UDC 575.633.15
https://doi.org/10.2298/GENSR1803023M
Original scientific paper

ANTIOXIDANT STATUS OF THE DIFFERENT SWEET MAIZE HYBRIDS UNDER HERBICIDE AND FOLIAR FERTILIZER APPLICATION

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Mesarović J., J. Srdić, S. Mladenović Drinić, V. Dragičević, M. Simić, M. Brankov and D. Milojković-Opsenica (2018): *Antioxidant status of the different sweet maize hybrids under herbicide and foliar fertilizer application*.- Genetika, Vol 50, No.3, 1023-1033.

The chemical method of weed control is an indispensable step in cropping practices of sweet maize. Application of the herbicides can induce the abiotic stress which affects the non-enzymatic antioxidants in the crops, especially on the sensitive one, like sweet maize is. Antioxidant profile, through the measurement of the soluble phenolic, carotenoids, phytic acid and glutathione concentration, in the grain of the three sweet maize hybrids after application of herbicides, foliar fertilizer, as well as their combinations, in field experiment, conducted over a two-year period, was determined. The content of tested antioxidant parameters was dependent on hybrids, growing season, as well as of the applied treatment. Sulfonylurea herbicides significantly increased the antioxidant status of sweet maize fresh grain, compared to the herbicide from triketone group, without affecting the fresh grain yield. Combination of herbicide plus foliar fertilizer expressed a various impact on antioxidant profile of the maize grain. Furthermore, significant correlations (positive and negative) between fresh grain yield and analyzed antioxidants in grain of three sweet maize hybrids were noticed.

Keywords: non-enzymatic antioxidant, *Zea mays* L. *saccharata*, abiotic stress, nicosulfuron, mesotrione

INTRODUCTION

In sweet maize, like in standard maize production, the need to use a chemical method as a tool for weed control is present (KOPSELL *et al*. 2011). A limiting factor of weeds controlling in sweet maize, compared to the standard maize, is its higher susceptibility (O'SULLIVAN *et al*.

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2000). Weeds elimination is of the crucial importance, in order to enable optimal plant growth, since the sweet maize is considered as a poor competitor due to shorter and less developed habitus (SIMIĆ *et al*. 2012). Furthermore, in order to improve the crop yield and quality, foliar fertilization is used as a supplement to soil fertilization, particularly when soil is deficient in some nutrients. By foliar fertilization plants are provided with essential nutrients (i.e., micro- and macro-elements, amino acids, etc.) whose transport *via* the root system can be hindered or disabled due to unfavorable soil condition (FAGERIA *et al*. 2009).

Herbicide application, as well as the temperature extremes, heavy metals, water deficit, etc. can lead to the abiotic stress (KOPSELL *et al*. 2011). Plant response could involve a different defense mechanisms such as metabolic detoxification or a various biochemical reactions which include synthesis of secondary metabolites, antioxidants as well as the enzymes such as glutathione S-transferases and cytochrome P450 monooxygenases (DE CARVALHO *et al*. 2009; NEMAT ALLA and HASSAN, 2006). Non-enzymatic antioxidants involved in plant defense system during stress condition are glutathione, carotenoids and polyphenols. Carotenoids protect the photosynthetic apparatus by scavenging the $^1O_2$, triplet states of chlorophyll molecules (chl^3) and other free harmful radicals. Glutathione (GSH), tripeptide with a sulfhydryl group, beside the important role of reactive oxygen species (ROS) removal, is involved in stress signaling, detoxification of toxic products of lipids and apoptosis (GILL and TUTEJA, 2010). Polyphenols, secondary plants metabolites, are capable to quench molecular species of active oxygen, chelate transition metal ions and can prevent the lipid peroxidation reacting with lipid alkoxyl radical (MATTOS and MORETTI, 2016). Phytic acid, the primary storage P form, as a good chelator of iron ions, can be considered as an antioxidant (AHN *et al*. 2004). The antioxidant systems have a significant role in prevention of oxidative stress caused by herbicide application (NEMAT ALLA and HASSAN, 2006). In response to the herbicide application, moreover, different changes of the antioxidants content in the sensitive and tolerant plant have been reported (KOPSELL *et al*. 2011; AGOSTINETTO *et al*. 2016; YANCHEVA *et al*. 2017). Generally, maize sensitivity (response) to the herbicides depends on herbicides selectivity; the amount of the applied herbicide; environmental conditions, as well as the genotype (SOLTANI *et al*. 2007; STEFANOVIĆ *et al*. 2010).

The worldwide increase in the usage of sweet maize leads to an increase of its production (FAOSTAT, 2013). In order to accomplish the high quality and yield, and in particular, to define sensitivity or tolerance, more data are needed regarding the interaction between various herbicide and different genotype of sweet maize. According to all mentioned, the aim of this study was to examine the response of three sweet maize hybrids to herbicides (from sulfonylurea and triketone groups), foliar fertilizer, as well as their combinations, through antioxidants content accumulation in grain (i.e. soluble phenolic, carotenoids, phytic acid and glutathione). Furthermore, correlation of these tested parameters with fresh grain yield was also evaluated.

MATERIALS AND METHODS

Plant material

Field experiment was performed at the experimental field of Maize Research Institute "Zemun Polje", Serbia, on a slightly calcareous chernozem soil type, during 2016 and 2017. Three sweet maize hybrids (ZP504su, ZP355su, and ZP553su) were sown according to the split-plot arrangement, in three replications. Five treatments were applied: C - control (without herbicide and foliar fertilizer application); FF - foliar fertilizer (formulation: L amino acids 6.5% w/w; total nitrogen 3.0% w/w; total organic matter 30.0% w/w; and seaweed extract 4.0% w/w;

applied in amount of 1.5 L ha^{-1}); H1 - mesotrione (120 g a.i. ha^{-1}); H2 - nicosulfuron (45 g ha^{-1}); H1+FF - mesotrione + foliar fertilizer; H2+FF - nicosulfuron + foliar fertilizer. Foliar fertilizer and herbicide treatments were applied at 5-6 leaves stage of maize. Maize ears were harvested 21 days after silking (pollination). Fresh ear yield without husk was measured from the elementary plot ($3.75m^2$) and calculated to t ha^{-1}. For each treatment shelling percentage – the percentage of fresh grain on the ear was determined, and it was used for the calculation of fresh grain yield. Grain samples were dried at 60°C in ventilation dryer to constant dry weight (DW) and milled into powder (Perten 120, Sweden, particle size <500 μm).

Chemical analysis

The content of the phytic acid (Pphy) from the samples was determined by the method DRAGIČEVIĆ *et al.* (2011); total yellow pigment (TYP) by method of AACC, (1995); total soluble phenolic compounds (SP) by the method of SIMIĆ *et al*. (2004); total glutathione content (GSH) by method of SARI-GORLA *et al*. (1993) and total antioxidant activity (TAA) measured as $DPPH^*$ scavenging activity by method of ABE *et al*. (1998).

Statistical data

Obtained data were subjected to the three-way analysis of variance (ANOVA – *F* test) by using M-STAT-C software (Michigan State University, 1989). The differences among the means were tested by the least significant difference test ($LSD_{0.05}$). Dependencies among kernel yield of sweet maize and the content of Pphy, TYP, SP, GSH, and $DPPH^*$ were assessed by correlation (Pearson's coefficients).

Principal Component Analysis (PCA) was achieved by employing PLS Toolbox software package v.6.2.1, for MATLAB 7.12.0 (R2011a). The singular value decomposition (SVD) algorithm was used at 0.95 confidence level for Hotelling T2 limits.

Meteorological condition

During two-year experiment, the trend in the meteorological conditions was opposite: 2016 had lower average temperature and approximately 1.5 times higher amount of precipitation compared to 2017 (Table 1). Major differences were noticeable in June (at flowering), when there were only 39.0 mm of precipitation in 2017, contrary to 107.4 mm in 2016. Similarly, average temperatures in May and June were for 1.0°C and 1.4°C, respectively, higher in 2017 in comparison to 2016.

Table 1. Average monthly air temperatures and precipitation sums for April - August at Zemun Polje in 2016 and 2017.

	Year	April	May	June	July	August	Average
Temeperature (^{0}C)	2016	15.3	17.6	23.0	24.2	22.3	20.5
	2017	12.4	18.6	24.4	25.5	25.8	21.3
							Sum
Precepitation (mm)	2016	51.9	47.4	107.4	33.6	43.2	283.5
	2017	47.1	49.2	39.0	26.7	23.7	185.7

RESULTS AND DISSCUSION

Antioxidants content

All the examined factors (i.e. genotype, treatment and year) and their interaction expressed significant influence on variation of antioxidants evaluated in sweet maize grain, including P_{phy}, SP, GSH, TYP concentration and DPPH* (Table 2). Among tested factors, the lowest variations were presented in TYP concentration (2.59%) and the highest variation was in P_{phy} content (10.39%). The highest values for TYP concentration and DPPH* was found in hybrid ZP553su, while hybrid ZP504su had the highest P_{phy} and GSH concentration.

Table 2. Analysis of variance for the effect of genotype, year, treatment and their interaction on antioxidants evaluated in grain of three sweet maize hybrids.

		MS[1]				
Source of variation		Pphy (mg/g)	SP (μg/g)	GSH (nmol/g)	TYP (μg/g)	DPPH* (%)
Genotype (G)		0.013**	327569.194**	38992.893**	378.019**	309.767**
Treatment (T)		0.078**	123176.073**	46967.348**	7.241**	170.605**
Year (Y)		0.336**	3077636.334**	992531.743**	153.173**	804.990**
G × T		0.018**	33797.203**	4058.309**	3.124**	279.768**
G × Y		0.091**	164350.483**	20149.341**	11.762**	635.590**
T × Y		0.049**	315264.749**	57368.423**	2.661**	97.699**
G × T × Y		0.009**	19024.293**	5529.878**	5.125**	295.611**
CV[2] (%)		10.39	7.17	4.10	2.59	2.72
LSD[3] $(G)_{0.05}$		0.02	46.79	10.66	0.18	0.46
Average	ZP504su	0.37	986.35	453.67	12.36	32.67
Min		0.11	568.65	246.64	9.83	14.45
Max		0.59	1335.18	873.68	15.80	45.90
Average	ZP355su	0.36	1138.14	399.33	8.24	25.59
Min		0.06	745.04	278.18	3.66	14.22
Max		0.56	1886.85	638.89	13.02	39.56
Average	ZP553su	0.33	1216.09 478.07	15.65	28.08	
Min		0.19	772.03	280.58	12.69	1.18
Max		0.52	1817.89	761.47	19.53	57.14

**- significant at $P \leq 0.01$ probability level; [1]MS- mean square; [2]CV- coefficient of variation; [3] Fisher's least significant difference test at $\alpha = 0.05$ level.

Pphy amount was lower mainly in 2017 compared to 2016, for all tested hybrids (Figure 1A). The highest Pphy content was noticed in hybrids ZP355su and ZP504su (0.52 and 0.45 mg/g dry weight (DW)) for 2016 and 2017, respectively. Compared to control, mesotrione and nicosulfuron significantly decreased the average Pphy concentration (Figure 1A) in all hybrids, which is opposite to the results obtained on fully ripened standard maize (BRANKOV *et al*. 2015).

In the treatment with nicosulfuron the greater reduction of Pphy content compared to the mesotrione was noticed. Treatment H2 + FF showed the higher average Pphy amount in all tested hybrids, in comparison to the control and H2 treatment.

GSH value was higher in 2017 in comparison to 2016, for all tested hybrids (Figure 1B). The highest GSH concentration was found in hybrids ZP553su (357.64 nmol/g DW) and ZP504su (507.12 nmol/g DW) for 2016 and 2017, respectively. In all hybrids, both applied herbicides increased the average GSH content (Figure 1B) compared to the control. Treatment with nicosulfuron showed the greater increasing trend in average GSH content compared to the mesotrione (Figure 1B). NEMAT ALLA and HASSAN (2006) and NEMAT ALLA *et al*. (2008) found that herbicides produce oxidative stress and that higher GSH content indicates the maize lines tolerance. Reduction of average GSH concentration for hybrids ZP504su and ZP553su was noticed in both treatments with herbicide plus FF, in comparison with H1, as well as with H2 treatment.

The SP content was lower in 2017 compared to the 2016, for all hybrids, except in the treatment H1 and H2 for hybrid ZP504su (Figure 1C). Opposite to our results, DRAGIČEVIĆ *et al*. (2012) found higher phenolic content in dry season. The highest SP content was found in ZP355su (1249.74 and 800.51 μg/g DW, respectively) for both tested years. In all evaluated hybrids, both applied herbicides increased the average soluble phenolic content (Figure 1C) compared to the control, except for ZP355su when mesotrione was applied. Oppositely, BRANKOV *et al*. (2017) reported a decrease in phenolic content after herbicide application. Treatment with nicosulfuron showed the greater increasing trend in average phenolic content compared to the treatment with mesotrione. Similarly, increased phenolic concentration in maize leaves after herbicides application was reported by DRAGIČEVIĆ *et al*. (2010). Maize lines with increased phenolic content after herbicide application can be considered as potentially tolerant (DRAGIČEVIĆ *et al*. 2012). In the treatment H2 + FF, significantly lower average SP concentration, in comparison to H2 treatment, was found for ZP504su, contrary to the hybrids ZP355su and ZP553su. Compared to the treatment H1, treatment H1 + FF significantly increased the average SP concentration in ZP504su and ZP355su that is opposite to the ZP504su.

Interestingly, in dry 2017, in treatment with combination of herbicides and foliar fertilizer, GSH and SP content in all hybrids (exception was SP content observed in hybrid ZP355su) was lower compared to the treatments with herbicides application only. Similar results were reported by BRANKOV *et al*. (2017). The results indicate that applied FF might act to reduce stress caused by herbicides, as well as drought observed in 2017 (Table 1).

Concentration of the TYP was higher in 2017 in comparison to 2016 for all tested hybrids, with the exception of ZP355su in control (Figure 2A). The highest TYP content, in both years, was found in ZP553su (12.90 and 16.55 μg/g DW, respectively). Compared to the control, significant increase of average TYP content (Figure 2A) was observed after herbicides application. Our results are contrary to those reported by KOPSELL *et al.* (2011), that mesotrione did not express significant influence on carotenoids concentration in sweet maize leaves.

To confirm the changes in the total antioxidant activity (TAA) of sweet maize grain, as response to applied treatments, the $DPPH^*$ scavenging assay was performed. Values for $DPPH^*$ scavenging activity were lower mainly in 2017, compared to 2016 for ZP504su and ZP553su, being opposite to ZP355su (Figure 2B). Application of herbicides significantly increased the average TAA value compared to the control in hybrids ZP504 and ZP355su, opposite to the ZP553su. Similarly, RADWAN *et al.* (2012) reported that the increase in total antioxidant capacity is a plant mechanism to minimize damage from free radicals obtained under herbicide application. In parallel to SP content, the treatment H2 + FF resulted in decrease of average TAA in comparison to H2 treatment for ZP504su, contrary to ZP355su and ZP553su. Compared to the H1, treatment H1 + FF increased average TAA value in ZP504su and ZP553su, opposite to ZP355su.

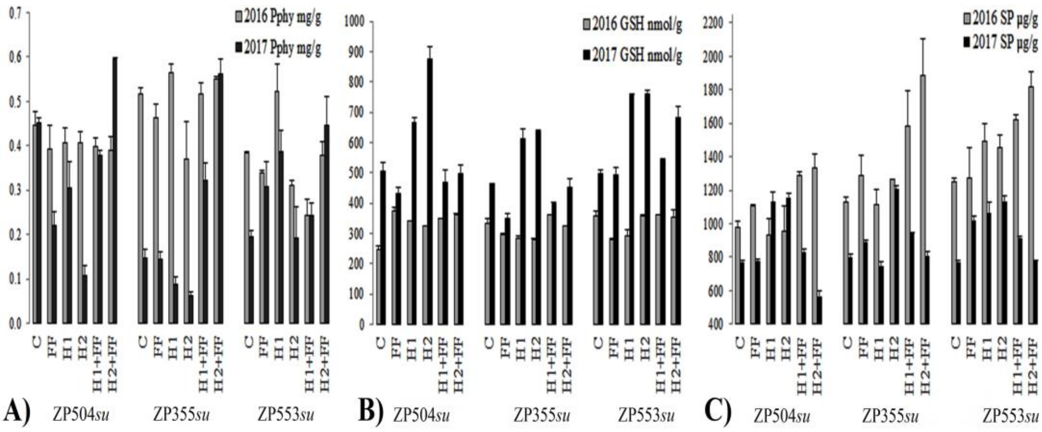

Figure 1. Effect of the applied treatments on the Pphy (A), GSH (B) and SP (C) content in grain of three sweet maize hybrids.

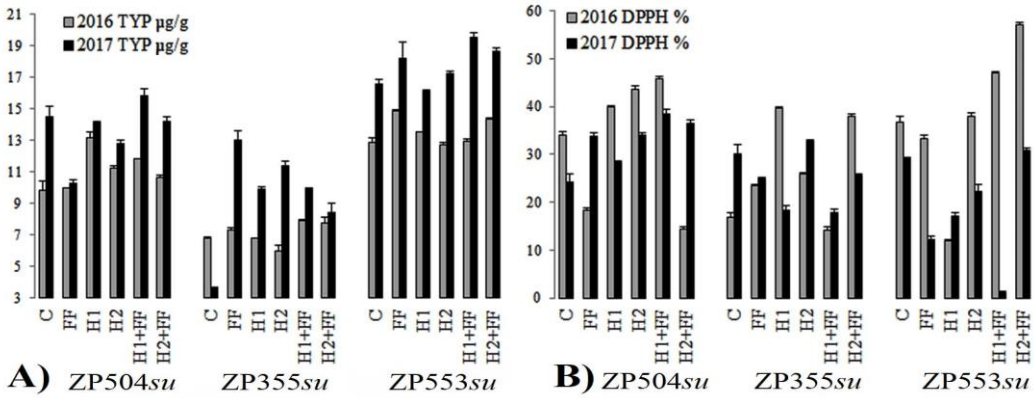

Figure 2. Effect of the applied treatment on the TYP content (A) and DPPH* (B) in grain of three sweet maize hybrids.

Fresh grain yield and its correlation with antioxidants parameters

From all examined factors significant variations in fresh grain yield were only obtained for genotype, year as well as their interaction (Table 3). Applied treatments had insignificant influence on the fresh grain yield of tested hybrids. In agreement with our results, GREY *et al*. (2000) reported no significant response of tolerant sweet maize to nicosulfuron application. Similarly, tolerant maize lines had shown the insignificant grain yield reduction after herbicides application (BRANKOV *et al*. 2015). In addition, O'SULLIVAN *et al.* (2000) suggested that nicosulfuron can be used only for tolerant sweet maize genotypes. Furthermore, it was noticed that sweet maize hybrids are more tolerant to the mesotrione than to the sulfonylurea herbicides (O'SULLIVAN *et al*., 2002). In opposite, positive impact of foliar fertilizer on maize grain yield was reported by BRANKOV *et al*. (2017). Nevertheless, H1 + FF were treatments with the highest achieved average grain yield (5.44 t ha^{-1}). The highest average yield among tested hybrids was obtained by hybrid ZP355su (6.30 t ha^{-1}). The same hybrids expressed the highest fresh grain yield (8.36 t ha^{-1}) in 2016, considered as optimal for maize production in temperate climatic conditions (Table 1). When all factors are taken into account, the highest grain yield was achieved by hybrid ZP355su, in control, in 2016.

Table 3. Variations in fresh grain yield (t ha^{-1}) of three sweet maize hybrids induced by year and application of herbicides and foliar fertilizer.

	ZP504su			ZP355su			ZP553su			2016	2017	Aver.
	2016	2017	Aver.	2016	2017	Aver.	2016	2017	Aver.			
C	5.75	3.91	4.83	8.80	3.88	6.34	6.15	3.70	4.92	6.90	3.83	5.36
FF	6.37	2.90	4.63	8.71	3.44	6.08	6.09	2.94	4.52	7.06	3.09	5.08
H1	4.53	2.52	3.53	8.58	3.43	6.01	5.04	3.94	4.49	6.05	3.30	4.67
H2	4.79	4.24	4.51	7.61	5.57	6.59	6.32	3.83	5.07	6.24	4.55	5.39
H1+FF	5.74	3.32	4.53	8.37	4.42	6.40	7.14	3.63	5.39	7.08	3.79	5.44
H2+FF	4.62	3.36	3.99	8.10	4.63	6.36	6.97	3.92	5.45	6.56	3.97	5.27
Aver.	5.30	3.37	4.34	8.36	4.23	6.30	6.28	3.66	4.97	6.65	3.75	5.20

	Year (Y)	Genotype (G)	Treatment (T)	Y × G	Y × T	G × T	Y × G × T
MS[1]	226.026**	35.956**	1.506	11.466**	2.598	0.604	1.158
$LSD^2_{0.05}$		0.52		0.74			

**- significant at $P\leq 0.01$ probability level; [1]MS - mean square; [2] Fisher's least significant difference test at α = 0.05 level.

According to the results presented in Table 4, significant and negative correlation between fresh grain yield and TYP was present in ZP504su, meaning that yielding potential was not supported with high antioxidants level in grain, such as phenolics and carotenoids. Meanwhile, positive correlation between fresh grain yield and Pphy and SP, was found in hybrid ZP355su. In the same hybrid negative and significant correlation between yield and GSH was noted. Positive and significant correlation between fresh grain yield and SP and DPPH* was found in ZP553su. Opposite, negative and significant correlation between yield and GSH and TYP was noticeable for the same hybrid. Such results indicate the importance of phenolics for better yielding potential, as well as the importance of antioxidative potential, generally, as was evidenced in ZP355su.

Table 4. Correlations between fresh grain yield and analyzed antioxidants in grain of three sweet maize hybrids

Antioxidant	Fresh grain yield		
	ZP504su	ZP355su	ZP553su
Pphy	0.200	0.744**	0.168
SP	0.465	0.740**	0.849***
GSH	-0.521	-0.647*	-0.706*
TYP	-0.616*	-0.433	-0.853***
DPPH*	-0.058	0.122	0.804**

*, **, ***The significant values at the *P*≤0.05; 0.01; 0.001, probability level, respectively.

Principal component analysis

In order to assess the possible relationship between tested antioxidants, sweet maize hybrid and applied treatment, Principal Component Analysis (PCA) was used. PCA resulted in a four-component model which explains 90.70% of total variance. The first two principal components, PC1 and PC2, explain 36.46 % and 22.96 % of the overall data variance, respectively. Mutual projections of factor scores and their loadings for these PCs have been shown in Figure 3A and 3B, respectively. The highest variability of phenolics and carotenoids were present mainly in kernel of hybrid ZP553su: phenolics in H1, FF and H1+FF, while carotenoids in H2, H2+FF, H1+FF and FF treatments. Variability in Pphy concentration was induced mainly by H1+FF and H2+FF, in kernel of hybrid ZP355su and to some extent in H2+FF treatment of hybrid ZP504su. DPPH* and GSH varied mostly in treatment: H1, H2 and H1+FF in ZP504su; control of ZP553su and H2 of ZP355su.

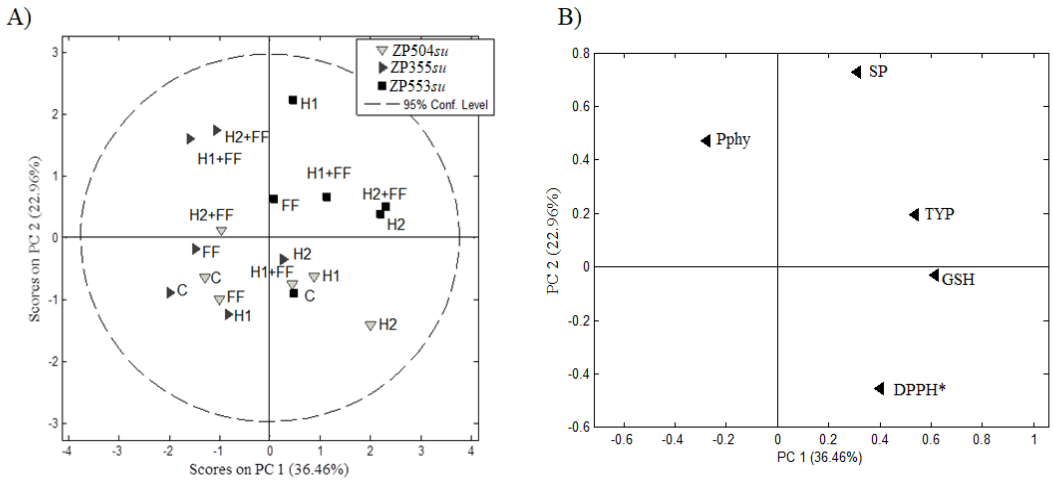

Figure 3. PCA: score plot (A) and loading plot (B) for sweet maize hybrids and applied treatments.

CONCLUSION

Although the applied treatments had no significant impact on fresh grain yield, significant alteration of antioxidant profile in sweet maize kernel was noticed. Growing seasons influenced the hybrids sensitivity reflected through the changes in the antioxidants content. In the dry year, mainly higher values for glutathione and total yellow pigments, as well as lower values for phytic acid, soluble phenolic and total antioxidant activity were noticed. Treatment with nicosulfuron, in general, increased the antioxidants values compared to the mesotrione in all tested sweet maize hybrids. Treatments with herbicides plus foliar fertilizer have shown a different impact on antioxidants parameters compared to the treatments with herbicides only. PCA highlighted the hybrid ZP553su according to the unique variability of total yellow pigments and soluble phenolic in response to the applied treatments. Furthermore, concentration of total soluble phenolic compounds was highly and positively correlated with fresh grain yield in ZP553su. Although relatively small number of genotypes was studied, high variability and different changes in antioxidant parameters after applied treatments were noticeable. This further indicates the importance of defining ~~the~~ sweet maize genotype sensitivity/tolerance to the herbicides, as well as the opportunities for developing new, more tolerant genotype, based on antioxidants level.

ACKNOWLEDGEMENTS

This research is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project Nos. TR31068, TR31037 and OI172017).

Received, May 15th, 2018

Accepted, October 18th, 2018

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ANTIOKSIDATIVNI STATUS RAZLIČITIH HIBRIDA KUKURUZA ŠEĆERCA NAKON PRIMENE HERBICIDA I FOLIJARNOG ĐUBRIVA

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Izvod

Hemijske mere u suzbijanju korova su neophodan korak u tehnologiji gajenja kukuruza šećerca. Kod useva, primena herbicida može dovesti do abiotičkog stresa koji negativno utiče na neenzimske antioksidanse, pogotovo kod osetljivijih genotipova, kao što je kukuruz šećerac. Antioksidativni profil je ispitivan merenjem koncentracije rastvorljivih fenolnih jedinjenja, karotenoida, fitinske kiseline i glutationa u svežem zrnu tri hibrida kukuruza šećerca nakon primene herbicida, folijarnog đubriva kao i njihovih kombinacija, u poljskom ogledu, tokom dve godine. Sadržaj antioksidanasa je varirao u zavisnosti od hibrida, klimatskih uslova tokom vegetacionog perioda, kao i od primenjenih tretmana. Sulfonilurea herbicid je značajno povećao antiokidativni status zrna u poređenju sa triketonskim herbicidom. Kombinacija herbicid plus folijarno đubrivo je značajno uticala na variranja antioksidativnog profila zrna. Pored toga, zabeležene su značajne korelacije (pozitivna i negativna) između prinosa i sadržaja ispitivanih antioksidanasa tri hibrida kukuruza šećerca.

Primljeno 15.V.2018.
Odobreno 18. X 2018.