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Title Effect of WGA on 24-Epibrassinolide-Induced Resistance of Wheat Plants and Cell Walls Reinforcement under the Influence of Cadmium Acetate †

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Abstract: The generally accepted approach to increase the plant resistance to heavy metals is the treatment by phytohormones of different nature. In particular brassinosteroids are highly effective in decreasing the level of toxic ions damage effects on plant growth. ABA and wheat germ agglutinin (WGA) contents were measured in the same plant with the help of enzyme immunoassay method. The localization of lignin and suberin on cross sections of wheat roots stained with berberine hemisulfate/toluidine blue. The mitotic index was determined as percentage of mitotic cells of total number of the cells. It was found that 0.4 μM 24-epibrassinolide (EBR) seed pretreatment under the influence of 1 mM cadmium acetate in the presence of fluridone (Fl) to inhibit the stress-induced accumulation of ABA, while the WGA content in plants stayed higher than in stressed EBR-untreated wheat roots. Moreover, EBR-pretreated wheat plants under cadmium stress form in the root cell walls Casparian bands and suberin lamellae, the critical places in the apoplastic barriers of endodermis and exodermis. Pre-sowing EBR-treatment promoted to acceleration Casparian bands and suberin lamellae formation without inhibiting growth processes. We have demonstrated the involvement of wheat lectin in the realization of EBR-induced protective effect on plants under cadmium stress. An important contribution to the EBR-induced strengthening the barrier properties of the cell walls of the studied tissues have the ability of EBR to induce ABA-independent accumulation of WGA, which further promotes the increasing lignin and suberin biopolymers deposition in the cell walls of wheat roots.

Keywords: wheat germ agglutinin; 24-epibrassinolide; ABA; mitotic index; Casparian bands

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1. Introduction

Wheat germ agglutinin (WGA) is a classical wheat lectin belonging to the RAB protein family [1]. WGA is an active participant in ABA-controlled reactions in response to biotic and abiotic stresses [2], some portion of this wheat lectin is excreted in the area of root apical meristem, apparently becoming an exogenous agent for the plants [3]. Lectins are carbohydrate-binding proteins known for their role in the regulation of innate immune responses in various organisms [4]. Their role in signaling pathways involved in adaptation to abiotic stress is also well understood recently.

The influence of cadmium ions has a toxic effect on plants, causes osmotic stress and induces the accumulation of ABA in plants [5]. In this regard, the actual question towards WGA involvement as an excreted protein in the process to wheat protection from the toxic cadmium effect arises. Some authors mention possible increase lectin genes expression in response to cadmium stress in the roots of plant species hypertolerant to such stress [6]. The key role of ABA in the induction of WGA synthesis and accumulation under the 1 mM cadmium acetate treatment was demonstrated. In turn this protein contributed to the maintenance of root cell mitotic activity under stress at higher level and indicated this lectin participation in the ABA-triggered defense reactions of wheat plants during cadmium stress [3].

The generally accepted approach to increase the plant resistance to heavy metals is the treatment by phytohormones of different nature. In particular brassinosteroids are highly effective in decreasing the level of toxic ions damage effects on plant growth [7]. Previously it was found that 24-epibrassinolide (EBR) treatment causes the significant WGA gene transcription activation and the increase in WGA content in wheat seedlings as well as the absence in changing in the endogenous ABA concentration [8]. These data, alongside with the previously obtained results on the WGA treatment possibility to enhance cell division of EBR-pretreated seedlings against the background of endogenous lectin deficiency, indicate the interaction of the wheat lectin system and EBR in the regulation of growth processes [9].

In this regard it was important to elucidate the WGA participation in EBR-induced wheat defense reactions in response to cadmium stress and to evaluate the endogenous ABA contribution to the regulation of WGA level in EBR-pre-sowing treated plants under cadmium acetate treatment.

2. Experiments

Experiments were performed with 7-day-old wheat seedlings of *Triticum aestivum* L., cultivar Bashkirskaya 26. Wheat seeds were sterilized in 96% ethanol and then they were soaked in a solution of 0.4 μM of 24-epibrassinolide or distilled water for 3 h. The 7-days-seedlings were preliminarily isolated from the endosperm and grown in glasses with 0.1 strength Hoagland-Arnon nutrient solution or 1 mM cadmium acetate (Cd $(CH_3COO)_2 \cdot 2H_2O$) for 7 h. Plants incubated in a Hoagland-Arnon solution served as control. To elucidate the ABA participation in the regulation of WGA content in wheat roots, the 5 mg/L inhibitor of ABA biosynthesis—fluridone (Fl) was used.

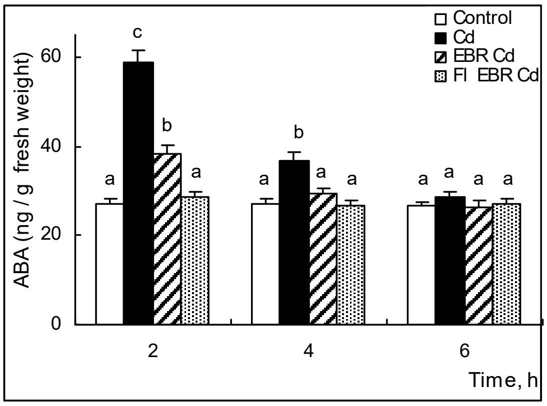
ABA and WGA contents were measured in the same plant with the help of enzyme immunoassay method [10] using specific polyclonal rabbit antibodies against ABA and WGA and anti-rabbit antibodies labeled with peroxidase.

The mitotic index was determined in the root apical meristems of the seedlings as percentage of mitotic cells of total number of the cells.

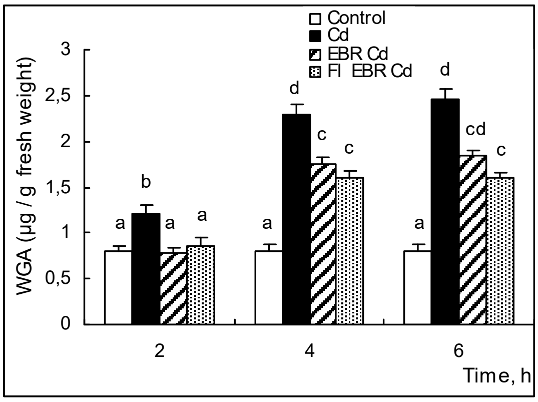
Lignin and suberin were identified using an aqueous solution of berberine hemisulfate (0.1% *w*/*v*) [11]. To enhance the fluorescence intensity, the sections were additionally stained for 15 min with toluidine blue (0.05% *w*/*v*) in 0.1 M phosphate buffer, pH 5.6. Stained sections were embedded in a 0.1% FeCl3/50% glycerol mixture and viewed with a confocal microscope (LSM 510, Germany), using a combination of filters BP 450-490/FT 510/LP 520.

3. Results and discussion

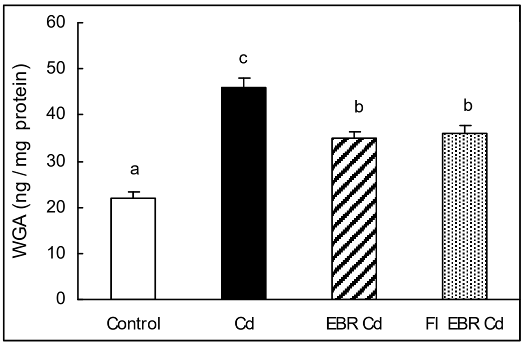
0.4 μM pre-sowing EBR-treatment twice reduced the level of cadmium-induced accumulation of ABA as well as WGA in wheat roots and in the root environment (Figure 1). These data indicate the less damaging effect of cadmium on EBR-pretreated plants. Fl pretreatment together with EBR-trearment led to inhibition of stress-induced ABA accumulation. However the WGA content in these seedlings was maintained at a level close to that in EBR-pretreated plants only that was also reflected in the WGA content in the external environment. This indicates the ABA-independent EBR ability to regulate the quantitative level of WGA in plant roots under cadmium stress and, therefore the presence of alternative pathways for hormonal regulation of lectin concentration, which is involved in the development of wheat resistance to cadmium. The EBR-protective effect is also indicated by prevention of the growth-inhibiting effect of cadmium on wheat plants and the maintenance of the mitotic index of EBR-pretreated plants at the control level. This protective effect of EBR on cell division was persisted in the presence of Fl, which confirms the independent nature of EBR action on wheat plants. Thus we have demonstrated the involvement of lectin, which plays an important role in the formation of nonspecific wheat resistance, in the realization of EBR-protective effect on wheat plants under cadmium stress.



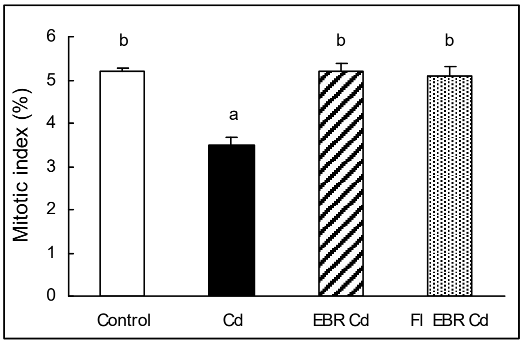
(**a**)



(**b**)



(**c**)



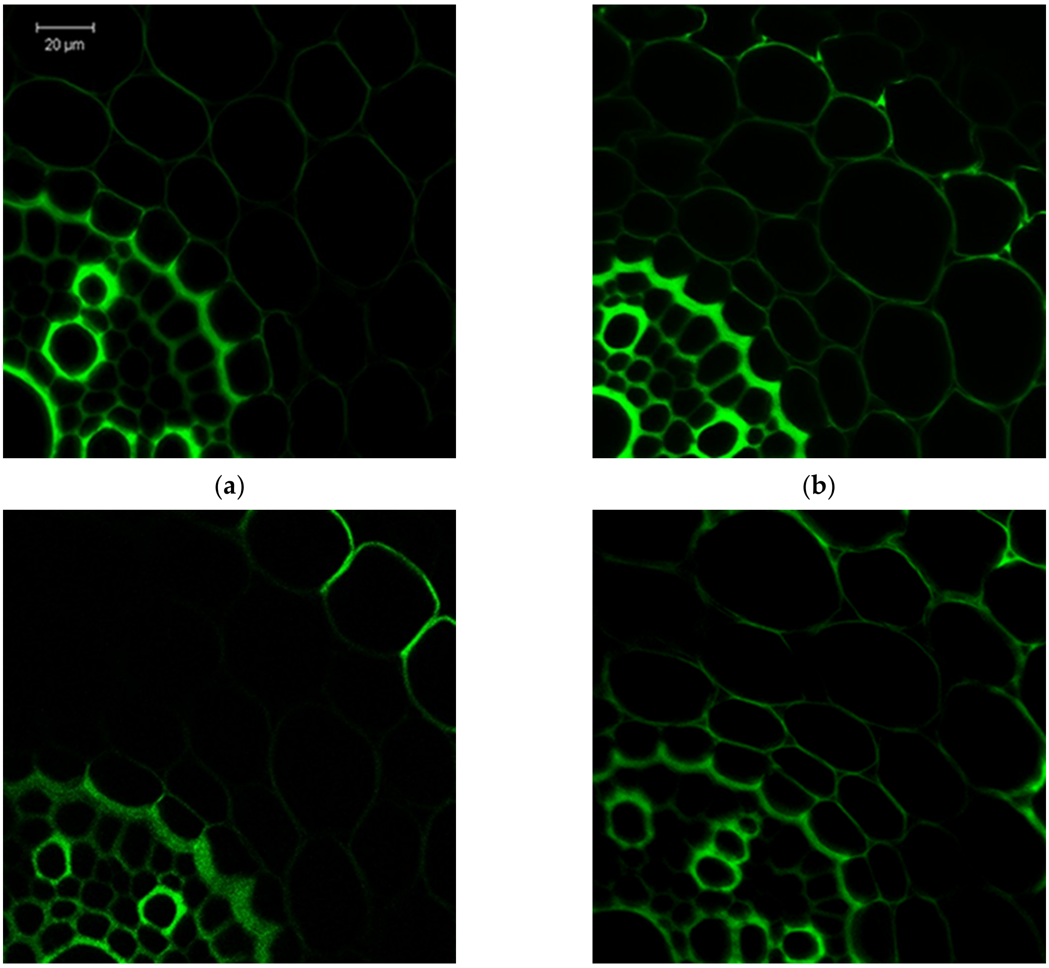
(**d**)

Figure 1. Effect of cadmium acetate stress on (**a**) ABA (ng/g fresh weight); (**b**) WGA (µg/g fresh weight); (**c**) WGA (ng/mg protein) contents and (**d**) Mitotic index (%). Reliably different values (n = 20) at the level $p < 0.05$ are indicated with different letters, t-тест.

The ability of EBR-pretreatment to activate the WGA synthesis and accumulation in wheat seedlings, their interaction in the growth processes regulation and the ability of this lectin to be excreted into the incubation medium obviously can make an important contribution to the realization of their protective effect on plants under cadmium acetate stress.

Since cadmium ions are mainly adsorbed at roots, therefore the anatomy and molecular structure of the root cell walls is critical. Endodermal and exodermal apoplastic barriers reinforcement plays an important role in protecting roots and plants in general from the toxic ions uptake [12]. Depending on the lignin and suberin biopolymers content as well as their localization, apoplastic barriers transport properties realizes. It was found that roots of various plant species with the higher suberin and lignin content might be less permeable to cadmium ions and, therefore, more resistant to its uptake and translocation [13]. The identified EBR ability to enhance the hydrogen peroxide production and peroxidase activity [14] probably contributes to this phytohormone participation in the lignin and suberin synthesis, which is involved in the reinforcement of the barrier properties of the cell walls, an effective barrier against the cadmium ions penetration [15] and limiting the diffusion of heavy metal throughout the plant.

It was revealed an increase of the fluorescence of Caspari belts in the exodermis and endodermis of cross sections of the root basal part under cadmium stress, which indicates an additional deposition of lignin and suberin, which limits apoplastic transport (Figure 2). The radial exodermis walls were especially thickened. In the endodermis there was an uneven thickening of the radial and significant reinforcement of the internal tangential cell walls near the pericycle border, the pericycle itself as well as the stelar parenchyma and in some degree the cortex. The EBR-pretreatment induced the plant preadaptive effects and led not only to additional, compared with the control, lignin and suberin deposition in the endodermis and the parenchyma of the root cylinder, but also in the exodermis, probably due to the acceleration of these plants development. The formation of Caspari bands and suberin lamellae is observed both in the endodermal and exodermal apoplastic barriers of phytohormone-pretreated roots under stress, while the thickening the cortex and stele cell walls was not as significant as under stress. At the same time, EBR-pre-sowing treatment of seeds promoted the acceleration of Casparian bands and suberin lamellae formation in the root cell walls in the critical places in the apoplastic barriers of endodermis and exodermis without inhibiting growth processes.



(**a**) (**b**)

(**c**) (**d**)

Figure 2. The lignin and suberin localization and detection of Casparian bands in root cross sections of the 7-day wheat seedlings stained with berberine sulfate/toluidine blue. (**a**) Control; (**b**) 7 h 1 mM Cd; (**c**) 0.4 μM EBR seed pretreatment; (**d**) 0.4 μM EBR + 7 h Cd. Bars = 20 μm.

Thus, it was revealed the lignin and suberin deposition in the root cell walls in the critical places of the endodermal and exodermal apoplastic barriers under EBR treatment and stress, which was reflected in the decrease in the negative effect degree of stress on growth processes.

4. Conclusions

An important contribution to the EBR-induced reinforcement the cell walls barrier properties of the studied tissues is made the EBR ability to induce ABA-independent accumulation of WGA, which in turn accelerates the phenolic biopolymers deposition, limiting the toxic ions uptake into the root and shoot inner tissues.

Author Contributions: M.B., A.L. and F.S. conceived and designed the experiments; M.B. and A.L. performed the experiments; M.B., A.L. and F.S. analyzed the data; M.B. and A.L. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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