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Presenter Information

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Production and analysis of transgenic white clover (*Trifolium repens*) plants over-expressing organic acid biosynthetic genes

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Introduction Aluminium (Al) toxicity is a major environmental limitation for plant production in acid soils, which represent more than one third of the world's agricultural land. Al-induced secretion in roots of organic acids (OA), such as malate and citrate, chelates the toxic Al cation excluding it from the root. This mechanism of Al-tolerance appears also to be associated with enhanced P-use efficiency. The development of transgenic plants for enhanced synthesis and secretion of OA from roots is a promising approach to confer Al-tolerance and enhanced P-acquisition efficiency. In order to understand the association between OA biosynthesis and secretion from roots in white clover (*Trifolium repens* L.), the physiological consequences of over-expressing 3 key white clover OA biosynthetic genes, individually and in combination, were assessed in transgenic plants.

Materials and methods White clover cDNAs encoding nodule-enhanced malate dehydrogenase (*TrneMDH*) and phosphoenolpyruvate carboxylase (*TrPEPC*) were isolated and sequenced. Transgenic white clover plants were generated by *Agrobacterium*-mediated transformation using binary vectors carrying chimeric OA biosynthetic genes from white clover under control of constitutive (CaMV35S) and/or root-prevalent (white clover phosphate transporter *TrPT1*) promoters.

Results and conclusions Nucleotide sequence analysis of *TrneMDH* revealed an open reading frame (ORF) of 1227 bp encoding for a protein of 408 amino acids. *TrPEPC* showed a 2904 bp ORF encoding a protein of 967 amino acids. Both *TrneMDH* and *TrPEPC* shared 93% sequence similarity with the respective *Medicago sativa* orthologues (*MsneMDH* and *MsPEPC*). A white clover mitochondrial citrate synthase (CS) cDNA (*TrCS*) was isolated and sequenced. Sequence analysis of *TrCS* revealed an ORF of 1419 bp encoding a protein of 472 amino acids. *TrCS* shared 74% identity to other dicotyledonous plant CS cDNAs. Molecular analysis of independent transgenic white clover plants confirmed the stable integration of the chimeric 35S::TrMDH, PT::TrMDH, 35S::TrPEPC, 35S::TrCS and PT::TrMDH-35S::TrPEPC transgenes. Transgenic white clover plants stably expressing the transgenes were identified by RT-PCR. Representative transformation events overexpressing *TrneMDH*, *TrPEPC* and *TrCS*, individually or in combination, were screened for OA synthesis and secretion. Selected transgenic lines were subjected to growth performance analysis under different aluminium and phosphorus levels, to allow for the identification of Al-tolerant transformation events with minimal disruption of carbon balance, and hence unaltered growth potential.

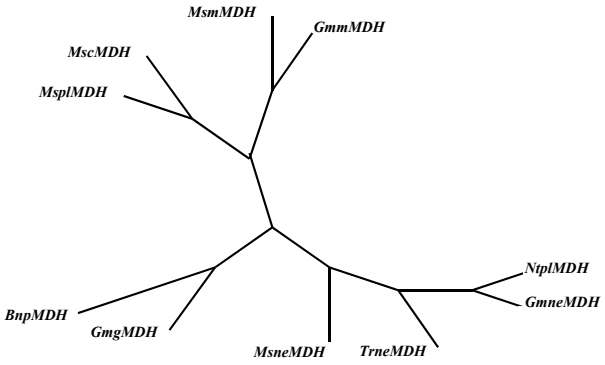

Figure 1 Unrooted phylogenetic dendrogram for deduced amino acid sequences of plant MDHs including white clover nodule-enhanced MDH (*TrneMDH*)