ACHIEVEMENTS OF SUNFLOWER BREEDING AT THE IFVC IN NOVI SAD

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Abstract

Novi Sad breeding materials and some wild species of the genus *Helianthus* were used to create new genetic variability in cultivated sunflower. Our breeding program during 1997-2003 had several directions, and greenhouses and winter generations (India and Argentina) were used. Artificial infection methods were utilized to develop new Rf and B-lines with resistance to diseases. Using different genetic materials and selection methods, 1,461 new restorers (Rf) and 217 new B-lines resistant to downy mildew (race 710) were developed during the 1997-2003 period. In the same period, a number of new B-lines were also developed that had high levels of tolerance to *Phomopsis*, *Sclerotinia* and *Macrophomina* simultaneously. Particularly valuable are the new lines with different oil quality (high-oleic plus different tocopherols). A total of 160 Rf lines with different oil quality and 157 new B-lines were developed. Furthermore, 260 new B-lines resistant to race E of broomrape (*Orobanche cumana*) were developed. Another important part of the breeding program is the incorporation of tolerance towards imidazolinone-based herbicides from the wild *H. annuus* into elite lines (B and Rf lines) and the development of the first hybrids tolerant to this herbicide group. The development of new lines tolerant to sulfonurea is in progress.

Introduction

International competition has been forcing breeders from all major sunflower-growing countries to try to develop more productive hybrids. This requires a more rapid application of the modern crop breeding methods to increase the genetic variability of the cultivated sunflower (Škorić, 2004).

Fick and Miller (1997) have provided an excellent review of the success made by sunflower breeders and geneticists in studying the genetic regularities controlling the mode of inheritance of major sunflower traits and in the achievement of breeding goals. The limiting factors in sunflower breeding have been described by Škorić (1992), Miller and Fick (1997) and Vranceanu (2000).

A large number of authors have been emphasizing the necessity of a more rapid and extensive utilization of the wild species of the genus *Helianthus* in the search for desirable genes (for resistance to diseases and insects and tolerance to stress and herbicides) and their incorporation into cultivated sunflower genotypes.

Since diseases are the main limiting factor in sunflower production, it is very important to use wild species in finding successful genes. In some wild species, Jan et al.

(2002) has found sources of resistance to the newly discovered broomrape race F in Spain. Several authors have achieved significant results in finding sources of resistance to downy mildew, rust and some other pathogens in the wild species.

The achievements of a number of authors in using induced mutations have made it possible to develop hybrids with different oil quality. The most important among them is Soldatov (1976), who treated sunflower seeds with a DMS (dimethyl sulfate) solution to obtain mutants with high oleic acid contents in the oil. If we add to this the achievements of Demurin (1993) in finding genes for different tocopherol content and type, we can conclude that it is possible for breeders to successfully develop hybrids with different oil quality.

The objective of this paper was to give an overview of the results of sunflower breeding at the Institute of Field and Vegetable Crops during 1997-2003.

Materials and Methods

In this cycle of selection, we used the rich breeding material developed in the preceding period (inbred and Rf lines, various hybrids, interspecific hybrids, and certain wild sunflower species). The subject of this paper is the new lines developed during the 1997-2003 period. The results on combining abilities are not presented because of their volume (every year over 3,000 new experimental hybrids are tested). In order to speed up the development of new inbred lines, we made use of the field as well as greenhouses (two generations per autumn/winter of every year) and winter generations in India and Argentina.

In developing restorer lines resistant to downy mildew, the donors of genes were RHA-340, resistant hybrids, the Rf population Kina-R, and interspecific hybrids between RHA-373 and a population of *H. argophyllus* T. & G. (1572-2). The initial crosses with the resistance sources were made using a number of NS restorers.

For developing new B-lines, we used Novi Sad (NS) lines JM-8 (race 710), Ha-336 and Ha-26-PR as donors of genes for resistance to downy mildew. The resistance was incorporated into a number of NS B-lines. Desirable genes were accumulated using single, double and fourfold convergent crosses.

In subsequent generations, Rf and B-lines were developed by inbreeding accompanied by testing of resistance using inoculation in a climatic chamber. Stable B-lines (S6 generation) were translated into the CMS form using backcrosses.

To develop lines highly tolerant of *Sclerotinia*, *Phomopsis* and *Macrophomina*, we used elite NS lines PR-ST-3, PH-BC-1-74, PH-BC-2-92 and Ha-48. A double convergent cross was made initially, after which a number of new B-lines were developed from it by inbreeding. In each selfed generation, the materials were divided into two parts and sown on two different plots. On one, which was irrigated (3 x 30 mm water/week), *Phomopsis*-infected stems from the year before were introduced and sclerotia were sown into rows by the plants. On the other, dry farming was used for *Macrophomina* assessment purposes.

In developing Rf lines with different oil quality, the following lines were used as donors of desirable genes: VK-66-1 (tph1tph2), VK-66-2 (OL + tph1) and VK-66-3 (OL + tph2). All three of these lines were crossed with RHA-583, RHA-SES and RHA-576. The F1 combinations and all subsequent generations of selfing were tested for their higher fatty acid composition by gas chromatography, while tocopherols were determined by paper chromatography.

When developing B-lines with different oil quality, the donors of desirable genes were the following lines: LG-21 (OL + tph_1), LG-25 (OL + tph2) and LG-24 (OL + tph1tph2). These lines were crossed with NS lines Ha-74. Ha-981 and CMS-3-8. Lines were developed by the same method as in the case of restorer lines. The newly developed B-lines translated into the CMS form using the BC method.

New B-lines resistant to broomrape race E were developed with several different sources as donors of genes (Table 6). With inbred lines resistant to race E, crosses were made with several elite NS lines. In the case of varietal populations, self pollination was used. The selection materials were tested each year on a plot infested by race E of broomrape.

When developing inbred lines resistant to imidazolinones, elite NS lines were crossed with a wild *H. annuus* from Kansas as the source of tolerance. After that, screening was done using Bolero herbicide in each generation of line development according to the procedure recommended by the BASF Company.

A wild *H. annuus* population was also used as the donor of desirable genes in the development of inbreds tolerant of sulfonurea. Elite NS Rf and B-lines were used for crossing. All backcross generations were tested for tolerance by treatment with sulfonurea and only resistant plants were used for further selection.

Results and Discussion

At the Institute of Field and Vegetable Crops, sunflower breeding began back in 1938, the year when the Institute was founded. Thus far, significant results have been achieved. Over 30 hybrids have been released in Serbia and Montenegro. NS sunflower hybrids have been grown in our country for more than 25 years on 98-100% of the total acreage of this crop. We have also released 82 hybrids abroad. In addition to this, our programs of joint hybrid development with various foreign partners have so far produced 55 registered joint hybrids. It is estimated that our NS and joint hybrids will be sown on over one million hectares worldwide in 2004.

The present paper describes the results for only those lines from the latest cycle of inbred line development (1997-2003) that have resistance to one or more diseases.

Sunflower Breeding for Resistance to Downy Mildew (Plasmopara). This part of our breeding effort had two main directions. One was the development of resistant B-lines and the other the development of resistant Rf (restorer) lines.

According to our results, our new inbred lines resistant to downy mildew originated from various sources of resistance. The largest numbers of restorer lines were obtained by crossing NS restorers with RHA-340. These restorers differ the most from each other in their morphological characteristics. The second largest group is restorers obtained from crosses between RHA-373 with the wild *H. argophyllus* (1572-2) and select NS restorers. These restorers definitely have the greatest breeding value. From the exotic Rf population Kina-R a group of medium-late and late restorer lines have been developed. The least numerous are restorers developed from well-known hybrids with resistance to downy mildew (Table 1).

Table 1. New restorer lines resistant to downy mildew.

Source of resistance	No. of resistant lines
510-1 Rha-373/Arg 1572-2 510-2 Rha-373/Arg 1572-2	199
RHA-340	1,072
Resistant hybrids	64
Varietal population Kina-R	126

The true value of the newly developed restorer lines will be known after their GCA and SCA with elite A-lines are investigated in a program we started in 2003.

The question still remains open as to the final list of downy mildew races the new restorers are resistant to, since we do not have the inocula of the most recently discovered races in some European countries. We can, however, say with certainty that they are resistant to races 100, 300, 700, 703 and 710.

In order to counter the appearance of the latest downy mildew races in our country, we have begun a new cycle of selection by crossing a group of select NS restorers with RHA-419 (Miller et al., 2002).

To make sure that our response to new downy mildew races domestically is as effective as possible, we have paid a great deal of attention to developing resistant B-lines as well.

It should be noted that in developing the new B-lines resistant to downy mildew we used lines resistant to broomrape race E (Ha-26-OR, CMS-1-90, VL-A-8, BT-VL-24 and BT-VL-30) and B-lines with field resistance to *Phomopsis* and simultaneous high tolerance of either *Phoma* (PH-BC-2-91 and PH-BC-2-92) or *Macrophomina* (UK-90 and UK-25) as well as lines with the highest tolerance of all the above pathogens and simultaneous high tolerance of *Sclerotinia* (Table 2).

Table 2. New B-lines resistant to downy mildew.

Source of resistance	No. of resistant lines
JM-8	6
Ha-336 and Ha-26-PR	211

The newly developed B-lines have a wider range of resistance to diseases, which is something many breeders strive for and something Škorić (2004) insists on strongly.

As with the new restorers, the breeding value of the new B-lines will be known once their GCA and SCA are tested.

Sunflower Breeding for Resistance to Sclerotinia, Phomopsis, and Macrophomina. The intended incorporation of a broader range of resistance to different major pathogens requires that new germplasm be developed, especially in the case of B-lines (Table 3).

Table 3. New B-lines highly tolerant of *Sclerotinia*, *Phomopsis* and *Macrophomina.*

	Sclerotinia			Phomopsis	Macrophomina
	Root	Stem	Head		
No. of lines	17	35	8	81	9

Complex tolerance towards the above pathogens has been found in six of the new B-lines, which are going to be of great value in the new cycle of hybrid development.

Sunflower Breeding for Different Oil Quality. The development of hybrids with different oil quality is one of the prime tasks of the NS breeding program. The goal is to simultaneously alter the composition of higher fatty acids and tocopherol types (Tables 4 and 5).

Table 4. New restorer lines with different oil quality.

Line	Number
Rf lines with high oleic acid content + different tocopherols	30
Linoleic-type Rf lines + different tocopherols	130

Table 5. New B-lines with different oil quality.

Lines	Number
High oleic B-new + different tocopherols	21
Linoleic B-new + different tocopherols	77
High oleic B-new	59

The new Rf and B-lines provide a rich pool of germplasm for the development of hybrids with different oil quality. Using the sources of different tocopherols discovered by Demurin (1993) that have been incorporated into elite NS Rf and B-lines, it is possible to develop hybrids containing: OL genes; OL + tph1; OL + tph2; OL + tph1tph2; and standard linoleic and oleic acid contents + tph1 or + tph2 or + tph1tph2 genes.

It is difficult to predict which type of oil will be dominant in the future, since that will depend a lot on the future trends in human nutrition and nutritionists' recommendations.

According to the findings of Demurin (1993) and Škorić (2004), it is to be expected that hybrids incorporating OL + tph2 genes will have an important role in the human diet in the future.

Sunflower Breeding for Broomrape (Orobanche cumana) Resistance. The appearance and rapid spread of broomrape race E in our country has made it necessary to develop hybrids resistant to this pathogen (Table 6).

Table 6. New B-lines resistant to race E of broomrape.

Source of resistance	No. of resistant lines
NS genfond	30
CMS_1-90, VL-A-8, PH-BC_1-54	78
Resistant varietal populations (Azovskiy, Donskoy 60 and Harkovski 7)	152

The large number of newly developed B-lines resistant to broomrape race E make up a rich germplasm pool that can be used for the development of resistant hybrids (Table 6). The most valuable among them are the 30 lines obtained from the NS gene pool resistance source, their utilization in resistant hybrid development is well under way.

The new B-lines developed based on resistance sources from CMS-1-90, VL-A-8 and PH-BC-1-54 have yet to be tested for their GCA and SCA with our elite and Rf lines, so only after that will their real value be known. The newly developed resistant B-lines based on varietal populations Azovskiy, Donskoy 60 and Harkovski 7 must be crossed solely with Rf lines that have high tolerance to *Phomopsis* and *Macrophomina*.

Results obtained while developing the B-lines resistant to race E of broomrape have shown that populations developed from certain wild species by Jan et al. (2002) do not contain sources of resistance to race E. The contribution of genes for resistance to the new race F recently discovered in Spain could not be determined in these populations. However, it has been determined that our B-line CMS-1-90 and restorers RHA-168 and RHA-N-187 do contain recessive genes for resistance to broomrape race F from Spain.

Sunflower Breeding for Herbicide Tolerance. Using a population of wild *H. annuus* from Kansas (Miller et al., 2000) as the donor of genes for tolerance towards herbicides from the imidazolinone group, we developed the first elite lines that have made it possible to develop the first IMI-resistant sunflower hybrids, which are now being introduced into commercial production in several European countries (Table 7).

Table 7. New inbred lines tolerant of imidazolinone-based herbicides.

Lines	Number
R_f lines	11
B-lines	20

With the aim of providing more genetic variability that will enable the development of highly productive hybrids and, especially, the attainment of a high seed oil content, 600 Rf restorers and 330 B-lines have been subjected to the inbreeding procedure. The resulting new hybrids are expected in the next few years.

Having high tolerance to just one herbicide group is not recommended; instead, the tolerance should be broadened to include other herbicides as well. For this reason, over 1,500 new Rf + B-lines with tolerance to sulfonurea (F3) are currently being developed using a population of wild *Helianthus annuus* as the source of resistance genes.

In light of the fact that progress in breeding can be made only by creating new genetic divergence in cultivated sunflower genotypes, we could say our goal has been accomplished. The real contribution of the Rf and B-lines will be known once their GCA and SCA have been tested. The importance of good combining abilities of newly developed lines has been stressed by a number of authors (Fick and Miller, 1997; Vranceanu, 2000; Škorić, 1992). It should be noted that the focus of the new inbred line development was to incorporate a wider range of disease resistance into new genotypes along with high tolerance towards herbicides or different oil quality.

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