Gene discovery and molecular dissection of lignin biosynthesis in perennial ryegrass (*Lolium perenne*)

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Keywords: perennial ryegrass, lignin biosynthesis, transgenic plants, herbage quality

Introduction Lignification of plant cell walls has been identified as a major factor limiting forage digestibility. It limits the amount of digestible energy available to livestock, resulting in an incomplete utilisation of cellulose and hemicellulose by ruminant animals. Modification of the lignin profile of ryegrasses (*Lolium* spp.) and fescues (*Festuca* spp.) is undertaken through modulating the expression of genes encoding enzymes involved in the biosynthesis of monolignols.

Materials and methods A targeted gene discovery program in perennial ryegrass (*L. perenne* L.) has led to the isolation and characterisation of cDNA and genomic clones encoding the following enzymes involved in the biosynthesis of the monolignol precursors and their extracellular polymerisation to yield lignins: phenyl alanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), ferulate-5-hydroxylase (F5H), caffeic acid *O*-methyltransferase (OMT), 4-coumarate-CoA ligase (4CL), caffeoyl-CoA 3-*O*-methyltransferase (CCoAOMT), cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), peroxidase (PER) and laccase (LAC) (Figure 1).

Results and conclusions Following sequence analyses, expression profiles (i.e. organ specificity, developmental regulation and wound-inducibility) of these lignification genes in perennial ryegrass were determined using microarray-based and northern hybridisation analyses. Gene organisation, copy number and genetic map location using RFLPs were determined for LpPAL, LpC4H, LpF5H, LpOMT, Lp4CL, LpCCoAOMT, LpCCR, LpCAD and LpPER. Functional analysis *in planta* was undertaken following production of transgenic model (i.e. arabidopsis, tobacco) plants for overexpression of chimeric target lignification genes under control of the CaMV35S promoter and the generation of transgenic ryegrass plants for overexpression and down-regulation of the chimeric genes under control of constitutive and xylem-specific promoters. This provided the basis for a molecular dissection of the biosynthesis of monolignol precursors in perennial ryegrass to enhance the understanding of lignin monomeric composition and properties in grasses. It furthermore underpinned the design of transgenic elite perennial ryegrass genotypes carrying modular vectors with a combinatorial modulation of key target lignification genes using exclusively ryegrass gene sequences for improved forage quality and nutritive value.

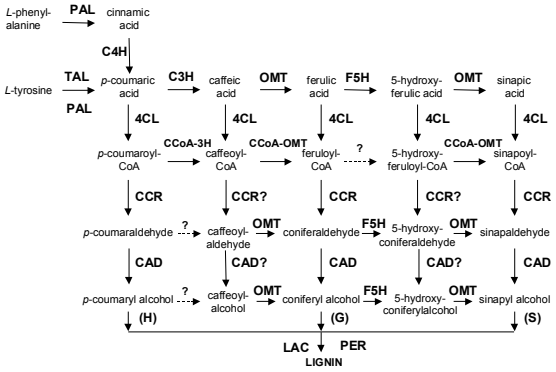

Figure 1 Hypothetical pathways of lignin biosynthesis in perennial ryegrass