



## Current environmental selection issues: selection of *h. annuus* L. for herbicide resistance

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### Abstract

This article is devoted to the creation of a new material of sunflower fertility reducing agents, having an optimal combination of tribenuron-methyl resistance and a sufficient vegetation period for the production of hybrids in zones with a short frost-free vegetation period. Lines used as a source of tribenuron-methyl resistance were purchased by Sativa LLC at the University of North Dakota Seed Fund (USA). The maximum resistance to aherbicide treatment was demonstrated by plants of hybrid combinations received by using the paternal line B0708VG, which line was significantly superior to the B0707VG line. Also, in the generation of the B0708VG line there is a tendency for increased productivity compared to the generation of the line B0707VG. The use of the method of intraspecific hybridization of the line - a carrier of the resistance gene SURES-2 followed by negative selection against the background of herbicide treatments makes it possible to create promising paternal forms with a sufficient period of vegetation and duration of the period «rise-start of flowering» for zones with a short frost-free period and having resistance to the tribenuron-methyl.

**Keywords:** SURES-2 resistance gene carrier; tribenuron-methyl; hybrids for short frost-free zones; intraspecific hybridization

Aglotkov MV, Ignatenko AI, Cherniavskih VI, Dumacheva EV, Korolkova SV, Koryakov DP (2020) Current environmental selection issues: selection of *h. annuus* L. for herbicide resistance. Eurasia J Biosci 14: 1505-1509.

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### INTRODUCTION

The adaptive capabilities of crop plants are very wide. The proof of this was found in 1996 among the weed plants of *H. annuus* L. in the soybean fields in Kansas, USA, individuals having natural resistance to the herbicide Imazethapyr-IMI-resistance. This herbicide is used on sunflower crops to control *Orobanchosumana* L. used on crops (Geier et al., 1996).

The obtained genetic material was transferred to a number of research centers, where active selection work was started with it. Already in 1998, the first public lines with resistance to herbicide IMISUN became available for free purchase at the University of North Dakota seed fund (Al-Khatib et al., 1998; Miller and Al-Khatib, 2000).

As a result, a *Clearfield*® complex product including *Euro-lightning* herbicide (imazamox 33 g/L + imazapir 15 g/L) and resistant sunflower hybrids was marketed. This was the beginning of an active research to identify genes for resistance to a number of other herbicides and to create complex products: herbicides and sunflower hybrids resistant to them (Gil et al., 2018; Gil and Nestares, 2019; Moschen et al., 2016; Balabanova, et al., 2018).

In 2008, a new source of resistance, CLHA-PLUS, was obtained by induced mutagenesis. On its basis the production system *Clearfield Plus*® was registered (Škoric et al., 2012; Breccia et al., 2017; Délye et al., 2015; Malik, 2016).

An active work is under the way to create sunflower hybrids resistant to herbicides with the active substance tribenuron-methyl. Their main advantage is the absence of a restriction on the seeding of crop rotation, as the herbicide is easily destroyed in the soil. A new resistance gene has been introduced in SURES-1 and SURES-2 populations that have been released for free sale by the University of North Dakota Seed Fund since 2001.

Tribenuron-methyl tolerance is controlled by a single dominant gene. Pioneer/DuPont and the Institute of Field and Vegetable Crops (Novi Sad, Serbia) were the first to market the corresponding commercial hybrids. This cultivation technology is known on the market as *ExpressSun*, *SUMO* (Jocić et al., 2008; Sala et al., 2012; Duhoux et al., 2015; Guo et al., 2017).

Received: June 2019

Accepted: May 2020

Printed: June 2020

On the Russian market, the first hybrid of PR64E83 compatible with the *ExpressSun* system was registered in 2011 (Demurin et al., 2013; Voronova, 2011). At present time, sunflower hybrids with two levels of resistance to herbicide *Express* (750 g/kg tribenuron-methyl, VDG) are obtained: 25 and 50 g/ha (Demurin et al., 2016; Dimitrijević and dHorn, 2018).

A work on ecological breeding and creation of various crops is actively carried out in the Belgorod region, such as alfalfa, soybeans, phacelia, clover, cereal grasses (Dumacheva et al., 2017; Dumacheva et al., 2018; Chernyavskikh et al., 2019). A work on ecological breeding and creation of new sunflower hybrids is actively carried out in the Belgorod region (Aglotkov et al., 2019; Ignatenko et al., 2017; Adepoju and Osunsanmi, 2018).

**The aim of the work** is to create a new material of sunflower fertility reducing agents, having an optimal combination of tribenuron-methyl resistance and a sufficient growing period for the production of hybrids in zones with a short frost-free vegetation period.

## MATERIAL AND METHODS

The lines used in the work as a source of tribenuron-methyl resistance were purchased by Sativa LLC at the University of North Dakota Seed Fund (USA). The line SURES-1 is an inbred sterility anchor line obtained after HA424/3/HA406//HA89/SU Res crossing. wild *H. annuus*. The SURES-2 line is a fertility reducer line based on the crossing RHA377/3/RHA392// RHA376/SU Res. wild *H. annuus*.

Selection study of the material has been carried out since 2008. Artificial pollination of sunflower was carried out. Castration of sunflower baskets for crossing was carried out with a solution of gibberellin at a concentration of 0.005% (50 mg/l, 10 ml solution was used per plant) in the sprocket phase when the sunflower plant lays down generative organs. On each plot, 3 to 5 plants were covered with a single insulator for forced plant inbred line. Sowing, ear behind crops and cleaning in the breeding nursery are carried out in accordance with standard methods. The results of the experiments are processed statistically using the methods of variation statistics (Dospikhov, 2012).

## RESULTS AND DISCUSSION

In the first stage of selection, the speed of SURES-1 and SURES-2 lines was compared with the varieties approved as standards for conducting state testing in the Belgorod region.

The lines of SURES-1 and SURES-2 were found to be too late ripening compared to the classes approved as standards and exceeded the official standard of Group 4 of the Darius ripeness by 7-8 days.

At the second stage, the SURES-2 line was compared with the parent hybrid forms of "Borey",

"Quin", "Darium", "Yason", "Kharkovsky 49" and promising father lines from the collection of parent forms used in the seed production of sunflower hybrids. Indicators such as the length of the period of vegetation, the period «sproutings– start of flowering» and their compliance with similar periods in the maternal forms used in the selection and seed program were evaluated.

It has been found that the SURES-2 form is limited for selection work in the Belgorod region. The main reason for this is that the father's SURES-2 line is significantly superior in length to the «sproutings–start flowering» period both father's (by 16-24 days) and mother's (by 14-22 days) lines.

The significant mismatch in flowering times revealed makes it impossible to use the SURES-2 line in the seed production of hybrids as a paternal one while simultaneously sowing with the maternal form. The delay in the seeding of the maternal line relative to the paternal line by 2-3 weeks carries the risk of decreasing the productivity of industrial hybridization sites and the quality of seeds due to the delay of the growing period and the increase in the probability of sunflower harvesting in the period with unfavorable weather conditions.

An important conclusion was made that in order to successfully create short-season sunflower hybrids having resistance to sulfonylurea herbicides for zones with a short frost-free period, the parent lines should have the following feature complex:

1. The growing period is 92-100 days at the length of the «sproutings-start flowering» period – 58-68 days to create hybrids of the first-second ripeness groups and 96-104 and 64-68 days, respectively, to create hybrids of the third-fourth ripeness groups.
2. Resistance to the tribenuron-methyl at 50 g/ha (treatment with preparation containing 750 g/kg tribenuron-methyl)
3. Guaranteed transmission of tribenuron-methyl resistance to first generation hybrids
4. Period of paternal form blooming is not less than 20 days, including due to side baskets blooming.

In the third stage, the method of intraspecific hybridization with paternal forms, as well as perspective lines from the collection of parental lines, which are used in industrial seed production of the Belgorod region, was used to create promising paternal forms.

With two-fold pollination, 28 pollinated baskets were obtained, with the binding capacity of the seeds being low - from 8 to 70 pcs per basket.

With two-fold pollination, 28 pollinated baskets were obtained, with the binding capacity of the seeds being low - from 8 to 70 pcs per basket. The obtained seeds were crossed by single-row divisions for three generations. Carried out a compulsory inbred line.

In the field, plants were rejected, in which flowering began both in early (less than 55 days since sprouts) and late (more than 70 days since sprouts) terms.

**Table 1.** Degree of the resistance of hybrid combinations to herbicide Express treatment at a consumption rate of 25 g/ha

No	Title	Degree of stability to herbicide, grade	Height of plants, cm	Duration of the period of vegetation, days	Crop productivity, t/ha	Fertility of plants of F <sub>1</sub> , %
<b>Hybrid combinations</b>						
1	B72A x B0707VG	7	140	102	2.61	100
2	B72A x B0708VG	9	160	102	2.83	100
3	B72A x B0711VG	9	150	108	2.42	0
4	(Cx808A x H1002B) x B0707VG	7	180	106	2.53	100
5	(Cx808A x H1002B) x B0708VG	9	190	110	2.67	100
6	(Cx808A x H1002B) x B0711VG	7	190	108	2.22	0
	NSR <sub>05</sub>	-	-	-	0.28	-
<b>Male parent forms</b>						
1	♂ x B0707VG	7.0	160	104	2.57	-
2	♂ x B0708VG	9.0	175	106	2.75	-
3	♂ x B0711VG	8.0	170	108	2.32	-
	NSR <sub>05</sub>	1.22	-	-	0.32	-

In the fourth step, the site with the selection samples seeded with the herbicide Express was treated at a consumption rate of 25 g/ha. Mass damage to plants was observed at this. The proportion of plants fully resistant to the effect of herbicide Express was 6.2%, and highly resistant – 17.2%.

From samples having stability at 7-9 points, 43 stable lines were selected, suitable for the period of vegetation, as well as for the duration of flowering for further selection study.

In the fifth stage, 43 selected lines were crossed for subsequent selection and re-treated with Express herbicide at a rate of 50 g/ha.

The increase in the dose of herbicide by half the number of resistant forms of sunflower has sharply decreased. As a result, three sunflower lines were completely stable.

For their preliminary evaluation, a crossing was performed by transferring pollen from the test lines: B72A (clean line) and Sch808A x X1002B (simple sterile hybrid) to establish their ability to transmit the stability sign. The choice of these lines as parents is related to the need to test generation resistance to tribenuron-methyl, in both simple and three-line hybrids.

In the sixth step, new hybrid combinations were prepared that needed to be tested for herbicide resistance. These hybrid combinations were seeded with single row plots and treated with the herbicide Express at a rate of 25 g/ha.

Results of the researchers are shown in **Table 1**.

The evaluation of the degree of injury of plant of hybrid combinations on the 7th and 14th days after herbicide treatment showed that all three promising lines inherited resistance to tribenuron-methyl treatment, but the resulting hybrid combinations differ in degree of

resistance. The maximum resistance to herbicide treatment was demonstrated by plants of hybrid combinations prepared using the paternal B0708VG line, which was significantly superior to the B0707VG line. Also in the generation of the B0708VG line there is a tendency for increased productivity compared to the generation of the line B0707VG.

In a phase of blossoming it was noted that the hybrid combinations received with the use of the B0711VG line were sterile, which means that this line does not restore fertility of plants in generation of F<sub>1</sub> that does it unsuitable for use as a fatherly form.

Two prospective paternal lines of B0707VG and B0708VG, having genetic resistance to tribenuron-methyl and transmitting this gene by inheritance, were selected for further selection work.

## CONCLUSION

1. On the basis of free-circulation selection line of SURES-2 and father's lines of different geographical origin, separated in the conditions of the Belgorod region, a new material of sunflower fertility reducing agents has been created, having an optimal combination of tribenuron-methyl resistance and a sufficient growing period for the production of hybrids in zones with a short frost-free vegetation period.

2. The use of the method of intraspecific hybridization of the line - a carrier of the resistance gene SURES-2 followed by the negative selection against the background of herbicide treatments makes it possible to create promising paternal forms with a sufficient period of vegetation and duration of the period «sproutings-start flowering» for zones with a short frost-free period and having resistance to tribenuron-methyl.

## REFERENCES

- Adepoju AO, Osunsanmi O (2018) Gender Differentials in Labour Market Participation of Rural Households in Non-Farm Activities in Oyo State, Nigeria. *International Journal of Sustainable Agricultural Research*, 5(4): 85-95.
- Aglotkov MV, Ignatenko AI, Chernyavskikh VI, Dumacheva EV (2019) Selection of high-oleic sunflower hybrids for regions with short frost-free period. *Innovations in life sciences: collection of materials of the International Symposium*. Belgorod, October 10-11, 2019. Under Ed. Spichak I.V. Belgorod: 15–17

- Al-Khatib K, Baumgartner JR, Peterson DE (1998) Imazethapyr resistance in common sunflower (*Helianthus annuus* L.). *Weed Science*, 46: 403–407.
- Balabanova D, Remans T, Vassilev A, Cuypers A, Vangronsveld J (2018) Possible involvement of glutathione S-transferases in imazamoxdetoxification in an imidazolinone-resistant sunflower hybrid. *Journal of Plant Physiology*, 221: 62–65.
- Breccia G, Gil M, Vega T, Altieri E, Bulos M, Picardi L (2017) Contribution of non-target-site resistance in imidazolinone-resistant Imisun sunflower. *Bragantia*, 76: 536–542.
- Chernyavskikh VI, Dumacheva EV, Sidelnikov NI, Lisetsky FN, Gagieva LCh (2019) Use of *Hissopus officinalis* L. culture for phytoamelioration of carbonate outcrops of anthropogenic origin the south of European Russia. *Indian Journal of Ecology*, 46 (2): 221–226.
- Délye C, Duhoux A, Pernin F, Riggins CW, Tranel PJ (2015) Molecular mechanisms of herbicide resistance. *Weed Science*, 63: 91–115.
- Demurin YaN, Tronin AS, Pikhtyareva AA (2016) Inheritance of Tribenuron-Methyl Tolerance in Sunflower. *Helia*, 39 (65): 183–188.
- Demurin YN, Pichtyaryeva AA, Tronin AS (2013) Transfer of tribenuron-methyl resistance gene to selection material of sunflower All-Russian Research Institute of Oil Crops (VNIIMK). *Oilseeds. Scientific and technical bulletin of the All-Russian Research Institute of Oil Crops*, 1: 153–154.
- Dimitrijević A, Horn R (2018) Sunflower hybrid breeding: From markers to genomic selection. *Frontiers in Plant Science*, 8: 2238.
- Dospekhov BA (2012) *Field experience methodology (with basic statistical processing of research results)*, M.: Book on Demand: 352.
- Duhoux A, Carrère S, Gouzy J, Bonin L, Délye C (2015) RNA-Seq analysis of rye-grass transcriptomic response to an herbicide inhibiting acetolactate-synthase identifies transcripts linked to non-target-site-based resistance. *Plant Molecular Biology*, 87: 473–487.
- Dumacheva EV, Cherniavskikh VI, Gorbacheva AA, Vorobyova OV, et al. (2018) Biological resources of the Fabaceae family in the Cretaceous South of Russia as a source of starting material for drought-resistance selection. *International Journal of Green Pharmacy*, 12 (2): 354–358.
- Dumacheva EV, Cherniavskikh VI, Tokhtar VK, Tokhtar LA, et al. (2017) Biological resources of the *Hyssopus* L. on the south of European Russia and prospects of its introduction. *International Journal of Green Pharmacy*, 11 (3): 476–480.
- Geier PW, Maddux LD, Moshier LJ (1996) Common sunflower (*Helianthus annuus* L.) interference in soybean (*Glycine max*). *Weed Technology*, 10: 317–321.
- Gil M, Nestares G (2019) Decoding Non-Target-Site Herbicide Resistance in Sunflower: The Beginning of the Story. *Helia*, 42 (70).
- Gil M, Ochogavia AC, Vega T, Felitti SA, Nestares G (2018) Transcript profiling of non-target-site imidazolinone resistance in Imisun sunflower. *Crop Science*, 58: 1991–2001.
- Guo S, Zuo Y, Zhang Y, Wu C, et al. (2017) Large-scale transcriptome comparison of sunflower genes responsive to *Verticilliumdahliae*. *BMC Genomics*, 18: 1–13.
- Ignatenko AI, Aglotkov MV, Cherniavskikh VI, Dumacheva EV (2017) Selection of quick-ripe sunflower hybrids in the Belgorod region. In the list: Innovative technologies in the crop production and ecology. *Proceedings of the International Scientific and Practical Conference. Gorsky GAU, Vladikavkaz* :197–198.
- Jocić S, Miklič V, Malidža G, Hladni N, Gvozdenović S (2008) New sunflower hybrids tolerant of tribenuron-methyl. *Proc. 17a1 Intl. Sunflower Conf.*, 2: 505–508.
- Malik VS (2016) RNA sequencing as a tool for understanding biological complexity of abiotic stress in plants. *Journal Plant BiochemBiotechnol*, 25: 1–2.
- Miller JF, Al-Khatib K (2000) Development of herbicide resistant germplasm in sunflower. *Proc. 15th Inter. Sunflower Conf. France, Toulouse; Paris: Inter. Sunflower Assoc.*, 2: 419–423.
- Moschen S, Bengoa Luoni JA, Di Rienzo JA, Caro MDP, et al. (2016). Integrating transcriptomic and metabolomic analysis to understand natural leaf senescence in sunflower. *Plant Biotechnology Journal*, 14: 719–734.
- Sala CF, Bulos M, Altieri E, Ramos ML (2012) Genetics and breeding of herbicide tolerance in sunflower. *Proc. 18th Int. Sunfl. Conf., Mar del Plata, Argentina*: 75–81.
- Škoric D, Seiler GJ, Liu Z, Jan CC, Miller JF, Charlet LD (2012) *Sunflower genetics and breeding: international monograph*. Novi Sad: Serbian Academy of Sciences and Arts. Branch.: 520.

Voronova O (2011) In the fight for a place under the sun. New agrarian magazine, 2 (2): 48–54.

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