INVESTIGATION OF DISEASE RESISTANCE AND OLEIC ACID CONTENT IN A NEW STOCK OF HIGH OLEIC SUNFLOWER

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Abstract

Only one high-oleic sunflower hybrid is registered in the Hungarian National Catalogue. Biodiesel fuel may be the main industrial target for high oleic acid sunflower oil. It is also a healthier cooking oil and has a superior stability over other vegetable oils presently on the market. In contrast to their economic interest, the National Institute for Agricultural Quality Control registers them in the group of conventional oilseed sunflower. Under Hungarian growing conditions, grain yield performance of the high-oleic sunflower candidate varieties is inferior to that of normal hybrids because of their sensitivity to the major pathogens. Healthy, competitive high-oleic sunflower hybrid production requires breeding of parental lines showing complex disease resistance. For this purpose, high-performing new inbred lines are necessary. In this connection, the aim of our research was to obtain putative candidate lines. A new stock of high oleic sunflower was created using a set of crosses. Reaction of cross combinations to many fungal pathogens was evaluated by scoring infection in natural field conditions as well as by artificial infections. Simultaneously, their oleic acid content was also compared. Presented results show that it is possible to improve the sanitary state and high oleic content of sunflower in our variety development programme. Some of the new candidate lines exhibit a stronger resistance than any other line studied so far. These materials were shown to be suitable for use in the breeding programme aimed at the creation of new hybrids.

Introduction

Sunflower (*Helianthus annuus* L.) is the most important oil crop in Hungary and covers a total area of 500,000 hectares followed by rape with 120,000 hectares. The major part of sunflower seed oil is traditionally processed to edible oil. During the past years interest in the industrial utilisation of the sunflower oils increased worldwide (Ahloowalia et al., 2004). Recently, the European Parliament and the Council voted for a directive COM 2003/30/EC (EC, 2003) to substitute 2% of all petrol and diesel for transport with biofuels by 2005, rising to 5.75% in 2010.

Vegetable oils can be converted by methyl esterification to biodiesel for transport uses. The potential of the high oleic sunflower for biodiesel production is significant. High oleic acid content ensures low susceptibility of vegetable oils to oxidative changes during processing for human nutrition and for industrial application (Fuller et al., 1967). In connection with their oxidative stability, Baldini et al. (2003) found high-oleic sunflower hybrids ideal for biodiesel production. Up to now only one high-oleic sunflower variety was registered in the Hungarian National Catalogue.

The Hungarian sunflower growing environment creates harsh conditions for breeding competitive hybrids. Long periods of drought and very high temperatures, interspersed with rainfall create ideal conditions for fungal pathogenesis. Pressure of fungal infection has a heavy influence on the yield performance of candidate hybrids. The relative importance of each fungal species is disputable. Several fungi including *Plasmopara halstedii, Sclerotinia sclerotiorum*, *Diaporthe helianthi,* and *Macrophomina phaseolina* frequently attack sunflower. Pacureanu-Joita et al.(2000) studied yield-reducing capacity of single fungi, exclusively that of *Phomopsis/Diaporthe helianthi.* In contrast to their principle, we propose an integrated breeding programme to investigate disease resistance simultaneously with high oleic acid content.

The Cereal Research Non-Profit Company (CRNPC) breeding programme for sunflower oil fatty acid composition was started in 1985. Further development since 1996 of our programme was aimed at the selection of new healthy high-oleic female (HO) and restorer lines resistant to all important races of *Plasmopara halstedii* as well. Two restorer lines resistant to many downy mildew races having normal acid content (N) were involved in this program. The objective of this paper was to present the first results of our work demonstrating the possibility of association of high oleic acid content with good field disease resistance.

Materials and Methods

Plant Materials. Two restorer lines of normal oleic acid content (N) showed good field disease resistance and three high-oleic (HO) CMS female lines showing susceptibility to diseases were used in crosses aimed at creation of four initial source populations. These lines were previously developed by CRNPC. Crosses were carried out in 1999 and F1 plants were self-pollinated in 2000. Further selfing of selected individual plants took place in 2001, 2002, and 2003 in the pathology nursery (in monoculture) at Kiszombor, Hungary. Natural fungal infection was enhanced by irrigation. Artificial inoculation with *Plasmopara halstedii* by the method of Gulya et al. (1991) was used to retest the presence of the PL6 resistance gene.

Evaluation of Field Disease Resistance. Reaction to diseases was scored on a scale from 1 to 9 (1=worst, 9=best) three times during the vegetation period.

Measurement of Oil Content. Ten to 15g of achenes were analyzed by nuclear magnetic resonance using an Oxford 4000 NMR oil analyzer.

Fatty Acid Composition Analysis. Samples for grinding contained from 10 to 20 achenes. A small portion of the ground sample (10 to 20 mg) was transferred to a glass tube and saponified with 3 ml 2M KOH dissolved in methanol by vortexing 20 seconds. Afterwards, 3ml distilled H2O was gently added to the reaction mixture followed by extraction of nonsaponifiable materials with 3 ml petroleum ether. The clear upper layer was transferred to a glass tube and incubated for 30 minutes at 70C. Concentrated methyl esters were eluted in 500 µl hexane. One µl of this sample was analyzed on a gas chromatograph (Hewlett-Packard model 5890 Series II) equipped with flame ionization detector (FID), on-column injector and 30 m long

SUPELCOWAXtm10 capillary column (Supelco INC, Bellefonte)using nitrogen as a carrier gas. The oven temperature was programmed for 195C, the injector and the detector were heated to 220C. After 8 minutes running time, fatty acids typically occurring in sunflower oil were determined: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2). Fatty acid peaks were identified by comparing the fatty acid methyl ester peaks and retention times of standards with sample peaks. An electronic integrator was used to calculate the total area under the peaks; the area of each fatty acid peak was expressed as a percentage of the total area. The peak of interest in the present study was monounsaturated oleic acid. Analysis of variance was used to compare the mean oil content, and oleic acid content of parental lines and that of selected self-pollinated plants.

Results and Discussion

Under actual Hungarian climatic and agronomic conditions, the most promising energy crop for biodiesel production seems to be the high-oleic sunflower. Selection for resistance to an individual fungal pathogen is a difficult way to reduce the effect of fungal damage in high-oleic sunflower. It is also difficult to determine which fungal pathogens are important components of infection in various field conditions. However, selection for complex field resistance to disease may be a possible way to be followed by the breeder. This study focuses on the better selection of healthy high-oleic restorer lines for this purpose.

In our program, the initial cross was made in 1999 between three newly developed high-oleic disease-sensitive CMS female lines and two normal, relatively resistant restorer lines. Four populations were created and harvested in monoculture at our pathology nursery. Oil content and fatty acid composition of the self-pollinated plants was determined in every year. In these four combinations, 159 F4 candidate lines were analyzed to determine influence of the high oleic acid content on the complex field disease resistance. In the case of all the four populations, 27% of analyzed entries were grouped in normal oleic content class. 23.3% of the material showed intermediate values and 49.7% of F4 lines selected in similar environmental conditions pooled in high oleic class (data not presented). According to the model of Fernandez-Martinez et al. (1989), the number of selected high-oleic F4 lines indicates a high degree of dominance of stable genetic factors controlling the high oleic acid content.

Table 1 shows the average of the parameters of four high-oleic populations in comparison with the population mean, and with that of parental lines. Significant differences between oil content, oleic acid content and field disease resistance were observed. A high oleic F4 line from the F931210 population had the highest oil content and oleic acid content. These higher values might be attributable mainly to better combining ability of the normal line with the high oleic line. However, the high oleic parent may also positively influence these parameters studied. Significant differences were found to be due to the high oleic parent in the case of F741185 population.

Disease symptoms on all the four populations were noted by visual observations of plants during the 2003 cultivation period in Kiszombor. Differences in complex responses of entries to fungal pathogens were observed in the field. The F741185 and F931210 populations showed the fewest diseased plants, consequently, the highest number of plants were selected with the best potential of field disease resistance. Present results indicate that in every four combinations it was possible to obtain better field-resistant pools than in the parental high-oleic lines. An important modification of field disease resistance was observed in these four populations. Retesting incorporation of the PL6 resistance gene against *Plasmopara halstedii* was made by artificial inoculation published by Gulya et al. (1991) and confirmed the presence of gene PL6.

Table 1. Mean performance of 159 F4 lines, of the line with highest oleic acid content and that of each parent of four populations.

Population identification	Character			
	Oil content %	Oleic acid content %	Sanitary state (bonification 1-9) 1=worst; 9=best	Date of flowering (days) **
F741185 population				
Population mean	55,9	62,9	4,94	VII.04. – VII.10. (63-69)
Line with highest oleic acid content	59,7	90,9	7	VII.08.(67)
High oleic female parent (1)	53,7	89,2	5	VII.09.(68)
Normal male parent (I)	49,8	23,4	7	VII.04. (63)
$LSD_{0,05}$*	2,1	6,5	0,61	not calculated
F861189 population				
Population mean	55,1	58,4	3,83	VII.04.-VII.08. (63-67)
Line with highest oleic acid content	58,4	91,3	5	VII.08. (67)
High oleic female parent (1)	53,7	89,2	5	VII.09. (68)
Normal male parent (II)	52,5	28,3	6	VII.06. (65)
$LSD_{0,05}$*	2,4	26,0	1,33	not calculated
F901192 population				
Population mean	53,1	75,6	3,56	VII.06. (65)
Line with highest oleic acid content	54,5	91,9	4	VII.06. (65)
High oleic female parent (2)	54,9	92,1	3	VII.07 (66)
Normal male parent (II)	52,5	28,3	6	VII.06. (65)
$LSD_{0,05}$*	4,1	8,8	0,78	not calculated
F931210 population				
Population mean	55,4	66,9	5,17	VII.06.-VII.11. (65-70)
Line with highest oleic acid content	62,1	93,3	6	VII.08. (67)
High oleic female parent (3)	59,5	92,3	5	VII.10. (69)
Normal male parent (II)	52,5	28,3	6	VII.06. (65)
$LSD_{0,05}$*	2,2	12,0	0,51	not calculated

* = Indicates the least significant difference between the line with highest oleic acid content and the population mean at the 0.05 significance level.
**= Indicates the number of days from emergence to 50% flowering.

Earlier flowering and maturity of high oleic hybrids may produce an advantage in Hungarian climatic conditions and may be related to a greater productivity of future hybrids. Fernandez-Martinez et al. (1993) studied flowering of near-isogenic high and low oleic acid hybrids. In their experiment high oleic content resulted in earlier flowering. In our study the number of days to 50% flowering was observed as mainly ±2 days.

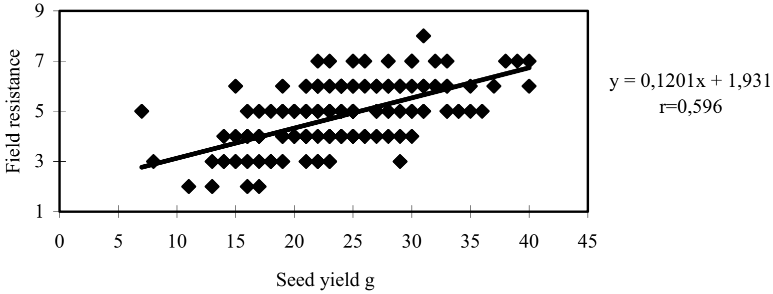

Figure 1. Correlation analysis between seed yield and complex field disease resistance.

Data of Figure 2 confirms our hypothesis that high oleic acid content may be associated with good field disease resistance. This study demonstrates no correlation between high oleic acid content and the sanitary state of candidate lines. This kind of selection would affect performance, mainly disease resistance of the candidate lines, since no correlation of oleic acid content with field disease resistance was detected.

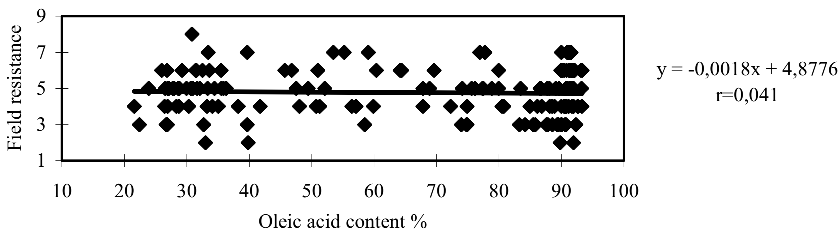

Figure 2. Correlation analysis between oleic acid content and complex field disease-resistance.

Conclusions

In summary, the present study revealed an important variability for oleic acid content and field disease resistance in four F4 sunflower populations. Data obtained in this study indicate that high oleic acid content may be associated with good field disease resistance. These results may appear in contrast to current opinion which associates high oleic acid content with highest field susceptibility to disease. It was demonstrated that it was possible to develop an effective breeding strategy to stabilize the high oleic acid content and to increase complex disease resistance at the same time. These results indicate a rational effective selection model for high oleic acid content under field pathogenic pressure. Further efforts to define relationships between high oleic content and complex field disease resistance are in progress.

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