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ARTICLE

Peroxidase activities in root segments of wheat genotypes under osmotic stress

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ABSTRACT One week old *Triticum aestivum* lines with different drought tolerance were subjected to polyethylene glycol (PEG 6000) treatment reaching 600 mOsm osmotic stress on the 13th day. Plant growth parameters, water status, soluble and cell wall-bound peroxidase (POD) activities were measured on the 15th day. While only small differences was found in the total peroxidase activities determined by either guaiacol or syringaldazine, the activities of some POD isoenzymes detected by benzidine substrate showed significant changes. The total density of soluble POD-containing fractions was higher in the drought stress tolerant Plainsman and Kobomugi cultivars due to osmotic stress than in controls. In Öthalom and Cappelle Desprez cultivars however, most of the soluble POD isoenzymes worked at lower level and only two isoenzymes showed enhanced activities after the PEG treatment. The changes of POD activities in the soluble fractions are in good correlation with the abiotic stress tolerance of wheat genotypes. The enzyme fraction bound covalently to cell wall showed a different isoenzyme pattern.
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KEY WORDS

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Plant peroxidases exist in numerous molecular forms. In *Arabidopsis*, more than 50 different sequences encoding peroxidase have been identified. Plants contain two classes of heme peroxidases: class I peroxidases are intracellular, whereas class III peroxidases are secreted into the cell wall or the surrounding medium. In the standard peroxidative cycle, the class III peroxidases catalyze the reduction of H_2O_2 by taking electrons from various donor molecules. These isoenzymes exist in a very large number and are responsible for a plethora of physiological functions, among which are: stiffening of the cell wall through the immobilization of extensin and cross-links of matrix polysaccharides, lignin and suberin deposition, defence against pathogen penetration, wounding and other abiotic stresses, auxin catabolism and biosynthesis of secondary metabolites (Tari and Csiszár 2003, De Gara 2004). Recently, an alternative, hydroxylic cycle has been described, in which native peroxidases can be converted to oxyferroperoxidases in two different ways, which can then lead to the formation of various reactive oxygen species (ROS) (Halliwell 1977, Liszkay et al. 2003). Peroxidases can therefore be considered as bifunctional enzymes that can oxidize various substrates in the presence of H_2O_2 but also produce ROS (Passardi et al. 2004). Their involvement in many physiological and developmental processes can be detected in plants. One of the features of peroxidases is that they are associated with cell elongation processes but also with reactions that restrict growth.

While the usual response of plants under different abiotic stresses is the decrease of growth, in some cases the root length can be higher than in controls: deficiences in water and nutrient supply can result in greater belowground allocation of physiological resources and increased root growth. The wide spectrum of biochemical reactions that peroxidases are able to catalyze and the great number of molecular isoforms explain the difficulty encountered in the study of plant peroxidases. Our aim was to investigate the activities of POD isoforms in both control circumstances and under osmotic stress treatment. The soluble fraction and peroxidases bound ionically and covalently to cell wall were prepared from the root segments containing the proliferation and elongation zones. In this study, we compared the peroxidase activities using guaiacol and syringaldazyne substrates in 1-cm-length root axis containing the proliferation and elongation zones of wheat cultivars with different drought tolerance. The impact of 600 mOsm osmotic stress on the growth, water content and peroxidase isoenzyme pattern was investigated.

Materials and Methods

Four wheat (*Triticum aestivum* L.) genotypes were used in our experiments: *T. a.* cv. Cappelle Desprez, a drought sensitive french wheat; Plainsman V, a drought resistant american cultivar; cv. GK Öthalom, a breeded hungarian genotype with an average drought tolerance; cv. Kobomugi, a drought-tolerant ancient line originated from inner part of Asia. Plants were grown in Hoagland solutions in greenhouse under controlled conditions for one week. Osmotic stress treatment was applied

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gradually from the 7th day reaching 600 mOsm polyethylene glycol (PEG 6000) concentration (ca. 25%) on the 13th day. Growth parameters and water status of plants were measured on the 15th day. The soluble, ionically cell wall-bound and covalently bound peroxidase fractions were prepared from 100 mg of root segments according to Hendricks et al. (1985). POD enzyme activities were determined using guaiacol (Upadhyaya et al. 1985) and syringaldazine substrates (Pandolfini et al. 1992). POD isoenzyme pattern in these fractions were investigated after native PAGE (Milone et al. 2003) with benzidine substrate (Shivakumar et al. 2003).

Results and Discussion

The effect of the 600 mOsm PEG resulted in the highest decrease in the water content of the drought sensitive Cappelle Desprez cultivar on the 15th day, while the lowest decrease was detected at the drought resistant Kobomugi plants. The applied osmotic stress inhibited both shoot and root growth at all investigated plants by this time, but on the 9th, 11th days (after 100 and 200 mOsm PEG treatment), the roots of drought tolerant Plainsman were even longer than those in controls. The inhibition of growth is a usual symptom of plants under unfavourable circumstances. However, deficiensis in water and nutrient supply can result in increased root length. Maintainance the growth of roots play an important role in plant survival during periods of drought. Changes in peroxidase (POD) activities and isoenzyme patterns have been shown in many cases in relation to growth and external stimuli.

In the total peroxidase activities of wheat root segments determined by either guaiacol or syringaldazine substrates, only small differences were found on the 15th day, two days after applying 600 mOsm osmotic stress. However, detecting the POD isoenzymes after native polyacrilamide gel electrophoresis by benzidine substrate showed significant changes in activities of some individual isoenzymes. The total density of soluble POD-containing fractions was higher in the drought stress tolerant Plainsman and Kobomugi cultivars in the osmotic stress-treated roots than in controls. In Öthalom and in the drought sensitive Cappelle Desprez cultivars most of the soluble POD isoenzymes worked at lower level and only two isoenzymes showed enhanced activities after the PEG treatment. The changes of POD activities in the soluble fractions are in good correlation with the abiotic stress tolerance of wheat genotypes. According to the results of Mišan et al. (2006) the level of POD activity is an important antioxidant parameter of different wheat lines, supposing that other oxidative stress scavenging enzymes (catalase, glutathione peroxidase) are working in an appropriate level.

Less isoenzyme was manifested in the covalently cell wall-bound fractions and their activities changed to less extent but in a different manner. Their activities showed some correlations with the growth responses of wheat roots.

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