Gene Discovery and Molecular Dissection of Fructan Metabolism in Perennial Ryegrass (Lolium Perenne)

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Gene discovery and molecular dissection of fructan metabolism in perennial ryegrass (*Lolium perenne*)

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Introduction Fructans are the main soluble carbohydrate stored in up to a third of the vegetation of the earth, including the economically important temperate grasses. Fructans are polymers of fructose attached to a sucrose precursor. Perennial ryegrass (*L. perenne* L.) accumulates fructans of the inulin series, inulin neoseries and levan neoseries. Four enzymes are required to produce fructans of this profile: 1-SST (sucrose:sucrose 1-fructosyltransferase), 1-FFT (fructan:fructan 1-fructosyltransferase), 6G-FFT (6-glucose fructosyltransferase) and 6-FFT (fructan:fructan 6-fructosyltransferase) or 6-SFT (sucrose:fructan 6-fructosyltransferase) (Figure 1). Fructan biosynthetic enzymes have evolved from invertases and thus it is argued that fructan metabolism is an extension of sucrose metabolism. A high fructan content is a valuable resource in perennial ryegrass as it can be readily mobilised to sustain regrowth immediately after defoliation as well as adding to the nutritive value of the feed. However, the physiological role of fructans in grasses is not fully understood.

Materials and methods A targeted gene discovery program in perennial ryegrass has led to the isolation and characterisation of cDNA and genomic clones encoding enzymes involved in sucrose and fructan metabolism: sucrose phosphate synthase (SPS), invertases (INV), sucrose synthase (SS), sucrose transporter (ST), 1-SST (sucrose:sucrose 1-fructosyltransferase), 1-FFT (fructan:fructan 1-fructosyltransferase) and fructan hexohydrolase (FEH).

Results and conclusions Following sequence analyses, expression profiles (i.e. organ specificity, developmental regulation, light/dark and temperature regulation) of these sucrose and fructan metabolism 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 LpSPS, LpINV, LpSS, Lp1-SST, LpFFT and LpFEH. Functional analyses of sucrose and fructan metabolism genes were undertaken in *Pichia pastoris* and *in planta*. Expression vectors were generated using sense, antisense and hpRNA technologies for overexpression, co-suppression or downregulation of target genes in transgenic perennial ryegrass plants. Transgenic perennial ryegrass plants have been generated and characterised at the molecular and biochemical level. This provided the basis for a molecular dissection of fructan metabolism to understand its physiological role in perennial ryegrass. It further underpinned the design of transgenic elite perennial ryegrass genotypes carrying modular vectors with a combinatorial modulation of key target fructan metabolism genes using exclusively ryegrass gene sequences for improved nutritive value, persistence and quality.

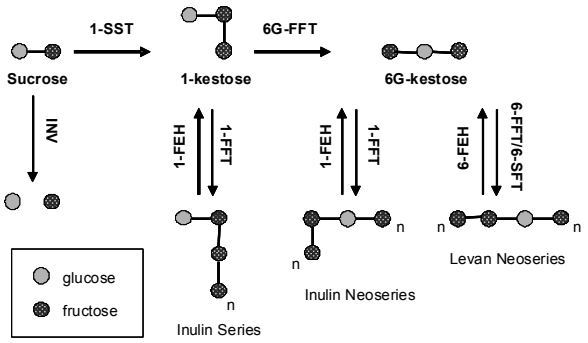

Figure 1 Hypothetical pathway of fructan metabolism in perennial ryegrass