Investigating the metabolomic response of *Lolium perenne* to a PEG induced drought stress.

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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 study was conducted to investigate the genetic basis of phenotypic and metabolic plasticity to drought for a set of perennial ryegrass lines.

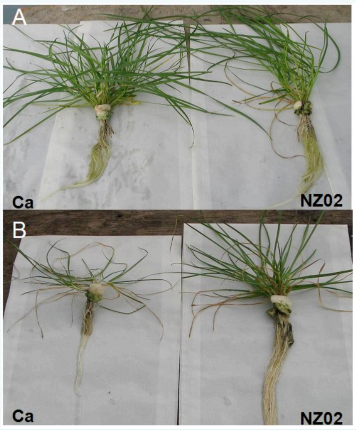

Figure 1 – Visual differences of 'Ca' and 'NZ02' when submitted to PEG induced drought. **A** represents both lines under control conditions after 1 week. **B** represents both lines grown in medium with 15% PEG 6000. **'NZ02'** drought tolerant line **'Ca'** commercial

Plant Material

Lolium perenne ecotype 'NZ02' and a commercial variety 'Ca' were grown in a hydroponics system supplied with either 1x MS medium or MS media containing 15% PEG6000 to induce osmostic stress over a period of 1 week. 'NZ02' was obtained from the GRIN (USDA) seed bank collection and has been documented as having drought tolerance.

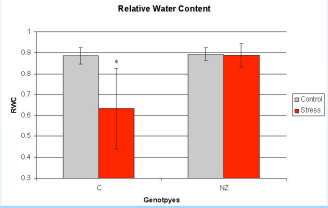

Figure 2 – Relative water content (%) measured after one week of growth in hydroponics, which corresponds to time of sampling for metabolomic analysis. * difference significant at $P<0.05$. n=6.

Materials and Methods

Electrolyte leakage and relative water content of leaves were measured and samples of both leaf and root tissue were collected. In order to quench the metabolism, collected 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 [1]. The polar and non-polar samples were analyzed similarly using a Thermo Finnigan Tempus GC–(TOF)–MS system using the XcaliburTM software package for data acquisition. Statistical analysis was performed using GenStat version 9.2.0.153.

Results

After one week of induced stress it was visually possible to distinguish differences between the two lines in the response to the different treatments (Fig 1). Root biomass was strongly increased in the 'NZ02' line compared to the 'Ca' line. This evidence along with measurements of relative water content performed (Fig.2) suggested that the lines selected have a differential tolerance to drought.

A principal component analysis (PCA) of the non-polar samples (Fig. 3) revealed a clear clustering 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.

The multivariate analysis of the polar samples has not revealed such a noticeable separation of clusters between tissue types among the two lines (Fig. 4), however the 'NZ02' line when submitted to stress appears to cluster further away from the commercial line 'Ca' under the same conditions.

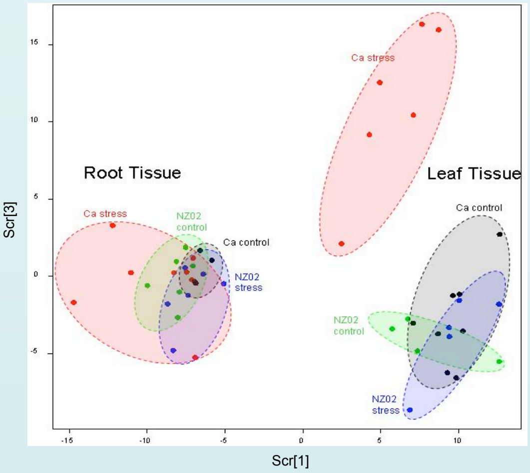

Figure 3 – Principal component analysis (PCA) plot of the non-polar extracts. Components 1 and 3 explained up to 28% and 11% of the variability, respectively.

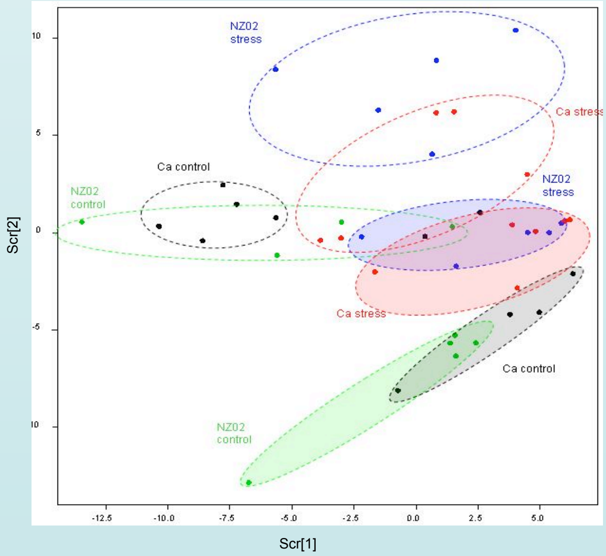

Figure 4 – Principal component analysis (PCA) plot of the polar extracts. Components 1 and 2 explained up to 19% and 16% of the variability, respectively. Filled clusters represent root tissue while blank clusters represent leaf tissue.

Conclusions

In this study it was possible to observe that the two lines used behaved differently when submitted to a PEG induced drought. The line documented as drought tolerant maintained its water content in the leaves where 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 its homeostasis. Further studies will be carried on to identify the metabolites driving these different responses as well as the mechanisms underlying the plant drought stress response.

Acknowledgements

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References

Shepherd, T; Dobson, G; Verrall, SR; Conner, S; Griffiths, DW; McNicol, JW; Davies, HV; Stewart, D -Metabolomics, Vol. 3, No. 4, 475-488 (2007)