RESPONSE OF TRANSGENIC COTTON TO OXIDATIVE STRESS

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The objectives of this study were to determine whether increasing the activity of anti-oxidant enzymes, primarily those associated with ascorbate and H_2O_2 metabolism, can improve cotton's response to oxidative stress with an emphasis on the initial recovery of photosynthesis following an exposure to chilling and high photon flux density (PFD) and to study the role of these enzymes in acclimation to high light. Three genotypes of transgenic cotton plants that expressed a chloroplast-localized chimeric gene encoding either manganese superoxide dismutase (Mn SOD), ascorbate peroxidase (APX), or glutathione reductase (GR) were generated for this study using *Agrobacterium*-mediated transformation.

Growing wild-type, Coker 312 plants at moderate PFD (600 μmol m^{-2} s^{-1}) reduced the activity of SOD by 50% compared to that for plants grown in full sun (1800 μmol m^{-2} s^{-1}). Moderate growth PFD also reduced the activities of enzymes associated with ascorbate regeneration, except for GR, as found for all transgenic genotypes. The APX activity was similar at both growth PFDs. When grown under moderate PFD, plants over-expressing Mn SOD showed a two-fold increases in total leaf SOD activity compared to wild-type Coker-312 plants. This activity is comparable to the activity measured in wild-type plants grown in full sunlight. Shade-grown transgenic APX plants exhibited a three-fold increase in total leaf APX activity and GR plants had roughly fifteen times the GR activity of wild-type plants. High light grown plants over-expressing Mn SOD showed a four-fold increase in SOD activity compared to wild-type plants. For plants grown under high PFD, total APX activity was at least five fold greater in APX plants compared to wild-type plants. Over-expressing GR resulted in a twenty to thirty fold increase in total leaf GR activity in all genotypes. Interestingly, transgenic plants over-expressing Mn SOD and the F_1 hybrid between SOD and GR over-expressing plants exhibited a slightly higher APX activity when grown under full sun in the greenhouse. SOD/APX and SOD/GR hybrids had significantly lower ($p=0.05$) monodehydro-ascorbate reductase activity and the SOD/GR hybrids showed lower dehydro-ascorbate reductase activity, while the GR activity in the SOD/APX hybrids was 35% higher than in wild-type plants.

The effect of increasing the anti-oxidant enzyme activities on photo-oxidative damage when plants were grown under moderate and high PFD conditions was tested. The ability of transgenic and control plants to recover CO_2-saturated rates photosynthesis after a period of chilling/high PFD (10 °C and 1700 μmol m^{-2} s^{-1} for 1, 2, or 3 hours) was used to assess the level of protection afforded photosynthesis by increased anti-oxidant enzyme activity. Our findings showed that the two-fold increase in total leaf SOD activity for shade plants was associated with a significantly higher recovery of photosynthesis following 2 and 3 hours of chilling in high light, compared to wild-type plants. In fact, the SOD plants were protected to the same extent that wild-type plants grown in full sun were. Over-expression of APX and GR in chloroplasts also conferred protection from suboptimal temperature stress for shade plants. For high PFD-grown plants, increasing SOD activity provided protection after short exposures to chilling temperatures only, while increasing APX and GR provided protection for exposures up to three hours. For shade-grown F_1 hybrids, combining high SOD and APX activities was the best combination for protecting photosynthesis, but recoveries of photosynthesis were essentially no better than having elevated SOD, APX, or GR alone. For high-light F_1 hybrids, combining high SOD with high APX or GR activity was best, but no better than high APX or GR activity alone.

Therefore, increases in SOD, APX, or GR activities for shade leaves can provide substantial protection from photo-oxidative damage during short-term (1-2 hours) chilling/high PFD stress. Although increasing the low SOD activity can, in itself, increase resistance to photo-oxidative damage, increasing APX or GR activities have a similar effect. However, leaves developed in full sun exhibit the best resistance to photo-oxidative damage with higher APX or GR activities. Increasing SOD activity is most effective for shade or sun plants when combined with higher activities of APX or GR in downstream reactions. We conclude that the protection of photosynthesis in cotton from the effects of chilling in high PFD can be enhanced by increasing the activities of anti-oxidant enzymes, particularly those of APX and GR. It remains to be determined whether this protection confers improved performance under field conditions.

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