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CYTOSKELETON-INDUCED ALTERATIONS OF LECTIN ACTIVITY IN MODIFICATION OF THE CALCIUM SIGNALING SYSTEM

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Summary. The roots of 7-d seedlings of two winter wheat cultivars differing in frost resistance were used to study changes in lectin activity using the inhibitor of microtubule polymerization oryzalin (15 μM) in unhardened (23 °C) and hardened (2–3 °C, 3–7 d) plants. Plants were grown with the antagonist of calmodulin, chlorpromazine (250 μM) or without chlorpromazine in medium. It was shown that the dynamic of lectin activity during cold hardening differed in two culrivars investigted. Disruption of the calcium signaling chain using chlorpromazine eliminates these differences in lectin activity. As a result we observed the same alterations in the activity of cell wall lectins. Pretreatment of plants with oryzalin increased the activity of cell wall lectins and decreased the activity of soluble lectins. It is supposed that an interaction exists between lectins of cell wall and microtubules. Plant cold hardening decreased the oryzalin effect on the lectin activity. These results could be explained by the appearance of tolerant microtubules with less affinity to the inhibitor. As with oryzalin, chlorpromazine decreased the activity of soluble lectins and increased the activity of cell wall lectins but to a greater degree. Apparently, injury of microtubule phosphorylation results in a more considerable microtubule disorganization than that observed after oryzalin effects. However, in cold hardened plants of Albidum 114, chlorpromazine promoted the oryzalin effect on lectin activity. It is possible that injury of Ca^{2+}-signaling system blocks the appearance of tolerant microtubules during cold hardening. Therefore, functioning of the Ca^{2+}-signaling system is necessary for microtubule stabilization. It is concluded that the activity of cell wall lectins depends on microtubule status which is regulated by the calcium signaling system.

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Key words: *calcium signaling system, cold hardening, microtubules, oryzalin, Triticum aestivum* L.

Abbreviations: cv – cultivar.

Introduction

Plant acclimation to low temperature in realized through step by step alterations in cell metabolism. Cell wall, plasma membrane and cytoskeleton are tightly interconnected into a complex system at the cell surface. During low temperature acclimation the number of contacts between plasma membrane and cell wall in alfalfa cells increased, which may depend on the changes in cell wall composition (Johnson-Flanagan, Singh, 1986). Lectins responsible for carbohydrate-protein interactions are critical compounds apparently involved in the interaction tween the cell wall and plasma membrane (Korolev, 1983). Because of their binding specificity, they have the capability to serve as recognition molecules within a cell, between cells, or between organisms. It is assumed that lectins play fundamental biological roles in plants because they are found in many different species and in many different organs and tissues. Nevertheless their physiological importance in plants is still discussed in the literature. It was established that drought, osmotic shock (Cammue et al., 1989), salinity, heat shock (Shakirova et al., 1993, 1996) and infection (Khairullin et al., 1993) enhanced the synthesis of lectins. The activity of lectins and their affinity to different soluble sugars was altered under low temperature conditions (Komarova et al., 1995). This data suggest that accumulation of lectins is a common response of plants to different kind of stresses.

However there are no data about mechanisms involving lectins in processes of adaptation. It is possible that this is achieved through participation of the cytoskeleton. It is assumed that one of reasons of cold resistance of plants is the stability of cortical microtubules (MT) (Akashi et al., 1990) and injury of plants by chilling is a result of MT polymerization (Rikin et al., 1983).The integrity and functional activity of cytoskeleton depends on the Ca^{2+} concentration in cells. Calcium signaling mechanisms are widely employed by eukaryotic organisms to regulate gene expression and a variety of other cellular processes such as microtubule formation, hormonal response, cell division and cell growh. Ca^{2+} mediates intracellular signaling and regulation of differentiation predominantly through the activity of Ca^{2+} dependent protein kinases and phosphatases. Ca^{2+} binds to a number of effector proteins including calmodulin, which, in turn can interact with myriad intracellular targets, including microtubules.

The aim of our work was to establish the dependence between microtubule stability, lectin activity and the Ca^{2+}-calmodulin system of second messengers during low temperature adaptation of winter wheat plants.

Materials and methods

Plant material

The roots of 7-day seedlings of two winter wheat (*Triticum aestivum L.*) cv which differed in the frost resistance: Mironovskaya 808 – resistant, Albidum 114 – very resistant were the objects of investigation. The plants were grown in water culture under illuminance of 100 W/m^2 and a photoperiod of 12 h. Hardening of plants was conducted in a T25/1.1 thermal chamber (Monsator, Germany) at 3ºC for 3–7 days. Inhibitor of microtubule polymerization (10 μM, Aldrich) was introduced in the excised roots by incubation in solutions, the exposure time was 3 h.The calmodulin antagonist chlorpromazine (250 μM, Serva) was added in the medium of plant cultivation before one day until hardening.

Extraction of soluble lectins

The extraction procedure was carried out at 4ºC. Plant tissues (roots or leaves) were homogenized in acetone and centrifuged at 6 000 for 5 min. The pellet was washed 3 times with acetone, ethanol, ethanol:ether (1:1) and ether. The the pellet was homogenized in 0,05 n HCl (1:10, w/v), containing 5 mM EDTA (Sigma) and 5 mM ascorbic acid for 1 h with constant stirring and then centrifuged at 15 000 g for 15 min. The pellet was washed for 10 min with 0.05 N HCl. Then the sample was centrifuged at 15 000 g for 15 min. The extracts were combined, neutralized and centrifuged. After that the supernatant was used for determination of lectin activity.

Extraction of cell wall lectins

The pellet after extraction of soluble lectins was homogenized in 20 mM K-phosphate buffer (pH 7.4), containing 0.2 M sucrose, 10 mM EDTA (Sigma), 50 μM dithiothreitol (Sigma) and 50 mM phenylmethylsulfonylfluoride (Sigma). The mixture was passed through nylon mesh and the cell wall was washed 3 times, 10 min each with K-phosphate buffer with end-over-end mixing, centrifuging each time for 15 min at 15 000 g. The purified cell walls were extracted with 0.9% NaCl and 0.05% Triton X-100 in K-phosphate buffer for 4 h at 4ºC with constant stirring and were then precipitated by centrifugation at 15 000 g for 15 min. The extract was used for further determination of lectin activity.

Lectin activity was defined by agglutination of trypsinized rabbit erythrocytes. In immunologic plates with U-like cavity a series of extract two-fold dilutions were prepared. Then erythrocytes (0.05 ml, 2%) were added in each sample. Results were determined after 2 h Lectin activity was estimated according to the minimal protein concentration which caused agglutination of erythrocytes.

Protein content was determined by the method of Bradford (1976).

Statistical analysis

Data presented in tables and figures are mean values of at least three independent biological replicates. The standard errors in the experiments did not exceed 5%.

Results

Figure 1 demonstrates alterations of lectin activity during cold acclimation. The dynamic of cell wall lectin activity during cold hardening of plant has a phased character. At first, the activity of cell wall lectins increased. This increase was temporary and the lectin activity decreased, then again increased and remained on this level until cold acclimation finished. Our data show that alterations of the lectin activity were different in 2 cultivars investigated. Cold treatment of plants caused a more rapid response in the lectin activity of the very frost resistant cultivar Albidum 114. For example, a decrease of the lectin activity was observed after 1 h and 3 h chilling in Albidum 114 and Mironovskaya 808 respectively. The lectin activity stabilized after 3 d in Albidum 114 and after 7 d in Mironovskaya 808. At first the activity of soluble lectins did not change and then increased in both Albidum 114 and Mironovskaya 808 (Fig. 1).

Pretreatment of plants with chlorpromazine changed the dynamic of lectin activity. The activity of cell wall lectins both in Mironovskaya 808 and Albidum 114 increased

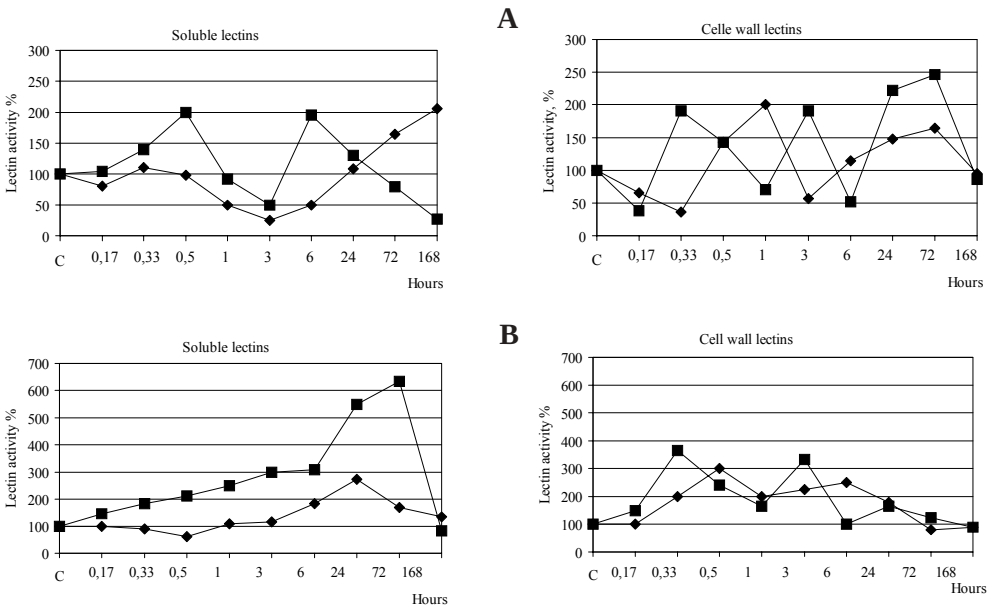

Fig. 1. Dynamic of the lectin activity in roots of Mironovskaya 808 (A) and Albidum 114 (B) during cold hardening of plantswith (-◆-) and without (-■-) chlorpromazine.

after 30 min, then after 3 h and after 1 d also (Fig. 1). At first, chlorpromazine increased the activity of soluble lectins and then decreased ones as compared with control (without chlorpromazine) (Fig. 1).

Pretreatment of plants with oryzalin increased the activity of cell wall lectins and decreased the activity of the soluble lectins (Fig.2). Plant cold hardening depressed the oryzalin effect on the activity of cell wall lectins. In cv. Mironovskaya 808 ory-

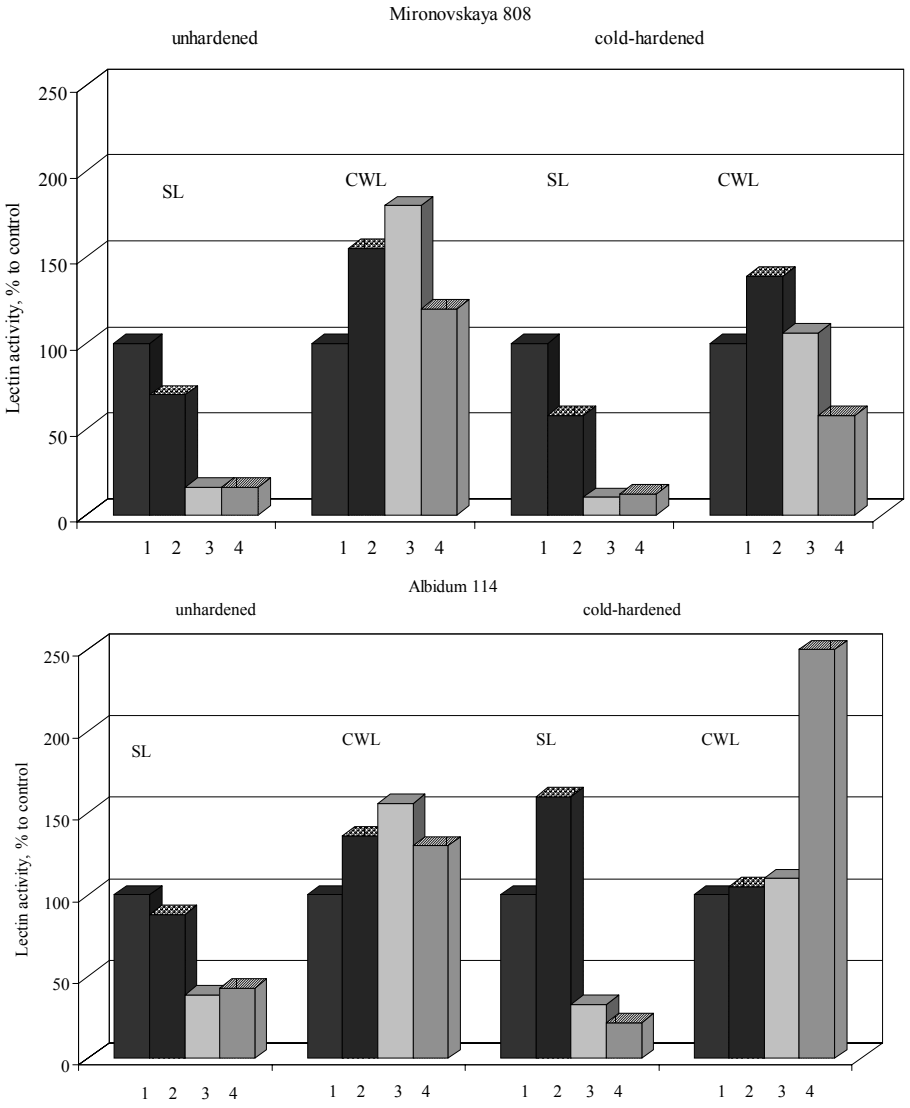

Fig. 2. Influence of oryzalin and chlorpromazine on the activity of soluble lectins (SL) and cell wall lectins (CWL) in roots of winter wheat seedlings. 1 –control, 2 – oryzalin, 3 – chlorpromazine, 4 – combined action of oryzalin and chlorpromaine.

zalin-induced increase of lectin activity was 155 and 139%, in cv. Albidum 114 – 136 and 105% in unhardened and cold-hardened plants, respectively (Fig. 2). However oryzalin increased the activity of soluble lectins in acclimated plants (Fig. 2).

The inhibitor of the Ca^{2+}-signaling system chlorpromazine decreased the activity of soluble lectins and increased the activity of cell wall lectins in unhardened plants (Fig. 2). After 7 days of low temperature adaptation chlorpromazine did not change the activity of cell wall lectins but decreased the activity of soluble lectins (by 89.6% in Mironovskaya 808 and 67% in Albidum 114), to greater degree than in unhardened plants (Fig. 2). Additional alteration of the lectin activity under the simultaneous action of oryzalin and chlorpromazine was not observed. However in hardened plants of Albidum 114, which were grown with chlorpromazine, the oryzalin effect on the activity of cell wall lectins increased (Fig. 2).

Discussion

The results reported here demonstrate that rapid alterations of the activity of cell wall lectins play an important role in low temperature adaptation processes. The role of cell wall modification during cold hardening is still poorly understood, although the responses of both winter and spring plants to chilling have been elucidated. It is possible that the rapid response of cell wall lectins under low temperature is necessary for activation of hydrolytic enzymes which destroy cell wall polysaccharides with the subsequent production of olygosaccharides. It is well known fact that olygosaccharides stimulate the protective reactions under pathogen and other stresses and regulate growth and differentiation of cell. It is reported that lectins can interact with enzymes such as β-glycosidase, β-mannosidase, β-acetylglycosamineidase. Carrutu et al. (1985) supposed that the decrease in lectin content during seed growth was a result of binding of lectin with hydrolases. We showed that the activity of cell wall lectin was decreased after 3 h of hardening. Our results agree well with data obtained by Zabotin et al. (1998). They observed the maximum activity of cell wall hydrolases after 3 h of low temperature adaptation of winter wheat seedlings.

On the other hand, lectins of the cell wall can bind with receptors on the plasmalemma, modify the structure of membranes and the activity of their enzymes with consequent alterations of cell metabolism. It has been suggested that exogenous lectins could bind with specific carbohydrates in plasma membrane of pea cells and induce production of phytoalexin associated with activity of signal-transduction pathway (Toyoda et al., 1995).

It is known that stress conditions induce the synthesis of stress proteins. However, together with stress proteins there are some proteins, which are contained in normal cells (Ericson and Alfinito, 1983) and increased during plant adaptation to stress. Soluble lectins are probably an example of such proteins, the quantitative increase in lectin

level is one of the universal nonspecific mechanisms of plant protection against the influence of unfavourable environmental conditions.

Results concerning the alterations of lectin activity under chlorpromazine demonstrate that lectin activity is regulated by the calcium signaling system. It is possible that there are specific time alterations of the activity of cell wall lectins in different cold resistance cultivars. Disruption of calcium signaling chain using chlorpromazine eliminates these differences in lectin activity. As a result we observed similar alterations of the activity of cell wall lectins in plants, which were grown with chlorpromazine (Fig. 1).

The dinitroaniline herbicide oryzalin affects the cytoskeleton structure via microtubule depolymerization. Oryzalin is a most potent antimitotic drug. It binds to microtubules, preventing their polymerization. As a result, microtubules disappear. We demonstrated that oryzalin increased the activity of cell wall lectins (Fig. 2). This implies an interrelation between cell wall lectins and integrity of the cytoskeleton. We believed that destruction of these interactions by depolymerization of cortical microtubules leads to the release of cell wall lectins followed by an increase in lectin activity. It was also demonstrated that the cell wall might stabilize MTs and prevent their depolymerization under freezing (Akashi et al.,1990). It is also possible that the increase of cell wall lectin activity under inhibitors of MT polymerization can be the compensatory mechanism which stabilizes the cytoskeleton structure in conditions of its destruction.

The decrease of the oryzalin effect on lectin activity in cold hardened plants as compared with unhardened ones (Fig. 2) points to the positive action of low temperature to cytoskeleton stability. This assumption that low temperature stabilizes microtubules is supported by the data showing decreased cell wall lectin activity in cold hardened plants.

In the literature different reasons for microtubule stability are discussed. Chu et al. (1993) observed that low temperature stress caused down-regulation of the b-tubulin gene in *Arabidopsis*. An increased stability of microtubules repolymerized from new tubulin isotypes was reported for chilled rye roots (Kerr and Carter, 1990). Microtubule stability under low temperature also depends on synthesis of new MAP. Cyr and Palevitz (1989) isolated microtubule-binding proteins from suspension-cultured cells of carrot . The addition of these proteins caused both bundling and cold stability of carrot and neuronal microtubules *in vitro*. Another reason for cold stability of microtubules is acetylation of b-tubulin and intensification of their interaction with plasmalemma (Astrom, 1992). Cortical microtubules are known to cross-link with each other and with the plasma membrane, so it may be anticipated that these interactions are important for their stability. Furthermore, transmembrane proteins, which were described by Akashi and Shibaoka (1991) in tobacco BY-2 cells, were involved in the associations of microtubules with the plasma membrane. Enzymatic digestion of these (and probably other) proteins caused microtubules to dissociate from the plasma mem-

brane, it also increased their sensitivity to depolymerization under low temperature. The microtubule-stabilizing effect of the plasma membrane is supported by the observation that endoplasmic microtubules, which lack extensive plasma membrane associations, depolymerized sooner than cortical microtubules in all the tissues investigated in the cold-treated roots of maize (Balluska et al.,1993). Besides tyrosine phosphorylation in addition to phosphorylation of threonine of both tubulin subunits on tyrosines can be involved in the reorganization of microtubules and the regulation of their interactions with other cellular structures (e.g. plasma membrane). It was shown that both α- and β-tubulins were intensively phosphorylated during activation of cAMP-, Ca^{2+}-calmodulin-dependent PKs and PK C (Blum et al., 1997).

In our experiments the calmodulin antagonist, chlorpromazine like oryzalin decreased the activity of soluble lectins and increased the activity of cell wall lectins but to a greater extent (Fig. 2). Apparently, injury of microtubule phosphorylation results in a more considerable microtubule disorganization than that observed after oryzalin treatment. However, in cold hardened plants of Albidum 114 chlorpromazine promoted the oryzalin effect on lectin activity (Fig. 2). It is possible that injury of the Ca^{2+}-signaling system blocks the appearance of tolerant microtubules during cold hardening, Therefore the affinity of microtubules is increased to oryzalin. Thus, the functioning of the Ca^{2+}-signaling system is necessary for microtubule stabilization.

It is concluded that activity of the cell wall lectins depends on microtubule status which is regulated by the calcium signaling system. As a result the cytoskeleton may be the mediator between external signals and cell responses.

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