OXIDATIVE STRESS RESPONSES O F TRANSGENIC COTTON THAT OVER-EXPRESSES SUPEROXIDE DISMUTASE, ASCORBATE PEROXIDASE, OR GLUTATHIONE REDUCTASE

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Abstract

The objective of this study is to determine the effects of oxidative stress on transgenic cotton plants that over-express SOD, APX, and GR in chloroplasts and determine potential limitations to improved scavenging of toxic oxygen species, as well as, any effects of the perturbation of oxygen metabolism may have on photochemistry. Three genotypes of transgenic cotton plants that each express a chimeric gene encoding chloroplast-localized manganese superoxide dismutase (Mn SOD), ascorbate peroxidase (APX), or glutathione reductase (GR) have been analyzed to determine whether they exhibit improved resistance to oxidative stress. The Mn SOD cotton plants exhibited a three-fold increase in the total leaf SOD activity and a 30% increase in APX activity and wild-type GR activity. Although photosynthetic recovery in leaves of these plants after 1 h at 10°C and a photon fluence rate of 1900 µmol m^{-2} s^{-1} was higher than for wild-type plants, recovery following longer exposure times or lower temperatures (5° - 7° C) was comparable for both genotypes. Consistent with photosynthetic recoveries, the oxidative state of the chloroplast, indicated by the deactivation of stromal fructose bisphosphatase, increased in both genotypes following severe chilling stress (5°C and 1700 µmol m^{-2} s^{-1}). In full sun prior to chilling stress, the Mn SOD leaves had a higher oxidation state, ratio of oxidized to reduced forms of ascorbate and total glutathione content compared to the controls. After 35 min. of chilling stress, total glutathione had risen in control leaves to 88% of Mn SOD values and oxidized to reduced ascorbate ratios were higher for both genotypes, but oxidized to reduced glutathione was unchanged for controls in contrast to an 80% increase in Mn SOD leaves. We postulate that the pool sizes of reduced ascorbate and glutathione may restrict the ability of the ascorbate-glutathione cycle enzymes to compensate for the increased activity of SOD in cotton over-expressing Mn SOD in chloroplasts during short-term chilling/high light stress. Transgenic plants that express a gene encoding chloroplast-localized APX exhibited a five-fold increase in total leaf APX activity. The immediate recovery of photosynthesis following exposure of leaf discs to 10°C and 1900 µmol m-2 s^{-1} was consistently higher for APX plants than all other genotypes tested to date, except for the 1 h time point where GR+ plants showed similar recoveries. GR over-expressing plants exhibited a ~30 fold increase in total leaf GR activity compared to wild-type and other transgenic lines. The recovery of photosynthesis after 1 h at 10°C and 1900 µmol m^{-2} s^{-1} was higher than that for wild-type and SOD plants but, the recovery after longer exposure times was comparable to that seen in wild-type plants. The manipulation of single enzymes or various combinations of enzymes should provide a means to mechanistically study reactive oxygen metabolism and the importance of these enzymes to the protection of photosynthesis during chilling/high light conditions.

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