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MICROSOMAL P450-RELATED ELECTRON TRANSFER COMPONENTS, GLUTATHIONE AND GLUTATHIONE S-TRANSFERASE CONTRIBUTION IN STRESS RESPONSE OF HERBICIDE-TREATED WHEAT TO DROUGHT AND WATERLOGGING

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Abstract

The activities of some plant microsomal P450-related electron transfer components (NADPH-cytochrome P450 reductase, CPR and NADH-cytochrome *b5* reductase, B5R), glutathione S-transferase (GST) together with the content of reduced (GSH) and oxidized (GSSG) glutathione were determined in wheat (*Triticum aestivum* L., cv. Sadovo 1) treated with herbicide Serrate® prior to drought or waterlogging. Both stresses, especially drought, increased the content of glutathione and the activities of CPR and B5R during the stress, while GST activity was rather decreased. Herbicide application alone increased substantially most of the parameters, especially at the beginning of the experimental period. Serrate®+drought/waterlogging (S+D/W), amplified the observed alterations (except for GST), which were more significant after drought. The decrease in ratio GSH to GSSG after 4 days of recovery for all treatment groups except Serrate® signifies disrupted buffer capacity in cells. After 4 days of recovery the activities of CPR and B5R dropped below the control, while the glutathione pool and GST activity remained increased in drought and S+D

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treated plants. Contrariwise, the levels of glutathione, CPR and B5R remained closer to control in waterlogged plants, while S+W kept higher levels of P450-related enzymes. The herbicide application prior to drought or waterlogging did not affect negatively detoxification and oxidative status of wheat plants compared to those subjected to water stress alone.

Key words: water stress, selective herbicide, herbicide detoxification

Abbreviations: CPR – NADPH-cytochrome P450 reductase, B5R – NADH-cytochrome *b5* reductase, GST – glutathione S-transferase, GSH – reduced glutathione, GSSG – oxidized glutathione, ROS – reactive oxygen species, S+D – Serrate®+drought, S+W –Serrate®+waterlogging

Introduction. Application of herbicides is an ordinary practice in agriculture to restrain weed growth. Serrate® (Syngenta) is a selective herbicide used in wheat cultivation. It contains two active compounds (clodinafop-propargyl and pyroxsulam) with different modes of action and inhibits the biosynthesis of fatty acids and amino acids, respectively. Serrate® is commonly used to cope with the annual grass and broadleaf weeds in cereal crops, especially in wheat, because of its tolerance. Drought and waterlogging are common worldwide abiotic stresses affecting negatively various aspects of plant growth and development and decreasing plant production. Oxidative stress is a common stress response leading to overproduction of reactive oxygen species (ROS) which may possess signalling or destructive functions depending on ROS type, cellular sites of production, concentration, etc. [1,2]. Depending on the balance between the production of ROS and their scavenging by the antioxidative defense systems plants may adapt or not. Antioxidative defense system consists of non-enzymatic (glutathione, ascorbate, phenolics, etc.) and enzymatic (superoxide dismutase, catalase, peroxidase, glutathione S-transferase, etc.) components acting together to sustain the homeostasis. NADPH:cytochrome P450 reductase (CPR) is a membrane-bound flavoprotein, localized primarily in the endoplasmic reticulum membrane. Its function is to transfer reducing equivalents from NADPH to diverse P450 monooxygenases, involved in various secondary metabolic reactions, including biosynthesis of phenylpropanoids, terpenoids, alkaloids, fatty acids, plant hormones, etc. [3–6]. NADH:cytochrome b_5 reductase (B5R) is localized in the endoplasmic reticulum, where it transfers electrons from NADH to cytochrome b_5 and then to various lipid-modification reactions, like fatty-acid and sterol precursors desaturation, and fatty acids hydroxylation as well as P450 mediated reactions [7,8]. Both enzymes are involved in plant defense mechanisms against diverse abiotic stresses [4,6]. The CPR and B5R, as well as glutathione and GST participate in Phase I and Phase II of xenobiotic catabolism, and herbicides detoxification in particular [3]. These defense compounds take part in antioxidative defense shield toward various stresses [4–6,9].

Recently multifactorial stress combinations have become more frequent within arable land but they are still scarcely studied in plants [10]. To our knowledge

there are limited reports on the physiological response of wheat to pretreatment with the selective herbicide Serrate® prior to drought [11] or waterlogging [12]. The aim of this study was to reveal how Serrate® influenced some detoxification (CPR, B5R, GST) and oxidative (glutathione pool) parameters in plants treated with drought or waterlogging.

Materials and methods. ***Model system.*** Seventeen-day-old wheat (*Triticum aestivum* L., cv. Sadovo-1) plants grown as soil culture were sprayed with the herbicide Serrate® according to the manufacturer's instructions. After 72 h part of the plants were exposed to drought or waterlogging. Drought stress was achieved by withholding watering for 7 days to reach water deficit of 60% below the control. Waterlogging was realized by relocating the pots into an exterior container filled with water at level 2 cm higher than the soil. The stress programme was ceased 7 days after the beginning and the plants were transferred to normal irrigation (recovery). The plant material was randomized within each treatment group, collected at 4 days and 7 days of stress and after 4 days of recovery, and kept at –70 °C until analysis.

Biochemical extraction and determination. For in vitro B5R (EC 1.6.2.2) activity assay, potassium ferricyanide was used as an artificial electron acceptor [13]. The rate of reduction was calculated by extinction coefficient of 1.02 $mM^{-1}cm^{-1}$ at 420 nm. For in vitro CPR (EC 1.6.2.4) activity assay, cytochrome *c* was used as an artificial electron acceptor [13]. The rate of reduction was calculated by extinction coefficient of 21.1 $mM^{-1}cm^{-1}$ at 550 nm. The endogenous glutathione levels were determined according to the enzymatic recycling procedure [14]. The amounts of total glutathione and GSSG were obtained from standard curves. GSH was calculated by subtracting the amount of GSSG from total glutathione. Soluble GST activity with 1-chloro-2,4-dinitrobenzene (CDNB, extinction coefficient 9.6 $mM^{-1}cm^{-1}$ at 340 nm) as substrate was determined [14]. A standard curve with BSA was used to determine the protein content [15].

Statistical analysis. The results are acquired from three independent experiments preformed in triplicate. The data are mean values ± standard error (SE). One-way ANOVA with post-hoc Duncan's multiple range test ($p < 0.05$) was used to evaluate the significant differences between the treatments.

Results. Herbicide and Serrate®+drought (S+D) increased GST activity (Fig. 1) at 4 days of stress and after 4 days of recovery.

Drought led to a reduction in GST activity at 7 days of stress but after 4 days of recovery the effect was quite opposite. Waterlogging slightly reduced GST activity at 4 days of stress but an increase was found after 4 days of recovery. Serrate®+waterlogging (S+W) increased GST activity after 4 days of recovery.

Herbicide increased significantly the levels of GSSG (Fig. 2A) at 4 days of stress and after 4 days of recovery. Drought increased gradually the content of GSSG with time up to 6.6-fold. The GSSG was increased even more due to S+D after 4 days of recovery.

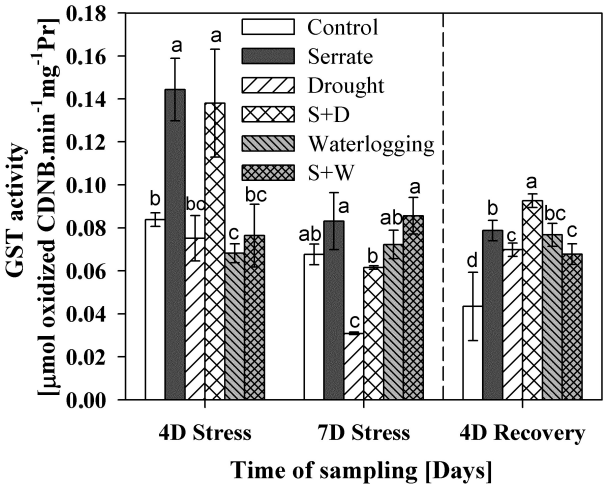


Fig. 1. Activity of glutathione S-transferase in wheat shoots treated with herbicide and exposed to drought or waterlogging. S+D – Serrate®+drought, S+W – Serrate®+waterlogging. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$

The increase of GSSG due to waterlogging was less pronounced than that caused by drought. The amount of GSH (Fig. 2B) tended to increase due to Serrate® and drought raised it gradually during the studied period. The level of GSH was additionally multiplied by S+D, and increased sharply with time. Waterlogging applied alone or after herbicide raised the amount of GSH only after 4 days of recovery. Total glutathione (Fig. 2C) is the pool of GSH+GSSG. Herbicide increased total glutathione levels at the beginning and at the end of the experiment. Drought raised the total glutathione content gradually with time up to 5-fold, and to 6.7-fold when Serrate® was applied in advance. Waterlogging and S+W increased total glutathione levels initially (4 days of stress) and after 4 days of recovery. Herbicide transiently reduced the ratio of GSH to GSSG (Fig. 2D) at 4 days of stress. Drought, waterlogging, and S+W, decreased the ratio GSH/GSSG at 4 days of stress and after 4 days of recovery but S+D led to increment at 7 days of stress.

Initially all treatment groups increased the activity of CPR (Fig. 3A). After 4 days of recovery the activity of CPR decreased in wheat plants subjected to drought or S+D but remained increased in Serrate® and S+W variants. Similarly, all treatment groups raised the activity of B5R at 4 and 7 days of stress (Fig. 3B). At 7 days of drought the induction of B5R activity was sharper than at 7 days of waterlogging. After 4 days of recovery B5R activity remained increased only in plants treated with Serrate® and S+W, but drought and S+D caused a reduction.

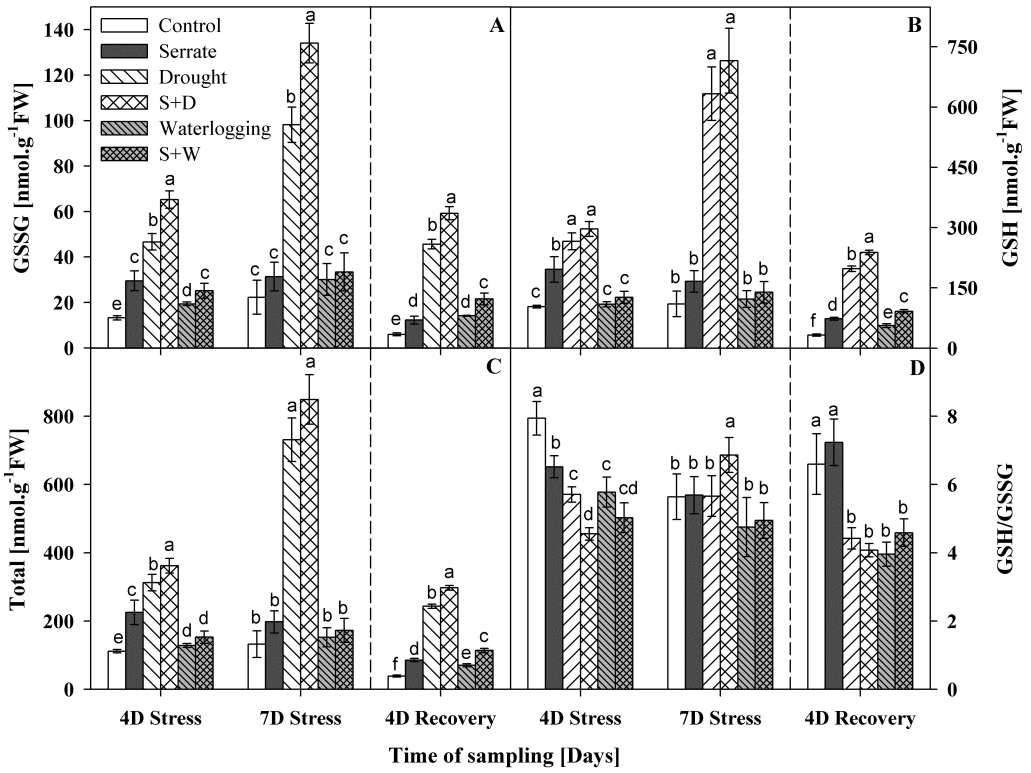


Fig. 2. Content of oxidized (A), reduced (B), total glutathione (C) and the ratio of reduced to oxidized glutathione (D) in wheat shoots treated with herbicide and exposed to drought or waterlogging. S+D – Serrate®+drought, S+W – Serrate®+waterlogging. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$

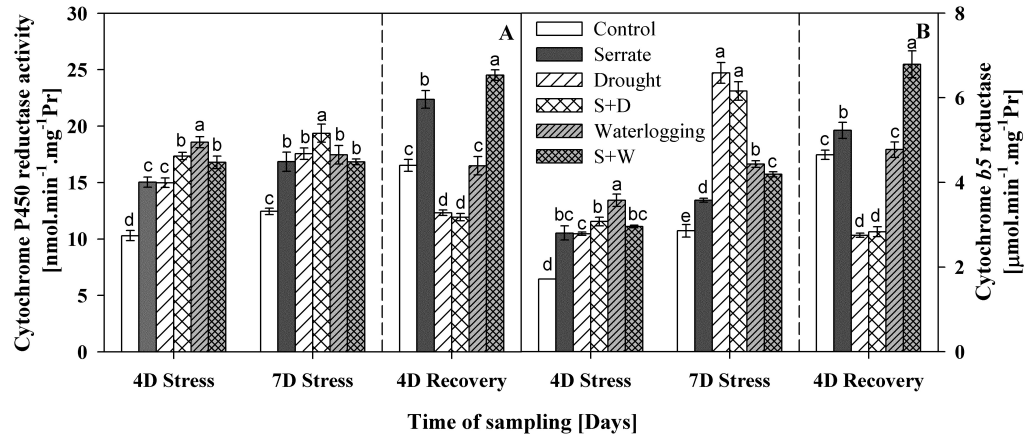


Fig. 3. Activity of cytochrome P450 reductase (A) and cytochrome b_5 reductase (B) in wheat shoots treated with herbicide and exposed to drought or waterlogging. S+D – Serrate®+drought, S+W – Serrate®+waterlogging. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$

Discussion. The focus of the current investigation is on the effects of the herbicide Serrate® on some detoxification antioxidants (GSH, GST) and on P450-related electron transfer components (CPR and B5R) in wheat plants subjected to water stress.

GSH-GSSG couple has multiple functions in plant cells. It maintains optimal cellular redox state. Glutathione may detoxify some ROS (lipid peroxides, reactive carbonyl species, etc.) and xenobiotics directly by conjugation via GST, or indirectly by signalling post-transcriptional and post-translational modifications, or by altering DNA binding characteristics [2,9,16,17]. When two molecules of GSH transfer an electron, glutathione disulphide (GSSG) is formed and the ratio of GSH to GSSG decreases, which usually happens during stress. The process is reversible and is part of the glutathione-ascorbate cycle. In general, the increased GSH content plays positive role in plant tolerance and helps to combat the oxidative stress, while a rise in GSSG has the opposite effect [9,18]. For example, a tolerant to waterlogging cultivar of watermelon was documented to have higher ratio of GSH to GSSG than the sensitive cultivar which experienced rigorous oxidative damage [18]. In our study, all treatments increased the GSSG more than GSH, so GSH to GSSG ratio dropped (except in plants subjected to herbicide only), leading to negative effect on cellular redox state. The significant decrease in GSH/GSSG ratio (especially after recovery), indicates that plants subjected to S+D, S+W or to water stresses applied alone had an impaired redox status. Importantly, the preliminary application of herbicide did not additionally aggravate the decrease of GSH to GSSG ratio. Therefore, Serrate® did not cause synergistic effect to drought or waterlogging. Expectedly, the GST activity was increased after recovery due to the necessity for ROS and/or xenobiotic detoxification. This increment seems crucial for plants subjected to drought and S+D to balance the decreased activities of CPR and B5R.

Overexpression of *NCR2* (a CPR gene) in cell lines of *Chlamydomonas* was reported to decrease ROS content under various stresses and to boost tolerance to multiple stresses [5]. Both activities of CPR and B5R were reported to increase in plants which are stress tolerant [4,19]. The decrease of CPR and B5R activities due to drought or S+D after recovery was similar, demonstrating that no additional harm was generated by the preliminary herbicide application. Both reductases are known to mediate lipid-modification reactions in the normal metabolism as well as under stress conditions. Increasing of lipid desaturation is a protective strategy for enhancing plant tolerance to waterlogged-induced hypoxia. The reduction of B5R activity after drought and S+D suggests altered biomembrane fluidity as the enzyme increases unsaturated fatty acids [8]. Probably the electron transfer systems as well as the oxidation-reduction status of wheat cells were disrupted, which was also shown by the low ratio of GSH to GSSG. Waterlogging alone and S+W sustained the activities of both CPR and B5R high during stress, which after recovery remained elevated only due to the combined stress. It is possible that

the herbicide provoked higher activities of the xenobiotic detoxification enzymes, which is evident in the plants, subjected only to Serrate®.

Our data present new information about the involvement of glutathione and xenobiotic-detoxifying enzymes in plants grown under multiple stress conditions. In conclusion, the herbicide application prior to drought or waterlogging did not affect negatively the detoxification and oxidative status of wheat plants compared to those subjected to water stress alone, as witnessed by glutathione-related compounds (GSH, GSSG, GST) and by P450-related electron transfer components (CPR and B5R).

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