Transcriptional and metabolic profiles of *Lolium perenne* L. genotypes in response to a PEG-induced water stress.

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Introduction

Perennial ryegrass is the principal forage grass species used in temperate grassland systems. Climate changes predictions suggest a shift towards warmer and dryer summers across the British Isles with greater temperature extremes, making drought tolerance an important target trait in breeding programmes. A combined transcriptomic and metabolomic study was conducted to investigate the genetic basis of phenotypic and metabolic plasticity to drought for a set of perennial ryegrass genotypes.

Plant Material and PEG-induced water stress

The *Lolium perenne* ecotype PI 462336 was selected from the Genomic Resources Information Network (GRIN) operated by the USDA (http://www.ars-grin.gov/npgs/). This accession was selected after a search for accessions being described as drought tolerant. Seeds were planted together with seeds from the cultivar Cashel. Clones of Cashel and PI 462336 were allowed to establish in an aerated hydroponics system supplemented with 4.4gl^{-1} MS medium (Duchefa) in two replicates. After two weeks of growth the solutions in both systems were replaced. In the first system a MS medium supplemented with 20% w/v PEG 6000 was applied to induce water stress and the second system contained MS salts only and acted as a control. Relative Water Content (RWC) was measured at mid-day after 24 hrs and 1 week according to the method described by Barr's and Weatherly [1]. Dry weights (DW) for above and below ground biomass were determined after two weeks of induced water stress.

'Omic' analysis

In order to quench the cellular activities, samples from leaf blades and roots were immediately flash frozen and freeze-dried for long term storage. Extraction and derivatization of polar and non-polar metabolites was performed as described by Shepherd *et al.* [2]. The polar and non-polar samples were analyzed using a Thermo Finnigan Tempus GC–(TOF)–MS system and the Xcalibur™ software package for data acquisition. Statistical analysis was performed using GenStat. Suppression Subtractive Hybridization (SSH) was performed to identify transcripts up-regulated in the documented drought tolerant accession PI 462336 after one week of a 20% PEG induced water stress. SSH was performed according to the protocol of Desai *et al*. [3] using stress and control material as tester and driver, respectively.

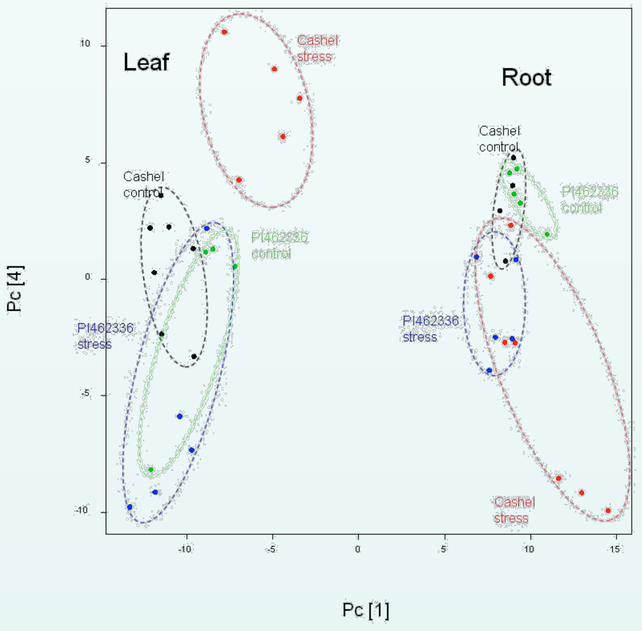

Figure 2: Principal component analysis (PCA) plot of all metabolite compounds found, following GC-TOF-MS analysis of the root and leaf tissues of Cashel and PI 462336. Components 1 and 4 explained up to 25% and 8% of the variability, respectively.

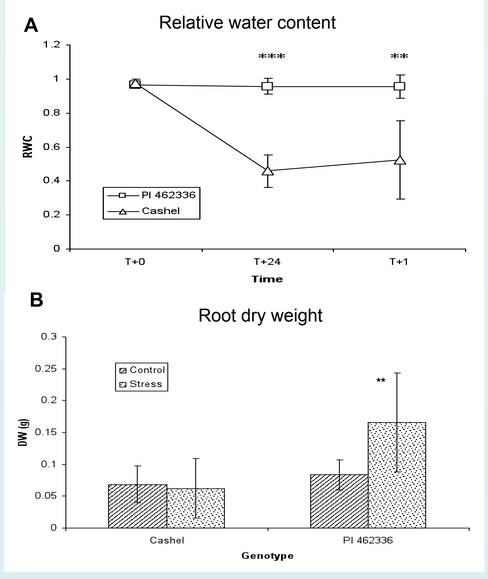

Figure 1: Physiological plant reactions to the PEG induced drought stress experiment **(A)** Relative water content (RWC) levels of Cashel and PI 462336 under PEG induced drought stress at the beginning of the experiment (T+0), after 24 hrs (T +24) and after 1 week (T+1) (** *P<0.01*. *** *P<0.001* N=6). **(B)** Root dry weight of Cashel and PI 462336 genotypes under control conditions and PEG induced drought stress after 2 weeks (** difference significant at *P<0.01* N=8). **(C)** Root biomass of PI 462336 in comparison to Cashel under PEG induced drought stress after 2 weeks.

Results and Discussion

- Physiological differences between the two genotypes (Fig. 1) suggests that the selected lines have differential tolerance to water stress
- There were clear differences in the metabolomes of depending on tissue, genotype and environmental condition (Fig. 2)
- Metabolite data seems to suggest an overall metabolic shift towards the production of sugars in the tolerant variety under stress conditions. (Fig. 3)
- A decrease in the levels of aminoacids seems to be common between the two genotypes (Fig. 3)
- SSH data revealed 39 upregulated transcripts (data not shown) including fructan:fructan 6G fructosyl transferase (f:f-6G-FFT)
- Expression of f:f-6G-FFT was quantified over 3 different sampling times and revealed significant differences between the two genotypes but not between stress conditions (Fig. 4A)
- Analysis of fructan oligosaccharides by HPAEC and further analysis by PCA has revealed differential clustering between the two genotypes (Fig. 4B) but not for different times of sampling nor stress conditions.
- The chromatograms of the fructan extracts did not reveal differences as pronounced as those in the genetic expression analyses (Fig. 4C)

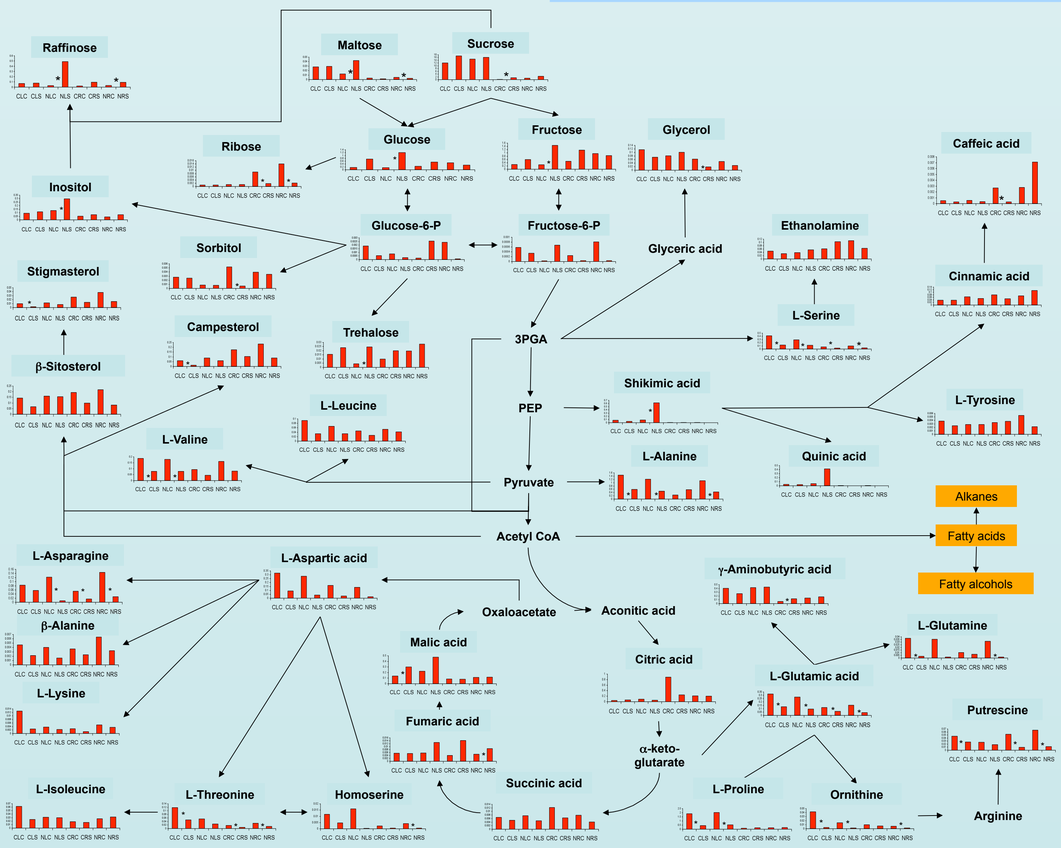

Figure 3 – Mapping of metabolite changes for known pathways in leaf and root material for both Cashel and PI 462336 grown under control and PEG-induced drought conditions. Represented values are relative to internal standard and represent the average values obtained. Significant differences ($p<0.05$) between control and samples from plants exposed to PEG-induced drought are represented with asterisk. CLC – Cashel leaf control CLS- Cashel leaf stress NLC- PI 462336 leaf control NLS- PI 462336 leaf stress CRC- Cashel root control CRS- Cashel Root stress NRC- PI 462336 root control NRS- PI 462336 root stress.

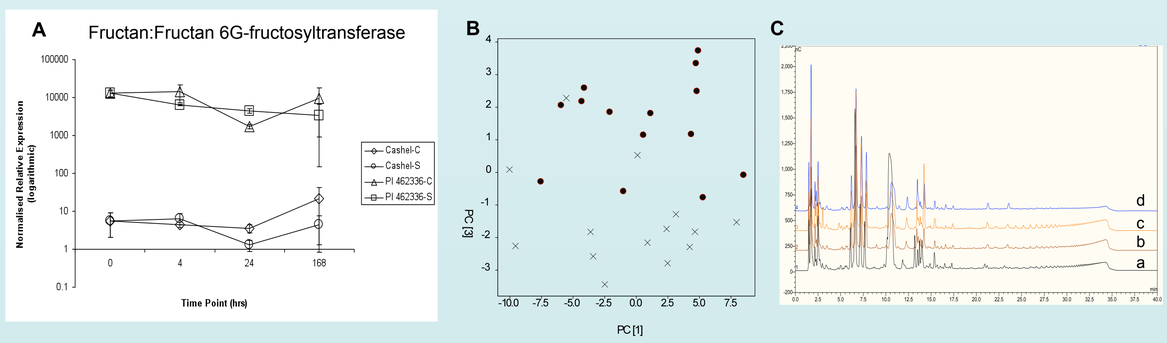

Figure 4 – **(A)** Relative expression of fructan:fructan 6G-fructosyltransferase during 1 week of PEG-induced water stress (S) and control conditions (C) in Cashel (Ca) and PI 462336 (NZ02) leaf material. Biological replicates were each tested in triplicate and normalized to LpGAPDH which was used as housekeeping gene and relative quantities were calculated using QBase (Hellemans *et al*., 2007). The graph shows the average of the two biological replicates with their standard deviation. **(B)** Principal component analysis (PCA) plot of the fructan extract, following HPAEC-PAD analysis of leaf blades of genotypes Cashel and PI 462336. Components 1 and 3 explained up to 45% and 7.5% of the variability, respectively. Circles represent PI 462336 and crosses represent Cashel**. (C)** Chromatogram of fructan extracts of leaf blades from 'PI 462336' and 'Cashel' analysed by HPAEC. Chromatogram a refers to samples collected from 'Cashel' at time 0, chromatogram b refers to 'Cashel' samples collected after 1 week of water stress treatment. Chromatogram c refers to samples collected from 'PI 462336' at time 0 while chromatogram d refers to 'PI 462336' samples collected after one week of water stress treatment

References

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[3] Desai, S. et al - *In.* "Functional Genomics: A Practical Approach" (Hunt, S. P., Livesey R., ed.) Oxford University Press, USA (2000)

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