



HORMONAL CHANGES IN SPRING BARLEY AFTER TRIAZINE HERBICIDE TREATMENT AND ITS MIXTURES OF REGULATORS OF POLYAMINE BIOSYNTHESIS

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ABSTRACT

Plants adapt to abiotic stress by undergoing diverse biochemical and physiological changes that involve hormone-dependent signalling pathways. The effects of regulators of polyamine biosynthesis can be mimicked by exogenous chemical regulators such as herbicide safeners, which not only enhance stress tolerance but also confer hormetic benefits such as increased vigor and yield. The phytohormones, abscisic acid (ABA) and auxin (IAA) play key roles in regulating stress responses in plants. Two years pot trials at Slovak University of agriculture Nitra were carried out with analyses of contents of plant hormones in spring barley grain of variety *Kompakt*: indolyl-acetic acid (IAA) and abscisic acid (ABA), after exposing of tested plants to herbicide stress, as well as the possible decrease of these stress factors with application of regulators of polyamine synthesis was evaluated. At 1st year in spring barley grain after application of solo triazine herbicide treatment in dose 0,5 L.ha⁻¹ an increase of all analyzed plant hormones was observed and contrary, at 2nd year there was the decrease of their contents. From our work there is an obvious influence of herbicide stress induced by application of certain dose of triazine herbicide at 1st year. Expect of the variant with mixture of triazine herbicide (in amount of 0,5 L.ha⁻¹) and 29,6 g.ha⁻¹ DAB, at this year all by us applied regulators of polyamine synthesis reduced the level of both plant hormones. Higher affect of stress caused by enhanced content of soluble macronutrients in soil where the plants of barley were grown was observed next year. Soil with increased contents of macronutrients (mg.kg⁻¹): N_{30.7} + P_{108.3} + K_{261.5} + Mg_{604.2} had reducing effect on contents of plant hormones in barley grain at variant treated with solo triazine herbicide (in dose at 0,5 L.ha⁻¹) in comparison to control variant. The mixtures of regulators of polyamine synthesis reduced the contents of IAA only in comparison to control variant. Decline in amount of ABA in barley grain was observed only after treatment with GABA, also in comparison to variant treated with water. Other mixtures of morphoregulators in combination with herbicide had not strong influence on contents of tested plant hormones in barley grain of variety *Kompakt*.

Keywords: barley; phytohormones; polyamines; herbicide

INTRODUCTION

Herbicides are chemicals used to manage unwanted plants in agriculture and horticulture which are usually referred to as „weeds“ (Piñol, 2011). Most herbicides are compounds that inhibit plant metabolic pathways or physiological processes by interacting with specific proteins (Veliny et al., 2010). The herbicides selectivity is based on the plant's ability to rapidly metabolize the herbicide, forming non-phytotoxic compounds. However, there is a differential selectivity between species, and the genetic makeup of the species or cultivar may determine varying degrees of tolerance or susceptibility to herbicides (Oliveira, et al., 2011). Weed management is an important practice in production systems that seek high productivity

and quality of agricultural products (Fehér et al., 2016). The most widely used weed management method is currently the chemical control because of its convenience and efficiency when compared to other methods (Agostinetto et al., 2016).

The major hormones produced by plants are auxins, gibberellins (GA), cytokinins (CK), abscisic acid (ABA), ethylene (ET), salicylic acid (SA), jasmonates (JA), brassinosteroids (BR) and strigolactones. Among these, ABA, SA, JA and ET are known to play major roles in mediating plant defense response against pathogens and abiotic stresses (Bari and Jones, 2009; Nakashima et al., 2013). Typically, ABA is responsible for plant defense against abiotic stresses because environmental conditions such as drought, salinity, cold, heat stress and wounding

are known to trigger increase in ABA levels (Lata et al., 2011, Verma et al., 2016). Contrastingly, salicylic acid, jasmonates and ethylene play major roles in response to biotic stress conditions as their levels increase with pathogen infection (Bari and Jones, 2009). However, the mechanism of stress-response is not solely restricted to these hormones. Recent studies have provided substantial evidence for the cross-talk of abscisic acid, salicylic acid, jasmonates and ethylene with auxins, gibberellins and cytokinins in regulating plant defense response (Bari and Jones, 2009; Navarro et al., 2008; Nishiyama et al., 2013). The key role of abscisic acid, salicylic acid and jasmonates as primary signals in the regulation of plant defense has been well established (Bari and Jones 2009; Pieterse et al., 2009). These hormones generate a signal transduction network that leads to a cascade of events responsible for the physiological adaptation of the plant to stress (Gaur and Sharma, 2014). The phytohormone ABA modulates many important plant development processes, such as the inhibition of germination, maintenance of seed dormancy, regulation of growth, fruit abscission and stomatal closure (Qin, 2011, Finkelstein et al., 2002, Parent et al., 2009, Raghavendra et al., 2010). In addition, ABA serves as an endogenous messenger in abiotic stress responses in plants; therefore, it is called a 'stress hormone'. Physiological experiments have shown that under abiotic stress, especially drought and salinity, plants accumulate high levels of ABA accompanied by major gene expression changes. The perception, signaling and transportation of ABA are some of the most central issues in plant science (Fujii et al., 2009, Ma et al., 2009, Park et al., 2009, Umezawa et al. 2009, Kang et al., 2010, Kuromori et al., 2010). The sites of ABA perception have intrigued plant biologists for many years, and the issue has reached some resolution with the recent identification of several ABA receptors.

Endogenous stimuli, such as plant hormones, coordinate and modulate the molecular and biochemical mechanisms that provide increased stress tolerance and adjust overall plant growth and development for greater survival (Peleg and Blumwald, 2011; Choudhary et al., 2012; Ha et al., 2012; Osakabe et al., 2013). Auxins regulate a wide variety of growth and developmental processes in higher plants, including cell elongation and thus the stimulation of shoot growth. However, roots are very sensitive to auxins and even low concentrations can inhibit root growth. Auxins also regulate differentiation of vascular tissue (phloem and xylem), and induce adventitious root initiation on shoot and root cuttings used for propagation. (Kurepin et al., 2013).

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).

γ -Aminobutyric acid (GABA) accumulates rapidly when plants are exposed to stress. Whether GABA accumulation represents the regulation of metabolism in response to stress or an adaptive response to mitigate stress is unknown. Genetic manipulation of GABA levels has

revealed that GABA accumulation functions in defense against drought and insect herbivory (Bown and Shelp, 2016).

Recent combined genetics and physiological studies of the GABA shunt indicate that its function is required for proper growth in response to abiotic stresses such as low light (Michaeli et al., 2011) and salt (Renault et al., 2013; Michaeli et al., 2015).

In addition, other polyamines, such as 1,3-diaminopropane (DAP), cadaverine (CAD), thermospermine (Ther-SPM), norspermidine (Nor-SPD) and norspermine (Nor-SPM) are found in many organisms as minor components of the cellular polyamine pool and are referred to as uncommon polyamines (Tavliadoraki et al., 2011). 1,3-Diaminopropane, an oxidation product of the naturally occurring polyamines, occurs in many cereal plants. Endogenous 1,3-diaminopropane level has been reported to decrease in attached oat leaves with increasing age of seedlings and in excised leaves with increasing time of dark incubation, suggesting that 1,3-diaminopropane, like polyamines, may be involved in the control of senescence (Kaur-Sawhney et al., 1982).

The theme of the research work was to analyze the contents of two plant hormones (auxin and abscisic acid) in barley grain after triazine herbicide treatment and its mixtures of regulators of polyamine biosynthesis.

MATERIAL AND METHODOLOGY

Two years pot trials were carried out in vegetation cage at Slovak University of agriculture in Nitra (coordinates of GPS: 48°18'13.38''S, 18°06'03.05''V, at height above sea level of 135 meters). To each pot 6 kg of substrate was weighed (soil: sand – 4: 2). 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 2. variant 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 (3. variant), in 4. variant with 1,3-propylenediamine (DAP) with dose of 59.2 g.ha⁻¹, and in 6. variant with the DAP in the amount of 29,6 g.ha⁻¹, in 7. variant was mixture triazine herbicide with GABA with dose of 100 g.ha⁻¹. In 5. variant we treated the plants with mixture herbicide with growth regulator Avit A +B. The plants were watered with constant volume in all pots. Crops were harvested in full ripeness.

Analysis of plant hormones

The concentrations of indole acetic acid (IAA) and abscisic acid (ABA) were determined using an HPLC (Waters 2695 Separations Module with 2487 Detector, Manufacturer: 34 Maple Street Milford, MA, USA) system following the method of Xu et al. (1998). The whole liquid chromatographic system was controlled by evaluating software HPLC System Manager. Quantitative determination was done by the setting of absorbance by wavelength 222 nm. Each sample (2 g of average sample of grain after milling) was homogenized in liquid nitrogen

with 15 mL 80% methanol, and the homogenate was stirred overnight at 4°C. After centrifugation the samples were filtrated through the filtration paper (company Niederschlad Germany – marked by violet ring (identification number 393)). The sample was adjusted to pH 2.5 with 1 M HCl and 0.5 M NaOH. Afterwards the solution was evaporated to dryness on rotating vacuum vaporiser RVO 400. The residue was dissolved in a solution (3 mL of 3% methanol and 97% acetic acid), and after filtration was applied onto a column (Agilent TC-C₁₈, 250 mm × 4.6 mm, 5 µm. The flow of mobile phase was 0.6 ml.min⁻¹, sensitivity 64, and temperature of the column was 25°C. Four replicated experiments were performed.

Standards were prepared as followed: 4 mg ABA was dissolved in 25 ml solution of 3% methanol and 97% acetic acid and 18 mg IAA was dissolved in 25 mL solution of 3% methanol and 97% acetic acid.

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

Plants adjust growth in response to internal and external stimuli through the activities of different hormones and simultaneous analysis of different hormones in the same plant provide a comprehensive picture of the stress induced rearrangement in plant metabolism (Shakirova et al., 2016). The phytohormones, abscisic acid (ABA), auxin, and ethylene play key roles in regulating stress responses in plants (Vanstraelen and Benkova, 2012). The effects of plant hormones can be mimicked by exogenous chemical regulators such as herbicide safeners, which not only enhance stress tolerance but also confer

hormetic benefits such as increased vigor and yield (Dashevskaya, 2013).

The contents of plant hormones of indolyl acetic acid (IAA) and abscisic acid (ABA) in spring barley grain of variety *Kompakt* were analyzed in pot trials at SUA Nitra. Solo application of herbicide induced mild extreme reactions in levels of phytohormones. In first year (table 3) of our experiment there was an increase of content of both tested organic compounds at 6th variant (statistically non-significant in comparison to control variant) after application of mixture triazine herbicide with regulators of polyamine biosynthesis DAP_{29.6} and the second highest value was found at 2nd variant where the stress situation was reached after application of solo triazine herbicide – statistically significant in comparison to 1st variant. Contrary to second experimental year (table 3), the second lowest level was found in contents of mentioned compounds, statistically non-significant at variant where the solo application of herbicide was applied. At the same year the highest content of IAA at control first variant and the highest content of ABA at variant with the mixture of triazine herbicide with morphoregulator DAP_{59.2} (4th variant) were found. It is substantial that in first year there was an increase of all analyzed phytohormones in spring barley grain and contrary, in second year there was their decrease at 2nd variant (after solo application of triazine herbicide). These extremes indicate mild stress effect of applied triazine herbicide with the consequence of arising condition called “hormonal chaos”. ABA concentrations synthesized in roots and shoots, and in particular in veins, seeds, and guard cells increase under stress conditions as a result of increased biosynthesis, decreased degradation, or release from conjugated forms (Boursiac et al., 2013; Song et al., 2014). These events could occur within the

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

Year	Soil reaction (pH/KCl)	Humus content (%)	N _{an} (mg.kg ⁻¹)	Content of nutrients		
				P (mg.kg ⁻¹)	K (mg.kg ⁻¹)	Mg (mg.kg ⁻¹)
1	7.08	3.36	10.8	46.3	198.4	502.7
2	7.49	2.79	30.7	108.3	261.5	604.2

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 +DAP 59.2 g.ha ⁻¹ : 1.0 mL water solution of triazine herbicide +3.8 mL 2 mM solution DAP +4.2 mL water
5	Triazine herbicide 0.5 l.ha ⁻¹ +Avit (A) 5.0 l.ha ⁻¹ +Avit (B) 0.5 l.ha ⁻¹ : 1.0 mL water solution of triazine herbicide +93 µL Avit A +7 µL Avit B +7.9 mL water
6	Triazine herbicide 0.5 l.ha ⁻¹ +DAP 29.6 g.ha ⁻¹ : 1.0 mL water solution of triazine herbicide +1.9 mL 2 mM solution DAP +6.1 mL water
7	Triazine herbicide 0.5 l.ha ⁻¹ +GABA 100 g.ha ⁻¹ : 1.0 mL water solution of triazine herbicide +1.0 mL 20 mM solution GABA +7.0 mL water

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

affected cell or in neighboring or remote cells resulting in ABA uptake by nonstressed cells (Mittler and Blumwald, 2015).

There were obvious mitigation stress effects of herbicide after application of regulators of polyamine biosynthesis. We presume that higher content of ABA (antagonist of IAA) in first year is the reaction on stress and this increased level reduced the negative influences of stress factors. All by us applied morphoregulators reduced the level of both phytohormones only in first experimental year (except of 6th variant where the mixture of herbicide with DAP_{29,6} was applied) only in comparison to variant with solo applied triazine herbicide – statistically significant only with mixture Avit A and B (5th variant) and GABA100 (7th variant) with triazine herbicide (Table 3). Not only the applied regulators of polyamine biosynthesis at optimal amounts eliminate the herbicide effect of triazine herbicide but they also act stimulating. Contrary, at second year the solo applied triazine herbicide statistically non-significant reduced the contents of both analyzed organic compounds in comparison to non-treated 1st variant. The mixtures of regulators of polyamine synthesis in this year reduced the contents of IAA only in comparison to control (just by water treated) variant. Statistically non-significant reduction of ABA in second year was found only after application of GABA but also only when compared to control variant. The mixtures of regulators at other variants in combination with herbicide do not show any dependence on amounts of plant hormones in grain of spring barley variety *Kompakt*. At this point we could consider the stronger influence of stress as the consequence of higher salinity of soil where the plants of barley were grown; where it could be seen from Table 1 even three-times higher content values of nitrogen in soil in second experimental year when compared to first year.

The same conclusions were published also by Islam et al. (2016) who found that saline stress differently affect the synthesis of endogenous hormones. Higher saline stress significantly enhanced the contents of IAA in two cultivars of rice *ZJ 88* and *XS 134*. The solo application of herbicide 2,4-dichlorophenoxyacetic acid without presence of saline stress also impacted higher values of IAA in cultivars of rice in comparison to control variant, the plants of sensitive cultivar of rice (*ZJ 88*) which were exposed to both stresses at the same time, had the values of IAA higher at 63% and 113% in comparison to variants without stress sources.

Saline stress also causes the enhanced content of phytohormone ABA. The similar results were found also by Fernando and Schroeder, 2016, who studied environmental stimuli and referred that salinity or drought stress cause dramatically increased cellular ABA levels. The plant cuticle has been shown to mediate stress signalling as well as ABA biosynthesis and signalling. In addition to its primary function, providing mechanical support to the cell wall and plasma membrane, the cuticle has been implicated in osmotic stress regulation. CED1 (9-cis epoxy-carotenoid deoxygenase defective 1) is an essential protein in cuticle biogenesis. CED1 mutants are sensitive to osmotic stress, as they are unable to induce ABA biosynthesis in response to osmotic stress (Wang et al., 2011).

Salinity stress causes osmotic stress and water deficit, increasing the production of ABA in shoots and roots (Cabot et al., 2009). The accumulation of ABA can mitigate the inhibitory effect of salinity on photosynthesis, growth, and translocation of assimilates (Jeschke et al., 1997). The positive relationship between ABA accumulation and salinity tolerance has been at least partially attributed to the accumulation of K⁺, Ca²⁺ and compatible solutes, such as proline and sugars, in vacuoles of roots, which counteract with the uptake of Na⁺ and Cl⁻ (Gurmani et al., 2011). ABA is a vital cellular signal that modulates the expression of a number of salt and water deficit-responsive genes. Abscisic acid mediates stomatal closure to prevent water loss caused by osmotic stress under high salt stress (Shinozaki and Yamaguchi-Shinozaki, 2007).

CONCLUSION

During two experimental years at Slovak University of agriculture in Nitra the contents of plant phytohormones (ABA and IAA) in barley grain of variety *Kompakt* after application of triazine herbicide (applied solo, or in combination with regulators of polyamine biosynthesis) were assessed. At 1st year the stress effect of herbicide on plants of barley and an increase of contents of both tested plant hormones were noticeable. Morphoregulators in mentioned year had reducing effect of stress influences of present herbicide (except of variant where the mixture of 0.5 L·ha⁻¹ herbicide with 29.6 g·ha⁻¹ DAB was applied).

At 2nd experimental year the solo treatment of triazine herbicide reduced the amounts of both organic substances in barley grain. The mixtures of regulators of polyamine synthesis at mentioned year reduced the contents of IAA only in comparison to control variant. Slight decline of ABA content in this year was evaluated only at variants treated with herbicide GABA. From the experiment it could be seen that for barley plants more stressful is the higher content of soluble salts in soil than herbicide stress induced by our treatment.

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