# A combined transcriptomic and metabolomic approach to understand the mechanisms underlying drought response in perennial ryegrass

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## Introduction

Perennial ryegrass is the principal forage grass species used in temperate grassland systems. Predictions for changing climate 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

*Lolium perenne* ecotype 'PI 462336' (NZ02), was obtained from the USDA seed bank collection and its phenotypic response was compared with the commercial variety 'Cashel' (Ca). NZ02 had been documented as drought tolerant. These ecotypes were grown in a hydroponics system supplied with either MS medium or MS media containing 20% PEG6000 to induce osmotic stress over a period of 1 week.

## Methods

Relative water content of leaves and dry weights of root material were measured and samples of both leaf and root tissue were collected. In order to quench the metabolism, Samples 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.* [1]. 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.

## Results

Following one week of induced drought it was possible to distinguish visible differences between the two lines in response to the different treatments (Fig 1). Root biomass was strongly increased in the NZ02 line compared to the Ca line suggesting together with relative water content data (Fig. 1) that the lines selected have a differential tolerance to drought.
Principal component analysis (PCA) of the all mass peaks (Fig. 2) revealed a clear segregation of metabolites of the different tissue types. Furthermore it was possible to see a clustering of the Ca stressed leaf material distinct from the Ca leaf samples.

Analysis of the metabolite changes in known pathways (Fig. 3 and 4) revealed an overall decrease in amino acid levels following drought exposure. Additionally, significant increases were observed in the sugar levels of NZ02 leaf material following drought exposure which do not occur in Cashel suggesting a possible role in enhanced drought tolerance. Furthermore Cashel experienced a decrease in secondary metabolites such as stigmasterol and campesterol, known to be implicated in membrane stabilization, which may suggest an involvement of these metabolites in drought tolerance.

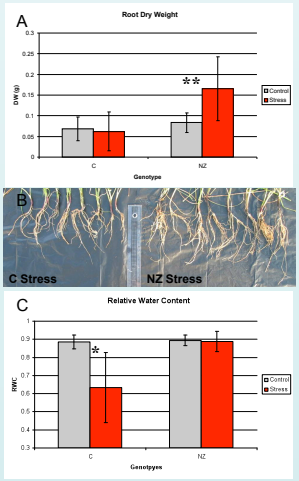


**Figure 1**: (A) Root dry weight of Cashel (C) and NZ02 (NZ) genotypes under control conditions and PEG-induced drought stress after 2 weeks. ** difference significant at *P<0.01* n=8 (B) Image illustrating increased root biomass of NZ genotype in comparison to C under PEG induced drought stress after 2 weeks. (C) Relative water content of genotypes under control and PEG induced drought stress after 1 week, which corresponds to time of sampling for transcriptome and metabolomic analysis. * difference significant at *P<0.05*. n=6.

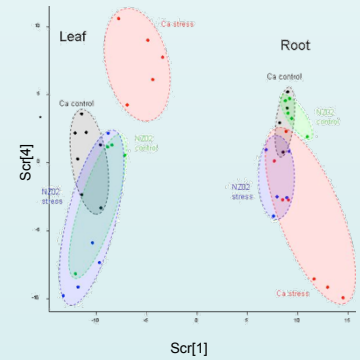


**Figure 2** – PCA plot of all the peak masses detected in the samples, following GC-MS analysis of the root and leaf tissues of NZ (NZ02) and Cashel (Ca) varieties. Components 1 and 4 explained up to 25% and 8% of the variability, respectively.

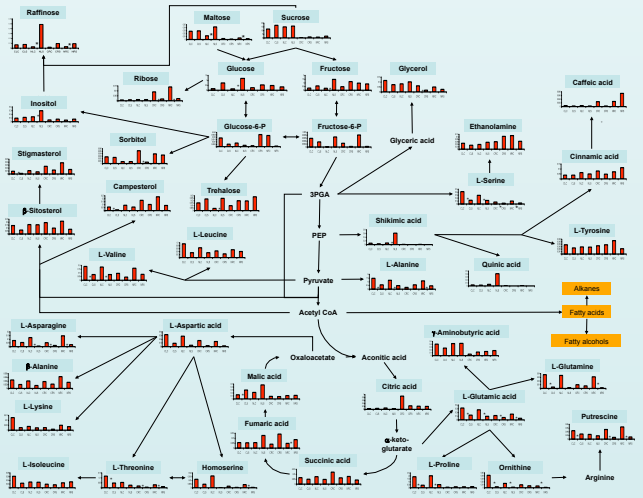


**Figure 3** – Mapping of metabolite changes for known pathways in leaf and root material for both Cashel and NZ02 grown under control and PEG-induced drought conditions. Represented values are relative to internal standard and represent the average values obtained. Significant differences (p<0.05) between control and samples from plants exposed to PEG-induced drought are represented with asterisk. CLC – Cashel leaf control CLS- Cashel leaf stress NLC- NZ02 leaf control NLS- NZ02 leaf stress CRC- Cashel root control CRS- Cashel Root stress NRC- NZ02 root control NRS- NZ02 root stress.

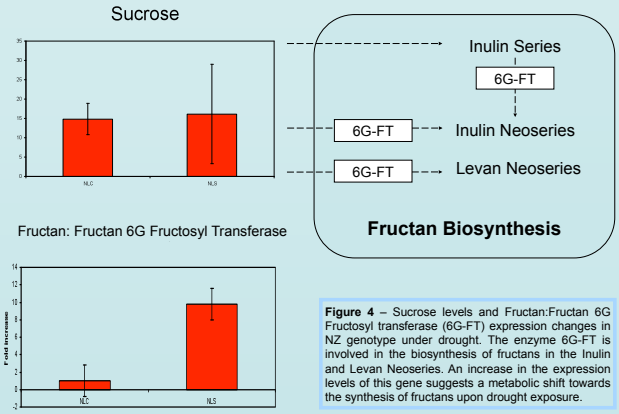


**Figure 4** – Sucrose levels and Fructan:Fructan 6G Fructosyl transferase (6G-FT) expression changes in NZ genotype under drought. The enzyme 6G-FT is involved in the biosynthesis of fructans in the Inulin and Levan Neoseries. An increase in the expression levels of this gene suggests a metabolic shift towards the synthesis of fructans upon drought exposure.

## Conclusions

The two plant lines used behaved differently when exposed to a PEG induced drought. The line documented as drought tolerant maintained its water content in the leaves whereas the commercial breeding line decreased its relative water content significantly. Besides differences in relative water content, the plants revealed a change in their metabolic profile suggesting that drought stress has triggered a metabolic response in order to maintain homeostasis. Analysis of the metabolite changes following drought exposure suggested a possible role of sugars (such as trehalose, raffinose, among others) and also secondary metabolites (such as stigmasterol and campesterol) in drought tolerance. Differences in the profile of fatty acids, fatty alcohols and also alkanes (data not shown) have been observed and may also account for enhanced drought tolerance.

## Acknowledgements

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## References

Shepherd, T *et al.* - Metabolomics, Vol. 3, No. 4, 475-488 (2007)