Spatial and temporal expression patterns of lolitrem biosynthetic genes in the *Epichloë festucae*-perennial ryegrass symbiosis

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Epichloë festucae systemically colonises the intercellular spaces of perennial ryegrass (*Lolium perenne*) aerial tissues forming a mutually beneficial association between the fungus and host plant. In association with its host grass, *E. festucae* synthesises a range of indole-diterpenes, including lolitrem B, which provide protection to the symbiotum against small animal herbivory. A gene cluster of at least ten *ltm* (lolitrem) genes is required for lolitrem B biosynthesis (Young *et al*. 2005; Young *et al*. 2006). RT-PCR analysis of RNA derived from mycelium and pseudostem tissue suggest that these genes are expressed only when the fungus is in association with the grass host (Young *et al*. 2006). The objectives of this study were, first, to test if *ltm* genes were subject to carbon and/or nitrogen catabolite repression, and secondly to examine the expression patterns of the *ltm* genes *in planta* using GUS reporter gene analysis.

The expression of the 10 *E. festucae ltm* genes was examined by reverse transcriptase (RT)-PCR analysis using RNA template derived from mycelium grown under a range of physiological conditions, including both N- and C-repressed and –derepressed conditions. While transcript could be detected for most genes under nearly all conditions tested, the level of transcript, with the exception of *ltmK*, was very low compared to transcript levels found *in planta*. These results demonstrate that *ltm* gene expression is not subject to either N or C-catabolite repression.

The *ltmM* gene, which encodes an enzyme for an early step in the lolitrem biosynthetic pathway, was selected to examine the patterns of *ltm* gene expression *in planta*. Five different *ltmM* promoter deletion fragments were generated, translationally fused to *gusA* and transformed into *E. festucae* protoplasts. The minimum promoter length required for *gusA* expression *in planta* was found to be 800 bp. In mature vegetative tillers, *gusA* was expressed in all infected aerial plant tissues, including epiphyllous hyphae. The *gusA* expression pattern of the positive control, a P*gpd-gusA* transformant, was similar to the P*ltmM-gusA* transformants, confirming that *ltmM* is expressed at all times in vegetative tillers. At pre-anthesis, *gusA* expression was observed in all floral organs except the immature gynoecium. Similar GUS activity patterns were observed in control plants, except the ovule was colonised, with hyphae restricted to the nucellar tissue. In post-anthesis florets, gene expression occurred almost exclusively in the anthers and the gynoecium with dense staining observed in the stigma. In contrast, for the control plants, GUS activity was observed in all tissues of the spikelet. In germinating seedlings P*ltmM* expression was observed in hyphae 24 h post-germination in seeds and 6 d post-germination in seedlings, from the mesocotyl to the tip of the emerging first leaf. These results suggest that during seed germination hyphae in the shoot apex have a significant role in further colonisation of the seedling.

REFERENCES

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