The Transformation of Tobacco MnSOD Gene into Baoding Alfalfa

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Abstract: Bio-engineering technologies are now routinely used for the genetic improvement of *Medicago sativa* L. However, breeding lines of alfalfa are not easily amenable to genetic transformation and therefore may not benefit from the molecular tools that have been developed for genetic manipulations. This paper describes a strategy developed to transfer DNA into a commercially important breeding line in China--Baoding alfalfa via *Agrobacterium* infection. To overcome the limitations to forage yield under environmental stress, especially higher temperatures(> 35°) and arid conditions, transgenic alfalfa plants have been generated that overproduce a *Nicotiana plumbaginifolia* L. manganese superoxide dismutase(MnSOD). To target the mitochondrial enzyme into the chloroplast, the cDNA sequence of *MnSOD* gene was fused to a chloroplast transit peptide from a pea Rubisco small subunit gene, with the chimeric gene as the CaMV 35S promoter. Putative transgenic plants were screened by NPTII specific and MnSOD-specific PCR amplification and southern hybridization. Most of the transgenic plants (83%)gave positive results. Results of MnSOD actitivy assay demonstrated that tobacco MnSOD genes contributed to 50% of the total MnSOD activity.

Key words: MnSOD; Genetic transformation; *Agrobacterium tumefaciens*; Baoding alfalfa