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REACTION OF GLUTATHIONE-DEPENDENT SYSTEM OF *ACER* TREES VEGETATIVE BUDS TO POLLUTANTS' ACTION

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The pollutants-induced changes of reduced glutathione content and glutathione dependent enzymes activity in vegetative buds of *Acer* trees were investigated. In buds of *A. platanoides* and *A. pseudoplatanus* increasing GSH pool and GST activity attached to high GR activity were established, whereas in *A. negundo* buds decreased GSH content but high induced GR and high constitutive GST activity were revealed. Specificity of vegetative bud's glutathione system changes points to variations of pathway of *Acer* species metabolic adaptation to pollutants' action.

Keywords: Acer L., vegetative buds, pollutants, glutathione, glutathione reductase, glutathione S-transferase.

Ontogenesis of plants in urban phytocenoses proceeds under condition of chronic combined action of different pollutants from industrial waste and exhaust fumes of transport. It is known that despite specificity of plant species stability to pollutants action, general effect of differing toxic compounds includes increasing of reactive oxygen species (ROS) content in plant cells [6]. Plant reactions are realized by means of enzymatic and non-enzymatic defense systems, which promote the subsequent formation of metabolic adaptation [14]. Glutathione-dependent system is one of the most effective parts of plants antioxidant defense against toxic action of heavy metals [10, 20], ozone [15], sodium chloride [17], and acidic rains [2], but its adaptive efficiency in case of integrated contamination of environment is not fully understood to date.

Systems assessment of plants functional state also requires the investigation of meristem-including organs which determine further development of plants. The suitable example of such organ is dormant vegetative bud as it's the shoot in an embryonic state, but comparatively little research has been directed toward revealing the metabolic characteristics of buds. For today scientific literature includes reports about effects of different environmental factors [8], and endogenous physiological and biochemical signals [1] to the vegetative buds status, whereas anthropogenic action is not paid much attention for. It was mentioned, for example, considerable increasing of heavy metals content in poplar vegetative buds from urban phytocenoses [16], but the reaction of plant defense systems was not studied.

The purpose of present study was to determine the species peculiarities of glutathione-dependent system changes in the vegetative buds under chronic combined action of pollutants, and to look deeper into the role of buds glutathione system in adaptation of *Acer* trees.

Materials and methods

Vegetative buds were collected in February 2013 from *A. platanoides*, *A. pseudoplatanus, A. negundo* trees in the Dnipropetrovs'k Botanical Garden (plot 1, control) and in contaminated urban phytocenoses: polluted with fume of transport (plot 2 and plot 3), industrial waste (plot 4 and plot 5), and waste of heat and power station (plot 6). It was taken on average 12–15 buds from 20-25-years-old trees of each species, and samples were stored in frozen state.

The reduced glutathione (GSH) amount was determined by photometrical method according to [11] in the no-protein fractions of buds, obtained by addition of 50% trichloroacetic acid to crude extracts with subsequent centrifugation (12000 g for 15 min and 4°C). The optical density of reaction mixture (2 ml of 0.4 M Tris-HCl buffer pH 8.9 and 1 ml of no-protein extract) was registered at 412 nm both before and after addition of Ellman's reagent and incubation for 1 h. The results were calculated by using calibration graph and expressed in μg GSH/g fresh weight (FW).

The activities of glutathione-dependent enzymes were determined in supernatant, obtained from bud's crude extracts (200 mg of fresh tissue homogenized with 1.0 ml of 0.1 M phosphate buffer contained 1 mM EDTA) by centrifugation (15000 g for 20 min and 4°C). All reactive mixtures were incubated at 30°C, and optical density change was detected for 4 min at 340 nm. The assay of glutathione S-transferase (GST, EC 2.5.1.18) activity was based on the method of Habig et al. [7] with phosphate buffer (pH 8.0), 20 mM GSH, and 20 mM CDNB as substrate, and enzyme activity was expressed in nM CDNB/sec·gFW. Glutathione reductase activity (GR, EC 1.6.4.2) was measured according to [5] in reactive mixture containing 50 mM Tris buffer (pH 7.8), supernatant, 2 mM GSSG and 2 mM NADPH, and was expressed in nM NADPH/min·gFW. All determinations were performed in three replicates, statistical analyses were achieved with an independent samples t-test, and significant differences between were indicated with $p \leq 0.05$.

Results and discussion

In this study substantial differences both control levels of GSH in *Acer* species buds and size of their pollution-induced changes were revealed (Fig. 1).

In contaminated buds of *A. platanoides* GSH content increased significantly as compared with control, amounting 431 and 523% in buds polluted with cars fume (plots 2 and 3, respectively), 393 and 164% with industrial waste (plots 4 and 5), and 152% in buds polluted with power station wastes.

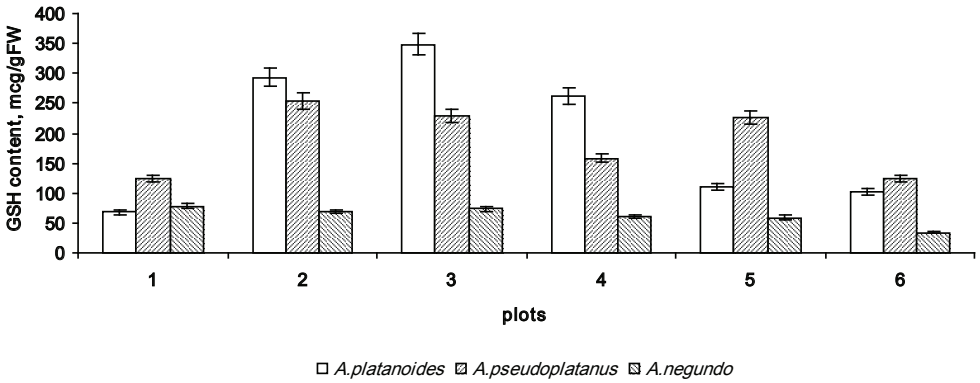

Fig. 1. GSH content in the *Acer* buds from control plot (1) and plots polluted with cars fume (2 and 3), industrial waste (4 and 5), and power station waste (6). All the values are the mean of three different samples ± standard error.

GSH content in buds of *A. pseudoplatanus* from plot 6 didn't differ from control, whereas on the other polluted plots it averaged out 150–203% as compared with control buds. GSH pool in all contaminated buds of *A. negundo* was below the control level: insignificantly (7–14%) in buds polluted with cars fume, average 22–25% in buds polluted with industrial waste, and 57% in buds from plot 6. That is, the largest enhancement of GSH pool was induced by cars fume in the buds of *A. platanoides* and *A. pseudoplatanus*; whereas in buds of *A. negundo* every pollutant induced decreasing level of GSH, most by the waste of power station.

It is known that intracellular GSH is involved in series of key metabolic processes [10], and therefore contributes to effective scavenging of ROS and rising of plant stress-stability [12], to maintaining of redox-state of the plant cells [9], and to performing of specific functions, such as determining of quantity and quality of embryos by way of GSH/GSSG ratio [18]. The recited facts offer a possibility of guessing, that sizable pollution-induced changes of GSH pool in the *Acer* buds must be assessed as the result of reaction not only of glutathione system but of the whole cells metabolism. Beside that, as the intracellular GSH level is determined by processes of biosynthesis, utilization, and reducing from oxidized form [13], so it's changing in buds reflects the pollutants action on glutathione-dependent enzymes as well.

Significant increasing of glutathione reductase (GR) activity in *A. platanoides* buds and little one in *A. negundo* buds were revealed on all polluted plots, whereas decreasing enzyme activity was revealed in all contaminated buds of *A. pseudoplatanus* (Fig. 2).

High activation of GR in *A. platanoides* buds was observed both owing to influence of cars fume (195–230% above control) and of industrial waste (186–199%), whereas rise of enzyme activity under the influence of power station waste achieved only 159%.

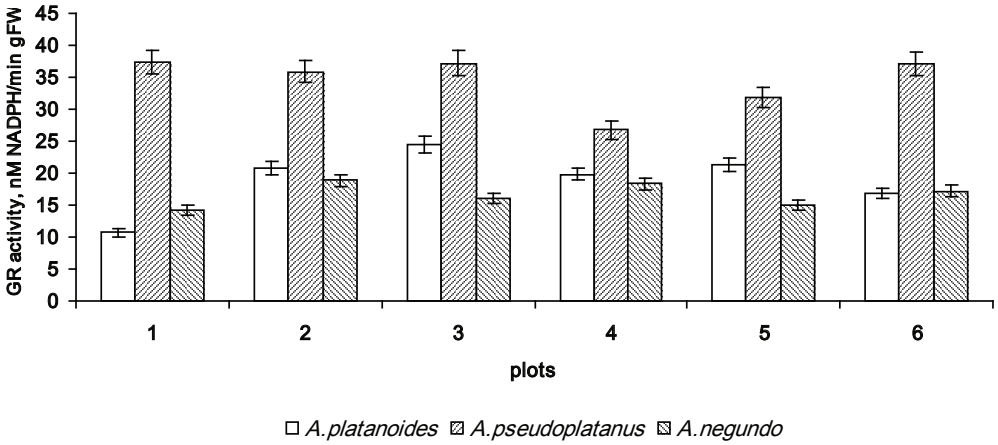

Fig. 2. GR activity in the *Acer* buds from control plot (1) and plots polluted with cars fume (2 and 3), industrial waste (4 and 5), and power station waste (6). All the values are the mean of three different samples ± standard error.

Against a background of first species rising GR activity in polluted buds of *A. negundo* was comparatively less reaching roughly 105–132% above control. Most decreased GR activity in *A. pseudoplatanus* buds was induced by influence of industrial waste (28 and 16% below control), but the enzyme activity was comparable to control in other polluted buds. It is known that GR catalyses glutathione reduction from oxidized form, and plant ability to induce the GR activity can make greater contribution to oxidative stress tolerance than increasing intracellular GSH pool [12]. That's why species peculiarities of GR changes in buds might indicate different adaptive mechanisms of *Acer* plants to pollutants action: high constitutive enzyme activity in the buds of *A. pseudoplatanus* (37.3 nM NADPH/min gFW), but on the contrary, low constitutive GR activity in the buds of *A. platanoides* and *A. negundo* (10.7 and 14.3 nM NADPH/min gFW, respectively) attached to high activation. Present supposition coincides with conclusion about adaptive direction of species differences in GSH biosynthesis and GR activity changes in Cd-treated water plants [4].

The highest glutathione S-transferase activity in *A. negundo* buds was founded on control plot and it was declined in buds from contaminated plots, whereas GST activities both in *A. platanoides* and *A. pseudoplatanus* buds from polluted plots were above control (Fig. 3).

In the buds of *A. platanoides* from polluted plots GST activity increasing reached 350–370% above control owing to industrial waste action, and 220–225% owing to influence of all other pollutants. In *A. pseudoplatanus* buds GST activity exceeding with spacing 150–260% above control was founded on all contaminated plots. Declining enzyme activity in polluted buds of *A. negundo* was negligible owing to influence of cars fume, whereas substantial owing to influence of industrial waste (38-62% below control), and power station waste coming 43% below control.

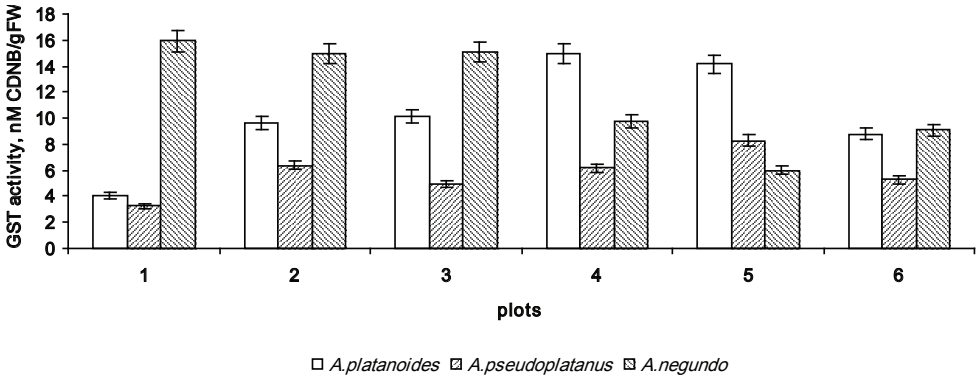

Fig. 3. GST activity in the *Acer* buds from control plot (1) and plots polluted with cars fume (2 and 3), industrial waste (4 and 5), and power station waste (6). All the values are the mean of three different samples ± standard error.

It's known that GST family is heterogeneous group of enzymes, that able to catalyze of variety toxic compounds conjugation with GSH [3] that is why increased level of GST activity in contaminated buds of *A. platanoides* and *A. pseudoplatanus* should enhancing detoxification. As for *A. negundo*, because some GST possesses glutathione peroxidase activity [13], decreasing GST activity in polluted buds might be conditioned not so much by inactivation of enzyme as by coordinated reaction of glutathione system to pollutants action, but certainly enhancing reduction of organic peroxides in buds requires future elucidation.

Correlative data analysis revealed in *A. platanoides* polluted buds significant positive dependents between GSH content and GR activity (r=0.76), and between GR and GST activities (r=0.70). In buds of *A. pseudoplatanus* and *A. negundo* the significant correlation was founded only between GSH content and GST activity (r=0.60 and r=0.68, respectively).

Thus, the results demonstrated that both level and direction of pollution-induced changes of GSH content, GR and GST activities in buds of *Acer* species characterized the specificity of the glutathione system reaction to pollutants action. The adaptive tendency of revealed coordinated changes of defense glutathione-dependent system in buds was confirmed by means of successful ontogenesis of *Acer* trees in the polluted phytocenoses. Developing buds accept assimilates from polluted leaves, so it looks as if the metabolic state of *Acer* dormant vegetative buds is a reflection of previous stressful experience of whole plants under conditions of contaminated phytocenoses. Present supposition coincides with views of Walter et al. [19] about an ecological stress-memory of individual plants, which includes accumulation of proteins and metabolites, and is regarded as one of prerequisites for high ecosystem stability under adverse environment conditions.

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РЕАКЦІЯ ГЛУТАТІОН-ЗАЛЕЖНОЇ СИСТЕМИ ВЕГЕТАТИВНИХ БРУНЬОК ДЕРЕВ РОДУ *ACER* НА ВПЛИВ ПОЛЮТАНТІВ

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Досліджено індуковані впливом полютантів зміни вмісту відновленого глутатіону та активності глутатіон-залежних ферментів у вегетативних бруньках дерев роду *Acer*. У бруньках *A. platanoides* і *A. pseudoplatanus* виявлено зростання пулу GSH і активацію GST за високої активності GR, тоді як у бруньках *A. negundo* виявлено зниження вмісту GSH за значної активації GR та високої конститутивної активності GST. Видова специфічність змін глутатіонової системи вегетативних бруньок вказує на варіативність шляхів метаболічної адаптації видів роду *Acer* до впливу полютантів.

Ключові слова: Acer L., вегетативні бруньки, полютанти, глутатіон, глутатіон-редуктаза, глутатіон-S-трансфераза.

РЕАКЦИЯ ГЛУТАТИОН-ЗАВИСИМОЙ СИСТЕМЫ ВЕГЕТАТИВНЫХ ПОЧЕК ДЕРЕВЬЕВ РОДА *ACER* НА ДЕЙСТВИЕ ПОЛЛЮТАНТОВ

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Исследованы индуцированные действием поллютантов изменения содержания восстановленного глутатиона и активности глутатион-зависимых ферментов в вегетативных почках деревьев рода *Acer*. В почках видов *A. platanoides* и *A. pseudoplatanus* выявлены возрастание пула GSH и активация GST при высокой активности GR, тогда как в почках *A. negundo* выявлено снижение содержания GSH при значительной активации GR и высокой конститутивной активности GST. Видовая специфичность изменений глутатионовой системы вегетативных почек указывает на вариативность путей метаболической адаптации видов рода *Acer* к влиянию поллютантов.

Ключевые слова: Acer L., вегетативные почки, поллютанты, глутатион, глутатион-редуктаза, глутатион-S-трансфераза.