Quantifying the Variation in Protein Content in White Clover (*Trifolium Repens* L.)

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Introduction White clover (*Trifolium repens* L.) is the main legume in temperate pastures. It has relatively low levels of water-soluble carbohydrate but produces forage of high quality with a high crude protein (CP) content and dry-matter digestibility (Beever, 1993). Some studies have suggested that the forage quality of white clover can be problematic because its high CP content may contribute to inefficient use of nitrogen in the rumen and exacerbate diffuse pollution via excreta (Waghorn & Caradus, 1994). The development of white clover germplasm with lower CP content would potentially benefit forage production and grassland management. A study was carried out to quantify the variation in CP content within an existing gene pool and develop high throughput techniques for protein determination appropriate to a plant breeding programme.

Materials and methods A medium and large leaf gene pool comprising 1000 genotypes (50 plants each of 10 medium and 10 large leaved varieties) grown as spaced plants in the field were sampled for protein content in summer 2003. The youngest fully expanded trifoliate leaf with petiole on three primary stolons per plant was sampled from each plant at peak flowering. Chlorophyll content of the middle trifoliate leaf was also measured using a SPAD meter. Protein content was measured using a modified version of the assay described by Bradford (1976), used by Kingston-Smith et al., 2003 and chlorophyll content by spectrophotometery. A sub-sample of 48 plants, across the range of measured protein content, was identified for further study. A clone of these plants was produced by removing a stolon core which was planted into pots in an unheated glasshouse. In summer 2004 protein content was measured on this subset of plants in the glasshouse and field as previously described.

Results Protein contents (mg/g fresh weight) in the medium leaf and large leaf gene pools were 0.48-8.09 and 0.29-4.99, respectively. There was no significant correlation between protein content and the chlorophyll content derived by the SPAD meter, or by assay, for either the medium or large leaf gene pools. The mean protein content of the sub-sample plants in the field did not differ significantly from that of the plants grown in the glasshouse (2.81 (range 0.92-8.53) vs. 2.97 (range 0.16-6.46) respectively). The correlation between the protein content of plants in the field and the cloned plants grown in the glasshouse was low and not significant. Similarly the correlation between the field and the glasshouse of the ranking of genotypes on the basis of their protein content was not significant (r=0.06; ns).

Conclusions Foliar protein content was lower than previously reported for white clover (Kingston-Smith et al., 2003), perhaps due to the fact that petioles were included in the assay. Although protein content varied widely among the medium and large leaf gene pools in the field, the non-significant correlation between protein and chlorophyll content confirmed the SPAD to be an inappropriate way to measure protein content. Although the wide range of variation in protein content between genotypes in the field was also observed in the glasshouse, there was no significant correlation between the protein content in the field and in the glasshouse. Similarly the correlation between the ranking of the genotypes in terms of their protein content in the glasshouse and field was not significant. This suggests that individual genotypes differed in their response to environment in terms of plant protein content, making identification of plants with consistently high or low protein content difficult to select. It also suggests that the environment where screening takes place is important and should be selected to represent the environments where white clover will be utilised if meaningful results are to be obtained. Further work is necessary, in terms of sampling plant material and in replication, if this technique is to provide reliable information on plant protein content that can be used in a plant breeding programme.

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