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DEVELOPMENT OF SUNFLOWER GENOTYPES RESISTANT TO DOWNY MILDEW

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SUMMARY

Sunflower is attacked by more than 40 different diseases of which only a certain number causes serious reduction of seed yield. One of the most damaging diseases is downy mildew, which is caused by the fungus *Plasmopara halstedii* (Farl.) Berl. et de Toni (syn. *Plasmopara helianthi* Novot.). Sunflower downy mildew has great economic importance in all countries where sunflower is grown. When the meteorological conditions during the vegetation period of sunflower become favourable for disease development, the damages produce considerable reducing of the seed yield and the oil content.

The best way of controlling the fungus is to grow resistant cultivars and because of that the major objective of this study was to develop sunflower genotypes genetically resistant to dominant races of downy mildew in Serbia. During this work two co-dominant CAPS markers for Pl_6 gene were developed which can also be used for Pl_7 gene. For introduction of these genes in breeding program marker assisted selection (MAS) was used. Developed commercial sunflower inbred lines exhibit resistance to all known races of downy mildew in Serbia indicated incorporation of resistance to downy mildew in well-known and widely produced hybrids. Besides that, *Pl*-genes were introduced to a large number of new inbred lines and new downy mildew resistant hybrids. These new hybrids reach higher seed and oil yields then hybrids widely produced.

Key words: sunflower, downy mildew, race, hybrid, inbred line

INTRODUCTION

Diseases are the main limiting factor in the production of sunflower (*Helianthus annuus* L.) and they cause poor realization of genetic yield potential of sunflower hybrids. Downy mildew is an economically significant disease. It is caused by the fungus *Plasmopara halstedii* (Farl.) Berl. et de Toni. (syn. *Plasmopara helianthi* Novot.). Downy mildew is widespread in all sunflower-growing countries with the exception of Australia. According to Tikhonov (1975), it was first discovered on

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sunflowers in the United States in 1883, and in 1892 it was found on *Helianthus tuberosus* in Russia. As the sunflower expanded to other countries, the disease followed it closely, especially after the World War II. The rapid expansion of the disease may be explained by its transfer with infected sunflower seed. In the former Yugoslavia, it was discovered by Perišić (1949) and described by Nikolić (1952).

Downy mildew has great economic importance in all sunflower-growing countries. Its occurrence depends on the growing and climatic conditions during the sunflower growing season. If the season is rainy, the number of diseased plants increases in proportion with the number of rainy days. The number of infected sunflower plants may vary from 1 to 100%. Extent of damage depends on infection type, *i.e.*, whether it is primary (systemic) or secondary infection. While the primary or systemic infection causes significant yield reductions, secondary infection has no importance for the production of sunflower (Aćimović, 1998).

Primary infection is effected during seed germination in the soil and the emergence of sunflower seedlings. It may be caused by fungus mycelium or oospores present on infected seeds, or by oospores present in infected soil into which healthy seeds were sown. The number of diseased plants depends on the amount of inoculums on seeds and in soil. No matter if primary infection starts from seeds or soil, the course of disease development in infected plants is identical. The fungus develops in unison with the development of young plants. It penetrates the root, stem, cotyledons and reaches the meristematic tissue at the top of young plants. The fungus develops inside the infected plants intercellularly, in all plant parts, pervading the young tissues and depriving the infected plants of assimilates and water. This is why infected plants lag behind healthy ones in growth and development. This way of fungus expansion inside the plant tissues is called a systemic infection. It begins with the infection of the germ and ends with the infection of the head and seeds. The fungus penetrates all parts of the seed (husk, endosperm and germ), which then produces a new infected seedling. In that way, conditions are created for the occurrence of the disease in the subsequent sunflower growing season. Infected plants, in addition to having stunted growth, *i.e.*, short internodes, are chlorotic and with a platform head which gives a smaller yield than the normal head. On the infected plant parts, the root, cotyledons, the stem, and especially the leaves, there occurs abundant white mycelium, which is typical for this disease. The mycelium occurs also on the reverse side of the leaves and it contains the vegetative organs of the fungus - conidiophores and conidia (zoosporangia). On the upper side of the leaf there occur chlorotic spots. Infected plants collapse and remain in the field after harvest. Numerous oospores that form on these prostrate plants are overwintering organs of the fungus, which are ready to start a new cycle of infection in the spring.

In the course of sunflower growing season, spots occur on the aboveground plant parts, especially on leaves. The spots are polygonal, with a characteristic white mycelium on the reverse side of the leaf and chlorosis on the upper side. These spots originate from summer conidia (zoosporangia) and represent a secondary infection. The intensity of their occurrence is typically low and they have no important impact on sunflower yield.

Measures of protection against downy mildew include cultivation practices, chemical measures and the use of resistant hybrids. The recommended cultivation practices are the use of healthy seeds for planting, seed treatment with fungicides against downy mildew, proper crop rotation, *i.e.*, intervals of 4-5 years between two sunflower crops in the same field, selection of fields for sunflower growing that are at least 500 m away from a field planted to sunflower the previous year because of infected harvest remains in that field, removal of volunteer ants, sowing at optimum time and avoiding late planting, and deep plowing of the field after sunflower harvest.

The most effective chemical measure of downy mildew control is seed treatment with metalaxyl-based preparations. This measure protects the sunflower crop at the time of the primary infection, *i.e.*, at early stages of development of sunflower. In addition, various chemicals for post-emergence treatment are available, but this practice raises the question of economic feasibility.

Use of genetically resistant hybrids is definitely the most effective way of controlling downy mildew in sunflower. Therefore, experiments have been set up with the objective of developing sunflower genotypes genetically resistant to dominant race of downy mildew in Serbia.

MATERIALS AND METHODS

In these experiments we used a part of sunflower breeding material developed at Institute of Field and Vegetable Crops in Novi Sad in the period from 2001 to 2009.

The following inbred lines developed in USDA-ARS Sunflower Research Program, Fargo, North Dakota, USA, were used as donors of downy mildew resistance genes:

1. B-lines: Ha-336 (Pl_6), Ha-338 (Pl_7)
2. *Rf*-lines: RHA-340 (Pl_8), RHA-419 (Pl_{arg})

Ha-26-PR (Pl_6) and JM-8 (Pl_6+), B-lines of sunflower developed in an earlier program at Institute of Field and Vegetable Crops were used as additional donors of downy mildew resistance genes.

The following donor lines developed at Institute of Field and Vegetable Crops had good GCA and SCA and high tolerance to *Phomopsis:*

1. B-lines: *cms*-1-90, Ha-48, VL-A-8, *cms*-1-50, PH-BC_1-92, PH-BC_1-74, Ha-981
2. *Rf*-lines: RHA-583, RHA-SES, RHA-N-49, RHA-168, RHA-SNRF, RHA-RU-3, RHA-576

In the first year of the experiment, the above mentioned sunflower inbred lines were crossed with each other, each B-line resistant to downy mildew with each B-line tolerant to *Phomopsis* and each *Rf*-line resistant to downy mildew with each *Rf*-line tolerant to *Phomopsis*. The plants that served as the female component in the crosses were manually emasculated in the early morning hours, before opening of anthers. After these initial crosses, the program of development of sunflower genotypes resistant to downy mildew was divided in two parts. The first part was aimed at the development of completely new inbred lines resistant to downy mildew using the head-to-row pedigree method of selection. The second part involved the conversion of commercial inbred lines into a form resistant to downy mildew using the backcross method. Winter greenhouse was used in both parts of the programs to speed up the selection process and obtain three sunflower growing seasons in a calendar year.

After harvest of each generation, individual plants were analyzed for resistance to downy mildew. The generations grown in field were also assessed for resistance to *Phomopsis*. Resistance to downy mildew was tested by a laboratory method (Lačok, 2008) using the mildew race 730. In the course of this work, two co-dominant CAPS markers for Pl_6 were developed (Panković *et al.*, 2007) which were later found to be applicable for Pl_7 too (Saftić-Panković *et al.*, 2007). Marker assisted selection (MAS) was used for introgression of these genes, which was of great importance especially in the program of backcrossing.

RESULTS AND DISCUSSION

For a long period of time there existed only two races of downy mildew. Race 100 was present exclusively in Europe and race 300 was present in North America. Fick and Zimmer (1974) found that gene Pl_1 controlled the race 100, while gene Pl_2 controlled both races 100 and 300. These two genes controlled the downy population in Europe until 1998 when new races emerged (710 and 703) in France (Tourvieille de Labrouhe *et al.*, 1991). Later research showed that these races were introduced from the USA via infected seeds (Roeckel-Drevet *et al.*, 2003). After the introduction of these races, there occurred a conflagration of new downy mildew races, especially in France. Those were race 304 (Tourvieille de Labrouhe *et al.*, 2000), and races 300, 307, 314, 700, 704 and 714 (Penaud *et al.*, 2003). According to Viranyi (2008), 35 different races of downy mildew have been discovered so far, of which only five are prevalent (300, 330, 710, 730 and 770). A special problem is the occurrence of new downy mildew races in France and Spain that are resistant to metalaxyl, the main active substance that controls downy mildew (Albourie *et al.*, 1998; Molinero Ruiz *et al.*, 2000).

In Serbia, only race 100 was present until 1990. However, the isolate sampled in 1991 under the name of NS 912 was identified as race 730 and at that moment it comprised about 10% of all isolates. Already in 1996, this race made about 50% of the total isolates (Maširević, 1998). Today, race 730 is definitely dominant in Serbia (Lačok, 2008).

Genes for resistance to new races of downy mildew have been determined in wild sunflowers and have been transferred into cultivated sunflower genotypes. Resistance to downy mildew is controlled by several single dominant genes called *Pl*-genes, which are racially specific and which provide vertical resistance. More than ten such genes have been discovered so far. The safest method of combating the fungus is entering resistance genes in sunflower hybrids.

As the *Pl*-genes are race-specific, there is a great probability that their resistance will be overcome by new races of downy mildew in a relatively short period of time. To prolong resistance duration, attempts have been made in breeding programs to combine as many different resistance genes as possible. Genetic studies of *Pl*-genes indicated that they are present in at least three gene clusters (Vear, 2004). Genes Pl_1, Pl_2, Pl_6, Pl_7 and Pl_7+ are in the first cluster, genes Pl_5 and Pl_8 in the second and genes Pl_4 and Pl_{arg} in the third. The following B-lines were selected donor lines from cluster 1: Ha-336 (Pl_6), Ha-338 (Pl_7) and JM-8 (Pl_6+). *Rf*-line RHA-340 (Pl_8) was selected from the cluster 2, and *Rf*-line RHA-419 (Pl_{arg}) from cluster 3. The last two lines are the donors of genes Pl_8 and Pl_{arg}, which for the moment provide resistance to all known downy mildew races. The above inbred lines were selected for donors in consideration of their genetic origin. Namely, the line Ha-336, the donor of gene Pl_6, had been transferred from wild *Helianthus annuus*, the line of Ha-338, the donor of gene Pl_7, had been transferred from *Helianthus praecox*, and the lines RHA-340 and RHA-419 are donors of genes Pl_8 and Pl_{arg} which had been transferred from different populations of *Helianthus agrophyllus*. By introducing genes Pl_6, Pl_7 and Pl_6+ into the female lines and genes Pl_8 and Pl_{arg} into the male lines, we shall be able to develop sunflower hybrids resistant to all races of downy mildew in the long run.

The inbred lines used in this research as donors of resistance to downy mildew, *i.e.*, the donors of genes Pl_6, Pl_7, Pl_8 and Pl_{arg}, are characterized by poor agronomic characteristics. In the first place, they are highly sensitive to *Phomopsis*. Therefore, these lines had to be crossed to inbred lines made at Institute of Field and Vegetable Crops, Novi Sad, which are characterized by good agronomic characteristics and high tolerance to *Phomopsis*. A portion of the obtained F_1 generation was selfed in order to obtain the F_2 generation, and the other portion was crossed with inbred lines known as donors of resistance to *Phomopsis*. The F_2 generation was then subjected to the pedigree method of selection in order to create new genetic variability and thus create the initial population for the selection of new inbred lines resistant to downy mildew. In the second part of the selection program, commercial B-lines were backcrossed in order that they can be converted into a form resistant to downy mildew.

Using the discovered co-dominant CAPS markers for Pl_6 gene allowed us to identify homozygous plants resistant to downy mildew in each generation of selfing.

In addition to the analysis of gene presence, resistance was also checked by analyzing the response to primary infection with fungus spores. Selfing and the mentioned analysis were done to a complete stabilization of this trait, *i.e.*, until the lines became homozygous for resistance to downy mildew. Additionally, each generation of selfing grown in field conditions was selected for resistance to *Phomopsis*. Oil content in seed was also analyzed. Based on the obtained results, we selected new inbred lines resistant to downy mildew and highly tolerant to *Phomopsis* (Table 1).

Table 1: Newly developed sunflower inbred lines possessing different *Pl*-genes.

Source of genes	No. of lines	Type
Ha-336 (Pl_6)	267	B-line
JM-8 (Pl_6+)	37	B-line
Ha-338 (Pl_7)	62	B-line
RHA-340 (Pl_8)	206	*Rf*-line
RHA-419 (Pl_{arg})	103	*Rf*-line

The multi-year work at the Novi Sad Institute on resistance to downy mildew has produced significant results. Resistance genes have been introgressed into a large number of lines in the program of backcrossing. As a result of these research efforts, in 2009, the hybrids Velja, Kazanova and Rimi-PR were commercially grown in the form resistant to all races of downy mildew present in Serbia.

Simultaneously we developed a new genetic variation, *i.e.*, lines resistant to downy mildew. New female and male lines possessing different *Pl*-genes have also been developed. These lines had already been crossed and new sunflower hybrids resistant to all races of downy mildew present in Serbia were developed, Sremac, Duško and the high-oleic hybrid Oliva. The hybrids Sremac and Duško deserve to be singled out for their high yields of seed which make them leading hybrids not only in Serbia but also in other European countries. The new lines with different *Pl*-genes make it possible to develop new sunflower hybrids, which may provide a long-term solution to the problem of downy mildew control.

CONCLUSION

A breeding program aimed at the development of hybrids resistant to downy mildew has produced genotypes with high values of agronomically important characteristics. The obtained results allowed us to draw the following conclusions.

- The discovered co-dominant CAPS markers for Pl_6 gene of resistance to downy mildew can be used for efficient identification of plants resistant to downy mildew race 730 in selection material originating from various genetic sources.
- Commercial sunflower lines have been converted into forms resistant to all downy mildew races present in Serbia, which allowed us to convert extensively grown sunflower hybrids Velja, Kazanova and Rimi-PR into forms resistant to downy mildew.

- *Pl*-genes have been incorporated in a number of new inbred lines and new hybrids have been developed which are resistant to downy mildew. These are: Sremac, Duško and Oliva. The new hybrids have higher yields of seed and oil compared with the currently most widely grown hybrids in Serbia.

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