UDC 575.633
https://doi.org/10.2298/GENSR1901287B
Original scientific paper

STUDIES ON GENE FLOW FROM HERBICIDE RESISTANT TO WEEDY SUNFLOWER

Dragana BOŽIĆ[1], Markola SAULIĆ[1], Aleksandra SAVIĆ[1,2], George GIBBINGS[3], Sava VRBNIČANIN[1]*

[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia
[2]Institut for Plant Protection and Environment, Belgrade, Serbia
[3]School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR, UK

Božić D., M. Saulić, A. Savić, G. Gibbings, S. Vrbničanin (2019): *Studies on gene flow from herbicide resistant to weedy sunflower*.- Genetika, Vol 51, No.1, 287-298.

Gene flow is a main concern associated with the use of herbicide resistant sunflower crops because it could transfer herbicide resistance traits to weedy sunflower. In order to estimate potential gene flow from imazamox and tribenuron-methyl resistant sunflower hybrids to weedy sunflower, field experiments and DNA analysis were conducted. The progeny of weedy sunflower which grown near imazamox (WS1) and tribenuron-methyl (WS2) resistant hybrid in previous experiments were used. In the field experiment, recommended rates of imazamox and tribenuron-methyl were applied to WS1 and WS2, respectively, and plants surviving were recorded. Herbicides effect on fresh weight of survived plants were also determined. The presence of mutations responsible for sunflower resistance to herbicides (imazamox and tribenuron-methyl) checked based on DNA analysis of selected survived plants. Percentage of survived plants in field experiment was recorded at maturity and depend on weedy sunflower accession (WS1or WS2) and distance of their mother plants from resistant sunflower hybrid in previous experiment and was higher for WS2 (50.25%) than for WS1 (24.50%). As DNA analysis were not confirmed the presence of the point mutations responsible for sunflower resistance to imazamox and tribenuron-methyl.

Keywords: DNA analysis, gene flow, HR sunflower, weedy sunflower

Corresponding author: Sava Vrbničanin, University of Belgrade, Faculty of Agriculture, Namanjina 6, Belgrade, Serbia, E-mail: sava@agrif.bg.ac.rs, Tel:+381114413307

INTRODUCTION

The main concern associated with cultivation of imazamox and tribenuron-methyl resistant sunflower hybrids is potential gene flow from crop to wild or weedy sunflower. Inter- and intraspecific hybridization between sunflower crops and its related species or volunteer plants were well documented (LINDER *et al*., 1998; BURKE *et al*., 2002; FAURE *et al*., 2002; MASSINGA *et al*., 2003; RIESEBERG *et al*., 1999; PRESOTTO *et al*., 2012). Also, new developed sunflower varieties are about 65% autogamous (ASTIZ *et al*., 2011) and weedy populations which are result of their hybridization with wild sunflower are self-incompatible (GANDHI *et al*., 2005). Widespread of huge weedy sunflower populations in sunflower growing areas in Serbia represent potential risk for gene flow from resistant crop plants to weedy sunflower.

Resistance to herbicides build in many crops using genetic engineering (genetically modified (GM) crops) or conventional breeding (non-GM crops) methods. Significant number of crop hybrids resistant to different ALS (acetolactate synthase; also known as AHAS-acetohydroxyacid synthase) inhibiting herbicides including sunflower were developed using conventional breeding methods (JOCIĆ *et al*., 2008; JOCIĆ *et al*., 2011; GREEN, 2012). These crops produced without the use of genetic engineering and due to that have been accepted in countries where genetically modified crops are not allowed (TAN *et al*., 2005). Three technologies of weed control in sunflower based on resistance to ALS-inhibiting herbicides, including Clearfield®, Clearfield Plus® and ExpresSun® system have been developed (SALA and BULOS, 2012; PENESCU *et al*., 2018).

Production of herbicide-resistant crops provides multiple benefits, including: 1) improved yield through high efficacy of control on a wide spectrum of grass and broadleaf weed species; 2) improved unit cost of food production; 3) improved quality through removal of existing volunteers of the same species; 4) the possibility of using low-tillage systems; 5) control of parasitic weeds; 6) low herbicide application rates; 7) low mammalian toxicity; 8) environmental benefits; etc. (KAYA *et al*., 2004; GREEN, 2012; MASLIIOV *et al*., 2018). In the case of resistant-sunflower hybrids, except above mentioned, this technology provides more effective control of *Cirsium arvense*, *Ambrosia artemisiifolia* and other species from *Asteraceae* family, and greater cost-efficiency in the suppression of some annual broadleaf weeds after sunflower emergence where no herbicides for post-emergence control (MALIDZA *et al*., 2016). On the other hand, there is a potential risk of growing herbicide-resistant crops associated with disadvantages such as: 1) potential for development herbicide-resistant weed populations; 2) potential for development of herbicide-resistant volunteers; 3) risks for cross-pollination; 4) gene flow from resistant to susceptible relatives (GRESSEL, 2015).

One of the main concerns to grow herbicide-resistant crops is the pollen mediated flow of genes responsible for resistance to the wild or weedy relatives. Generally, gene flow have continuously occurred between related species including cultivated, wild or weedy relatives and have important role in evolution of many weed species (MORJAN and RIESEBERG, 2004). However, this phenomenon can play important role in movement of resistance between weed population/species (ZELAYA *et al*., 2007; MOLIN *et al*., 2016), between herbicide-resistant and –susceptible individuals of the same crop (DEVOS *et al*., 2005) and between herbicide-resistant crops and related wild/weedy species (MESSEGUER *et al*., 2004; ZHANG *et al*., 2006; BOZIC *et al*., 2015; PRESSOTTO *et al*., 2012; GRESSEL, 2015). Transfer of genes from herbicide resistant crops to their relatives depend on many factors including coexistence of pollen donor and receptor plant, overlapping flowering, presence of pollen vector and could be favoured by presence of

feral population and volunteers, out-crossing, self-incompatibility, large pollen source, large amounts of produced pollen, lightweight pollen, strong winds (for wind pollinated species), large insect populations (for insect pollinated species), long pollen viability etc. (MALLORY-SMITH and ZAPIOLA, 2008). The highest risk was estimated for sorghum, followed by oilseed rape, sugar beet, wheat, sunflower and alfalfa, while low level of risks for gene flow associated with maize, rice, potato, soybean, barley and bean (STEWART *et al*., 2003).

The first sunflower hybrids resistant to imidazolinone and tribenuron-methyl in Serbia developed in 2004 (JOCIĆ *et al*., 2004) and 2007 (JOCIĆ *et al*., 2011), respectively. Soon after that, these hybrids became dominant in the commercial sunflower cultivation. At the same time, weedy populations of the same species (*Helianthus annuus*) occupy sunflower growing areas, reaching up 1 000 ha in Southern Srem and around 7-8 000 ha in Southern Banat (STOJIĆEVIĆ *et al*., 2017). Weedy sunflower is troublesome weed for cultivated sunflower for many reasons including yield reduction (GEIER *et al*., 1996; MULLER *et al*., 2009; CASQUERO *et al*., 2013); quality and quantity reduction of oil (CASQUERO *et al*., 2013) and potential for gene flow from crop to weedy and contrary (ZHANG *et al*., 2006; PRESOTTO *et al*., 2012; BOZIC *et al*., 2015). The aim of the study is to check and quantify the potential gene flow from herbicide resistant sunflower hybrids to weedy sunflower.

MATERIALS AND METHODS

Seed source

Two independent experiments set up in 2009 in order to collect seeds for investigation potential gene flow from imazamox (WS1) and tribenuron-methyl (WS2) resistant sunflower hybrids to weedy plants (unpublished data). Experiments were located at two experimental sites (E1 and E2) in central part of Serbia in area without sunflower crops to avoid gene flow from any source out of experiment. The distance between experiments was about 4 km in order to avoid gene flow between experiments as well. In both experiments sunflower hybrids were sown at row long 50 m with interrow spacing 24 cm not regarding to hybrid type, which was imazamox resistant hybrid at E1 and, tribenuron-methyl resistant hybrid at E2. At distances 1, 2, 3, 4 and 5 m from hybrid row, weedy sunflower rows of equal length were sown with the same inter row distance. Mature seeds were collected from randomly selected plants and heads from every distance, cleaned by hand and stored indoor until used in the experiment.

Field experiment

Field experiment was conducted in 2012, near Belgrade (Serbia) at the Experimental Farm of the Faculty of Agriculture "Radmilovac" (44.75°N, 20.57°E). Seedlings were produced in a nursery, and when the seedlings were at the growth stage of two true leaves, they were transplanted in the field by hand during the first week of May. The experimental area was managed according to the traditional agronomic practices. Humidity was provided daily by watering the experiment until plants rooted. The meteorological conditions during the field experiment are given in Table 1. Growing degree days (GDD) were calculated using the formula (GILMORE and ROGERS, 1958):

$$GDD\ (°C) = \Sigma\ [(T_{max} + T_{min}) / 2 - T_{base}] \qquad [1]$$

where $T_{base} = 10°C$.

The experimental plot were divided into two subplots, one for WS1 (seeds collected from E1) and the other for WS2 (seeds collected from E2) plants. The experimental model in each subplot was a randomized complete block design with four replications. One hundred sixty plants (4 replication x 20 plants for herbicide application + 4 replication x 20 plants for control) for per distance for both WS1 and WS2 were transplanted in rows with interrow spacing 24 cm and distance between rows 70 cm. Plants were treated with herbicides at the four true leaves growth stage using a knapsack sprayer and TeeJet 1004 flat-fan nozzles to deliver a spray volume of 300 L water per hectare. WS1 plants were treated with recommended rate (48 g ai ha^{-1}) of imazamox (Pulsar-40, 40 g ai L^{-1}, SL, BASF, Germany) and WS2 were treated with recommended rate (22.50 g ai ha^{-1}) of tribenuron-methyl (Express 50-SX, 500 g ai kg^{-1}, WG, Du Pont, Switzerland). Control plants were not treated. Surviving weeds were removed by hand hoeing. Fresh weight of survived plants was recorded 15, 30, 45 and 60 days after treatment (DAT). Four plants per replication were measured. Differences between treated and control plants were tested using t-test. Final number of survived plants was recorded at maturity and cut off plants were included in calculation. The seeds from survived plants were collected and stored at room temperature until use for DNA analysis.

Table 1. Precipitation and GDD in 2012 at the experimental site.

Month	Precipitation (mm)	GDD
May	104.00	289.25
June	49.30	336.50
July	624.00	436.65
August	669.00	476.60
Total	1446.30	1539.00

DNA analyses

In order to check the presence of mutations responsible for sunflower resistance to herbicides (imazamox and tribenuron-methyl) in weedy sunflower accessions and confirm gene flow from resistant hybrids to weedy sunflower, DNA analysis were performed. Seeds collected from survived plants from field experiment (20 seeds per treatment; total 10 treatments = two WS accessions x 5 distances) were sown in pots (38cm^2 surface area) containing a commercial potting mix (Flora Gard TKS1, Germany). Pots were placed in greenhouse and manually irrigated as needed. For PCR analysis randomly selected 40 plants (20 WS1 and 20 WS2). Young leaf tissue (at the four leaves growth stage) was harvested and lyophilized after 24 h storage at -80°C for DNA isolation. Lyophilised samples were storage at room temperature until use.

Genomic DNA was isolated using a DNeasy ® Plant Mini Kit following the standard protocol for isolation of DNA from plant leaf tissue outlined in the DNeasy Plant protocol handbook (Qiagen Inc.). The quality and concentration of extracted DNA samples were determined using a Nanodrop® 1000. DNA extracts were stored at -20°C when not in use.

For amplification approximately 700bp fragments of *ALS* gene, the primers Hel ForA (CAATGGAGATCCACCAAGCT) and Hel RevA (AACGCAAGCAACAAATCACT) were used. These oligonucleotide primers were designed using DNA sequences from several sources (WHITE *et al*., 2003; KOLKMAN *et al*., 2004) and software Primer 3. Each PCR reaction contained

19 µl of mastermix (10 units Biomix, 7 units DEPC water, 1 unit forward primer and 1 unit reverse primer) and 1µl of DNA sample. Cycling conditions were: 2 min incubation at 94°C; 35 cycles of 30 sec denaturation at 94°C, 20 sec annealing at 53°C and 45 sec extension at 72°C; and 5 min final extension at 72°C. Visualization of PCR products were done on 2% low-melt agarose gel containing ethidium bromide.

Purified PCR products were sent together with the corresponding primer (Hel ForA) to Sorce Bioscience (Osford, UK) for sequencing. Analysis of obtained sequences were done based on comparison with sequences of the amplified region of *ALS* gene located in GenBank using a multiple sequence alignment program Clustal Omega.

RESULTS

Weedy sunflower response to herbicides

Response of WS1 and WS2 plants treated with imazamox and tribenuron-methyl, respectively, 15 DAT were similar for all distance treatments. Namely, all treated plants (based on visual estimation, data not shown) were strongly damaged by herbicides, which confirmed by fresh weight reduction (Figure 1 and 2). Recommended rates of imazamox (48 g ai ha^{-1}) and tribenuron-methyl (22.50 g ai ha^{-1}) reduced fresh weight of weedy sunflower plants significantly ($P<0.01$, Table 2) in comparison with control. At different treatments (distance), fresh weight of WS1 treated plants were less 78-83% than fresh weight of untreated plants, while fresh weight reduction of WS2 plants were between 79.50 and 85.50%. Some damaged plants were recovered 30 DAT and their fresh weight was less reduced (WS1: 43-50%; WS2: 30-35%) in comparison to untreated plants than two weeks before. Fresh weight of WS1 and WS2 treated plants 45 DAT was 15-24% and 3-12% less than fresh weight of untreated plants, respectively. At the last assessment (60 DAT) differences in fresh weight between treated and untreated plants in almost all treatments for both, WS1 and WS2, were less significant ($P>0.05$ or $0.01<P<0.05$) than in previous assessments (Table 2).

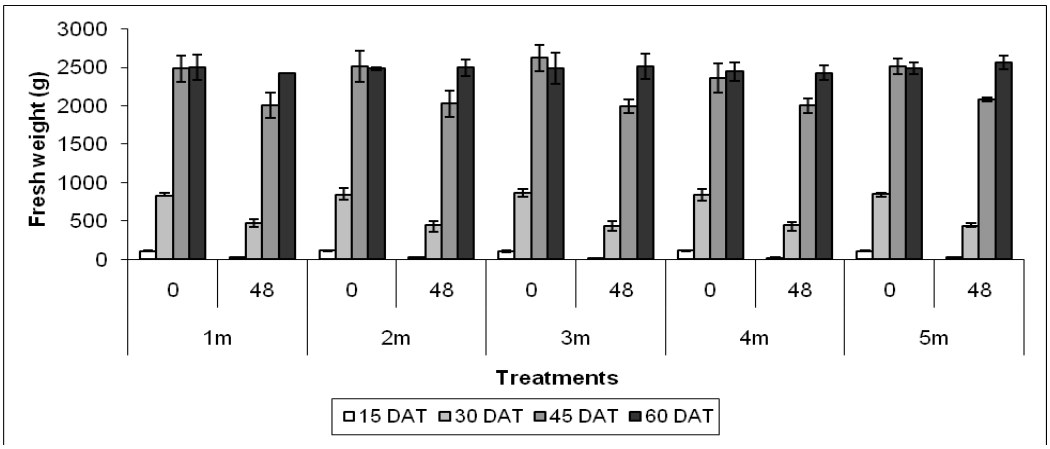

Figure 1. WS1 response to recommended rate of imazamox

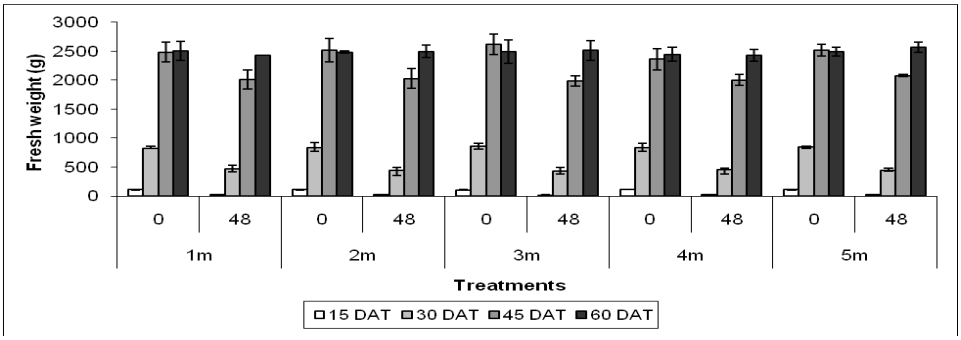

Figure 2. WS2 response to recommended rate of tribenuron-methyl

Table 2. Significance of differences between treated and untreated weedy sunflower plants

Distance	Assessment	Treated : untreated	
		WS1	WS2
1 m	15 DAHA	0.000000**	0.000000**
	30 DAHA	0.000000**	0.000000**
	45 DAHA	0.000561**	0.000051**
	60 DAHA	0.738626ns	0.017513*
2 m	15 DAHA	0.000061**	0.000320**
	30 DAHA	0.000079**	0.000543**
	45 DAHA	0.000717**	0.000065**
	60 DAHA	0.354553ns	0.11200ns
3 m	15 DAHA	0.000028**	0.0000051**
	30 DAHA	0.000000**	0.000000**
	45 DAHA	0.000079**	0.000016**
	60 DAHA	0.049280*	0.007252**
4 m	15 DAHA	0.000061**	0.000005**
	30 DAHA	0.000029**	0.000034**
	45 DAHA	0.000717**	0.000008**
	60 DAHA	0.032368*	0.044368*
5 m	15 DAHA	0.000000**	0.000000**
	30 DAHA	0.000020**	0.000012**
	45 DAHA	0.000542**	0.000000**
	60 DAHA	0.023689*	0.170047ns

** - $p < 0.01$; * - $p < 0.05$; ns- $p > 0.05$

Percentage of survived plants were different depend on weedy sunflower accession (WS1 or WS2) and distance of their mother plants from resistant sunflower hybrid in previous year (Table 3). General, higher percentage was recorded for WS2 (50.25%) than for WS1 (24.50%). In particular, the number of WS1 survived plants ranged from 6.25% (at distance 1 m)

to 40% (at distance 4 and 5 m). The highest percentage of WS2 survived plants were recorded for distance 1 m, while the smallest was recorded for distance 5 m.

Table 3. Percentage of survived plants in progeny of WS1 and WS2 after application recommended rate of imazamox and tribenuron-methyl, respectively.

	Survived plants (%)	
Distance	WS1	WS2
1 m	6.25	85
2 m	17.50	45
3 m	18.75	41.25
4 m	40.00	51.25
5 m	40.00	28.75
Total	24.50	50.25

DNA analysis

Fragments of *ALS* gene approximately 700bp were obtained by PCR (Figure 3), but their sequencing (Figure 4 and 5) were not confirmed the presence of the point mutations responsible for sunflower resistance to imazamox and tribenuron-methyl. Namely, mutations responsible for resistance to imazamox (alanine (GCG) to valine (GTG) in codon 205) and to tribnuron-methyl (proline (CCC) to leucine (CTC) in codon 197) were not detected. TNucleotide alignment of all sequences obtained from amplified fragments of *ALS* gene from WS1 and WS2 plants was shown full match between all samples. Examples of alignment for five randomly selected sequences from WS1 and WS2, were presented in Figures 4 and 5, respectively.

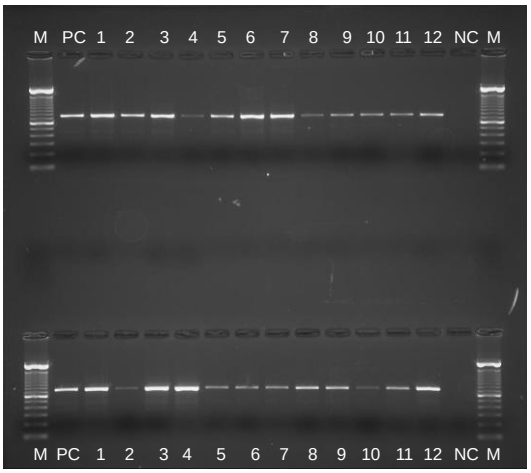

Figure 3. Amplification products of *ALS* gene fragments generated by the specific-primers Hel ForA and Hel RevA: M= DNA ladder; PC= positive control; upper lines 1-12= WS1; bottom lines 1-12= WS2; NC= negative control.

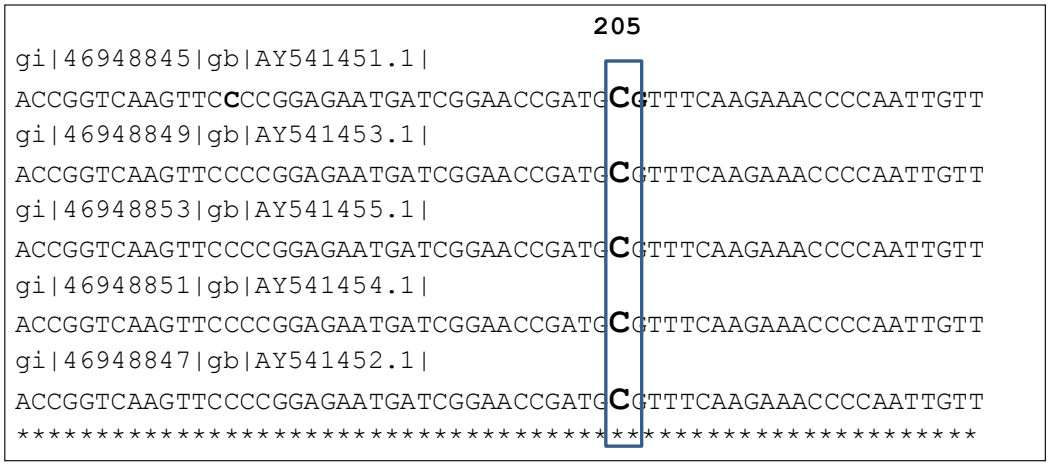

Figure 4. Nucleotide alignment of five randomly selected sequences of ALS fragment from WS1

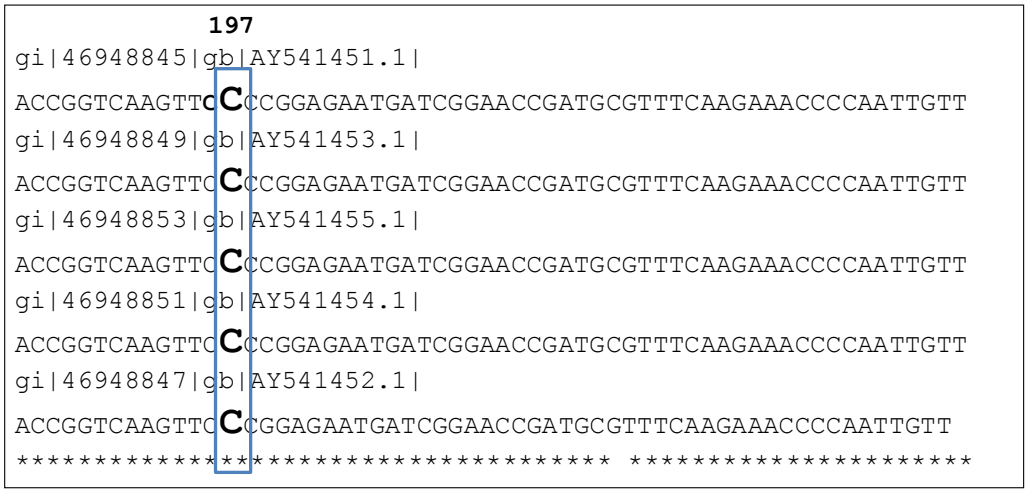

Figure 5. Nucleotide alignment of five randomly selected sequences of ALS fragment from WS2

DISCUSSION

The first step in estimation of gene flow between herbicide resistant crop and weedy plants could be assessment of the response of weedy plants progeny to herbicide. In our study, 15 DAT all treated plants (WS1 and WS2) were strongly damaged by recommended rates of herbicides (imazamox: 48 g ai $ha^{-1;}$ tribenuron-methyl: 22.50 g ai ha^{-1}) indicating susceptibility of WS1 and WS2 progeny to applied herbicides (Figure 1 and 2). Namely, statistically very significant reduction of treated plants fresh weight in comparison to untreated plants were recorded (Table 2). But, two weeks later (30 DAT) some damaged plants were recovered and kept growing, while some completely died independently to distance. At the last assessment (60 DAT) difference between survived treated and untreated plants was not so pronounced in almost all distances for both WS1 and WS2 (Table 2). Percentage of survived plants was less for WS1 than for WS2. Namely, at distances 1,2,3,4 and 5 m survived 6.25, 17.50, 18.75, 40 and 40% of WS1 plants, respectively, while surviving of WS2 at the same distances were 85, 45, 41.25, 51.25 and 28.75% of treated plants (Table 3). This is in accordance with previous studies which shown that progeny of weedy sunflower which were grown near tribenuron-methyl resistant sunflower survived this herbicide application in prominent percentage (BOZIC *et al*., 2015). The lack of gene flow could be explained as consequence of the short period of overlapping flowering time between the resistant hybrid, which pollination period (from July 1st to July 9th) was shorter than weedy sunflower pollination period (from July 7th to July 21th). Obtained percentage of progeny surviving in our study (from 6.25, to 40% for WS1 and from 28.75 to 85% for WS2 depend on distance) was much higher than percentage of hybridization between cultivated sunflower and *Helianthus petiolaris*, which was in rage 0.3 to 0.5% depend on flowering period and presence of common pollinators (GUTIERREZ *et al*., 2010). Also, it was higher than surviving of progeny of weedy sunflower grown near tribenuron-methyl resistant sunflower hybrid after this herbicide application, which was between 5 and 25% depend on distance and wind direction (BOZIC *et al*., 2015). On the other hand, level of surviving was in line with wild sunflower progeny surviving (33%) after exposure to gene flow from cultivated sunflower (BURKE *et al*., 2002). Also, the percentage of the surviving progeny of wild sunflower growing near imidazolinones-resistant hybrid was 27% at 3 m distance (MASSINGA *et al*., 2003), while WHITTON *et al*. (1997) estimated that 42% of the wild sunflower progeny at 3m distance from the cultivated sunflower represent its hybrids.

The amino acid substitution in ALS enzyme, represented by 28 possible substitutions identified in different weed species, is the main mechanism of target-site based resistance to ALS inhibiting herbicides (POWLES and YU, 2010; BROSNAN *et al*., 2016). Therefore, molecular analyses are necessary to confirm transfer of genes responsible for resistance from cultivated to weedy plants. It is well known, resistance to imidazolinones in sunflower resistant hybrids correlated with mutation alanine (GCG) to valine (GTG) in codon 205, whereas resistance to sulfonylurea herbicides correlated with mutation proline (CCC) to leucine (CTC) in codon 197 (KOLKMAN *et al*., 2004). Although all treated plants were strongly damaged 15 DAT, high percentage of WS1 and WS2 progeny were recovered 30 DAT. Due to that high percentage of surviving were recorded at maturity indicating possibility of gene flow. But, DNA analysis shown opposite results. Namely, mutations in codons 205 and 197 were not confirmed in DNA sequences from survived weedy sunflower plants (Figure 4 and 5). Therefore, it is clear that plant surviving after imazamox and tribenuron-methyl application in the field conditions was not consequence of gene flow and possible explanation could be plant regrowth from lateral buds.

Similar to that, recovery of common and *H. petiolaris* after imazamox application MASSINGA *et al*. (2003) attributed to regrowth development from lateral buds.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. III46008). No conflicts of interest have been declared.

Received, April 28th, 2018
Accepted November 18th, 2018

REFERENCES

ASTIZ, V., L.A., IRIARTE, A., FLEMMER, L.F., HERNANDEZ (2011): Self compatibility in modern hybrids of sunflower (*Helianthus annuus* L.) fruit set in open and self-pollinated (bag isolated) plants grown in two different locations. Helia, *34*: 129-138.

BURKE, J., K., GARDNER, L., RIESEBERG (2002): The potential for gene flow between cultivated and wild sunflower (*Helianthus annuus*) in the United States. Am. J. Bot., *89*: 1550-155.

BOZIC, D., D., PAVLOVIC, V., BREGOLA, A., DI LORETO, S., BOSI, S., VRBNICANIN (2015): Gene flow from herbicide-resistant sunflower hybrids to weedy sunflower. JPDP, *122*: 183-188.

BROSNAN, J.T., J.J., VARGAS, G.K., BREEDEN, L., GRIER, R.A., APONTE, S., TRESCH, M., LAFOREST (2016): A new amino acid substitution (Ala-205-Phe) in acetolactate synthase (ALS) confers broad spectrum resistance to ALS-inhibiting herbicides. Planta, *243*: 149-159.

CASQUERO, M., A., PRESOTTO, M., CANTAMUTTO (2013): A case study of intraspecific/interbiotype interference promoted by human activity. Field Crop Res., *142*: 95-101.

DEVOS, Y., D., REHEUL, A., DE SCHRIJVER (2005): The co-existence between transgenic and non-transgenic maize in the European Union: a focus on pollen flow and cross-fertilization. Environ. Biosafety Res., *4*: 71–87.

FAURE, N., H., SERIEYS, A., BERVILLÉ (2002): Potential gene flow from cultivated sunflower to volunteer, wild Helianthus species in Europe. Agric., Ecosys. Environ., *89*: 183–190.

GANDHI, S.D., A.F., HEESACKER, C.A., FREEMAN, J., ARGYRIS, K., BRADFORD, S.J., KNAPP (2005): The self-incompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. TAG, *111*: 619-629.

GEIER, P.W., L.D., MADDUX, L.J., MOSHIER, P.W., STAHLMAN (1996): Common sunflower (*Helianthus annuus*) interference in soybean (*Glycine max*). Weed Techn., *10*: 317-321.

GILMORE, E.C., R.S., ROGERS (1958): Heat units as a method of measuring maturity in corn. Agr. J., *50*: 611–615.

GREEN, J.M. (2012): The benefits of herbicide-resistant crops. Pest Manag. Sci., *68*: 1323–1331.

GRESSEL, J. (2015): Dealing with transgene flow of crop protection traits from crops to their relatives. Pest Manag. Sci., *71*: 658–667.

GUTIERREZ, A., A., CARRERA, J., BASUALDO, R., RODRIGEZ, M., CANTAMUTTO, M., POVERENE (2010): Gene flow between cultivated sunflower and *Helianthus petiolaris* (Asteraceae). Euphytica, *172*: 67-76.

JOCIĆ, S., D., ŠKORIĆ, G., MALIDŽA (2004): Sunflower tolerant to imidazolinone herbicides. J. Sci. Agric. Research., *65 (229)*: 81-89. (In Serbian)

JOCIĆ, S., G., MALIDŽA, N., HLADNI, S., GVOZDENOVIĆ (2008): New hybrids of sunflower tolerant to tribenuron-methyl. Zbornik radova Naučnog instituta za ratarstvo i povrtarstvo, *45*: 89-95. (in Serbian)

JOCIĆ, S., G., MALIDŽA, S., CVEJIĆ, N., HLADNI, V., MIKLIČ, D., ŠKORIĆ (2011): Development of sunflower hybrids tolerant to tribenuron-methyl. Genetika, *43(1)*: 175-182.

KAYA, Y., M., DEMIRCI, G., EVCI (2004): Sunflower (*Helianthus annuus* L.) breeding in Turkey for broomrape (*Orobanche cernua* L.) and herbicide resistance. Helia, *27*: 199–210.

KOLKMAN, J.M., M.B., SLABAUGH, J.M., BRUNIARD, S., BERRY, B.S., BUSHMAN, C., OLUNGU, N., MAES, G., ABRATTI, A., ZAMBELLI, J.F., MILLER, A., LEON, S.J., KNAPP (2004): Acetohydroxyacid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower. TAG, *109*: 1147-1159.

LINDER, C.R., I., TAHA, G.J., SEILER, A.A., SNOW, L.H., RIESEBERG (1998): Long-term introgression of crop genes into wild sunflower populations. TAG, *96*: 339–347.

MALIDZA, G., S., VRBNICANIN, D., BOZIC, S., JOCIC (2016): Integrated weed management in sunflower: challenges and opportunities. Proc 19th International Sunflower Conference, Edirne, Turkey, 88-97.

MALLORY-SMITH, C. and M., ZAPIOLA (2008: Gene flow from glyphosate-resistant crops. Pest Manag. Sci., *64*: 428–440.

MASLIIOV, S.V., N.J.U., MACAI, O.O., BESEDA, V.V., STEPANOV (2018): Control of Broomrape Orobanche Cumana Wallr. Ukrainian Journal of Ecology, *8*: 74-80.

MASSINGA, R.A., K., AL-KHATIB, P.ST., AMAND, J.F., MILLER (2003): Gene flow from imidazolinone-resistant domesticated sunflower to wild relatives. Weed Sci., *51*: 854-862.

MESSEGUER, J., V., MARFA, M.M., CATALA, E., GUIDERDONI, E., MELE (2004): A field study of pollen-mediated gene flow from Mediterranean GM rice to conventional rice and the red rice weed. Mol. Breed., *13*:103–112.

MOLIN, W.T., V.K., NANDULA, A.A., WRIGHT, J.A., BOND (2016): Transfer and Expression of ALS Inhibitor Resistance from Palmer Amaranth (*Amaranthus palmeri*) to *A. spinosus* x *A.palmeri* Hybrid. Weed Sci., *64*: 240-247.

MORJAN, C.L. and L.H., RIESEBERG (2004): How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Mol. Ecol., *13*: 1341-1356.

MULLER, M.H., F., DELIEUX, J.M., FERNANDEZ MARTINEZ, B., GARRIC, V., LECOMTE, G., ANGLADE, M., LEFLON, C., MOTARD, R., SEGURA (2009): Occurence, distribution and distinctive morphological traits of weedy *Helianthus annuus* L. populations in Spain and France. Gen. Res. Crop Evol., *56*: 869-877.

PENESCU, A., M., BRAN, M., NICHITA, N., IONESCU, D., SANDOIU, C., CIONTU, T., SCHIOPU, M., GADEA, C., SONEA, C., BOLOHAN (2018): Comparative study regarding the influence of herbicides on the yield of sunflower crops, the crops being obtained with conventional, clearfield and expressun technologies in the field conditions of Moara Domestica. Agronomy, *LXI*: 367-374.

POWLES, S.B., Q., YU (2010): Evolution in Action: Plants Resistant to Herbicides. Ann. Rev. of Plant Biol., *61*: 317-347.

PRESOTTO, A., M.S., URETA, M., CANTAMUTTO, M., POVERENE (2012): Effects of gene flow from IMI resistant sunflower crop to wild *Helianthus annuus* populations. Agric. Ecosyst. Environ., *146*: 153-161.

RIESEBERG, L.H., J., WHITTON, K., GARDNER (1999): Hybrid zones and genetic architecture of barrier to gene flow between two sunflower species. Genetics, *152*: 713-727.

SALA, C.A. and M., BULOS (2012): Inheritance and molecular characterization of broadrange tolerance to herbicides targeting acetohydroxyacid synthase insunflower. TAG, *124*: 355-364.

SAULIC, M., D., STOJICEVIC, A., MATKOVIC, D., BOZIC, S., VRBNICANIN (2013): Population variability of weedy sunflower as invasive species. Proc 4th ESENIAS Workshop: International Workshop on IAS in Agricultural and Non-Agricultural Areas in ESENIAS Region, Çanakkale, Turkey, 79-85.

STEWART, C.N., M.D., HALFHILL, S.I., WARWICK (2003): Transgene introgression from genetically modified crops to their wild relatives. Nat. Rev. Genet., *4*: 806–817.

STOJIĆEVIĆ, D., A., ILIĆ, T., SEKULIĆ, V., STUPAR, D., BOŽIĆ, S., VRBNIČANIN (2017): Distribution of weedy sunflower on territory of Republic of Serbia and potential risks for agriculture. J. Hortic., Forestry and Biotech., *21(2)*: 132-137.

TAN, S., R.R., EVANS, M.L., DAHMER, B.K., SINGH, D.L., SHANER (2005): Imidazolinone-resistant crops: history, current status and future. Pest Manag. Sci., *61*: 246-257.

VRBNICANIN, S., D., BOZIC, D., PAVLOVIC, M., SARIC- KRSMANOVIC, D., STOJICEVIC, A., ULUDAG (2017): Fitness studies on invasive weedy sunflower populations from Serbia. Rom. Biotech. Lett., *22*: 12464-12472.

WHITE, A.D., M.A., GRAHAM, M.D.K., OWEN (2003): Isolation of acetolactate synthase homologs in common sunflower. Weed Sci., *51*: 845-853.

WHITTON, D., E., WOLF, D.M., ARIAS, A.A. SNOW, L.H., RIESEBERG (1997): The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. TAG, *95*: 33-40.

ZELAYA, I.A., M.D.K., OWEN, M.J., VANGRESSEL (2007): Transfer of glyphosate resistance: evidence of hybridization in *Conyza* (Asteraceae). Am. J. Bot., *94*: 660-673.

ZHANG, W., S.D., LINSCOMBE, E., WEBSTER, S., TAN, J., OARD (2006): Risk assessment of the transfer of imazethapyr herbicide tolernce from Clearfield rice to red rice (*Oryza sativa*). Euphytica, *152*: 75-86.

POTENCIJALNI TRANSFER GENA SA SUNCOKRETA TOLERANTNOG PREMA HERBICIDIMA NA HIBRIDNE FORME DIVLJEG SUNCOKRETA

Dragana BOŽIĆ[1], Markola SAULIĆ[1], Aleksandra SAVIĆ[2], George GIBBINGS[3], Sava VRBNIČANIN[1]*

[1]Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd, Srbija
[2]Institut za zaštitu bilja i životnu sredinu, Beograd, Srbija
[3]Fakultet za poljoprivredu, agrarnu politiku i razvoj, Univerzitet u Redingu, Reding RG6 6AR, UK

Izvod

Gajenje suncokreta tolerantnog prema herbicidima povezano je sa rizikom od transfera gena odgovornih za rezistentnost sa ovih useva na hibridne forme divljeg suncokreta. Radi procene potencijalnog transfera gena sa hibrida suncokreta tolerantnih prema imazamoksu i tribenuron-metilu na hibridne forme divljeg suncokreta obavljena su istraživanja u polju i na molekularnom nivou. Potomstvo hibridnih formi divljeg suncokreta koji je u prethodnim istraživanjima rastao u blizini hirida tolerantnog na imazamoks (WS1) i hibrida tolerantnog na tribenuron-metil (WS2) je korišćeno kao polazni materijal za istraživanje. Biljke WS1 i WS2 su u poljskom ogledu tretirane preporučenim količinama imazamoksa i tribenuron-metila i praćeno je njihovo preživljavanje. Takođe, utvrđen je efekat herbicida na svežu masu biljaka. Prisustvo mutacija odgovornih za rezistentnost na herbicide (imazamoks i tribenuron-metil) je provereno na osnovu DNK analize slučajno odabranih biljaka koje su preživele primenu herbicida. Procenat biljaka koje su preživele primenu herbicida zavisio je od toga da li se radilo o WS1 ili WS2 biljkama i od rastojanja biljaka od čijeg semena potiču u odnosu na toleratni hibrid suncokreta u prethodnim eksperimentima. Veći procenat preživljavanja je utvrđen za WS2 (50,25%) nego za WS1 (24,50%). S obzirom da analizom DNK nije potvrđeno prisustvo tačkastih mutacija odgovornih za rezistentnost suncokreta prema imazamoksu i tribenuron-metilu, preživljavanje tretiranih biljaka se može objasniti kao posledica oporavka biljaka aktiviranjem bočnih pupoljaka.

Primljeno 28.IV.2018.
Odobreno 18. XI. 2018.