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GENOTYPE DEPENDENT TOLERANCE TO HERBICIDES OF MAIZE (*Zea mays,* L) INBRED LINES

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Herbicide application in seed maize requires special attention due to their larger sensitivity than hybrid maize. The aim of study was to examine and define the sensitivity/tolerance of the five maize inbred lines with different susceptibility to herbicides (belonging to different heterotic groups), based on alterations of secondary metabolites (phenolics, protein sulfhydryl groups, phytic and inorganic phosphorus). Two groups of herbicides: triketons (mesotrione and topramezone) and sulfonylureas (rimsulfuron and foramsulfuron) were tested. Lines from independent heterotic group, which were sensitive to herbicides expressed visible damages together with significant reduce in grain yield, mainly induced by sulfonylurea herbicides. Parallel with that, significant increase in phenolics, phytic and inorganic phosphorus, as well as drop in protein sulfhydryl groups were observed in their leaves. Tolerant lines (belonging to Lancaster group) had mainly insignificant grain yield reduce, also with lesser variations in sulfhydryl groups, content of phytic and inorganic phosphorus, as well as increase in phenolics content. Among examined secondary metabolites, phytate is the main factor, contributing to herbicide tolerance in maize lines. Owing to lesser yield decrease and variation in content of examined secondary metabolites, expressed in treatments with triketone herbicides, they usage could be safe in maize lines.

Key words: herbicides, maize lines, secondary metabolites, tolerance

INTRODUCTION

Seed maize production is one of the most profitable activities in agriculture. Many negative effects can limit maize production. First of all, due to homozygous, maize lines have often smaller habitus, with uneven growth than hybrid maize. Such conditions create a specific

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microclimate and weeds fits the best in those conditions (STEFANOVIĆ *et al*., 2007), what could lead to complete absence of maize yield, if weeds are not suppressed. From that point, great attention should be paid to weed control in maize seed production.

Also, homozygosis of maize lines carries susceptibility to various biotic and abiotic parameters, including herbicides (STEFANOVIĆ *et al*., 2000). Furthermore, herbicides are not register for use in seed maize. So, on one hand we have a situation that is simply necessary to apply herbicides (to control weeds in maize seed production) and on the other hand, special attention is required in order to prevent possible crop damages. Negative effects of herbicides are usually associated with growth delay, reduction in plant height, leaf area, while in the worst case it can lead to total destruction of the plants. When plants are able to overcome the effect of herbicides – it is a temporary stress, unlike permanent stress includes greater plant damages, reducing grain yield and in the worst case complete plant destruction and death is occurred (de CARVALHO, 2007). Visual assessment can be useful to express damage caused by herbicides, although it may be subjective.

There are many natural mutations which include severe mechanisms of maize tolerance to herbicides, like mutations on AHAS gene, CTM mutation, etc. (ZHU *et al.,* 2000; TAN *et al.,* 2005; VANČETOVIĆ *et al*., 2014). Secondary metabolites play an important role in various metabolic processes. Thus, they vary by the influence of various biotic and abiotic factors. Furthermore, secondary metabolites could also vary with herbicide uptake. In the detoxifying processes large number of metabolites is involved, mostly referred as antioxidants (ROBERTS, 1998). This group of compounds include various compounds such as glutathione, phenolic compounds, compounds containing phosphorus – phytate, etc. Numerous studies have stated a significance of antioxidant systems in prevention of oxidative stress, which could be connected to herbicide toxicity (NEMANT ALLA and HASSAN, 2006; NEMAT ALLA *et al*., 2008).

From the point of successful seed maize production is to safely protect crops from weeds. The aim of this study was to examine and define the sensitivity/tolerance of the five maize inbred lines with different susceptibility to herbicides (belonging to different heterotic groups), based on alterations of secondary metabolites.

MATERIALS AND METHODS

Field experiment was set up on a slightly calcareous chernozem soil type, in experimental field of the Maize Research Institute "Zemun Polje", Serbia (44˚52´N, 20˚19´E, 81 m asl), during 2010, 2011 and 2012. Five maize lines from different heterotic groups (parental components of commercial ZP hybrids) and with different susceptibility to herbicides were tested to two sulfonylurea and two triketone herbicides: independent source (PL38, PL39), BSSS (L355/99) and Lancaster group (L375/25-6, L155/18-4/1). Herbicides were applied in recommended (RD) and double dose (DD): for mesotrione – 120 and 240 g a.i. ha^{-1}; for topramezone – 67,2 g a.i. ha^{-1} and 134,4 g a.i. ha^{-1}; for rimsulfuron – 15 g a.i. ha^{-1} and 30 g a.i. ha^{-1} and for foramsulfuron 45 a.i. ha^{-1} and 90 l a.i. ha^{-1}. Herbicides were applied in 15-16 leaf stage of maize according to BBCH scale. The four-replicate trial was set up according to the split-plot arrangement. The main plots encompassed one 10 m row of each inbred line in 4 replications, while the subplots included herbicide treatments and a control (without herbicide application).

Plant samples (leaves from 4 plants per replication) for chemical analysis were collected 21 days after herbicide application. At the same time, visual damages were estimated according to EWRC scale (0 - no damages, 10 - plant death; FELDFERSUCHE MANUAL, 1975). After drying at 60

°C, alternations in content of secondary metabolites were determined: free thiolic groups (PSH), by the method of de KOK *et al*. (1981), phenolics, by the method of SIMIĆ *et al*. (2004), phytic and inorganic phosphorus by the method of DRAGIČEVIĆ *et al*. (2011a). The obtained data (three year averages) were statistically processed by ANOVA (F test) and differences between means were tested by the least significant difference test ($LSD_{0.05}$). The differences among tested secondary metabolites were evaluated using Principle Component Analysis (PCA) and dependences between EWRC values and the contents of phenolics, PSH, phytic and inorganic phosphorus were obtained by regression analysis. Statistical analysis was performed by SPSS 15.0 for Windows Evaluation version.

RESULTS AND DISCUSSION

All tested lines had more intense damages when sulfonylurea herbicides were applied, compared to triketone herbicides (higher average EWRC values; Table 1). EWRC values were significantly influenced by year, herbicide and their interaction. On lines PL38 and PL 39, as sensitive ones, both doses of foramsulfuron caused moderate to tolerable damages, while rimsulfuron in both doses induced light to moderate damages, what is present in significantly higher EWRC values. In other lines, very light to light damages were recorded. Introduction of sulfonylurea into maize production have led to lower crop tolerance to this group of herbicides (STEFANOVIĆ *et al*., 2007; MALIDŽA, 2007), irrespective to their good efficiency in weed control. According to STEFANOVIĆ *et al*. (2000) maize lines have significantly higher yield losses owing their higher susceptibility to sulfonylureas than hybrid maize. Parallel with that, and in accordance to higher EWRC values, grain yield of examined maize lines significantly varied by the influence of year, herbicide and their interaction, particularly in treatments with sulfonylurea herbicides, too (Table 1). For PL38 and PL39 significant decreases in grain yield were observed in both doses with rimsulfuron and foramsulfuron, down to 64% (PL38 in DD foramsulfuron treatment). Foramsulfuron and rimsulfuron in DD significantly decreased grain yield of L335/99 (BSSS) and L155/18-4/1 (from Lancaster group), as relatively tolerant lines. BRANKOV *et al*. (2012) also stated that sulfonylurea herbicides affect maize grain yield in higher extent, while that was not case when triketone herbicides, which haven't induced significant variations in EWRC and grain yield.

Furthermore, the content of secondary metabolites varied significantly under the influence of year, herbicide and their interaction, what was underlined in treatments with sulfonylureas (Table 2). In the leaves of PL38 and L335/99 significant increase of phenolics was observed on both doses of rimsulfuron, as well as on double dose of foramsulfuron. NEMAT and YOUNIS (1995) also find that phenolic metabolism in maize and soybean was significantly affected by rimsulfuron. On the other hand, in more tolerant lines, such L375/25-6 and L155/18-4/1 from Lancaster group, both doses of foramsulfuron and rimsulfuron in DD significantly decreased phenolics content. Relative high values of phenolic compounds in control, present in the leaves of these lines could be linked with herbicide resistance (BRAIZER *et al*., 2002). PSH content was significantly reduced mainly in leaves of sensitive lines (PL38 and PL39) on treatments with sulfonylureas. However, foramsulfuron significantly and rimsulfuron insignificantly increased PSH content in tolerant line L375/25-6, up to 60% (foramsulfuron in DD). Considering that PSH present group of compounds that belongs to safeners and detoxicants on cellular level (RIECHER *et al,* 2010) it is likely that their variation is under the considerable impact of herbicide toxicity (DRAGICEVIC *et al*., 2010a).

Table 1. Influence of applied herbicides on EWRC values and grain yield of maize inbred lines (three year mean).

Herbicide	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
Genotipe	RD	DD	RD	DD	-	RD	DD	RD	DD
Estimated EWRC values									
PL38	1.33	1.83	1.83	1.75	1	4.42*	5.58*	5.58*	6.42*
PL39	1.42	1.83	1.67	2	1	3.08*	3.67*	3.5*	4.58*
L335/99	1.5	1.75	1.75	1.83	1	2.42	2.25	2.58	2.75
L375/25-6	1.58	1.75	1.67	1.75	1	2.5	2.25	2.83	2.42
L155/18-4/1	1.83	1.5	1.42	1.5	1	2.33	2.17	2.5	2.25

F test	Herbicide		Year		H x Y	
	F	p	F	p	F	p
PL38	225.9	0.00	150.8	0.00	48.24	0.00
PL39	146.9	0.00	43.51	0.00	52.88	0.00
L335/99	6.69	0.07	59.83	0.00	8.71	0.00
L375/25-6	11.03	0.21	50.28	0.00	20.27	0.00
L155/18-4/1	19.18	0.52	113.19	0.00	19.73	0.00

Grain yield ($t\ ha^{-1}$)									
PL38	1.01	0.99	1.05	0.98	1.13	0.78*	0.69*	0.63*	0.51*
PL39	1.92	1.73	1.72	1.95	1.90	1.59*	1.58*	1.58*	1.52*
L335/99	4.35	4.21	4.25	4.31	4.07	4.19	3.76*	4.26	3.63*
L375/25-6	3.97	3.73	3.67	3.93	3.90	3.64	3.82	3.76	4.02
L155/18-4/1	2.64	2.52	2.55	2.63	2.68	2.36	2.09*	2.44	2.15*

F test	Herbicide		Year		H x Y	
	F	p	F	p	F	p
PL38	69.46	0.00	2232.32	0.00	16.61	0.00
PL39	16.52	0.00	3027.60	0.00	7.54	0.00
L335/99	14.320	0.00	6836.20	0.00	14.32	0.00
L375/25-6	33.56	0.14	4680.38	0.00	10.55	0.00
L155/18-4/1	15.92	0.00	4083.73	0.00	10.68	0.00

* significantly different at 0.05 level, ($p<0.05$), RD – recommended herbicide dose, DD – double herbicide dose

Variations in content of phytic phosphorus were also observed with application of both herbicides, mainly increasing P_{phy}, what was particularly emphasized by sulfonylurea herbicides. In lines PL38 and PL39, P_{phy} was also significantly increased under the influence of foramsulfuron and rimsulfuron, applied in DD and what is more important, DD of topramezone showed the similar impact (Table 2). Only, when rimsulfuron, mesotrione and topramezone were applied in RD, P_{phy} was decreased to some extent, but this difference was insignificant. This could be tied to positive impact of phytate to stress cushion, leading to permanent stress (de CARVALHO *et al.*, 2009; DRAGIČEVIĆ *et al*., 2010b) what was reflected on insignificant variations in grain yield (Table 1). It is also important to underline that L375/25-6 and L155/18-4/1 from Lancaster group, had the highest phenolics and P_{phy} content among examined lines. In case of inorganic phosphorus, significant variations were also recorded after sulfonilurea herbicides application (Table 2). In leaves of PL38 foramsulfuron in both applied doses significantly increased P_i content, as well as in L155/18-4/1 DD of foramsulfuron and rimsulfuron increased P_i content, similarly to results of DRAGIČEVIĆ *et al*. (2011b) who detected increase in P_i induced by herbicides.

Table 2. Influence of applied herbicides on content of phenolics, PSH, P_{phy} and P_i in leaves of maize inbred lines (three year mean).

Herbicide	Mesotrione		Topramezone		Control	Rimsulfuron		Foramsulfuron	
Genotipe	RD	DD	RD	DD	-	RD	DD	RD	DD
					Phenolics content (µg g leaf^{-1})				
PL38	445.3	493.4	453.3	483.7	503.4	585.4*	593.3*	539.7	662.7*
PL39	523.5	582.7	529.8	562.9	518.5	582.3	552.8	488.2	537.4
L335/99	504.2	511.2	509.2	551.2	500.6	619.5*	617.2*	508.8	633.0*
L375/25-6	558.2	434.5	498.4	478.1	567.5	580.2	496.4*	494.1*	498.1*
L155/18-4/1	775.7	727.5	742.9	711.2	768.6	739.3	656.9*	680.8*	769.8
F test		Herbicide			Year			H x Y	
	F		p	F		p	F		p
PL38	394.95		0.00	9147.94		0.00	622.77		0.00
PL39	27.66		0.07	1398.28		0.00	125.00		0.00
L335/99	414.03		0.00	7118.19		0.00	478.90		0.00
L375/25-6	247.32		0.00	6378.85		0.00	100.95		0.00
L155/18-4/1	121.05		0.00	9234.67		0.00	748.36		0.00
					P-SH content (µg g leaf^{-1})				
PL38	208.5	193.3	203.8	198.6	257.2	218.9	242.4	143.0*	254.8
PL39	150.6	232.7	178.5	232.7	244.0	265.3	162.4*	176.3	163.4*
L335/99	217.8	247.2	216.7	263.3	226.5	284.3	248.4	229.9	294.7
L375/25-6	161.7	201.6	196.9	209.3	185.9	228.3	187.7	185.2	289.5*
L155/18-4/1	253.5	233.2	230.7	231.9	267.0	264.6	111.4	194.1	222.7
F test		Herbicide			Year			H x Y	
	F		p	F		p	F		p
PL38	394.95		0.00	2996.74		0.00	49.30		0.00
PL39	38.97		0.06	346.80		0.00	69.79		0.00
L335/99	17.61		0.052	676.35		0.00	66.54		0.00
L375/25-6	52.15		0.00	718.22		0.00	122.53		0.00
L155/18-4/1	20.88		0.052	3105.81		0.00	43.66		0.00

Phytic phosphorus content (µg g $leaf^{-1}$)									
PL38	2.28	2.16	2.19	2.59*	2.22	2.33	2.53*	2.55	2.86*
PL39	2.45	2.10	2.42	2.13	2.18	2.10	2.47*	2.37	2.59*
L335/99	2.33	2.22	2.22	2.37	2.18	2.24	2.10	2.29	2.14
L375/25-6	2.37	2.44	2.30	2.49	2.41	2.34	2.51	2.37	2.39
L155/18-4/1	2.50	2.46	2.43	2.60	2.39	2.30	2.41	2.40	2.47

F test	Herbicide		Year		H x Y	
	F	p	F	p	F	p
PL38	47.82	0.00	2089.21	0.00	29.28	0.00
PL39	48.50	0.06	2562.02	0.00	39.13	0.00
L335/99	9.29	0.07	2637.84	0.00	19.37	0.00
L375/25-6	11.32	0.06	6408.70	0.00	27.49	0.00
L155/18-4/1	48.52	0.06	8222.11	0.00	56.27	0.00

Inorganic phosphorus content (µg g $leaf^{-1}$)									
PL38	370.6	374.8	342.3	378.1	393.9	386.9	373.4	434.8*	430.9*
PL39	405.2	422.6	389.7	459.8	414.2	422.6	398.2	428.5	407.8
L335/99	422.8	429.3	407.6	424.3	415.1	413.9	386.4	435.7	454.1
L375/25-6	391.7	400.0	381.1	422.8	398.6	410.5	428.0	403.8	392.0
L155/18-4/1	458.1	472.7	432.7	436.6	452.3	434.9	490.8*	435.1	498.1*

F test	Herbicide		Year		H x Y	
	F	p	F	p	F	p
PL38	93.31	0.00	755.15	0.00	99.44	0.00
PL39	45.04	0.12	183.26	0.00	73.65	0.00
L335/99	45.04	0.50	1950.55	0.00	43.85	0.00
L375/25-6	20.23	0.81	725.22	0.00	22.81	0.00
L155/18-4/1	31.91	0.00	592.60	0.00	33.26	0.00

* Significantly different at 0.05 level, ($p<0.05$), RD – recommended herbicide dose, DD – double herbicide dose

Among examined secondary metabolites in leaves of susceptible and tolerant maize lines, the significant and the highest positive correlation was observed between EWRC values and phytic phosphorus ($R^2 = 0.199$; Figure 1), what could mean that this metabolite plays crucial role in tolerance to herbicides (DRAGIČEVIĆ *et al*, 2011b).

Principal component analysis (PCA) of investigated maize lines revealed that phenolics and P_i contributed to the first axis (PC1), which explained 44.9% of the total variability (Table 3). The second axis (PC2), which explained 32.6% of the variation, was defined with PSH and P_{phy}. Such results could indicate that phenolics and P_i content commonly vary by the phytotoxic effect of herbicides, while variations in PSH and P_{phy} content are independent of phenolics and P_i, with their inverse correlation, signifying that increasing trend of PSH is followed by P_{phy} decrease. That could mean that these two antioxidants could play protective role in phytotoxic defence by switching each other.

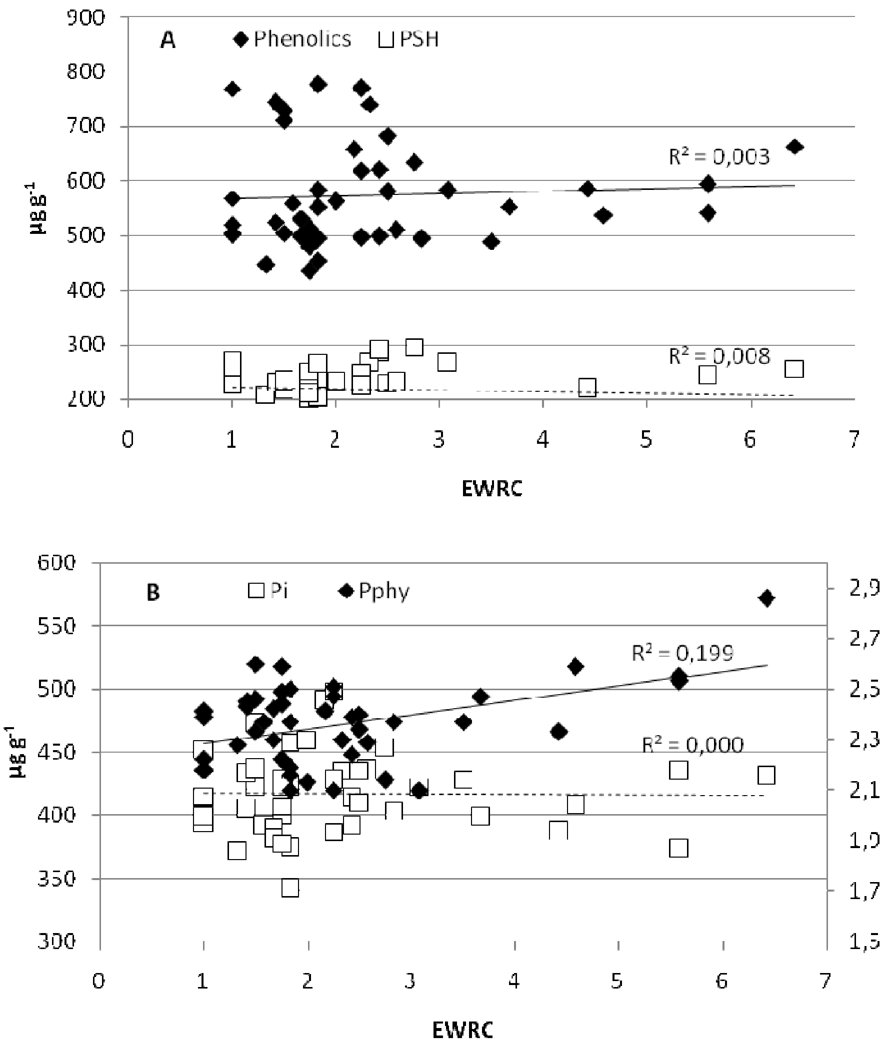

Figure 1. Interdependence between EWRC values and phenolics and PSH (A) and P_{phy} and P_i (B) in maize leaves

Table 3. Results of PCA for phenolics, PSH, P_{phy} and Pi (synthetic variables: PC1 - principal component axis 1 and PC2 - principal component axis 2)

Variable	PC1	PC2
Phenolics	-0.688	0.028
PSH	-0.258	0.704
P_{phy}	-0.224	-0.707
P_i	-0.640	-0.066
Explained variance	1.797	1.305
Proportion of total variance (%)	44.9	32.6

CONCLUSIONS

According to obtained data, maize lines which were sensitive to herbicides (PL38 and PL39, belonging to independent group) expressed visible damages together with significant reduce in grain yield, mainly induced by sulfonylurea herbicides. Parallel with that, significant increase in phenolics, P_{phy} and P_i, as well as PSH drop was observed in their leaves. Tolerant lines, such L375/25-6 and L155/18-4/1 (belonging to Lancaster group) had mainly insignificant grain yield reduce, also with lesser variations in PSH, P_{phy} and P_i content, as well as increase in phenolics content. Among examined secondary metabolites, phytate is the main factor, contributing to herbicide tolerance in maize lines, what could be supported by the further investigations, which include large number of maize lines. Owing to lesser yield decrease and variation in content of examined secondary metabolites, expressed in treatments with triketone herbicides, they usage could be safe in maize lines.

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TOLERANTNOST LINIJA KUKURUZA PREMA HERBICIDIMA ZAVISI OD GENOTIPA

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Izvod

Primena herbicida u usevu linija kukuruza zahteva posebnu pažnju zbog veće osetljivosti u poređenju sa hibridnim kukuruzom. Cilj ovog rada je bio da se ispita i definiše osetljivost/tolerantnost pet inbred linija kukuruza, na osnovu promena sekundarnih metabolite (fenola, sulfhidrilnih grupa, fitina i neorganskog fosfora). Primenjeni su triketoni (mezotrion i topramezon) i sulfoniluree (rimsulfuron i foramsufuron). Linije koje pripadaju nezavisnoj heterotičnoj grupi, koje su osetljive prema herbicidima su imale značajne fitotoksične simptome sa smanjenim prinosom, uglavnom primenom sulfonylurea. U vezi sa tim, značajno povećanje sadržaja fenola, fitina i neorganskog fosfora kao i smanjenje sadržaja sulfhidrilnih grupa je zabeleženo u njihovim listovima. Kod tolerantnih linija (Lancaster heterotične grupe) zabeleženo je smanjenje prinosa, ali ne značajno. Takođe, manja variranja sadržaja sulfhidrilnih grupa, sadržaja fitina i neorganskog fosfora, ali i povećanje sadržaja fenola je zabeleženo kod tih linija. Na osnovu svih posmatranih parametara, sadržaj fitata je glavni factor koji doprinosi tolerantnosti prema herbicidima. Zahvaljujući manjem smanjenju prinosa i variranja sadržaja sekundarnih metabolita na tretmanima sa triketonima, njihova primena u semenskom kukuruzu je bezbedna.

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