

## MODIFICATION OF OXIDATIVE STRESS TOLERANCE IN TRANSGENIC PLANTS

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### Introduction

Oxidative stress causes tissue damage in plants exposed to a wide range of stressful conditions including high light intensity, extreme temperatures, water drought, salt stress, nutrient deficiency and exposure to a variety of herbicides.

Oxidative stress results from the reduction of molecular oxygen (O<sub>2</sub>) to produce reactive oxygen intermediates (ROIs) that include superoxide radicals (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (OH). These ROIs can damage cellular membranes, proteins, and nucleic acids (1).

To determine the importance of ROI-scavenging enzymes in the oxidative stress protective mechanisms of plant cells, we have developed transgenic plants that contain gene constructs for various forms of superoxide dismutase (SOD) and ascorbate peroxidase (APX). We have found that expression of certain of these antioxidative enzymes in transgenic tobacco plants can provide significant protection from oxidative stress (2).

### Materials and Methods

Gene constructs were developed that encode pea chloroplast-localized Cu/Zn SOD (chl Cu/ZnSOD), a modified form of pea Mn SOD in which the native mitochondrial transit peptide was replaced with a chloroplast-specific transit peptide (chlMnSOD), pea cytosolic APX (cytAPX) or an APX with an added chloroplastic-transit peptide (chlAPX). These constructs were introduced into tobacco (*Nicotiana tabacum* cv., Xanthi) using *Agrobacterium*-mediated transformation. Plants that expressed high levels of the introduced transgene products were identified and self-pollinated to produce lines of expressing and nonexpressing plants. These plant lines were tested for increased resistance to the ROI-generating herbicide methyl viologen (MV) using a membrane permeability assay (3).

### Results

Tobacco plants that expressed transgene constructs for chlCu/ZnSOD and chlMnSOD had approximately three-fold higher levels of total SOD activ-

ity than nonexpressing or Xanthi control plants. Plants that expressed cytAPX had approximately five-fold higher levels of total APX activity than control plants while those expressed chlAPX had nearly 15-fold higher total APX activity than non-expressing plants.

Transgenic tobacco plants that expressed chlMnSOD showed a significant reduction in membrane permeability, compared with control plants, following treatment with MV. Although chlCu/ZnSOD expressing plants also had increased protection against MV-associated damage, the levels of membrane protection in these plants were less substantial than in chlMnSOD plants.

Expression of cytAPX in transgenic tobacco plants also led to a significant reduction in MV-induced membrane damage. However, plants that expressed chlAPX showed no significant decrease in membrane damage after MV-exposure.

### Discussion

The susceptibility of plants to oxidative stress can clearly be affected by modification of the levels of ROI-scavenging enzymes. We have found that expression of chlMnSOD and cytAPX can provide protection against MV-induced membrane damage, while chlCu/ZnSOD is somewhat less effective, and chlAPX provides no detectable protection. The differential performance of chlCu/SOD and chlMnSOD in providing protection from MV is probably due to inactivation of Cu/Zn SOD by H<sub>2</sub>O<sub>2</sub>. The ability of cytAPX to protect membranes during MV-exposure, indicates that scavenging of cytosolic H<sub>2</sub>O<sub>2</sub> is an important factor in oxidative stress protection, and the failure of chlAPX to provide significant levels of protection may indicate that levels of SOD activity, rather than APX activity, limit ROI-scavenging in chloroplasts of MV-treated tissues.

1. Bowler C et al. *Crit Rev. In Plant Sci.* 1994;13:199-218.

2. Allen RD *Plant Physiol Plant Physiol.* 1995;107:1049-1054.

3. Sen Gupta A et al. *Proc Natl. Acad. Sci. USA* 1993;90:1629-1633.