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GENERAL BIOLOGY

The Priming of Potato Plants Induced by Brassinosteroids Reduces Oxidative Stress and Increases Salt Tolerance

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Abstract—This is the first study to show that brief pretreatment of potato plants with two brassinosteroids differing in structure causes in plants the ability to react to delayed salt stress by accumulation of compounds with antioxidant activity and by increased salt tolerance.

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The priming of plants is the process of acquisition by them, after the primary contact with a stress factor, of the ability to increase stress tolerance in response to action of a certain damaging factor in the future. Inducers (stimuli) of transition of a plant into the state of priming may be natural stressors of chemicals [1]. Mechanisms underlying the transition of a plant from the normal state into the state of priming have been poorly investigated. Especially interesting is the use as priming signals of chemicals, e.g., phytohormones, as a brief pretreatment of seeds (priming of seeds) or plants to considerably increase the tolerance to various abiotic and biotic damaging agents [2].

Among phytohormones, steroid hormones of plants, brassinosteroids (BRs), are the most promising as priming inducers. Brassinosteroids possess a clear pleiotropic action. They are regulators of numerous molecular and integrated physiological processes, from stimulation of synthesis of DNA, RNA, and proteins, activation of cell division, of biosynthesis of components of cell wall to photosynthesis, respiration, donor–acceptor relationships, growth, ontogenesis, and productivity [3–6].

In addition, BRs are effective stress-protector compounds whose protective action much depends on their capacity of mobilization or synthesis of components of the cell antioxidant system [4, 7]. The majority of papers published until now have been aimed at investigation of the protecting mechanisms of BR under condition of stress. Their ability to induce the state of priming resulting from brief hormonal pretreatment of plants before the stressing has been poorly investigated. The present study is aimed at investigation of mechanisms of priming of potato plant under the action of BSs.

We used plants of *Solanum tuberosum* L. of a midripening cultivar Lugovskoy (identifier, 8301891) widely distributed in the central areas of Russia and in Siberia. This cultivar of potato yields a stable high crop, its tubers are characterized by a high storability and tolerance to certain diseases, including phytophthora (late blight) infection. Sanitized regenerants of potato plant in vitro were obtained from the apical meristem. Then they were cultivated for 25 days on agarized Murashige and Skoog (MS) medium containing half of the normal content of macro- and microelements. In the end of cultivation the roots of plants were washed off the agarized medium, and microclones were adapted to a liquid MS medium and to conditions of aerial medium during two weeks under L36W/77 Fluora luminescent lamps (Osram, Germany) at a density of quantum flux of photosynthetically active radiation of 200–250 μmol m^{-2} s^{-1} in a phytotron with a 16-h photoperiod and temperature of 20 ± 3°C. After two weeks of growth in MS medium in a hydroponic installation the plants were transferred for 4 h to the same medium in the absence (control) or in presence of brassinosteroids within concentration range from 10^{-11} to 10^{-8} M. As active brassinosteroids 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) were used, differing both in the number of C atoms in the molecule—C28 (24-epibrassinolide) and C29 (28-homobrassinolide)—and in configuration of lateral substituents—24R-methyl (24-epibrassinolide) and 24S-ethyl (28-homobrassinolide).

After 4-h hormonal treatment the plants were transferred to an MS solution without BRs for 20 h. Then, they were placed into MS nutrient medium in

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Table 1. Influence of 4-h treatment of potato plants with 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) on the main growth parameters

Variant of treatment	Number of stolons		Sum area of leaves		Sum weight of plants	
	pcs	%	cm^2	%	g	%
Control	5.00 ± 0.38	100	45.52 ± 4.36	100	5.45 ± 0.31	100
10^{-11} M EBL	6.63 ± 0.46*	132	62.10 ± 4.32*	136	6.35 ± 0.53	117
10^{-10} M EBL	5.78 ± 0.64	116	65.49 ± 5.46*	144	6.26 ± 0.63	115
10^{-9} M EBL	4.89 ± 0.54	98	55.76 ± 4.65	122	5.67 ± 0.39	104
10^{-8} M EBL	5.18 ± 0.81	104	50.92 ± 5.22	112	5.78 ± 0.50	106
10^{-11} M HBL	6.36 ± 0.58*	127	57.57 ± 4.19	126	5.92 ± 0.40	109
10^{-10} M HBL	5.00 ± 0.47	100	53.03 ± 5.30	116	5.27 ± 0.28	97
10^{-9} M HBL	3.70 ± 0.90	74	47.11 ± 6.34	103	5.03 ± 0.55	92
10^{-8} M HBL	4.63 ± 0.97	93	49.97 ± 5.36	110	5.62 ± 0.54	103

Here and in Figs. 1 and 2, $M \pm m$, $n = 3$; * $p < 0.05$ compared with the control.

the absence (control) or presence of 100 mM NaCl (experimental variants). After six days, the plant material was fixed in liquid nitrogen and used in analyses. Growth and physiological parameters were assessed as described previously [4]. The experiments were performed in at least three replicates. Significance of differences between experimental and control samples was estimated by Student's *t* test.

Brief treatment of plants with EBL and HBL at concentrations of 10^{-11} and 10^{-10} M somewhat stimulated their growth after 164 h of hormone action (Table 1). This was manifested in the increase in fresh weight of a potato plant by 15–17% of the control value in response to treatment with EBL and HBL, of the sum leaf surface (by 36–44%), and of the number of stolons (by 27–32%). However, the dry weight of plants, the sum leaf surface, and the content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) did not change in response to action of BRs (data not shown).

Growing of potato plants during 144 h on MS medium containing 100 mM NaCl resulted in a decrease in the sum area of leaves by a factor of 1.6, in the number of stolons by a factor of 3.8, and in the number of layers (Fig. 1). As compared to the control, fresh and dry weight of plants decreased by about 1.4 and 1.7 times, respectively (data not shown). The level of photosynthetic pigments (chlorophyll *a* and carotinoids) in leaves of potato decreased by a factor of 1.6 (Fig. 2b).

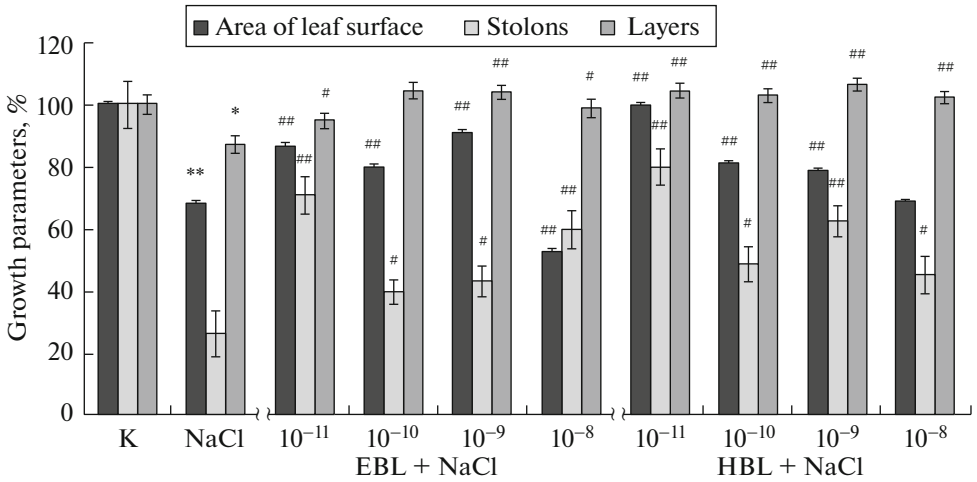

Fig. 1. Influence of salinization (100 mM NaCl, 144 h) and 4-h pretreatment with the brassinosteroid 24-epibrassinolide (EBL) or 28-homobrassinolide (HBL) followed by "delayed" salinization (100 mM NaCl, 144 h) on the main growth parameters of potato plants. * $p < 0.05$, ** $p < 0.01$ (control variant vs. influence with NaCl); # $p < 0.05$, ## $p < 0.01$ (NaCl vs. influence with brassinosteroids against the background of salinization).

It is known that NaCl at high concentrations renders not only a direct toxic effect on cell metabolism and causes osmotic stress, but also stimulates generation reactive oxygen species (ROS) and development of oxidative stress. In this case, the main cause of oxidative stress is related to closure of stomata, decrease in access to CO_2, and increase in energy of excitation of electrons accompanied by intensive generation of ROS [8]. Another cause of increase in production of ROS upon salinization is disturbance of respiration [9].

For assessment of the level of oxidative stress in potato plants at salinization the intensity of peroxide oxidation of lipids was estimated which was measured by the content of malonic dialdehyde (MDA) in reaction with thiobarbituric acid. The value of this parameter in the reaction medium increased by 58–60% when plants of *S. tuberosum* were used exposed to salt in comparison with the control (Fig. 2a).

It is known that, for alleviation of negative influence of oxidative stress, plants activate antioxidant protective systems whose action is aimed at suppression of ROS. In this connection, of special interest are the enzyme systems (superoxiddismutase, catalase, peroxidase, etc.) and non-enzyme systems (carotinoids, low-molecular phenolic compounds, prolin, etc.) of antioxidant protection [10].

We did not record any significant changes in the activities of key antioxidant enzymes (superoxide dismutase, peroxidase) in response to salinization (data not shown), but there was a fivefold increase in comparison with the control plants of prolin, the key component of the non-enzyme system of antioxidant protection (Fig. 2c).

A short-term introduction of steroid hormones in the nutrient medium decreased negative influence of salinization (100 mM NaCl), which occurred 20 h after the end of the hormonal treatment, on growth parameters, including the number of stolons, sum leaf surface, and wet weight of plants. This indicates an increase in salinity tolerance. The number of stolons and size of leaf surface increased by factors of 3.0–3.2 and 1.3–1.5, respectively, at the minimal concentration of BRs (10^{-11} M), while the number of layers increased by 25–26% in comparison with plants exposed to saline stress alone (Fig. 1).

Exogenous introduction of hormones in the entire range of investigated concentrations against the background of salinization contributed to a decrease in the MDA level. This indicated a decrease in peroxide oxidation of lipids and intensity of oxidative stress (Fig. 2a) and was a probable cause of increase in the plant tolerance to salinity.

The content of photosynthetic pigments (chlorophyll *a* and carotinoids) also increased in the case of treatment of plants with EBL (from 10^{-10} to 10^{-8} M) and with HBL (10^{-9} and 10^{-8} M), attaining in some cases the control values (Fig. 2b). It is known that accumulation of carotenoids is a strategy decreasing intensity of oxidative stress [11]. Carotenoids participate in extinction of $^1O_2^*$ radicals and oxygen peroxide generated in excessive excitement of chlorophyll.

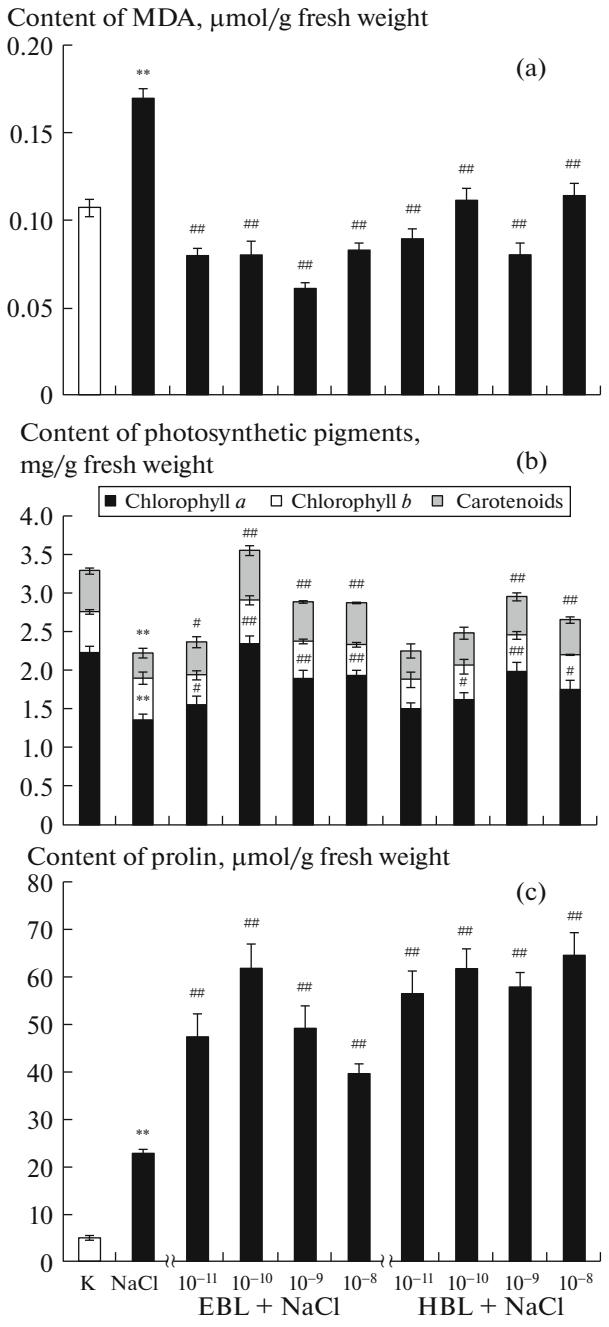

Fig. 2. Influence of salinization (100 mM NaCl, 144 h) and 4-h pretreatment with the brassinosteroid 24-epibrassinolide (EBL) or 28-homobrassinolide (HBL) followed by "delayed" salinization (100 mM NaCl, 144 h) on (a) the MDA content, (b) the content of main photosynthetic pigments, and (c) the content of prolin.

In addition to the accumulation of carotenoids in adaptation of plants to water deficit and toxicity of the excess of inorganic ions, an important role is played by

the amino acid proline, a compatible osmolyte with distinct properties of an antioxidant and a chemical chaperon [12, 13].

Our data imply that a brief pretreatment with BRs modifies metabolism in the cells of potato so that they can response to "delayed" action of saline stress by a more (2.0- to 2.7-fold) active accumulation of proline in comparison with the plants exposed only to 100 mM NaCl (Fig. 2c).

Thus, our results demonstrate that transient treatment with BRs induces transition of potato plants into the state of priming. This state manifests itself in the capacity of plants to react to "delayed" salt stress by a more efficient accumulation of prolin and carotenoids possessing strong antioxidant and stress-protecting properties. Evidently, this fact provides the basis of the decrease in the level of oxidative stress and increase in salt tolerance in potato cells.

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