DEVELOPMENT OF INBRED LINES OF SUNFLOWER WITH VARIOUS OIL QUALITIES

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SUMMARY

The cultivated sunflower (*Helianthus annuus*) is one of most important oil crops in the world. Although sunflower is primarily grown for extraction of oil there is a limited production of non-oilseed types used for confections or as a bird feed.

The objective of this research was the development of B- and R-lines with stable and high oleic acid content and modified tocopherol composition, with high GCA and SCA values for the two most important agronomic characters (seed yield and oil yield) and high tolerance to Phomopsis.

B-lines [Lg-21 (tph1, Ol), Lg-24 (tph1, tph2) Lg-25 (tph2, Ol)] and R-lines (near-isogenic lines for three genes, i.e., Tph1, Tph2 and Ol–VK 66) were used as donors of tph1 and tph2 recessive alleles and Ol gene, in a backcross program with lines for high GCA and SCA values and high tolerance to Phomopsis: B-lines (Ha-74, Ha-98 CMS-III-8) and R-lines (RHA-583, RHA-576, RHA-SEL, RHA-SNRF). Tocopherol composition was determined by thin-layer chromatography followed by the Emmerie-Engel reaction and densitometer quantification. The screening for oleic acid content was performed by gas liquid chromatography. The program of incorporation of tph1 and tph2 alleles was based on the backcross method for the recessive traits of sunflower seeds, which includes the selfing of backcrossed plants and simultaneous individual crossing with the recurrent line.

As a result of this investigation we made a set of new inbred lines with high or normal content of oleic acid and modified tocopherol composition which may be used for developing materials with certain levels of oleic and linoleic acids and a desirable content of alpha, beta, gamma and delta-tocopherol. As a final result, a set of specialty oils might be obtained for human consumption and industrial purposes.

KEY WORDS: sunflower, tocopherol composition, oleic acid, oil quality

INTRODUCTION

The cultivated sunflower (*Helianthus annuus*) is counted among most important oil crops in the world. Sunflower oil is in a group of plant oils of highest quality. Composition of higher fatty acids and contents of tocopherol, sterol, carotenoids and other compounds determine oil quality. Sunflower is considered a promising species for genetic modification of oil quality (*Scharp*, 1986).

Fatty acids composition and mutual ratios are basic parameters determining oil quality. Standard sunflower oil contains linoleic acid, oleic acid, palmitic acid, stearic acid, and small amounts of other higher fatty acids. Linoleic and oleic acid take 90% of the total content, 55-65% the former and 20-30% the latter (*Škoric et al*., 1994). The first source of high oleic acid was developed by *Soldatov* (1976) who treated seeds of the variety VNIMK 8931 with a 0.5% solution of dimethylsulphate (DMS). In the M_3 generation, *Soldatov* selected individual plants that contained over 50% of oleic acid which, after further selection, gave rise to the variety Pervenetz containing 80-90% oleic acid in oil. Pervenetz had been extensively used for the development of high oleic inbred lines and hybrids although genetic studies have not provided a definite answer on the mode of inheritance and the number of genes that control this characteristic. *Fick* (1984) reported that the high oleic acid content is controlled by a single gene for partial dominance (Ol), *Urie* (1985) and *Miler et al.* (1987) reported one dominant (Ol) and one recessive modifier gene (ml), while *Fernadez-Martinez et al.* (1989) reported three complementary genes (Ol_1, Ol_2, Ol_3).

Not only the content of higher fatty acids but also the composition of tocopherol may be modified by genetic manipulations. The standard sunflower oil contains about 95% of alpha-tocopherol, 3% of beta-tocopherol, 2% of gamma-tocopherol and delta-tocopherol in traces. By selfing the variety VNIIMK 8931, *Demurin* (1988) obtained an inbred line that contained 50% of alpha-tocopherol and 50% of beta-tocopherol. The high content of beta-tocopherol was controlled by a single recessive gene, tph_1. Further searching through the VIR gene bank, the same author came across the population No. 44 containing the recessive gene tph_2 which controls high content of gamma-tocopherol (5% of alpha-tocopherol and 95% of gamma-tocopherol). By combining these two recessive genes, *Demurin* (1993) obtained an inbred line with a high content of delta-tocopherol (8% of alpha-tocopherol, 84% of gamma-tocopherol and 8% of delta-tocopherol).

The objective of this study was to develop B- and Rf-inbred lines with a high content of oleic acid and modified tocopherol composition, good GCA and SCA for seed and oil yields and high tolerance to Phomopsis. These lines may be subsequently used for the development of hybrids with modified oil content.

MATERIAL AND METHOD

In this study, the donor lines for oil quality were inbred lines developed at VNIIMK, Krasnodar, Russia:

1. B-lines: Lg-21 (Ol, tph_1), Lg-25 (Ol, tph_2) and Lg-24 (tph_1, tph_2)
2. Rf-lines:VK-66-1 (tph_1, tph_2), VK-66-2 (Ol, tph_1) and VK-66-3 (Ol, tph_2)

The donors for good GCA and SCA and high tolerance to Phomopsis had been developed at the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia:

1. B-lines: Ha-74, Ha-981 and CMS-3-8
2. Rf-lines: RHA-583, RHA-576, RHA-SEL and RHA-SNRF-b

In the first year of the experiment (1993), each B-line tolerant to Phomopsis was crossed to all B-lines - donors for quality, and each Rf-line tolerant to Phomopsis to all Rf-lines - donors for quality. During the autumn/winter of 1993/94, backcross with the lines tolerant to

Phomopsis (F_1BC_{P1}) and selfing of F_1 hybrids to produce the F_2 generation were completed in a greenhouse. The female plants were emasculated by hand, in early morning, before anthers open up. In 1994, 1995, 1996 and 1997, the selfing program was continued for development of new inbred lines. The conversion of selected B-lines into the cms-sterile form started in the summer of 1995. Three generations were produced each year, first in the period January-April in the greenhouse, second in the period May-September in the field and third in the period September-January in the greenhouse.

After harvest of each generation, individual plants were analyzed for the composition of tocopherols, oleic acid content and oil content in seed. Field generations were also assessed for resistance to Phomopsis. The composition of tocopherols was determined by the half-seed method (*Demurin,* 1994) and thin-layer chromatography (TLC), the composition of higher fatty acids by gas chromatography (GC), the oil content in seed by the method of nuclear magnetic resonance (NMR). Phomopsis tolerance of the newly developed material was rated on the scale 0-5 (0-resistant, 5-susceptible).

RESULTS AND DISCUSSION

The inbred lines from VNIIMK, Krasnodar (Russia) used in this study as donors for oil quality, i.e., donors of genes Ol, tph_1 and tph_2, have poor agronomic characteristics, in the first place high susceptibility to Phomopsis. This is why they had to be crossed to inbred lines from the Institute of Field and Vegetable Crops in Novi Sad which have good agronomic characteristics and high tolerance to Phomopsis. A part of the obtained F_1 generations was selfed for the production of the F_2 generation, the other part was backcrossed to the donors for Phomopsis resistance. In this way we made an initial population for the development of inbred lines with modified oil quality and high tolerance to Phomopsis.

After each generation of selfing, the materials were analyzed for the content of oleic acid in oil in order to identify the material with completely stable and high oleic acid content, i.e., the material heterozygous for this characteristic. Also, each generation selfed in the field was selected for Phomopsis resistance. Analyses of the composition of tocopherols and oil content in seed were done too. Inbred lines with increased content of oleic acid and high tolerance to Phomopsis were then selected on the basis of the obtained results.

Among the high oleic B-lines (Table 1), particularly promising are the inbreds UK-18 and UK-26, with high oil content in seed (> 50%), high tolerance to Phomopsis and the ratio alpha vs. beta tocopherol 50:50. These inbreds also have a stable and high content of oleic acid (> 80%). They have been converted to the sterile form, which is the basic prerequisite for the development of high oleic sunflower hybrids. Among the high oleic Rf-lines, there is a group of restorers distinguished for the oil content in seed over 50 %, high tolerance to Phomopsis and the ratio alpha vs. beta tocopherol 50:50 (Table 2). Some of these Rf-lines have a high and stable content of oleic acid, as indicated by low values of standard deviation. There is also a large set of high oleic restorers possessing medium tolerance to Phomopsis and various levels of oil content in seed (Table 3). In some of them, the oil content exceeds 55%, and in the restorer Rus-rf-ol-154 it reaches 63.9 %. Regarding tocopherol, the set may be subdivided into a subset with dominant Tph_1 gene (100% alpha-tocopherol) and a subset with recessive tph_1 gene (50% alpha- tocopherol and 50% beta-tocopherol).

The newly developed B- and Rf-lines may be used for the development of high oleic sunflower hybrids possessing a high oil content in seed and different compositions of tocopherols. Taking in consideration the high tolerance to Phomopsis in some of these lines, it seems reasonable to expect of them to render resistant hybrids. Combining ability tests that started this year produced preliminary results which indicate that we are close to developing high oleic sunflower hybrids which match the conventional hybrids regarding the important agronomic traits. According to *Friedt et al*. (1994), agronomic inferiority has been the main obstacle to a large-scale commercial production of high oleic sunflower hybrids.

The results of *Demurin et al.* (1994) and *Škorića et al*. (1996) indicate that the stability of high oleic sunflower oil is threefold that of the conventional oil. Combining high oleic content with increased contents of beta-, gamma- and delta-tocopherol may further increase oil stability. *Demurin* (1996) states that the presence of gamma-tocopherol in oil and a high proportion of oleic acid increase the stability of sunflower oil 16.4 times over that in the conventional sunflower oil.

CONCLUSION

Following conclusion were drawn on the basis of the obtained results.

- We developed a large set of high oleic B-lines that are tolerant to Phomopsis and that possess various compositions of tocopherols.
- Also, we developed high oleic and high oil Rf-lines that are highly tolerant to Phomopsis and that possess various compositions of tocopherols.
- The newly developed B- and Rf-lines will be used for the development of new sunflower hybrids with modified oil quality.

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Table 1. B-lines with increased oleic acid content and various compositions of tocopherols

No.	Line	Fatty acids composition (%)		Tocopherols (%)		Oil content (%)	Phomopsis resistance (scale 0-5)
		oleic	linoleic	alpha	beta		
1.	UK-4	88	3	50	50	43.86	4
2.	UK-8	85	5	100	/	46.90	3
3.	UK-9	86	3	50	50	48.36	4
4.	UK-18	86	4	40	60	52.72	2
5.	UK-26	88	3	50	50	53.05	2
6.	UK-29	86	3	50	50	41.64	2
7.	UK-56	88	2	50	50	42.80	2
8.	UK-57	89	2	50	50	47.96	2
9.	UK-58	86	4	50	50	43.92	2
10.	UK-87	88	3	100	/	47.10	2
11.	UK-88	86	4	50	50	/	2
12.	UK-89	87	3	50	50	/	2
13.	UK-90	88	3	50	50	40.90	2
14.	UK-91	88	2	50	50	40.17	2
15.	UK-96	86	4	100	/	49.10	2

Table 2. Selected high oleic Rf-lines having high tolerance to Phomopsis and oil content in seed over 50%

No.	Line	Oleic acid content (%)			
		Average	Max	Min	Std.Dev.
1.	Rf-kv-1	85.4	87	80	3.05
2.	Rf-kv-2	88.8	89	88	0.45
2.	Rf-kv-3	88.6	91	88	1.34
4.	Rf-kv-4	88.0	90	86	1.41
5.	Rf-kv-5	85.6	88	78	4.28
6.	Rf-kv-6	87.6	89	85	1.67
7.	Rf-kv-7	89.2	91	88	1.30
8.	Rf-kv-8	88.2	89	87	0.84
9.	Rf-kv-9	81.2	90	49	18.01
10.	Rf-kv-10	83.0	88	70	7.55
11.	Rf-kv-11	87.8	89	87	0.84
12.	Rf-kv-12	87.4	89	86	1.34
13.	Rf-kv-13	71.8	89	41	19.59
14.	Rf-kv-14	82.4	87	71	6.54
15.	Rf-kv-15	85.2	89	73	6.87
16.	Rf-kv-16	88.2	89	87	0.84

Table 3. High oleic Rf-lines with various compositions of tocopherols

No.	Line	Fatty acids composition (%)		Tocopherols (%)		Oil content (%)	Phomopsis resistance (scale 0-5)
		oleic	linoleic	alpha	beta		
1.	Rus-rf-tph-ol-8	86	4	50	50	51.21	4
2.	Rus-rf-tph-ol-10	86	3	90	10	40.50	2
3.	Rus-rf-tph-ol-14	90	3	50	50	54.37	4
4.	Rus-rf-tph-ol-15	82	10	50	50	54.05	4
5.	Rus-rf-tph-ol-16	87	3	50	50	54.22	4
6.	Rus-rf-tph-ol-23	91	2	100	/	53.87	4
7.	Rus-rf-tph-ol-25	89	2	50	50	55.38	3
8.	Rus-rf-tph-ol-26	90	2	50	50	56.68	3
9.	Rus-rf-tph-ol-35	87	3	50	50	50.05	3
10.	Rus-rf-tph-ol-37	84	6	50	50	52.08	3
11.	Rus-rf-tph-ol-39	89	2	50	50	42.32	4
12.	Rus-rf-tph-ol-43	88	3	50	50	/	5
13.	Rus-rf-tph-ol-51	87	5	50	50	49.59	4
14.	Rus-rf-tph-ol-69	85	7	50	50	39.54	4
15.	Rus-rf-tph-ol-81	83	5	50	50	55.92	4
16.	Rus-rf-tph-ol-83	86	6	50	50	49.93	4
17.	Rus-rf-tph-ol-87	81	9	50	50	51.70	4
18.	Rus-rf-tph-ol-93	84	4	50	50	51.40	4
19.	Rus-rf-tph-ol-96	87	4	50	50	33.73	4
20.	Rus-rf-ol-27	85	4	100		47.68	4
21.	Rus-rf-ol-38	89	4	100		54.70	4
22.	Rus-rf-ol-39	87	5	100		44.87	4
23.	Rus-rf-ol-54	90	2	100		51.71	3
24.	Rus-rf-ol-67	89	2	100		49.41	4
25.	Rus-rf-ol-68	87	4	100		47.88	4
26.	Rus-rf-ol-70	85	5	100		43.71	4
27.	Rus-rf-ol-77	91	1	100		58.39	4
28.	Rus-rf-ol-78	88	2	100		56.00	4
29.	Rus-rf-ol-80	87	4	100		45.98	4
30.	Rus-rf-ol-91	88	3	100		55.26	3
31.	Rus-rf-ol-94	86	3	100		43.55	4
32.	Rus-rf-ol-134	85	6	100		43.46	4
33.	Rus-rf-ol-140	84	4	100		46.43	4
34.	Rus-rf-ol-142	88	2	100		59.13	3
35.	Rus-rf-ol-154	84	4	100		63.90	3
36.	Rus-rf-ol-206	88	3	100		42.02	4
37.	Rus-rf-ol-207	90	2	100		53.46	4
38.	Rus-rf-ol-209	90	1	100		52.83	4
39.	Rus-rf-ol-222	90	2	100		52.57	4
40.	Rus-rf-ol-242	91	3	100		52.57	4