Microarray-based transcriptome analysis of the interaction between perennial ryegrass (*Lolium perenne*) and the fungal endophyte *Neotyphodium lolii*

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Introduction *Neotyphodium lolii*, *Neotyphodium coenophialum* and *Epichloë festucae* are common symbiotic fungal endophytes of the temperate pasture grasses perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and red fescue (*Festuca rubra*), respectively. A genomic resource of 13,964 expressed sequence tags (ESTs), representing 7,585 unique endophyte genes, has been established for *Neotyphodium* and *Epichloë* fungal endophytes.

Materials and methods The endophyte genomic resource established has enabled the design and fabrication of two endophyte-specific cDNA microarrays (Nchip™ microarray and EndoChip™ microarray). The Nchip™ and EndoChip™ microarrays have been applied to comparative transcriptome analyses of different asexual (*N. coenophialum* and *N. lolii*) and sexual (*E. festucae*) endophyte taxa under various saprophytic growth conditions, leading to the identification of differentially expressed genes.

Results and conclusions The Nchip™ and EndoChip™ microarrays permit the interrogation of 3,806 *Neotyphodium* genes (Nchip™ microarray), and 4,195 *Neotyphodium* and 920 *Epichloë* genes (EndoChip™ microarray), respectively. They represent tools for high-throughput transcriptome analysis, including genome-specific gene expression studies, profiling of novel endophyte genes, and investigation of the host grass-fungal symbiont interaction. Microarray-based transcriptome analysis was also undertaken 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] compared to untransformed control, confirming the power of these tools for applications in characterising genetically modified endophytes. A recent extension of this proof-of-concept research has included the transcriptome analysis of transgene-expressing endophytes to assess gene expression responses to specific genetic modifications (e.g. gain-of-function, knock-outs and knock-downs). Within the context of a comprehensive spatial and temporal systems biology approach, a transcriptomics study of the mutualistic interaction between perennial ryegrass (*L. perenne* L.) and its fungal endophyte (*N. lolii*) was undertaken. In combination with a 15K ryegrass unigene microarray, the EndoChip™ microarray was applied to the detailed analysis of in planta gene expression in different ryegrass organs using endophyte-infected and endophyte-free ryegrass plants in an isogenic host genetic background. Data derived from endophyte microarray analysis has been validated using both northern hybridisation and RT-PCR analyses.

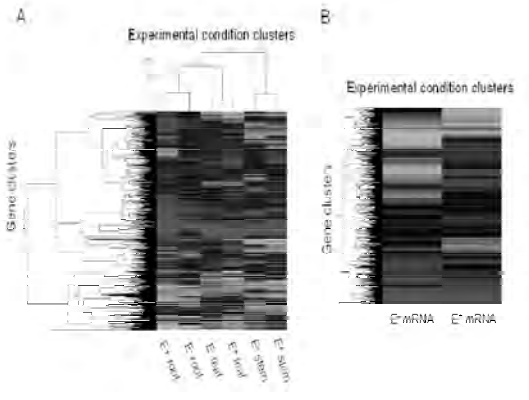

Figure 1 *Neotyphodium in planta* gene expression microarray-based transcriptome analysis. A) Hierarchical clustering of mean signal values (Euclidean distance) from infected and uninfected ryegrass organs on the Endophyte unigene microarray. B) Endophyte-infected stem prevalent gene expression

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.