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Fructan biosynthesis
in *Lolium perenne*

**Tissue, cultivar and temperature effects
on gene expression and protein accumulation profiles**

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ABSTRACT

Cultivars of *Lolium perenne* with high concentrations of water soluble carbohydrates (WSCs) offer opportunities to mitigate greenhouse gas emissions (nitrous oxides) from grazed pastures and improve meat and milk production in livestock. Our previous studies demonstrated that fructan accumulation in the blades of high WSC grasses involves a strong gene x environment interaction. To identify the temperature effects on the expression of high sugar trait in the high sugar cultivars, we conducted a pot trial in climate chambers with temperature regimes set at 10/10, 20/10 and 20/20°C (day/night), respectively. Water soluble carbohydrate concentrations, the expression of the key genes and proteins: 1-SST (sucrose: sucrose 1-fructosyltransferase), 1-FFT (fructan: fructan 1-fructosyltransferase), 6G-FFT (fructan: fructan 6G-fructosyltransferase) and 1-FEH (1-fructan exohydrolases) involved in the fructan biosynthetic pathway of *L. perenne* were compared in blades and sheaths of three selected high sugar cultivars (P, A and H) and a common cultivar (F) grown under the three temperature regimes. We found that amongst the selected 3 high sugar cultivars, high molecular weight (HMW) WSC content was significantly higher in P and A cultivars, regardless of the temperature regimes. As expected, sheaths contained significantly higher concentrations of HMW WSCs (fructans) compared to leaf blades. The highest WSC contents in both leaf and sheath tissues accumulated at 10/10°C while the lowest accumulated at 20/20°C. Gene expression profiles demonstrated that all four genes studied were more significantly expressed in sheaths compared to blades, and the expression levels were highly correlated with fructan accumulation in this tissue. Low temperature resulted in significant up-regulation of 1-SST in sheaths, but not in blades. 1-FFT was highly expressed in blades of A and P cultivars. Unexpectedly, 6G-FFT was expressed more significantly in the control F cultivar, but not in the high sugar cultivar P. Protein expression profiles showed that 1-SST protein accumulated to high levels in sheaths, whereas protein levels of 1-FFT and 1-FEH were higher in blades. 1-SST protein levels in both blades and sheaths generally increased in plants grown at low temperatures, whereas 1-FFT protein was not affected by low temperatures in blades and sheaths, furthermore, in both tissues there was no consistent effect observed between the different cultivars and temperature regimes on 1-FEH protein levels.

ABBREVIATIONS

1-FEH:	1-fructan exohydrolases
1-FFT:	Fructan: fructan 1-fructosyltransferase
1-SST:	Sucrose: sucrose 1-fructosyltransferase
6G-FFT:	Fructan: fructan 6G-fructosyltransferase
6-SFT:	Sucrose: fructan 6-fructosyltransferase
AP:	Ammonium persulfate
bp:	Base pair
cDNA:	Complementary deoxyribonucleic acid
C_T:	Threshold cycle
C-terminus:	Carboxyl-terminus
DEPC:	Diethylpyrocarbonate
DM:	Dry mass
DNA:	Deoxyribonucleic acid
DNase:	Deoxyribonuclease
dNTPs:	Dinucleotide triphosphates
DP:	Degree of polymerisation
EDTA:	Ethylenediaminetetraacetic acid
FEHs:	Fructan exohydrolases
g:	Relative centrifuge force
h:	Hour(s)
His:	Histidine
HMW:	High molecular weight fructans
HSD:	Honestly Significant Different test
HSG:	High sugar grass
IPTG:	Isopropyl- β -D-thiogalactopyranoside
kDa:	Kilodaltons
LB:	Luria-Bertani
LMW:	Low molecular weight
mA:	Milli ampere
min:	Minute(s)
NCBI:	National Center for Biotechnology Information

Ni-NTA:	Nickel-nitrilotriacetic acid
PBS:	NaH ₂ PO ₄ - NaCl buffer
PCR:	Polymerase chain reaction
PMSF:	Phenyl methyl sulfonyl fluoride
PVDF:	Polyvinylidene difluoride
qPCR:	Quantitative polymerase chain reaction
RNA:	Ribonucleic acid
RNase:	Ribonuclease
rpm:	Revolutions per minute
rRNA:	Ribosomal RNA
RT-PCR:	Reverse transcription polymerase chain reaction
Rubisco:	Ribulose 1,5-bisphosphate carboxylase
SDS:	Sodium dodecyl sulfate
SDS- PAGE:	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
sec:	Second(s)
TAE:	Tris-acetate-EDTA buffer
Taq:	<i>Thermus aquaticus</i>
TBST:	Tris-HCl-NaCl-Tween 20 buffer
TEMED:	N,N,N',N'-tetramethylethylenediamine
Tris:	Trishydroxymethylaminomethane
UV:	Ultraviolet light
V:	Voltage
WSCs:	Water soluble carbohydrates
X-gal:	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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