DEVELOPMENT OF SUNFLOWER HYBRIDS WITH DIFFERENT OIL QUALITY

Dragan Škorić, Siniša Jocić, Nada Lečić and Zvonimir Sakač, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
E-mail: skoricd@ifvcns.ns.ac.yu

Abstract

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

Objectives of this research was development of hybrids with stable high oleic acid content and modified tocopherol composition, with high values for the two most important agronomic characters (seed yield and oil yield) and high tolerant to Phomopsis.

The incorporation of the gene $Ol+tph_1$ into these genotypes has led to the development of high-oleic hybrids with altered tocopherol profiles. Oil of these hybrids can be sustained for much longer than standard sunflower oil can.

The process of incorporating the genes $Ol+tph_2$ and $Ol+tph_1tph_2$ into highly productive sunflower genotypes is under way.

The most important products of this line of research are the newly developed female lines the oil of which has an oleic acid content of over 90% and the male lines (restorers) with an oleic acid content in the 89-93% range. Using these lines, hybrids will be developed whose oil is going to have an oleic acid content of over 90%.

Introduction

The sunflower is one of the most important oil crops globally and its oil is among the highest quality vegetable oils on the market. Oil quality is determined by the higher fatty acid composition and the levels of tocopherols, sterols, carotenoids, and other compounds. The sunflower is regarded as one of the most promising crops when it comes to the genetic alteration of oil quality (Scharp, 1986.). Standard sunflower oil is predominantly composed of linoleic acid (C 18:2) and oleic acid (C 18:1). These two acids account for about 90% of the total higher fatty acid content of sunflower oil. The remaining 8-10% are comprised of palmitic and stearic acids (C 16:0 and C 18:0, respectively). Conventional sunflower oil also contains several other higher fatty acids, but these are usually found only in traces (C 14:0, C 16:1, C 14:1, C 20:0, C 22:0) (Friedt et al., 1994).

The first significant alteration of sunflower oil was carried out by Soldatov (1976), who treated the seed of the cultivar VNIIMK 8931 with a 2.5% solution of dimethyl sulphate (DMS) to obtain an induced mutation for a high oleic acid content, which he then used to develop the first high-oleic sunflower, Pervenec. Sunflower breeders worldwide have since used this cultivar as their source of genes for developing the present-day high-oleic hybrids of this crop. The mode of inheritance of high oleic acid content has been studied by a number of authors (Fick, 1984; Urie, 1985; Fernandez-Martinez et al. 1989;

Demurin and Škorić 1996, etc). According to Fernandez–Martinez et al. (2004), different modes of inheritance of a high oleic acid content have been reported, including partial dominance of a single gene; dominance of either one, two or three dominant genes; one dominant gene + one recessive one acting as the modifier; a single recessive gene, and so on. Molecular studies revealed a strong positive correlation between Δ12 RFLP and the high-oleic mutant (Lacombe et al. 2000).

Besides the genetic manipulation of the higher fatty acid composition, it is also possible to change the tocopherol composition of the sunflower oil. Standard sunflower oil is around 95% α-tocopherol, 3% β- tocopherol, 2% γ- tocopherol i trace amounts δ-tocopherol. Demurin (1988) selfed the cultivar VNIIMK 8931 to obtain an inbred line containing 50% α- tocopherol and 50% β- tocopherol. The increased β- tocopherol content is controlled by a single recessive gene called tph_1. In population No. 44 from the VIR gene bank, the same author discovered tph_2, a recessive gene that controls increased γ-tocopherol content (5% α- tocopherol and 95% γ- tocopherol). By combining these two recessive genes, Demurin (1993) obtained an inbred line with an increased δ- tocopherol content (8% α- tocopherol, 84% γ- tocopherol and 8% δ- tocopherol).

Analyzing the achievements of sunflower breeding for different oil quality, Demurin et al. (1994), Demurin and Škorić (1995), and Škorić et al. (1996 and 1998) found positive synergy between genes for a high oleic acid and those for different tocopherol levels (Ol + tph_1; Ol + tph_2; Ol+ tph_1tph_2). The objective of this paper was to examine the possibility of developing sunflower hybrids that have different oil quality (primarily increased oleic acid levels) and altered tocopherol composition as well as good agronomic characteristics and high tolerance to Phomopsis.

Materials and Methods

Used in this study was part of the breeding material developed at the Institute of Field and Vegetable Crops in Novi Sad during the 1993-2005 period.

For donor lines of sunflower oil quality we chose inbred lines developed at VNIIMK, Krasnodar, Russia, namely:

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 donor lines of good GCA and SCA and high tolerance of Phomopsis were the following inbred lines developed at our institute::

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 study (1993), the above lines were subjected to crossing according to a system where every B-line tolerant of Phomopsis was crossed with every B-line donating quality and every Rf-line tolerant of Phomopsis was crossed with every Rf-line used as a donor of quality. In the autumn of 1993, under greenhouse conditions, backcrosses were made with the lines tolerant of Phomopsis and the hybrids were sellfed to obtain the F2 generation. Plants used as the maternal component in the crosses were manually emasculated in the early morning hours before the anthers had opened. In the next phase, pedigree selection was used to develop inbreds with the desired characteristics, and the conversion of the selected B-lines into their cms variants was started in the summer of 1996. In the year 2000, testing began of the general (GCA) and special (SCA) combining abilities by the line x tester method, while in 2003 the best hybrid combinations began to be tested in a network of small- and large-plot trials.

After harvesting in each generation, individual plants were tested for their tocopherol composition, oleic acid content, and seed oil content and, in the case of generations grown under field conditions, for resistance to Phomopsis. Tocopherol content was determined by the half-seed method (Demurin, 1994) using thin-layer chromatography (TLC), while higher fatty acid composition and seed oil content were determined using gas chomatography (GS) and nuclear magnetic resonance (NMR), respectively. Tolerance to Phomopsis of the newly developed breeding materials was graded on a scale of 0 to 5 (0-resistant, 5-susceptible).

Results and Discussion

The inbred lines developed at VNIIMK, Krasnodar, Russia that were used in the present study as donors of oil quality (Ol, tph_1 and tph_2 genes) are characterized by very poor agronomic traits, most notably by very high susceptability to Phomopsis. For this reason, they were crossed to inbreds developed at the Institute of Field and Vegetable Crops in Novi Sad that have very good agronomic characteristics and high tolerance of Phomopsis. One part of the F1 generations obtained was subjected to selfing to produce the F2 generation, while another was backcrossed to the inbreds used as donors of resistance to Phomopsis. This way a starting population was developed for the selection of new inbred lines with different oil quality and a high tolerance of Phomopsis.

Each of the selfed generations was analysed for increased oleic acid content in the oil until this trait stabilized itself completely, i.e. until the line being developed became homozygous for the trait. Additionally, each of the generations grown under field conditions was subjected to selection for resistance to Phomopsis and tests were done to determine tocopherol composition and seed oil content. Based on the results, new inbreds were developed with an increased oleic acid content and high tolerance towards Phomopsis.

Years of work on altering sunflower oil quality at the Novi Sad Institute have produced significant results. Genes for a high oleic acid content (Ol genes) have been incorporated into a number of existing lines. In parallel with this, new genetic variability for increased oleic acid content has been created and new male and female lines with extremely high oleic acid levels have been developed. Compared with the existing high-oleic hybrid Olivko (75-85 %), the newly developed inbred lines have significantly higher oleic acid levels (89-93%) (Table 1).

Using these lines it is possible to rapidly develop new high-oleic (HO) sunflower hybrids. The only thing needed is to expeditiously convert the female (DOP) lines into their cms variants under field and greenhouse conditions using backcrosses. It is important to note that the male lines from this group have oleic acid levels ranging from 89 to 93% (Table 1, B). Use of these materials most definitely makes it possible to quickly develop high-oleic sunflower hybrids with oleic acid levels of over 90% and usethem for large-scale introduction into commercial production. According to preliminary studies of the combining abilities of said lines, the new high-oleic hybrids will have yields rivalling those of the highest-yielding existing sunflower hybrids. At the same time, these inbreds have been selected with a broad range of disease resistance in mind, so the new high-oleic hybrids are going to have a high degree of resistance to the dominant diseases of sunflower.

The first cycle of breeding for oil quality has been completed at the Novi Sad Institute , producing hybrids that now incorporate the Ol genes for a high oleic acid content and the tph_1 genes for tocopherol content alteration (50% alpha + 50% beta) (Table 2). The newly developed hybrids are as productive as the most productive standard NS hybrid in existence, NS-H-111. Several of these hybrids are currently undergoing registration and some of them are expected to enter commercial production within the next two years.

Unfortunately, the breeding projects for incorporating Ol+tph_2 and Ol+$tph_1 tph_2$ genes into productive sunflower genotypes have not finished yet. Inbred lines possessing these combinations of genes have not been fully fixed yet and the process of their development is still in progress. These projects are expected to be completed within the next few years. Once they are, we will have new sunflower hybrids that have a high oleic acid content and are predominantly made up of either beta+gamma, gamma or gamma+delta tocopherols. This will put on the market new top quality sunflower kernels that can be used to obtain high-value final products from this crop.

Conclusion

Our breeding program on developing sunflower hybrids with different oil quality (1992-2005 godine) has produced genotypes with high values of agronomically important traits:

- A number of female lines with oleic acid levels of over 90% have been developed. At the same time, a numbver of restorer (male) lines have been developed with oleic acid levels in the 89-93% range. These lines are now being used to develop sunflower hybrids with an olec acid content of 90% and more.
- Ol+tph_1 genes have been incorporated into a number of inbreds and hybrids have been developed that possess a high oleic acid content and (>80%) and altered tocopherol composition (50% alpha + 50% beta). The newly developed hybrids have considerably higher oil stability than the standard ones;
- The incorporation of Ol+tph_2 and Ol+$tph_1 tph_2$ genes into productive sunflower genotypes is in progress.

Table 1. Sunflower inbred lines with the highest levels of oleic acid in the oil

A) female lines

No.	Genotype	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
1	DOP-2	3.01	1.57	92.66	2.32
2	DOP-5	3.33	1.32	92.79	2.26
3	DOP-13	3.32	1.33	92.40	2.63
4	DOP-14	3.41	2.11	91.63	2.55
5	DOP-15	3.59	1.19	92.56	2.35
6	DOP-25	3.74	1.92	92.43	1.90
7	DOP-35	3.95	1.63	91.61	2.52
8	DOP-36	3.95	1.66	91.65	2.36
9	DOP-51	3.30	1.47	91.91	2.97
10	DOP-56	3.58	1.40	92.94	1.73

B) male lines

No.	Genotype	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
1	RHA-S-OL-9	4.10	3.53	89.28	2.46
2	RHA-S-OL-11	3.42	2.24	92.17	1.83
3	RHA-S-OL-12	4.28	2.26	91.80	1.41
4	RHA-S-OL-18	3.06	4.47	89.60	2.48
5	RHA-S-OL-21	3.46	4.52	89.78	1.81
6	RHA-S-OL-22	3.88	4.24	89.70	1.76
7	RHA-S-OL-23	3.48	3.82	89.77	2.59
8	RHA-S-OL-25	3.46	2.61	91.43	2.50
9	RHA-S-OL-26	3.48	3.62	90.04	2.24
10	RHA-S-OL-30	3.77	3.22	89.32	3.69

Table 2. Oleic acid and tocopherol contents in oil of new sunflower hybrids

No.	Designation	Oleic acid content %	Tocopherol composition (%)			
			α	β	γ	δ
1	NS-H-2031	78.56	100	-	-	-
2	NS-H-2032	81.13	100	-	-	-
3	NS-H-2033	81.30	50	50	-	-
4	NS-H-2034	82.47	50	50	-	-
5	NS-H-2072	80.34	50	50	-	-
6	NS-H-2073	82.56	50	50	-	-
7	NS-H-2074	83.37	50	50	-	-
8	NS-H-2075	81.90	50	50	-	-
9	NS-H-2076	82.29	50	50	-	-
10	NS-H-256	83.36	40	60	-	-
11	NS-H-2510	84.25	80	20	-	-
12	NS-H-2526	85.32	40	60	-	-
13	NS-H-2527	84.11	40	60	-	-
14	NS-H-2031	86.13	50	50	-	-
15	NS-H-9024	85.11	40	60	-	-

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