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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_2PO_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: *Thermus aquaticus*

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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