	2.325	10.569	43.841
PRIN 4	1.970	8.956	52.797
PRIN 5	1.398	6.352	59.149
PRIN 6	1.222	5.554	64.703
PRIN 7	1.110	5.047	69.750
PRIN 8	0.874	3.971	73.721

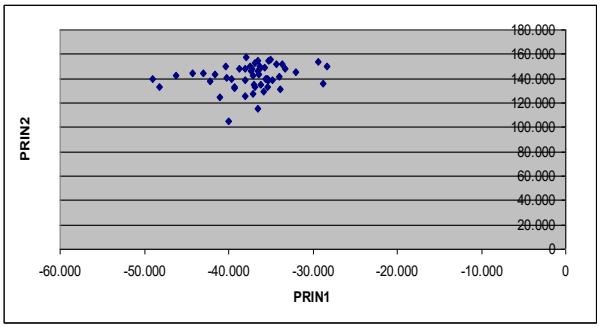


Figure 2. Distributions and grouping of the samples on PRIN1 and PRIN2 (Tan *et. al*., 2013).

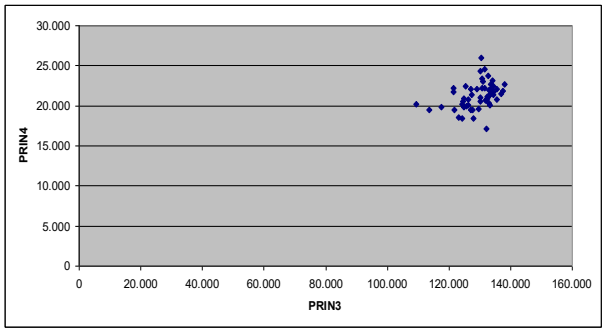


Figure 3. Distributions and grouping of the samples on PRIN3 and PRIN4 (Tan *et. al*., 2013).

Second pairs of Principal Components (PRIN3 and PRIN4) accounted with 52.797% of total variance. Leaf shape of cross section, leaf width, physiological maturity and plant height were effective character on PRIN3 whereas leaf width, leaf length, and leaf blistering were effective

characters on PRIN4. In this scatter one compact group was formed and some accessions of Erzurum province with tall plant heights and with long vegetation period were split out this group (Figure 3). The third pairs of Principal Components (PRIN5 and PRIN6) accounted with 64.703% of total variance were formed by the influence of effective variables leaf blistering on PRIN5 and head attitude on PRIN6. In this scatters, one group were observed as in the other principal component pairs. Pattern was almost same with other scatters and some accessions were scattered outside of the group (Figure 4) (Tan *et. al*., 2013).

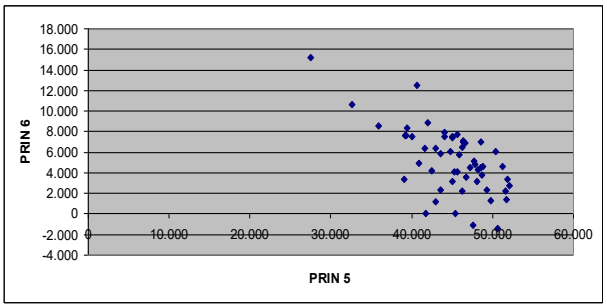


Figure 4. Distributions and grouping of the samples on PRIN5 and PRIN6 (Tan *et. al*., 2013).

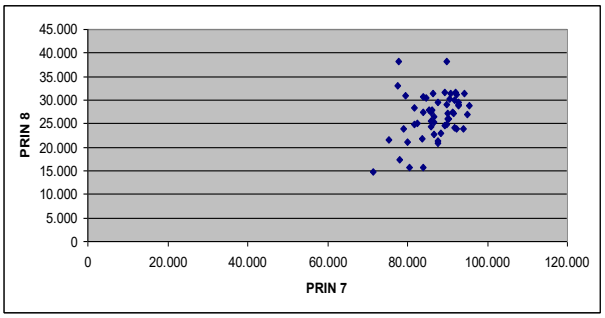


Figure 5. Distributions and grouping of the samples on PRIN7 and PRIN8 (Tan *et. al*., 2013).

The accessions in this group were very distinct from each other mainly with their very variable characteristics of head attitude. Some of the Erzurum and Denizli accessions were scattered from the group in different directions.

The fourth pairs of Principal Components (PRIN7 and PRIN8) accounted with 73.721% of total variance were formed by the influence of effective variables, leaf distribution on stem, plant height, and seed stripes on PRIN7 and leaf distribution on stem, leaf blistering, seed shape and plant height on PRIN8. In this scatters one group were observed (Figure 5). The distribution pattern was very diverse (Tan *et. al*., 2013).

II. Characterization of Oilseed Sunflower Genetic Resources of Turkey

The morphological variation on the observed characters was found highly variable for most of the characters. There was no variation on pollen fertility, type of phyllotaxis, external petal color, number of head, Seed hairiness. All accessions have released the fertile pollen, with hairless seeds, dark yellow ray flower, and alternate leaf arrangements. Plants were mostly vigor. Stems were mostly pubescence. Leaf shape was observed mostly as triangular, but cordate and rounded leaves were also observed and recorded. Head angle was very variable at maturity, and all types were observed (0°, 45°, 90°, 135°, 180° and 225°). Head shapes were also presented as concave, flat, convex and misshapen. Type of branching was another diverse character, but mostly basal branching and top branching were observed. The fully branched with central head were also observed in some plants of some accessions. The variation on quantitative characters was shown in Table 4. In case of plant height, they exhibited high range (157.0-273.5) of variation. Similar pattern were observed in the 1000 seed weight (78.4-142.25 g).

Table 4. Statistical values of the quantitative characters (Tan and Tan, 2012).

Statistical values	Days to flowering	Days to physiological maturity	Plant height (cm)	Head diameter (cm)	1000 seed weight (g)	Husk percentage (%)
Mean	56.00	110.97	185.43	20.61	96.26	28.69
Min.	52.00	108.00	157.00	16.40	78.40	20.95
Max.	71.00	121.00	273.50	27.00	142.25	50.79
S^2 (Variance)	24.74	8.54	878.36	5.01	168.34	57.48
S (Standard error)	4.97	2.92	29.64	2.24	12.97	7.58
$SE_{\bar{x}}$ (Standard error of the mean)	0.83	0.49	4.94	0.37	2.16	1.26
CV (%)	8.88	2.63	15.98	10.86	13.48	26.42

Statistical values	Stem width (cm)	Seed length (mm)	Seed width (mm)	Leaf width (cm)	Leaf length (cm)	Number of leaf
Mean	12.19	6.21	30.69	23.41	22.46	2.38
Min.	10.66	4.92	24.10	16.60	18.00	1.70
Max.	16.70	8.00	45.40	33.00	30.90	3.40
S^2 (Variance)	2.22	0.59	24.44	18.72	15.39	0.17
S (Standard error)	1.49	0.77	4.94	4.33	3.92	0.41
$SE_{\bar{x}}$ (Standard error of the mean)	0.25	0.13	0.82	0.72	0.65	0.07
CV (%)	12.22	12.38	16.11	18.48	17.47	17.39

Table 5. Result of Principal Component Analysis (Tan and Tan, 2012).

PRINs	Latent Roots (Eigen values)	Percentage variance	Cumulative variance
PRIN 1	8.766	35.062	35.062
PRIN 2	3.507	14.029	49.091
PRIN 3	2.194	8.776	57.867
PRIN 4	1.675	6.700	64.567
PRIN 5	1.451	5.803	70.370

Principal component analysis (PCA) showed that the first five principal components (PRINs) accounted for 70.370 % of the total variation. The detailed result of principal component analysis with Latent Roots (Eigen values), Percentage Variance and Cumulative Variance values is given in Table 5. First two Principal Components (PRIN1 and PRIN2) accounted with 49.091 % of total variance. Plant height, leaf length, leaf width, seed length, Stem width and husk percentage were effective variables on PRIN1, and head size, pubescence on leaf and plant vigority were effective variables on PRIN2 to form the groups and the scattering the accessions. Only one group was formed which consist of oil types and confectionary types were separated from this groups (Figure 6). Second pairs of Principal Components (PRIN3 and PRIN4) accounted with 64.567% of total variance. Leaf shape is effective character on PRIN3 and seed length, head flower color, leaf edge and leaf shapes are effective characters on PRIN4. In this scatter one group was formed which consists of oil types and all confectionary types and some oil types with large seed were outside of this group (Figure 7). The third pairs of Principal Components (PRIN4 and PRIN5) accounted with 70.370 % of total variance were formed by the influence of effective variables seed length, head flower color, leaf edge and leaf shape on PRIN4 and type of branching, head flower color and Pubescence at stem on PRIN5. In this scatters one group were observed as in the other principal component pairs. Pattern was almost same the confectionary types were outside of the group (Figure 8) (Tan and Tan, 2012).

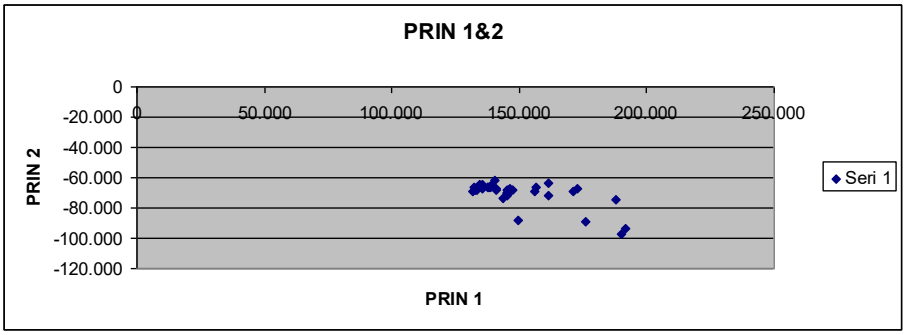


Figure 6. Distributions and grouping of the samples on PRIN1 and PRIN2 (Tan and Tan, 2012).

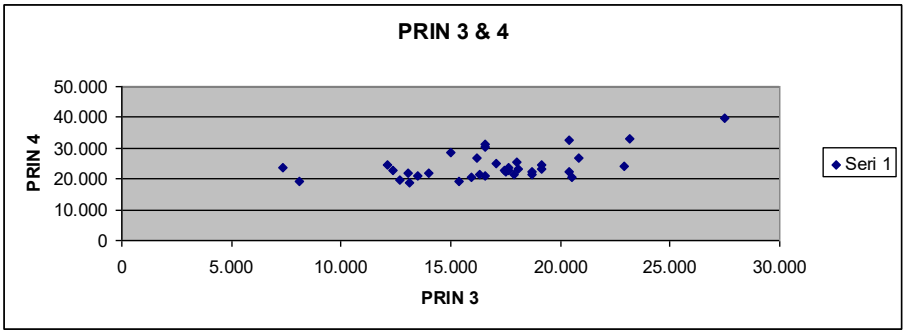


Figure 7. Distributions and grouping of the samples on PRIN3 and PRIN4 (Tan and Tan, 2012).

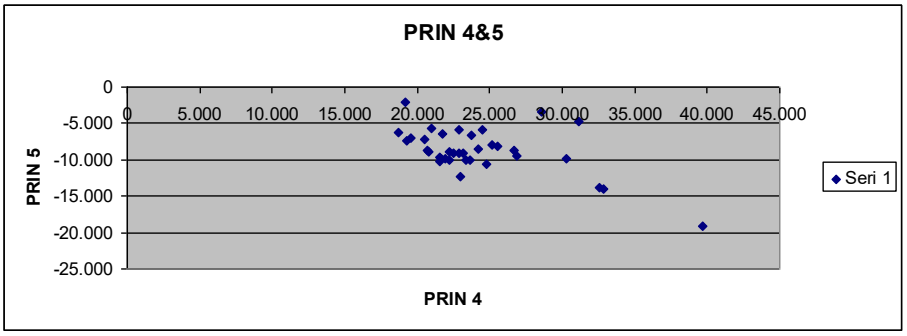


Figure 8. Distributions and grouping of the samples on PRIN4 and PRIN5 (Tan and Tan, 2012).

CONCLUCION

Sunflower land races, especially the confectionary types were very variable for morphological characters. The distinct separation on the morphology of accessions mostly depended on the types of accessions whether oilseed or confectionary types. The variation was also observed not only among accessions but also within the accessions.

Although locality separation by germplasm origin was observed in the accessions, but in general, the origin was not corresponded closely with the grouping pattern. The variation of the land races among and within the provinces and even in the villages on some characters brings up the consideration of the adaptation to different ecological conditions and also the different pLITERATURE of the farmers selection. The some degree of similarity of the some accessions collected from different localities of different provinces may result of the informal seed exchange mechanism among the farmers (Tan and Tan, 2010; Tan and Tan, 2012; Tan *et. al*., 2013).

Landraces show varying degrees of morphological and genetic integrity and may change with time, but they are recognized by farmers on the basis of a number of morphological and agronomic criteria. However, scientists and breeders may look to preserve particular genetic resources of crops, as a means of ensuring that the maximum possible range of genetic variability is available for today and future. Therefore the landraces, before the replacement with modern varieties should be collected, conserved and evaluated for source of breeding. For this purposes the existing sunflower land races still growing by farmers are collected and characterized and used in the sunflower breeding programs.

The genetic diversity plays an important role in plant breeding. Hybrids of parental lines with diverse origin, generally display a greater heterosis than those between closely related parents (Tan, 1993; Tan, 2005). The characterization of existing sunflower collection is essential for the breeders. Thus, the existing confectionary and oilseed sunflower genetic resources collections are started to characterize and evaluate for utilization at the breeding program at AARI.

Sunflower genetic resources have also been using in sunflower breeding program to develop new varieties. Improved germplasm, and breeding lines (A, B and Rf lines) of oilseed and confectionary type of sunflower germplasm, hybrids (TURAY, SUN 2235), open pollinated variety (EGE 2001), hybrids parental lines have been developed by conventional breeding techniques. New oilseed and confectionary type of sunflower hybrids improved for registration (Tan, 2010c; Tan *et. al*., 2015a) for the direct benefit of the countries agricultural sector.

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ABSTRACT

Part of the "Genomic & Genetic of Sunflower" research team in the Laboratory of Plant-Microbe Interactions (INRA, Toulouse, France), the French *Helianthus* Biological Resources Center (BRC) is dedicated to the conservation, multiplication and distribution of seeds of *Helianthus annuus* and its wild or relative species. The germplasm collection is composed of patrimonial and scientific resources, collected or created by INRA since the early 1960's. The patrimonial part of the collection is represented by open pollinated varieties, R-line and B-line varieties and wild ecotypes, for a total of 2250 accessions. The preservation and the availability of this diversity are important for the breeding of new cultivated hybrids and the improvement of the oilseed crop. The French *Helianthus* BRC is also involved in the development, maintenance and diffusion of genetic material dedicated to scientific studies, such as mapping populations, EMS-mutant population or interspecific lines, which represents a total of 2860 entries. Finally, the *Helianthus* BRC is also implicated in the molecular characterization of the patrimonial germplasm by high-throughput SNP genotyping. This helps us to evaluate the genetic diversity and to improve the management of these biological resources.

Key Words : Sunflower; Helianthus; plant genetic resources; ex situ conservation; genetic diversity

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EVALUATION OF VARIATION ON SUNFLOWER SINGLE CROSSES

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ABSTRACT

Sunflower is the main crop in the rotation system in Bulgaria. Sunflower yield is strongly dependent on the cultivar. A two-year experiment with 3 repeats was carried out to study the effects of climatically difference on yield and 1000 seeds weight. 24 genotypes: 4 *A* lines, 4 *R* lines and 16 hybrids combinations were compared over two years (2013 - drought and 2014 - wet conditions). Variation was highly significant. Two-year experiment for five characteristics (as yield and 1000 seeds weight) hybrids show good adaptation of test hybrids. The *boxplot*-diagram indicated that the hybrid *A3* x *R3* was the best.

Key words: 1000 seeds weight, adaptation, genotypes, hybrid, sunflower, yield.

INTRODUCTION

Agriculture is linked with the development of heterosis in sunflower breeding and the production of a new variety resistant and adapted to the dynamically changing climatic conditions. The creation of hybrids depends on the choice of starting material. The choice of parental forms based on their high combining ability and a complex of economically valuable signs that output components possess. Between plants and environmental conditions there are complex relationships. Under the influence of environmental factors is observed modification variability within a given rate of reaction. These changes characterize the adaptive capacity of the individual genotype to conditions during their development.

Sunflower is economically important oil plant in Bulgaria. Sowing of this trench culture helps the proper crop rotation. In recent years the area planted with sunflower increases and reaches 800,000 ha with an average yield of 2.3 t/ha ("Agrarian Report" by years of MZH). Economic significance is determined by the fact that international trade in sunflower seed is very successful. Important role in seed production of hybrid sunflower varieties separated maternal line with cytoplasmic male sterility (*CMS*), i.e. creation of sterile parent used to avoid castrated stamens.

The existing genetic variability of the cultivated sunflower makes it possible to develop hybrids with a genetic potential for seed yield of over 6 t/ha and seed oil content of over 55 %. However, most often sunflower yields obtained in large-scale commercial production are in the range of 1.5-3.0 t/ha. There are multiple limiting factors preventing the realization of the high genetic potential of this crop. Their removal will enable commercial sunflower yields to stabilize at level of 4 t/ha and above (Skoric, 2012). Competitive characteristic of plants plays an important role in yield formation. They claim that if in agrophytocenosis a potential genetic productivity of individual plants is realized only 10-20 % and 50 % of general phenotypic variance is caused by genetic and ecological differences in the competitive ability, it is difficult to identify the genotype according to its phenotype which leads to the decrease in the efficiency of selection. In the practical selection which is a part of the production of hybrids with a high potential of production, as well as high adaptive potential, a strong influence belongs to the autonomy of epigenetic systems of adaptive reactions to the ecological environment they are located in, or to the dynamics of changes that happen in the environment on the location of cultivation (Kirichenko, 2005).

The aim of the study is to evaluate the genetic potential, using statistical methods, of economically valuable lines in a complex of 5 characteristics and effect of environmental factors on the results obtained in 16 hybrid combinations.

MATERIAL AND METHODS

Four sterile analogues of *B* lines (*A* lines, with *CMS Pet-1*), four *R* lines and 16 hybrids combinations (table 1) were compared over two years. The *B* and *R* lines are received by selection of varieties (*B2* and *B4*) and intraspecific (*B1*) and interspecific (*B3* and all *R*) hybridization (Hristova-Cherbadzhi, 2012, 2009; Hristova-Cherbadzi et al., 2007).

Table 1. Origin of plant material.

I. Lines:	
B1 - *H.annuus* line HA89B x H.annuus line 2607B	*R1* - *H.annuus* line HA89A x *H.neglectus*
B2 - *H.annuus* variety Peredovik	*R2* - *H.annuus* line 2607A x *H.neglectus*
B3 - *H.annuus* line HA89B x *H.nuttallii* ssp. *rydbergii*	*R3* - *H.annuus* line HA89A x *H.nuttallii* ssp. *rydbergii*
B4 - *H.annuus* variety Birimirec	*R4* - *H.annuus* line HA89A x *H.pauciflorus* ssp. *subrhomboideus*

II. Hybrids:			
A1 x *R1*	*A1* x *R2*	*A1* x *R3*	*A1* x *R4*
A2 x R1	*A2* x *R2*	*A2* x *R3*	*A2* x *R4*
A3 x *R1*	*A3* x *R2*	*A3* x *R3*	*A3* x *R4*
A4 x *R1*	*A4* x *R2*	*A4* x *R3*	*A4* x *R4*

Experiences are displayed on the black-earth without fertilization and one hoeing. Experimental hybrids are included in the competitive variety trials ordered in the scheme by the principle of randomized block method, in triplicate, with reporting land area - 20 m^2. The results are represented by boxplot-diagrams in R multiplier for five quantitative traits - yield (kg/dka), 1000 seeds weight (*g*), seed oil (%), diameter head (*cm*) and plant height (*cm*). A two-year experiment with 3 repeats was carried out to study the effects of climatically difference on yield and 1000-seeds weight. *Correlation* and *cluster analysis* were realized. *Correlation analysis* (R Core Team, 2014) is to calculate the correlation coefficient between the values of characteristics yield and weight of 1000 seeds for two consecutive years (2013 - drought and 2014 - wet conditions) and subsequent verification of its statistical significance to evaluate the influence of environmental factors on the values in hybrids and parents. *Bootstrap*-intervals are represented by a histogram, which is designated the primary value of the correlation coefficient as a vertical line, QQ-diagram (Canty and Ripley, 2015; Davison and Hinkley, 1997), visualizing the location of the correlation coefficient and limits of 95 % confidence interval. For *cluster analysis* (R Core Team, 2014) was used the *hclust* function.

RESULTS AND DISCUSSION

***Correlation analysis*.** Zero hypothesis H_0 was that the correlation coefficient is zero, i.e. the value of characteristics were not correlated. The results from correlation analysis are presented on table 2.

For five characteristics hybrids show *very good adaptation* to changing environmental conditions and correlations are high significant- p <0.001, i.e. different climatic conditions in both years (drought and wet conditions) no significantly impact on the appearance of traits. A statistically significant correlation coefficient indicates that the values change synchronously over the years and hybrids. The exception is the trait "diameter head" which also exists statistically proven strong correlation between its values, but it is with hybrids evaluation 0.01 <p <0.05.

This is an evaluation of the total tolerance of the hybrids by traits, i.e. combinations are tolerant to environmental changes. Similar findings were reported for the parental lines, which is the result of their good uniformity. The change in climatic conditions affected most of the "diameter head" in hybrids.

Table 2. *Correlation analysis* of repeatability in years for five quantitative traits.

Quantitative traits	Estimates of correlation coefficient	t-distribution	Degrees of freedom	Lower limit	Upper limit	p-value
yield (hybrids)	0.9683	26.297	46	0.944	0.982	< 2.2e-16
yield (parents)	0.983	13.119	6	0.906	0.997	1.21e-05
1000 seeds weight (hybrids)	0.966	25.27	46	0.94	0.98	< 2.2e-16
1000 seeds weight (parents)	0.998	50.4	6	0.993	0.999	4.096e-09
Seed oil (hybrids)	0.998	66.74	14	0.995	0.999	< 2.2e-16
Seed oil (parents)	0.962	8.73	6	0.803	0.993	0.0001
Diameter head (hybrids)	0.53	2.34	14	0.047	0.812	0.034
Diameter head (parents)	0.979	11.61	6	0.882	0.996	2.449e-05
Plant height (hybrids)	0.834	5.66	14	0.577	0.94	5.883e-05
Plant height (parents)	0.978	11.61	6	0.881	0.995	2.453e-05

***Bootstrap*-intervals for correlation between observations of two traits in years**

Confidence interval on 0.9525 - 0.9855 at hybrids and 0.9288 - 1 at parents for characteristics yield (Fig.1, A and B), and on 0.9485 - 0.9826 at hybrids and 0.9907 - 1 at parents for characteristics 1000 seeds weight (Fig.1, C and D) is observed.

In figure 1A thick vertical line corresponds to the experimental value of 0.968 correlation coefficient between the values for 2013 and 2014 for hybrids for trait 'yield' and in figure 1B value

0.983 of the correlation of parental lines. Since the QQ-diagram (Fig. 1B) can be seen that the distribution of the correlation coefficient is significantly different from normal.

In figure 1C thick vertical line corresponds to the experimental value of 0.966 correlation coefficient between the values for two years for hybrids for trait '1000 seeds weight' and in figure 1D value 0.998 of the correlation of parental lines. Since the QQ-diagram (Fig. 1D) can be seen that the distribution of the correlation coefficient is significantly different from normal.

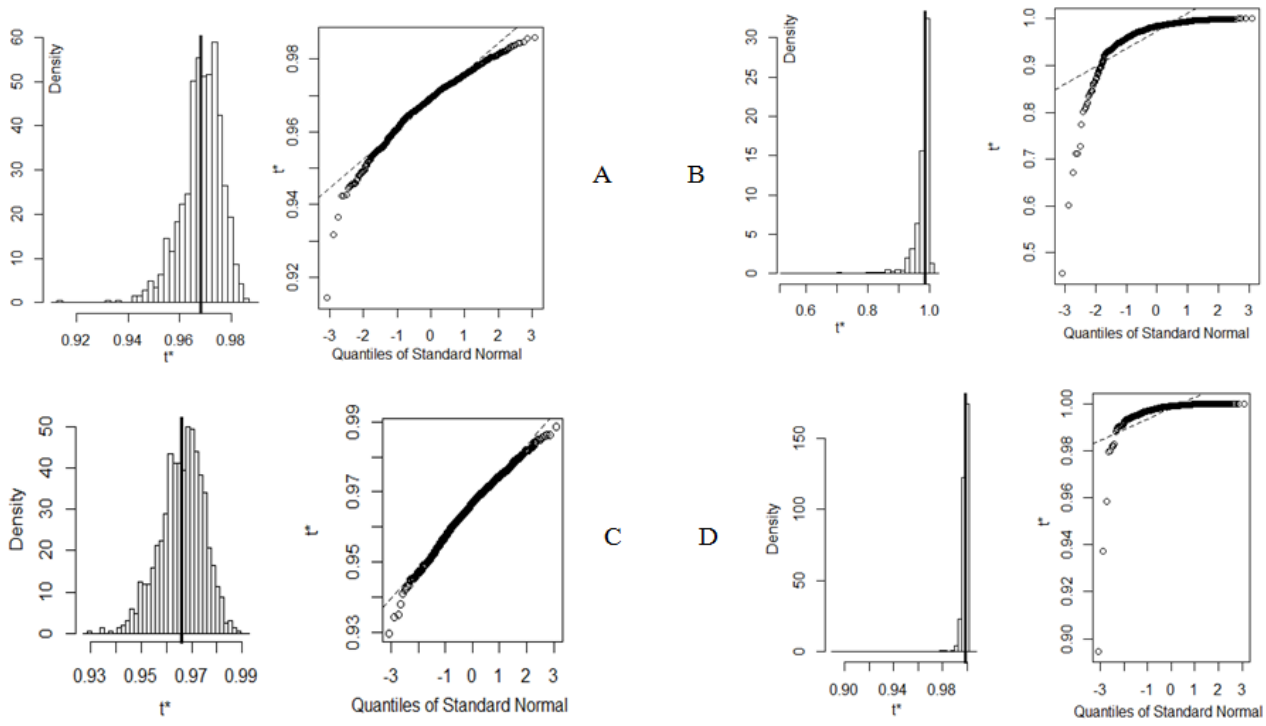

Fig.1. Histogram of the distribution of the bootstrap-values of the correlation coefficient for the characteristic 'yield' in hybrids (A) and in parental lines (B) and the characteristic '1000 seeds weight' in hybrids (C) and in parental lines (D) and their corresponding QQ-diagram.

Relationship does not have significantly change over the years, i.e. studied materials are tolerant to environmental changes with very good adaptability of hybrids, resulting in the statistically significant correlation coefficient. The results obtained from the displayed field experience vary depending on the year and the hybrid combination:

- The average *yield* of hybrids (from 347 kg/dka - in 2013 and 316 kg/dka - 2014 for cross *A2* x *R1* to 503 kg/dka - in 2013 and 509 kg/dka - 2014 for cross *A3* x *R3*);

- For the average *weight of 1000 seeds* in hybrids (from 70.1 g - 2013 and 70.3 g - 2014 for cross *A2* x *R2* to 88.9 g - 2013 and 90.3 g - 2014 for cross *A3* x *R3*).

Selection aim is to create new highly productive hybrids to realize their potential in different agro-ecological conditions. To achieve this, both parent lines of hybrid combinations must have high combining ability. The increase seed yield of new varieties remains one of the most important directions in the selection of the sunflower.

Seed yield is determined by the number and weight of fertile seeds per head (Robinson, 1983). The sunflower head has the ability to compensate for seed damage by increasing the weight of the individual seed Seiler (1997). According Charlet and Miller (1993) 10 % reduction in the number of flowers meet the 20 % reduction in the number of fertile seeds, 18 % of the total weight

of the seed per head to 22 % of the total volume of the seed. Seed yield is a complex character that results from the influence of a large number of traits, which can exert effects individually as well as jointly. The genetic basis of this character is polygenic in nature. Sunflower seed yield is a product of interactions between the genotype and environmental factors that take place throughout the growing season (Skoric, 2012). 1000-seed weight varies depending on genotype and environmental factors, too. The prevailing mode of inheritance of 1000 seed weight is partial dominance, although complete dominance and positive heterosis occur frequently as well.

Yan (2002) declared that typically environment (E) explains most (up to 80 % or higher) of total yield variations, and genotypes (G) and GE are usually smaller. Partitioning of variances revealed the significance of the environmental variance, compared to the genotype and GE interaction variances. It shows that in spite of the same location over the years there were big differences between environments due to different precipitation, temperature and 1-time irrigation in different years (Pourdad and Moghaddam, 2013).

Study the effects of abiotic conditions on plant growth and development, and yield of sunflower is an important prerequisite for the creation of many high-yield crops. Interaction of genotype and environment is important moment to the realization of the genetic potential of many crops, and stability in their production. The potential of sunflower is very high but average yield of sunflower is low and depending on weather conditions. One of reasons is inappropriate selection of varietal composition - parents.

Boxplot-diagram

Boxplot-diagrams for hybrids visually represent variations of maternal (*cms* sterile) and father (fertile) lines regarding on the relevant traits. On the graphs (Fig.2), due to high significant correlation coefficient for the two years, shows the variation of the characteristics. For each trait is evaluated and visualized the genetic potential of parental components. The main range of the relevant parent is defined and different colored rectangle. The solid line within the rectangle is the median, and it is perpendicular to the variation range for the each trait.

The obtained results indicate variation of the five traits in individual lines in different degrees. On the graph shows that the largest potential for trait "yield" means the lines *A1*, *R3* and *R4* for "weight" - *A1*, *A4*, *R2* and *R3*, for "oil" - a *A2* and *R2*, a "diameter" - a *A4* and *R1* and "height" - a A3 and R3. The highest values are around that range maternal *A3* and paternal *R3* line. Combining them to produce hybrid (*A3* x *R3*) is successful and leads to the highest expression of the trait "yield" - 506 kg/dka, due to the overlapping of their main interval of variation (IQR). This is subject to verification by the following analyzes. The lowest values ranging around maternal *A2* and paternal *R1*. Combining these two lines of hybrid (*A2* × *R1*) can be the lowest value for the same trait. In the case in hybrid yield is 332 kg/dka, the lowest in comparison with other results for the remaining hybrid combinations. Some lines for trait "oil", "diameter" and "height" have values close to the median. These lines have very low genetic potential for the trait. For example, the variation of the trait "oil" at *A3* and *A4* is highly asymmetric, which can lead to narrowing of the variation, but this feature may not be an indication of heterosis. Similar asymmetry was observed in the indicators "diameter" of the lines *A1* and *R4*, and "height" of line *A2*.

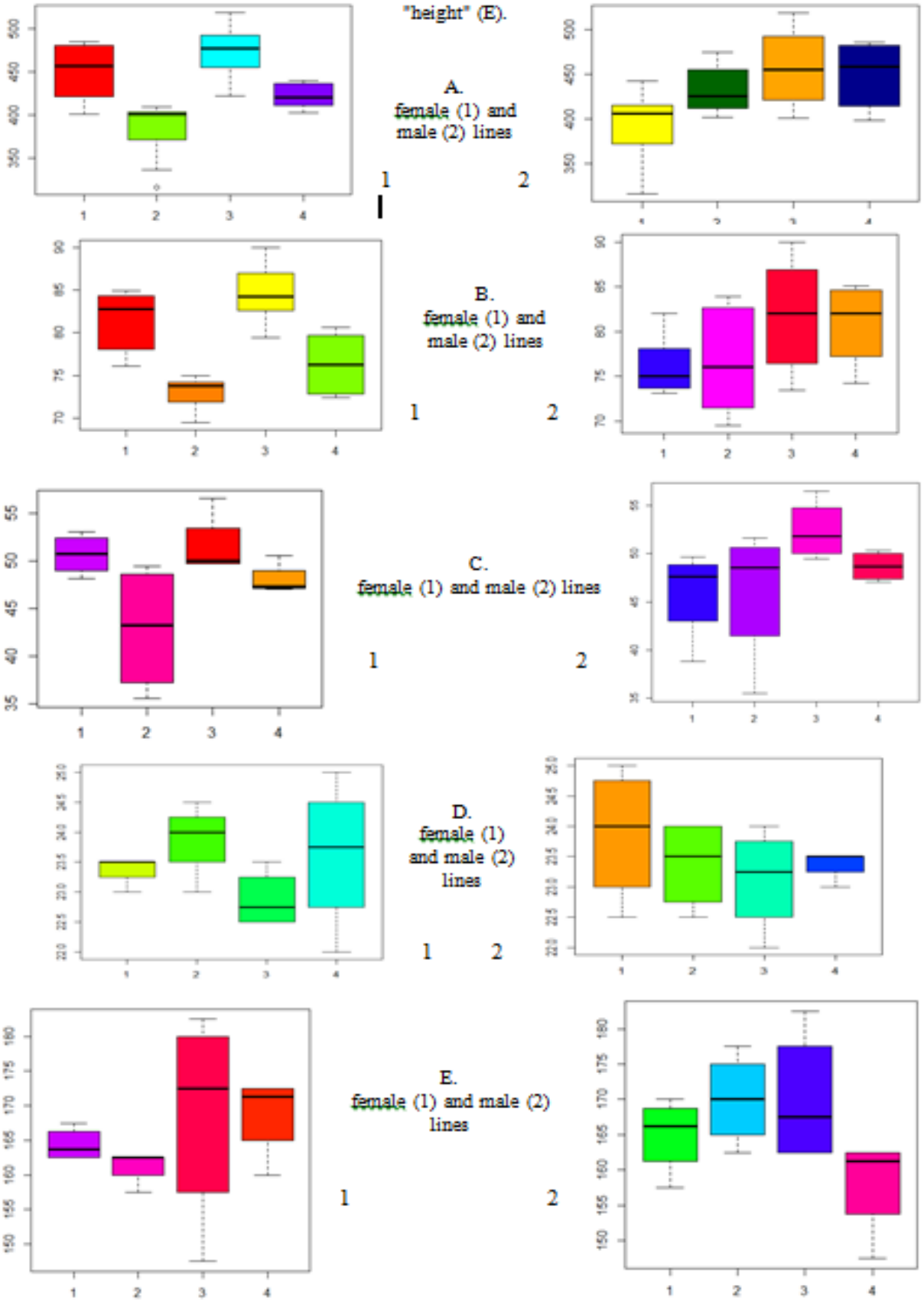

Fig.2. Variation of the characteristics “yield” (A), "1000-seeds weight" (B), "oil" (C), "diameter"

Combining statistical results facilitates breeder in his choice of starting materials in the creation of new hybrids. For example the trait "diameter" in lines *A3* and *R3* values around that range are the lowest, i.e. median or middle observation in the corresponding variation order is the smallest. These same two lines are the highest values for the "oil" and "weight" in hybrid combination (*A3* x *R3*) is the highest manifestation of these traits, i.e. hybrid with the smallest diameter of the heel is the highest yield and the weight of 1000 seeds.

Hybrid combining ***A3*** × ***R3*** is the highest manifestation of traits "oil" and "weight" even the smallest "diameter" and is the most promise, combining the most important economic business qualities.

Cluster analysis

Interspecific hybridization or detection of desirable genes in wild species of the genus *Helianthus* and their insertion into cultivated sunflower genotypes occupies a special place in sunflower production. Heterosis in the sunflower hybrids is highly linked to the genetic distance between the parental lines. With great economic importance for the sunflower is cytoplasmic male sterility - with the including of *CMS* in sunflower (Leclercq, 1969) *Rf* and the identification of genes (Enns et al, 1970; Kinman, 1970) and the creation of lines *R*. Carrying these genes it is possible to use a heterosis breeding for increasing the yield of hybrid seed. Of the hybrid seeds obtained after crossing the two parental forms - *A* (sterile analogues of sterile lines) and *R* (fertility restorer) lines are obtained 100 % fertile F_1 hybrid plants (Putt, 1997). *CMS* system for the production of sunflower hybrid seeds, for first time was used in 1972 (Fick and Miller, 1997).

The *B* and *R* lines, using in this research are received by selection of varieties (*B2* and *B4*) and intraspecific (*B1*) and interspecific (*B3* and all *R*) hybridization (Hristova-Cherbadzhi, 2012, 2009, 2007; Hristova-Cherbadzi and Christov, 2008; Hristova-Cherbadzi et al., 2007). The sterile analogues of *B* lines (*A* lines) were with *CMS Pet-1*.

The results from cluster analysis are presented on figures 3 and 4. Each cluster dendrogram shows the diversity (remoteness) of the materials by grouping parents (Fig.3A) or hybrids (Fig.4A) for 5 traits (Fig.3B, 4B).

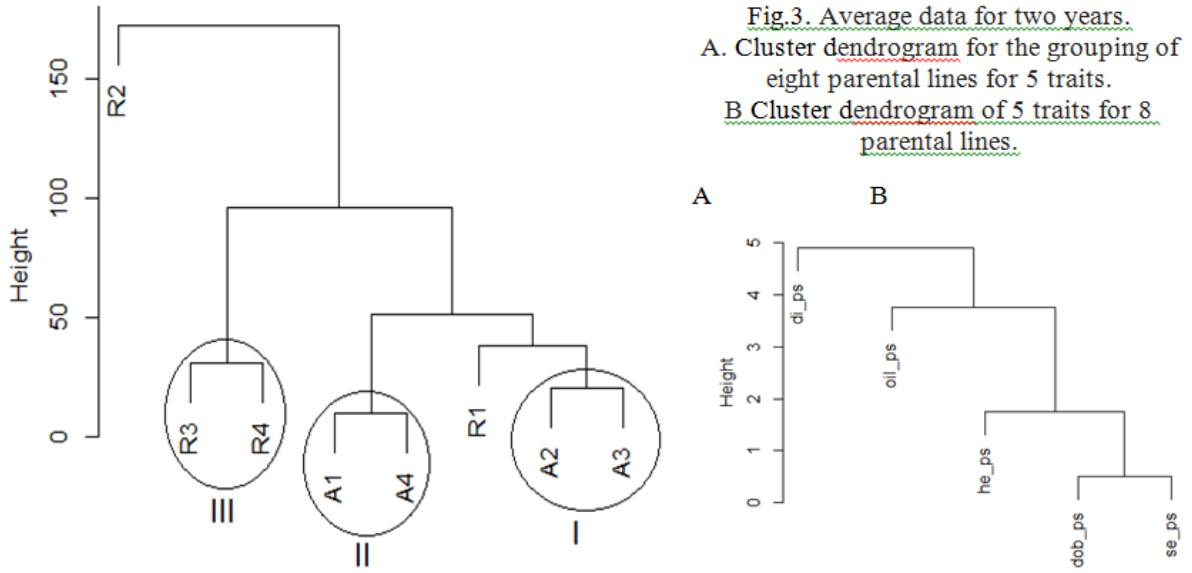

Fig.3. Average data for two years.
A. Cluster dendrogram for the grouping of eight parental lines for 5 traits.
B Cluster dendrogram of 5 traits for 8 parental lines.

Figure 3A shows a design space on the 5 characteristics, where lines *A2* and *A3* are the closest (group I). Next to them is a line *R1*. The lines *A1* and *A4* are in the next near group (II). Group III includes lines *R3* and *R4*. Line *R2* is most remote from all other lines.

Line *B2* is selected from variety Peredovik and line *B3* - after interspecific hybridization *H.annuus* line HA89B x *H.nuttallii* ssp. *rydbergii*. Many years ago line HA89B is selected from variety Peredovik (from J. Miller, Fargo, ND, USA), too.

The result is very interesting because the lines *A3* and *R3* are genetically distant, but at the same time received hybrid combination had strong positive heterosis for traits "1000 seeds weight" and "yield". These two lines (*A3* and *R3*) are received by interspecific hybridization. They are unique in that, that they are obtained after selection of the initial cross *H. annuus* (line HA89*B/A*) x *H. nuttallii* ssp. *rydbergii*. Until 2007 (Hristova-Cherbadzi and Christov, 2008) successful hybridization with this wild perennial deploid subspecies had not yet been reported. The subspecies *subrhomboideus* of the perennial hexaploid species *Helianthus pauciflorus* (*rigidus*), that was crossed with the cultivated sunflower, was studied less, too. Genes that controlled such characters as resistance to *Plasmopara helianthi*, *Phomopsis helianthi*, *Phoma macdonaldii* and *Orobanche cumana*, *Rf* gene for *CMS Pet-1*, suitable type of branching for *R* lines, high oil content (53.43 % for line *R3*) and high combining ability (line *A3*) were transferred.

Differences in expression of the characteristics “yield” and "1000-seeds weight” are the small (Fig.3B). To them next is a "height". In remove of these is the "oil", and finally "diameter".

Figure 4A shows a design space on the 5 characteristics for hybrid combinations. The 16 hybrids can group in three near close groups, too. Only one hybrid stay single, remove from other.

The closeness between the characteristics at hybrids keeps like this at the lines, but it has one difference - places of "oil" and "height" are exchanged. Here the “oil” is closer to the characteristics "yield" and "1000-seeds weight.

Maintaining the relationship between the closeness of the characteristics in parental lines and hybrids is confirming the genetic factor.

The use of statistical analysis in evaluation of the genetic properties of genotypes of *CMS* system together with the use of the selection method - remote hybridization, is practicable and can be further developed.

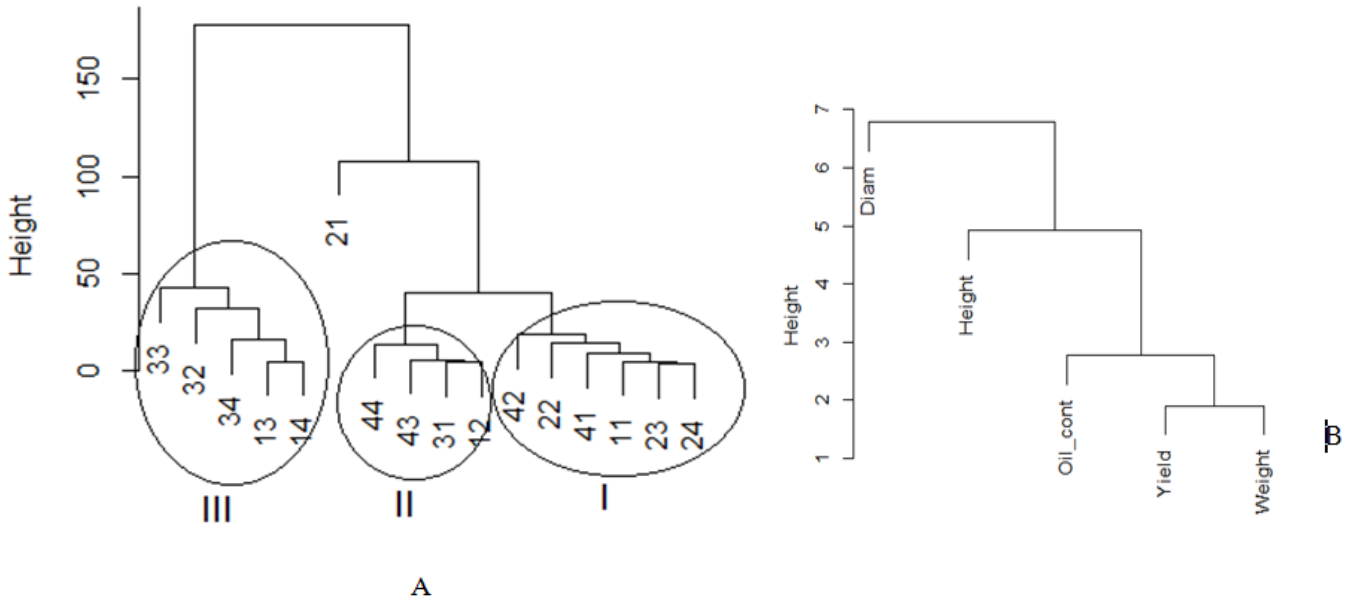

Fig.4. Average data for two years.
A. Cluster dendrogram for the grouping of 16 hybrids for 5 traits.
B. Cluster dendrogram of 5 traits for 16 hybrids.

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HYBRIDIZATION BETWEEN SUNFLOWERS (*HELIANTHUS ANNUUS* L.) AND LESS STEM ROSETTE (*CARLINA ACANTHIFOLIA* ALL.). CHARACTERIZATION OF RECEIVED INTERGENERIC FORMS

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ABSTRACT

Hybridization between sunflower (*Helianthus annuus* L.) and less stem rosette (*Carlina acanthifolia* All.) were made. These were two plants with different habitat in northern Bulgaria. Intergeneric hybrid plants and seeds were obtained in both directions of crossing. There were some outstanding differences between the two intergeneric hybrid groups. The first is related with the pollination and the viability of hybrid plants. Another interesting difference was in the seeds. Seeds of second group plants were larger, with 2-3 times longer size than those from first group, though the maternal parent form was the same. Line had small seeds. New intergeneric hybrids were carriers of *Rf* genes for CMS Pet-1 transferred from *Carlina acanthifolia* All. In crosses with sterile sunflower lines showed 100 % restoration ability.

Key words: *Carlina acanthifolia*, *Helianthus annuus*, intergeneric hybrid, sunflower

INTRODUCTION

Application of intergeneric hybridization in sunflower is hardly realizable work. Under certain conditions and well-chosen parental components enables the creation of rich source material for selection of sunflower (Christov and Panajotov, 1991; Christov et al., 1994, 2004, 2009; Christov and Vassilevska-Ivanova, 1999; Hristova-Cherbadzi, 2007, 2012; Christov, 2013 and etc.). Of particular interest are the plants used in folk medicine. One of these plants is less stem rosette (*Carlina acanthifolia* All.).

MATERIAL AND METHODS

The investigation was carried out at the Vrachantsi, Dobrich, Bulgaria, during the period 2013 - 2015.

For maternal parent form used sterile analogue of line HA-821 and as pollinator *Carlina acanthifolia*: accession №1, found near Vrachantsi, Dobrich (Fig.1) and accession №2 by region Balgarevo, Kavarna.

Fig.1. *Carlina acanthifolia*.

Method of intergeneric hybridization (crossing between cultivated sunflower line HA-821A and *Carlina acanthifolia*), crossing between A lines and intergeneric hybrids, selection,

self-pollination and sib-pollination were used. Morphological characteristics were based on phenotypic observation and biometric measurements during the vegetation period and on laboratory studies of whole plants and seeds. *Rf* genes, in crosses of sterile sunflower lines x *Carlina acanthifolia* and sterile sunflower lines x intergeneric hybrid - F_1 and F_2, were searched. The taste of the kernel is tested in wax and full maturity and after short roasting seeds.

RESULTS AND DISCUSSION

As a result of hybridization between sunflower (*H. annuus*) - line HA-821A and *Carlina acanthifolia* - accessions №1 and №2 total 31 seeds and from them 7 F_1 plants were obtained (Table 1). The number of received seeds for separate inflorescences was from 1 to 8.

Table 1. Crossability of cultivated sunflower *H. annuus* and *Carlina acanthifolia*.

Crosses	Pollinated inflorescences			Total number seeds	Hybrid plants	
	total number	with seed				
		number	%		number	%
H. annuus x *C. acanthifolia* №2 - "first group"	23	5	21,74	12	2	16,67
H. annuus x *C. acanthifolia* №1 - "second group"	23	3	13,04	19	5	26,32

In early March 2014 the seeds were sown in separate containers in a special room with enough light and warm (21-25^{0}C). Then they were seedlings in 30 cm distance from place, where was the *C. acanthifolia* accessions №1. The plants developed normally until flowering. Their height was from 30 to 55 cm. The disk flowers of all plants digressed pollen. One plant from the "second group" (*H. annuus* x *C. acanthifolia* №1) died 3 days after full of blooms. Another plant from the "first group" (*H. annuus* x *C. acanthifolia* №2) died 21 days after blooming. After self-pollination of the plant from the "first group" and two plants from the "second group" are received in total 11 (4 + 7) seeds. Two plants from the "second group" were pollinated with one another. After that 17 seeds were obtained. With pollen from the last two plants was pollinated one sterile plant from the line 92A. After this bekkros number of seeds was 21. In 2014, a second generation plants (2 and 1) were received also. From each group are left spare seeds. Results of the seeds' viability from the first hybrid generation and the viability of hybrid plants from the next generation are presented in Table 2.

Table 2. Viability of seeds and hybrid plants from the second generation, 2014.

Type of pollination	Sown seeds, n	Received plants, n	Vital plants	
			number	%
Self-pollination				
- "first group"	3	2	2	100,00
- "second group"	5	4	4	100,00
Sisterly pollination				
- III group	12	11	10	90,91
Bekkros				
- IV group	15	15	15	100,00

From sown total 8 seeds after self-pollination of F_1 plants, were received 6 F_2 plants. The disk flowers of all plants digressed pollen. After self-pollination of the two plants from "first group" 32 seeds were obtained. Their dimensions were closed to these of line HA-821A. Some of the seeds had gray-black color of the peel and the others were light- motley colored. After self-pollination of 4 F_2 plants from the "second group", 89 seeds were received. The seeds are characterized by large size in length (12-13 mm) and light- motley colored. From III group (since the sisterly pollination of the F_1 plants) 12 seeds were sown. Eleven F_2 plants were obtained. At the beginning of flowering one plant dies from them. The other 10 F_2 plants were fertile. Seven from them were self-pollinated and 201 seeds were obtained. The remaining three plants were left open pollinate. Since all plants the seeds were obtained, but at about 1/3 of the disc florets on each head had not seeds. The best result was obtained at BC_1 plants from the IV group.

From 15 sown seeds, 15 vital plants were received. One from them was male sterile and the other 14 plants - fertile. Eight plants were self-pollinated and 7 (6 + 1) plants were left open pollinate. From all 15 plants, the seeds were obtained. By two inflorescences of lines HA-821A and 92A were pollinated with pollen from F_2 plants (cross *H. annuus* x *C. acanthifolia* №1, "second group"). Seeds were obtained from the 4 pollinated heads. Twenty one seeds from all the numbers of different groups' intergeneric hybrids were sown on April 2015 to obtain plants from third generation. Some results are presented in Table 3.

Table 3. Characteristics of F_2, BC_1, F_3, F_1, BC_1 and F_4 hybrids.

Groups	Sown seeds, n	Received plants, n	Vital plants, n	Inflorescences with seeds, n	Male sterility plants, n
I group	21	17	17	9	-
II group	21	19	19	19	-
III group	21	16	16	16	1
IV group	21	20	20	20	1
V group:					
1 head - HA-821A x II group	21	20	20	20	3
2 head - HA-821A x II group	21	19	19	19	2
1 head 92A x II group	21	21	21	21	2
2 head - 92A x II group	21	20	20	20	1

Six plants from all numbers (groups) were self-pollinated, to receive next generation.

Some of the F_3 plants originating from a cross HA-821A x *C. acanthifolia* №2 had not seeds, although the plants were fertile. Seven of the 8 plants that have not received seeds were open pollinated. This may mean that the work with accession №2 will be difficult. Probably the two accessions №1 and №2 were different. The seeds from all plants II group are distinguished with their length (12-13 mm) and light-motley colored. The seeds from more of plants III group were similar to those of the II group. From other plants, seeds larger than the line HA-821A were received. All plants from the IV group have large seeds, because line 92A was with large seeds. Some of the seeds are colored gray-brown. The seeds of the first two numbers from the V group were larger than those of the line HA-821A, but were smaller than those of the plants from the II group. Seeds from the other two numbers were long and similar to the seeds of plants from the II group. The received fertile plants from all groups showed that in both accessions were established *Rf* genes for CMS Pet-1.

The taste of the kernel from the F_3 plants II group is tested in wax and full maturity and after short roasting seeds. As standards are used variety Favorit and hybrid XL-4337. Taste of nut plants from II group compared with this from nuts on both standard was more similar to hybrid XL-4337, but different in wax maturity.

CONCLUSION

Hybridization between sunflower (*H. annuus*) and two accessions of *C. acanthifolia* was successful. The received interspecies hybrids with the both accessions were differ in some characteristics, such as size of the seeds, in the plant growth and the next generation multiply, carriers of *Rf* genes for CMS Pet-1 and others. Many of the characteristics of the received material to be studied for the future.

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SUNFLOWER VERTICILLIUM WILT: BEHAVIOUR OF COMMERCIAL HYBRIDS IN QUICK TESTS PERFORMED AT CONTROLLED CONDITIONS.

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Introduction and objective. *Verticillium dahliae* is a major pathogen of many important crops. Verticillium sunflower wilt is an important disease in Argentina. The symptoms tend to be rather unreliable for assessing resistance under field condition, since the interaction with environment is often present. There is poor information about the behaviour of Argentinean sunflower hybrids in quick tests performed at controlled conditions. The objective of this work was to quantify the sunflower wilt incidence and severity of hybrids inoculated with two variants of *V. dahliae*.

Materials and methods. Eighteen commercial hybrids from Argentina were tested, including a susceptible and resistant control. Fourteen days old sunflower seedlings were inoculated by root immersion in a conidial suspension of *V. dahliae* (two isolates representative of Argentinean V. dahliae variants inoculated separately). Plants were planted in pots with a mixture of pasteurized soil and perlite and incubated in a growth chamber (12 h, photoperiod, 25 ± 2°C). Forty-five days from the inoculation, the severity and incidence of Verticillium wilt was recorded. The variance of data was analysed and means were compared by turkey test.

Results. The susceptible control had significantly higher *Verticillium wilt* severity and incidence than the resistant one ($P<0.0001$). The two isolates caused differential levels of disease severity and incidence to sunflower hybrids ($P<0.0001$). There was differential behaviour between hybrids in severity and incidence of Verticillium wilt.

Conclusion. The commercial sunflower hybrids show variability in their resistant level against *Verticillium wilt*. One hybrid show a good resistance level to both isolates

Discussion. This information adds knowledge to the breeding for resistance to sunflower *Verticillium wilt*.

Key Words : Sunflower; Verticillium Wilt; Commercial hybrids; Quick tests

ARGENTINEAN AND EUROPEAN SUNFLOWER HYBRID PERFORMANCE IN A *VERTICILLIUM* INFECTARIUM

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Introduction and objective. *Verticillium dahliae* is a major pathogen of a number of economically important crop species. In Sunflower is an important disease in Argentina and increase the importance in the rest of the sunflower world area (special Europe). The expansion to non-traditional areas of disease exposure hybrids to this pathogen. The objective of this work is to compare the resistance level of sunflowers from Argentina (verticillium endemic) and Europa (non *Verticillium)* in monoculture high field pressure (infectarium).

Materials and methods. Fourteen commercial hybrid from Argentina and Europa were tested five years, (including a susceptible and resistant control) in a verticillium infectarium with more than 15 years of continuous sunflower history in Miramar (38°11'14.41"S, 57°55'15.97"W, Province of Buenos Aires, Argentina). The experimental unit were two row of 5m long, the inferctarium have additional irrigation. From R6 to R8 growth stages, severity of Verticillium was quantified. Mix model analysis were used to analyzed the results.

Results. The susceptible control had significantly higher verticillium severity than the resistant one ($P<0.0001$). None of the European materials show similar resistance level of the control (local hybrid). Most of the European materials were highly susceptible. The Argentinean hybrids show a contrasting level of resistance

Conclusion. The European and Argentinean materials show different level of resistance to Verticillium. A higher resistance level were shown by Argentinian hybrids. Some European hybrids had a good level of resistance to Verticillium.

Discussion. This information add knowledge to the breeding for resistance to Verticillium.

Key Words : Sunflower hybrid; Verticillium infectarium

CHARACTERIZATION OF HELIANTHUS TUBEROSUS L. ACCESSIONS FROM VIR COLLECTION

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ABSTRACT

The Jerusalem artichoke *Helianthus tuberosus* L. is distributed throughout the European part of Russia up to about 630 N in gardens and orchards. The population uses its tubers as food (for diabetes prevention), for fodder and as an ornamental plant. Varieties of grown material are not known for sure. Seven accessions of *H. tuberosus* collected in Pskov, Leningrad regions and St. Petersburg were collected and planted in the nursery of perennial wild species in Kuban experimental station of VIR in the Krasnodar region (KES). In the same nursery five accessions of *H. tuberosus* obtained in different years from various habitats in the USA are maintained. We had an opportunity to compare the development of Jerusalem artichoke plants from different places of origin in 2014 and 2015. Spring growth of plants originating from the North-Western of Russia started a little later (April, 15-25) compared with accessions from the USA (April, 4-10), but they flowered earlier: on June, 15 - 26 in 2014 and on June, 20 – July, 14 in 2015. In the maternal populations of these accessions in Leningrad and Pskov regions flowering started on August, 5-10. Accessions of Jerusalem artichoke, grown in KES started flowering on September, 4-20. All the accessions had normally formed flowers. Cytological study showed that anthers produced more than 70% of normal pollen both in Krasnodar region and in the North-West of Russia. For their size the pollen grains were divided into three groups, which may be a consequence of their different ploidy. Study of ovules was not carried out. The seeds were formed only in the conditions of Krasnodar region. In the North-West of Russia plants of Jerusalem artichoke are able to reproduce only vegetatively by tubers.

Key Words : Helianthus tuberosus, Krasnodar region, North-West of Russia, flowering, cytological study

GENETIC RESOURCES FOR THE BREEDING OF LARGE FRUIT SUNFLOWER

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ABSTRACT

On the bases of many years maintenance and several re-sowings among 2230 accessions from VIR collection we have selected 34 genotypes which have not lost the character of a large fruit. Very long fruit accessions from China also were included in this group. These accessions were evaluated in the field at the Kuban experimental station of VIR in Krasnodar region for 3 years for the weight of 1000 seeds. The implementations of achenes was estimated using the microfocus x-ray. The radiation load on the seeds during the investigation, is extremely low and has no mutagenic effect. Three-year observations selected 15 sunflower accessions with weight of 1000 seeds more than 100 grams. Among them modern confectionery varieties bread in all-Russia Institute of Oil crops (VNIIMK) can be marked: Donskoy Krupnoplodniy k-3510, Konditerskiy k-3426, Lakomka k-3526 and also Zaporozhskiy konditerskiy k-3516. High rates of the character show landrace varieties, such as Stadion k-2642 from Bulgaria, Ger-Ger k-1589 from Armenia and the local accessions collected during the expeditions in Primorye kk-2817, 2818, 2835, 2836, 2843 and Argentina k-3583. The highest weight of 1000 seeds is typical for 2 accessions from China k- 3633, k-3586 and line VIR 846 k-3683. The last ones are characterized by light coloration of the achene with gray stripes. Accessions of oil and confectionery use, differing in origin, size of achenes, and the degree of implementation were analyzed with the use of x-ray (Fig). Mathematical analyses of the obtained radiographs were made using the specialized computer program. The largest achenes (fetus' area more than 4,00 mm2) are typical for accessions kk-3586, 3516, 3619, but their implementation is less than 50 %. Accessions with the largest implementation (over 50%) are: kk-1693, 1960, 1961, 2051, 3315, 3351, 3447, 3455, 3553, 3621. Therefore, the largest achenes are typical for one groupe of accessions, and the most implemented – for the other. The weight of 1000 seeds is not always corresponded to the size of the seed and kernel. But accession from China, k-3586 has achenes larger and heavier than all other analyzed accessions. Thus, it is shown a new opportunity for individual selection of genotypes during the creation of the initial material for breeding varieties and hybrids of large-fruited and confectionary sunflower. The method of lifetime estimation of implementation of sunflower achenes using microfocus radiography and specialized computer program for mathematical analyses of x-rays pictures.

Key Words : large fruit sunflower,the implementations of achenes, the microfocus x-ray

CAN GENOTYPE X ENVIRONMENT MANAGEMENT INTERACTIONS (GEMI) BE PREDICTED IN SUNFLOWER MULTI-ENVIRONMENT TRIAL?

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ABSTRACT

Climate change and input reduction in agriculture lead to a diversification of cropping environments with a higher expression of biotic and abiotic stresses. In this context, adapting the choice of cultivars according to their cropping environment is of special importance to increase sunflower productivity. Crop cultivar assessment programs aim at evaluating the performance of new cultivars in multi-environment trials (MET). These are a series of field trials conducted across a range of geographic locations and sometimes over several years. However, choosing a cultivar according to its global performance can be risky because of GEMI, which induce significant variations in the relative performance of cultivars when they are assessed in different environments and submitted to various crop management practices. The analysis of GEMIs could enrich the current information on commercial cultivars, and therefore improve the recommendations on cultivar according to the farmers cropping environment. This study aimed at evaluating the predictive value of statistical methods that model GEMI on cultivar MET. Those methods use environmental covariates quantifying major abiotic stresses. Two approaches were evaluated: the model is performed either directly on the yield variable or on the interaction terms first estimated by a mixed model. For both approaches, several methods are evaluated: factorial regression, PLS regressions, Random Forest and Lasso regression. These models are assessed on a "virtual dataset" generated by SUNFLO, a dynamic model simulating genotype-specific performance of a sunflower crop in contrasted environments. The predictive quality of the statistic models was assessed by cross-validation and their predictive values were compared to the one of an additive model in which GEMI is not taken into account. Then a diagnosis of error of prediction was performed to identify which kind of environment is more difficult to predict. The results obtained showed that the best predictive approach is to model directly GEMIs with the Random forest statistical method. However, compared to an additive model, the improvement of the predictive value achieved by modeling GEMI's remains limited. This improvement is all the worst that the stresses generating GEMI are early in the cropping season. This study shows clearly the inadequacy of the classic statistic methods to model the GEMI in the MET even in an optimistic context (data generated without error on the yield and the environmental covariates).

Key Words : Genotype-environment-management interactions (GEMIs), multi-environment trials (MET), Sunflo

SUNRISE PHENOTYPING DATABASE : A TOOL FOR THE SUNFLOWER COMMUNITY TO SHARE AGRONOMIC, PHYSIOLOGICAL AND MOLECULAR DATA

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ABSTRACT

In the SUNRISE project, a combination of several approaches is developed including the establishment of appropriate and high-throughput phenotyping strategies to characterize the molecular, physiological and agronomical responses of sunflower to variation of the abiotic environment. For instance, large-scale experiments are conducted in multi-environment by the different partners resulting in the acquisition of millions of molecular, physiological, agronomical data points, all associated e.g. through genotypes, and environments. In this context, our community needs to develop a resource to integrate, maintain, store, manage, connect and visualize these valuable data. Our team constituted of agronomists, physiologist, molecular biologist and bioinformaticians developed an information system composed of a database, a query system and a user interface allowing comprehensive data integration and fast interpretation of results. As a first step, the SUNRISE archive was created for collection, storage and long-term accessibility of raw data. From this step on, the collection protocol, description of the environment and data description (metadata) are bound to phenotypic data. Data archiving is made through a secure web portal with user access to identify responsible persons and property. Then, a second step generates from this archive a database for data exploitation. The database schema was built in collaboration between bioinformatics and biologists to structure different type of data and their properties (genotypic information, statistical design, environmental factors, geographical informations, partners,…). A web interface accessible at sunrise.toulouse.inra.fr/phenoDB allows querying the data, viewing them and exporting them. Data access is restricted to authorized users on a file-based system and is therefore very flexible. Importantly, the generic architecture of the archive and the database allows its potential expansion to other types of phenotypic data on sunflower and other species and its use at a larger scale for other public or private projects.

Key Words : Database ; Transcriptomics ; metabolomics ; proteomics ; agronomics ; phenomics

NEW TECHNICAL AND METHODOLOGICAL DEVELOPMENTS FOR SUNFLOWER FIELD PHENOTYPING

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ABSTRACT

INRA is co-developping a set of tools for sunflower phenotyping from leaf to plot level using sensors and imagery based techniques. At the plant level, Mobi-Leaf is a smartphone based application co-developed with Terres Inovia and designed to estimate individual leaf area using the built-in imaging capabilities of the device while collecting the relevant metadata. At plot level, PIETON® is a system designed to measure light transmission using LED based sensors mounted on a stick for simultaneous acquisition of light over and under the canopy to estimate LAI. At the field level, the PHENOME project aims to develop a UAV system for crop phenotyping. The vector is a hexacopter with a payload of 800g. Two sensors combination are available, 1) a high resolution RGB camera, ii) a specially developed 6 band VIS-NIR multispectral camera that can be combined with a thermal infra-red FLIR Tau camera. On sunflower, the first development targeted plant and flower counting and we will now focus on LAI and water stress tolerance using the multispectral camera and thermal IR camera. To overcome the size and weight limitations of UAV, the Phenomobile is an automatic ground vector under development with a 10 m long arm designed to carry a wider range of sensors such as Lidar and spectroradiometers over the canopy. This system, which has active lighting capabilities, will be able to collect data for detailed organ level structure and optical properties and thus complement the canopy approach of the UAV system.

Key Words : high throughput phenotyping, sensor, UAV, image analysis

DIVERSIFICATION OF SUNFLOWER GERMPLASM FOR DIFFERENT IMPORTANT CHARACTERISTICS

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ABSTRACT

Sunflower is a very important crop in the world agriculture. Taking into consideration the high seed yield and oil yield, thanks to the extension of sunflower cultivated varieties and hybrids having a high oil content, this crop has a good place in the hierarchy of dominant crops over the world. Sunflower wild species are the most rich and varied source of favorable genes for the important characteristics of cultivated species. Sunflower interspecific hybrids are very important in breeding, thanks to a very good genetic variability. As the result of our research work, we have obtained many interspecific populations, after crossing sunflower wild species with*Helianthus annuus* cultivated varieties. There have been studied different characteristics, in two years of experiments for the wild species, for the cultivated varieties and for the interspecific populations. Observations regarding flowering duration and vegetation period were recorded. There have been analyzed different morphological characteristics (plant height, number of leaves, petiole length, head diameter, seed wide, seed length, and number of branch) as well as other characteristics, including the seed oil content. Testing resistance to the pathogen *Plasmopara halstedii* and the parasite *Orobanche cumana,* we found that, after 5 generations of selfpollination, some hybrid populations have a good resistance to the pathogen and to the parasite. The obtained data has shown that in the most cases, the differences referring to the cultivated sunflower are statistically significant. Similar results were obtained with the hybrid populations for all analyzed characteristics.

Key Words : sunflower, genetic resources, wild species, analyzed characteristics

CURRENT STATUS OF SUNFLOWER CROP MANAGEMENT IN MOLDOVA

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ABSTRACT

Broomrape (*Orobanche cumana* Wallr.) is one of the main risk factors in sunflower production, causing significant quality and quantity crop damages. Extension of sunflowers land and its irrational exploitation contributes to the increasing of frequency and intensity of pathogen attack. This work presents the results of integrative study of sunflower farm fields from different geographical areas of Moldova, with the focus on cultivated hybrids of *Helianthus annuus* L., crop rotation and the frequency and intensity of the broomrape attack in natural conditions. Observation and sociological survey of sunflower growers were used as study methods. The investigations were conducted in the period of July-August, 2014, in 80 locations from center, south and north of Moldova. It was found that *Orobanche cumana* Wallr. is preferentially widespread in the central and southern part of country, frequency and intensity of the broomrapes attack, also, being higher in these regions. Around eleven hybrids, especially belonging from Pioneer Seed, Saaten Union and Syngenta companies, were cultivated on analysed fields. One from these hybrids, was found to be resistant (ARENA PR), three tolerant (SY SUBTYL, PARAISO 102 CL and P63 LE10) and others were susceptible and high susceptible to broomrape infection. It was established that efficient and programmed culture of sunflower in a well-organized rotation (using maize and wheat as a proceeding crops, with a return of sunflower to the same field at least after a period of 4 years) decreases the number of plants affected by *Orobanche*.

Key Words : Helianthus annuus L., broomrape, Orobanche cumana Wallr., crop rotation, hybrids

EFFECT OF GIBBERELLIC ACID ON POLLEN DEVELOPMENT IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Differentiation of meiotic cells requires temporally coordinated interactions with anther wall layers and any their defects result in aborted microgametogenesis. Light and electron microscopy investigations highlighted the crucial role of the tapetum in the pollen development. In sunflower degeneration of the tapetal cells is considered to be the result of programmed cell death and aborted microspores may be cause of poor nutrition or defects in pollen coatings secreted by these cells. However, there are many aspects such as signal triggers of cell death, checkpoint factors, signal transduction ways that has not been described in detail and which may provide a clearer picture of tapetum function. More recently, an intensive progress of meiosis and pollen viability studies are associated with use of interspecific hybridization in sunflower breeding. The divergence and heterogeneity of the genus cause difficulties, such as cross incompatibility, high percentage of meiotic abnormalities, resulting in sterility or reduced fertility of interspecific hybrids. These particularities have advantage in gametocides identifying for male sterility induction to achieve hybrid seeds (F1) or model systems for fundamental aspects of pollen development research. The most effective for induction of male sterility is considered to be GA3, but, is known that various genotypes respond differently to the GA3 treatments. Obtained date demonstrated alteration of cellular organization during microspore development in GA3 induced male sterility. Such investigations furnish useful information about the tissues most sensitive to gametocide and contribute as complimentary approaches in highlighting the role of gibberellins in transcriptional regulatory network for anther development.

Key Words : gibberellic acid, Helianthus annuus L., microgametogenesis, GA3 induced male sterility

GENETIC VARIABILITY OF BROOMRAPE POPULATIONS FROM REPUBLIC OF MOLDOVA

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ABSTRACT

Sunflower broomrape (*Orobanche cumana* Wallr.) is one of the most devastating sunflower pathogens in Eastern Europe and Mediterranean region, which annually causes significant yield losses and negative influence oil quality. Virulence of pathogen directly depends on physiological race. Actually, eight races (A-H) of broomrape are described. Knowledge regarding pathogen race could help farmers and breeders to make correct choice for cultivation and breeding of sunflower varieties. Thus, the aim of this study was determination of genetic variability, race and geographical distribution of different broomrape populations from Republic of Moldova. Forty one broomrape populations from Republic of Moldova and one of each from Romania, Ukraine and Spain were investigated. For evaluation of genetic variability were screened 15 SSR primers (Ocum-52, -59, -70, -74, -75, -81, -87, -108, -122, -141, -160, -174, -196, -197 and -206), which are reported previously as the most polymorphic. PCR results were visualized in 8 % polyacrylamide gels. SSR analysis demonstrated different level of polymorphism for investigated populations. The highest polymorphism level was detected for three primer pairs: Ocum-197, -160 and -59. Correlation of obtained data with information about the racial status of populations and their geographical distribution will contribute to identification of specific markers for physiological races of pathogen and will reveal phylogenetic position of populations and their spread.

Key Words : broomrape, physiological race, geografical distribution, genetic variability

MICROSPORE CULTURE RESPONSE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS

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ABSTRACT

Androgenic methods are used for the production of haploid plants such as anther and microspore culture. The microspore culture technique plays an important role for efficient production of haploid plants and genetic potential of cultivars is very important for being successful in this method. In this research, different hybrid sunflower cultivars obtained from Trakya Agricultural Research Institute have been selected and their responses to isolated microspore culture were evaluated. Meanwhile the effects of different plant growth regulators and media on androgenic microspore culture were studied in selected cultivars. Effects of microspore developmental stages and sterilization methods on isolation of uninucleate, pure and viable microspores and their culture were examined with the aim of optimizing culture conditions. Capitulum containing anthers were collected when the microspores were at the late uninucleate stage from the field. The florets containing uninucleate microspore were detached from the sterilized capitulum were blended with a blender in 30 ml of cold microspore isolation solution containing 13% sucrose at pH 6 and transfer to modified NLN- medium. After the last centrifugation the microspore density in the pellet was determined. Further studies with isolation method, different media compositions and culture conditions will be necessary in order to develop an efficient microspore isolation and culture technique in sunflower. This research has been supported by TUBITAK KBAG (Project No: 214O274).

Key Words : Sunflower, microspore culture, haploid.

GENOTOXIC EFFECTS OF IN VITRO TISSUE CULTURE CONDITIONS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

In vitro culture of plant cells is useful tool to study the adaptive mechanisms of plants living in adverse environments. In view of these facts, the objective of this work was to understand the genotoxic effects of plant growth regulators and the other parameters in sunflower callus tissues. Hybrid *Helianthus annuus* L. genotypes were grown at field and used as plant material in this study. The capitulums were collected when the microspores were at uninucleate stage for anther culture. Plating density was around 20 anthers per control and media supplemented with plant growth regulators petri dishes. Plated anthers were pretreated with cold for 24, 48 or 72 hours at 4°C and heat for 0, 2, 4, 8 or 12 days at 35 0C in the dark. Obtained calli from the control and supplemented with different plant growth regulators media (0,5 mg/l IAA+0,5 mg/l BAP; 0,5 mg/l NAA+0,5 mg/l BAP; 0,5 mg/l 2,4D+ 0,5 mg/l BAP) were used as a material to detect the DNA damage levels by Comet assay. In our preliminary studies, the highest frequency of calli induction (95%) was obtained at 350C for 2 days on the MS medium supplemented with 0, 5 mg/l 2,4D and 0, 5 mg/l BAP. Examples of nuclei with different levels of DNA damage in the comet assay were evaluated. Preliminary screening suggest that callus tissues of sunflower could be useful for studying the genotoxic damage due to different pretreatment applications. This research has been supported by TUBITAK KBAG (Project No: 214O274).

Key Words : Sunflower, callus, DNA damage, plant growth regulators.

NEW RACE OF BROOMRAPE IN SOUTH REGION OF UKRAINE

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ABSTRACT

Nearly all plants of well-known hybrid Brio (Syngenta) which were sown in 2013 in the fields of Izmail district of Odessa region were infected with broomrape. There were 10 to 15 sprouts of parasite on each plant of hybrid. We collected seeds from this broomrape plants and infected, in controlled conditions (greenhouse) the differential lines of sunflower LC-1093 (F) and RO-1-1(G). Susceptibility of these lines was 92% and 36% with the intensity of the defeat 5.7 and 0.6 respectively. So there was appearing the new virulent race H of broomrape. The results of this research show that there is a big risk to grow hybrids of sunflower with the stability to F race of broomrape in South region of Ukraine.

Key Words : broomrape, virulent race H

TISSUE CULTURE STUDIES IN SUNFLOWER

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ABSTRACT

Unfortunately, tissue culture studies on sunflower in Turkey are not sufficient when we compare them with similar studies on Earth. Tissue culture techniques offer important approach about sunflower breeding and germplasm conservation. This report was written for unroll the scientific knowledge on this subject in our country and encourage the researchers to study on tissue culture of sunflower. According to the literature, a study which was made in the first half of the 90s on anther culture of sunflower was the first investigations in Turkey. At the same time, hypocotyl and cotyledon explants of sunflower were cultured in another research. In the second half of the 90s, developing a regeneration procedure from different type of explants and determining transformation protocols studies were made. Following these studies in the early 2000s, micropropagation protocol via direct somatic organogenesis from hypocotyl and cotyledon explants of sunflower was carried out. In the same period, shoot and then whole plant regeneration was formed from mature and immature somatic embryos. Gene transfer studies with *Agrobacterium tumefaciens* were also seen at the same period. Finally in 2010s, anther culture technique and germplasm conservation by slow growth technique were used in sunflower tissue culture. Although these researches established an important scientific knowledge about sunflower tissue culture in Turkey, this is not sufficient yet. Therefore, there is an urgent need to make more *in vitro* studies on sunflower which is an important agricultural plant for Turkey. Also, it is essential in terms of sectoral that the information obtained in this studies should be transferred to agricultural practice.

Key Words : in vitro, *Helianthus annuus*, Breeding, Germplasm Conservation

WIDE (INTERSPECIFIC AND INTERGENERIC) HYBRIDIZATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.): A TOOL FOR CREATION OF GENETIC VARIABILITY AND SELECTION OF DESIRED TRAITS

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ABSTRACT

Hybridization is a reunion between differentiated genetic materials. The domesticated sunflower (*Helianthus annuus* L.) is one of the most important oil species with a comparatively narrow genetic base. Most of the important agronomic characters of a sunflower cultivar such as yield, oil content, oil quality, response to abiotic stress, and also many of pathogen –resistance traits would benefit from wild *Helianthus* species. The strategy using wide hybridization between *H. annuus* and its wild relatives (annual and perennial sunflowers), and also some species from related genera of Compositae (Asteraceae) has proved to be successful approach for development of introgressed plants with a wide range of variability. Herewith, we describe several advanced sunflower lines developed after wide hybridization *H. annuus* x *H. mollis*, *H. annuus* x *Verbesina encelioides*, *H. annuus* x *Verbesina helianthoides*, *H. annuus* x *Tithonia rotundifolia*, and *H. annuus* x *Echinacea purpurea*. Analyses of morphological and agronomic traits that characterized the phenotype of these lines as well as some biochemical parameters are presented.

Key Words : wild species, *Helianthus*, productivity, inheritance, Compositae, cultivated sunflower, wide hybridization

AGRO-MORPHOLOGICAL DIVERSITY OF TUNISIAN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is a summer crop in Tunisia. All Tunisian sunflower seed production is sold for snack food and marketed as roasted dry fruits (glibettes) in the kiosks. The morphological variation and the systematical status of 30 accessions of sunflower collected from different geographical areas of Tunisia were assessed based on 19 qualitative and 8 quantitative traits. Turkich sunflower was used as a reference variety. The data underwent an analysis of variance and a discriminant analysis. Significant differences ($p<0.05$ and $p<0.001$) among accessions within and between origins were revealed for the majority of the qualitative and quantitative traits. The genetic diversity within accessions (Shannon and Weaver's index) was high (H'= 502) and varied according sites of collection. The major proportion of the variation was attributable to individual differences within accessions. The Accessions A22 from the Beja locality were more polymorphic and exhibited the highest genetic diversity (= 0,787). The discriminant UPGMA dendrogram performed on all measured traits showed that most accessions clustered independently to their geographical origin and the variation within the same sites was extremely important. The geographic location did not seem to be the main factor structuring the variability of the studied accessions. There proved to be high phenotypic variability in the Tunisian material for the vigor descriptors.

Key Words : *Helianthus annuus*, accessions, genetic variation, morphological traits, UPGMA

MOLECULAR STUDIES OF RESISTANCE MECHANISMS IN SUNFLOWER AGAINST *OROBANCHE CUMANA* WALLR.

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ABSTRACT

Sunflower is one of the most important annual oilseed crops in the world. Sunflower broomrape *(Orobanche cumana* Wallr.) is a holoparasitic plant and infects sunflower roots. This parasitic infection is a crucial problem in all countries, but it is mainly observed in south Europe and Turkey. In order to decrease the infection problem, chemical and biological treatments were used in the past. However, herbicide utilization is not economical and causes health problems in humans whereas biological control treatments do not give trustable results. Therefore, molecular studies are necessary to understand the resistance mechanism of sunflower against *O. cumana* for a long-term solution to this parasitic plant problem. *Orobanche* spp. (broomrapes) cause significant damage by depriving organic nutrients, minerals and water from the host through a special organ called haustorium. Some monogenically and dominantly inherited resistance genes are identified as *Or1, Or2, Or3, Or4* and *Or5* that are controlled by a root-specific promoter as expected. In order to hinder the parasitic infestation, at least two independent resistance components were suggested to be activated simultaneously in sunflower roots. Here, we present recent molecular studies of resistance mechanisms in sunflower against *Orobanche cumana* genotypes.

Keywords: *Orobanche cumana*, resistance, sunflower, molecular studies

SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA* WALLR.)

Orobanche (*Phelipanche*) spp. (broomrapes) are pathogenic agents attacking sunflower with parasitic angiosperms, and they are a significant threat to sunflower crops worldwide (Parker, 2013). Diploid (2n) *O. cumana* species have chromosome number of 38 and an estimated genome size of 1.42 Gb (Piednoël et al., 2012). Unfortunately, the complete transcriptome and genome sequences are not available for this species (Molinero-Ruiz 2015). In the Parasitic Plant Genome Project website, only Expressed Sequence Tags (ESTs) from *Phelipanche aegyptiaca* are publicly accessible (PPGP, 2015).

There are several species of *Orobanche* that cause major economic losses in the world; e.g. *P. aegyptiaca* (Pers.) Pomel, *O. crenata* Forsk, *P. ramosa* (L.) Pomel, *O. minor* Sm., *O. cernua* L. (Parker, 2013). *O. cumana* limits the range of sunflower species in the wild and sunflower is parasitized by *O. cumana* crop. These species are found in a wide range in the wild, and they damage sunflower species economically (Kreutz, 1995, Parker, 2013). While the resistance of host range in other parasitic systems is non-complete, horizontal and regulated by many genes, sunflower resistance to broomrape is vertical, race specific and regulated by a few

single dominant genes (Pérez-Vich et al., 2013). Vertical resistance means the genes are mainly controlled by the gene-for-gene interactions between the host resistance genes and related parasite avirulance genes (Flor, 1971). Generally, these interactions are defined by physical characters of parasite and dominant avirulance genes that are regulated by dominant resistance genes (Fernández-Martínez et al., 2015). Kaya (2014) reported a total of eight races (namely, A to H) of *O. cumana*. However, terminology of race identification is confusing. Because, the same race classification is used without corresponding other studies in different geographical areas. Velasco et al. (2016) reported that there were some marked differences between the classified populations of F from Romania and Spain about virulence against in a set of differential lines. Sunflower resistance against broomrape is reported as a trait controlled by a few single dominant genes. This resistance was reported against the races of E (Sukno et al., 1999; Vranceanu et al., 1980; Lu et al., 2000; Ish-Shalom-Gordon et al., 1993; PérezVich et al., 2004), G (Velasco et al., 2012) and F (Pacureanu-Joita et al., 2004; Pérez-Vich et al., 2002).

In the parasitizing process, while parasite cells move towards host vessels, a specialized endophytic organ called haustorium is formed in the development of vascular connections (Hibberd&Jeschke, 2001). The haustorium is used for water or nutrient (nitrogen compounds and carbohyrates) uptake. While host factors for the haustorium development (haustorium-inducing factors or HIF) are determined in some *Orobanchaceae* species, those factors are not characterized in *Phelipanche* and *Orobanche* species (Yang et al., 2015). Studies on developing molecular markers related with avirulence genes in *O. cumana* plants are advancing. To provide accurate information about race classification of *O. cumana* populations and individual tolerant plants, markers developed from avirulence genes will be used as extremely impressive tools even though there are difficulties in studying the gene-for-gene interactions between sunflower host genes and parasite genes of *O. cumana*. Molecular marker studies concerning avirulance genes involve the development of new lines, crossing between them and selfing segregated broomrape populations (Velasco et al., 2016). Further studies on genetic diversity of *O. cumana* and its interaction with different sunflower populations will give breakthrough for the understanding of resistance mechanism of *O. cumana* in sunflower diversity (Pineda-Martos et al., 2013). The gene-for-gene interaction of *O. cumana*-sunflower was reported by Pineda-Martos et al. (2013). They showed that *O. cumana* race E avirulence in the presence of resistance gene *Or5* was inherited as a single dominant gene. Additionally, in gene-for-gene relationships between dominant resistance genes for *Or5* gene in sunflower, resistance to broomrape E but not to race F, avirulance is determined in presence of presence of the dominant *Or5* gene by avirulence gene Avr_{Or5}.

Unfortunately, genetic diversity of *O. cumana* has not been studied on global scale. In a recent study conducted by Pineda-Martos et al. (2013), two separate gene pools of *O. cumana* were identified in either south or central Spain according to their distribution as weedy forms in sunflower crops instead of distribution in the wild. The two gene pools included very low internal variability, and they were genetically very distant from each other. This phenomenon is known as the "founder effect", where two genetically distant gene pools are introduced simultaneously in two separate emergence events. However, this interpretation depends on low diversity (Pineda-Martos et al., 2013). Gene transfer was found to continue between *O. cumana* populations parasitizing sunflower populations in the wild as well as in the agricultural fields (Pineda-Martos et al., 2014). In any case, gene transfer from parasitizing wild plants to weedy populations may suggest additional mechanisms. That may create genetic diversity for latter populations. A large genetic diversity in *O. cumana* populations may contribute to the mechanisms that cope with the resistance barriers. Gene transfer in the opposite direction (from weedy populations to parasitizing wild plants) shows ecological significance because the

potential movement of new alleles of wild *O. cumana* virulence may be seen under selection pressure of agricultural fields (Velasco et al., 2016).

Inheritance of resistance to broomrape race F in sunflower line K-96 was studied and quantitative trait loci (QTL) of potential value for marker assisted pyramiding of resistance genes were characterized (Akhtouch, 2016). To search inheritance of broomrape resistance, susceptible line P-21 and the line P-96 were analyzed with oligogenic recessive resistance. K-96 and P-96 were found to have a minor effect on broomrape resistance. Therefore, they could be suitable donors in marker-assisted sunflower breeding programs against broomrape resistance. Broad transgressive segregation is observed in crosses with P-96 for susceptibility in F2 generation. Molecular analyses of both lines have identified different resistance alleles in each line. Crosses with P-21 proposed that the resistance trait(s) could be controlled by dominant-recessive epistasis at two loci. Five QTLs of linkage groups of 2, 3, 4, 5 and 6 control the broomrape resistance traits in susceptible line P-21 (Akhtouch, 2016). Additionally, it is found that the linkage groups (LG) of 4 and 5 control the plant height. This suggests a pleiotropic effect of plant height in broomrape resistance (Akhtouch, 2016).

STRATEGIES FOR SUSTAINABLE RESISTANCE

The use of reduced number of sunflower sources of resistance to broomrape, which are dominant and monogenic, increasingly contribute to the continuous development of resistance. Huge efforts are devoted by companies to create commercial hybrids with new resistance genes that are identified so far. Unfortunately, broomrape is distributed to new areas that were not infected by the virus before, such as Tunisia (Amri et al., 2012), France (Jestin et al., 2014), and northern Spain (Pineda-Martos et al., 2013). Very virulent populations of the races G and H are prevalent in several areas (Antonova, 2014; Pacureanu, 2014). Their use in strategy of broomrape control for genetic resistance will support the development of new races even though most of the broomrape populations are controlled as vertical resistance sources. In sustainable genetic control of broomrape, major genes controlling different resistance mechanisms are needed to take into consideration. Molecular markers can be developed with major genes to sustain the effects of resistance.

Vrânceanu et al. (1980) in Romania identified a set of differential lines having resistance to five successive broomrape races A, B, C, D, and E. They are controlled by the dominant genes of *Or1*, *Or2*, *Or3*, *Or4* and *Or5*, respectively. Molecular studies carried on resistance to race E indicated that its resistance is controlled by the major dominant *Or5* gene. *Or5* gene is probably localized in the telomeric region. and is related with linkage group 3 (LG3) of the sunflower genetic map (Lu et al., 1999; Tang et al., 2003; Pérez-Vich et al., 2004; Márquez-Lema et al., 2008). Regarding to this knowledge, four QTLs associated with the number of broomrape shoots per plant were determined (Pérez-Vich et al., 2004). Imerovski et al. (2013) indicated that resistance genes *Or2*, *Or4* and *Or6* are strongly associated with simple sequence repeat (SSR) markers of LG3. Regardless of *O. cumana* race populations, it is possible to develop resistant sunflower genotypes (Škorić & Pacureanu, 2010). A mutation of *ACETOLACTATE SYNTHASE* (*AHAS*, EC 4.1.3.18,) gene in maize (*Zea mays*, var Black Mexican Sweet) (Shaner et al., 1984) and pea (*Pisum sativum* L. var Alaska) (Ray, 1984) was shown to be used in the control of broomrape infestation. Therefore, similar strategies may also work in sunflower. Based on the introduction of different *AHAS* alleles to obtain resistance in sunflower, three different herbicidal technologies are available (Sala et al., 2012). They are Clearfield® technology based on Imisun sunflowers, Clearfield Plus® technology based on CLPlus sunflowers and and Sures sunflowers (Sala et al., 2012). The first commercial herbicide tolerance (HT) in sunflower is called as

'Imisun'. Crossing of imidazolinones-tolerant plants with cultivated sunflower lines results in IMI-tolerant populations (Al-Khatib et al., 1998). The partially dominant allele *Ahasl1-1* and the other a modifier or enhancer factor controls the inheritance of Imisun (Miller and Al-Khatib, 2002; Bruniard and Miller, 2001). The second IMI tolerance trait in sunflowers as CLPlus is regulated by partially dominant nuclear allele Ahasl1-3. Its expression is developed by seed mutagenesis and selection with imazapyr (Sala et al., 2008). The third one is Sures sunflowers. They are developed from wild sunflower populations discovered in USA (Al-Khatib et al., 1999). Forward crossing and selection with the herbicide tribenuron accesses the tolerance allele *Ahasl1-2* and result in the trait known as Sures (Miller and AlKhatib, 2004).

RPG01 is one of the sunflower lines that carry *Or5* gene conferring resistance to race E of *O. cumana*. Identification of sequence characterized amplified region (SCAR) markers regarding to broomrape resistance gene *Or5* could be a significant tool in breeding resistance against broomrape race E (Lu, 2000). Lu et al. (2000) found five SCAR markers significantly linking to the *Or5* locus. They are RTS05, which is at 5.6 cM from the *Or5* locus, RTS28, RTS40 and RTS29 that are in distance interval of about 20 cM from the resistance gene, and UBC120_660 that is the only one in opposite side of the *Or5* locus. Identification of *Or5*-linked SCAR markers in broomrape resistance studies will help selection of new resistant lines in molecular genetics of broomrape resistance genes in sunflower (Lu, 2000).

MECHANISMS OF RESISTANCE AGAINST *OROBANCHE CUMANA* WALLR.

Conducting physiology-based breeding and pyramiding resistance genes underlying different resistance mechanisms will support to obtain information on the physiological basis of the different resistance sources on broomrape resistance (Pérez-Vich et al., 2013). Importance of the pH in root system for broomrape resistance is indicated in early studies of sunflower. Rihter (1924) mentioned that susceptibility to this parasite is promoted by low pH values in the roots of the host plant. On the other hand, Lozano-Cabello et al. (1999) notified that basic soils with pH 8.14 gave lower number of broomrape stems per sunflower plant compared to acid soils with pH 6.17 in Spain.

Plant defensins are basic peptides around 5-10 kDa and show antifungal activity (De-Zelicourt et al. 2007). After attachment and before necrosis of *O. cumana*, it is shown that roots of resistant sunflower genotype (LR1; *Helianthus annuus* x *Helianthus debilis debilis*) encodes a putative defensin by the gene *HaDef1* (Letousey et al. 2007). It supports that defensins could play significant roles in the resistance of sunflower genotype LR1 by leading to parasite death (De-Zelicourt et al. 2007). De-Zelicourt et al. (2007) confirmed that *HaDef1* activates cell death in *Orobanche* parasitic plants.

CONCLUSION

In summary, the parasitic broomrape (*Orobanche* spp.) is a major global issue that acts as a huge risk in sunflower production in Southern and Eastern European countries every year, causing 50% losses in the yield (Molinero-Ruiz 2015). In broomrape resistance of sunflower, next generation sequencing technologies, metabolomics and their applications on parasitic weeds will be necessary to exploit. Application of different bioinformatics approaches with known *O. cumana* target genes obtained from model plants of sunflower will lead to a rapid and effective characterization of broomrape (*Orobanche* spp.) resistance mechanisms in sunflower. Although there are several sunflower genes characterized in resistance against broomrape (*Orobanche* spp.), molecular basis of resistance has not been completely comprehended. Broomrape resistance in sunflower can be managed by the use of molecular techniques such as marker-

assisted selection, QTL identification and associating mapping. Additionally, the application of different molecular methods such as transcriptomics will help the identification of new sunflower broomrape resistance genes. Therefore, the use of molecular markers, RFLP, SSR, QTL, RAPD, TRAOP, and physiological markers will be the most reliable and the most easily applied methods to screen sunflower breeding materials for broomrape resistance.

LITERATURE

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THE RESISTANCE OF ADVANCED HIGH OLEIC RESTORER LINES AND THE EVALUATION OF THEIR HYBRDS' YIELD TRAITS

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ABSTRACT

Downy mildew is the most serious disease in sunflower production in Turkey. To determine the adaptation and combining abilities of inbred lines is key issue in sunflower hybrid breeding. F8 and F9 stage restorer lines having high oleic acid content crossed with 9661-A (CMS) female line used as female tester for general combination ability were used in the study. These restorer lines developed in National Sunflower Hybrid Breeding Project conducted by Trakya Agricultural Research Institute (TARI) were selected as resistant to the broomrape parasite *(Orobanche cumana)* which is the major problem in most of sunflower growing areas in Turkey among other inbred lines in TARI. Mildew tests were conducted in artificial inoculation collected from infected plants as a mixture at various locations across Thrace region in 2014. In downy mildew tests, plant samples having a blend of all the mildew races were dried under shadow as 24-48 hours then they were preserved in the cooler at -80 Cº. Based on downy mildew test results, the hybrids with F8-R SN: 1, 2, 7, 8, 17 and F9-R SN: 9, 10, 12, 13 restorer lines were found fully resistant and others were found mid resistant. To determine their performances of these 18 hybrids, yield trial was conducted as randomized complete blocks design with 3 reps in 4 rows and with three commercial controls at Edirne location in 2015. For seed yield, the number of 5, 15 and 12 inbred line hybrids exhibited higher performances than commercial checks. While the numbered of 4, 5, 12, 15 and 16 line hybrids were found as resistant to downy mildew whereas other hybrids were found as mid resistant. As a result, high oleic, resistant to downy mildew and broomrape restorer lines and their hybrids and also exhibited higher yield and quality performance ones were determined. If they keeps higher performances in this year, they will send registration trials and the same testers will be used for developing sunflower hybrids for downy mildew and high oleic types.

Key Words : Sunflower, High Oleic, Downy Mildew Resistant, Tester, Restorer Line