New sunflower hybrids tolerant of Tribenuron-Methyl

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ABSTRACT

Discovery of a tribenuron-methyl resistant wild *Helianthus annuus* L. population (ANN-KAN) created an opportunity for expansion of a sunflower herbicide resistance breeding program. The aim of this work was the creation of sunflower hybrids resistant to tribenuron-methyl. Creation of tribenuron-methyl resistant hybrids would enable the use of a wider palette of herbicides for sunflower, more efficient chemical control of *Cirsium arvense* and more economically profitable post-emergence control of some annual broad-lea weeds in sunflower. Original populations SURES-1 and SURES-2 are homozygous for resistance to tribenuron-methyl. F_1 generations produced from the crossings are completely resistant to tribenuron-methyl, pointing to the dominant way of inheritance of this trait. Studies on the exact number of genes controlling the resistance are in progress. Tribenuron-methyl resistance was transferred from original populations into a number of mother and restorer inbred lines of cultivated sunflower. These inbred lines could enable creation of a number of hybrids resistant to tribenuron-methyl. Hybrids NS-H-2017-SU, NS-H-2018-SU and NS-H-2019-SU are resistant to doubled application dose of tribenuron-methyl. Agronomical characteristics of these hybrids are on the level with the leading conventional sunflower hybrids.

Key words: hybrid – sunflower – tolerance – tribenuron-methyl.

INTRODUCTION

The main aim of plant breeding is to develop new varieties and hybrids to meet the needs of people and domestic animals. Due to the rapid growth of the human population, loss of arable land, global climate change, and water supply problems, the production of sufficient amounts of food will be a challenge in the future. The increase of yields of cultivated plants requires not only the development of new, more productive genotypes but the advancement of growing technology as well. Plant breeding for tolerance to herbicides covers both of these aspects.

The development of plants with herbicide tolerance has been made possible by the latest insights into the mechanism and target place of herbicide action at the molecular level and by the development of new biotechnology methods. In the 1990s, a number of crop genotypes resistant to herbicides have been developed as a result (Table 1). Although it is theoretically possible to develop a plant tolerant of any kind of herbicide, only combinations of major economically important crops and herbicides possessing favorable characteristics (glyphopsate, glufosinate ammonium, sulfonylurea, imidazolinones, etc.) have found an actual commercial application (Malidza et al., 1999).

Table 1. Year of first registration of herbicide tolerant crops (Malidza et al., 1999).

Year	Company	Crop
1992	Cyanamid	IMI, IR, IT Maize
1992	Du Pont	STS Soybeans
1995	Calgene	BXN Cotton
1995	AgrEvo	Liberty Link Canola
1996	Monsanto	Roundup Ready Soybeans
1996	Monsanto	Roundup Ready Canola
1997	Monsanto	Roundup Ready Cotton
1997	AgrEvo	Liberty Link Maize
1997	AgrEvo	Liberty Link Soybeans
1999	Monsanto	Roundup Ready Maize

The initial stages of plant breeding for herbicide resistance did not include any work on sunflower. Crop species for which herbicide-tolerant genotypes had been developed began to be grown more widely thanks primarily to the improved economy of their production. A result of this was a decrease of area in sunflower in South and North America, where the new technologies had been accepted without any legal limitations. Additionally, weed killing herbicides are developed less rapidly in sunflower than in the rest of field crops. Weeds cause significant yield losses in sunflower due to a lack of effective herbicides for the suppression of broadleaf weeds and use after crop emergence. The currently existing chemical measures are ineffective against large-seeded broadleaf weeds, while the present soil herbicides are often not effective enough in the suppression of small-seeds weed species, especially in years with rainfall deficits occurring after herbicide application (Malidza et al., 2004). All this prompted sunflower researchers to begin working on the crop's tolerance to herbicides. The first major breakthrough came when Al-Khatib et al. (1998) found a population of wild *Helianthus annuus* L. (ANN-PUR) originating from Rossville, Kansas (USA) that was resistant to imidazolinone-based herbicides. Once the genetics of the resistance were studied and understood (Miller and Al-Khatib, 2000; Jocić et al., 2001), this population was used to develop the first sunflower hybrids tolerant of imidazolinone herbicides. These were developed in the USA in 2003 and Serbia and Turkey in 2004 (Jocić et al., 2004).

The discovery in Kansas, USA of a wild *Helianthus annuus* L. (ANN-KAN) population (Al-Khatib et al., 1999) resistant to a sulfonylurea herbicide (tribenuron-methyl) opened up the possibility of expanding the scope of sunflower breeding for tolerance to herbicides. The present study was aimed at the development of sunflower hybrids possessing tolerance of tribenuron-methyl. The introduction of such hybrids provides multiple benefits, including a broadened range of available herbicides in sunflower, more effective control of Canada thistle (*Cirsium arvense*), and greater cost-efficiency in the suppression of some annual broadleaf weeds after sunflower emergence (Zollinger, 2003; Malidza et al., 2006).

MATERIALS AND METHODS

The herbicide Granstar 75 WG was used in the study in two doses, the normal, recommended one (30 g/ha) and twice that (60 g/ha). In the latter years of the program, another herbicide was also used in the study to test the tolerance of the newly developed hybrids. This was Express 50-SX (500 g/kg tribenuron-methyl), a new and improved tribenuron-methyl-based herbicide manufactured by Du Pont. Express 50-SX was applied at 45 g/ha (standard dose) and 90 g/ha (double dose).

The sources of genes for tolerance to tribenuron-methyl were the populations SURES-1 and SURES-2. SURES-1 is a population of B lines obtained from the cross HA 424/3HA 406 // HA 89/ ANN-KAN, while SURES-2 is a population of restorer lines originating from the cross RHA377/3 RHA 392 // RHA 376/ ANN-KAN (Miller and Al-Khatib, 2004). Of cultivated sunflower genotypes, we used the self-pollinated B lines HA-26, VL-A-8 and HA-48 for crosses with SURES-1 and the restorer lines RHA-583, RHA-SES and RHA-N-49 for crossing with SURES-2.

The tolerance of SURES-1 and SURES-2 towards tribenuron-methyl was tested in the greenhouse during September through December 2000. In parallel with this, initial crosses were made between the two populations and the self-pollinated lines chosen for the study. During the 2001 growing season, the tolerance of the resultant F_1 generations was tested under field conditions using the double dose of tribenuron-methyl. After determining the mode of inheritance, pedigree selection was employed, with each inbred generation being treated with the double dose of Granstar 75-WG (60 g/ha). The most tolerant plants from the most tolerant progenies were selected for further breeding work. Treatment with herbicides was performed at the stage of 2-6 leaves using the knapsack sprayer Solo, 350 l/ha of water and a pressure of 2 bars. Twenty days after the treatment, phytotoxicity was assessed visually on a scale of 0 to 100% (0% - no symptoms, 100% - complete plant necrosis). Thanks to the use of a greenhouse, three inbred generations were obtained per year, which enabled us to develop the first experimental hybrids as early as 2004 and to test the general (GCA) and specific (SCA) combining abilities of the newly developed restorer lines. The testing was done using line x tester method (Singh and Choudhary, 1976). The comparative trial was carried out on a well-prepared chernozem soil at the Rimski Sancevi Experiment Field of the Institute of Field and Vegetable Crops using a randomized block design with three replications. The planting dates were optimal, intensive cultural practice was implemented during the growing season, and harvesting was done manually. The best hybrid combinations were selected and tested for tolerance to tribenuron-methyl and performance characteristics in a network of small-plot trials in 2005.

RESULTS AND DISCUSSION

Tribenuron-methyl is a herbicide that inhibits the acetolactate synthase enzyme (ALS), which is responsible for the synthesis of the amino acids valin, leucine and isoleucine. It is also one of the oldest sulfonylurea herbicides in existence (Ferguson et al., 1985) and has been among the most important herbicides in small grains for the past two decades. In Serbia, it is used in wheat crops and is the active ingredient of the Granstar 75-WG formulation (75% tribenuron-methyl) (Mitic, 2004). According to

Kolkman et al. (2004), the SURES-1 and SURES-2 populations have been found to contain the Pro197 mutation. This mutation is one of the most common mutations found in crop species tolerant of herbicides inhibiting ALS. It provides several-fold tolerance towards such herbicides compared with the susceptible genotypes. During the 2001 growing season, progenies of the source populations were found to possess full tolerance to tribenuron-methyl, meaning these populations are fully homozygous for this trait. Full susceptibility of the conventional inbred lines was confirmed as well. The F_1 generations exhibited full tolerance along with slight chlorosis, but there was absolutely no lagging behind in growth of any sort relative to the control treatment, which indicates the dominant mode of inheritance of tolerance to Granstar 75-WG. Determining the genetic basis of herbicide tolerance is a very sensitive kind of research. The first requirement is to use the double dose of the active ingredient. Environmental factors have a great influence on the expression of herbicide tolerance, as does the genetic basis of the lines receiving the tolerance genes. Because the donor populations possess many traits characteristic of the source population of wild *Helianthus annuus*, the determination of the genetics of the tolerance requires prior development of inbred lines tolerant of tribenuron-methyl. Pedigree selection was used to develop 52 inbred lines from crosses between SURES-2 and the restorer lines RHA-583, RHA-SES and RHA-N-49 as well as 46 female inbreds obtained by crossing SURES-1 and the lines Ha-26, VL-A-8 and Ha-48. All these self-pollinated lines are tolerant of the double dose of tribenuron-methyl, since the herbicide was applied at the 2-6-leaves stage in each generation during their development. Besides the herbicide tolerance, the newly developed selfed lines also have other favorable agronomic characteristics (most importantly tolerance to *Phomopsis helianthi*), as these were selected for these characteristics as well during the selection process.

The development of these lines also enabled the development of the first hybrids tolerant of tribenuron-methyl. The GCA and SCA of the new lines were tested and then the experimental hybrids were developed in 2004. All the hybrids were tested for performance characteristics and resistance to the common diseases and treated each year with the double dose of tribenuron-methyl. Based on the results, three of the hybrids were chosen for commercial production.

Due to the large volume of this research program, the present paper shows only the results for the newly developed SU hybrids NS-H-2017-SU, NS-H-2018-SU and NS-H-2019-SU. Table 2 shows the results produced by the three hybrids in two years of testing. The main requirement these hybrids must meet is to have a sufficient level of tolerance to tribenuron-methyl. What this means in concrete terms is that they have to be able to withstand the double dose of the standard, recommended dose of the active ingredient per unit area without showing any signs of phytotoxicity or any significant losses of yield or yield components. The results achieved by our hybrids have shown that they have a sufficient level of tolerance, as there were no statistically significant yield losses or reductions in the other studied traits in the treatment with the double dose of tribenuron-methyl relative to the treatment in which no herbicide was used (Table 2). Additionally, there were no visible signs of phytoxicity either. The only thing observed was that there was some slight chlorosis seven days after the treatment, but these symptoms disappeared completely after two weeks. The second important condition the new hybrids have to fulfil is to have good performance characteristics in addition to tolerance to tribenuron-methyl. Thus, they have to have a high yield potential, a high oil content, and resistance to the common diseases so as to be able to compete with the standard sunflower hybrids used in commercial production. The check hybrids in our trials were NS-H-111, the leading sunflower hybrid in Serbia, and NS-H-43, which is a hybrid that domestic sunflower growers are well familiar with, as it has been present in Serbian sunflower production for a considerable number of years already. The results of the trials have shown that the new SU hybrids are completely on a par with the standard ones in terms of performance. The performance of NS-H-2017-SU and NS-H-2019-SU completely matched that of the class-leading NS-H-111 in terms of seed yield, oil content and oil yield, while NS-H-2018-SU performed as well as NS-H-43 despite being two weeks earlier in terms of maturation (Table 2).

Our results indicate that the new SU hybrids NS-H-2017-SU, NS-H-2018-SU and NS-H-2019-SU will find their niche in the domestic sunflower market very soon. This has been confirmed by their results in the official variety trials of the Serbian Variety Commission and their subsequent registration in the Serbian Variety List.

The source populations SURES-1 and SURES-2 are homozygously tolerant of tribenuron-methyl. The F_1 generations produced in the program are completely tolerant of tribenuron-methyl, indicating the presence of the dominant mode of inheritance. Studies to determine the exact number of genes controlling this resistance are in progress. Resistance to tribenuron-methyl has been transferred from the source populations to a number of female and self-pollinated sunflower lines. This makes it possible to develop a larger number of hybrids tolerant of tribenuron-methyl. The hybrids NS-H-2017-SU, NS-H-2018-SU and

NS-H-2019-SU are tolerant of twice the recommended dose of tribenuron-methyl per hectare and are also as good as the leading sunflower hybrids in the domestic market in terms of agronomic performance.

Table 2. Mean values of several traits in tribenuron-tolerant sunflower hybrids

Hybrid	Treatment	Plant height (cm)	Maturity (days)	Seed yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)
NS-H-2017	Untreated	176.43	122.5	4 265.33	46.36	1 976.43
	Tribenuron-methyl (45 g/ha)	178.29	123	4 230.57	46.25	1 956.20
NS-H-2018	Untreated	161.40	110.4	3 702.26	47.18	1 747.45
	Tribenuron-methyl (45 g/ha)	162.30	109.9	3 806.34	48.53	1 847.28
NS-H-2019	Untreated	189.55	127.8	3 926.57	48.30	1 896.53
	Tribenuron-methyl (45 g/ha)	188.75	127.5	4 157.52	49.25	2 046.11
NS-H-43	Untreated		129	3 938.04	46.49	1 830.35
NS-H-111	Untreated		123	4 258.12	48.53	2 066.36
			LSD	476.93	4.27	260.65

ACKNOWLEDGEMENTS

This work was supported by Ministry of Science of Republic of Serbia.

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