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POLYAMINE ALTERATIONS OF TRITICALE IN RESPONSE TO HERBICIDE, DROUGHT AND WATERLOGGING TREATMENTS

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Abstract

Drought and waterlogging are environmental stress factors that havoc normal plant growth and development. Polyamines are plant growth regulators, which participate in plant growth and development, as well as in the physiological responses to stress conditions. The polyamines fractions were evaluated in triticale plants pretreated with the selective herbicide Serrate®, and exposed to drought or waterlogging for 7 days. The herbicide applied alone provoked a slight decrease in polyamine content. A decrease of polyamine content was found also after drought, while waterlogging provoked an increase in polyamine levels. These data correlate with plant fitness and phenotype characteristics of treated seedlings. Our data provide new information about the role of plant polyamines in the physiological responses of triticale plants to water stress after herbicide application.

Key words: putrescine, spermidine, spermine, ×*Triticosecale* Wittm., water stress

Abbreviations: PAs – polyamines, Put – putrescine, ROS – reactive oxygen species, Spd – spermidine, Spm – spermine, TCA – trichloroacetic acid

Introduction. Putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant polyamines (PAs) in plants. They are among the most extensively studied plant growth regulators. PAs might be detected in free form

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or coupled with different small molecules as phenolic acids – (conjugated form) and with macromolecules (bound form) because of their net positive charge at physiological pH [1]. In plants, PAs participate in many growth and developmental processes –in the physiological response to unfavourable environmental conditions, they possess antioxidant features and free radical scavenging abilities [2]. Drought and waterlogging are widespread environmental issues, which alter negatively plant physiology, development and productivity of crops. In modern agriculture, herbicide usage is a widespread practice to withhold weed growth. Serrate® (Syngenta) is one of the systemic and selective herbicides used for wheat, rye and triticale, and control annual grass and broadleaf weeds. It consists of two active ingredients: clodinafop-propargyl – an inhibitor of fatty acids biosynthesis, and pyroxsulam – an inhibitor of branched-chain amino acids biosynthesis. In the field, plants are usually exposed to combinations of different environmental issues but the resultant effect is impossible to be foreseen [3].

Hura et al. [4] reported alterations in the content of free and cell wall-bound PAs: agmatine, cadaverine, Put, Spm and Spd in triticale subjected to drought at different stages of the crop development. Recently, we reported data on the involvement of polyamine fractions Spm, Spd and Put on physiological reaction of wheat seedlings treated with Serrate® and subsequently subjected to water stress [5]. Now we aimed to investigate the combined effects of the same herbicide and subsequent water stress on the polyamine profile in Bulgarian triticale cultivar Rhozen as triticale is expected to be more stress tolerant than wheat. The polyamine content in triticale seedlings subjected to Serrate® applied alone or in combination with water stress conditions (drought or waterlogging) have not been studied up to now.

Material and methods. ***Plant material and treatments.*** Young triticale seedlings ×*Triticosecale* Wittm., cv. Rhozen) were leaf sprayed with the herbicide Serrate® (water solution plus adjuvant Adigor) following the instructions of the producer (Syngenta). Three days later the treated seedlings were exposed for 7 days to drought by restraining irrigation; or to waterlogging by placing the pots into vessel filled with water 2 cm above the soil level [5]. Triticale plants were grown under controlled conditions as follows: 22/19 °C day/night temperatures, 16/8 h light/dark regime providing 250 µmol.m^{-2}.s^{-1} photon flux density. The leaf material was collected after 7 days of stress and after 4 days of recovery. Photos of treated plants were taken on the same days.

Polyamine assay. The free Put, Spd and Spm were extracted with 5% TCA following the method of Smith and Best [6] and measured in supernatant by direct dansylation. The conjugated PAs were assayed in supernatants, and bound PAs were analyzed in pellets after hydrolysis with concentrated HCl following the method of Torrigiani et al. [7]. Then, the HCl-hydrolysed PAs fractions were subjected to further dansylation and separation procedure by thin layer chromatography on Silicagel G 60 plates (Merck) using solvent system (cy-

clohexane:ethylacetate, 3:2 v/v). The spots were marked under UV light, scraped off, and then eluted in 2 ml anhydrous acetone. The sample fluorescence (excitation of 360 nm and emission of 505.5 nm) was recorded using Spectrofluorophotometer RF-1601 (Shimadzu, Japan). The obtained data were quantified against dansylated PA standards loaded on the same TLC plate and handled as samples.

Statistical analysis. The results are from two independent experiments performed in triplicate. The data are average values with standard error (SE). One-way ANOVA with Duncan's multiple range test was used to evaluate the significant differences at a level of 0.05.

Results. Profiles of the different polyamine fractions and the total amounts are presented in Fig. 1 (Spm), Fig. 2 (Spd), and Fig. 3 (Put).

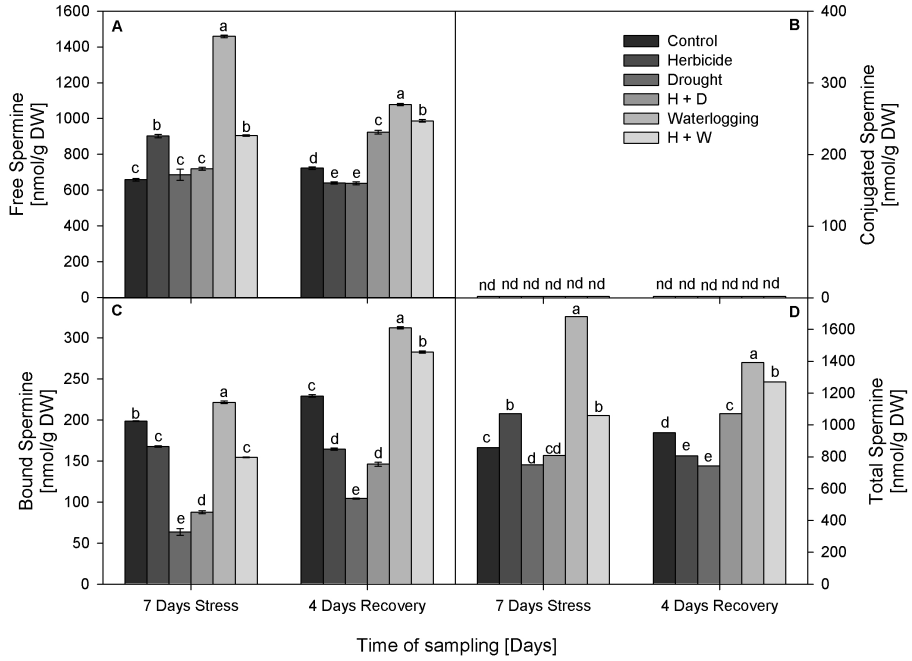


Fig. 1. Content of free (A), conjugated (B), bound (C), and total (D) spermine in leaves of triticale plants treated with herbicide (H) and exposed to drought (D) or waterlogging (W). Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$. Letters 'nd' in panel (B) stand for 'not detected'

The applied herbicide (H) provoked predominantly slight decrease in polyamine levels except those of free spermine (Fig. 1) and spermidine (Fig. 2) which were increased on the 7th day of stress. In general, drought when applied alone (D) or in combination with herbicide (H + D) decreased the endogenous content of polyamines during the estimated period (Fig. 1–3). Greatest reduction was detected in bound fractions and in free spermidine (Fig. 2C).

In contrast to drought, waterlogging provoked an increase in polyamines,

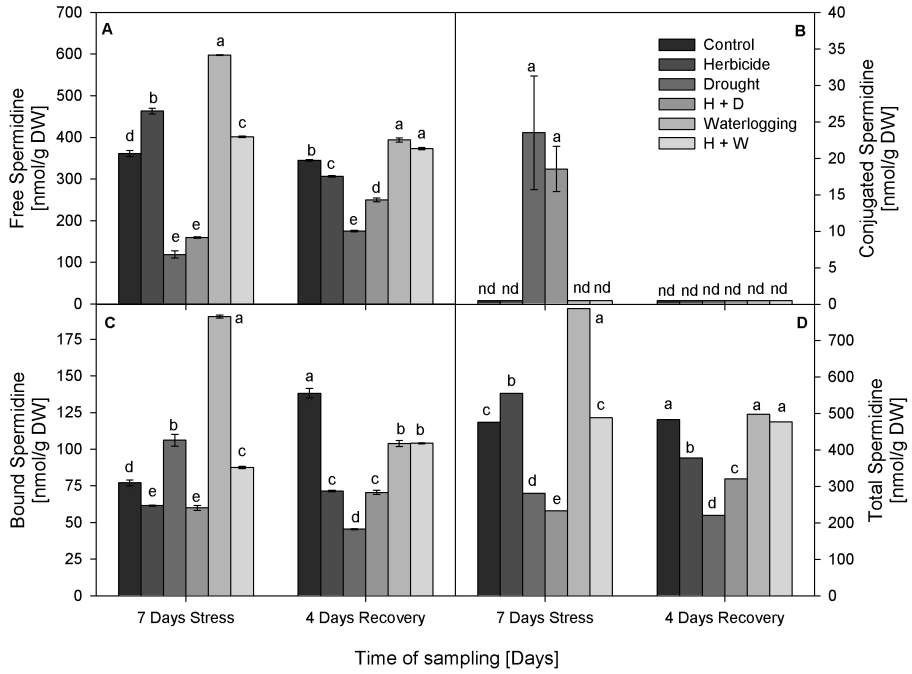


Fig. 2. Content of free (A), conjugated (B), bound (C), and total (D) spermidine in leaves of triticale plants treated with herbicide (H) and exposed to drought (D) or waterlogging (W). Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$. Letters 'nd' in panel (B) stand for 'not detected'

and especially of the free putrescine (Fig. 3C), which was augmented 6–7-fold up as compared to the control levels. This reflected on the increased total Put content (Fig. 3B). Interestingly only the amounts of soluble conjugated Spd (Fig. 2B) and conjugated putrescine (Fig. 3B) were detected after drought and H + D treatments, while no conjugated polyamines were found in control or after waterlogging during the stress period. No conjugated polyamines were found also during the recovery period.

The sums of all polyamines measured are presented in Fig. 4. It correlated with the aforementioned observation that drought caused a decrease in polyamines which is noteworthy clearly expressed during the recovery period, while waterlogging led to a significant increase in polyamine levels. Figure 4 evidenced that the herbicide did not induce discernible alteration in plants phenotype except for the yellowing of the older leaves at recovery. Drought evoked typical phenotypic alterations in triticale – obvious wilting was observed after 7 days of stress (Fig. 4A). After 7 days of waterlogging (including H + W), only a slight leaf yellowing was observed. After 4 days of recovery, the negative outcomes of drought were stronger than those after waterlogging in triticale plants (Fig. 4B).

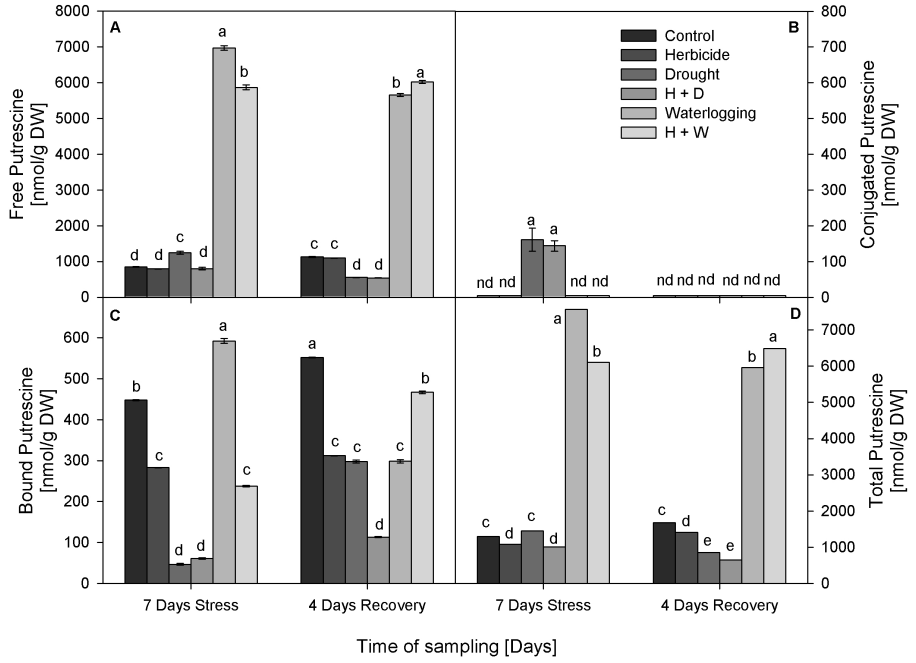


Fig. 3. Content of free (A), conjugated (B), bound (C), and total (D) putrescine in leaves of triricale plants treated with herbicide (H) and exposed to drought (D) or waterlogging (W). Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$. Letters 'nd' in panel (B) stand for 'not detected'

Discussion. Generally, under harsh environment plant polyamines rise in order to avoid the negative impact of stress and to sustain plant survival and growth [2]. For example PAs are increased due to water deficit [8–10] or water excess [11,12]. However, a decline in PAs after such water stresses is also reported [13–15]. Our results show that contrasting water supply caused differential response in the endogenous polyamines (Fig. 1–4). While drought predominantly decreased endogenous PAs, waterlogging mainly led to their augmentation. Recently, we reported that these stresses (especially drought) increased the content of free proline [16] in triticale. Since PAs share a common precursor with proline [10], it could be suggested that the increased biosynthesis of the compatible solute proline under drought stress corresponded to the decreased amount of PAs. The opposite to drought, the proline's increase in triticale under waterlogging stress was not huge [16], and PA levels upheld increased. This observation supports the suggestion that plants exposed to harsh environmental conditions redirect different biosynthesis pathways depending on plants vulnerability to specific stresses applied. In addition, plants that grow under waterlogging conditions are exposed to hypoxia (O_2 insufficiency) or anoxia (lack of O_2), inhibition of aerobic respiration, energy deprivation, accumulation of toxic end products of anaerobic

processes and reactive oxygen species (ROS) [17]. However PAs, and especially Put are increased in response to hypoxia [18, 19]. Earlier, it was reported that several *Gramineae* species, maize, rye, barley, and rice accumulated Put as a part of adaptation mechanisms of plants subjected to low oxygen conditions [18, 19]. Possible explanation of this fact is that Put could function as a part of homeostasis buffering mechanism to eliminate cellular ionic imbalance, and as a free scavenger, that removes hypoxia-induced ROS [18, 19]. Therefore, our findings of the immense accumulation of free Put, which caused the increased quantity of total Put (Fig. 3) in triticale exposed to waterlogging are in accordance with the aforementioned statements.

The free form of PAs is the most ample but they can form amide bonds with carboxylic groups of different soluble compounds or with cellular macromolecules and cell walls [1, 19]. While soluble conjugates are important components of the radical scavenging mechanisms, insoluble-bound PAs could serve as agents that modify the stability and permeability of cellular membranes [18, 19].

We found that the conjugated PAs were not presented during the recovery period in triticale. During the stress period, conjugated Spd and Put were only found in drought-stressed seedlings, which assumes the involvement of conjugated fraction in radical scavenging mechanism to cope with ROS generated due to drought stress. However, according to some earlier reports [19] conjugation reactions could regulate the functions of PAs by affecting their binding and interaction with cellular macromolecules. These interactions of bound PAs stabilize cell membranes, and increased content of amine conjugation might lead to membrane destabilization and thus contribute to senescence. Accordingly, bound PAs lower than the controls in drought treated plants probably indicated poor potential for stabilization of membrane systems and accelerated senescence as documented in Fig. 1–4. In addition, previous studies established that high levels of Put conjugate inhibited cell proliferation and thus retarded plant growth and development [19], and our findings concerning droughted triticale (including H + D) are in good correlation with the preceding observations. Disparately, the lack of conjugated PAs after waterlogging stress probably suggests that triticale tolerates the stress caused by water excess better than that of water scarcity, and plant growth continued. This could be a result of the active involvement of Put in ROS scavenging reactions as proposed above. Further, bound PAs in triticale exposed to waterlogging maintained levels close or higher than the controls, which corresponds well to the aforementioned hypothesis and could confirm cell membrane stability of plants.

In living organisms, PAs are versatile players such as the intracellular growth factors that increase the cell growth rate and further influence biomass accumulation [20]. The molecular mechanism of polyamine-enhanced growth revealed that PAs stimulate the synthesis of numerous key factors including several transcription factors [20]. The increased content of PAs in triticale exposed to waterlogging

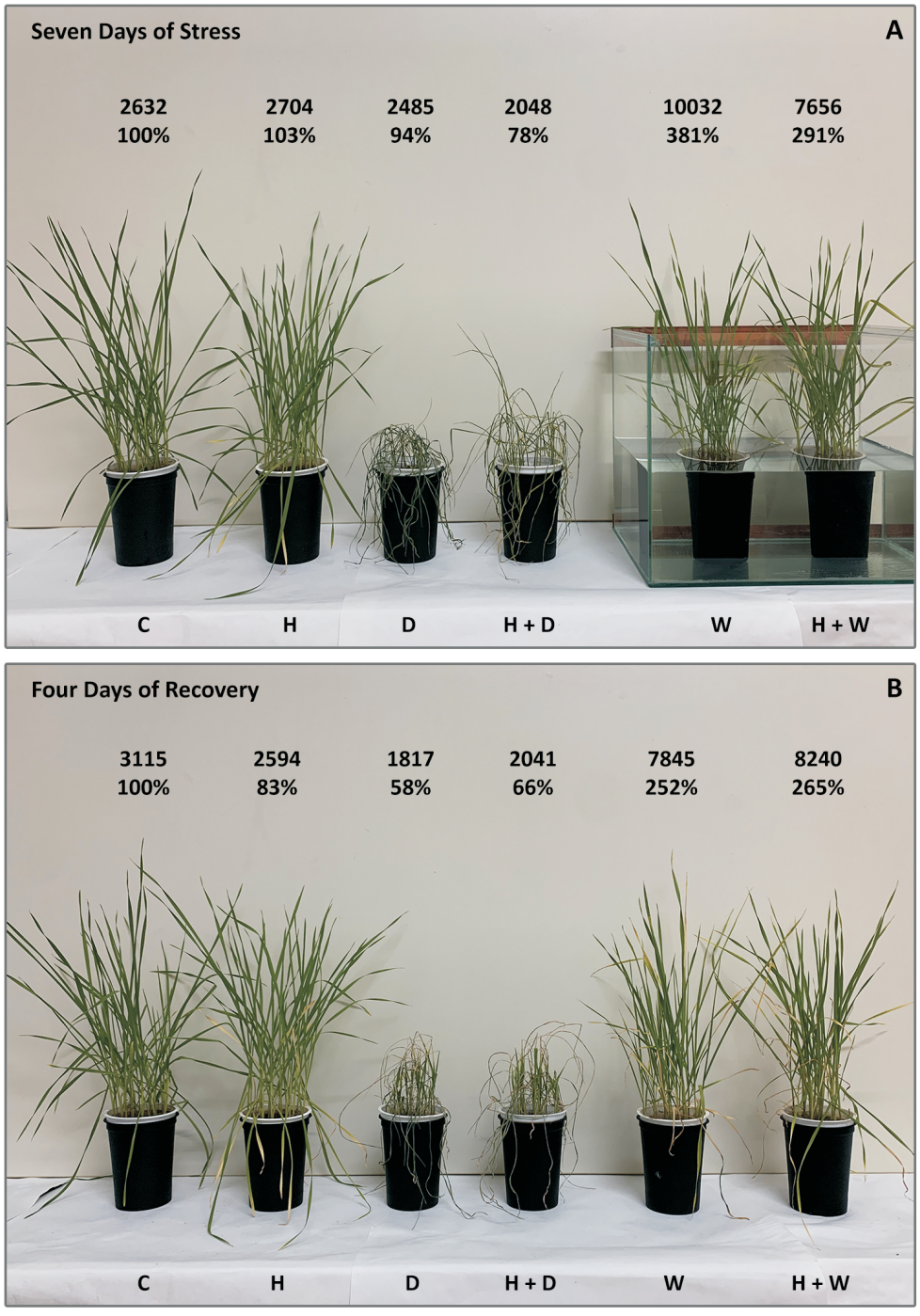


Fig. 4. Phenotypic alterations of triticale plants treated with herbicide (H) and exposed to drought (D) or waterlogging (W) after 7 day of stress (A), and 4 days of recovery (B). Numbers above the pots present the sum of the polyamines measured in leaves, expressed as nmol/g DW and as a percentage of the control

could theoretically act vigorously in plant growth, even under unfavourable growth conditions (Fig. 4A). On the contrary, the reduced PAs in drought-treated plants probably did not assist enough plants to sustain growth under water deficit and after rehydration (Fig. 4B). Direct relation between decreased PAs and retarded plant growth under drought evidences the key role of PAs in plants growth and development.

Usually, herbicide application causes modifications of endogenous PAs content – either decrease or increase, which depends on plant species, age, organs, developmental stage, sensitivity to herbicide, etc. [1]. We found that Serrate® slightly lowered PAs content than that of the respective controls (Fig. 1–3), which did not affect plant growth and seedlings showed normal phenotypic features (Fig. 4).

In conclusion, our study is a first report that triticale responded in a different way to drought and waterlogging stress regarding the alterations of endogenous polyamines. Drought-treated plants exhibited decreased quantity of polyamines, which reflected the stunted growth even after water supply restoration. Waterlogging increased polyamine pool and this was of benefit to the sustained plant growth. The herbicide caused lesser alterations in polyamine profiles than the water stresses applied. Our study expands the information about the role and functions of the endogenous polyamines in plant responses to different environmental conditions.

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