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Cytoskeleton-dependent changes in the structural organization of reticuloplasmins in Triticum aestivum cells during cold acclimation and treatment with abscisic acid

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Abstract

We studied the effects of the anti-microtubule drug, oryzalin, on the content and spatial organization of reticuloplasmins (Ca2+-binding marker proteins of the endoplasmic reticulum) in winter wheat seedlings after their cold acclimation (3°C, 7 days) and treatment with ABA (30 μM). For identification and visualization of reticuloplasmins, we applied one-dimensional SDS-PAGE with subsequent Western blotting and indirect fluorescent microscopy. We used polyclonal HSP70 and CRH antibodies against BiP and calreticulin (Cal), respectively. On immunoblots, the brightest bands corresponded to polypeptides with mol wts of 58 kD (calreticulin) and 79 kD (BiP). The content of calreticulins in roots was shown to be higher than in leaves. Cold acclimation enhanced, and ABA treatment reduced, the concentration of calreticulins in root cells. Both treatments increased the BiP concentration in roots. Oryzalin (10 μM, treatment for 3 h) did not affect the level of reticuloplasmins in roots of unhardened, cold acclimated, treated with ABA and with a combination of cold and ABA plants. However, both oryzalin and low-temperature treatments resulted in the accumulation of reticuloplasmins in the two spherical structures in the vicinity of the plasmalemma and nuclear envelope. After the combined action of oryzalin and low temperature, the conical sphere of BiP proteins was shifted into the endoplasm and calreticulins appeared in the nuclear matrix. We believe that these changes in the reticuloplasmin localization are related to the rearrangement of the endoplasmic reticulum determined by the cytoskeleton modification. They result in the improved capacity of reticuloplasmins to control Ca2+ behavior and/or to the function as chaperones. The results obtained permit the conclusion that cytoskeletal proteins interact with reticuloplasmins, and this interaction might be involved in the transduction of the external and internal signals.

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Keywords

ABA, BiP, Calcium, Calreticulin, Chaperones, Cold acclimation, Cytoskeleton, Oryzalin, Reticuloplasmins, Triticum aestivum