Isolation and Characterisation of Genes Encoding Malate Synthesis and Transport Determinants in the Aluminum-Tolerant Australian Weeping-Grass (*Microlaena Stipoides*)

R. M. Polotnianka
La Trobe University, Australia

E. Ribarev
La Trobe University, Australia

L. Mackin
La Trobe University, Australia

K. A. Sivakumaran
Australian Centre for Plant Functional Genomics, Australia

G. D. Nugent
La Trobe University, Australia

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Presenter Information

R. M. Polotnianka, E. Ribarev, L. Mackin, K. A. Sivakumaran, G. D. Nugent, U. P. John, and G. C. Spangenberg

Isolation and characterisation of genes encoding malate synthesis and transport determinants in the aluminium-tolerant Australian weeping-grass (*Microlaena stipoides*)

R.M. Polotnianka[1,2,3], E. Ribarev[1], L. Mackin[1], K.A. Sivakumaran[1,2], G.D. Nugent[1,3], U.P. John[1,2,3] and G.C. Spangenberg[1,2,3]

[1]Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Victoria 3086, Australia [2]Australian Centre for Plant Functional Genomics, Australia [3]Victorian Centre for Plant Functional Genomics, Australia Email: renata.polotnianka@dpi.vic.gov.au

Keywords: weeping grass, aluminium tolerance, malate dehydrogenase, aluminium-induced malate transporter, aluminium exclusion

Introduction Acid soils cover some 40% of the Earth's arable land where they represent a major limitation to plant production. Plant growth on acid soils is primarily limited due to aluminium (Al) solubilized by acidity into toxic Al^{3+} cations which will inhibit root growth resulting in poor uptake of water and nutrients. Many important pasture species lack sufficient Al tolerance within their germplasm to allow effective breeding for this character.

Materials and methods A gene discovery and functional genomics program was undertaken in the Australian weeping grass (*Microlaena stipoides*) in order to determine the molecular basis of its enhanced tolerance to toxic Al^{3+} species associated with acidic soils, as a "Xenogenomics" approach that seeks to isolate and characterise determinants of abiotic stress tolerance in indigenous and exotic non-model and non-crop plants. *M. stipoides* has a highly efficient, rapidly induced aluminium exclusion mechanism in its roots whereby within hours of exposure to elevated Al levels at low pH it ceases to assimilate Al.

Results and conclusions EST sequencing of *M. stipoides* cDNA libraries derived from root material of plants exposed to 1mM Al revealed an enrichment of clones encoding malate dehydrogenase (MDH) orthologues (0.58%), relative to root EST collections of other grasses (< 0.1%). Northern hybridisation analysis showed that steady state levels of mRNA encoding the predominant cytoplasmic MDH (cMDH) form are not elaborated in response to Al or low pH but are enriched 4-fold in root tips relative to the mature portion of the root (Figure 1). Thus *cMDH* transcripts are preferentially localised to the region of the root most vulnerable to Al toxicity. A 1.4 kb region of upstream sequence has been isolated by adaptor PCR and cloned into promoter-reporter gene vectors for analysis in transgenic plants.

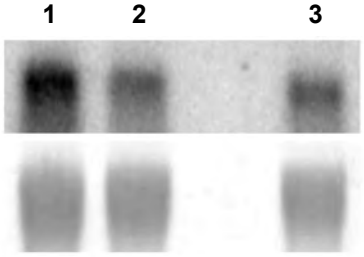

Figure 1 Northern hybridisation analysis of cytoplasmic MDH transcripts in root tips (1), mature part of root (2), and leaves (3) of *M. stipoides*. Lower panel shows rRNA as loading control.

In order to exploit malate exudation to confer aluminium tolerance, *M. stipoides* must not only be able to synthesise malate but it must efficiently secrete it. To investigate transport of malate, orthologues of the aluminium-induced malate transporter (*ALMT1*) gene of wheat that confers malate efflux and aluminium tolerance (Sasaki *et al*., 2004), were isolated and characterised. The genome of *M. stipoides* contains a small family of *ALMT1* related sequences. Adaptor PCR has been used to isolate two distinct genomic clones encoding putative orthologues with 71.4 and 70.4% identity to wheat ALMT1. Further analysis of the expression and function of these candidate malate transporters is in progress.

Reference

Sasaki, T., Yamamoto, Y., Ezaki, B., Katsuhara, M., Ahn, S.J., Ryan, P.R., Delhaize, E., Matsumoto, H., (2004) A wheat gene encoding an aluminium-activated malate transporter. *Plant Journal,* 37,645-53.