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Original scientific paper

REACTION OF SUSCEPTIBLE MAIZE INBRED LINES TO HERBICIDES

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Maize production is inconceivable without herbicide application, and certainly depends on crop susceptibility. Some injuries could be induced by herbicides, what could result in yield losses. This is especially prominent in maize seed production, due to the lines susceptibility to various stressful conditions, including herbicides. Crop response to herbicide application could include whole range of different biochemical reactions such as alterations in content of various metabolites and antioxidants. The experiment was conducted to examine the response of three sensitive maize lines (sugary, popcorn and white kernel maize) to herbicides from sulfonylurea and triketone groups, during the period after herbicide application, when visual injuries are the most obvious and in correlation with grain yield. Variations in soluble proteins, phytic and inorganic phosphorus content, as important metabolites, were followed. The variations in soluble proteins and particularly phytic and inorganic phosphorus content are linked to the expression of susceptibility to herbicides in examined maize lines. Growing season had significant influence on susceptibility. In 2015, as unfavourable season, line ZPT165*b* expressed the highest susceptibility, having the highest values of examined metabolites at the beginning of experiment. All applied herbicides increased grain yield in 2014, but in 2015 nicosulfuron expressed the lowest selectivity, by decreasing grain yield and soluble proteins up to the 21th day after herbicide application, when compared to control.

Key words: susceptible maize lines, herbicide stress, phyticphosphorus, inorganic phosphorus, soluble proteins.

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INTRODUCTION

Specificity of maize seed production reflects in careful application of cropping measures, particularly when weed control is considered. It is well known that maize production is inconceivable without herbicide application, and significantly depends on crop susceptibility. Some injuries could be induced by herbicides, what could result in yield losses (STEFANOVIĆ *et al*., 2007; STEFANOVIĆ *et al*., 2010; TESFAY *et al*., 2014). SUBEDI and MA (2009) emphasized that weed infestation is the most important yield-limiting factor, reducing grain yield by 27–38%, so weed control is one of the most important measures in the whole cropping technology.

Irrespective to herbicides mode of action, crop response to their presence could include whole range of different biochemical reactions. Some mechanisms include metabolic detoxification, increasing content of metabolizing enzymes, such as the glutathione *S*-transferases, cytochrome P450 monooxygenases, etc. (RIECHERS *et al*., 2010), while the others include secondary metabolites and various antioxidants (NEMAT ALLA and HASSAN, 2006; NEMAT ALLA *et al*., 2008a). Sometimes, the available tolerance mechanisms aren't enough to prevent injuries. Negative consequences of herbicides biological activity are reflected through reduction in growth, height, weight, dry matter accumulation, leaf area, as well as yield reduction and in some cases even crop failure (STEFANOVIĆ *et al.*, 2010). Herbicide stress could be temporary, when plants are able to rapidly restore energy necessary for growth and yielding, with less damage expressed; or it could be permanent, when irreversible changes are present and yield losses are greater (de CARVALHO, 2007).

Herbicide efficacy in weed control is especially important for specific genotypes such as sweet, white and popcorn maize genotypes which could be directly consumed in the human nutrition (SIMIĆ *et al*., 2012). They are more sensitive than standard maize genotypes (BRANKOV, 2016). During stress presence plants behave as if they are grown under suboptimal conditions, when their metabolic flexibility, based on activation of alternative metabolic pathways, plays an important role in adaptation to stressful conditions (DOBROTA, 2006). This is particularly important when plant energy is considered. For instance, stressful conditions like salinity or water logging affect photosynthesis and depress ATP synthesis (ZHENG *et al*., 2009) and also could increase phosphorus accumulation in leaves (UYGUR and YETISIR, 2009). Stressful conditions induced by environmental factors could enhance crop susceptibility to herbicides, decreasing yield and increasing phytic acid and soluble proteins content in maize leaves (DRAGIČEVIĆ *et al*., 2011a; DRAGIČEVIĆ *et al*., 2012; BRANKOV *et al*., 2014). In some cases, herbicides could decrease phytic and inorganic phosphorus content in maize leaves, as in case of sulfonylurea herbicides (BRANKOV *et al*., 2015). NEMAT ALLA *et al*. (2008a) ascertained that herbicides, particularly metribuzin, could cause a deficiency in ammonia uptake, having as a consequence reduced protein formation. On the other hand, the elevation of soluble nitrogen forms and amino acids is linked to breakdown of the pre-existing protein (NEMAT ALLA *et al*., 2008a). Such opposite results could be connected to the crop growth stage (herbicide application time), as well as variability in environmental factors.

The aim of this research was to examine the response of three sensitive maize lines (sugary, popcorn and white kernel maize) to herbicides from sulfonylurea and triketone groups, during the period after herbicide application, through measurements of soluble proteins content, phytic and inorganic phosphorus, when injuries are mainly expressed and to correlate the results with grain yield.

MATERIALS AND METHODS

To examine the reaction of susceptible maize lines (ZPKŠ 8/1-161*su* – L1, ZPP608-2/11112111*k* – L2 and ZPT165*b* – L3) to herbicides predominantly used in hybrid production the following treatments were used: H1 – nicosulfuron[1] (60 g l^{-1}), H2 – mesotrione[2] (480 gl^{-1}), H3 – tembotrione+isoxadifen-ethyl[3] (420 + 210 g l^{-1}), and C – control without herbicide application. Field experiments were performed on a slightly calcareous chernozem soil type at the experimental field of Maize Research Institute "Zemun Polje", Serbia (44˚52´N, 20˚19´E) at the rain-fed conditions, during 2014 and 2015. Herbicides were applied in 5–6 leaves phase (BBCH 15-16) at recommended doses: H1 – 0.75 L ha^{-1} (45 g ha^{-1}a.i.), H2 – 1.2 l ha^{-1} (756 g ha^{-1}a.i.) and H3 – 2 l ha^{-1} (840 + 420 g ha^{-1}a.i.). The three-replicate trial was set up according to the split-plot arrangement. The main plots encompassed maize inbred lines, while the subplots included herbicide treatments and control (without herbicide application). Plant samples (leaves from 3 plants per replication) for chemical analysis were collected 2 days (BBCH 15-16) – phase I, 7 days (BBCH 17-18) – phase II and 21 days (BBCH 31-32) – phase III, after herbicide application. After drying at 60 °C, alternations in plant metabolites content were determined: soluble proteins, by the method of LOWRY *et al.* (1951), phytic (P_{phy}) and inorganic phosphorus (P_i) by the method of DRAGIČEVIĆ *et al.* (2011b). At the end of vegetation, the maize grain yield was measured and calculated to 14 % of moisture.

The data were statistically processed by ANOVA (F test) and differences between means were tested by the least significant difference test ($LSD_{0.05}$). Results of metabolite variations are present with standard deviation (SD) and the dependencies between grain yield of maize and the contents of soluble proteins, phytic and inorganic phosphorus were obtained by correlation (Pearson's coefficients).

IUPAC:

[1] 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-N,N-dimethylpyridine-3-carboxamide

[2] 2-(4-methylsulfonyl-2-nitrobenzoyl)cyclohexane-1,3-dione

[3] 2-{2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}-1,3-cyclohexanedione

Meteorological conditions. Two experimental years showed opposite trends in meteorological conditions: 2014 had lower average temperature and more than doubled the amount of precipitation in relation to 2015 (Table 1). Such conditions were emphasized in July, when only 7.2 mm of precipitation reached surface in 2015 as opposed to187.4 mm in 2014. Also, in July and August average temperatures were for 3.2 and 3.1°C higher in 2015 in regard to 2014.

Table 1. Average monthly air temperatures and precipitation sums for vegetative period (April-September) in 2014 and 2015 at Zemun Polje

Month		IV	V	VI	VII	VIII	IX	Aver./Sum
Temperature (°C)	2014	13.7	17.4	21.1	23.2	22.6	18.0	19.3
	2015	12.9	19.1	22.1	26.4	25.7	20.2	21.1
Precipitation (mm)	2014	84.8	192.5	71.2	187.4	41.0	75.6	652.5
	2015	19.7	97.8	31.1	7.2	56.0	73.6	285.4

RESULTS AND DISCUSSION

The significant variation of grain yield was influenced by year and interactions genotype × year, herbicide × year and genotype × herbicide × year (Table 2). In accordance to higher precipitation and lower temperature values, significantly higher grain yield was achieved in 2014, of about 5.6 times higher than in 2015. Interactions highlighted ZPP608-2/11112111*k*, as a genotype with no negative consequences to application of tembotrione+isoxadifen-ethyl and to a lesser extent to nicosulfuron, resulting in increased grain yield, particularly in 2014. In the same year, ZPKŠ 8/1-161*su* and ZPT165*b* also had increased grain yields in all herbicide treatments, while in 2015 nicosulfuron showed reducing effect on grain yield of all three genotypes. The highest average yield was obtained by ZPP608-2/11112111*k* after application of herbicide tembotrione+isoxadifen-ethyl while ZPT165*b* had the lowest yield values in 2015, mainly in H1 treatment. STEFANOVIĆ *et al.* (2007) and STEFANOVIĆ *et al.* (2010) underlined that expression of maize line susceptibility lays mainly in genotype characteristics and meteorological conditions. Particular attention was given to sulfonylureas which could seriously affect maize growth, leading to the yield lack (STEFANOVIĆ *et al.*, 2010; BRANKOV *et al.*, 2015).

Table 2. Variability in grain yield (t ha^{-1}) of examined maize lines induced by applied herbicides

Treat.	2014				2015				Average
	L1	L2	L3	Aver.	L1	L2	L3	Aver.	2014/2015
H1	1.74±0.17	3.21±0.20	2.57±0.25	2.51±0.20	0.31±0.07	0.51±0.16	0.30±0.06	0.38±0.10	1.44±0.15
H2	1.80±0.15	2.81±0.14	2.40±0.24	2.33±0.18	0.40±0.12	0.47±0.16	0.31±0.09	0.39±0.12	1.36±0.15
H3	1.83±0.24	3.32±0.15	2.56±0.26	2.57±0.22	0.45±0.07	0.60±0.05	0.32±0.10	0.46±0.07	1.51±0.14
C	1.00±0.27	2.79±0.18	1.74±0.30	1.84±0.25	0.42±0.10	0.53±0.12	0.34±0.10	0.43±0.11	1.14±0.18
Aver.	1.59±0.21	3.03±0.17	2.32±0.26	2.31±0.21	0.40±0.09	0.53±0.12	0.32±0.09	0.41±0.10	1.36±0.16
lines	1.03±0.15	1.86±0.14	1.44±0.17						
$LSD_{0.05}$	Gen.	Year	Herb.	G×Y	G×H	H×Y	G×H×Y		
	1.06	0.54	1.11	0.35	1.13	0.53	0.30		

L1 – ZPKŠ 8/1-161*su*, L2 – ZPP608-2/11112111*k*, L3 – ZPT165*b*, H1 – nicosulfuron, H2 – mesotrione, H3 – tembotrione+isoxadifen-ethyl, C – control

The significant differences among genotypes were expressed for P_{phy} in all three examined phases (F= 21.25, F=22.43 and F=8.86, respectively for phases I, II and III), as well as for soluble proteins and P_i seven days after herbicide treatment, in phase II (F=16.54 and F=4.04, respectively) (Table 3). Year had significant impact on variability of soluble proteins and P_i content in phase I (F= 29.57 and F=24.37, respectively) and P_i in phase II (F=14.44). Interaction genotype x herbicide was significant for P_{phy} variation, in all three phases (F=1.55, F=1.46 and F=1.54, for phases I, II and III, respectively), as well as for soluble proteins in phases II and III (F=1.60 and F=2.81, respectively). Significant impact of herbicide × year interaction was evident only for soluble protein and P_i content two days after herbicide application, in phase I (F=0.81 and F=3.15, respectively). Among factors examined, only interactions genotype × year and genotype × herbicide × year expressed significant variability in soluble proteins, P_{phy} and P_i content. DRAGIČEVIĆ *et al.* (2011a) indicates that there is significant influence of season on variation of P_{phy} and P_i content in maize leaves under the under the herbicides treatment, inducing P_{phy} increase and P_i decrease during unfavourable conditions.

Table 3. Analysis of variance for the effect of genotype, year and herbicide treatment on soluble protein, phytic and inorganic phosphorus (P_{phy} and P_i) contents leaves of 3 maize inbred lines

		Phase	I			II			III			Grain yield
	d.f.^{1}		Prot.	P_{phy}	P_i	Prot.	P_{phy}	P_i	Prot.	P_{phy}	P_i	
Genot.	2	LSD 0.05	48.17	0.18	0.02	23.68	0.25	0.10	28.39	0.32	0.08	1.06
		F	0.49	21.25*	1.07	16.54*	22.43*	4.04*	3.49	8.86*	1.37	3.8
		p	0.61	0.00	0.35	0.00	0.00	0.02	0.04	0.01	0.27	0.04
Year	1	LSD 0.05	37.58	0.25	0.10	29.03	0.33	0.09	29.67	0.36	0.07	0.544
		F	29.57*	0.00	24.37*	5.94	4.31	14.44*	1.58	2.36	5.88	219.62*
		p	0.00	0.99	0.00	0.02	0.04	0.00	0.22	0.13	0.02	0
Herb.	3	LSD 0.05	49.11	0.25	0.12	31.29	0.35	0.11	30.06	0.38	0.08	1.112
		F	0.08	0.96	0.05	0.24	0.24	0.12	0.79	0.13	0.61	0.39
		p	0.97	0.42	0.99	0.87	0.87	0.95	0.51	0.94	0.61	0.762
Gen. × Year	5	LSD 0.05	9.17	0.16	0.08	13.85	0.17	0.02	10.73	0.23	0.03	0.348
		F	245.31*	15.28*	629.17*	37.23*	28.76*	183.39*	64.39*	8.03*	51.64*	128.49*
		p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Gen. × Her.	11	LSD 0.05	55.41	0.25	0.13	27.81	0.35	0.12	24.22	0.41	0.08	1.128
		F	0.02	1.55*	0.06	1.60*	1.46*	0.20	2.81*	1.54*	0.75	0.66
		p	1.00	0.16	1.00	0.14	0.19	1.00	0.01	0.16	0.98	0.77
Herb. × Yr.	7	LSD 0.05	40.00	0.25	0.10	29.77	0.35	0.10	30.93	0.38	0.08	0.526
		F	0.81*	0.80	3.15*	1.34	0.77	1.91	0.54	0.44	1.11	32.06*
		p	0.00	0.59	0.01	0.26	0.62	0.09	0.80	0.87	0.38	0
G × H × Y	23	LSD 0.05	7.49	0.50	0.02	10.20	0.12	0.02	7.01	0.06	0.02	0.304
		F	81.54*	46.66*	79.91*	17.24*	15.37*	78.77*	35.99*	85.55*	52.00*	38.29*
		p	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Dynamics of soluble proteins content expressed similar trend for all three genotypes in 2014, having maximum in phase II (Figure 1), but with the highest values obtained in ZPKŠ 8/1-161*su* (from 201.6 mg g^{-1} in H1 treatment to 237.5 mg g^{-1} in control), irrespective to the applied treatment. NEMAT ALLA *et al*. (2008a) also noticed increase in soluble-N and amino acids content in seedlings treated with metribuzin, chlorimuron-ethyl and butachlor, resulting from break of the pre-existing protein with recovery present in butachlor treatment. Similarly, the lowest values of soluble proteins content were observed in ZPT165*b*, in H1 treatment (127.9 mg g^{-1}) and the highest values in control (135.6 mg g^{-1}). In 2015, as a year with higher temperatures even at the beginning of vegetation, i.e. time of herbicide treatment (May and June), maximum values of soluble proteins were also recorded mainly in phase II for ZPKŠ 8/1-161*su*and ZPP608-2/11112111*k* (170.2-216.5 mg g^{-1}) in all treatments, with slightly higher values obtained in second genotype. Susceptibility of maize genotypes is closely related to the growing season, and in unfavourable seasons, higher level of injuries and lower yields, induced by sulfonylureas are correlated positively with increased content of soluble proteins (DRAGIČEVIĆ *et al*., 2012; BRANKOV *et al*., 2014). Hence, during the same growing season, 2015, the highest values of soluble proteins were observed in phase I in ZPT165*b*, with rapid drop till the phase III for all treatments. The highest reduction of soluble protein content between phases I and III, of about 83.60 and 86.09 mg g^{-1}, respectively, was recorded with H1 and H3 application treatments. Similar reduction in soluble proteins content under the influence of nicosulfuron and s-metolachlor + terbuthylazine + mesotrione was established (DRAGIČEVIĆ *et al*., 2010). Such results could indicate reduced ammonia assimilation which affects the protein synthesis in maize seedlings exposed to the herbicides (NEMAT ALLA *et al*., 2008b).

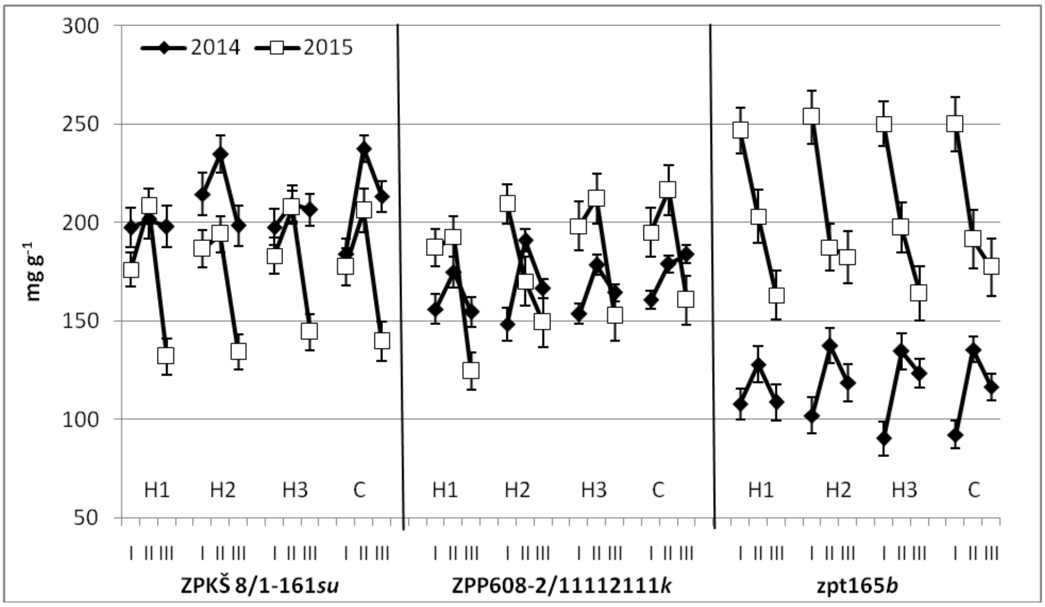

Figure 1. Dynamics of soluble protein content (phases I, II and III) in leaves of three maize lines induced by herbicide treatments

P_i content showed the similar trend as the soluble protein dynamics – maximum values for all genotypes and treatments were recorded seven days after herbicide application, in phase II (Figure 2). Slight differences between examined years in variation of P_i content were noticed in ZPKŠ 8/1-161*su* and ZPP608-2/11112111*k*, with higher amplitude between phases present in ZPKŠ 8/1-161*su*, having subtraction of 0.20 mg g^{-1} between phases II and III in both years in H1 treatment. ZPT165*b* showed great differences in P_i content between years examined, with higher values and amplitude obtained between phases II and III in 2015, and the lowest values of grain yield, as well (Table 1). Such trend was also observed for susceptible genotypes during unfavourable season, when decrease in P_i values induced by herbicides was followed by parallel yield decrease, when compared to control (DRAGIČEVIĆ *et a*l., 2011a). The greatest variation between phase II and III was observed in H3 treatment, with difference of 0.08 mg g^{-1} and 0.22 mg g^{-1}, recorded in 2014 and 2015, respectively.

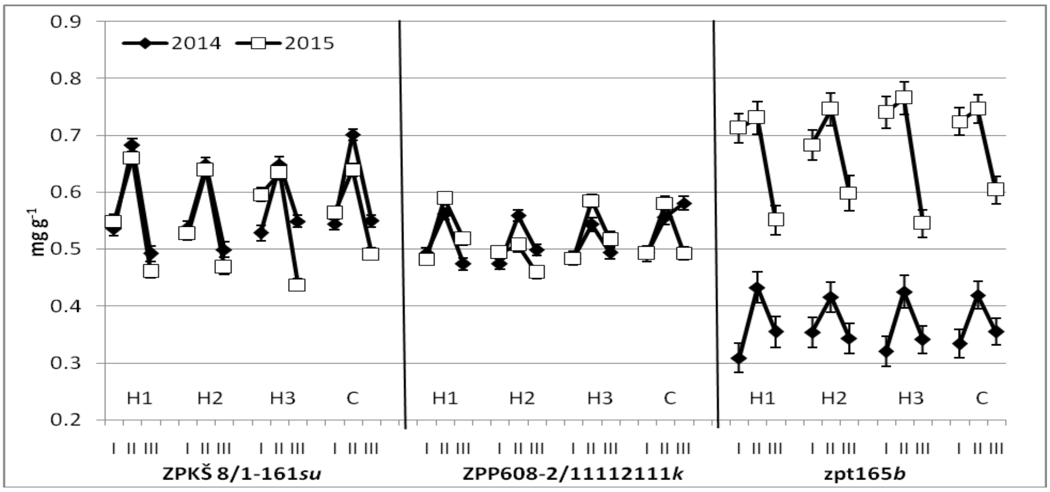

Figure 2. Dynamics of inorganic phosphorus content (phases I, II and III) in leaves of three maize lines induced by herbicide treatments

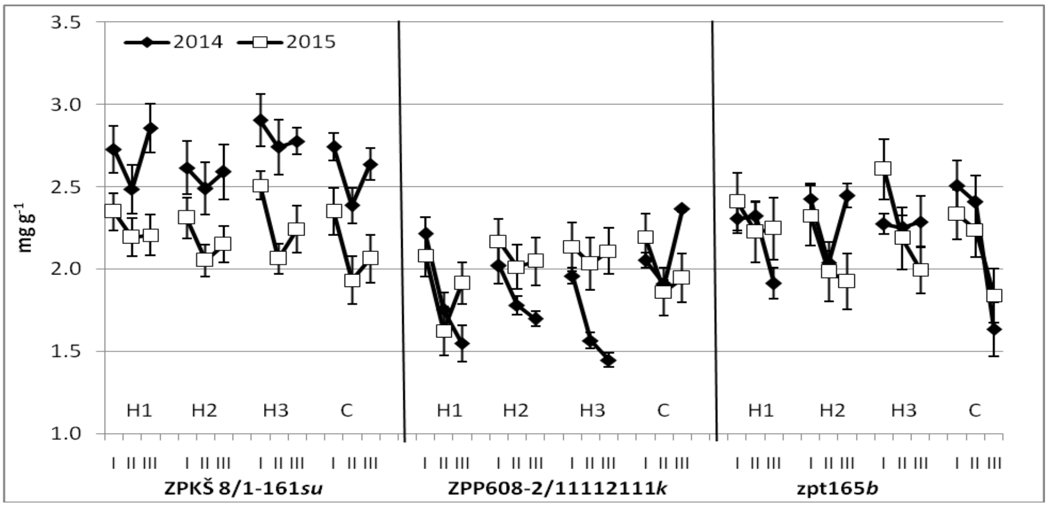

Figure 3. Dynamics of phytic phosphorus content (phases I, II and III) in leaves of three maize lines induced by herbicide treatments

Among parameters tested, P_{phy} content showed the greatest variability and inconstancy during all phases (Figure 3). Opposite to soluble proteins and P_i, greater variability between genotypes and applied herbicides was noticed in 2014. In general, higher P_{phy} values were measured in ZPKŠ 8/1-161*su*, particularly in 2014, in relation to 2015. Dynamics of P_{phy} content expressed decreasing trend from phase I to phase II in ZPKŠ 8/1-161*su* in both years and increase to phase III. In ZPP608-2/11112111*k* and ZPT165*b*, further decrease to phase III was observed. Irrespective to variations obtained between phases, the highest average P_{phy} values (Figure 3) and grain yield (Table 2) were achieved by H3 treatment in 2015 in all three lines, as agreement with findings of DRAGIČEVIĆ *et al*. (2011a) and BRANKOV *et al*. (2015) regarding the role of phytate in reducing the stress intensity and increasing the restoration ability.

The significant and negative correlation between grain yield and soluble proteins, P_{phy} and P_i determined in phases I and II, as well as between grain yield and soluble proteins and P_{phy} determined in phase III was noticed (Table 4). BRANKOV *et al*. (2015) determined decrease in P_{phy} and P_i parallel with yield reduction induced by application of sulfonylureas in susceptible maize lines, in comparison to the treatment without herbicide application. Similar to the previous study, P_{phy} and P_i values found in our research were mainly lower in herbicide treatments in regard to control (particularly in phase III), but alterations correlated negatively to the grain yield. Moreover, greater injuries and lower yields induced by herbicides correlated positively with increased values of soluble proteins (DRAGIČEVIĆ *et al*., 2012; BRANKOV *et al*., 2014). It was interesting to underline that significant and high correlation (> 0.80) between grain yield, P_{phy} and P_i at first two phases was present, indicating connection of these parameters to susceptibility expressed in examined lines. This could also indicate their importance and/or involvement in expression of irreversible changes induced by herbicides (de CARVALHO, 2007).

Table 4. Correlation between grain yield and metabolites: soluble proteins, phytic and inorganic phosphorus

Phase	I	II	III
		Grain yield	
Soluble proteins	-0.45*	-0.36*	-0.42*
Phytic phosphorus	-0.81*	-0.84*	-0.53*
Inorganic phosphorus	-0.89*	-0.80*	0.05

*The significant values at the level of significance of 0.05.

CONCLUSION

Based on the results obtained, it could be concluded that variations in soluble proteins and particularly content of phytic and inorganic phosphorus are linked to the expression of susceptibility to herbicides in maize lines. Growing season has significant influence on susceptibility. All applied herbicides increased grain yield in 2014. In 2015, as unfavourable season, line ZPT165*b* expressed the highest susceptibility, having the highest values of examined metabolites at the beginning of experiment. In the same year nicosulfuron expressed the lowest selectivity, by decreasing grain yield. In parallel, it decreased soluble proteins during all three examined phases and increased P_i in phase II, as well as P_{phy} in phase III, when compared to control.

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REAKCIJA OSETLJIVIH INBRED LINIJA KUKURUZA NA HERBICIDE

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Izvod

Gajenje kukuruza je nezamislivo bez primene herbicida, međutim zavisno od osetljivosti kukuruza moguća je pojava oštećenja od herbicida, što može negativno da utiče na prinos. To je najizraženije u proizvodnji semenskog kukuruza, zahvaljujući osetljivosti linija na razne stresne uslove uključujući i herbicide. Reakcije biljaka na herbicide je praćen velikim brojem biohemijskih reakcija koje uključuju i različite metabolite i antioksidante. Eksperiment je postavljen u cilju ispitivanja tri osetljive linije kukuruza (linije šećerca, kokičara i belog zrna) i variranja sadržaja rastvorljivih proteina, fitinskog i neorganskog fosfora kao značajnih metabolita na uticaj herbicida iz grupa triketona i sulfonilurea u periodu posle primene herbicida, kada su vizuelni simptomi najizraženiji i najviše koreliraju sa prinosom zrna. Variranja u sadržaju rastvorljivih proteina i naročito fitinskog i neorganskog fosfora ukazuju na osetljivost linija prema primenjenim herbicidima. Godina kao faktor je imala uticaja na ispoljavanje osetljivosti, kada su sadržaj rastvorljivih proteina i neorganskog fosfora u fazi 2-7 dana posle primene herbicida, kao i u fazi 21 dan posle primene bili praćeni smanjenjem prinosa zrna. Najveća osetljivost je zabeležena kod linije ZPT165b, u vidu najviših vrednosti ispitivanih metabolita u početnim fazama, naročito u nepovoljnoj godini za gajenje kukuruza. Od svih primenjenih herbicida, nikosulfuron je pokazao najmanju selektivnost utičući na smanjenje sadržaja ispitivanih metabolita.

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