

## **DIMINISHING HERBICIDE STRESS IN MAIZE INBRED LINES BY APPLICATION OF FOLIAR FERTILISER**

M. BRANKOV<sup>a\*</sup>, V. DRAGICEVIC<sup>a</sup>, M. SIMIC<sup>a</sup>, M. FILIPOVIC<sup>a</sup>,  
M. KRESOVIC<sup>b</sup>, V. MANDIC<sup>c</sup>

<sup>a</sup>*Maize Research Institute, 1 Slobodana Bajic Street, 11 185 Zemun Polje, Belgrade-Zemun, Serbia*

*E-mail: mbrankov@mrizp.rs*

<sup>b</sup>*Faculty of Agriculture, University of Belgrade, 6 Nemanjina Street, 11 000 Belgrade, Serbia*

<sup>c</sup>*Institute for Animal Husbandry, Zemun, Serbia*

**Abstract.** Dominance of grass weeds in maize crop occurs due to a lack of selective herbicides for their control. With sulphonylurea herbicides this problem became under control, but a problem with selectivity was developed, particularly in maize seed crop. The effect of sulphonylureas and foliar fertiliser on maize lines was evaluated by visual estimation, grain yield, as well as the alterations in the content of antioxidants: free thiolic groups, phenolics and soluble proteins in the leaves. The proteins content did not vary significantly under the influence of herbicides, compared to the control, opposite to free thiolic groups and phenolics. The differences in the content of phenolics and thiolic groups in the treatments with herbicides plus foliar fertiliser indicated that herbicide stress was more rapidly overcome. Most of the genotypes expressed significant increase of grain yield in the treatments with foliar fertiliser, compared to control and analogous treatments with herbicides.

*Keywords:* maize lines, antioxidants, sulphonylurea, fertilising.

### **AIMS AND BACKGROUND**

The aims of this study were to examine the possible phytotoxic effect of sulphonylurea herbicides and the potential anti-stress effect of an amino acid foliar fertiliser on maize inbred lines. These goals were realised by measuring the visual damages and grain yield, alterations in the content of soluble proteins and antioxidants, such as free thiolic groups (PSH) and phenolics, in the leaves of five maize inbred lines.

The technology of maize production, particularly seed production, involves the use of herbicides. Before the introduction of the sulphonylureas, there were no herbicides for the control of grass/narrow weeds in maize crop. Sulphonylurea herbicides are used in low quantities per area unit and have favourable ecotoxicological properties<sup>1</sup>, with excellent results in weed control in maize hybrid

---

\* For correspondence.

crop<sup>2</sup>. However, their use in maize seed crop, which is generally more sensitive than hybrid crop<sup>3</sup> has created new problem.

The purpose of herbicide use is to eliminate weeds, but sometimes they could express phytotoxic effects and lead to yield loss<sup>4</sup>. The herbicides can cause temporary or permanent stress in plants, depending on the genotype, applied herbicide and agro-meteorological conditions<sup>5</sup>. In case of temporary stress, plants can recover and resume normal growth and development, while in the case of permanent stress, the yield is reduced plant could die. Plant tolerance to herbicides could be located at the site of herbicide action or else. This type of tolerance, i.e. tolerance located away from the site of action involves detoxification systems, such as antioxidants, like glutathione<sup>6</sup>, phenolic compounds<sup>7</sup>, phytate<sup>8</sup> and many others, which are used to eliminate free radicals produced by stress.

Mineral fertilisation is one of the essential cropping practices, providing optimum plant growth and development. Foliar fertilising enables rapid absorption and quick plant response to the applied fertiliser<sup>9</sup>. Stress in plants can lead to the production of various reactive molecules which could damage the cellular components, and increase plant energy consumption for stress eradication<sup>10</sup>. The direct supply of amino acids and other nutrients facilitates protein synthesis and plant growth. An additional impact of foliar N application is reflected through better maize yields<sup>11,12</sup>. Brankov et al.<sup>13</sup> also stated that a combination of amino acid fertilisers with agro-chemicals, such as herbicides, could have positive effects on plant status and could help plants to overcome stress.

## EXPERIMENTAL

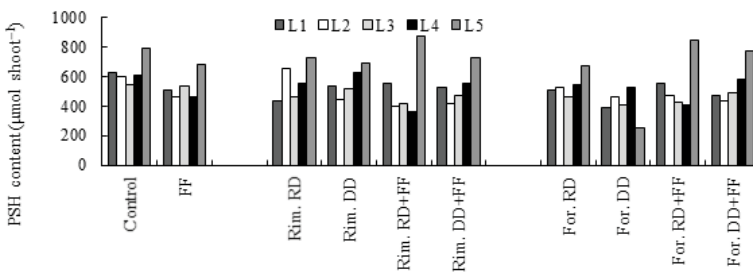
The field experiment was conducted during 2010 and 2011 in the experimental field of the MRI, in Zemun Polje. The influences of two sulphonylurea herbicides in recommended (RD) and double dose (DD) (i.e. rimsulphuron 15 g active ingredients ha<sup>-1</sup> and 30 g active ingredients ha<sup>-1</sup> and foramsulphuron 45 g active ingredients ha<sup>-1</sup> and 90 g active ingredients ha<sup>-1</sup>) and the foliar fertiliser (FF), (formulation: 12N:4P<sub>2</sub>O<sub>5</sub>:6K<sub>2</sub>O + 0.2 MgO + microelements + amino acids) were examined in the experiment. Foliar fertiliser was applied together with the herbicides in an amount of 4 l ha<sup>-1</sup>. The four-replicate trial was set up according to the split-plot arrangement. Plant samples were taken 48 h and 2–3 weeks after herbicide and foliar fertiliser application and dried at 40°C in ventilation dryer. Samples were analysed for the content of free thiolic groups (PSH) by the method of de Kok et al.<sup>14</sup>, phenolics – by the method of Simić et al.<sup>15</sup> and soluble proteins (SP) = by the method of Lowry et al.<sup>16</sup> In addition, herbicide toxicity<sup>17</sup> was visually evaluated 2–3 weeks after treatments. The grain yield was measured at the end of vegetation.

The obtained data were statistically processed by ANOVA and differences between means were tested by the least significant difference test (LSD<sub>0.05</sub>). The

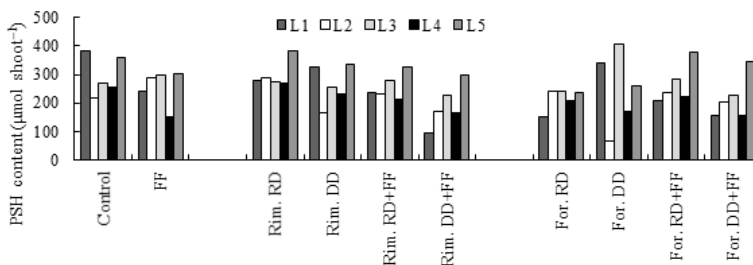
dependences between the contents of SP, phenolics and PSH under the influence of the herbicides and FF were obtained by regression analysis.

## RESULTS AND DISCUSSION

Generally, a decrease in the PSH content was obtained 48 h after application of the herbicide and FF in all applied treatments, compared to the control (Fig. 1), which could represent its consumption in the defence against oxidative attack, induced by herbicide stress<sup>18</sup>. The RD of herbicides resulted in a significant decrease in the PSH content in L1, L2, L3 and L4 inbred lines. Only in the line L5 increased PSH content was recorded in treatments with both: herbicides + FF, as well as in line L2 in rimsulphuron treatment, when compared to the control. A DD of the herbicides reduced the PSH content to a higher degree, compared to treatment with the RD. In lines L1 and L5, a double dose of the both herbicides significantly decreased the PSH content (38.2 and 68.3%, respectively), indicating higher oxidative stresses<sup>19</sup>. It is interesting to emphasise that in foramsulphuron + FF treatment, PSH content was increased in lines L1, L3, L4 and L5.



**Fig. 1.** Influence of applied herbicides and FF on the PSH content in the shoots of 5 maize inbred lines, 48 h after treatments of ( $LSD_{0.05} = 98.06$ )

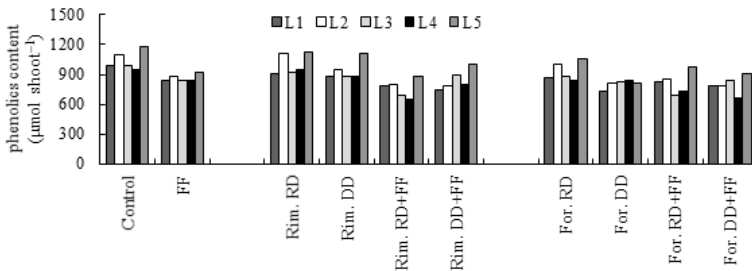


**Fig. 2.** Influence of applied herbicides and FF on the PSH content in the shoots of 5 maize inbred lines, 2–3 weeks after treatment ( $LSD_{0.05} = 67.23$ )

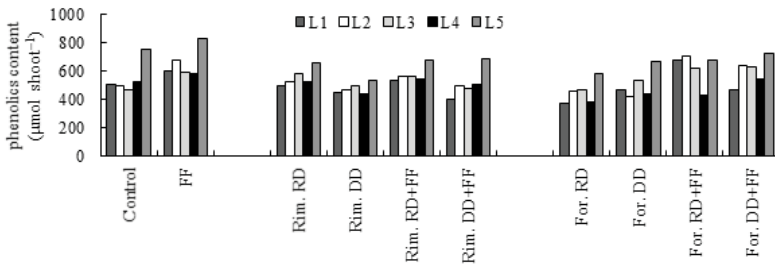
In period of 2–3 weeks after treatments a significant reduction of the PSH content was observed, particularly in the DD treatments (Fig. 2). At RD, a signifi-

cant reduction of the PSH content was noticed in lines L1, L4 and L5 compared to the control (down to 60.5%). The lowest variations in the PSH content were recorded in line L5 under DD treatment, while in the other lines, the applied treatments caused significant variation in the PSH content, the highest in L2 (down to 69.1%) and L1 (down to 74.8%) compared to the control.

Phenolics content also varied among the treatments (Fig. 3). It is well known that phenolic compounds have antioxidant functions and represent a part of the detoxification mechanisms<sup>20</sup>. Both applied herbicides decreased the content of phenolics, compared to the control. Higher values of phenolics were recorded in the treatments with only an herbicide, compared to herbicide plus FF treatment. Dragicevic et al.<sup>18</sup> also indicated that the application of various herbicides increased the content of phenolics in maize leaves.



**Fig. 3.** Influence of applied herbicides and FF on the phenolics content in the shoots of 5 maize inbred lines, 48 h after treatment ( $LSD_{0.05} = 114.7$ )

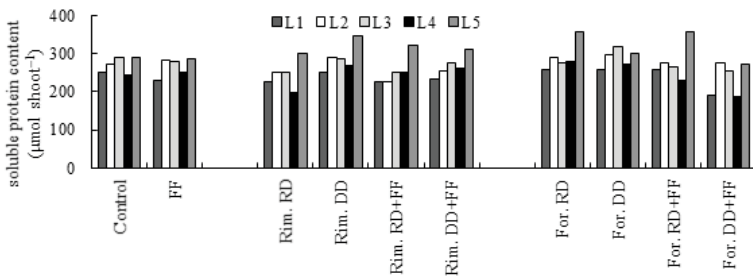


**Fig. 4.** Influence of applied herbicides and FF on the phenolics content in the shoots of 5 maize inbred lines, 2–3 weeks after treatment ( $LSD_{0.05} = 93.23$ )

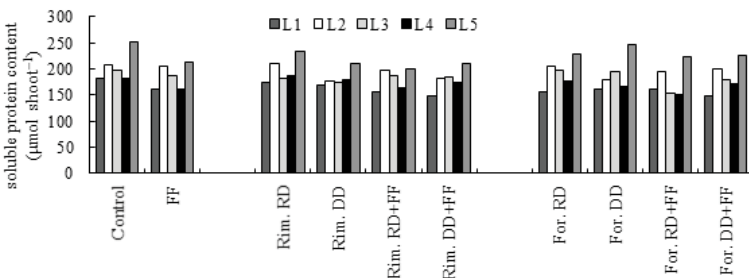
Similar to the variations in PSH, the content of phenolics was decreased during the period from 48 h to 2–3 weeks after herbicide application (Fig. 4). Moreover, in the second measuring in the herbicide plus FF treatments at the RD, higher values of phenolics were recorded in all lines, compared to treatment with the herbicides alone. This may indicate a greater expenditure of phenolics during this period in the leaves of the lines that were not treated with FF. Similar results were recorded

at the DD of herbicides when all lines, except line L1, had higher phenolics values compared to the treatment with herbicides only.

The SP content had the least variation, compared to the two previously tested parameters. The SP represent short polypeptide chains and their content in the leaves of susceptible maize genotypes, was increased under the influence of sulphonylureas<sup>3</sup>, possibly as a consequence of the obstruction of polypeptide polymerisation. These herbicides have a very specific site of action, inhibiting the synthesis of essential amino-acids and polypeptide chain polymerisation, blocking strongly cell division<sup>21</sup>. Among genotypes, tested, the largest increase in the SP content was observed in L5 (18.4%) and in L3 in foramsulphuron treatment (16.1%), compared to the control (Fig. 5). It is interesting that the application of DD of herbicide together with FF also induced increases in SP, but to a smaller extent than the treatments with herbicides alone. The largest reduction of SP content was recorded in lines L2 (up to 17%) and L4 (up to 19%) at the RD of herbicide. 2–3 weeks after treatment, a slight decrease in the SP content was observed in all genotypes after application of the treatments with herbicides plus FF. At the same phase, significantly higher content of SP was observed in lines L2 and L5, with the DD treatments (Fig. 6).



**Fig. 5.** Influence of applied herbicides and FF on the SP content in the shoots of 5 maize inbred lines, 48 h after treatment ( $LSD_{0.05} = 25.63$ )



**Fig. 6.** Influence of applied herbicides and FF on SP content in shoots of 5 maize inbred lines, 2–3 weeks after treatment ( $LSD_{0.05} = 14.95$ )

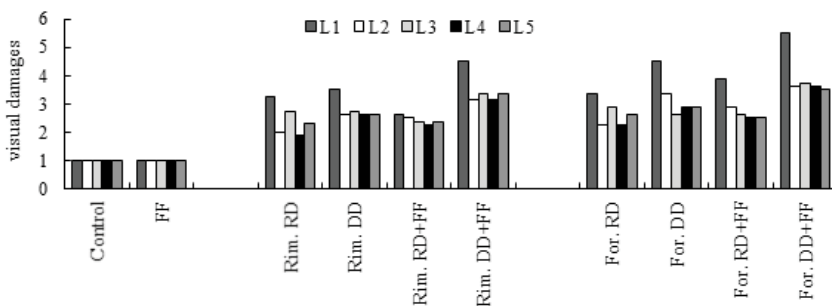
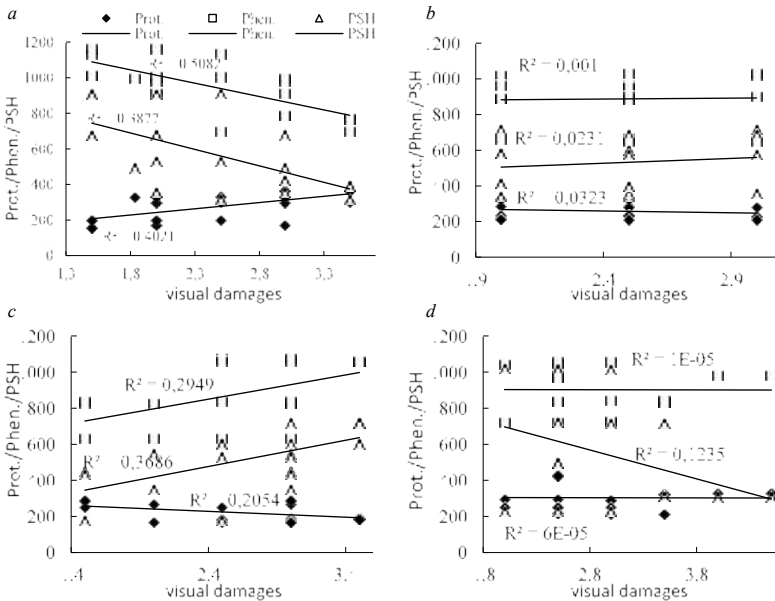


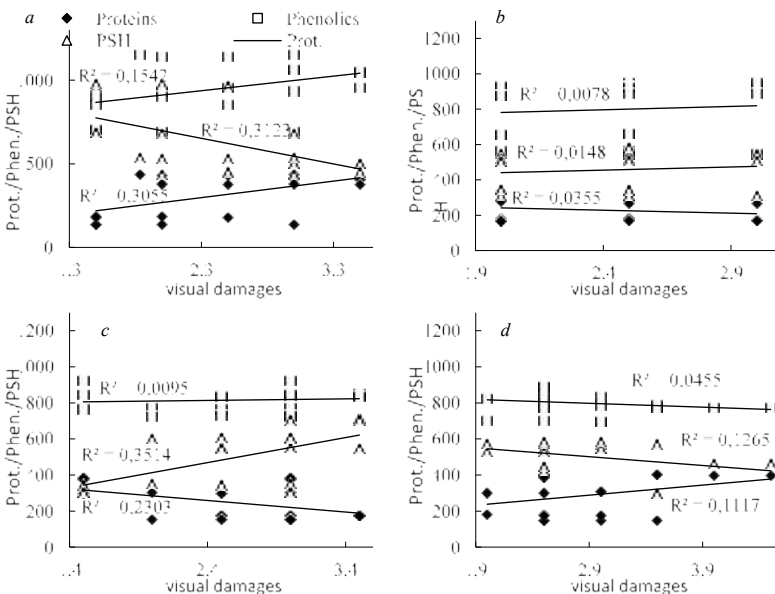
Fig. 7. Visual damages of the 5 maize inbred lines (LSD<sub>0.05</sub> = 1.13)

The maize lines showed different sensitivity to herbicides according to the estimated visual damages (Fig. 7). Stefanovic et al.<sup>22</sup> also indicated that sulphonylureas caused significant damages in maize lines. As was expected, line L1 was the most sensitive to both doses of sulphonylurea herbicides, where light to moderate damages were recorded, while very light to light damages were recorded in the other genotypes. In the DD treatments, higher damages were recorded than in the RD treatments. Less visual damages were registered for lines L1, L3 and L5 in the treatments with RD + FF, when compared to the control. DD induced somewhat higher damages in comparison to RD.

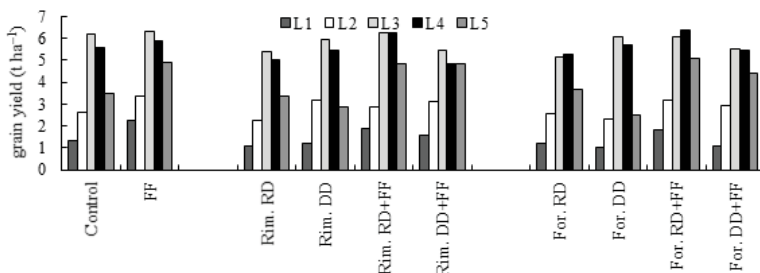
The application of rimsulphuron induced significant increases in the amount of SP, parallel with increase of visual damages (Fig. 8a). On the other hand, the contents of phenolics and PSH significantly decreased with increase of visual damages at RD. The variation of SP and phenolics at DD of rimsulphuron were insignificant (Fig. 9a). The application of foramsulphuron induced inversely proportional trends in the examined parameters: parallel with increase of visual damages, the contents of phenolics and PSH increased and the SP content decreased at RD (Fig. 8c). The application of FF diminished the impact of rimsulphuron on plants to an insignificant level (Fig. 8b). In the foramsulphuron treatments, FF induced significant decrease of PSH with raise of visual damages at both doses (Figs 8d and 9d). The results could indicate that the examined antioxidants could diminish rimsulphuron toxicity and that they could be mitigate toxic effects of this herbicide<sup>19</sup>. The increase in the contents of the examined antioxidants under foramsulphuron treatment could indicate that their amount was not sufficient to diminish the herbicide stress, or some other detoxification mechanism could be involved in toxicity suppression.



**Fig. 8.** Interdependence between the contents of SP (mg shoot<sup>-1</sup>), phenolics (µg shoot<sup>-1</sup>), PSH (µmol shoot<sup>-1</sup>) 48 h after treatment and visual damages in treatments with RD of the herbicides: *a* – rimsulphuron, *b* – rimsulphuron + FF, *c* – foramsulphuron and *d* – foramsulphuron + FF



**Fig. 9.** Interdependence between the contents of SP (mg shoot<sup>-1</sup>), phenolics (µg shoot<sup>-1</sup>), PSH (µmol shoot<sup>-1</sup>) 2–3 weeks after treatment and visual damages in treatments with DD of herbicides: *a* – rimsulphuron, *b* – rimsulphuron + FF, *c* – foramsulphuron, and *d* – foramsulphuron + FF



**Fig. 10.** Influence of applied herbicides and FF on the grain yield of 5 maize inbred lines ( $LSD_{0.05} = 0.65$ )

A positive effect of the FF on grain yield was observed in all maize lines and in all treatments, particularly in DD (Fig. 10). Positive effects of nitrogen fertilisation in maize is also reported by Shirazi et al.<sup>23</sup> Overall, the herbicides significantly decreased the grain yield of all lines, even at RD. On the other hand, the application of FF increased the grain yield, as a result of the stress reduction caused by the herbicides. Brankov et al.<sup>13</sup> stated in previous research that treatments with herbicide plus FF had higher yield in comparison to herbicide treatments alone. The highest yields of all examined genotypes were achieved when FF was applied in RD treatments, compared to the control. Among tested lines, line L5 had the highest grain yield in treatments with rimsulphuron plus FF (up to 40.9%) and foramsulphuron + FF (up to 42.6%) at both levels of applied herbicides. Also, here is very significant to emphasise that maize seed crop is more than ten times valuable production than basic maize production, and possibility of obtaining the higher grain yields is very important.

## CONCLUSIONS

Based on the obtained results, it could be concluded that application rimsulphuron and foramsulphuron in seed maize could be safe. However, an initial testing of the sensitivity of each inbred line is required. An increase in the content of SP could be a good indicator of sulphonylurea stress in the early stages of lines development. Nevertheless, the possibility of herbicide stress mitigation by application of foliar fertiliser with micronutrients and amino acids have practical importance and it is based probably on the alteration of antioxidant status.

**Acknowledgements.** This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Projects TR 31037).



## REFERENCES

1. H. M. BROWN: Mode of Action, Crop Selectivity and Soil Relations the Sulphonylurea Herbicides. *Pestic Sci*, **29**, 263 (1990).
2. C. L. FOY, H. L. WITT: Johnsongrass Control with DPX-V9360 and CGA-136872 in Corn (*Zea mays*) in Virginia. *Weed Technol*, **4**, 615 (1990).
3. L. SREFANOVIĆ, M. SIMIĆ, V. DRAGIČEVIĆ: Studies on Maize Inbred Lines Susceptibility to Herbicides. *Genetika*, **42** (1), 155 (2010).
4. M. BRANKOV, V. DRAGIČEVIĆ, M. SIMIĆ, S. VRBNIČANIN, I. SPASOJEVIĆ: The Sensitivity of Maize Lines to Different Herbicides. In: Proc. of the International Symposium on Current Trends in Plant Protection, Belgrade, September 25–28, year???, 178–182.
5. S. J. P. de CARVALHO, M. NICOLAI, R. RODRIGUES FERREIRA, A. V. de OLIVEIRA FIGUEIRA, P. J. CHRISTOFFOLETI: Herbicide Selectivity by Differential Metabolism: Consideration for Reducing Crop Damages. *Science Agriculture (Piracicaba, Brazil)*, **66** (1), 136 (2009).
6. L. L. van EERD, R. E. HOAGLANG, R. M. ZABLOTOWICH, J. C. HALL: Pesticide Metabolism in Plants and Microorganisms. *Weed Sci*, **51**, 472 (2003).
7. M. D. R. PIZZIGALO, R. MININNI, P. RUQQIERO: Adsorption of Triasulfuron on Different Soils and Humic Acids. *Fresen Environ Bull*, **10** (2), 221 (2001).
8. E. GRAF, J. W. EATON: Antioxidant Function of Phytic Acid. *Free Radic Biol Med*, **8**, 61 (1990).
9. D. OOSTERHIUS: Foliar Fertilization: Mechanisms and Magnitude of Nutrient Uptake. Paper for the Fuidl Fertiliser Foundation Meeting in Scottsdale, Arizona, 2009.
10. E. T. MERTZ, V. L. SINGLETON, C. L. GAREY: The Effect of Sulfur Deficiency on the Amino Acids of Alfalfa. *Arch Biochem Biophys*, (38), 139 (1952).
11. H. SHIRVANI SARAKHSI, M. YARNIA, R. AMIRNIYA: Effect of Nitrogen Foliar Application in Different Concentration and Growth Stage of Corn (Hybrid 704). *Advantages in Environmental Biology*, (4), 291 (2010).
12. M. BRANKOV, M. SIMIĆ, S. VRBNIČANIN, V. DRAGIČEVIĆ, I. SPASOJEVIĆ, B. KRESOVIĆ: The Influence of Foliar Fertilisers on Morphological Traits of Maize Inbreds. In: Proc. of the 47th Croatian and 7th International Symposium on Agriculture, Opatija, February 13–17, 2012, 40–44.
13. M. BRANKOV, V. DRAGIČEVIĆ, M. SIMIĆ, S. VRBNIČANIN, I. SPASOJEVIĆ: The Foliar Application of Herbicides and Mineral Fertiliser in Maize Inbred Lines. In: Proc. of the Vth Symposium with International Participation: Innovations in Crop and Vegetable Production, Belgrade, October 20–22, 2011, p. 63.
14. L. J. de KOK, P. J. L. de KAN, G. TANCZOS, J. C. KUPIER: Sulphate Induced Accumulation of Glutathione and Frost-tolerance of Spinach Leaf Tissue. *Plant Physiol*, **53**, 435 (1981).
15. A. SIMIĆ, S. SREDOJEVIĆ, M. TODOROVIĆ, L. ĐUKANOVIĆ, Č. RADENOVIĆ: Studies on the Relationship between Content of Total Phenolics in Exudates and Germination Ability of Maize Seed during Accelerated Aging. *Seed Sci Technol*, **32**, 213 (2004).
16. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, R. J. RANDAL: Protein Measurement with the Folin-Phenol Reagent. *J Biol Chem*, **193**, 265 (1951).
17. FELDFERSUCHE MANUAL: Ciba-Geigy AD, Basel, Switzerland, 1975.
18. V. DRAGIČEVIĆ, M. SIMIĆ, L. STEFANOVIĆ, S. SREDOJEVIĆ: Possible Toxicity and Tolerance Patterns towards Post-emergence Herbicides in Maize Inbred Lines. *Fresen Environ Bull*, **19** (8), 1499 (2010).
19. K. J. KUNERT, A. D. DOGDE: Herbicide-induced Radical Damage and Antioxidative Systems. In: *Target Sites of Herbicide Action* (Eds P. Boger, G. Sandmann). CRC Press Inc, 1989, 45–64.
20. M. A. SOOBRAATE, S. V. NEERGHEENA, A. LUXIMON-RAMMAA, I. O. ARUOMAB, T. BAHORUN: Phenolics as Potential Antioxidant Therapeutic Agents: Mechanism and Action. *Mutat Res Fundam and Mol Mech of Mutagen*, **579** (1–2), 200 (2005).

21. T. B. RAY: The Mode of Action of Chlorosulfuron: The Lack of Direct Inhibition on Plant DNA Synthesis. *Pestic Biochem Physiol*, **181**, 22 (1982).
22. L. STEFANOVIĆ, M. SIMIĆ, M. ROŠULJ, M. VIDAKOVIĆ, J. VANČETOVIĆ, M. MILIVOJEVIĆ, M. MIŠOVIĆ, D. SELAKOVIĆ, Z. HOJKA: Problems in Weed Control in Serbian Maize Seed Production. *Maydica*, **52**, 277 (2007).
23. S. M. SHIRAZI, ZULKIFLIYUSOP, F. A. SALMAN: Response of Yield Contributing Characters of Maize to Irrigation and Nitrogenous Fertiliser. *J Environ Prot Ecol*, **13** (3), 1412 (2012).

*Received 25 September 2017*

*Revised 9 October 2017*