Changes in gene expression during acclimation to cold temperatures in white clover (*Trifolium repens* L.)

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Keywords: abiotic stress, acclimation, cDNA-AFLP

Introduction White clover is an important component of many temperate pastures and improved winter hardiness is a major objective of breeding programmes in many countries. Exposure to cold and fluctuations in temperature are components of winter stress and although some studies have investigated the agronomic and physiological mechanisms of cold tolerance, little research has been carried out to identify the genes involved. We are complementing mapping of quantitative trait loci (QTL) responsible for cold tolerance with studies of variation in gene expression between plants growing at different temperatures. In particular we are initially focusing on the process of acclimation by analysing plants subjected to low but above zero temperatures.

Materials and methods Three sets of 25 plants each of the variety Olwen were used. The first set (A) was kept at 2C for 24days before RNA was extracted. The second set (B) was kept at 2C for 24days then at a regime of 20C day/10C night with a 12hr day length for 14days. The final set (C) was kept at 20C/10C day/night for 38days with a 12hr day length before RNA was extracted. RNA from leaf and stolon tissue was separately extracted from all plants using TRIzol ™ from Invitrogen. The purification process was carried out according to the manufacturer's instructions (RNAse free kit from Ambion ™). Comparisons of gene expression were made using the cDNA-AFLP technique. Initial comparisons were carried out on RNA from each treatment separately bulked from leaf and stolons. To confirm the pattern of polymorphisms between plants from the different treatments subsequent analysis with the same primers was carried out on individual genotypes, again comparing stolon and leaf samples separately.

Results A large number of bands were obtained in this analysis. For the bulked leaf samples, for instance, more than 100 bands from six primer combinations were seen. Similar numbers were observed for all tissue/ treatment combinations. Polymorphisms were observed for both tissue types between all treatments. These represented a small proportion of the total, facilitating further analysis. Bands present in the bulks were present in individuals and polymorphisms observed in the bulks are now being identified in the individual plants. Bands which were observed in Treatment A only are being sequenced and already some interesting similarities have been observed e .g. with a vernalisation control gene in *Arabidopsis thaliana*.

Discussion Despite its preliminary nature, this work has demonstrated the utility of cDNA-AFLP to identify changes in gene expression, consistent across a range of genotypes, during acclimation. In particular, the bands scored in the bulks represented only approx. 10% of those present when all individuals were analysed separately. Thus polymorphisms identified in bulks represented a suitable means of reducing the overall differences to a more manageable number which could subsequently be confirmed on individual genotypes and sequenced. This work is being extended in a number of directions. Clones of the genotypes used have been subjected to a test of survival under sub-zero condition and these results are being related to changes in gene expression during acclimation. Fatty acid profiles, implicated in cold tolerance in a range of species, are also being analysed in the same plants. Candidate genes identified after further analysis will be tested for co-location to QTL for cold tolerance. We will also confirm differences in expression by RT-PCR and carry out, in conjunction with the laboratory of G. Spangenberg, PBC, Victoria, Australia, microarray analysis to complement the results obtained by cDNA-AFLP.

Acknowledgements
We are grateful for the financial support of the Biotechnological and Biological Sciences Research Council and the Department for Environment, Food and Rural Affairs.

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