Discovery, Isolation and Characterisation of Promoters from Perennial Ryegrass (*Lolium Perenne*)

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Introduction The availability of a suite of promoters with a range of spatial, temporal and inducible expression patterns is of significant importance to enable targeted expression of genes of interest for molecular breeding of forage species. A range of resources and tools have been developed for promoter isolation and characterisation in perennial ryegrass (*Lolium perenne* L.) including genomic lambda and BAC libraries and a 15 K unigene microarray.

Materials and methods Discovery, isolation and characterisation of heterologous and endogenous promoters were undertaken in perennial ryegrass. Expression patterns of chimeric *gusA* reporter genes encoding bacterial β-glucuronidase (GUS) with two pollen-specific promoters from maize (Zm13, CDPK) were evaluated in transgenic ryegrass plants generated by biolistic transformation of embryogenic cells. The promoter of the gene encoding a Lolp2 pollen allergen from perennial ryegrass was isolated and its pollen-specificity was demonstrated in transgenic tobacco plants.

Results A perennial ryegrass BAC library consisting of 50,304 BAC clones with 113 kb average insert size, corresponding to 3.4 genome equivalents and 97% genome coverage was established. Upstream regulatory sequences of genes involved in lignin biosynthesis and fructan metabolism in perennial ryegrass were identified, isolated and characterised *in planta* as *gusA* transcriptional fusions. Chimeric *gusA* genes under control of the 5' regulatory sequences isolated from perennial ryegrass genes encoding caffeic acid *O*-methyltransferase (*LpOMT1*) and 4-coumarate-CoA ligase (*Lp4CL2*) revealed strong GUS activity in vascular tissue of transgenic tobacco plants. The promoter from the perennial ryegrass sucrose:sucrose 1-fructosyltransferase (*Lp1-SST*) conferred strong constitutive expression in leaves while the regulatory sequences from the perennial ryegrass fructosyltransferase homologue *LpFT1* showed weaker expression in stem bases. Conserved regulatory elements identified in genes showing inducible (e.g. by light, drought and sucrose) or cell-type specific (e.g. pollen cells) expression were identified in promoter sequences from perennial ryegrass genes (Figure 1).

Conclusions This research provides a toolbox of promoters with a range of specificities for targeted gene expression as part of a molecular breeding approach in perennial ryegrass deploying exclusively ryegrass genes and promoters for transgenic product development.

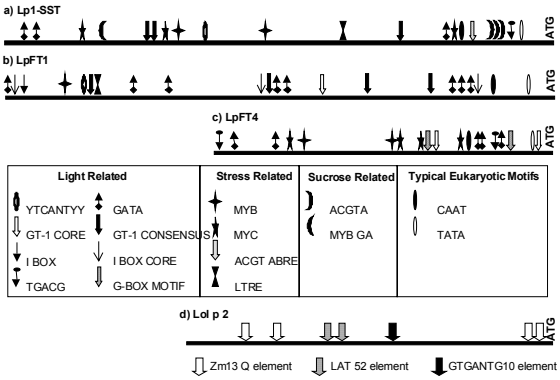

Figure 1 Analysis of perennial ryegrass regulatory sequences. a) 1.6 kb *Lp1-SST* promoter; b) 1.6 kb *LpFT1* promoter; c) 0.9 kb *LpFT4* promoter; and d) 0.95 kb *Lol p 2* promoter