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Inheritance of Tribenuron-Methyl Tolerance in Sunflower

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Abstract: The genetic stocks of SURES-1 and SURES-2 showed complete tolerance to tribenuron-methyl. No resistance were obtained after observation on 200,000 plants of four VNIIMK breeding lines grown on an experimental field with total area of 5 ha treated with Express at 1x rate. Potential frequency of a dominant gene for tolerance to tribenuron-methyl was estimated less than 5×10^{-6}. Moreover screening both of 700 plants of 39 inbred lines of the genetic collection and 800 plants of M_2 four breeding lines did not reveal any resistance. Tolerance to tribenuron-methyl in F_1 and F_2 in the crosses of SURES-1 and SURES-2 with VNIIMK breeding lines was controlled by one dominant gene. All recessive homozygous hybrid plants of *sur sur* genotype in the cross were dead with phytotoxicity index of 9 after Express treatment at any rate of tribenuron-methyl from 0.5x to 4x. Heterozygous hybrid plants of *Sur sur* genotype showed no injury symptoms at 0.5x rate and increasing level of phytotoxicity index of 4, 6 and 7 for 1x, 2x and 4x rates correspondingly. Dominant homozygous hybrid plants of *Sur Sur* genotype possessed no injury symptoms from 0.5x to 2x rates whereas the phytotoxicity index of 4 was observed at the 4x rate. Consequently there was no difference between dominant homozygote *Sur Sur* and heterozygote *Sur sur* with phytotoxicity index of 0 at 0.5x rate. Allelic interaction in a heterozygote *Sur sur* can vary from complete to partial dominance in accordance with increasing rate of tribenuron-methyl treatment estimating with the phenotypic scale of phytotoxicity index.

Keywords: screening, herbicide, gene, dominance degree, heterozygote

Introduction

The quest for genetic resistance to herbicides in the populations both of cultivated plants and their wild relatives has drawn attention of the researches from

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the point of view of the trait genetics and to develop appropriate innovations in the modern agro technologies.

Presently there are two similar approaches to control the annual broad-leaves weeds with the post-emergence tribenuron-methyl treatment of herbicide tolerant sunflower (Sala *et al*., 2012; Skoric, 2012). The Sumo technology is based on a SURES genotype found in the wild sunflower population from Kansas (Al-Khatib *et al*., 1999) whereas the ExpressSun^{TM} uses an EMS-mutant of a breeding line (Streit, 2012).

Introduction of the resistant gene to tribenuron-methyl from wild *H. annuus* into the gene pool of cultivated sunflower resulted in the development of two public sources of this trait. SURES-1 is the B-line obtained after crossing HA424/3/HA406// HA89/SU Res. wild *H. annuus* and Sures-2 is the Rf-line derived from the cross RHA377/3/ RHA392//RHA376/SU Res. wild *H. annuus* (Miller and Al-Khatib, 2004).

From molecular-genetic point of view, the resistance to sulfonylureas of the SURES genotype is the point mutation C-T in codon 197 of the *Ahasl1-2* gene controlling AHAS (ALS) enzyme (Bulos *et al*., 2013; Kolkman *et al*., 2004). The original lines SURES-1 and SURES-2 were shown to be homozygous for resistance to tribenuron-methyl and the mode of inheritance this trait in the F_1 was dominant (Jocic *et al*., 2008, 2011).

The main aim of our study was focused on inheritance of tribenuron-methyl tolerance in the crosses with VNIIMK lines including preliminary screening of VNIIMK genotypes for herbicide resistance and the dose-response effect on dominance degree of a *Sur* gene.

Materials and methods

Forty five of sunflower inbred lines of VNIIMK collection including elite breeding B-lines (VK276, VK678) and Rf-lines (VK580, VK585), the accessions of different origin, the M_2 of inbred lines after chemical mutagenesis with DMS and introduced from the USA two genetic stocks of SURES-1 and SURES-2 were used in the research since 2007 year. Screening for resistance to tribenuron-methyl herbicide was done with Granstar 75 WG or Express 750 g/kg (the same of a.i. tribenuron-methyl, DuPont). The plants were treated at the stages of four-six leaves (V4–V6) according to ExpressSun technology with 20 g a.i. ha^{-1} (1x) as a recommended dose of tribenuron-methyl. There were two types of application with a manual sprayer (water volume of five liters) or a field tractor sprayer (water volume of 200 liters).

Plants were scored phenotypically using a phytotoxicity index (Sala and Bulos, 2012) with our modifications (including description of each triple scores for leaf chlorosis, curling abnormalities and necrosis) at 10–14 days after

herbicide treatment. Phytotoxicity index presents a phenotypic scale from 0 to 9 in accordance to an injury rate. Plants without any symptoms were recorded as 0, increasing levels of leaf yellowing chlorosis with respect to the untreated check were recorded as 1 (low), 2 (middle) and 3 (high), increasing levels of leaf curling abnormalities were recorded as 4, 5 and 6, increasing levels of plant tissue necrosis were recorded as 7, 8 and 9 (dead).

Plant spacing was 70 × 35 cm. In case of hybridization the plants were manually emasculated to produce of the F_1 seeds of VK876 × SURES-1, VK580 × SURES-2, VA93 × SURES-1 and VA325 × SURES-2. The F_2 seeds of these crosses were obtained by self-pollination of the F_1 plants with individual bag isolation.

In dose-response analysis the susceptible lines (VK876, VK195, VK680) and resistant lines (VK876Sur, VA317Sur) were used to produce an homo- and heterozygote F_1 plant set. CMS lines were crossed as female. The parent inbred lines did not include in this set because of the smaller biomass in compare with hybrid heterozygous plants. The tribenuron-methyl rate (Express) was 10 (0.5x), 20 (1x), 40 (2x) and 80 (4x) g a.i. ha^{-1}.

Results and discussion

The genetic stocks of SURES-1 and SURES-2 showed complete tolerance to tribenuron-methyl herbicide of Granstar at 1x rate of 20 g of a.i. ha^{-1}. These populations were used as a source of tribenuron-methyl in the further research.

No resistance were obtained after observation on 200,000 plants of four breeding lines grown on an experimental field in 2013 year with total area of 5 ha treated with Express at 1x rate. Potential frequency of a dominant gene for tolerance to tribenuron-methyl was estimated less than 5×10^{-6} (1:200,000) for germplasm of VNIIMK lines. Moreover screening both of 700 plants of 39 inbred lines of the genetic collection and 800 plants of M_2 four breeding lines did not reveal any resistance (Table 1).

Tolerance to tribenuron-methyl in F_1 and F_2 in the crosses of the genetic stocks of SURES-1 and SURES-2 with VNIIMK breeding lines was controlled by one dominant gene if two phenotypic classes “resistant” and “susceptible” were discriminated at the 1x rate (Table 2).

All recessive homozygous hybrid plants of *sur sur* genotype in the cross of VK876 × VK195 were dead with phytotoxicity index of 9 after Express treatment at any rate of tribenuron-methyl from 0.5x to 4x (Table 3). Heterozygous hybrid plants of *Sur sur* genotype in the cross of VK680 × VA317Sur showed no injury symptoms at 0.5x rate and increasing level of phytotoxicity index of 4, 6 and 7 for 1x, 2x and 4x rates correspondingly. Dominant homozygous hybrid plants of

Table 1: Screening for herbicide tolerance to tribenuron-methyl in sunflower inbred lines.

Type of treatment	Genotype	No. of plants	
		Resistant	Susceptible
Field sprayer	VK276	0	40,000
	VK678	0	40,000
	VK580	0	60,000
	VK585	0	60,000
Manual sprayer	39 inbred lines	0	700
	M_2 4 breeding lines	0	800

Table 2: Inheritance of herbicide resistance to tribenuron-methyl in F_1 and F_2.

Generation	Cross	No. of plants		$\chi^2_{3:1}$
		Resistant	Susceptible	
F_1	VK876 × SURES-1	41	0	–
	VK580 × SURES-2	20	0	
	VA93 × SURES-1	48	0	
	VA325 × SURES-2	13	0	
F_2	VK876 × SURES-1	36	11	0.06*
	VK580 × SURES-2	30	11	0.07*
	VA93 × SURES-1	30	7	0.72*
	VA325 × SURES-2	29	12	0.39*

Note: *$p > 0.05$.

Table 3: Dose-response to tribenuron-methyl with phytotoxicity index for three genotypes of sunflower hybrids in 10 days after treatment.

Tribenuron-methyl (rate), g a.i. ha^{-1}	VK876 × VK195, *sur sur*		VK680 × VA317Sur, *Sur sur*		VK876Sur × VA317Sur, *Sur Sur*	
	No. of plants	Phytotoxicity index	No. of plants	Phytotoxicity index	No. of plants	Phytotoxicity index
0	43	0	30	0	42	0
10 (0.5x)	19	9	12	0	20	0
20 (1x)	37	9	21	4	44	0
40 (2x)	40	9	26	6	37	0
80 (4x)	40	9	23	7	38	4

Sur Sur genotype in the cross of VK876Sur × VA317Sur possessed no injury symptoms from 0.5x to 2x rates whereas the phytotoxicity index of 4 was observed at the 4x rate.

Consequently there was no difference between dominant homozygote *Sur Sur* and heterozygote *Sur sur* with phytotoxicity index of 0 at 0.5x rate only. This type of allelic interaction for the *Sur* gene is considered as a complete dominance. On the other hand dominant homozygote *Sur Sur* possessed the higher level of herbicide tolerance compering with heterozygote *Sur sur* from 1x to 4x rates. This type of allelic interaction corresponds to partial dominance.

Conclusion

The genetic stocks of SURES-1 and SURES-2 are a reliable source of monogenic tribenuron-methyl tolerance in the breeding programs. The germplasm of sunflower inbred lines of VNIIMK collection did not contain any dominant allele *Sur*. Allelic interaction in a heterozygote *Sur sur* can vary from complete to partial dominance in accordance with increasing rate of tribenuron-methyl treatment estimating with the phenotypic scale of phytotoxicity index.

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