RESEARCH ARTICLE

# Construction of intersubspecific molecular genetic map of lentil based on ISSR, RAPD and SSR markers

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## Abstract

Lentil (*Lens culinaris* ssp. *culinaris*), is a self-pollinating diploid ($2n = 2x = 14$), cool-season legume crop and is consumed worldwide as a rich source of protein (~24.0%), largely in vegetarian diets. Here we report development of a genetic linkage map of *Lens* using 114 $F_2$ plants derived from the intersubspecific cross between L 830 and ILWL 77. RAPD (random amplified polymorphic DNA) primers revealed more polymorphism than ISSR (intersimple sequence repeat) and SSR (simple sequence repeat) markers. The highest proportion (30.72%) of segregation distortion was observed in RAPD markers. Of the 235 markers (34 SSR, 9 ISSR and 192 RAPD) used in the mapping study, 199 (28 SSRs, 9 ISSRs and 162 RAPDs) were mapped into 11 linkage groups (LGs), varying between 17.3 and 433.8 cM and covering 3843.4 cM, with an average marker spacing of 19.3 cM. Linkage analysis revealed nine major groups with 15 or more markers each and two small LGs with two markers each, and 36 unlinked markers. The study reported assigning of 11 new SSRs on the linkage map. Of the 66 markers with aberrant segregation, 14 were unlinked and the remaining 52 were mapped. ISSR and RAPD markers were found to be useful in map construction and saturation. The current map represents maximum coverage of lentil genome and could be used for identification of QTL regions linked to agronomic traits, and for marker-assisted selection in lentil.

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## Introduction

Lentil (*Lens culinaris* Medik. ssp. *culinaris*), a self-pollinated diploid ($2n = 2x = 14$) with a relatively large genome size of 4063 Mbp (Arumuganathan and Earle 1991), is an important cool-season legume crop grown widely throughout the Indian subcontinent, northern Africa, western Asia, southern Europe, North and South America and Australia (Erskine 1996). Lentil originated in the Fertile Crescent of the Near East and later spread to northern Africa and eastern Ethiopia, central and southern Europe, North America, Oceania and South Asia (Duke 1981). *L. culinaris* spp. *culinaris* encompasses two physio-morphological cultivated lentil types (Barulina 1930), small-seeded (microsperma) and large-seeded (macrosperma), while *L. culinaris* spp. *orientalis* is considered to be the progenitor of cultivated lentil (Ladizinsky *et al.* 1984). Lentil is an important source of dietary protein for both human diet and animal feeds and it helps in the management of soil fertility. India ranks second after Canada in lentil production, whereas Canada and Turkey are the world's largest lentil exporters (FAO 2010, http://faostat.fao.org/site/339/default.aspx).

Legumes in general and lentil in particular suffer from poorly developed molecular tools for genetic and genomic analyses, thus limiting the application of enabling technologies for crop improvement. There is a significant need to increase availability of high-quality molecular genetic markers that can be used for marker-assisted selection (MAS) in breeding programme and to improve the capacity for biotechnology by developing information resources in key legume species like lentil. Biochemical and molecular markers have revealed that lentil has relatively low levels of genetic

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variation compared to many other plant species (Álvarez *et al.* 1997; Eujayl *et al.* 1997; Ford *et al.* 1997; Ferguson *et al.* 1998; Sonante and Pignone 2001). Molecular markers can serve as the basis for development of a high-density genetic map which facilitates MAS, positional cloning of resistance genes, and mapping of QTLs of agronomic interest in many crops (Paterson *et al.* 1988; Winter and Kahl 1995).

In lentil, the majority of genome maps have been created with anonymous and dominant random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat (ISSR) markers (Eujayl *et al.* 1997, 1998; Rubeena *et al.* 2003; Durán *et al.* 2004; Hamwieh *et al.* 2005; Tullu *et al.* 2008; Saha *et al.* 2010). Nevertheless, these first-generation maps served as foundations upon which to build more detailed genetic maps with greater utility.

To maximize polymorphism for map construction in lentil, intersubspecific hybrid populations have been used (Paterson *et al.* 1990; Eujayl *et al.* 1997; Durán *et al.* 2004). Such populations have also been used to map quantitative traits related to plant structure, growth habit and yield in lentil (Fratini *et al.* 2007). The genetic map presented here is based on $F_2$ intersubspecific progenies between *L. culinaris* ssp. *culinaris* and *L. c.* ssp. *orientalis*, and includes ISSR, RAPD and SSR markers. It can serve as the basis for development of a high-density map that can be used for mapping quantitative trait loci (QTLs) of agronomic interest and MAS, and is a first step in map-based cloning of disease resistance genes and other genes of interest.

## Materials and methods

### *Plant material*

An $F_2$ population of 114 individuals derived from an intersubspecific cross of *L. culinaris* ssp. *culinaris* cv. L 830 as female parent and *L. culinaris* ssp. *orientalis* ILWL 77 as pollen donor was used for construction of the genetic map. A single $F_1$ plant was selfed to produce the $F_2$ population. The $F_2$ plants were grown in a greenhouse for collecting leaf samples for DNA extraction. The parents were chosen on the basis of their reaction to lentil rust caused by *Uromyces vicia fabae* (Pers.) and variation for other agro-morphological and seed traits. Genomic DNA was then isolated from harvested young leaf tissue (0.5–1 g) of the parents and $F_2$ individuals using CTAB method (Murray and Thompson 1980).

### *ISSR analysis*

Thirty ISSR primers, universal in nature (15 to 23 nucleotides in length), were used to screen the parents for polymorphism. Only five of the primers that produced amplification pattern with scorable polymorphic bands were chosen for genotyping. The ISSR technique was performed following Rana *et al.* (2012). The reaction products were mixed with 2 $\mu$L of gel loading dye (0.25% bromophenol blue and 40% sucrose), and resolved on 1.8% agarose gel at 100 V for 90 min in 1× Tris acetate EDTA (TAE) buffer (40 mM Tris, 40 mM glacial acetic acid, 1 mM EDTA, pH 8.0). The gels were stained with ethidium bromide (0.5 $\mu$g/mL) and the PCR products were visualized and photographed using the Gel-Unit (Bio-Rad, Hercules, USA).

### *RAPD analysis*

Of a total of 523 RAPD decamer primers tested (Operon Technologies, Alameda, USA), only polymorphic primers, which produced easy-to-score and distinct fragments on the parents were used for genotyping the $F_2$ mapping population. For amplification of genomic DNA with RAPD, a reaction mixture of 12.5 $\mu$L volume was prepared using 7.15 $\mu$L of sterilized distilled water, 1.0 $\mu$L template DNA (25 ng/$\mu$L), 1.0 $\mu$L primer (RAPD/ISSR), 1.0 $\mu$L $MgCl_2$ (25 mM), 1.25 $\mu$L 10× PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.0 $\mu$L dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 $\mu$L *Taq* polymerase (5 U/$\mu$L). The amplification was carried out in a 2720 Thermal Cycler (Applied Biosystems, Carlsbad, USA). The PCR condition for RAPDs was optimized as initial cycle of 94°C for 5 min, 39 cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 2 min with final extension at 72°C for 5 min, before cooling to 4°C. The PCR products were mixed with 2 $\mu$L of gel loading dye (0.25% bromophenol blue and 40% sucrose), and separated on 1.5% agarose gel at 100 V for 90 min in 1× Tris acetate EDTA (TAE) buffer (40 mM Tris, 40 mM glacial acetic acid, 1 mM EDTA, pH 8.0). The gels were stained with ethidium bromide (0.5 $\mu$g/mL) and the bands were visualized and photographed using the Gel-Unit (Bio-Rad, Hercules, USA).

### *SSR analysis*

SSRs, including 68 published (Durán *et al.* 2004; Hamwieh *et al.* 2005, 2009; Saha *et al.* 2010) and 76 unpublished, were surveyed for polymorphism analysis at different annealing temperatures using DNA from both parental lines. Previously mapped SSRs that were polymorphic in the present study were used as anchor markers. The polymorphic microsatellites were used for genotyping of $F_2$ mapping family. A total of 622 cross-genera microsatellite primers including 122 *Medicago truncatula* SSRs (http://medicago.org/genome), 280 *Trifolium pratense* SSRs (Kölliker *et al.* 2005; Sato *et al.* 2005), 142 *Pisum sativum* SSRs (Loridon *et al.* 2005) and 78 intron-targetted amplified polymorphic (ITAP) markers (Phan *et al.* 2007) derived from *M. truncatula* were used for assessing transferability on the parental set of lentil genotypes. SSR amplification was performed in a total volume of 12.5 $\mu$L which was prepared using 7.15 $\mu$L of sterilized distilled water, 1.0 $\mu$L template DNA (25 ng/$\mu$L), 0.5 $\mu$L of forward and 0.5 $\mu$L of reverse primer (5 $\mu$M), 1.0 $\mu$L $MgCl_2$ (25 mM), 1.25 $\mu$L 10× PCR buffer (10 mM Tris-Hcl, 50 mM KCl, pH 8.3), 1.0 $\mu$L dNTP mix

(0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 $\mu$L *Taq* polymerase (5 U/$\mu$L). The amplifications were carried out in Gene Amp PCR System 9700® (Applied Biosystems) and 2720 Thermal Cycler (Applied Biosystems) by using protocols suggested by authors above. The Touch Down PCR profile was used for the amplification of *L. culinaris* SSRs following an initial denaturing cycle of 95°C for 2 min, followed by 18 cycles of 95°C for 50 s, 65°C for 20 s, 72°C for 50 s, followed by 25 cycles of 95°C for 20 s, 55°C for 50 s, 72°C for 50 s and 72°C for 10 min before cooling to 4°C. The amplification products were electrophoresed in 3% agarose gel (HIMEDIA, Mumbai, India) and stained with ethidium bromide (0.5 $\mu$g/mL). The gels were visualized and photographed using the Gel-Documentation Unit (Bio-Rad, Hercules, USA).

### *Generation of data*

Presence and absence of each band of a particular molecular weight in RAPD, ISSR and SSR profiles of all the individuals were scored manually. For deducing relationship among individuals, each band of a specific molecular weight in the DNA profile of an individual was treated as a locus/marker. A data matrix with 'A' indicating the presence or absence of band in parent L 830 and 'B' the presence or absence of a particular band in parent ILWL 77 was generated. In case of codominant SSRs, heterozygotes were denoted as 'H'.

### *Linkage analysis*

The markers were analysed by chi-square test for goodness of fit to the expected Mendelian segregation ratio for codominant (1 : 2 : 1) and dominant (3 : 1) inheritance in the lentil $F_2$ population for SSR and RAPD/ISSR markers, respectively. Linkage analyses were performed using GMENDEL/iMAS 2.0 software (http://www.icrisat.org/bt-biomatrics-imas.htm). Preliminary grouping was performed using the 'Compute Groups' command with an LOD of 4.0 and a maximum recombination value of 0.25. Simulating annealing and multiple pairwise methods were used for locus ordering. Linkage groups (LGs) were established by two-point analysis using the 'group' command. The best order of markers for each LG was established using the 'map' command and then verified by the more stringent linkage threshold criteria of the 'ripple' command. The Kosambi mapping function (Kosambi 1944) was used to convert the recombination fractions into additive genetic distance (centiMorgans), provided by the program.

## Results

### *Cross-genera marker transferability*

Of the 622 cross-genera SSRs tested, only 226 (36.33%) were transferable in lentil. The maximum transferability of SSR primers to amplify across species expressed as the total mean percentage of successful amplification was shown by *T. pratense* SSRs (48.92%), followed by *M. truncatula* SSRs (24.59%, including SSRs with tetranucleotide and hexanucleotide motifs) and *P. sativum* SSRs (8.45%). *M. truncatula* SSRs with dinucleotide repeats had higher levels of amplification (25.27%) as compared to 19.23% shown by primer pairs with trinucleotide repeat motifs. By contrast, *T. pratense* SSRs with dinucleotide repeats had lower levels of amplification (38.35%) than those with other motifs (trinucleotide 50.58% and tetranuceotide 62.85%).

### *Mapping of markers*

Of the 235 markers used for genotyping the $F_2$ population, 199 (84.68%) were assigned to different LGs (table 1; figure 1), while 36 (30 RAPD, 1 ISSR and 5 SSR) markers remained unlinked. The considerable proportion of unlinked markers (36) reflects the need for screening a large number of markers to cover the entire lentil genome evenly. The map comprising a total of 199 markers includes 162 RAPDs, 9 ISSRs and 28 SSR markers.

In our study, ISSR primers produced fewer polymorphic markers than RAPD. Of the 523 RAPD primers screened, only 79 (15.1%) were polymorphic generating a total of 192 reproducible and segregating markers (2.4 markers/primer). A total of 162 (84.37%) RAPD markers were mapped on the lentil genome and the markers were distributed on all LGs. The remaining 30 (15.62%) RAPD markers were found to be unlinked. The size of the markers varied from approximately 300 to 3000 bp. The analysis revealed 11 LGs (LGI to LGXI) spanning a total length of 3843.4 cM, with the length

**Table 1.** Molecular markers used for construction of genetic linkage map.

| Marker type | Number of primers screened | Number of polymorphic primers | Number of polymorphic markers | Polymorphism (%) | Number of mapped markers |
|---|---|---|---|---|---|
| SSR | 766* | 34 (4.43%) | 34 (1.0 marker/primer) | 4.43 | 28 (82.35%) |
| ISSR | 30 | 5 (16.7%) | 9 (1.8 markers/primer) | 30.0 | 9 (100%) |
| RAPD | 523 | 79 (15.1%) | 192 (2.4 markers/primer) | 36.71 | 162 (84.37%) |
| Total | 1319 | 118 (8.94%) | 235 (1.99 markers/primer) | 17.81 | 199 (84.68%) |

*This includes cross-genera SSRs as well.

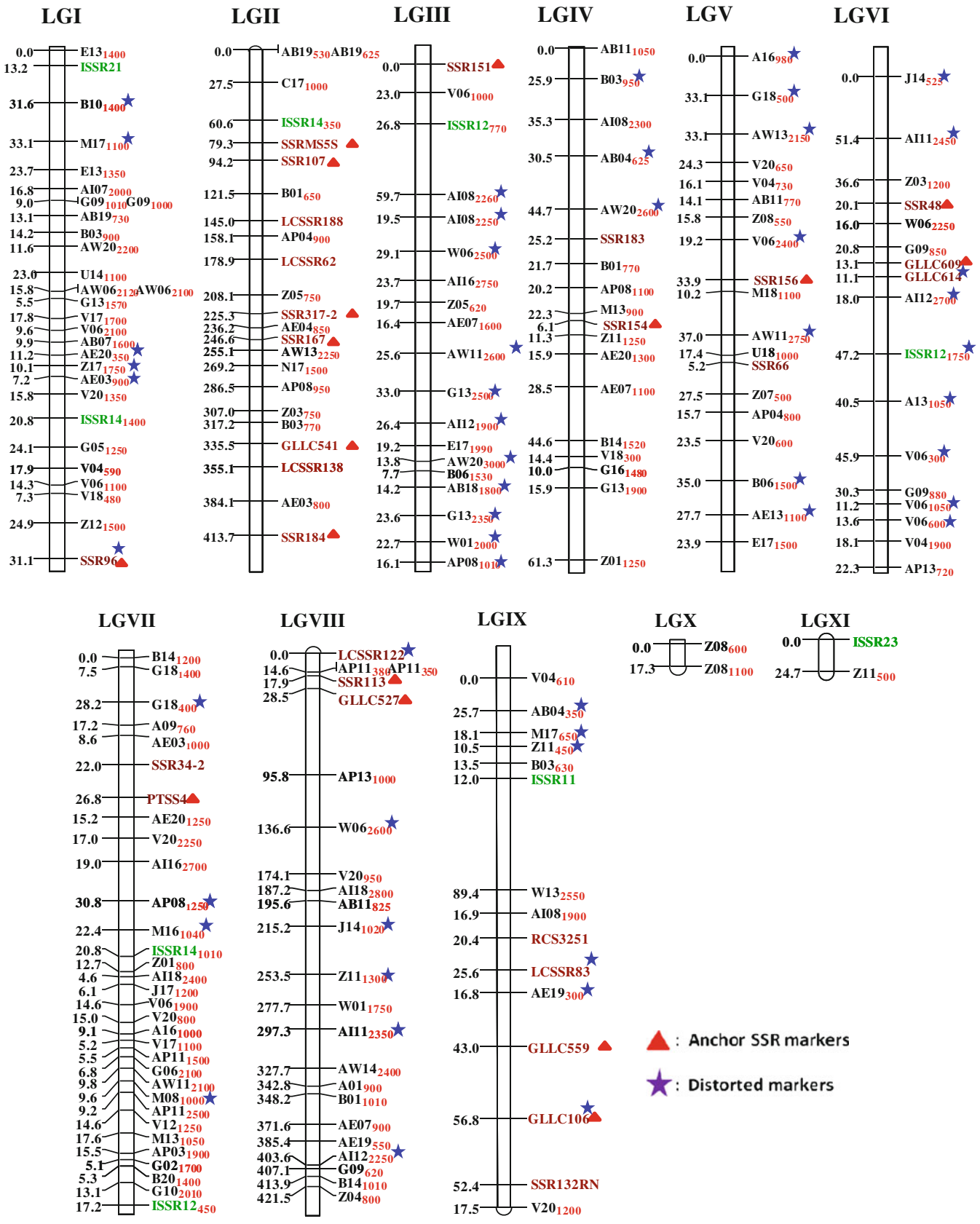


**Figure 1.** Genetic linkage map of lentil showing the distribution of different markers at LOD 4.0. Genetic distances, on the left side on the maps are in centiMorgans (Kosambi function). Loci names are indicated on the right side of vertical bars.

**Table 2.** Number and type of markers assigned to different linkage groups.

| Linkage group | Marker | | | Total |
|---|---|---|---|---|
| | RAPD | ISSR | SSR | |
| LGI | 26 | 2 | 1 | 29 (14.57%) |
| LGII | 13 | 1 | 9 | 23 (11.55%) |
| LGIII | 17 | 1 | 1 | 19 (9.54%) |
| LGIV | 16 | – | 2 | 18 (9.04%) |
| LGV | 17 | – | 2 | 19 (9.54%) |
| LGVI | 13 | 1 | 3 | 17 (8.54%) |
| LGVII | 28 | 2 | 2 | 32 (16.08%) |
| LGVIII | 20 | – | 3 | 23 (11.55%) |
| LGIX | 9 | 1 | 5 | 15 (7.53%) |
| LGX | 2 | – | - | 2 (1.00%) |
| LGXI | 2 | – | - | 2 (1.00%) |
| Total | 162 (81.40%) | 9 (4.52%) | 28 (14.07%) | 199 |

of LGs varying from 17.3 cM to 433.8 cM. The number of marker loci per LG ranged from 2 to 32 (table 2) and the average distance between markers was 19.3 cM. Nine LGs (LGI to LGIX) were longer than 400 cM, whereas two LGs (LGX and LGXI) were less than 25 cM in length. RAPD markers were distributed throughout the genome (table 2). However, ISSRs and SSRs were mapped on six (LGI, LGII, LGIII, LGVI, LGVII and LGIX) and nine of the LGs (LGI to LGIX), respectively. Five of the LGs (LGIII, LGIV, LGVI, LGVIII and LGIX) contained gaps between adjacent markers of more than 35 cM. The distribution of markers between LGs was unequal. There were nine large groups with length varying from 412.7 to 433.8 cM that contained markers in the range of 15–32, and two small groups that contained only two markers each (table 3). The distance between markers on the current map also varied greatly across the different LGs, and the size of the LG did not necessarily reflect the number of linked markers. For instance, LGVII with the highest number of markers (32) (16.08%) covered 432.1 cM with an average marker spacing of 13.50 cM, whereas LGIX spanning a distance of 418.6 cM was covered by only 15 markers.

Variation in marker density was observed as many markers showed pronounced clustering. Four LGs (LGI, LGIII, LGVII and LGVIII) showed clustering of mainly RAPD markers at or near the middle of the LGs (figure 1).

In the present study, 28 of 34 SSRs were mapped on the genetic map. Out of 28 mapped SSRs, 13 (46.42%) produced a single band in both parents, whereas five SSRs produced multiple bands (table 4). It was observed that 10 of the 14 SSRs had amplicon sizes similar to expected size of bands reported earlier. Even if the sizes are the same, the knowledge of allelic relationship of these marker loci to the reported loci (Hamwieh *et al.* 2005, 2009) needs to be verified through sequence information of the SSR allele isolated from one of the parents (ILL 5588) used by Hamwieh *et al.* (2005) and parental lines used in present study. One *T. pratense* SSR also had similar amplicon size as given by Sato *et al.* (2005). Assuming that the 10 loci are allelic, SSR 113 mapped to LGVIII, whereas SSR 107 and SSR 184 were present on LGII as reported in the previous map (Hamwieh *et al.* 2005). Similarly, SSR 107 and SSR 184 mapped to LGII (Phan *et al.* 2007) and SSR 184 also mapped to LGII in the genetic map of Tullu *et al.* (2008). In the present map, SSR 156 mapped to LGV which is in complete agreement with Phan *et al.* (2007) and Gupta *et al.* (2011). However, this locus was mapped to LGVII in the map developed by Hamwieh *et al.* (2005). Out of total 10 allelic loci, SSR 66 and SSR 183 which were previously unmapped were assigned to LGV and LGIV, respectively whereas SSR 132RN and *T. pratense* SSR RCS3251 mapped to LGIX. In addition to these, seven new SSRs—namely LC SSR 62, LC SSR 138 and LC SSR 188 (LGII); GLLC 614 (LGVI); SSR 34–2 (LGVII); LC SSR 122 (LG VIII); LC SSR 83 (LGIX)—have also been assigned map positions in the present linkage map. So the study reports assigning of 11 new SSRs on the present linkage map.

**Table 3.** Characteristics of the intersubspecific genetic linkage map of lentil.

| Linkage group | Length of LGs (cM) | No. of markers | No. of loci | Average marker spacing (cM) |
|---|---|---|---|---|
| LGI | 432.6 | 29 | 27 | 14.91 |
| LGII | 413.7 | 23 | 22 | 17.98 |
| LGIII | 420.2 | 19 | 19 | 22.11 |
| LGIV | 433.8 | 18 | 18 | 24.10 |
| LGV | 412.7 | 19 | 19 | 21.72 |
| LGVI | 416.2 | 17 | 17 | 24.48 |
| LGVII | 432.1 | 32 | 32 | 13.50 |
| LGVIII | 421.5 | 23 | 22 | 18.32 |
| LGIX | 418.6 | 15 | 15 | 27.90 |
| LGX | 17.3 | 2 | 2 | 8.65 |
| LGXI | 24.7 | 2 | 2 | 12.35 |
| Total | 3843.4 | 199 | 195 | Average: 19.3 |

Unlinked markers, 36; total markers, 235.

### *Marker distortion*

Chi-square analysis revealed that 66 markers (59 RAPD, 1 ISSR and 6 SSR) did not segregate according to the expected Mendelian ratio of dominant (3 : 1) and codominant (1 : 2 : 1) markers in $F_2$ progeny. However, 52 of the 66 markers (46 RAPD, 1 ISSR, 5 SSR) were mapped, whereas the remaining 14 (13 RAPD and 1 SSR) remained unlinked. Out of the 66 distorted markers, five RAPDs and two SSRs deviated significantly ($P < 0.05$) and 54 RAPDs, one ISSR and four SSRs deviated very significantly ($P < 0.01$). Of the 52 mapped distorted markers, five, including four RAPD and one SSR, deviated significantly from the expected segregation ratio ($P < 0.05$), and 47, including 42 RAPD, one ISSR and four SSR markers, were highly distorted ($P < 0.01$). Of the 14 unlinked distorted markers, one SSR and one RAPD showed significant deviation ($P < 0.05$) and the other 12, all RAPDs, showed very significant distortion ($P < 0.01$).

**Table 4.** Features of SSR markers assigned on the linkage map.

| Marker name | No. of amplified products | | Expected size (bp) | Nature of inheritance | Location in previous maps | Location in current map |
|---|---|---|---|---|---|---|
| | L830 | ILWL77 | | | | |
| SSR 48 | 1 | 1 | 165 | Codominant | LG 3, LG 1 | LGVI |
| SSR 66* | 2 | 2 | 253 | Codominant | Unmapped | LGV |
| SSR 96* | 2 | 1 | 210 | Dominant | LG 8 | LGI |
| SSR 107* | 1 | 1 | 168 | Codominant | LG 2 | LGII |
| SSR 113* | 1 | 3 | 211 | Codominant | LG 8 | LGVIII |
| SSR 151 | 3 | 3 | 134 | Codominant | LG 3 | LGIII |
| SSR 154 | 1 | 2 | 272 | Dominant | LG 4, LG 5 | LGIV |
| SSR 156* | 1 | 1 | 176 | Dominant | LG 7, LG 5 | LGV |
| SSR 167* | 1 | 3 | 160 | Codominant | LG 5 | LGII |
| SSR 183* | 4 | 4 | 119 | Dominant | Unmapped | LGIV |
| SSR 184* | 2 | 1 | 250 | Dominant | LG 2 | LGII |
| SSR 34–2 | 2 | 1 | 185 | Dominant | Unmapped | LGVII |
| SSR132RN* | 1 | 1 | 330 | Codominant | Unmapped | LGIX |
| SSR 317-2* | 1 | 1 | 120 | Codominant | LG 1 | LGII |
| PTSS4 | 1 | 1 | NA | Dominant | LG 6 | LGVII |
| RCS 3251* | 4 | 3 | 113 | Codominant | Unmapped | LGIX |
| SSR MS 5S | 2 | 2 | 181 | Dominant | LG 5 | LGII |
| GLLC 106 | 1 | 1 | NA | Codominant | LG 2 | LGIX |
| GLLC 527 | 1 | 1 | NA | Codominant | LG 8 | LGVIII |
| GLLC 541 | 1 | 2 | NA | Dominant | LG 11 | LGII |
| GLLC 559 | 1 | 1 | NA | Codominant | LG 12 | LGIX |
| GLLC 609 | 2 | 1 | NA | Codominant | LG 2 | LGVI |
| GLLC 614 | 2 | 1 | NA | Codominant | Unmapped | LGVI |
| LC SSR 62 | 1 | 1 | NA | Codominant | Unmapped | LGII |
| LC SSR 83 | 1 | 1 | NA | Dominant | Unmapped | LGIX |
| LC SSR 122 | 1 | 1 | NA | Codominant | Unmapped | LGVIII |
| LC SSR 138 | 1 | 2 | NA | Dominant | Unmapped | LGII |
| LC SSR 188 | 1 | 1 | NA | Codominant | Unmapped | LGII |

Markers with polymorphic bands similar to expected size as reported by Hamwieh *et al.* (2005, 2009) and Sato *et al.* (2005) are highlighted with an asterisk; NA, not available.

In general, the aberrant markers were located on all the LGs, except LGX and LGXI, and were positioned at the end or middle of the LGs. The distribution of distorted markers revealed that RAPD markers showed the maximum distortion. The highest and least numbers of distorted markers were present on LGIII and LGII respectively, and the most distorted RAPD markers were mapped to LGIII. In the present study, out of 17 anchored SSRs, seven—namely SSR 107 and SSR 184 (LGII); SSR 113 and GLLC 527 (LGVIII); SSR 151 (LGIII); SSR 154 (LGIV); SSR 156 (LGV)—were mapped to be same LG and 10 were mapped to different LGs compared to previous studies. As the physical size of the lentil genome was estimated to be 4063 Mbp, one cM of the present map translates on average to 1.05 Mbp.

## Discussion

One of the major objectives in lentil research remains development of detailed linkage maps. The use of molecular markers for developing maps and other breeding applications, however, has been limited in lentil by the low levels of genetic variation at species level and the lack of marker resources available. A pressing need in legume genomics is to integrate knowledge gained from the study of model legume genomes with the biological and agronomic questions of importance in the crop species. A high level of colinearity has been found between the faba bean, lentil and *M. truncatula* genomes based on commonly mapped orthologous markers (Ellwood *et al.* 2008). Using cross-genera SSRs, a low level of polymorphism was observed among parental genotypes as detected by differences in the size and number of amplification products obtained. At the same time, variation in number and size of the amplified fragments with *M. truncatula* and *T. pratense* SSRs was also observed. Transferability of microsatellites on a panel of six lentil accessions was 36.0%, 62.0% and 25.0% for *M. truncatula*, *P. sativum* and *T. pratense*-derived markers (Reddy *et al.* 2009). A high level of cross-genera SSR transferability from *M. truncatula* to lentil has been reported for EST-SSR (82.60%) and genomic SSRs (98.14%) with polymorphism of 7.9% and 26.4%, respectively (Gupta *et al.* 2011). The present study indicated the potential for transfer of genomic and EST-SSR markers from closely related taxa, circumventing laborious cloning and screening procedures involved in characterizing SSR loci for target species.

Some of the previously developed *Lens* maps were constructed using a wild relative as one of the parents of the mapping population for increasing the level of polymorphism available for mapping (Havey and Muehlbauer 1989a; Paterson *et al.* 1990; Eujayl *et al.* 1997; Durán *et al.* 2004). Previous *Lens* maps differ from our map with respect to marker type and number, genome coverage and marker density (Havey and Muehlbauer 1989b; Weeden *et al.* 1992; Eujayl *et al.* 1998; Rubeena *et al.* 2003; Durán *et al.* 2004; Hamwieh *et al.* 2005; Tullu *et al.* 2008; Tanyolac *et al.* 2010; Gupta *et al.* 2011). The differences in the crossing-over frequency can influence marker density in LGs. The uneven marker distribution has been ascribed to the presence of centromeric heterochromatin, and in some instances telomeres experience up to 10-fold less recombination (Tanksley *et al.* 1992). Heterogeneity in recombination along the genome has implications for 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 recombination-suppressed regions requires much larger progeny sizes to allow for rare recombination events, which is necessary for construction of fine maps.

Our genomic map comprises 199 markers covering 3843.4 cM with average marker spacing of 19.3 cM. In comparison, the earlier maps (Havey and Muehlbauer 1989a; Weeden *et al.* 1992) were only sparsely covered with markers spanning 330 and 560 cM of genome with average marker distance of 12.8±3.2 cM and 8.75 cM respectively. The first intersubspecific lentil map obtained by Eujayl *et al.* (1998) covered 1073 cM with average marker spacing of 6.0 cM, whereas the lentil linkage map produced by Durán *et al.* (2004) based on intersubspecific population contained 10 LGs spanning a map distance of 2172.4 cM. A comprehensive intersubspecific map was developed by Hamwieh *et al.* (2005) by enriching the previous map of Eujayl *et al.* (1998) with 39 new lentil-specific SSRs and 50 new AFLP markers. They generated a lentil linkage map using 283 DNA markers covering the genome with an average marker distance of 2.6 cM and total coverage of about 751 cM. Recently, Gupta *et al.* (2011) enhanced the existing lentil linkage map of Phan *et al.* (2007) with the addition of EST-SSR/SSR markers from *M. truncatula*, and the relative location(s) of QTL governing seedling or pod maturity and resistance to *Aschochyta lentis* were determined. The map comprised 11 LGs varying in length from 359.4 cM to 2.4 cM and covered a total distance of 1392.5 cM with an average marker density of 7.1 cM.

In interspecific crosses, reduced recombination (Paterson *et al.* 1990) or chromosomal rearrangements (Tadmor *et al.* 1987) may lead to segregation distortion and nonrepresentative genetic distances and linkage relationships. Segregation distortion in intraspecific crosses can be explained by small chromosomal rearrangements with little impact on fertility and chromosome pairing during meiosis (Slocum *et al.* 1990) or linkage to a lethal allele in gametes (Pillen *et al.* 1992; Wagner *et al.* 1992). A clustering of distorted loci has often been reported within the LGs constructed in several species (Graner *et al.* 1991; Kammholz *et al.* 2001). A similar trend of clustering of markers was previously observed in field pea and chickpea (Laucou *et al.* 1998; Winter *et al.* 2000). Even though segregation distortion can be seen in intraspecific crosses, use of the *Lens culinaris* ssp. *orientalis* accession as one of the parents might have elevated the probability of reduced recombination in the mapping population and hence segregation distortion. Durán *et al.* (2004) reported 24.5% marker distortion in $F_2$ intersubspecific population with 17 RAPDs, 13 ISSRs, 41 AFLPs and one SSR deviating from Mendelian segregation. Different levels of segregation distortion in lentil mapping populations have been observed (Hamwieh *et al.* 2005; Rubeena *et al.* 2006; Phan *et al.* 2007; Tullu *et al.* 2008; Tanyolac *et al.* 2010; Saha *et al.* 2010; Gupta *et al.* 2011).

## Conclusions

Lentil has poor genomic resources and development of SSR markers is both cost and labour intensive compared to other marker systems. Comparative genomics and synteny analyses with closely related legumes promises to advance knowledge of the lentil genome and provide lentil breeders with additional genes and selectable markers for use in MAS. In this study, a successful attempt has been made to examine the cross-species transferability of SSRs from related legume species into the *Lens* genome. Transferability of SSR loci across species has been found to be advantageous in lentil because this legume suffers from lack of polymorphism and genomic resources. The study has implications in large-scale development and identification of markers which can be used for construction of saturated genetic maps and further genetic studies in lentil.

In this study, it was found that RAPD, ISSR and SSR (including cross-genera SSRs) markers were useful for constructing a genetic linkage map of lentil. We also report assigning of 11 new SSRs on the linkage map, including one red clover SSR, five newly developed genomic SSRs, and five previously developed unmapped SSRs. The $F_2$ population produced for linkage map construction exhibited sufficient polymorphism of DNA markers, including variation for rust resistance and other agromorphological traits. A population of $F_6$ recombinant inbred lines (RILs) has been produced from this $F_2$ mapping family which will be further exploited for mapping of traits of agronomic value. $F_2$ and RIL maps can be further joined to develop a saturated linkage map of lentil for use in QTL mapping and marker-aided breeding. However, there is dire need to develop and map more functional markers like EST-SSRs and SNPs on such maps to enhance their relevance in lentil genetics and breeding.

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