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ORIGINAL PAPER

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A genetic linkage map of *Lens* sp. based on microsatellite and AFLP markers and the localization of fusarium vascular wilt resistance

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Abstract Microsatellites have currently become the markers of choice for molecular mapping and marker-assisted selection for key traits such as disease resistance in many crop species. We report here on the mapping of microsatellites which had been identified from a genomic library of lentil (*Lens culinaris* Medik.). The majority of microsatellite-bearing clones contained imperfect di-nucleotide repeats. A total of 41 microsatellite and 45 amplified fragment length polymorphism (AFLP) markers were mapped on 86 recombinant inbred lines derived from the cross ILL 5588 × L 692-16-1(s), which had been previously used for the construction of a random amplified polymorphic DNA and AFLP linkage map. Since ILL 5588 was resistant to fusarium vascular wilt caused by the fungus *Fusarium oxysporum* Shlecht. Emend. Snyder & Hansen f.sp. *lentis* Vasud. & Srini., the recombinant inbreds were segregating for this character. The resulting map contained 283 markers covering about 751 cM, with an average marker distance of 2.6 cM. The fusarium vascular wilt resistance was localized on linkage group 6, and this resistance gene was flanked by microsatellite marker SSR59-2B and AFLP marker p17m30710 at distances of 8.0 cM and 3.5 cM, respectively. These markers are the most closely linked ones known to date for this agronomically important *Fw* gene. Using the information obtained in this investigation, the development and mapping of microsatellite markers in the existing map of lentil could be substantially increased, thereby providing the possibility for the future localization of various loci of agronomic interest.

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Introduction

Microsatellites or simple sequence repeats (SSRs) are small tandem repeats of DNA, usually 2–5 bp in length, that occur in most eukaryotic genomes. They are being widely applied in plant genome mapping and phylogenetic analysis because of their co-dominant inheritance and high degree of polymorphism. Microsatellite markers had been used in various plants for genome mapping (Röder et al. 1998; Winter et al. 1999; Cho et al. 2000) and tagging various traits of agronomic importance.

Lentil (*Lens culinaris* Medik.) is an important crop throughout Western Asia and Northern Africa, the Indian subcontinent and North America (Webb and Hawtin 1981; Erskine 1996). It is a diploid ($2n = 2x = 14$ chromosomes), self-pollinating annual crop, with a haploid genome size of 4,063 Mbp (Arumuganathan and Earle 1991). Lentil is an important source of dietary protein in both the human diet and in animal feed, and it also helps in the management of soil fertility. A major constraint in its production in Western Asia and Northern Africa is fusarium vascular wilt caused by the soil borne fungus *Fusarium oxysporum* Shlecht. Emend. Snyder & Hansen f.sp. *lentis* Vasud. & Srini., which can account for a total yield loss in heavy infested areas. As the most effective and economical ways to manage this disease is to cultivate resistant varieties (Beniwal et al. 1993), current breeding programs aim at developing high-yielding cultivars which carry resistances against

fusarium wilt. Even though very little preliminary work had been carried out on the genetics of this pathosystem, Komboj et al. (1990) and Abbas (1995) reported that inheritance of the resistance to vascular wilt is controlled by the monogenic dominant gene, *Fw*. Abbas (1995) based his conclusions that this single dominant gene conferred resistance on crosses studied at the International Centre for Agricultural Research in the Dry Areas (ICARDA). Although resistance to fusarium wilt is simply inherited, the present field-based screening methods for resistance to fusarium vascular wilt are time-consuming and yield inconsistent results, and they cannot be performed everywhere because of the variability of the pathogen. The screening tests always have to be repeated to ensure accuracy. Therefore, there is a need to explore more efficient and effective breeding strategies to develop fusarium vascular wilt-resistant varieties.

In recent years, the use of DNA markers has improved the efficiency and effectiveness of breeding for disease resistance in various crops (Baum et al. 2000). The availability of molecular maps has facilitated gene tagging, marker-assisted selection and the positional cloning of resistance genes. The first genetic maps of lentil consisted of a small number of markers, mainly of isozymes, restriction fragment length polymorphisms (RFLPs) and some morphological markers, that covered a relatively small portion of the genome (Havey and Muehlbauer 1989a; Weeden et al. 1992; Tahir et al. 1993). The *Lens* sp. map constructed by Eujayl et al. (1998a) was based on 86 recombinant inbred lines (RILs) and consisted primarily of 89 random amplified polymorphic DNAs (RAPDs) and 79 A FLPs together with six co-dominant markers, most of the latter being RFLPs. The lentil linkage map developed by Rubeena et al. (2003) using an F_2 population encompassed 100 RAPDs, 11 ISSRs (inter-simple sequence repeats) and three resistance gene analog (RGA) markers. The most recent *Lens* sp. map contains 62 RAPDs, 29 ISSRs 65 AFLPs and four morphological and one microsatellite marker (Durán et al. 2004). To date, this latter marker is the only microsatellite marker reported for lentil. We report here the development of microsatellite markers and their application with some 50 AFLP markers to enhance the existing *Lens* sp. linkage map (Eujayl et al. 1998a) and to localize the fusarium vascular wilt resistance gene.

Materials and methods

Plant material and DNA isolation

The 86 RILs used in this study were developed from the cross ILL 5588 × L 692-16-1(s) using the random single seed descent (SSD) method (Eujayl et al. 1998a). Total genomic DNA was isolated from young seedling of the parents and their RILs according to Edwards et al. (1991).

Development of microsatellite markers

Genomic DNA (200 µg) from ILL 5588 was digested to completion with *Sau*3AI and electrophoretically fractionated on a 0.75% agarose gel. Fragments varying between 500 bp and 1,500 bp length were eluted from the gel using the gel extraction kit (QIAGEN, Valencia, Calif.) cloned into the *Bam*HI site of the plasmid vector *pUC*18. The recombinant plasmids were electroporated into *Escherichia coli*, and resulting transformants were plated onto 22×22-cm plates containing Luria broth (LB) agar medium supplemented with 50 mg/l ampicillin), 50 mg/l X-gal and 24 mg/l IPTG and incubated overnight at 37°C. Approximately 200,000 colonies were transferred onto Hybond-N+ membrane (Amersham Pharmacia Biotech, Piscataway, N.J.) and lysed; their DNA was then fixed on the membranes and hybridized with radiolabelled y-[^{32}]ATP oligonucleotide repeats, namely $(GT)_{10}$, $(GA)_{10}$, $(GC)_{10}$, $(GAA)_8$, $(TA)_{10}$ and $(TAA)_5$. The filters were hybridized overnight at 45°C, washed twice at 55°C with 2× SSC and 1% sodium dodecyl sulfate (SDS) for 10 min each time and exposed to X-ray films (Amersham Pharmacia Biotech) overnight at −70°C. All colonies giving strong signals were picked out and transferred to small petri dishes filled with LB agar containing with ampicillin (in two replications) and re-screened as before. The GFX Micro Plasmid Prep kit 250 (Amersham Pharmacia Biotech) was used for plasmid DNA isolation and purification according to the protocol of the kit. Approximately 200 clones were sequenced with M13 primers (both forward and reverse) on either a LI-COR DNA sequencer (model 4000;LI-COR Biosciences, Lincoln, Neb.) or on a MegaBACE 1000 DNA Sequencing System (Amersham Pharmacia Biotech). The contig analysis was performed to avoid duplicate clones, if any, using SEQMANII-DNASTAR software (DNASTAR, Madison, Wis.). Functional primers for 124 sequences were produced, of which 56 produced products of the expected size. The primers were further tested on the parents of the mapping populations: about 30 primers were polymorphic and are described in detail in this article. Primers that failed to produce amplification products were re-designed. Re-designed primers and monomorphic primers were tested extensively on a panel of germplasm, including cultivated and wild relatives. A detailed description of the methodology applied in isolating the microsatellites in this crop, including details on the abundance and polymorphism of microsatellites in a mini-core collection of lentil germplasm, will be published in the near future, once the methods have been sufficiently tested. SEQMANII-DNASTAR software was used to design primers at the flanking sites of the microsatellite motifs. The length of the primers varied between 18 and 23 bases. The melting temperatures of the primers were determined as presented in Baldino et al. (1989), using SEQMANII-DNASTAR software.

Microsatellite marker analysis

The primers (Table 1) were used for PCR amplification of corresponding microsatellites in both the parents and the RIL populations. The PCR mixture (20 µl) contained 10 ng genomic DNA and 10 pmol of each primer, 0.2 m *M* dNTP, 1× PCR buffer and 1 U *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif.). PCR was performed in a thermocycler (PE 9600; Perkin-Elmer, Foster City, Calif.) with the following profile: an initial denaturing cycle of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s (denaturation), a specific temperature depending upon the primer pair (listed in Table 1) for 35 s (annealing) and 72°C for 60 s (extension); this was followed by a final extension at 72°C for 5 min. The PCR products were electrophoresed on 6% polyacrylamide gels and the fragments were visualized by silver staining using a silver staining kit (Promega) as described by the supplier.

AFLP analysis

AFLP procedures were performed as outlined by Vos et al. (1995) with minor modifications (von Korff et al. 2004). Initially 24 *Tru*9I (*Mse*I) and *Pst*I selective PCR primers combinations were tested, and eight primer combinations (p20m95, p11m43, p100m43, p101m43, p104m43, p11m95, p16m95 and p294m43) were identified for mapping using the RILs. AFLP fragments were separated on 6% denaturing polyacrylamide gels and the fragments visualized by a silver staining using silver staining kit (Promega) as described by the supplier.

Screening for fusarium vascular wilt resistance

Screening for disease resistance and rating of disease reaction were performed according to Eujayl et al. (1998b) in a well-established wilt-sick plot.

Linkage analysis and integration with existing map of lentil

Genotypic data from the existing lentil map (Eujayl et al. 1998a) with 89 RAPD markers, 79 AFLP markers and four morphological markers [pod indehiscence (*Pi*), seed coat pattern (*Scp*) and flower color (*W*) and radiation frost tolerance (*Rft*)] were used together with the new SSR and AFLP markers and fusarium vascular wilt resistance scoring data to construct a linkage map by means of JOINMAP ver. 3.0 (Van Ooijen and Voorrips 2001). For linkage analysis, LOD scores of 3.00–6.00 and maximum distance between markers of 25 cM were used. Final map distances were calculated by applying the Kosambi function (Kosambi 1944).

Results

Characteristics of microsatellite markers

Table 1 summarizes the marker names, the primer sequences, the repeat sequence, T_m (°C) and the expected size of the amplified loci. The three types of repeats defined by Weber and May (1989) were found among the 30 microsatellite loci sequences shown, with the imperfect repeat being the most common (12), followed by the compound repeat (11) and lastly by the perfect repeat (7). All of the microsatellites except for four (SSRs 151, 309, 317-1 and 336) had di-nucleotide repeat units.

Primers were designed from sequences flanking the SSRs, and PCR was performed using the DNA of the two parents [ILL 5588 and L 692-16-1(s)] of the mapping population. Following polyacrylamide gel electrophoresis, all PCR fragments revealed polymorphism between the two parents. Most of the PCR fragments from ILL 5588 were of the same length, as was expected from sequence analysis (Table 1). While most of the primer pairs (70%) amplified single fragments, four amplified two fragments, two amplified four fragments and one amplified three fragments. When two or more fragments were amplified, one of them showed co-dominant inheritance and the other(s) showed dominant inheritance, with exception of two loci, SSRs 233 and 154, for which all of the fragments showed dominant inheritance.

Construction of a genetic map

Following a screening for the highest polymorphism rates between both parents, eight *Pst*I/*Mse*I primer combinations (p20m95, p11m43, p100m43, p101m43, p104m43, p11m95, p16m95 and p294m43) were identified. Each primer combination generated up to 60 bands, of which on average six were polymorphic. A total of 50 polymorphic AFLP bands were generated and used for mapping.

In addition, 30 primer pairs from microsatellite flanking loci were used for PCR with 86 RILs from the mapping population; this gave rise to 42 polymorphic fragments. Table 2 provides a summary of microsatellite and AFLP marker distribution on different linkage groups.

Chi-square analysis indicated that the segregation rates of four microsatellite loci and eight AFLP loci developed in this study deviated significantly ($P < 0.05$) from the expected 1:1 ratio. Of these distorted loci, three microsatellite loci and three AFLP loci showed highly significant ($P < 0.01$) deviation, and these were excluded from linkage analysis. Among the distorted microsatellites, the locus SSR 199C on linkage group (LG) 1 was skewed towards the L 692-16-1(s) parental allele, whereas loci SSR 113 and SSR 213 on LG 8 were skewed towards the ILL 5588 parental alleles. The dis-

Table 1 Lentil microsatellite flanking primer sequences, expected PCR product size in ILL 5588, annealing temperature used for PCR amplification and observed size of amplified PCR product

Clone no.	Primer sequence (5′ → 3′)	Annealing temperature (T_m) used for PCR (°C)	Expected size (bp)	Repeats	Locus name	Amplified fragment size (bp)	Nature of inheritance
SSR13	GAAACAACACCGAAATACAC CGAAGTCAGATGAAGTTTG	53	150	$(CA)_6$	SSR13A SSR13B	150[d] 130[e]	Co-dominant Dominant
SSR 19	GACTCATACTTTGTTCTTAGCAG GAACGGAGCGGTCACATTAG	58	250	$(TG)_{14}$	SSR19	250[d]	Co-dominant
SSR 33	CAAGCATGACGCCTATGAAG CTTTCACTCACTCAACTCTC	56	289	$(CA)_{21}(GA)_{25}$	SSR33	289[d]	Co-dominant
SSR 48	CATGGTGGAATAGTGATGGC CTCCATACACCACTCATTCAC	57	165	$(TG)_{13}$	SSR48	165[d]	Co-dominant
SSR 59-2	CCAAATACTGCAACACACCG GTTCCCATCAGGCAGAAGG	58	175	$(CA)_{19}(TA)_{19}$	SSR59-2A SSR59-2B	175[d] 210[d]	Co-dominant Dominant
SSR 80	CCATGCATACGTGACTGC GTTGACTGTTGGTGTAAGTG	56	155	$(TC)_{14}(AC)_{12}(AT)_2$	SSR80	155[d]	Co-dominant
SSR 96	GTTATCTTCCAGCGTC GATATACAATCAGAGATG	49	210	$(TG)_{10}$	SSR96	210[d]	Co-dominant
SSR 99	GGGAATTTGTGGAGGGAAG CCTCAGAATGTCCCTGTC	57	161	$(TG)_8TC(TG)_2$	SSR99	161[d]	Co-dominant
SSR 107	GCGGCGAGCAAATAAAT GGAGAATAAGAGTGAAATG	51	168	$(TC)_9 + (AT)_5C(AT)_3(GT)_{14}A(TG)_7^a$	SSR107	150[d]	Co-dominant
SSR 113	CCGTAAGAATTAGGTGTC GGAAAATAGGGTGGAAAG	51	211	$(AC)_{17}(AT)_{13}$	SSR113	211[d]	Co-dominant
SSR 119	GAACTCAGTTTCTCATTG GAACATATCCAATTATCATC	49	266	$(TA)_4TT(TA)_{11}(TG)_{19}$	SSR119	250[d]	Co-dominant
SSR 124	GTATGTGACTGTATGCTTC GCATTGCATTTCACAAACC	52	174	$(TGC)_3 + (GT)_9TA(TG)_2$	SSR124	174[d]	Co-dominant[c]
SSR 130	CCACGTATGTGACTGTATG GAAAGAGAGGCTGAAACTTG	55	196	$(GT)_9$	SSR130	196[d]	Co-dominant
SSR 151	GGTAGGTGAGATAGTTG GGAGCAAGAAGAAGCAG	51	134	$(TG)_4(TGTGTA)_7(TG)_4$	SSR151	134[d]	Dominant[c]
SSR 154	GGAATTTATCACACTATCTC GACTCCCAACTTGTATG	51	272	$(AC)_3ATAG(AC)_7(AT)_2$	SSR154A SSR154B SSR154C SSR154D	360[e] 272[d] 230 [e] 130[e]	Dominant Dominant[c] Dominant Dominant
SSR 156	GTACATTGAACAGCATCATC CAAATGGGCATGAAAGGAG	53	176	$(TC)_2(TG)_{13}$	SSR156	176[d]	Co-dominant
SSR 167	CACATATGAAGATTGGTCAC CATTTATGTCTCACACACAC	54	160	$(TA)_{16}(TG)_{21}$	SSR167	160[d]	Co-dominant
SSR 184	GTGTGTACCTAAAGCCTTG GTAAGTTGATCAAACGCCC	55	250	$(GT)_{10}(AT)_{15}(GT)_{19}$	SSR184	260[d]	Co-dominant
SSR 199	GTGTGCATGGTGTGTG CCATCCCCCTCTATC	51	182	$(GT)_4GC(GT)_8GC(GT)_3$	SSR199A SSR199B SSR199C	160[d] 250[e] 100[e]	Co-dominant Dominant Dominant
SSR 204	CACGACTATCCCACTTG CTTACTTTCTTAGTGCTATTAC	53	186	$(TG)_4 + (AC)_7^b$	SSR204	186[d]	Co-dominant
SSR 212-1	GACTCATTGTTGTACCC GCGAGAAGAATGGTTG	50	181	$(AT)_2(TC)_{26}(AC)_8$	SSR212-1	181[d]	Co-dominant

SSR 213	CACTCGCACCTCTTATG GAAATTGTCTCTTAGCAAG	51	151	$(TA)_8(TG)_5TA(GT)_5$	SSR213	151[d]	Co-dominant
SSR 215	CATTAATATTTCTTTGGTGC CTTTTCTTCTCTTCCCC	50	331	$(CA)_{15}(TA)_{25}$	SSR215A SSR215B	380[d] 331[d]	Co-dominant Dominant
SSR 233	CTTGGAGCTGTTGGTC GCCGCCTACATTATGG	52	111	$(GT)_9$	SSR233A SSR233B SSR233C SSR233D	380[d] 180[e] 150[e] 111[d]	Dominant Dominant Dominant Dominant
SSR 302	CAAGCCACCCATACACC GGGCATTAAGTGTGCTGG	56	261	$(TA)_{15}(CA)_{11}$	SSR302A SSR302B	197[d] 215[d]	Co-dominant Dominant
SSR 309-2	GTATGTCGTTAACTGTCGTG GAGGAAGGAAGTATTCGTC	50	182	$(AT)_3GT(TA)_3T(TAT)_6$	SSR309-2	182[d]	Co-dominant
SSR 317-1	GTGGGTGTAATTATTGCTAC GTATCAAACTTATGGTGAAATC	53	308	$(AT)_4(GT)_{16}(GC)_6GTGGC(GT)_5$ $A(TG)_8+(TAA)_5$	SSR317-1	308[d]	Co-dominant
SSR 317-2	CACGTAACATCTTGCTTATG GTAGCAATAATTACACCCAC	53	120	$(TTG)_2(AT)_2A(AT)_2G(TA)_{14}ATC(GT)_4$	SSR317-2	120[d]	Co-dominant
SSR 323	AGTGACAACAAAATGTGAGT GTACCTAGTTTCATCATTG	51	250	$(AT)_{22}(CA)_4$	SSR323	250[d]	Co-dominant
SSR 336	GTGTAACCCAACTGTTCC GGCCGAGGTTGTAACAC	54	253	$(TAA)_6AGA(TAA)_4$	SSR336	253[d]	Co-dominant

[a] $(TC)_9TATCGATCATCTG(AT)_5C(AT)_3(GT)_{14}A(TG)_7$
[b] $(TG)_4CTTAAGCCTAGGTAGGAGGCTTATCTCTCAAGTAAAACACCCATAACCTAACAAT(AC)_7$
[c] Ungrouped marker
[d] Allele size of ILL 5588
[e] Allele size of L 692-16-1(s)

torted AFLP markers P100m43k (on LG 1) and p11m43f (on LG 5) were skewed towards the L962-16-1 parental alleles, whereas the distorted AFLP markers p104m43i (on LG 6) and P100m43i (on LG 5) were skewed towards the ILL 5588 parental alleles.

All marker data were used for developing an integrated map of lentil. This map comprises a total of 283 markers, which includes 49 AFLPs and 39 SSRs markers of this study and 194 loci (110 RAPDs and 80 AFLPs and four morphological markers) previously mapped by Eujayl et al. (1998a).

Among the four morphological markers mapped, seed coat pattern (*Scp*) and flower color (*W*) were located on LG 3 flanked by two AFLP markers, p11m43h and p16m4315 at a distance of 3.5 cM and 3.4 cM, respectively. The Pod indehiscence (*Pi*) locus was located on LG 2 linked at a distance of 0.6 cM to two new AFLP markers, p294m43c and p100m43c. There was a good correspondence between this map and that of Eujayl et al. (1998a) with respect to the genetic location of these morphological markers. The radiation frost tolerance locus (*Rft*) (Eujayl et al. 1999) was linked at a distance of 8.4 cM to RAPD marker OPS-16b but was not assigned to any of the major linkage groups.

Localization of fusarium vascular wilt resistance

Eujayl et al. (1998b) screened 71 RILs for fusarium vascular wilt resistance in the wilt sick plot along with their parents, ILL 5588, which was resistant, and L 962-16-1 (s) which was susceptible to fusarium vascular wilt. The disease reaction of each RIL was scored as either resistant or susceptible, as described by Bayaa et al (1995). The population segregated for 37 resistant versus 34 susceptible RILs, which is in accordance with a 1:1 segregation rate ($\chi^2 = 0.1$; $df = 1$; $P > 0.05$), indicating that the fusarium vascular wilt resistance is controlled by a single gene (*Fw*). The microsatellite and AFLP marker data (Eujayl et al. 1998a and current study) were then used to determine the genetic linkage of the marker and its position to the locus conferring resistance. Our linkage analysis revealed that fusarium vascular wilt resistant locus (*Fw*) was located on LG 6 and that this locus was linked to microsatellite marker *SSR59-2B* and AFLP marker p17m30710 at distances of 8.0 cM and 3.5 cM, respectively (Fig. 1).

Discussion

The purpose of this study was to isolate microsatellites from lentil and to map them using an existing mapping population (Eujayl et al 1998a). We have isolated a set of 30 microsatellite clones from which 30 primer combinations were derived. Using these primer pairs, we amplified 42 PCR fragments between both parents of the mapping populations, out of which we mapped 39 onto the linkage map. The high degree of microsatellite

Table 2 Summary of the marker distribution on different linkage groups of the *Lens* sp. genome

Linkage groups	Length (cM)	Number of markers	Number of SSRs	Number of new AFLPs	LOD
1	93.3	50	12	12	4
2	63.5	47	4	6	5
3	171.9	41	4	10	5
4	90.1	45	5	5	4
5	69.6	19	2	3	4
6	98.3	35	4	8	4
7	47.4	21	1	1	4
8	41.6	10	5	0	5
9	26.5	3	1	1	4
10	13.4	3	0	2	4
11	8.5	2	0	0	4
12	3.5	2	0	1	3
13	7.7	3	0	0	3
14	15.2	2	1	0	3
Total	750.5	283	39	49	

polymorphism that we observed in lentil is not surprising and is comparable to the results of related legumes such as chickpea (Udupa et al. 1999; Winter et al. 1999) and *Medicago truncatula* (Eujayl et al. 2004). The degree of polymorphism that we have detected using microsatellites in lentils is relatively high compared to previous mapping efforts using isozymes, RFLPs, RAPDs and AFLPs (Havey and Muehlbauer 1989a; Weeden et al. 1992; Eujayl et al. 1998a; Rubeena et al. 2003).

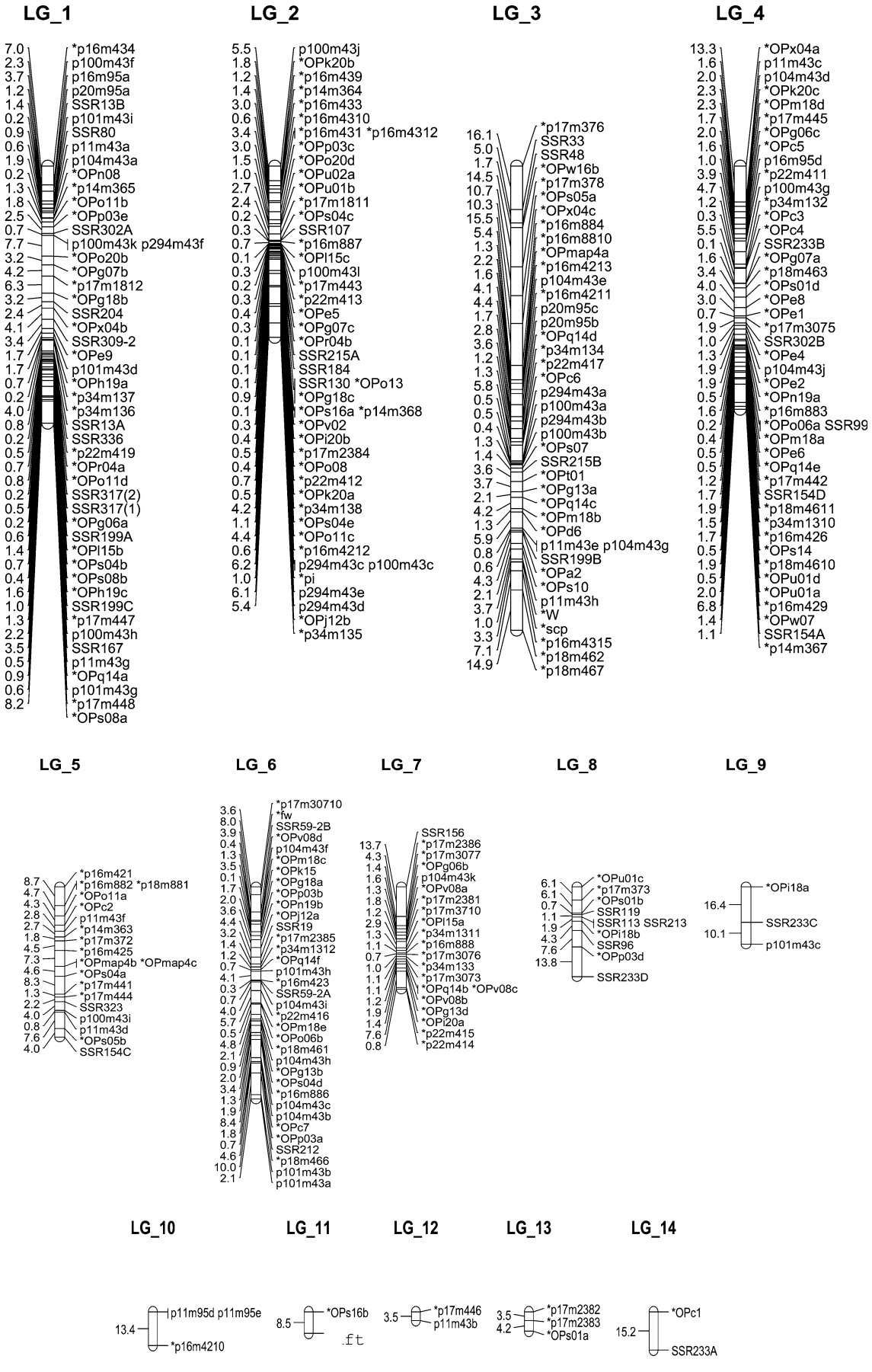
On the basis of χ^2 analysis ($P > 0.05$), our results showed that four microsatellite loci (9.5%) and eight AFLP loci (17.8%) followed a distorted segregation. In the same population with fewer markers, Eujayl et al. (1997, 1998a) previously observed only 8.4% segregation distortion ($P > 0.05$). Rubeena et al. (2003) observed a segregation distortion of about 14% in an F_2 population of lentil. The segregation distortion in $F_{6:8}$ population that we observed is comparatively low when compared with that of the RILs derived from an interspecific cross of *Cicer* sp. (38.4% segregation distortion; Winter et al. 2000) and other crops such as rice (39.4% segregation distortion; Xu et al. 1997). Our results are based on the selection of a population with a low amount of segregation distortion in early generations (Eujayl et al. 1997).

The clustering of markers in the middle or upper middle or lower middle part of the linkage groups in this study may also indicate the location of centromeres. The centromere regions experience up to tenfold less recombination than other areas of the genome (Tanksley et al. 1992). Heterogeneity in recombination along the genome has implications on the development of high-resolution linkage maps as the latter are much easier to develop for regions of higher recombination. On the other hand, mapping of suppressed recombination requires much larger progeny sizes in order to allow the rare recombination events to occur, which is necessary for the construction of fine maps (Tanksley et al. 1992).

Previous maps of this species differed with respect to marker type and number, genome coverage (span) and marker density (Havey and Muehlbauer 1989b; Weeden et al. 1992; Eujayl et al. 1998a; Rubeena et al. 2003; Durán 2004). The most recently constructed maps based on an F_2 population developed by Rubeena et al. 2003 (784.1 cM, 114 markers) and Durán et al. 2004 (2,172.4 cM, 161 markers including one microsatellite) were developed with AFLP, RAPD, ISSR and morphological markers. In addition to the addition of microsatellite markers, our map sees the addition of 50 new AFLP markers to the old one (Eujayl et al. 1998a) using a RIL population. This has resulted in an increase in the total number of markers to 289. The map spans 792 cM and is distributed over 14 LGs. Seven of these 14 LGs have more than 18 markers each, while the others contain only ten or fewer markers. The average distance between the markers in our map is 2.7 cM, which is shorter than those reported in the previous maps (6.0 cM, Eujayl et al. 1998a; 6.9 cM, Rubeena et al. 2003; 15.87 cM, Durán et al. 2004).

The linkage distance between the markers on our map also varies greatly across the different linkage groups, and the size of the LG does not necessarily reflect the number of linked markers. For instance, LG 1, with a total linkage distance of 95.25 cM, was covered by 50 markers, whereas in LG 3, a 172-cM distance was covered by only by 41 markers. Linkage distances of more than 10 cM were observed only between 12 different loci, with the maximum distance being 16.1 cM (on LG 3). Similar results were also reported by Winter et al. (2000) in the *Cicer* sp. map.

Fig. 1 A genetic linkage map of *Lens* sp. based on microsatellite, AFLP, RAPD and morphological markers. The map shows the position of microsatellite (denoted as SSR_), AFLP (denoted as p_m_), RAPD (OP_) and morphological markers, namely seed color pattern (*Scp*), flower color (*W*) pod indehiscence (*Pi*), Fusarium wilt resistance (*Fw*) and radiation frost tolerance locus (*Rft*) distributed on 14 linkage groups (*LG*) at LOD score ≥3. The marker names beginning with an *asterisk* are those markers mapped by Eujayl et al. (1998a). The values on *left side* of the individual linkage groups represents distance in centiMorgans calculated using the Kosambi mapping function


LG_1
7.0 *p16m434
2.3 p100m43f
3.7 p16m95a
1.2 p20m95a
1.4 SSR13B
0.2 p101m43i
0.9 SSR80
0.6 p11m43a
1.9 p104m43a
0.2 *OPn08
1.3 *p14m365
1.8 *OPo11b
2.5 *OPp03e
0.7 SSR302A
7.7 p100m43k p294m43f
3.2 *OPo20b
4.2 *OPg07b
6.3 *p17m1812
3.2 *OPg18b
2.4 SSR204
4.1 *OPx04b
3.4 SSR309-2
1.7 *OPe9
1.7 p101m43d
0.7 *OPh19a
0.2 *p34m137
4.0 *p34m136
0.8 SSR13A
0.2 SSR336
0.5 *p22m419
0.7 *OPr04a
0.8 *OPo11d
0.2 SSR317(2)
0.5 SSR317(1)
0.2 *OPg06a
0.6 SSR199A
1.4 *OPl15b
0.7 *OPs04b
0.4 *OPs08b
1.6 *OPh19c
1.0 SSR199C
1.3 *p17m447
2.2 p100m43h
3.5 SSR167
0.5 p11m43g
0.9 *OPq14a
0.6 p101m43g
8.2 *p17m448
*OPs08a
LG_2
5.5 p100m43j
1.8 *OPk20b
1.2 *p16m439
1.4 *p14m364
3.0 *p16m433
0.6 *p16m4310
3.4 *p16m431 *p16m4312
3.0 *OPp03c
1.5 *OPo20d
1.0 *OPu02a
2.7 *OPu01b
2.4 *p17m1811
0.2 *OPs04c
0.3 SSR107
0.7 *p16m887
0.1 *OPl15c
0.3 p100m43l
0.2 *p17m443
0.3 *p22m413
0.4 *OPe5
0.3 *OPg07c
0.1 *OPr04b
0.1 SSR215A
0.1 SSR184
0.1 SSR130 *OPo13
0.9 *OPg18c
0.1 *OPs16a *p14m368
0.3 *OPv02
0.4 *OPi20b
0.5 *p17m2384
0.4 *OPo08
0.7 *p22m412
0.5 *OPk20a
4.2 *p34m138
1.1 *OPs04e
4.4 *OPo11c
0.6 *p16m4212
6.2 p294m43c p100m43c
1.0 *pi
6.1 p294m43e
5.4 p294m43d
*OPj12b
*p34m135
LG_3
16.1 *p17m376
5.0 SSR33
1.7 SSR48
14.5 *OPw16b
10.7 *p17m378
10.3 *OPs05a
15.5 *OPx04c
5.4 *p16m884
1.3 *p16m8810
2.2 *OPmap4a
1.6 *p16m4213
4.1 p104m43e
4.4 *p16m4211
1.7 p20m95c
2.8 p20m95b
3.6 *OPq14d
1.2 *p34m134
1.3 *p22m417
5.8 *OPc6
0.5 p294m43a
0.5 p100m43a
0.4 p294m43b
1.3 p100m43b
1.4 *OPs07
3.6 SSR215B
3.7 *OPt01
2.1 *OPg13a
4.2 *OPq14c
1.3 *OPm18b
5.9 *OPd6
0.8 p11m43e p104m43g
0.6 SSR199B
4.3 *OPa2
2.1 *OPs10
3.7 p11m43h
1.0 *W
3.3 *scp
7.1 *p16m4315
14.9 *p18m462
*p18m467
LG_4
13.3 *OPx04a
1.6 p11m43c
2.0 p104m43d
2.3 *OPk20c
2.3 *OPm18d
1.7 *p17m445
2.0 *OPg06c
1.6 *OPc5
1.0 p16m95d
3.9 *p22m411
4.7 p100m43g
1.2 *p34m132
0.3 *OPc3
5.5 *OPc4
0.1 SSR233B
1.6 *OPg07a
3.4 *p18m463
4.0 *OPs01d
3.0 *OPe8
0.7 *OPe1
1.9 *p17m3075
1.0 SSR302B
1.3 *OPe4
1.9 p104m43j
1.9 *OPe2
0.5 *OPn19a
1.6 *p16m883
0.2 *OPo06a SSR99
0.4 *OPm18a
0.5 *OPe6
0.5 *OPq14e
1.2 *p17m442
1.7 SSR154D
1.9 *p18m4611
1.5 *p34m1310
1.7 *p16m426
0.5 *OPs14
1.9 *p18m4610
0.5 *OPu01d
2.0 *OPu01a
6.8 *p16m429
1.4 *OPw07
1.1 SSR154A
*p14m367
LG_5
8.7 *p16m421
4.7 *p16m882 *p18m881
4.3 *OPo11a
2.8 *OPc2
2.7 p11m43f
1.8 *p14m363
4.5 *p17m372
7.3 *p16m425
4.6 *OPmap4b *OPmap4c
8.3 *OPs04a
1.3 *p17m441
2.2 *p17m444
4.0 SSR323
0.8 p100m43i
7.6 p11m43d
4.0 *OPs05b
SSR154C
LG_6
3.6 *p17m30710
8.0 *fw
3.9 SSR59-2B
0.4 *OPv08d
1.3 p104m43f
3.5 *OPm18c
0.1 *OPk15
1.7 *OPg18a
2.0 *OPp03b
3.6 *OPn19b
4.4 *OPj12a
3.2 SSR19
1.4 *p17m2385
1.2 *p34m1312
0.7 *OPq14f
4.1 p101m43h
0.3 *p16m423
0.7 SSR59-2A
4.0 p104m43i
5.7 *p22m416
0.5 *OPm18e
4.8 *OPo06b
2.1 *p18m461
0.9 p104m43h
2.0 *OPg13b
3.4 *OPs04d
1.3 *p16m886
1.9 p104m43c
8.4 p104m43b
1.8 *OPc7
0.7 *OPp03a
4.6 SSR212
10.0 *p18m466
2.1 p101m43b
p101m43a
LG_7
13.7 SSR156
4.3 *p17m2386
1.4 *p17m3077
1.6 *OPg06b
1.3 p104m43k
1.8 *OPv08a
1.2 *p17m2381
2.9 *p17m3710
1.3 *OPl15a
1.1 *p34m1311
0.7 *p16m888
1.0 *p17m3076
1.1 *p34m133
1.1 *p17m3073
1.2 *OPq14b *OPv08c
1.9 *OPv08b
1.4 *OPg13d
7.6 *OPi20a
0.8 *p22m415
*p22m414
LG_8
6.1 *OPu01c
6.1 *p17m373
0.7 *OPs01b
1.1 SSR119
1.9 SSR113 SSR213
4.3 *OPi18b
7.6 SSR96
13.8 *OPp03d
SSR233D
LG_9
16.4 *OPi18a
SSR233C
10.1 p101m43c
LG_10
13.4 p11m95d p11m95e
*p16m4210
LG_11
8.5 *OPs16b
LG_12
3.5 *p17m446
p11m43b
LG_13
3.5 *p17m2382
4.2 *p17m2383
*OPs01a
LG_14
15.2 *OPc1
SSR233A

With respect to the morphological markers, seed coat pattern (*Scp*) and flower color (*W*) were localized on LG 3 flanked by the new AFLP markers p11m43h and p16m4315 at a distance of 3.5 cM and 3.4 cM, respectively, while in the earlier map (Eujayl et al. 1998a), these morphological markers were linked to RAPD marker OPs10 at a distance 10.6 cM on the same linkage group (LG 3). Pod indehiscence (*Pi*) was located on LG 2 linked at a distance of 0.6 cM from two new AFLP markers, p294m43c and p100m43c, while previously the *Pi* marker was linked to RAPD marker OPp03c at 19.5 cM (Eujayl et al. 1998a). We were not able to integrate the radiation frost tolerance (*Rft*) locus into any of the major linkage groups (Eujayl et al. 1999). More markers are needed to cover the entire genome and to localize this locus onto a linkage group.

The expected number of seven linkage groups for a comprehensive linkage map of lentil ($2n = 2x = 14$) was exceeded by seven linkage groups, out of which three linkage groups had only two markers and three linkage groups had only three markers. Since some linkage groups are extremely small, it is safe to conclude that the apparent excess of linkage groups might be due to incomplete coverage of the genome with the marker loci.

One of the major aims of molecular mapping in lentil is the tagging of genes for resistance to fusarium vascular wilt. Eujayl et al. (1998b) reported earlier that the fusarium vascular wilt in lentil is controlled by a single dominant gene. We were able to successfully localize the resistance gene on LG 6. This resistance gene is flanked by microsatellite marker SSR59-2B and AFLP marker p17m30710 at a distance of 8.0 cM and 3.5 cM, respectively. Further fine mapping of the locus and the use of the SSR59-2B marker will greatly help in the marker-assisted breeding of lentils for fusarium wilt resistance.

The microsatellite markers developed in this study are highly polymorphic and locus-specific and the majority of them are co-dominant. This type of marker is highly useful for mapping because they allow for the differentiation of homozygous and heterozygous genotypes. In marker-assisted breeding (both in selection and backcross breeding), co-dominant markers are effective in identifying desirable genotypes as homozygous at early stages of selection. Therefore, the co-dominant markers developed in this study could be an important asset for lentil, which until recently has lacked co-dominant markers. Future research should be aimed at developing more microsatellite markers for this crop and using these for tagging various traits of agronomic importance and marker-assisted selection.

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