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Effect of osmotic stress on antioxidant enzyme activities in transgenic wheat calli bearing *MsALR* gene

Jolán Csiszár[1]*, Erzsébet Fehér-Juhász[2], Éva Kótai[2], Orsolya Ivankovits-Kiss[2], Gábor V Horváth[3], Antal Mai[3], Ágnes Gallé[1], Irma Tari[1], János Pauk[2,4], Dénes Dudits[3], László Erdei[1]

[1]Department of Plant Physiology, University of Szeged, Szeged, Hungary, [2]Cereal Research Non-Profit Co., Szeged, Hungary, [3]Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary, [4]Research Group for Molecular Plant Breeding, Hungarian Academy of Sciences, Szent István University, Gödöllő, Hungary

ABSTRACT The antioxidant enzyme activities were studied in transgenic wheat calli bearing alfalfa *MsALR* gene. The effect of 14% PEG treatment was studied measuring the activities of some hydrogen peroxide related enzymes (SOD, CAT, POD) and the glutathione related GR, GST and GS-PX enzymes. Induction of the antioxidant enzymes is usually a complex process, one enzyme alone supposedly can not ensure enough protection under stress conditions. Our results showed that the changes of antioxidant enzyme activities are characteristic for the cultures. Some calli had higher activities of antioxidant enzymes than untransformed controls even in control circumstances. There are transgenic wheat calli with elevated SOD, CAT and/or POD activities, while in some other calli the activities of the glutathione related enzymes (GR, GS-PX) were increased comparing to the control.

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KEY WORDS

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The growth and productivity of plants depend on the environmental conditions. Extreme circumstances can limit CO_2 fixation and enhance the generation of active oxigen species (AOS), such as superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxil radical ($OH^{\cdot}$). Incompletely reduced AOS can be extremely reactive and oxidize biological molecules (DNA, RNA, proteins and lipids), inactivate enzymes, decrease the rate of protein synthesis. AOS levels are determined by the rates of production and metabolism. The plants have developed different scavenge mechanisms to control the level of AOS. Several low molecular weight antioxidants - such as ascorbic acid, reduced glutathione (GSH), α-tocopherol - and antioxidative enzymes take part in the scavenging of reactive radicals and molecules. When more AOS are produced than metabolized, oxidative stress can occur. However, the superoxide and mainly the relatively long-lived and diffusible hydrogen peroxide can also function as signalling molecules that mediate responses to different stresses (Desikan et al. 2001). The interaction between the elements of defence mechanisms is very complex and not well-understood yet.

A full-length cDNA encoding an alfalfa aldose/aldehyde reductase (ALR) was identified among several stress-induced cDNAs from a somatic embryo-derived library by Oberschall et al. (2000). Tobacco plants overproducing the alfalfa enzymes provided considerable tolerance against oxidative damage caused by paraquat, UV-B, cold and heavy metal treatment, they could resist a long period of water deficiency (Oberschall et al. 2000; Hideg et al. 2003; Hegedűs et al. 2004). Aldose reductases catalyze the NADPH dependent reduction of different aldoses, aliphatic and aromatic aldehydes and detoxify toxic compounds in different stresses. The enzymes are reported to be involved also in the synthesis of sugar alcohols (e.g. sorbitol and mannitol) which may function as compatible solutions in high concentrations (Bagnasco et al. 1987). The aim of our work was to study the antioxidant enzyme activities of wheat calli containing *MsALR* gene and compare the changes of control and transgenic lines after osmotic stress treatment.

*Corresponding author. E-mail: csiszar@bio.u-szeged.hu

Materials and Methods

Osmotic stress treatment was carried out using 14% polyethylene glycol (PEG 6000) solutions applied on three-week-old wheat (*Triticum aestivum* L.) calli containing *MsALR* gene under controlled conditions. Enzyme activities were measured after three days of PEG treatment. One gram of plant tissue was homogenized in 2 ml extraction buffer (50 mM phosphate buffer pH 7.0, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride and 1% polyvinyl-polypirrolidone). After centrifugation the supernatant was used for enzyme activity assays. Superoxide dismutase (SOD) activity was determined by measuring the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin in light (Dhindsa et al. 1981). Catalase (CAT) activity was determined by the decomposition of H_2O_2 and was measured spectrophotometrically by following the decrease in absorbance at 240 nm (Upadhyaya et al. 1985). Guaiacol peroxidase (POD) activity was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol (Upadhyaya et al. 1985). Glutathione reductase (GR) activity was determined by measuring the

absorbance increment at 412 nm when 5,5'-dithio-*bis*(2-nitrobenzoic acid; DTNB) was reduced by GSH, generated from glutathione disulfide (GSSG; Smith et al. 1988). Glutathione S-transferase (GST) activity was measured spectrophotometrically by using an artificial substrate, 1-chloro-2,4-dinitrobenzene (CDNB), according to Habig et al. (1974). Glutathione peroxidase (GS-PX) activity was measured by the method of Awasthi et al. (1975), with cumene hydroperoxide as substrate. The protein contents of the extracts were determined by the method of Bradford (1976).

Results and Discussion

The levels of antioxidants and the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione reductase (GR) are generally increased in plants under stress conditions and in several cases correlate well with the enhanced tolerance. In our experiments two control and 15 transformant wheat calli were exposed to PEG treatment and SOD, CAT, POD, GR, glutathione S-transferase (GST) and glutathione peroxidase (GS-PX) activities were measured. Comparing to the control, the activity of antioxidant enzymes were elevated in some transgenic lines. The results of independent experiments showed that changing of antioxidant enzyme activities are characteristics for the lines. Especially the activities of the glutathione related enzymes (GR, GS-PX) increased under osmotic stress, and these lines showed enhanced tolerance in other stress treatments as well (Pauk et al., unpublished results). The activities of SOD, CAT and POD were usually lower in these lines as in the control. However, there are some calli with elevated SOD, and/or POD activities as well. Our results indicated, that measuring of antioxidative enzyme activities in calli can be a tool for characterization of transgenic tisssue cultures.

Acknowledgments

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