Development and Validation of Molecular Markers for *Phytophthora medicaginis* Resistance in Lucerne

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Development and validation of molecular markers for *Phytophthora medicaginis* resistance in lucerne

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Abstract. Resistance to *Phytophthora medicaginis* is an essential attribute to incorporate into lucerne (*Medicago sativa*) cultivars which are likely to be grown on heavy soils or in conditions where the soil remains excessively wet for prolonged periods. Current breeding strategies rely on recurrent phenotypic selection to maintain adequate levels of resistance in newly developed synthetic cultivars. However, little is known about the source or mechanism(s) of genetic resistance operating in the cultivar. A genetic linkage map was generated from a tetraploid *M. sativa* population using SSR markers anchored to existing genetic and physical maps. Large effect QTL were identified on linkage groups 2, 5, 6 and 7, each of which contributed between 11-30% of the phenotypic variation. Evaluation of the marker-trait associations in another sampling of the same population was undertaken, using a different isolate of *P. medicaginis*. The findings indicate that in the lucerne genotype examined in this study, a network of interactions involving at least three common loci, contribute to resistance to *P. medicaginis*. An alignment of the resistance loci identified in this study with those previously identified provided a framework for cataloguing the diversity of resistance loci present in lucerne, and will be used to guide future lucerne breeding efforts.

Keywords: Alfalfa, multi gene resistance.

Introduction

Cultivated alfalfa (lucerne) is autotetraploid (2n = 4x = 32), and belongs to the *Medicago sativa* complex, which includes subspecies *sativa* and *falcata*. The biology of lucerne has up to now necessitated its commercialisation as broadly based synthetics derived from about 100 S_0 clones. Lucerne cultivars are generally a heterogeneous mixture of genetically distinct, out crossing individuals produced by random mating of a large number of genetically diverse parent plants (genotypes) (Busbice 1969; Stanford 1951). Thus, each cultivar usually consists of a diverse population of individuals, each differing in its proportion of plants resistant to each individual disease. Improving disease resistance levels through recurrent phenotypic selection is routinely undertaken, however when working on a population basis, it is possible that this occurs in the absence of an understanding of the genetic basis of the resistance mechanism(s) operating in the breeding material.

It is likely that in the pursuit of higher productivity, the number of S_0 parents used in breeding synthetic varieties will be reduced from around 100 to 4-16. This will lead to a greater chance of new pathotypes evolving, unless steps are taken to deploy a diversity of resistance mechanisms against individual high risk pathogens. This can only be achieved when genetic mechanisms conferring resistance to a pathogen have been identified including their chromosomal locations, allowing deployment of multiple resistances even in a narrowly based synthetic.

An important attribute to incorporate into lucerne cultivar development is resistance to the soilborne pathogen *Phytophthora medicaginis*. Under conditions favouring disease development, significant plant losses and reductions in yield potential of up to 100% can occur. The expression of resistance to *P. medicaginis* in lucerne is complex. At least six independent loci conferring resistance to *P. medicaginis* have been identified through inheritance studies involving individual plants (genotypes) across different *M. sativa* genetic backgrounds (Havey *et al.* 1987; Irwin *et al.* 1981a, 1981b). Inheritance of resistance in this material is dependent on the host genetics, with both dominant and complementary, incompletely dominant loci identified. Gene dosage was important in one genetic background, with resistance expressed only when two loci were present at a dosage of at least simplex at one loci, and duplex at the second. Despite the identification of numerous loci with varying degrees of dominance, and quantitative loci conferring resistance to *P. medicaginis* in a further study, the genomic location of these loci and identity of the underlying genes remains unresolved.

This work sought to determine the genetic basis for resistance to *P. medicaginis* in an autotetraploid lucerne population segregating for *P. medicaginis* resistance, and for the first time to ascertain the genomic location of resistance loci using molecular markers of known chromosomal location.

Methods

A tetraploid *M. sativa* intercross population of 182 individuals was generated from the cross WA272 x D, which are *P. medicaginis* resistant and susceptible genotypes respectively. Phenotypic characterisation was undertaken across multiple infection courts with established *P. medicaginis* inoculation methods involveing both seedling-based inoculation of cotyledons and soil infestation of clonal propagules of each individual. Phenotypes were obtained for cotyledon reaction, root reaction, and root disease severity assessments.

The intercross population, and parental genotypes, were genotyped using microsatellite markers obtained from existing *M. sativa* genetic linkage maps, and *M. truncatula* genetic and physical maps. A genetic linkage map was developed for each parental genotype, using TetraploidMap. Marker-trait associations and composite interval mapping analyses where marker density permitted were used to identify QTL associated with *P. medicaginis* resistance in WA272.

Evaluation of the marker-trait associations identified in the mapping population was undertaken on a second sampling of the same tetraploid intercross population (WA272 x D) using the cotyledon inoculation method with a new *P. medicaginis* isolate.

Results and Discussion

Phenotypic characterisation data for *P. medicaginis* reaction in the tetraploid WA272 x D F_1 population was generated across root, cotyledon and stem infection courts. This is the first time that these phenotyping methods involving different infection courts have been studied on the same individuals in a segregating population. The segregation data (at $P>0.05$) fitted a single gene model (cotyledon reaction phenotype), and either a single gene or a 2 complementary, independently segregating gene model (root reaction phenotype). However a larger F_1 population size than the one developed would be necessary to critically test these and other multi-gene models. A combination of the segregation data and the molecular marker data described below were used to further elucidate the genetic basis for the inheritance of resistance to *P. medicaginis*

Analysis of the molecular marker data identified four interacting, large-effect QTL for resistance to *P. medicaginis* on WA272 linkage groups 2, 5, 6 and 7 (Table 1). This work has identified for the first time, the genomic location of resistance loci to *P. medicaginis* in lucerne. Data generated included single marker-trait associations, the generation of a composite genetic linkage map for WA272 and D, QTL analyses, and interaction analyses. The location of the QTLs identified on linkage groups 2, 5, 6 and 7 indicate that at least the same co-segregation groups, if not the same region on each of these contribute large effect QTL for resistance to *P. medicaginis* in WA272 in both cotyledon and root infection courts, with significant interactions detected between loci.

The identification of large effect QTLs for resistance in WA272, and interactions between these resistance loci

Table 1. Percentage of the phenotypic variation for resistance in WA272 explained by the largest single marker from the four major loci identified

Linkage group	Plant reaction phenotype		
	Cotyledon[a]	Root reaction[b]	Root severity[b]
2	19	17	22
5	23	11	15
6	21	9	14
7	11	7	7

[a] Reaction of lucerne cotyledons to inoculation with a 10µl drop of *P. medicaginis* zoospores; [b] Reaction of root system of clonal lucerne propagules to soil infestation with *P. medicaginis* mycelia

is an important finding for breeding for *Phytophthora* resistance. These findings strongly support previous work in this field, where in the absence of DNA-based molecular data, inheritance studies determined *Phytophthora* resistance in diploid *M. sativa* subsp. *sativa* and subsp. *falcata* genotypes was under the control of up to six independently segregating loci across the genetic backgrounds investigated (Havey *et al.* 1987). In the current study with tetraploid *M. sativa*, we identified four major loci on linkage groups 2, 5, 6 and 7 contributing resistance to *P. medicaginis* in the WA272 x D mapping population. Three of these loci, on linkage groups 2, 5 and 6, contributed to resistance when another population from the WA272 x D background was assessed for *P. medicaginis* reaction, indicating that common regions on linkage groups 2, 5 and 6 appear to contribute to resistance in WA272. A preliminary alignment of the location of the resistance loci identified in this study with those from the previously developed W116 x D map using common microsatellite markers (data not shown), indicates that with the exception of linkage group 7, separate loci confer resistance to *P. medicaginis* in the WA272 and W116 lucerne backgrounds.

The use of microsatellite markers of known chromosomal location from *M. truncatula* genetic and physical maps allowed the alignment of the resistance loci identified in this study with those identified in *M. truncatula*. *M. truncatula* and *M. sativa* share common chromosomal regions on linkage groups 2 and 6 for resistance to *P. medicaginis*. However, the involvement of additional loci and the identification of significant interactions between resistance loci in *M. sativa* highlights the additional complexity present in an outbreeding tetraploid relative to that observed in the inbreeding diploid *M. truncatula*.

The resistance loci on WA272 linkage groups 2, 5, 6 and 7 are of considerable interest for further research due either to their association with resistance across multiple *P. medicaginis* isolates in the same genetic background, or across different *Medicago* genetic backgrounds. By anchoring the W116 and WA272 *M. sativa* linkage maps to the *M. truncatula* physical map, this research will allow increased utilisation in future research of the genomics resources available from the sequenced and annotated *M. truncatula* genome to investigate the gene space underlying the QTL for *P. medicaginis* resistance in lucerne and the development of functional markers for

lucerne breeding.

There was marker saturation in this *M. sativa* linkage map to identify markers suitable for further evaluation in additional lucerne genotypes. Furthermore, an increased density of markers derived from the *M. truncatula* physical map in this *M. sativa* linkage map would allow investigation of the corresponding region in *M. truncatula* for candidate gene identification and functional marker development.

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