Application of Molecular Markers Derived from *Medicago Truncatula* in White Clover (*Trifolium Repens* L.)

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Introduction White clover is the major forage legume of temperate areas. Genome maps have been produced recently (Jones *et al*., 2003; Barrett *et al*., 2004) and the location of QTL for important agricultural traits reported (Abberton *et al*., 2004). White clover is closely related to the model legume *Medicago truncatula* and there is likely to be considerable benefit in applying genomic resources from model to crop. However, the extent of synteny between the species must be established. Here we present preliminary results detailing progress towards this goal.

Materials and methods An F_1 mapping family of white clover has been developed. Parents were derived from material that was produced from three generations of divergent selection for stolon characteristics, variation in which largely governs persistence. The primary traits selected were stolon profuseness and thickness. Molecular markers derived from *M. truncatula* were applied to this F_1 mapping family. Simple sequence repeat (SSR) markers were used from expressed sequence tags (ESTs) of *M. truncatula* based on information kindly supplied by T. Huguet, INRA, France. DNA was extracted from 350 plants of the mapping family including the two parents using either a modified 2x CTAB or Qiagen DNA extraction kit. A total of 205 primer pairs were used with the following amplification conditions: a 25 µl reaction volume using an ABI GeneAmp PCR System 9700 of 50ng DNA, 1x buffer with 1.5µM $MgCl_2$ (Roche), 80µM dNTP, 50pM primers and 0.4U Taq DNA polymerase (Roche), initial denaturation 94°C, 5 min, denaturing step 94°C, 1 min, 55°C annealing for 1 min, 72°C elongation 1 min, and a final elongation step of 72°C.

Results 23 polymorphic primers (see Table 1) were re-assessed on polyacrylamide gels and 17 were chosen for further analysis. The mapping population was screened with each of these primers on an ABI 3100 Genetic Analyser. The primers detected from two to ten peaks in the white clover DNA, however the molecular weights were not always exactly those observed in *M. truncatula*.

Table 1 Structure of SSRs from *M. truncatula*, their amplification and the extent of polymorphism in the white clover F_1 mapping family.

Structure	Number	Efficient amplification (%[a])	Polymorphic primer pairs (%[b])
Perfect	189	169 (82)	22 (12)
Compound	12	10 (5)	1 (0.5)
Imperfect	1	1 (0.5)	0 (0)
TOTAL	202	180 (89)	23 (13)

[a] total number of primer pairs. [b] primer pairs showing efficient amplification.

Discussion Markers developed in *M. truncatula* are able to amplify products in white clover, showing a degree of conservation of microsatellite flanking sequences between the two species. The proportion of primers showing amplification was high, although polymorphism in this family was limited. The SSR data are now being supplemented by twenty AFLP markers. Alongside the molecular work, a range of characteristics were measured on each plant of the mapping family including thickness, length and number of stolons and other agronomically important traits. These measurements will be combined with the molecular data to identify the number and location of QTL for key stolon morphology traits. The framework map will also allow preliminary observations on the degree of synteny between white clover and *M. truncatula*.

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