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Oxidative stress tolerance in transgenic orchardgrass plants that express combinations of ascorbate peroxidase and superoxide dismutase

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Objectives

Plants have evolved an array of antioxidant enzymes, such as ascorbate peroxidase (APX) and superoxidized dismutase (SOD) to scavenge reactive oxygen species (ROS) and detoxify them. In the present study, we have over expressed apx and sod genes were placed under the stress inducible SWPA2 promoter. Transgenic orchardgrass plants were obtained by *Agrobacterium tumefaciens* mediated transformation. At least twenty transgenic lines were identified using PCR. These plants were evaluated for protection against methyl viologen (MV, paraquat), hydrogen peroxide (H₂O₂) oxidative damage in leaf discs.

Materials and Methods

1. Plant cultivars: Orchardgrass cv. Roughrider, Frontier.
2. Expression vectors: *Agrobacterium* EHA105 carrying vector pCAMBIA1300 encoding the SWPA2::SOD/APX genes and hygromycin phosphotransferase (HPT) in the T-DNA region.
3. Stress treatment: Leaf squares (4 mm in size) prepared from leaves of 4-week-old plants grown in greenhouse were placed in a petridish containing 10 ml of 0.4 M sorbitol with various concentrations of methyl viologen (MV). The tissues were incubated in the dark for 12 h and then exposed to continuous light (250 $\mu\text{mol m}^{-2}\text{s}^{-1}$) for various time periods.
H₂O₂ in the same way with MV treatment but without dark incubation.
4. Determination of ion leakage: Ion leakage from leaf squares during different stress treatments was determined periodically by measurement of ion leakage into bathing medium using a conductivity meter (Inolab, UK).

Results and Discussion

We developed transgenic orchardgrass (*Dactylis glomerata* L.) plants expressing both Cu/Zn SOD and APX in chloroplasts under control of stress inducible promoter, SWPA2

PCR and Southern blot analyses revealed that transgenes were successfully integrated into genome of regenerated plants. Leaf squares from transgenic orchardgrass plants were subjected to abiotic stresses such as methyl viologen (MV) and hydrogen peroxide (H₂O₂) stresses and cellular damages are measured. Transgenic plants showed significant reduction in cellular damages induced by oxidative stresses than non-transgenic plants.

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