Integration of EST-SSR markers of *Medicago truncatula* to intraspecific linkage map of lentil and identification of QTL conferring resistance to ascochyta blight at seedling and pod stages

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Summary:

Microsatellite markers have been extensively utilised in the leguminosae for genome mapping and identifying major loci governing traits of interest for eventual marker assisted selection (MAS). The lack of available lentil-specific microsatellite sequences instigated the mining and transfer of EST-SSR/SSR sequences from the model genome *Medicago truncatula*, to enrich the existing intraspecific lentil linkage map. A total of 196 markers, including 15 Mt EST-SSR/SSR, were mapped using a population of 94F₅ RIL produced from the cross cv. Northfield (ILL5588) x cv. Digger (ILL5722) and clustered into 11 linkage groups (LG) covering 1156.4 cM. Subsequently, the size and effects of QTL conditioning *Ascochyta lentis* resistance at seedling and podding/maturity stages were characterised and compared. Three QTL were detected for seedling resistance (LG1 and LG9) and a further three were detected for pod/maturity resistance (LG1, LG4 and LG5). Together, these accounted for 34% and 61% of the total estimated phenotypic variation, respectively and demonstrated that resistance at the different growth stages is potentially conditioned by different genomic regions. Several of the closest flanking markers may be useful for MAS and for the future pyramiding of potentially different resistance genes into elite backgrounds that are resistant throughout the cropping season.

Key words: ascochyta blight, EST-SSR/SSR, lentil, linkage map, *Medicago truncatula*, QTL

Introduction

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an important cool-season annual food legume crop grown from the Near East to the Mediterranean area, and in Asia, Europe, USA, Canada and Australia. The world lentil production was 1.86 million tonnes (Mt) during 2008-2009 (Market Outlook Report, 2010). India ranks first in lentil production and consumption, whereas Canada and Turkey are the world's largest lentil exporters.

A major constraint affecting lentil production is the incidence of diseases and especially ascochyta blight, caused by the ascomycete *Ascochyta lentis*, a serious fungal disease affecting all lentil growing regions of the world. Seedling infection causes stem girdling and plant death, whereas infection at podding and maturity affects seed quality and finally yield (Morrall and Sheppard 1981). The most effective and economical way to manage this disease is to cultivate sustainably resistant cultivars, a major aim of many breeding programs.

Resistance is reported to be governed by genes with major effects (Ford et al. 1999; Nguyen et al. 2001; Ye et al. 2001; 2002), which can be deployed in combinations as multiple gene barriers (Parlevliet 1993). Ye et al. (2001) reported two recessive genes with additive effect and a single recessive gene control foliar resistance in cvs Indianhead and Laird, respectively. Foliar resistance in cv. Northfield (ILL 5588) was reported to be governed by a single dominant gene (Ford et al. 1999); or by one dominant gene for high resistance and one dominant gene for moderate resistance (Ye et al. 2001). Ali (1995) found that ILL5588 was resistant to both seed and foliar infection. These studies suggested that major genes are involved in resistance, however, different levels of resistance at different growth stages have been reported, which may affect the sustainability of the resistance trait throughout the growing season. For example, seedling resistance in Laird was reported to break down during the late podding stage (Gossen and Morrall 1983), Pedersen and Morrall (1994) also reported difference in disease severity among seedling and podding stage. To aid in determining if the resistance

mechanisms at different growth stages within the same genotype are conserved or different, a trait mapping approach is appropriate. Locating the position of major quantitative resistance effects using genome linkage mapping would also enable the potential to pyramid multiple different resistance genes into one superior agronomic background. The use of molecular markers linked to the major different resistance quantitative trait loci (QTL) would improve efficiency and speed of conventional resistance breeding strategies.

In order to identify molecular markers significantly associated with resistance to *A. lentis*, several genomic maps have been developed (Havey and Muehlbauer 1989; Eujayl et al. 1998; Rubeena et al. 2003; Hamwieh et al. 2005; Tullu et al. 2008; Tanyolac et al. 2010). Although several major genomic regions controlling seedling resistance to *A. lentis* have been reported (Ford et al. 1999; Chowdhury et al. 2001; Ye et al. 2001; 2002; Rubeena et al. 2003), those governing resistance at the podding/maturity stage have not been identified. Also, the majority of genome maps were created with mostly arbitrary and dominant RAPD, AFLP and ISSR markers (Eujayl et al. 1997; 1998; Rubeena et al. 2003; Duran et al. 2004; Hamwieh et al. 2005). Nevertheless, the first generation maps served as foundations upon which to build denser maps with greater ultimate application.

A relatively saturated linkage map is required so that a QTL mapping approach can be employed to help determine if separate or common genomic location(s) condition *A. lentis* resistance in the same genomic background at the different crucial developmental stages of seedling and maturity/podding. Once the major QTL have been uncovered, tightly linked markers may be validated for use in marker assisted selection and potentially even as a starting point for the positional cloning of the underlying functional resistance gene(s) (Haley and Andersson 1997). In recent years, considerable progress has been made for positional cloning of several QTL in major crops (Salvi et al 2002; Lui et al. 2008; Fu et al. 2009), however, in legumes most of the positional cloning work has been done with *Medicago truncatula* and *Japonica lotus* where several genetic loci responsible for

symbiosis have been characterized (Stracke et al. 2002; Ane et al. 2004; Zhang et al. 2010).

The number of characterised lentil-specific and co-dominant markers is relatively low and the dearth of lentil-specific genomic information has meant that resources from related species have been used. The potential to transfer genome sequences from the model temperate legume *Medicago truncatula* to lentil, with clear evidence of conserved genome orders, was demonstrated by Phan et al. (2007). In particular, intron-targeted amplified polymorphic (ITAP) markers were used together with SSR markers to produce a comprehensive lentil genome map. The map was produced using cv. Northfield x cv. Digger F₅ RIL and other dominant and co-dominant markers. However, this map remains incomplete with marker intervals >25 cM and larger than 2n=14 representative chromosomal linkage groups. Transfer of EST-SSR/SSR markers from *M. truncatula* would provide further saturation and with markers of functional association since their transferability relies on conservation in the exon regions.

EST-associated and sole SSR primer sequences have been identified from the international genomic sequencing program for *M. truncatula*, for which the transcript database contains more than 230,000 EST (<http://www.medicago.org/genome/>). Therefore, the aims of the study were to 1) enrich the current map (Phan et al. 2007) through transfer of *M. truncatula* (Mt)-originating EST-SSR and sole SSR sequences; 2) identify the genome locations of the major QTL conditioning both seedling and maturity/podding resistance and 3) identify markers close and flanking the individual QTL that could be used for future validation for the pyramiding of differential genes/QTL towards sustainable resistance.

Materials and Methods

Plant and fungal materials and bioassays

The RIL population of 94 F₅ individuals, developed from a cross of cv. Northfield (ILL 5588, resistant) x cv. Digger (ILL 5722, susceptible), and the parental seed

were provided by Dr Michael Materne of the Victorian Department of Primary Industries (VicDPI), Horsham, Victoria, Australia.

The *Ascochyta lentis* AL4 isolate was originally sourced from the VicDPI Horsham disease nursery in 1996, stored freeze dried and passaged through the susceptible host genotype ILL6002 prior to use. For disease bioassays, parental and F₅ RIL seedlings were raised in 20 cm diameter pots. These were sown at a density of five plants/pot per RIL or parental genotype with a single replication per bioassay of each RIL and triplicate pots per bioassay of each parent. All plants were raised in a growth room maintained at $20 \pm 2^{\circ}\text{C}$ with a 16 hr day length.

Seedling bioassays were conducted and disease score data was collected on a 1-9 scale as previously described (Ford et al. 1997) at 14, 21 and 28 days after inoculation (DAI). For maturity/podding stage bioassays, All plants were inoculated (*A. lentis* or water) using a hand pump until run-off and pots were covered with sealed plastic sheets to maintain humidity and left in the dark for 48 hours to induce optimal spore germination and infection. Then, the plastic sheets were removed and plants were left to grow under artificial epiphytotic conditions. At 14, 21 and 28 DAI the tagged pods were assessed for percentage of total pod infection (Moore et al. 2002).

Genomic DNA extraction, PCR and transferability of SSR primers from *Medicago truncatula*

Total genomic DNA was extracted from approximately 200 mg of young leaf tissue of each individual using the CTAB method described in Ford et al. (1997). Genomic DNA was quantified with a spectrophotometer and the quality was assessed on agarose gel (1.8%), stained with ethidium bromide and visualised under uv-light. DNA was diluted to a working concentration of 20ng/μl for PCR analysis.

To saturate the existing linkage map, ten EST-SSR primer pairs from Eujayl et al. (2004) and 90 EST-SSR/SSR primer pairs from the *Medicago truncatula* sequencing project (http://www.medicago.org/genome/genetic_map_table.html) were used. Of the 100 *M. truncatula* primer pairs, 46 were EST-derived SSR and 54 were sole SSR sequences, these were screened for their transferability and

amplification in cv. Northfield. Primer pairs that amplified a clear and reproducible product were then assessed for size polymorphism among the parental accessions and across the entire RIL mapping population.

The PCR reaction (25 µl volume) was conducted in a MyCycler Personal Thermal Cycler (Bio-Rad, Australia) and consisted of PCR buffer (Scientifix, Australia), 3mM MgCl₂ (Scientifix, Australia), 0.24 mM of each dNTP (Scientifix, Australia), 0.24 µM of each primer (Sigma, Australia), 0.5 units of *Taq* polymerase (Scientifix, Australia) and 20 ng of template DNA. The PCR conditions were as follows: Initial denaturation at 94 °C for 5 min, followed by 39 cycles at 94 °C for 15 s, annealing at 50-62 °C for 30 s (dependant on the primer T_m), extension at 72 °C for 30 s and final extension at 72 °C for 5 min. Amplification products were resolved on 2% agarose or Metaphor (2.5%) gel and visualised against known molecular weight standards (Lambda DNA - Hind III digested, Invitrogen, Australia) as described above.

Construction of genetic map

Segregating markers were scored as co-dominant or dominant and the data were added to the existing dataset and used to construct a revised cv. Northfield x cv. Digger genome map. Locus and allele-specific marker data were supplied for the existing map that was produced using the same RIL and parental genomes (Phan et al. 2007). The final map comprised four types of molecular markers; ISSR, RAPD, SSR and ITAP markers. Goodness-of-fit to the expected 1:1 segregation ratio for marker segregation within an RIL population was tested by χ^2 analysis ($P < 0.05$) and the map was constructed using Map Manager QTX (Manly et al. 2001). For this, markers were assigned to linkage groups using the 'make linkage groups' command at a LOD score of 3.0 and a maximum recombination fraction (θ) of 0.5. Kosambi's function was applied to convert recombination percentages to centiMorgan (cM) map unit distance units (Kosambi 1944).

Marker nomenclature

In the existing map, markers were named as follows: RAPD - capital letter with two digit number; ISSR - 'UBC' with three digit number, SSR - 'ILM' with two digit number; lentil SSRs - 'SSR' with two and three digit number for those developed by Hamwieh *et al.* (2005) and ITAP - the name as previously presented in Phan *et al.* (2007). The 21 newly mapped EST-SSR/SSR markers from *M. truncatula* were named using their original names with the last three digits only. Primer pairs with more than one marker size were named by the primer name with a lower case letter (a, b or c).

QTL analysis

No prior assumptions of phenotypic classes were made regarding the raw disease scores of the F₅ RIL and parents at 14, 21 and 28 DAI. Initially, single point analysis was performed on all markers using the 'Links Report' command to determine any markers significantly associated with the resistance traits ($p < 0.05$). Subsequently, simple interval mapping was conducted to detect QTL effects within marker intervals with a likelihood of odds ratio of 3.0, and without controlling for the effects of other/background QTL. Empirical determination of experiment-wise error rates was performed with 1000 permutation tests to provide a 95% confidence on the location of QTL within marker intervals (Churchill and Doerge 1994). The effect at each marker locus was estimated by calculating the difference between the total trait variance and the residual variance, expressed as a percentage of the total variance, and was used to estimate the percentage of phenotypic variation explained. Multiple QTL peaks detected within 10 cM regions were regarded as a single QTL.

Results

SSR marker transferability and map revision

Three of the 10 EST-SSR primer pairs from Eujayl *et al.* (2004), and 88 (35 EST-SSR and 53 sole SSR) of the 90 primer pairs obtained from the *M. truncatula* sequencing project amplified a clear and reproducible product of the approximate expected size in cv. Northfield. However, only three of the 38 EST-SSR markers

(7.9%) and 14 from 53 SSR markers (26.4%) were polymorphic among the parental genomes (Table 1). From these, 21 clear and reproducible markers were produced for addition to the existing linkage map (Table 2).

Table 1. Summary of usefulness of *M. truncatula* EST-SSR/SSR markers used for the saturation of the existing lentil linkage map (cv. Northfield x cv. Digger).

Marker type	No. of primers screened	No. of primers showing transferability	No. of polymorphic primers	No. of polymorphic Markers	No. of mapped markers
EST-SSRs	46	38	3 (7.9%)	3 (1 marker/primer)	3 (100%)
SSRs	54	53	14 (26.4%)	18 (1.3 markers/primer)	12 (66.7%)
Total	100	91	17	21	15

Table 2: List of polymorphic primers between parents cv. Northfield and cv. Digger

S. No.	Primer Name	Type of marker	Forward primer sequence	Reverse primer Sequence	Expected band Size	Annealing temperature
1	BE325495	EST-SSR	CAGCCACATTTTG CTGTAAAGA	AGTAACCTTTGA CCCCAGCAT	300-330	50.2
2	BE323614	EST-SSR	GCACCAGGAATAA TCCAATAACA	AGCCGTCCAGTA CCTTTGAC	350	58.0
3	TC16680	EST-SSR	TGGAGCCATCAGA ATTCCTC	ATTACGATCCAC CAGGCAAC	75	50.2
4	AC123571	SSR	CTGATCCTTTCCA AGAAGCG	CGCTAATTGCTG GCTTCAA	190	56
5	AC139354	SSR	TGAGAGAGAGAG GGCGAGAG	AGGGGCTTTTGC CTATTGTT	275	52.2

6	AC139748	SSR	ATCTGGTAGGAGA TGGTGCG	ATGCAGAGGGGT GATTCAAG	150	58.0
7	AC143341	SSR	CACGTGGGATGTC ACCACTA	GCCTTGCTGCAG AAGCTATT	400	59
8	AC146569	SSR	GACAAACGTTCAA TGCCACA	GGCTCCCTCCAC TTGTAATG	270	55.0
9	AC146588a	SSR	GGGTTCTATGCAT TCTTCGC	CCTCCCTCCCTC TCTCTCTC	410	56
10	AC146588b	SSR	GGGTTCTATGCAT TCTTCGC	CCTCCCTCCCTC TCTCTCTC	800	56
11	AC146588c	SSR	GGGTTCTATGCAT TCTTCGC	CCTCCCTCCCTC TCTCTCTC	850	56
12	AC148097a	SSR	TTGGTGCACCGTA TTTTGAG	CCAGGCATCCTT TTCTTTTC	700	50.2
13	AC148097b	SSR	TTGGTGCACCGTA TTTTGAG	CCAGGCATCCTT TTCTTTTC	200-220	50.2
14	AC149127a	SSR	GGCTGATTTGAAA CATGCCA	GGTGGTTGTGGG ACACTTTT	330	55.0
15	AC149127b	SSR	GGCTGATTTGAAA CATGCCA	GGTGGTTGTGGG ACACTTTT	100	55.0
16	AC149208	SSR	GTTACACCTAGCC CCATCCA	CACCAGAGTTAT GCCAGGGT	175	56.0
17	AC153128	SSR	GTTCCAAAAACGC ACCAAGT	CATGACAGCAGT ACATTGCC	550	55.0
18	AC152551	SSR	TCAGCTTCATCAG CCAAAGA	CCAAACAGGGCC ATAGACTC	220	52.2
19	AC157537	SSR	GCGTGGGATCAC GTACTTC	CTCATCCATTGAT CTTTCCG	500-525	50
20	CR538722	SSR	GGGTTTGTGGTA GTCGGTT	TCGAAAAGATGG GTGGAGTC	950	52.1
21	AC168149	SSR	GGCTGATTTGAAA CATGCCA	GGTGGTTGTGGG ACACTTTT	475	52.1

In total, 196 polymorphic markers were linked within the revised genome map (cv. Northfield x cv. Digger), comprising 68 ISSR, 21 RAPD, 71 ITAP and 21 SSR

markers from the previously published map and 15 new Mt EST-SSR/SSR derived markers. However, 6 of 21 EST-SSR/SSR markers remained unlinked. The 11 linkage groups (LG) varied in length from 359.4 cM to 2.4 cM and covered a total of 1392.5 cM with an average marker density of 7.1 cM. The EST-SSR/SSR markers from *M. truncatula* were placed on LG1, LG3, LG5, LG6 and LG7 (Table 3).

Table3. Characteristics of the revised genetic linkage map (cv Northfield x cv Digger)

Linkage groups	No of Markers	Length (cM)	EST-SSR/SSR Marker from <i>M. truncatula</i> /LG
LG1	27	208.9	2
LG2	37	188.4	None
LG3	47	359.4	4
LG4	22	158.9	None
LG5	17	146.4	5
LG6	12	103.6	2
LG7	14	117.6	2
LG8	4	16.5	None
LG9	8	54	None
LG10	2	2.4	None
LG11	6	36.4	None
Total	196	1392.5	15

The addition of new markers to the existing linkage map caused several rearrangements in map order and linkage group size. For example, LG3 was increased from 187.3 cM (37 markers) to 359.4 cM (47 markers) and LG11 was reduced from 137.7 cM (13 markers) to 36.4 cM (6 markers).

Segregation distortion

The inheritance of most of the markers fitted a 1:1 segregation ratio ($P < 0.05$). However, five of the 21 EST-SSR/SSR markers (AC146588b, AC139354,

AC148097a, AC148097b and BE323614) from *M. truncatula* showed distorted segregation from the expected Mendelian ratio for RIL F₅ progeny (1:1) ($P < 0.05$) (Table 4). The segregation distortion did not correlate with the number of unlinked markers as none of the unlinked marker showed distorted segregation. All of the distorted markers were mapped and concentrated in LG1, LG3, LG5 and LG6.

Table 4: Segregation distortion and unlinked markers on lentil linkage map (cv. Digger x cv. Northfield) from *M. truncatula* markers

Marker type	No. of markers	Distorted segregation (χ^2 at $P < 0.05$) (%)	Unlinked markers
EST-SSR	3	1	-
SSR	18	4	6
Total	21	5	6

QTL governing resistance to *A. lentis*

Single point analyses detected up to 22 markers significantly associated with resistance following 14, 21 and 28 DAI of seedling or maturity/pod infection. The majority associated with seedling resistance (10) were located on LG1 and 3 and those associated with pod/maturity resistance were located on LG1, 2, 3, 4 and 5 (Fig. 1). The phenotypic variation explained by individual markers was from 3% to 6% (seedling) and from 3% to 8% (pod/maturity). None of the 19 markers for seedling resistance was consistently associated across all three scoring periods, indicating that potentially different resistance mechanisms may operate at different DAI. However, seven markers were commonly associated with seedling resistance at 21 and 28 DAI scoring periods. On the other hand, among the markers associated with pod/maturity resistance, 10 were consistent across all of the scoring periods, potentially indicating a more restricted genomic involvement in defence at this later growth stage. One RAPD marker, V14a, was associated with seedling resistance at 21 and 28 DAI as well as pod resistance at all of the scoring periods, which may indicate that the defence gene(s) at this locus are stable among growth stages and disease progress times.

Simple interval mapping detected six genomic regions associated with seedling and/or pod/maturity resistance. Three conditioning seedling resistance were QTL-1 at 14 DAI, QTL-2 at 21 DAI and 28 DAI and QTL-3 at 14, 21 and 28 DAI (Fig. 1). Together, they accounted for 34% of the total estimated phenotypic variance. Three different genomic regions were consistently detected for pod/maturity resistance at every scoring period; QTL-4, QTL-5 and QTL-6. Together, they accounted for 61% of the total estimated phenotypic variance (Table 2). QTL-3 conferring seedling resistance and QTL-4, 5 and 6 conferring resistance at podding/maturity were detected at every scoring period, potentially indicating stable expression of resistance during early to late reproductive stage at this locus.

Figure1. Genetic linkage map of the lentil genome (cv. Northfield x cv. Digger) showing locations of putative QTL conditioning ascochyta blight resistance at seedling and pod/maturity stage at various scoring periods detected by simple interval mapping.

Stars indicate markers significantly associated with resistance at seedling and dots indicate markers significantly associated with resistance at pod/maturity stage by single point analysis ($P < 0.05$).

All resistance QTL were flanked by one or more significant markers detected by single point analysis. ITAP marker DK 225 was collocated with QTL-1 (at 14 DAI scoring period) and ISSR marker UBC 890a was collocated with QTL-3 at all scoring periods. QTL-2 was flanked by SSR marker AC097a from *M. truncatula* at 10cM and RAPD marker V20a at 16cM (at 21 and 28 DAI; Table 5). SSR marker

ILMs 25 was collocated with QTL-4 at all scoring periods. QTL-5 was flanked by ISSR markers, UBC 855a and UBC 830b and UBC 807a was associated with QTL-6 at all scoring periods (Table 5).

Table 5: Putative QTL for *Ascochyta lentis* resistance in the cv. Northfield (ILL5588) x cv. Digger (ILL5722) population at two developmental stages (seedling and pod/maturity) at 14, 21 and 28 DAI.

Assessment period	QTL	Linkage group	Interval length (cM)	Flanking markers	Additive effect	R^2 (%)
Seedling stage						
14 DAI	QTL 1	LG1	10.3	DK 225-UBC825c	0.26	6
	QTL 3	LG9	2.3	UBC890-ARG10	0.18	3
21 DAI	QTL 2	LG1	26.4	AC097a-V20a	0.54	8
	QTL 3	LG9	2.3	UBC890-ARG10	0.33	6
28 DAI	QTL 2	LG1	26.4	AC097a-V20a	0.69	7
	QTL 3	LG9	2.3	UBC890-ARG10	0.33	4
Total variance						34
Pod/maturity stage						
14 DAI	QTL 4	LG1	7.6	ILMs25-UBC857b	0.50	7
	QTL 5	LG4	20.1	UBC855a-UBC830b	-0.64	7
	QTL 6	LG5	3.9	UBC807a-Lup91	0.52	7
21 DAI	QTL 4	LG1	7.6	ILMs25-UBC857b	0.64	8
	QTL 5	LG4	20.1	UBC855a-UBC830b	-0.76	7
	QTL 6	LG5	3.9	UBC807a-Lup91	0.58	6
28 DAI	QTL 4	LG1	7.6	ILMs25-UBC857b	0.51	6
	QTL 5	LG4	20.1	UBC855a-UBC830b	-0.67	7
	QTL 6	LG5	3.9	UBC807a-Lup91	0.52	6
Total						61

variance

Discussion

The existing lentil linkage map (Phan et al. 2007) was enhanced with the addition of EST-SSRs/ SSRs from *M. truncatula* and the comparative location(s) of QTL governing seedling or pod/maturity resistance to *A. lentis* was determined. Among the 100 EST-SSR and SSR markers tested for transferability to lentil from *M. truncatula*, many that were transferred were SSR sequences associated with EST. This is not surprising since EST-SSR markers are derived from transcribed genomic regions and hence are expected to be conserved (Scott et al. 2000). Ultimately, there is potential to associate such gene-specific loci with functionality, particularly since the *M. truncatula* genome is soon to be completely sequenced (Young and Udvardi 2009).

The newly mapped EST-SSR/SSR markers from *M. truncatula* filled marker intervals and formed clusters in the centre or upper central or lower central part of the LG, possibly indicating their association with centromeres. A similar trend of clustering of markers was previously observed in lentil (Eujayl et al. 1998; Rubeena et al. 2003; Durán et al. 2004; Hamwieh et al. 2005; Phan et al. 2007) and in the related legume chickpea (Winter et al. 2000). Although the newly mapped markers were genetically linked to the existing mapped markers (Phan et al. 2007) in each LG, suggesting that all previously mapped chromosomes were represented (Paterson 1996), there still remained nine unlinked markers and the number of LG was too many for the haploid chromosomal number of lentil ($n=14$). A map is considered complete when the number of linkage groups is equal to the haploid chromosome number of the plant species and no unlinked markers are left. Although incomplete, this map was of adequate density to determine locations of genomic loci governing *A. lentis* resistance.

Similar to the previous study by Rubeena et al. (2006), multiple QTL associated with seedling resistance were identified, which was in contrast to the single

dominant gene identified in Northfield (Ford et al. 1999). Comparison between the population derived from Northfield (ILL5588)/Digger (ILL5722) and ILL5588/ILL7537 from Rubeena et al. (2006) revealed QTL 1 to be in the same genomic region as QTL 5 on LG1 conferring seedling blight resistance, and QTL 5 for pod resistance adjacent to the genomic region of QTL 3 for 21 DAI seedling resistance on LG 4. Two markers, P06a and V14a, were found significantly associated with seedling resistance in both the populations. This comparison helped to validate the seedling resistance in cv. Northfield.

Further validation among different genetic backgrounds is required to determine if these QTL are unique to the ILL5588 genome or represent common resistance loci in lentil. The statistically significant markers that flanked the QTL for seedling (ITAP marker DK 225, *M. truncatula* SSR marker AC097a, ISSR marker UBC 890a and RAPD marker V20a) and pod blight (SSR marker ILMs 25 and ISSR markers UBC 855a, UBC 830b and UBC 807a) resistance, will provide an opportunity for MAS in blight resistance breeding. These also offer possibility of introduction or pyramiding of these QTL into elite germplasm, to develop improved lentil cultivars.

The QTL for blight resistance accounted for 34% and 61% of the total estimated phenotypic variance at seedling and podding stage, respectively. The remaining and unaccounted variance may be due to the possible involvement of additional unidentified loci, which have not been detected either because of incomplete genome coverage or the small RIL population size as well as experimental and environmental variation.

Different QTL contributed towards seedling resistance at 14 DAI and common QTL were present at 21 and 28 DAI. However, the presence of three QTL was consistent at different DAI for pod resistance, exhibiting the involvement of relatively few genomic regions for resistance at maturity. This can be explained with the view that the pod stage was almost senescent, so potentially lower numbers of active defence genes would have been expected and hence potentially fewer differences in genomic regions involved in defence at this stage.

Phenotypic analysis of recombinants carrying different combinations of the resistance QTL is required to determine potential additive effects that may help to

slow down the rate of disease development which will reduce the area under the disease progress curve (AUDPC). This will eventually provide greater sustainability of resistance, also given that the *A. lentis* population is heterothallic and hence diverse (Galloway et al. 2004). The development of such elite lentil cultivars with combinations of QTL for seedling and pod resistance will contribute substantially to the management of this disease and help reduce losses.

In conclusion, this study has provided insight towards transfer and integration of EST-SSR/SSR markers from *M. truncatula* into the lentil linkage map. The newly mapped EST-SSR/SSR markers filled the marker interval gaps on several of the previously identified LG. This map will also be useful in the future location of gene loci governing desirable traits. This will lead to the selection and pyramiding of different resistance gene combinations and result in more durably resistant cultivars. Furthermore, markers that flank the gene(s) could be used in marker-assisted selection, potentially reducing the time for breeding for improved varieties.

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