PRODUCTION, STUDY AND UTILIZATION OF SUNFLOWER INTERSPECIFIC HYBRIDS.

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Summary:

Results of this research confirmed the possibility of interspecific hybridization within *Helianthus* genus. between cultivated sunflower (*H. annuus*) and most of perennial wild species (except *H. ciliaris*) regardless of their relationship or genome composition. Interspecific hybrids successfully come to existence in the field without any biotechnological techniques. In the course of this work large percentage of successful progeny was ensured with cultivated sunflower as a female form. Plant materials obtained from interspecific hybridization are extremely diverse. Results of interspecific hybridization are unpredictable.

INTRODUCTION

10 years of our experiments resulted in obtaining the following types of interspecific hybrids:

1. Perennial plants, fertile and capable of seed development, without any likeness to either male, or female forms. F1 and F2 were obtained.
2. Annual plants very similar by their phenotype to wild-growing *H. annuus*. F1, F2 and F3 were obtained.
3. Hybrids with segregation in F1 and F2 into cultivated and wild phenotypes, fertile and sterile, single-head and branching.
4. Hybrids without segregation in F2 F7, bearing likeness to cultivated sunflower lines, but having a distinctive and specific branching feature. It seems more proper to call these hybrids introgressive lines. They bear genes of self-fertility, genes of pollen fertility restoration, of resistance to downy mildew and could be used for breeding industrial heterotic hybrids producing high quality oil.

Wild-growing sunflower species are sources of CMS, pollen fertility restorer genes, resistance to pathogens and unfavourable factors, and possess an original composition of fatty acids (Anashchenko, Popova, 1985). In this relation, studies of crossability and production of hybrids between the cultivated sunflower and wild species are of interest to researchers.

It has been established that annual wild species cross with the cultivated sunflower easily and produce fertile progeny (Anashchenko, Popova, 1985). Perennial wild species cross with the cultivated sunflower much less easily than the annual ones.

MATERIALS AND METHODS

Most North American perennial species are represented in the VIR's collection and are maintained at the quarantine nursery of the Kuban Experiment Station in the Krasnodar Territory. Crosses and analysis of the obtained hybrids were made in 1990 through 1999. As female forms, CMS lines of the cultivated sunflower VIR 114, VIR 151 and HA 232 were used. Twenty-two perennial wild species were used as male forms (in accordance with the Heiser nomenclature. From one to three populations of each species were involved in the crosses. Interspecific hybrids F2, F3, F4, F5, F6 and F7 were obtained through individual self-pollination of F1, F2, F3, F4, F5 and F6 plants, respectively. Twenty-two interspecific F2 hybrids harvested in 1992 have been studied for their chemical composition. Protein content was determined by Kjeldal, that of oil by the defatted residue, and fatty acids by GLC. Resistance to downy mildew has been evaluated in laboratory conditions at VNIIMK (Krasnodar, 1992) and at the Veidelevsky Institute (Belgorodsky Region, 1999). In Krasnodar, the non-segregating F2 hybrids have been evaluated for resistance to downy mildew races 1 and 2 according to Panchenko (1975) against cv. Peredovik which is resistant to both races. The same material has been studied biochemically.
In Veidelevka, the same interspecific hybrids have been studied, only after 7 generations of inbreeding. The race 6 of pathogen was determined by using the method proposed by Gulya et al. (1991) application of which showed 100% susceptibility of the lines RHA265, DM2 and cv. Voskhod that is susceptible to all downy mildew races, as well as resistance of the lines HA335, HAR4, HAR5, 803-1 and RHA274.

RESULTS AND DISCUSSION

Nineteen out of 22 perennial species hybridized with the cultivated sunflower regardless of the chromosome number. The attempt to obtain hybrids between *H. annuus* and genome C species from the *Ciliares* section failed. Crosses were more successful, in comparison to reciprocal combinations, when CMS lines of the cultivated sunflower were used as female forms.
The hybrids of HA89 x *H. strumosus* were perennial and had a phenotype intermediate between the parental forms; they did not look like an annual wild species, but reminded more a perennial one. Hybrids with a similar phenotype were produced by Georgieva-Todorova (1984) from crosses of *H. annuus* and *H. resinosus*.

The majority of our F1 hybrids were annual, morphologically reminded the cultivated sunflower or the annual wild one, and did not look like the female CMS line. The hybrids revealed features inherited from the wild parent, namely strong stem pubescence, anthocyan colouring of the stem, petioles, tubular flowers and stigma lobes, as well as all types of branching, i.e. lower, upper and along the whole stem; plants with the 1st and the 2nd branching orders have been described. As it follows, the plants obtained through crosses are not the apomictic progeny of the female line, which does not possess all of the above characters. Besides, the female line possessed CMS, while in most cases with hybrids the restoration of pollen fertility was observed. By all morphological characters, as well as by plant height, vegetative period and fertility, segregation in F1 was observed in some cross combinations, and was not in others. Notable is that no regularities in presence/absence of segregation could be noted (Gavrilova et al. 1994). When analyzing the following F2, F3 and F4 generations, progenies of some combinations showed segregation by all the characters in question, while progenies of other combinations did not segregate.

No segregation has been recorded in F2 through F7 of the hybrids of *H. annuus* x *H. floridanus* and *H. annuus* x *H. angustifolius*. Segregation occurred in progenies of some hybrid plants from the interspecific crosses of *H. annuus* x *H. giganteus*, *H. annuus* x *H. californicus*, *H. annuus* x *H. occidentalis*, *H. annuus* x *H. maximiliani*, *H. annuus* x *H. rigidus*, *H. annuus* x *H. tuberosus*, and did not occur in progenies of other hybrid plants. F5, F6 and F7 have been produced only for the cross combinations that did not segregate in F4. Notable is that plants of the non-segregating hybrids from different combinations had morphologically the same appearance (Gavrilova, 1998; Gavrilova et al. 1997).

In progeny of plants resulting from free pollination and grown encircled by the cultivated sunflower, segregation into single-head and branching plants was observed. Recurrent crosses with a CMS line produced single-head, tall (180-190 cm), uniform hybrid plants with large heads about 25 cm in diameter. The weight of 1000 hybrid seeds varied from 31 to 80 g. Hybrids from the combinations of *H. annuus* x (*H. californicus* x VIR 227), F2 *H. annuus* x *H. californicus*, HA 232 x (*H. annuus* x *H. giganteus* p1) had sufficiently large seed (1000 seed weight of 68 to 80 g). The percentage of husk in the studied hybrids varied from 23% to 43%. In 6 hybrids the percentage of husk was less than 30 %, for instance in HA232 x (*H. annuus* x *H. californicus* p3), F2 (*H. annuus* x *H. californicus*), F2 (*H. annuus* x *H. maximiliani*). In seed size and huskness, hybrids ranked below cv. Peredovik (Table 1).

The content of oil and protein fluctuated in hybrids. Oil content ranged from 48 % to 56.3 %, and on the average this quality index was by 1.9 % higher for hybrids than for the standard. Oil percentage in the kernel was over 50 % in hybrids, with exception for 2 combinations, and

in 4 hybrids was 55 % and above, that is in F2 *H. annuus* x *H. giganteus* p2, *H. annuus* x *H. giganteus* p1, *H. annuus* x *H. californicus* p1, and Fb HA232 x (*H. annuus* x *H. giganteus*). Protein content in the kernel varied from 13.1 % to 21.3 %. The highest protein content of above 20 % has been recorded for hybrids from the following combinations: Fb HA232 x (*H. annuus* x *H. giganteus* p1), *H. annuus* x (*H. californicus* p2 x VIR227) and F2 (*H. annuus* x *H. californicus*).

The content of fatty acids in oil varied as follows: palmitic (C 16:0) from 5.3 . % to 8.5 %, stearic (C 18:0) from 3.5% to 6.1 %, oleic (C 18:1) from 27.9 % to 44.7 %, linoleic (C 18:2) from 43.3 % to 60.2 %. The concentration of oleic acid in the oil from hybrids was on the average no lower than in that from the standard cultivar. Two hybrids, F2 (*H. annuus* x *H. californicus* p1) and Fb HA232 (*H. annuus* x *H. giganteus* p1) should be mentioned for the highest percentage of oleic acid, 44.7 % and 41.9 %, respectively. Also, for food purposes they had a nearly optimal ratio of oleic and linoleic acids (Table 1). In the 4 hybrids with oil content over 55 %, the oleic acid concentration varied from 27.9 % to 33.6 %.

Table 1. Chemical composition of seed from sunflower interspecific hybrids.

Combination	**1000 seed weight, g**	**Husk, %**	**Protein (N x 5.5), %**	**Oil, %**	**Fatty acids, % from the total**			
					C 16:0	**C 18:0**	**C 18:1**	**C 18:2**
H. annuus x H. giganteus p3	46	39	17.6	53.9	7.7	4.0	32.3	56.0
H. annuus x H. giganteus p1	43	34	15.6	56.0	7.3	4.0	32.7	56.0
HA 232 x (*H. annuus x H. giganteus*)	34	40	15.3	55.2	7.6	3.7	29.3	59.4
H. annuus x H. californicus p4	50	33	16.5	54.3	6.3	4.5	36.9	52.3
H. annuus x H. giganteus p2	39	35	14.8	56.3	7.5	3.5	33.6	55.4
HA232 x (*H. annuus x H. giganteus* p1)	68	37	20.0	51.2	6.6	4.6	41.9	46.9
H. annuus x H. giganteus p3	36	32	17.0	53.8	7.1	3.8	34.7	54.4
H. annuus x H. angustifolius	41	30	17.6	53.8	8.5	4.5	32.5	54.5
H. annuus x H. californicus p1	59	39	18.3	53.0	6.1	5.9	44.7	43.3
H. annuus x (*H. californicus* p2 x VIR 227)	80	29	21.3	49.7	5.3	5.4	37.2	52.1
H. annuus x H. californicus p3	47	34	17.4	53.1	6.0	5.0	39.7	49.3
HA 232 x (*H. annuus x H. californicus* p3)	60	27	17.8	52.8	6.5	5.0	39.8	48.7
H. annuus x H. maximiliani	36	28	16.9	53.8	6.6	4.4	38.2	50.8
H. annuus x H. californicus p1	40	27	13.9	55.5	7.1	3.8	30.4	58.7
H. annuus x H. californicus p2	37	30	14.5	54.3	7.6	4.3	33.6	54.5
H. annuus x H. californicus p2	31	29	14.0	54.2	7.4	4.2	32.9	55.5
H. annuus x H. californicus p1	34	30	13.1	54.6	7.7	4.3	28.9	59.1
H. annuus x H. occidentalis	58	33	19.6	48.0	5.8	6.1	35.7	52.4
H. annuus x H. californicus	78	34	20.9	49.2	6.7	4.9	34.1	54.3
Average for all hybrids	47	33	16.8	53.5	7.0	4.4	34.6	54.0
cv. Peredovik	99	23	19.3	51.6	6.2	4.6	35.5	54.3

Out of 12 non-segregating F7 inbred progenies, the hybrid combinations VIR 151 x *H. angustifolius*, HA232 x *H. angustifolius* showed no signs of affection with race 1, 2 and 6 of downy mildew, HA232 x *H. lactiflorus* was resistant to race 6 (Table 2). In some hybrid combinations (e.g. *H. annuus* x *H. giganteus* and *H. annuus* x *H. californicus*) significant variation has been noted for oil content, fatty acid composition and resistance to downy mildew (Tables 1 & 2). This fact points to plant heterogeneity within the wild species population.

Table 2. Crossability of cultivated sunflower with wild perennial species and evaluation of resistance to downy mildew in the obtained interspecific hybrids.

Crossing combination	F0 seeds, pcs.	F1 plants, pcs.	Last progeny	Degree of susceptibility with downy mildew, %	
				race 1 & 2	race 6
H. annuus x *H. angustifolius*	18	5	F8	0	0
H. annuus x *H. californicus*	19	4	F8	0	33
H. californicus x *H. annuus*	54	6	F4	-	0
H. annuus x *H. decapetalis*	60	1	-	-	-
H. decapetalis x *H. annuus*	75	3	F2	--	-
H. annuus x *H. floridanus*	6	2	F8	-	-
H. floridanus x *H. annuus*	55	4	F2	-	-
H. annuus x *H. giganteus*	60	15	F8	P1 0 P5 100 P6 33	100 100 100
H. annuus x *H. grosseserratus*	20	3	F3	-	88
H. grosseserratus x *H. annuus*	25	0	0	-	-
H. annuus x *H. lactiflorus*	17	10	F8	-	0
H. annuus x *H. maximiliani*	45	13	F8	50	100
H. maximiliani x *H. annuus*	37	2 *	F1	-	-
H.microcephalus x *H. annuus*	15	3	F2	-	-
H. annuus x *H. mollis*	2	2	F8	-	88
H. mollis x *H. annuus*	6	1	F2	-	-
H. annuus x *H. nuttalli*	42	10	F2	-	-
H. nuttulli x *H. annuus*	12	0	0	-	-
H. annuus x *H. occidentalis*	22	3	F7	50	100
H. annuus x *H. rigidus*	3	1	F8	-	33
H. rigidus x *H. annuus*	2	1	F2	-	-
H. annuus x *H. strumosus*	14 30	7 (4 + 2 *)	F8 F1	- -	65 -
H. strumosus x *H. annuus*	15	2 *	F1	-	-
H. annuus x *H. tomentosus*	31	5	F8	-	-

* perennial

The present research resulted in obtaining new interspecific hybrids that meet requirements to the quality of oil and are resistant to downy mildew. Besides, these hybrid combinations bear genes of pollen fertility restoration, are uniform by all morphological characters, they are branching, and may be used in breeding male forms for the production of heterotic hybrids.

There exist the notions of "false hybrids" and "introgressive hybrids" of sunflower (Pustovoit, 1975) which apparently denoted this unclear phenomenon – the absence of segregation in remote crosses within gen. *Helianthus*. The analysis of hybrids from the recurrent cross of *H. annuus* x (*H. annuus* x *H. mollis*) using RAPD markers has revealed 1:1 segregation by the character presence/absence of the perennial wild species specific marker (Faure et al. 1999).

When analyzing the storage protein helianthinine in such interspecific hybrids, Anisimova (1998) established the presence of specific markers of the cultivated sunflower and markers of helianthinine which is characteristic of the annual wild species genome. It is probable that during interspecific hybridization of the cultivated sunflower and wild perennial species inclusion of separate parts of the wild species genome into that of the cultivated sunflower and formation of introgressive material take place in F1 meiosis. In our case, we can speak of introgressive lines, since progeny from self-pollination in F8 has been obtained. Further research should reveal genetic constitution of these lines, however, this material can be used in breeding process right now.

Results of interspecific hybridization are unpredictable. When crossing the cultivated sunflower with wild perennial species, in F1 it is possible to observe variation that would range from the cultivated to the wild perennial type with a multitude of intermediate forms that bear likeness to wild annual species. Results of these tests are reproducible. In field conditions, we have obtained hybrids similar to those produced in Bulgaria by Georgieva-Todorova (1984), Christov (1996) and others.

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