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REDUCTION OF HERBICIDE AND WATER STRESS IN SPRING BARLEY BY REGULATORS OF POLYAMINE BIOSYNTHESIS

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ABSTRACT

The experiment was carried out under artificial light of fluorescent lamps starting with 60 % full water capacity which was afterwards decreased on 40 % and finally the plants of barley were not watered. 30 plants of this cereal after plant emergence were thinned on 22 pieces. Experiment was treated by triazine herbicide, as well as its mixtures of regulators of polyamine synthesis: γ-aminobutyric acid, 1.3-propylenediamine dihydrochloride and salicyl acid. Solo application of triazine herbicide during water stress had negative balance on formation of root and above ground biomass. Addition of regulators of polyamine synthesis had positive effects on mentioned parameters, but not in comparison to control variant. These stress factors were eliminated most significantly only the application of GABA (100 $g.ha^{-1}$) in mixture with herbicide.

Keywords: herbicide, barley, polyamine, γ-aminobutyric acid

INTRODUCTION

Herbicides are xenobiotics that are usually used to control growth and reproduction of weeds. They differ in their structure but all exert unfavorable effects after penetration the plant. Triazine herbicides are among primary common agrochemicals applied to pre- and post-emergence weed control for agricultural and non-agricultural purposes (**Avramides and Gkatsos, 2002; Ji *et al.*, 2008; Gao *et al.*, 2010; Shah *et al.*, 2011**; **Wang *et al.*, 2012**). The high herbicide concentrations induce suppression of seed germination, root and shoot growth retardation, chlorosis, and disturbances in physiological functions. In addition, herbicide effects on different plant species are very selective; therefore, a comparison between weed and cultivated plants to treatment with a herbicide allow us to elucidate the contribution of the antioxidant defense systems in the mechanisms of selectivity**. (Gar'kova *et al.*, 2011).**
Water stress in plants reduces the plant-cell's water potential and turgor, which elevate the solutes' concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases leading to growth inhibition and reproductive failure. This is followed by accumulation of abscisic acid (ABA) and compatible osmolytes like proline, which cause wilting. At this stage, overproduction of reactive oxygen species (ROS) and formation of radical scavenging compounds such as ascorbate and glutathione further aggravate the adverse influence. (**Lisar *et al.*, 2012**).
Studies on plant polyamine research pointed to their involvement in responses to different environmental stresses. During the last few years, genetic, transcriptomic and metabolomic approaches have unravelled key functions of different polyamines in the regulation of abiotic stress tolerance. Nevertheless, the precise molecular mechanism(s) by which polyamines control plant responses to stress stimuli are largely unknown. In the early stages of polyamine research, **Richards and Coleman, (1952)** observed the presence of a predominant unknown ninhydrin positive spot that accumulated in barley plants exposed to potassium starvation **(Alcázar *et al.*, 2009)**.
The objective of our study was to characterize the effects of water and herbicide stress of barley and their minimizing with regulators polyamine biosynthesis.

MATERIAL AND METHODS

Experiment was carried out at Slovak University of Agriculture in Nitra at Department of Chemistry under light platform with linear fluorescent lamp with power wattage 28 W and colour rendering index 80 (neutral white). Weight of initial substrate was 1.5 kg (soil : sand 1 : 0.5) and weight of this substrate filled with water was kept on weight 1.638 kg (60 % of full water capacity). During three days (after foliar treatment) full water capacity was amended on value 40 % (1.616 kg) and afterwards the experiment was not watered for 6 days till the harvest (to simulate week water stress). In our experiment 30 grains of spring barley were sown (variety Kompakt) which were after plant emergence thinned on 22 pieces. 7 variants (Table 1) were carried out with four repetitions, where the plants were foliar treated after 17 days by substituted triazine herbicide (TAH) where the main component is cyanazine - its chemical scheme is 2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropiononitrile. Other following morphoregulators for pot trial were used: PDA – propylendiamine dihydrochlorid, p.a. (Sigma – Aldrich, Germany), GABA - γ-aminobutyric acid, p.a. (Sigma – Aldrich, Germany), KS – salicylic acid, p.a. (Lachema a.s., Brno, Czech republic).
Agrochemical traits of soil are mentioned in Table 2. The soil was extracted using the Mehlich III method (CH_3COOH, NH_4NO_3, NH_4F, HNO_3 and EDTA). The content of available phosphorus (P) in the extract was determined by colorimetric method and the content of available calcium (Ca) and magnesium (Mg) by atomic absorption spectrometry (AAS). The ion-selective electrode (ISE) method was used to determine the pH value after extraction in 0.01 M KCl (Table 2). The values of pH/KCl in soil were slightly acidic, the content of nitrogen (N_{an}) in soil was medium, the content of phosphorus was lower, and the content of humus, calcium and magnesium is under standard values.

Table 1 Variants of pot trial under light bar in substrate soil : sand (4 : 1); (herbicide and contemporary herbicide and water stress)

VARIANT NUMBER	FOLIAR TREATMENT
1	Control: 8.4 ml water
2	Triazine herbicide 0.5 $l.ha^{-1}$: 0.25 ml water solution of triazine herbicide + 8.2 ml water
3	Triazine herbicide 0.5 $l.ha^{-1}$ + GABA 100 $g.ha^{-1}$: 0.25 ml water solution of triazine herbicide + 0.25 ml 20 mM solution GABA + 7.9 ml water
4	Triazine herbicide 0.5 $l.ha^{-1}$ + GABA 10 $g.ha^{-1}$: 0.25 ml water solution of triazine herbicide + 25.0 µl 20 mM solution GABA + 8.15 ml water
5	Triazine herbicide 0.5 $l.ha^{-1}$ + PDA 59.2 $g.ha^{-1}$:

	0.25 ml water solution of triazine herbicide + 1.00 ml 2 mM solution PDA + 7.15 ml water
6	Triazine herbicide 0.5 $l.ha^{-1}$ + PDA 10 $g.ha^{-1}$: 0.25 ml water solution of triazine herbicide + 166.0 µl 2 mM solution PDA + 8.0 ml water
7	Triazine herbicide 0.25 $l.ha^{-1}$ + salicylic acid 50 $g.ha^{-1}$: 0.25 ml water solution of triazine herbicide + 1.00 ml 2.5 mM solution of salicylic acid + 7.15 ml water

Table 2 Characteristics of soil used in pot trial under light bar

Soil reaction	**Humus content**	**Nutrients**			
		N_{an}	**P**	**Ca**	**Mg**
(pH/KCl)	**(%)**	**($mg.kg^{-1}$)**	**($mg.kg^{-1}$)**	**($mg.kg^{-1}$)**	**($mg.kg^{-1}$)**
6.46	2.43	10.50	18.1	1440.3	413.0

RESULTS AND DISCUSSION

The tested plants reacted more negative under conditions with herbicide and water stress (60 % of full water capacity). These conditions significantly affected root part of barley. With reducing of water content in soil substrate in plants treated by triazine herbicide the intensity of root biomass formation was reduced (Table 3). Roots are often a primary target of environmental stresses, such as soil flooding or drought, which in turn have considerable impact on root development (**Vartanian *et al.*, 1994; Couée *et al.*, 2004**). A lot of these responses to stress involve changes in the balance of phytohormones which are known to have effects on root development (**Ross and O'Neill, 2001**). Treatment with solo application triazine herbicide caused the reduction of root biomass by 29.5 % (strong statistically significant) in comparison to control variant. Under these conditions the synergism of regulators of polyamine biosynthesis in comparison to variant where the solo triazine herbicide was applied, an increase of root dry matter by 7.4-28 % was observed. The most effective influence (statistically non-significant) on mentioned parameter had additive using of GABA in dose 100$g.ha^{-1}$.

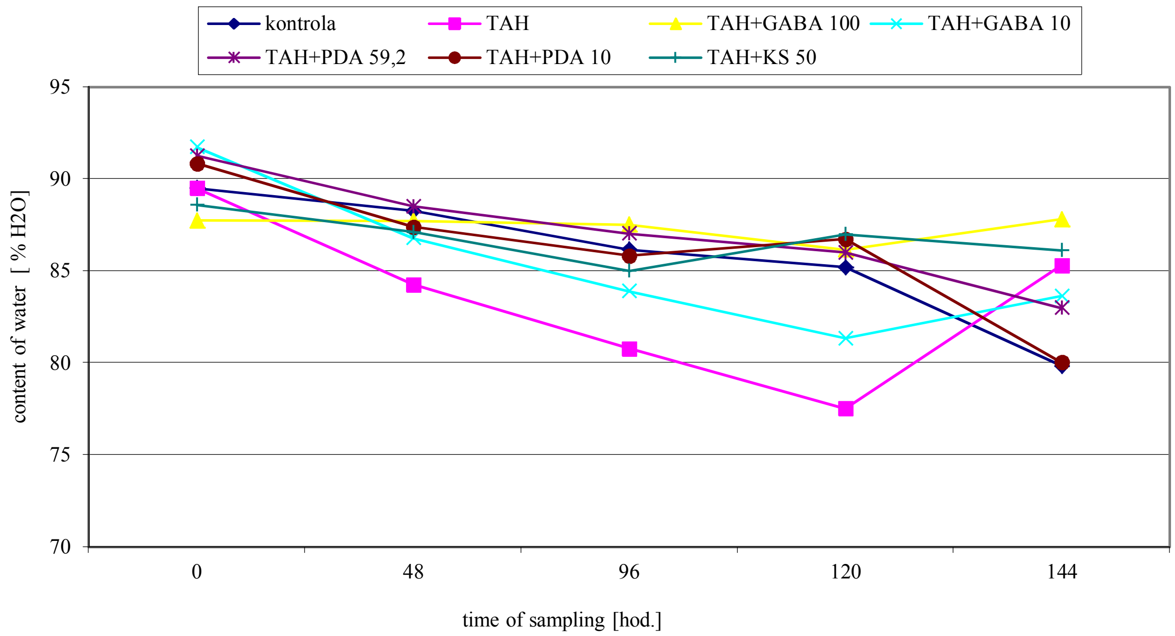
Similar results were also evaluated also in formation of above ground biomass where solo application of triazine herbicide had also negative influence on its increased weight of fresh matter, as well as dry matter. Common application of regulators of polyamine synthesis with triazine herbicide had statistically non-significant more positive effect on amount of dry matter from above ground biomass and except of PDA in dose of 59.2 $g.ha^{-1}$ also on production of fresh above ground biomass. Only applied GABA had the consequence in elimination of herbicide and water stress in this short-time experiment (mostly statistically high non-significant). Indeed, decrease of polyamine level is often associated with ageing and senescence whereas accumulation, more precisely spermidine spermine accumulation, is associated with growing tissue activity and organogenesis **(Perez-Amador *et al.*, 1995; Couée *et al.*, 2004).**

In practice, applied herbicide is more effective in two lower doses than the application at once. **Krawczik and Kaczmarekova, (2009)** mentioned in their studies that the application of herbicides in the split dose system on spring cereals, using preparations designed for early weed growth stages, made it possible to obtain high effectiveness of weed control. Further they referred that in those plantations the experimental application of a mixture of herbicides at 10 – 20 % recommend dose effectively reduced weeding in a degree comparable with the standard object, in which the recommended herbicide dose was applied during one application.

In final part of our experiment the tested cereal was not watered for 6 days. There was the most significant reducing of above ground fresh biomass, its dry matter and also of root dry matter in plants treated with triazine herbicide. Only common application of GABA in amount of 100 $g.ha^{-1}$ in mixture with herbicide eliminated in short-time experiment herbicide and partially water stress (graph 1). The effect of long-time drought was emphasised by negative effect of treatment with herbicide. Application of herbicide during long-time drought significantly reduced the processes of plant development. All regulators slightly reduced negative influence of triazine herbicide (from the viewpoint of following plant development it is necessary to consider this fact mainly in root part), but only GABA application in dose 100 $g.ha^{-1}$ eliminated stress effect of herbicide under conditions of water stress (graph 1). The results support known fact about level of polyamines that are in strong relation to various stress influences and that exogenous polyamines improve the plant development in unfavourable conditions of environment **(Želeva and Karanov, 1994).** Dynamics of slight decrease of water in control plants was observed after 120 h water stress. Such a disruption was examined also in plants treated by mixture of triazine herbicide with PDA. In contrary, in plants treated by solo herbicide application (the most vehement reducing of water content), as well as in plants treated its mixture with GABA, meant as a reaction on gradual adaptation of plants to extraneous conditions, adverse point after 120 hours was examined as increase of water content in final 24 hours of carrying out the experiment (Graph 1).

Table 3 Common effect of short time herbicide and water stress on formation of above ground and root matter of spring barley, variety Kompakt and its regulation by regulators of polyamine biosynthesis. Capital letters in table stand for statistical significance in columns (P<0.01) and their conformity means that the values are statistically non-significant and different letters characterize statistically strong significance

Treatment	**Overground matter**				**Root matter**	
	fresh [g]	% to control	dry [g]	% to control	dry [g]	% to control
Control, water	21.84^{A}	100	4.40^{A}	100	2.71^{A}	100
Herbicide (0.5 $dm^{-3}.ha^{-1}$)	17.18^{B}	78.66	3.60^{A}	81.82	1.91^{B}	70.48
Herbicide (0.5 $dm^{-3}.ha^{-1}$) + +$GABA_{100}$	22.09^{A}	101.14	4.58^{A}	104.09	2.67^{AB}	98.52
Herbicide (0.5 $dm^{-3}.ha^{-1}$) + +$GABA_{10}$	24.76^{AB}	113.37	4.40^{B}	100	2.16^{B}	79.70
Herbicide (0.5 $dm^{-3}.ha^{-1}$) + +$PDA_{59.2}$	16.45^{B}	75.32	3.75^{A}	85.23	2.11^{AC}	77.86
Herbicide (0.5 $dm^{-3}.ha^{-1}$) + +PDA_{10}	18.52^{B}	84.80	3.92^{AB}	89.09	2.28^{A}	84.13
Herbicide + +KS_{50}	22.84^{A}	104.58	4.04^{A}	91.82	2.24^{A}	82.66



Graph 1 Dynamics of water content in above ground matter of spring barley variety Kompakt after common foliar application of triazine herbicide (TAH) with regulators of polyamine synthesis and water stress

CONCLUSION

In experiment with initial 60 %, following 40 % full water capacity for improved initial plants development and non-watering (week water stress) the formation of fresh above ground and dry matter was reduced, the most significant in variant with solo herbicide application and it was evaluated as statistically high significant. Only the application of GABA (100 $g.ha^{-1}$) in mixture with herbicide (tendency towards statistical non-significance) eliminated these stress factors, because it reduced the decrease of water in plants. Lower dose of PDA in mixture also seems to inhibit the reducing of formation of above ground and root matter caused by herbicide and concurrently also with water stress. It means that in extreme moisture soil conditions (lack of water) regulating effect of inhibitors of polyamine synthesis was shown.

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