Dryland clovers: a phytochemical resource

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Introduction Recent developments in the utilisation of phytoestrogens of red clover (Wuttke *et al*., 2002) have encouraged us to investigate a wider range of *Trifolium* species for metabolites which could provide new product opportunities. The phytochemistry of the agronomically-important *Trifolium* species white (*Trifolium repens)* and red (*T. pratense*) clovers has been investigated in some detail (Foo *et al*., 2000; Sivakumaran *et al*., 2004). However numerous other clover species have been neglected in agriculture due to agronomic issues or the fact they are annuals and require more intensive management. While some of these clover species have been studied for their genetic diversity (Marshall *et al*., 2002), investigations of the chemical composition of these specific species has not been reported.

Materials and methods Alsike (*T. hybridium*), ball (*T. nigrescens*), balansa (*T. michelianum*), Caucasian (*T. ambiguum*), strawberry (*T. fragiferum*) and subterranean (*T. subterraneum*) clover plants were grown at Grasslands Research Centre or Lincoln University. Aqueous acetone (3:7 v/v) extraction of leaves and flowers was performed on frozen plant material. The Sephadex LH-20 purified proanthocyanidin polymers of flowers were studied by acid catalyzed degradation with benzyl mercaptan and mass spectrometry. Flavanoid metabolites were identified from purified LH-20 fractions by analysis with liquid chromatography (LC)-ion trap mass spectrometry (MS).

Results and conclusions Analysis of proanthocyanidin polymers by thiolytic cleavage provides evidence of the identity of the terminal and extender units that make up the proanthocyanidin polymer, information on the mean degree of polymerisation (mDP), and the procyanidin to prodelphinidin unit ratio. Negative ion electrospray ionisation (ESI) and positive ion matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry provides evidence of the compositional dispersion (range of DP) for oligomeric proanthocyanidin components. Methanolic fractions from LH-20 chromatography yielded a range of flavanoids and flavanoid glycosides, with some apparent contrasts between the clover species studied as determined by LC-MS.

The white clover floral prodelphinidins (Foo *et al*., 2000) consist of terminal and extender units with nearly equal proportions of epigallocatechin and gallocatechin. The red clover floral procyanidins (Sivakumaran *et al*., 2004) consist of extension units with epicatechin as the abundant flavan-3-ol and the terminating units dominated by catechin. The dramatic difference in the stereochemistry of the terminal and extender units observed for the red clover floral procyanidins contrasts with the mixture of *cis* and *trans* stereochemistry observed for white clover floral prodelphinidins. Other floral clover proanthocyanidin polymers were determined to be composed of either prodelphinidins or procyanidins exclusively, or possibly a mixture. The results of this study will present the biosynthetic trends for floral proanthocyandins from a range of clover species.

The LC-MS results indicate there is diversity amongst the quercetin, kaempferol and isorhamnetin derivatives extracted from the leaves of these dryland clovers. The targeted identification of clover metabolites such as the red clover isoflavones, could lead to future product opportunities.

References

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