DEVELOPMENT OF THE ß-GLUCURONIDASE REPORTER GENE SYSTEM TO STUDY *ACREMONIUM* ENDOPHYTE INTERACTIONS WITH PERENNIAL RYEGRASS

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Karyn Saunders

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ABSTRACT

A transformant of the fungal endophyte *Acremonium lolii*, strain Lp19, containing the *gusA* gene under the control of the constitutive P*gpd* promoter was generated, and assigned the name KS1. Analytical digests and Southern hybridisation showed that this transformant contained a single chromosomally integrated copy of the *gusA* gene. The transformation frequency of Lp19 was found to be very low, and attempts to increase the transformation frequency were unsuccessful.

KS1 was used to artificially infect seedlings of several different genotypes of *Lolium perenne*, all of a single cultivar, 'Nui'. These seedlings were grown into mature plants, and the endophytically produced GUS enzyme was extracted from individual plant tissues. Assays were performed on the enzyme extracts, and the levels determined were used as a measure of endophyte metabolic activity. Alterations of the *gusA* gene in some plants was detected by Southern hybridisation. One alteration was found to result in loss of GUS activity, the other did not appear to alter *gusA* expression.

Levels of transformed endophyte GUS activity were initially compared between clonal plant material of a single genotype. Statistical analysis revealed that no significant differences were detectable for a particular tissue between the different plants. This showed that plant material of identical genotype could be pooled for analysis without the pooling of the individual plants having an affect on the outcome of the analysis.

Next, levels of the transformed endophyte GUS activity were compared between genetically diverse perennial ryegrass plants of cultivar 'Nui'. Significant differences in GUS activity were detected in most tissues tested between the different genotypes, with only the most mature tissue displaying no detectable differences.

Finally, a single plant of each of two individual genotypes was divided into several clonal plants, and the resulting mature plants were pooled in their genotypes for analysis of GUS, peramine, ergovaline and lolitrem B levels. The F test was not particularly sensitive in this experiment, and only one major difference between genotypes could be detected. Despite this, some trends emerged which were found to be consistent with those found in other studies. Metabolic activity and peramine levels were shown to be highest in the leaf sheath tissue, with levels generally decreasing with increasing tissue age. Lolitrem B was found to be highest in leaf sheath tissue also, but with levels increasing in general with tissue age. Ergovaline levels were very low in all tissues. The results presented show the potential of the use of the GUS reporter gene system to study endophyte gene expression *in planta*, and pooling of plants can be carried out to allow simultaneous study of toxin expression.

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