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Effects of low temperature stress on ferritin or aldose reductase overexpressing transgenic tobacco plants

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ABSTRACT The alfalfa ferritin overproducing CaMVF9 transgenic tobacco and the alfalfa aldose reductase overexpressing ALR1/9 line showed higher F_v/F_m ratios than SR1 plants during ROS generating low temperature stress. The chlorophyll content of CaMVF9 and ALR1/9 lines proved to be much more favourable than that of SR1 plants whereas there was no difference in the electrolyte leakage. Activities of all the tested antioxidant enzymes (POD, CAT, APX, GR, GST) in the leaves were significantly higher in CaMVF9 and ALR1/9 lines, the malondialdehyde content was significantly lowered in the transgenic lines. The antioxidant enzyme systems induced through several redox-signalling mechanisms have simultaneously contributed to the greater tolerance of CaMVF9 and ALR1/9 lines. **Acta Biol Szeged 46(3-4):97-98 (2002)**

KEY WORDS

aldose reductase
antioxidant enzymes
ferritin
low temperature photoinhibition
reactive oxygen species
stress tolerance

Severe effects on the photosynthetic apparatus of low temperature and high irradiance may be mediated by reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals (Wise and Naylor 1987b). H_2O_2 is eliminated by catalase (CAT), guaiacol peroxidase (POD) and the enzymes of the ascorbate-glutathione cycle (Elstner 1982).

If H_2O_2 has not been successfully scavenged hydroxyl radicals – one of the most potent oxidants – can be generated via the Fenton reaction that is catalysed by free Fe^{2+} cations (Halliwell and Gutteridge 1984). Most of intracellular nonmetabolic iron is sequestered in ferritin (Theil 1987). The formation of highly toxic lipid aldehydes, such as 4-hydroxynon-2-enal (HNE) is an inevitable process in cellular damage (Srivastava et al. 1995). Detoxification of HNE may take place by glutathione S-transferase (GST) or by the action of aldose/aldehyde reductase (ALR) enzymes (Srivastava et al. 1995).

A ferritin and a novel ALR gene was identified from alfalfa somatic embryo-derived library, and ectopically overexpressed in tobbacco. Previous studies indicated that these transgenic plants exhibited greater tolerance to a wide range of oxidative stress factors (Deák et al. 1999; Oberschall et al. 2000).

Materials and Methods

Transgenic tobacco plants were created as described by Deák et al. (1999) and Oberschall et al. (2000). Seedlings were grown on kanamycin containing medium. At four-leaf-stage plants were transferred to a growth chamber (Conviron PGV-36) operating at 0°C with continuous light, PAR was 200 µmol m^{-2} s^{-1}.

Chlorophyll content was determined as described by Arnon (1949). Plant tissue extraction and determination of POD, CAT and APX activities as well as the level of lipid peroxidation were carried out as described previously (Hegedűs et al. 2001). GR and GST activities were determined according to Smith et al. (1988) and Mozen et al. (1983), respectively. Electrolyte leakage measurement was carried out according to Szalai et al. (1996). Chlorophyll fluorescence was recorded using a Plant Efficiency Analyser (Hansatech Ltd.) after 50 min of dark adaptation (Horváth et al. 1996).

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Results and Discussions

Chlorophyll fluorescence induction provides rapid, non-invasive methods for detecting changes in the activity of the photosynthetic apparatus (Janda 1998). Figure 1. shows that low temperature caused a marked decrease in the F_v/F_m ratios. Plants overexpressing ferritin homologues (CaMVF9) or those accumulating ALR homolouges (ALR1/9) showed significantly higher F_v/F_m values than SR1. The recovery of the transgenic plants was quicker than that of the SR1. Similar results were obtained during drought stress, Fe-EDTA or paraquat treatment (Deák et al. 1999; Oberschall et al. 2000).

Chlorosis is one of the most obvious symptoms of low temperature induced damage in leaves (Wise and Naylor 1987b). Chlorophyll content of CaMVF9 and ALR1/9 lines proved to be much more favourable than that of SR1 plants whereas their electrolyte leakage was not different. The irreversible increase in membrane permeability can be attributed to the lipid phase transition (Lyons 1973).

Low temperature photoinhibition leads to ROS generation in plant cells (Wise and Naylor 1987a). ALR1/9 and CaMVF9 transformants showed much lower rate of MDA formation than the SR1 and it reached the initial values in the recovery phase.

As the antioxidant enzyme system has an important role in the plant natural defense mechanism (Elstner 1982) we investigated the activity of its major contributors (data not

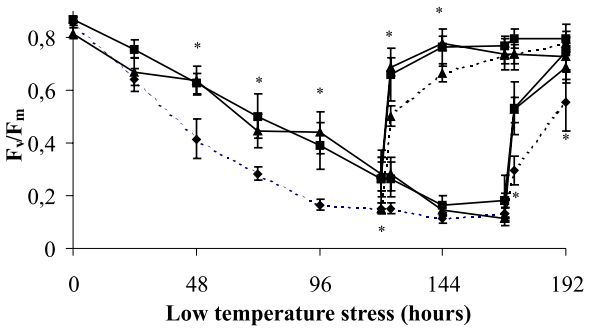

Figure 1. The influence of low temperature photoinhibition on PS2 efficiency.
SR1, ferritin and ALR overexpressing tobacco plants were treated at 0°C with continuous light (200 µmol m^{-2} s^{-1}). Filled rhombuses represent data from control SR1 plant, squares and triangles correspond to data from transgenic plants, CaMVF9 and ALR1/9, respectively, * p<0,05.

shown). Activities of POD, CAT, APX and GR were elevated by cold treatment in the ALR1/9 and CaMVF9 lines, while there was no significant change in SR1. Increased levels of activity were preserved during the recovery phase. ALR1/9 showed the greatest increase in GST activity and it was not even lessened during the recovery phase. GST may have a crucial role in the defense system as ALR is a more efficient catalyst for the reduction of glutathiolated aldehydes (Ramana et al. 2000).

Detoxification of lipid aldehydes in the ALR overproducing lines (Srivastava et al. 1995), as well as inhibition of the Fenton reaction in the ferritin overproducing lines (Halliwell and Gutteridge 1984) result in restricted cellular damage. The antioxidant enzyme system induced through several redox-signalling mechanisms has simultaneously contributed to the greater tolerance of CaMVF9 and ALR1/9 lines.

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