A High-Throughput Gene Silencing Approach for Studying the Interaction Between Perennial Ryegrass (*Lolium Perenne*) and the Fungal Endophyte *Neotyphodium Lolii*

S. Felitti
La Trobe University, Australia

P. Tian
Molecular Plant Breeding Cooperative Research Centre, Australia

D. Edwards
Molecular Plant Breeding Cooperative Research Centre, Australia

G. C. Spangenberg
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

A high-throughput gene silencing approach for studying the interaction between perennial ryegrass (*Lolium perenne*) and the fungal endophyte *Neotyphodium lolii*

S.A. Felitti[1,2], P. Tian[1,2], D. Edwards[1,2] and G.C. Spangenberg[1,2]
[1]*Primary Industries Research Victoria, Plant Biotechnology Centre, La Trobe University, Bundoora, Victoria 3086, Australia* [2]*Molecular Plant Breeding Cooperative Research Centre, Australia*
Email: silvina.felitti@dpi.vic.gov.au

Keywords: fungal endophytes, perennial ryegrass, expressed sequence tags (ESTs), gene silencing, siRNAs

Introduction Perennial ryegrass (*Lolium perenne* L.) and its fungal endophyte (*Neotyphodium lolii*) are known to establish a mutualistic association that impacts on the agronomic productivity of endophyte-infected ryegrass pastures. To study this interaction at the molecular level, a genomic resource consisting of 13,964 endophyte ESTs has been generated. However, the functions of a large proportion of these genes remain to be elucidated. Recent work has demonstrated the potential for RNA-mediated gene silencing to suppress gene expression in a sequence specific manner thus allowing for the subsequent analysis of gene function.

Materials and methods Vectors for RNA-mediated gene silencing in grass endophytes based on the Multisite Gateway™ recombination system were developed. These vectors can be used to readily create large numbers of genetically modified endophytes, for high-throughput gene characterisation. In order to validate this system, RNA silencing was performed in transgenic *Neotyphodium* endophyte expressing a chimeric *gusA* reporter gene [strain FM13 (Lp1/pNOM-101 and pAN7-1) (Murray *et al*., 1992) kindly provided by B. Scott, Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand]. Vectors expressing sense and hairpin RNAs were produced and introduced into the *gusA*-expressing transgenic endophyte (Figure 1).

Results and conclusions Efficient gusA gene silencing was achieved through the expressed *gusA* hairpin RNA. Furthermore, small interfering RNAs (siRNAs) designed from the *gusA* sequence and used in endophyte transfection, were effective in silencing the expression of the *gusA* transgene. This high-throughput gene silencing technology has now been applied to characterise the function of a large number of endogenous endophyte genes corresponding to functionally annotated and unannotated sequences represented in the EST resource established. Overall, these results indicate that RNA silencing effectively operates in grass endophytes and this technology provides a new tool for the high-throughput functional characterisation of endophyte genes.

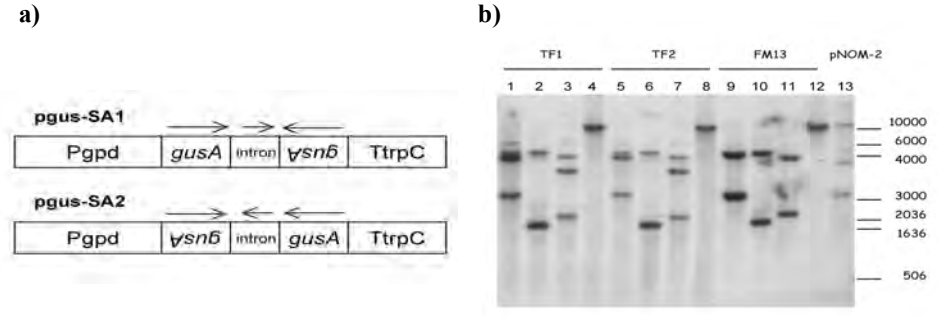

Figure 1 a) Maps of hpRNA *gusA* gene silencing vectors. Arrows indicate the orientation of the *gusA* gene. All the constructs were under control of the *Aspergillus nidulans gpd* promoter (Pgpd) and included *trpC* terminator (TtrpC). b) Southern blot analysis of endophytes transformed with hairpin RNA *gusA* gene silencing vectors pgus-SA1 or pgus-SA2. Genomic DNA was digested with *Hind*III (lanes 1, 5 and 9), *Nco*I (lanes 2, 6 and 10) and *Xba*I (lanes 3, 7 and 11) and was probed with a 250 bp *gusA* gene fragment. Lanes 4, 8 and 12 show undigested genomic DNA

Reference

Murray, F.R., Latch, G.C.M. and Scott, D.B. (1992). Surrogate transformation of perennial ryegrass, *Lolium perenne*, using genetically modified *Acremonium* endophyte. *Mol. Gen. Genet*., 233, 1-9.