Potravinarstvo® Scientific Journal for Food Industry

Potravinarstvo, vol. 10, 2016, no. 1, p. 114-119
doi:10.5219/535
Received: 3 October 2015. Accepted: 20 January 2016.
Available online: 24 January 2016 at www.potravinarstvo.com

ISSN 1337-0960 (online)

HERBICIDAL EFFECT IN RELATION TO THE ACCUMULATION OF MACROELEMENTS AND ITS REGULATION BY REGULATORS OF POLYAMINE SYNTHESIS

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ABSTRACT
Stress effects of triazine herbicide on cumulating of important macroelements (phosphorus, potassium, calcium and magnesium) into the grain of barley variety Kompakt, as well as the elimination of its negative effect through the addition of regulators of polyamine synthesis (γ-aminobutyric acid and propylenediamine) were investigated in pot trial. These morphoregulators are degrading products of polyamines and hypothetically after foliar application they should support their biosynthesis which increased level act against stress in plants. Application of the herbicide alone in comparison to control variant reduced the contents of all mentioned macroelements in grain of barley and also in variants, where the mixtures of herbicide with regulators of polyamine biosynthesis were applied, also the values of contents of all macroelements (except of magnesium) in barley grain were reduced (in comparison to this variant). It could be summarized that the presence of regulators in mixtures with triazine herbicide in comparison to control variant had not positive effects on contents of these biogenic elements in grain. By the comparison of variant with the applied herbicide with variants, where also regulators of polyamine synthesis were applied, there was the most positive influence of these mixtures of morphoregulators on statistically non-significant accumulation of phosphorus into generative organs of spring barley and in the case of positive accumulation of magnesium into these plant tissues there was statistically significant relation only after application of mixtures of herbicide with propylenediamine. Positive influence on accumulation of calcium was evaluated only after using of mixtures of herbicide with propylenediamine (statistically significant relation was recorded at the dose 29.6 $g.ha^{-1}$).

Keywords: barley; polyamines; triazine herbicide

INTRODUCTION
Triazine herbicides are widely used against broad leaf weeds and crops and in tree seedling nurseries. Triazines are primarily soil applied herbicides. Further research showed that triazine herbicides when taken up by the root move rapidly to the top by apoplastic movement. It concentrates first in the internal veinal areas and finally in the margin of the leaf **(Parveen et al., 2002)**. They are photosynthetic inhibitors and cause chlorosis and desiccation of green tissues. However all these effects are observed in light and not in the dark. Atrazine is a chloroamino triazine herbicide. It is a selective, pre-emergence herbicide for control of many grasses and broad leaf weeds in maize, sorghum, sugar cane and many table crops and increases the yield of crop **(Shah et al., 2000)**.

The great majority of herbicides act by inhibiting a specific plant enzyme essential for metabolism, whereas the remainder, including auxinic herbicides, act as general inhibitors **(Powles and Yu, 2010; Cabrito et al., 2011)**.
Polyamines are small positively charged aliphatic molecules ubiquitous in almost all life forms. These compounds have been implicated in a wide range of life processes in plants including seed germination, growth, floral initiation, floral development, pathogen defenses, and environmental stress responses **(Martin-Tanguy and Aribaud, 1994; Walters, 2003; Palavan-Unsal, 1995)**. Despite extensive studies on polyamine metabolism, the exact role that these compounds play in plant physiology remains unclear **(Tiburcio et al., 1997)**. In plants, polyamines are involved in various physiological events such as development, senescence and stress responses. **(Gill and Tuteja, 2010; Ramakrishna and Ravishankar, 2011)**. Endogenous polyamines could contribute to plant stress tolerance as part of defense mechanisms or adaptation programs that help plant organism to cope with the negative stress consequences **(Todorova et al., 2015)**.

High cellular levels of polyamines correlate with plant tolerance to a wide array of environmental stresses. Moreover, as compared with susceptible plants, stress-tolerant ones generally have a large capacity to enhance polyamine biosynthesis in response to abiotic stress **(Gill and Tuteja, 2010)**.

Conversely, treatments with polyamine biosynthesis inhibitors reduce stress tolerance, but this effect is reversed by concomitant application of exogenous polyamines. The influence of polyamines on in vitro morphogenetic response and caffeine biosynthesis were reported in Coffea canephora. Apart from primary metabolic functions, external feeding of certain polyamines are known to act as elicitors **(Kumar et al., 2008)**.

In addition, uncommon polyamines, like homospermidine, 1,3-diaminopropane, cadaverine and canavalmine have been detected in a large number of biological systems, including plants, animals, algae and bacteria. At the physiological pH, polyamines are found as cations. This polycationic nature of polyamines is one of their important properties effectuating their biological activities. Large body of evidence suggested that plant transformation with genes of polyamines biosynthetic enzymes or the exogenous application of polyamines such as putrescine, spermidine and spermine results in abiotic stress tolerance in various plants **(Valero et al., 2002)**.

Ali (2000) reported that exogenous application of putrescine reduced the net accumulation of Na^+ in different organs of *Atropa belladonna* subjected to salinity stress. Putrescine alleviated the adverse effect of NaCl during germination and early seedling growth and increased the alkaloids as well as endogenous putrescine of *A. belladonna*. **Lutts et al., (1996)** reported that putrescine increased the growth and the leaf tissue viability of salttreated plants in all cvs. of *Oryza sativa*. They suggested that this positive effect was associated with an increase in ethylene biosynthesis through an increase in ACC content and a suppression of NaCl-induced inhibition of ACC conversion to ethylene and suggested the involvement of putrescine in salinity tolerance in rice. **Ndayiragije and Lutts (2006)** studied the possible role of exogenous application of polyamines on *Oryza sativa* and noted that addition of polyamines in nutritive solution reduced plant growth in the absence of NaCl and did not afford protection in the presence of NaCl. Polyamine-treated plants exhibited a higher K^+/Na^+ ratio in the shoots, suggesting an improved discrimination among monovalent cations at the root level, especially at the sites of xylem loading. Putrescine induced a decrease in the shoot water content in the presence of NaCl, while spermidine and spermine had no effects on the plant water status. In contrast to spermidine, spermine was efficiently translocated to the shoots.

GABA is a non-protein amino acid with some functional properties for human health such as lowing blood pressure and regulating heart rate **(Mody et al., 1994)**. GABA is widely present in prokaryotic and eukaryotic organisms **(Yang et al, 2015)**. In recent years, GABA-enriched foods have become popular, such as GABA-tea **(Syu et al., 2008)**, GABA-brown rice **(Komatsuzaki et al., 2007)**, GABA-soy bean sprouts **(Guo et al., 2012)**. In plant cells, GABA is synthesized via the α-decarboxy1ation of glutamate (Glu) in an irreversible reaction which is cata1yzed by glutamate decarboxylase (GAD) **(Bown et al., 1997)**. This metabolic pathway is called GABA shunt. In addition, GABA can also be formed via γ-aminobutyraldehyde intermediate from polyamine degradation reaction where diamine oxidase (DAO) is the key enzyme **(Wakte et al., 2011)**. Researches on GABA accumulation in germinating seeds focus on GABA shunt **(Bai et al., 2009; Mae et al., 2012)**, but little information is available on polyamine degradation pathway **(Xing et al., 2007)**. In the majority of germinating seeds, stressful conditions such as hypoxia **(Guo et al., 2011)**, salt stress **(Widodo et al., 2009)** and drought **(Kramer et al., 2010)** can strongly increase GABA content. During fava bean germination under non-stress condition, GABA content increased slightly **(Yang et al., 2011)**, but it increased significantly when germinating under hypoxia stress **(Yang et al., 2013)**. Under these stressful conditions, the relationship between GABA shunt and polyamine degradation pathway is still not clear.

MATERIAL AND METHODS

In pot experiment 6 kg of substrate (soil:sand – 4:2) was weighed. Analyses done in soil used in experiment are shown in Table 1. It was sown 30 plants which were thinned into 20 pieces after post-emergence. At the phase of early tillering plants were foliar treated (after 25 days) in the control treatment with the water (Table 2), in other

Table 1 Agrochemical characteristics of soil (horizons 0 – 0.2m).

Soil reaction	Humus content	Content of nutrients			
		N_{an}	P	K	Mg
(pH/KCl)	(%)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)
7.03	2.34	8.7	54.3	178.35	407.8

Table 2 Variants of the pot experiment.

VARIANT NUMBER	FOLIAR TREATMENT
1	Control: 9.0 mL water
2	Triazine herbicide 0.5 $l.ha^{-1}$: 1.0 mL water solution of triazine herbicide +8.0 mL water
3	Triazine herbicide 0.5 $l.ha^{-1}$ +GABA 500 $g.ha^{-1}$: 1.0 mL water solution of triazine herbicide +4.7 mL 20 mM solution GABA +3.3 mL water
4	Triazine herbicide 0.5 $l.ha^{-1}$ +PDA 59.2 $g.ha^{-1}$: 1.0 mL water solution of triazine herbicide +3.8 mL 2 mM solution PDA +4.2 mL water
5	Triazine herbicide 0.5 $l.ha^{-1}$ +PDA 29.6 $g.ha^{-1}$: 1.0 mL water solution of triazine herbicide +1.9 mL 2 mM solution PDA +6.1 mL water

NOTE: PDA – 1,3-propylenediamine, GABA – γ-aminobutyric acid.

variants with triazine herbicide alone (the active ingredient is cyanazine with chemical formula 2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropiono-nitrile), or its mixture with γ-aminobutyric acid (GABA) with dose 500 $g.ha^{-1}$, in another variant with 1,3-propylenediamine (PDA) with dose of 59.2 $g.ha^{-1}$, and in last variant with the PDA in the amount of 29,6 $g.ha^{-1}$. The plants were watered with constant volume in all pots.

Crops were harvested in full ripeness, 2 g of barley grain after homogenization were mineralized in 20 mL of nitric acid with 5 mL of perchloric acid and after filtration the filtrate was afterwards filled to volume 50 mL. Then the contents of potassium, calcium and magnesium were determined by method of flame AAS with VARIAN (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA, manufacturer Varian Australia Pty Ltd, A.C.N. 004 559 540, Mulgrave, Australia). Phosphorus was determined by method of Gonzáles **(John, 1970)** – 0.5 mL of above mentioned solution in 50 mL volumetric flask was filled with water till mark, 1 mL of ascorbic acid was added and 4 mL of solution with extraction agent containing sulphuric acid, ammonium molybdate and potassium antimonyl tartrate hemihydrate. Solution was mixed and after two hours the absorbance at 670 nm on UVmini-1240, UV-VIS Spectrophotometer, SHIMADZU, Japan (UV-1800), was measured against distilled water. Final values of phosphorus content in barley grain were defined from calibration curve of standards absorbance.

Results were evaluated by statistical program Statgraphics 4.0 (Statpoint Technologies, Inc., Czech republic), the data were analyzed by means of one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Application of herbicide alone in comparison to control variant (Table 3) reduced the contents of all macroelements in barley grain (values of our tested macroelements in barley grain percentually declined in interval 13 – 29%) – statistically significant in the case of phosphorus and potassium and also in variants with applied mixtures of herbicide with regulators of polyamine biosyntheis; the values of all macroelements contents (except of magnesium) in grain of barley were also reduced (in comparison to first variant.

It could be summarized that the presence of regulators in mixtures with triazine herbicide in comparison to control variant had not positive effects on contents of these biogenic elements (P, K, Ca) in grain (Table 3). Evaluating of magnesium cumulating had following summarise: the most positive statistically significant influence was in variants with applied propylenediamine (PDA). By the comparison of variant with the applied herbicide with variants, where also regulators of polyamine synthesis were applied, there was the most positive influence of these mixtures of morphoregulators on statistically non-significant accumulation of phosphorus into generative organs of spring barley and in the case of positive accumulation of magnesium into these plant tissues there was statistically significant relation only after application of mixtures of herbicide with PDA. Positive influence on accumulation of calcium was evaluated only after using of mixtures of herbicide with PDA (statistically significant relation was recorded at the dose 29.6 $g.ha^{-1}$). Only the uptake of potassium into barley grain was not affected positively by regulators of polyamine synthesis when compared to variant, where triazine herbicide was used alone (in mentioned cases this influence was statistically non-significant).

Cereals in Slovak republic, as well as in European Union have important representation in structure of plant production **(Kračmár et al., 2014; Tomka et al., 2010)**. Cereal agricultural production is limited by a wide array of abiotic and biotic stress factors including weeds, drought, cold, heat, salinity, imbalances in mineral nutrition, viral, and others, often acting in combinations under field conditions.

Since pesticide stress has not yet been extensively examined at the grain macroelements level, no data on this topic are currently available in the literature. Therefore, this study provides the first data and a framework for further investigation. Several researches have suggested that crop selectivity to triazine herbicides or their residues might be improved by exploiting the natural variability that is clearly preset in plants, either by searching for varietal differences in triazine tolerance or by altering the genetic structure of the crop by repeated artificial selection for genuine resistance.

Treatment of spring barley variety Kompakt with triazine herbicide and its mixtures with regulators of polyamine biosynthesis affected the accumulation of macroelements. From the theoretical point of view it could be presumed that applied amounts of regulators of polyamine synthesis will not directly affect the content of nutrients in plant, because they do not contain mentioned inorganic macroelements (P, K, Ca, Mg), because they are organic compounds. Changes in accumulation of macroelements in spring barley grain are probably caused by influencing of translocation of nutrients in plant, or by influencing of

Table 3 Contens of macroelements in grain of barley.

Variant Number	Content of nutrients ($mg.kg^{-1}$)				Dry matter (%)
	P	K	Ca	Mg	
1	4672.9B	8627.7B	122.6A	1452.1A	94.10B
2	3652.6A	6316.9A	87.0A	1275.4A	94.00B
3	3926.5A	5990.5AB	80.2A	1406.7AB	93.85B
4	3674.7AB	5378.3AB	96.7AB	1481.1B	93.42A
5	3825.7B	4490.3A	106.1B	1622.3B	93.52B

NOTE: Letters in table stand for statistical significance in columns (p <0.01). Their conformity means that the values are statistically non-significant and different letters characterize statistically significance.

metabolism of compounds groups, or physiology of plant and subsequent change in ability of plant to uptake nutrients from soil solution.

Triazine herbicides affect biochemical processes in plant, also energetic processes in cells which with their presence in tissues are obviously inhibited. Macroelement phosphorus is the part of structures $H_2PO_4^-$ and HPO_4^{2-} that are the part of important compounds NADP and NADPH$^+$, as well as phosphate fragments are in macroergic bonds. Our experiment confirmed this fact and also there was minimal influence of PDA and GABA on reduction of stress induced by herbicide presence.

Function of potassium in metabolism of spring barley plants is versatile: affects managing with water and improves health state and grain quality. Triazine herbicide which acts in plants destructive has great impact also on its uptake into barley grain.

Similarly, Pakistani authors **(Perveen et al.**, **2002)** found out that the contents of potassium, phosphorus and sodium were in roots and in shoots of bean plants (*Vigna radiate* (L.)) decreased after the application of triazine herbicide. The authors have explained it by injuries of tissues in plants after application of these pesticides. Potassium is involved in the protein synthesis, cell membrane and ionic balance, opening of stomata and other plant movements **(Hale and Orcutt, 1987)**.

Not only macroelements contents have decreasing tendency after the application of triazine herbicide, but this decrease was evaluated also by other important organic compounds in plants. In experiment carried out by Indian scientists **(Khan et al, 2006)** it was obvious that the application of herbicide isoproturon significantly decreased the values of protein in grain of wheat *Triticum aestivum*. Also significant decline of chlorophyl content (with bound magnesium) was also recorded **(Yin et al, 2008**; **Nemat et al., 2008)**, even by low concentration of isoproturon in plants.

As well as in the case of phosphorus, also the cumulating of potassium into barley grain the morphoregulators have not reducing effect on stress induced by triazine herbicide presence.

The role of Ca^{2+} as one of the nutrients and as a key ion in maintaining the structural rigidity of the cell walls as well as in membrane structure and function has been known for a long time **(Reddy et al., 2011; Hepler, 2005)**. During the last three decades, numerous studies have shown that Ca^{2+} is an important messenger in eliciting responses to diverse signals, including many biotic and abiotic signals **(McAinsh and Pittman, 2009; DeFalco et al., 2010)**. It appears that plants use Ca^{2+} as a messenger more than any other known messengers in plants. This is evident from the fact that nearly all signals (developmental, hormonal, and stresses) cause changes in cellular Ca^{2+}, primarily in the cytosol and, in some cases, in the nucleus and other organelles.

These herbicides belong to groups of photosynthesis inhibitors – their effectiveness lies in inhibition of photosynthetic electron transfer by disabling of photochemic reaction II. level known as Hill reaction. The most probable place of chlorophyll inhibition by photosynthesis is 5-membered ring of chlorophyll. By the bond of herbicide on keto-, resp. enol- form of five 5-membered ring, the chlorophyll inhibits the transfer of electrons. Changes as consequence of destruction of photosynthetic apparatus (inhibition of photosynthetic electron process) in plant are induced. Main photoreceptors of green plants are chlorophylls a + b which contain magnesium complex of reduced porfirine. This fact could explain our decline in magnesium that is the part of these important organic structures. The experiment revealed more positive influence of regulators of polyamine biosynthesis on increase of magnesium content in generative organs of tested cereal, because they have more positive influence on protection of photosynthetic structure by inhibiting of chemical bonds of triazine herbicide formation with elements from chlorophyll.

Recently, GABA acts an important function in plant stress responses **(Saito et al., 2008)**. This compound inhibits not only the influence of herbicide on plants, but also reduces harm pathogens. In experiment of **Okada and Matsubara (2012)** where added GABA and arginine (0.1, 1% w/v) into the Fusarium root rot (Fusarium oxysporum f. sp. asparagi, MAFF305556, SUF1226) in vitro suppressed further rot extension. GABA was also patented in USA as important antistressor **(Plant Health Care Inc., 2009)**.

CONCLUSION

Negative influence of triazine herbicide on accumulation of tested macroelements (P, K, Ca, Mg) into barley grain variety Kompakt was recorded. The application of herbicide mixtures with regulators of polyamine biosynthesis in comparison to control variant did not improve this accumulation (except of macroelement magnesium content). In our experiment there was more positive influence of used PDA and GABA in combination with triazine herbicide only in comparison to variant, where the herbicide alone was applied and in the case of statistically non-significant accumulation of phosphorus, statistically significant relation of macroelements uptake into barley grain was evaluated, in the case of magnesium accumulation into barley grain with mixtures of herbicide with PDA and calcium only at the dose of this morphoregulator 29.6 g.ha^{-1}. The uptake of potassium into barley grain was not positively affected by regulators of polyamine synthesis in comparison to variant where triazine herbicide was used alone.

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Acknowledgement

The work was supported by grants KEGA 014SPU-4/2013.

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