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ORIGINAL PAPER

Extensive macrosynteny between *Medicago truncatula* and *Lens culinaris* ssp. *culinaris*

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Abstract The first predominantly gene-based genetic linkage map of lentil (*Lens culinaris* ssp. *culinaris*) was constructed using an F_5 population developed from a cross between the cultivars Digger (ILL5722) and Northfield (ILL5588) using 79 intron-targeted amplified polymorphic (ITAP) and 18 genomic simple sequence repeat (SSR) markers. Linkage analysis revealed seven linkage groups (LGs) comprised of 5–25 markers that varied in length from 80.2 to 274.6 cM. The genome map spanned a total length of 928.4 cM. Clear evidence of a simple and direct macrosyntenic relationship between lentil and *Medicago truncatula* was observed. Sixty-six out of the 71 gene-based markers, which were previously assigned to *M. truncatula* genetic and physical maps, were found in regions syntenic between the *Lens* c. ssp. *culinaris* and *M. truncatula* genomes. However, there was evidence of moderate chromosomal rearrangements which may account for the difference in chromosome numbers between these two legume species. Eighteen common SSR markers were used to connect the current map with the most comprehensive and recent map that exists for lentil, providing the syntenic context of four important domestication traits. The composite map presented, anchored with orthologous markers mapped in *M. truncatula*, provides a strong foundation for the future use of genomic and genetic information in lentil genetic analysis and breeding.

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Introduction

Comparative genomic mapping is the analysis of the chromosomal organization of genetic information based on gene content and gene order between species belonging to different taxa. Comparative genomic mapping has demonstrated that plants have retained different levels of conservation in their genomes during evolution depending on their phylogenetic separation (Choi et al. 2004a, b; Paterson et al. 2000; Zhu et al. 2005). These conserved regions, so called syntenic or orthologous regions, have collinear gene contents when compared either genetically or physically.

There has been a long history of comparative genetic mapping in legumes dating back to "The law of homologous variation" (Vavilov 1922). Comparative mapping is entirely dependent on the use of markers with demonstrable orthology. As morphological and isozyme markers are generally unsuitable, the introduction of molecular markers was critical. Early pioneering studies suggested a simple genetic relationship between pea and lentil (Weeden et al. 1992) and within

grasses and crucifers shortly thereafter. Subsequent comparative genetic studies of increasing sophistication have demonstrated differing degrees of synteny between chickpea (*Cicer arietinum*) and pea (*Pisum sativum*, Simon and Muehlbauer 1997), mungbean (*Vigna radiata*) and cowpea (*V. unguiculate*, Menancio-Hautea et al. 1993), mungbean and lablab (*Lablab purpureus*, Humphry et al. 2002), and alfalfa (*Medicago sativa*) and pea (Kalo et al. 2004).

Building on these foundations has required the generation of abundant genomic and genetic resources focussed around model species. In the case of legumes, the two models are *M. truncatula* and *L. japonicus.* Early work tended to use RFLP probes based on the genome sequence of model species. Although RFLP probes are more polymorphic and better at detecting duplications, they are laborious and have therefore been superseded by PCR techniques. PCR-based, co-dominant marker systems for comparative genomics have markedly increased the efficiency of transferring genetic information across species. In this approach, oligonucleotide primers are designed from sequences of conserved regions, for example in gene exons that span polymorphic regions such as introns or microsatellites. Examples include the comparison of *Medicago truncatula* with alfalfa, pea, chickpea and lupins (Aubert et al. 2006; Choi et al. 2004a, b; Nelson et al. 2006); reviewed in Zhu et al. (2005).

Lentil (*Lens culinaris ssp. culinaris*) is an important legume crop grown widely throughout the Indian subcontinent, western Asia, northern Africa, southern Europe, North and South America and Australia (Erskine 1996). It is an important source of dietary protein in both human diet and animal feed and helps in the management of soil fertility. Lentil is a diploid ($2n = 2x = 14$ chromosomes) self-pollinating annual crop, with a haploid genome size of 4,063 Mbp (Arumuganathan and Earle 1991). Initially, genetic maps consisted of small numbers of morphological, isozyme and restriction fragment length polymorphism (RFLP) markers which covered only a small proportion of lentil genome (Havey and Muehlbauer 1989; Tahir et al. 1993; Weeden et al. 1992). More recently, dominant molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeats (Durán et al. 2004; Eujayl et al. 1997; Rubeena et al. 2003) have been used. The most recent map was produced using 41 genomic SSR and 45 AFLP markers (Hamwieh et al. 2005).

Comparative genetic maps not only reveal patterns of chromosomal evolution between species, but are also valuable tools for crop improvement as they allow the comprehensive resources of a model species, in this case *M. truncatula*, to be applied to the crop species. The main objectives in the current study were: (1) to develop a gene-based genetic map of lentil; (2) to characterise syntenic relationships with *M. truncatula*; and (3) to integrate the resulting genic and comparative map with the most recent and comprehensive genetic map of lentil.

Materials and methods

Genetic mapping population

A population of 94 F_5 RILs was used to create a genetic map of the cultivated lentil genome. The population was produced via single seed descent from a cross between the Jordanian landrace Northfield (ILL5588) and the ICARDA cultivar Digger (ILL5722; made from a cross between the ILL883 and ILL470 landraces). The population was made at the Grains Innovation Park of the Victorian Department of Primary Industries, Horsham, Australia. Northfield is moderately resistant to *Ascochyta lentis* (Erskine 1996; Ford et al. 1999) and Digger is broadly adapted to the Australian lentil growing regions and contains desirable seed quality characteristics (Brouwer 1995). Both accessions were included in the Australian lentil breeding program at its inception in 1994. Total genomic DNA was isolated from 100 mg of young leaf material of 10-day-old seedlings of each parent and F_5 individual (Ellwood et al. 2006).

Primer sets

Two types of intron-targeted amplified polymorphic sequence (ITAP) primers were utilised in this study: the 'ML' primers were developed from alignment of *M. truncatula* and *Lupinus* spp. database EST sequences, as described in Nelson et al. (2006). The 'MLG' primers were designed from alignment of *M. truncatula*, *Lupinus albus*, and *Glycine max* EST sequences available from the NCBI dbEST in September 2005, together with an additional 1140 *L. albus* root EST sequences provided by Prof. Carroll Vance (USDA-ARS). Five hundred ITAP markers were produced. The majority of the primers were designed from genes in characterized chromosomal regions, so that the resultant ITAP markers could be physically mapped using *M. truncatula* BACs. In addition, 126 cross-species 'MP' markers developed by the Department of Plant Pathology, University of California, Davis, USA, were included in this study (Choi et al. 2004a).

Polymorphism detection

Each primer pair was screened for ability to amplify a clear unilocus amplicon in both lentil parental DNAs. PCRs were carried out as described in Nelson et al. (2006). Different methods were used to genotype the F_5 population depending on types of polymorphism identified (Table S1). Amplicons with distinct length (>10 bp) polymorphisms were separated directly on 2% agarose gels and visualised under UV-light after staining with ethidium bromide. Small differences in amplicon size (<10 bp) were resolved on an AB 3730 capillary sequencer (AB, Applied Biosystems USA), using fluorescently labelled primers and the GeneScan™-500 LIZ® size standard (AB). Genotyping was performed using the AB GeneMapper program. Amplicons exhibiting the same size in each parent by agarose gel electrophoresis were purified and sequenced directly as described in Nelson et al. (2006). Sequence polymorphisms were identified by manual inspection of alignments using Vector NTI software (Invitrogen, Carlsbad, CA, USA). Restriction enzyme sites were also identified using Vector NTI software. Amplicons were digested with the appropriate restriction enzyme in accordance with the manufacturer's instructions (http://www.rebase.neb.com), run on 2% agarose gels, and visualised under UV-light after staining with ethidium bromide. Where no differential restriction enzyme sites were available, single nucleotide polymorphism (SNP) marker primers were designed and genotyping performed using SNaPshot (AB), and fluorescent products analysed on an AB 3730 capillary sequencer. Alternatively, bi-directional allele-specific PCR was performed as described by Delye et al. (2002). For this, two internal allele-specific primers were designed, where the 3′ end of the primers corresponded to the SNP site. Together with the two external primers, the PCR reaction produces three amplicons (two specific for each parental genotype and one in common). All resultant amplicons were resolved on 2% agarose gels, stained with ethidium bromide, and visualised under UV-light.

Map construction

Chi-square analysis ($P < 0.05$) was applied to test the segregation of the mapped markers against the expected Mendelian segregation ratio for co-dominant inheritance in the lentil F_5 RIL population. Genetic linkage mapping was conducted with MultiPoint software (MultiQTL Ltd, Institute of Evolution, Haifa University, Israel), with a recombination fraction (rf) of 0.32 (LOD = 9.0). Map distances were calculated in cM by applying the "Kosambi" function.

Integration of an existing map with the current lentil genic and comparative map

To integrate with the lentil genetic map developed by Hamwieh et al. (2005), 30 lentil SSR primer pairs used in that study were deployed to screen the two lentil parental lines, Digger and Northfield. Length polymorphisms, detected by 3.5% agarose gels, were used to genotype all 94 F_5 RIL individuals. The PCR conditions for these markers were as described in Hamwieh et al. (2005).

Results

Gene-based marker amplification and genetic map properties

Three hundred and eighty of the 626 ITAP markers that were screened amplified PCR products in the *L. c.* ssp. *culinaris* genome. Seventy-four percent (281) of these products were unambiguous single band amplicons (Table 1). Ninety-eight polymorphic markers were identified, of which 79 were used to genotype the 94 individuals of the F_5 RIL population. GenBank accession numbers, primer sequences, and detection methods are given in Table S1. Of the three genic marker groups, the 'MLG' markers represented the highest amplification rate, followed by the 'MP' markers, the lowest being the 'ML' markers. However, all three marker types produced a similar level of polymorphism (approximately 35%; Table 1).

In addition, 18 of 30 lentil SSR primer pairs developed by Hamwieh et al. (2005) were polymorphic between the Digger and Northfield parental genomes and were used to map the F_5 RIL population (Table 2). The data obtained was used together with the 79 genic markers to develop a composite genetic map of lentil. Table 2 provides the marker name, number of amplified PCR products, estimated size of mapped/polymorphic markers, nature of inheritance, and the location in each map for each SSR marker. Sixty-seven percent of the SSR markers produced single band in both the parents which is similar to that reported by Hamwieh et al. (2005). In comparison to that study, 14 of the 18 SSR markers mapped here exhibited amplicons of the same size. Three markers produced multiple bands, and the polymorphic bands were different in size to those reported (Table 2). Four markers were not assigned to any LG in the previous map. These markers were

Table 1 Efficiency of genic markers used to construct the comparative genetic linkage map of lentil

Marker type	Screened	Amplification[a]	Sequenced	Polymorphism[b]	Mapped
MP	126	102 (81%)	95	31 (33%)	24
ML	350	146 (42%)	100	37 (37%)	30
MLG	150	132 (88%)	86	30 (35%)	24
Total	626	380	281	98	79

[a] Figures in parentheses are percentages of amplified markers of the total markers screened

[b] Figures in parentheses are percentages of polymorphic markers of the total sequenced markers

Table 2 Lentil SSR markers mapped in the F_5 RIL population developed from Digger (D) × Northfield (NF)

Marker name[a]	No. of amplified products		Estimated size of mapped markers (bp)		Nature of inheritance	Location in previous map	Location in current map
	D	NF	D	NF			
SSR 33	1	1	274	289	Co-dominant	LG_3	LG-I
SSR 48	1	1	185	165	Co-dominant	LG_3	LG-I
SSR 59*	4	4	145	155	Co-dominant	Unmapped	LG-IV
SSR 99	1	1	152	161	Co-dominant	Unmapped	LG-I
SSR 107	1	1	180	168	Co-dominant	LG_2	LG-II
SSR 119	1	1	255	266	Co-dominant	LG_8	LG-III
SSR 151	1	3		134	Dominant	Unmapped	LG-III
SSR 156	1	1	188	176	Co-dominant	LG_7	LG-V
SSR 184	1	1	215	250	Co-dominant	LG_2	LG-II
SSR 199	2	2	190	182	Co-dominant	LG_1	Unlinked
SSR 204	1	1	177	186	Co-dominant	LG_1	LG-IV
SSR 212	1	1	170	181	Co-dominant	LG_6	LG-VI
SSR 215A*	2	2	425	410	Co-dominant	LG_2	LG-II
SSR 233	3	2	170		Dominant	Unmapped	LG-IV
SSR 302	2	2	250	261	Co-dominant	LG_1	LG-IV
SSR 317-1	1	1	230	308	Co-dominant	LG_1	LG-IV
SSR 323*	1	1	225	330	Co-dominant	LG_5	LG-III
SSR336	1	1	263	253	Co-dominant	LG_1	Unlinked

[a] Markers with polymorphic bands different in size to those previously found by Hamwieh et al. (2005) are highlighted with an asterisk

either unmapped in that study (SSR 99 and SSR 151) and produced multiple products (SSR 59), or originated from Digger (SSR 233) which was not a parent in the previous map.

Twelve of the mapped markers, including two SSR and 10 genic markers, deviated significantly ($P < 0.05$) from the expected Mendelian inheritance ratio of 1:1. Of these, one SSR and two genic markers were highly distorted ($P < 0.01$). With the exception of the 'GLUT' marker in LG 2, all of the distorted markers segregated in favour of the Northfield parent.

The general features of the comparative map are summarised in Table 3 and Fig. 1. The maximum recombination fraction (rf) was 0.32 (LOD = 9; Purcell 2001). Of the 91 mapped markers, 75 were genic and 16 were SSR markers. These comprised seven linkage groups (LG) that varied in length from 80.2 to 274.6 cM. Six markers remained unlinked. The map spanned a total of 928.4 cM, and linkage groups were assigned Roman numerals (LG I to LG VII) to distinguish them from the Arabic numbering used by Hamwieh et al. (2005). The number of markers per LG ranged from five to 25 (Fig. 1 and Table 3). Twenty-two markers co-segregated at eight loci, ranging from two to six markers per locus (three loci in LG-I, three in LG-II, two in LG-III, Fig. 1 and Table 3). The maximum distances between markers ranged from 20.1 cM in LG-III to 41 cM in LG-I with an overall mean gap distance of 13.5 cM (Table 3).

Comparison of the position of genic markers in the lentil map with *M. truncatula*

Sixty-six of the 71 genic mapped markers were previously assigned to the *M. truncatula* genetic and physical maps (http://www.medicago.org/genome/). Comparison of the genetic map locations of these markers revealed clear evidence of a simple and direct macrosyntenic relationship between the *L. c.* ssp. *culinaris* and *M. truncatula* genetic maps (Fig. 2). In particular, extensive collinearity was observed between lentil LG-II and Mt LG-8. All of the orthologous markers mapped to lentil LG-I were in syntenic regions of either Mt LG-4 or Mt LG-7. Five genic markers with

Table 3 Properties of the lentil comparative genetic map

Linkage group	Length of LGs (cM)	No. of markers	No. of loci	Average marker spacing (cM)	Largest distance b/t markers (cM)	No. of orthologous markers/Mt LG
LG-I	274.6	25	21	13.7	41	18/4,7
LG-II	119.1	21	12	10.8	30.6	18/8
LG-III	81.7	17	15	5.8	20.1	14/1,3,6
LG-IV	217.3	10	10	24.1	37.4	3/2
LG-V	72.5	7	7	12.1	30	5/5
LG-VI	83	6	6	16.6	37.6	4/3
LG-VII	80.2	5	5	20.1	34	4/3
Total	928.4	97	76			

known locations in the *Medicago* LG-5 were mapped in lentil LG-V (Fig. 2).

However, there was also evidence of moderate chromosomal rearrangement: for example, the merging of *M. truncatula* chromosomes 4 and 7 as well as 6 and 1 to form the *L. c.* ssp. *culinaris* LG-I and LG-III, respectively, and the splitting of *M. truncatula* chromosome 3 into LG-VI and LG-VII in *L. c.* ssp. *culinaris*. Inversions and insertions were observed among the orthologous markers within each syntenic pair of lentil and Mt LGs. Of the seven lentil LGs, all of the genic markers mapped in LG-II and LG-III were in syntenic regions with Mt LG-8, 6 and 3. The remaining LGs each had one or two genic markers which were either unmapped in Mt or not within a syntenic region (Table 3 and Fig. 2).

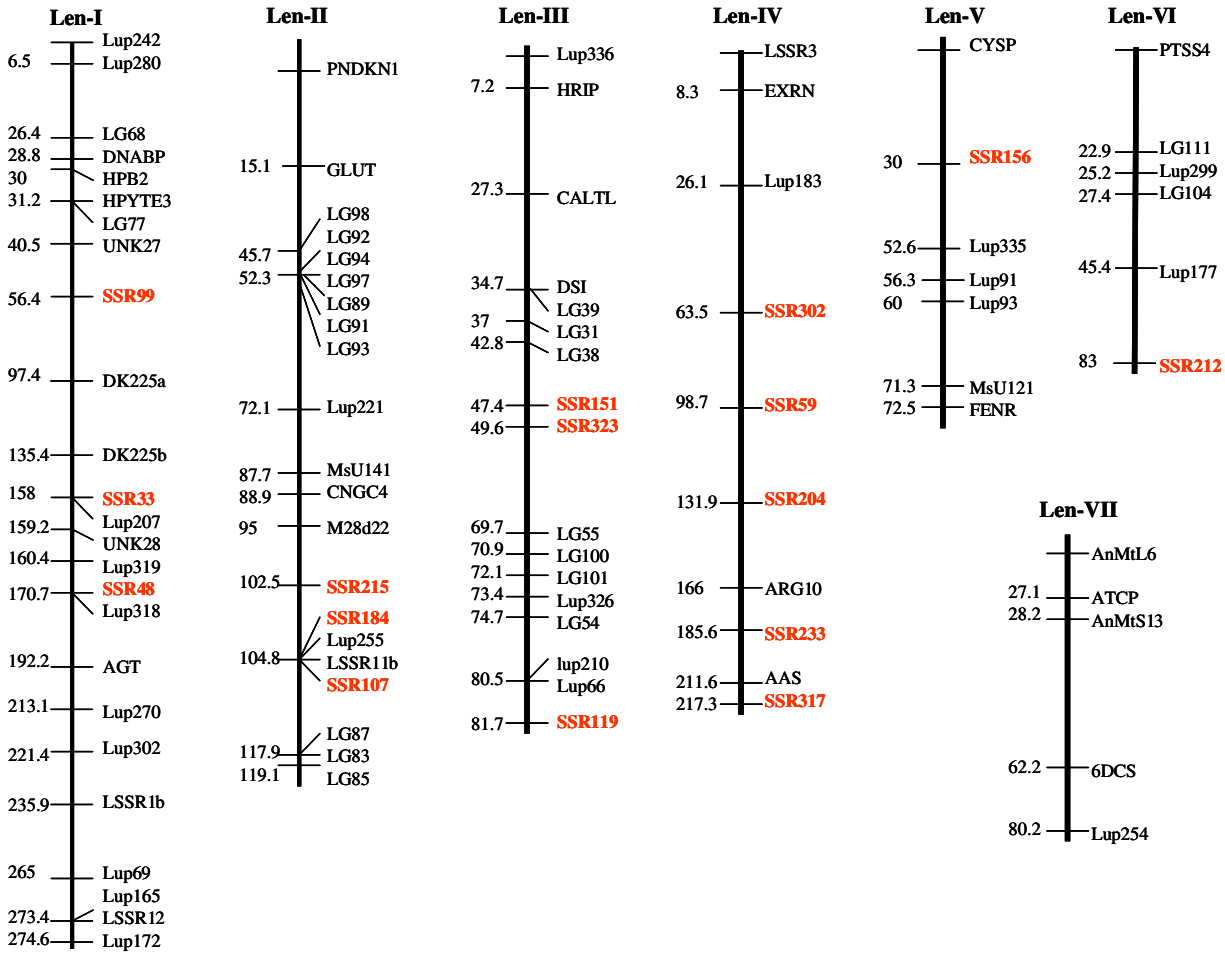

Fig. 1 A gene and SSR-based genetic linkage map of lentil (*Lens culinaris* ssp *culinaris*). SSR markers are shown in *red* and *bold*. Marker distances are given in cM

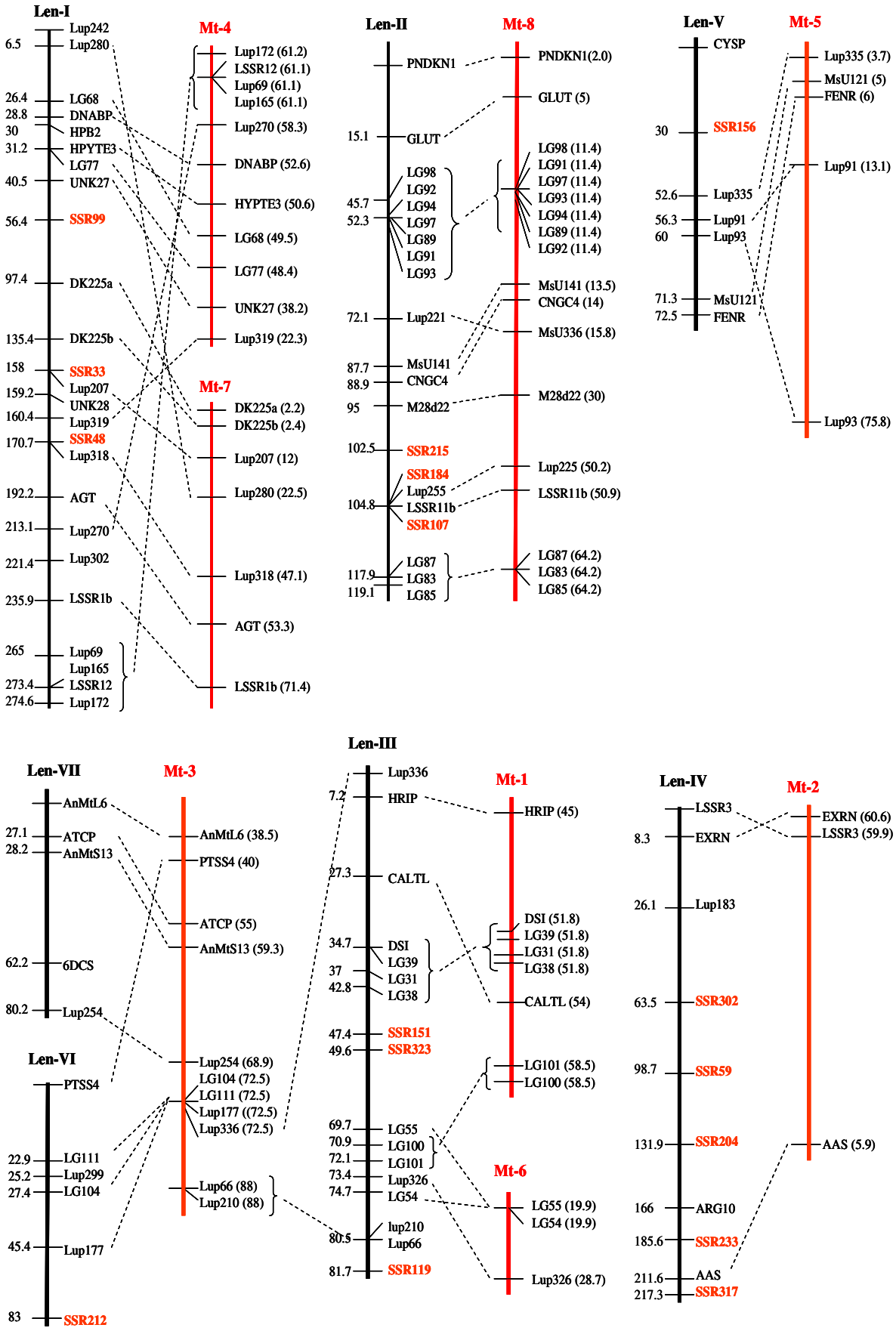
Len-I
Lup242
6.5 Lup280
26.4 LG68
28.8 DNABP
30 HPB2
31.2 HPYTE3
LG77
40.5 UNK27
56.4 SSR99
97.4 DK225a
135.4 DK225b
158 SSR33
159.2 Lup207
UNK28
160.4 Lup319
170.7 SSR48
Lup318
192.2 AGT
213.1 Lup270
221.4 Lup302
235.9 LSSR1b
265 Lup69
Lup165
273.4 LSSR12
274.6 Lup172
Mt-4
Lup172 (61.2)
LSSR12 (61.1)
Lup69 (61.1)
Lup165 (61.1)
Lup270 (58.3)
DNABP (52.6)
HYPTE3 (50.6)
LG68 (49.5)
LG77 (48.4)
UNK27 (38.2)
Lup319 (22.3)
Mt-7
DK225a (2.2)
DK225b (2.4)
Lup207 (12)
Lup280 (22.5)
Lup318 (47.1)
AGT (53.3)
LSSR1b (71.4)
Len-II
PNDKN1
15.1 GLUT
LG98
LG92
45.7 LG94
52.3 LG97
LG89
LG91
LG93
72.1 Lup221
87.7 MsU141
88.9 CNGC4
95 M28d22
102.5 SSR215
SSR184
Lup255
104.8 LSSR11b
SSR107
LG87
117.9 LG83
119.1 LG85
Mt-8
PNDKN1(2.0)
GLUT (5)
LG98 (11.4)
LG91 (11.4)
LG97 (11.4)
LG93 (11.4)
LG94 (11.4)
LG89 (11.4)
LG92 (11.4)
MsU141 (13.5)
CNGC4 (14)
MsU336 (15.8)
M28d22 (30)
Lup225 (50.2)
LSSR11b (50.9)
LG87 (64.2)
LG83 (64.2)
LG85 (64.2)
Len-V
CYSP
30 SSR156
52.6 Lup335
56.3 Lup91
60 Lup93
71.3 MsU121
72.5 FENR
Mt-5
Lup335 (3.7)
MsU121 (5)
FENR (6)
Lup91 (13.1)
Lup93 (75.8)
Len-III
Lup336
7.2 HRIP
27.3 CALTL
34.7 DSI
LG39
37 LG31
42.8 LG38
47.4 SSR151
49.6 SSR323
69.7 LG55
70.9 LG100
72.1 LG101
73.4 Lup326
74.7 LG54
lup210
80.5 Lup66
81.7 SSR119
Mt-1
HRIP (45)
DSI (51.8)
LG39 (51.8)
LG31 (51.8)
LG38 (51.8)
CALTL (54)
LG101 (58.5)
LG100 (58.5)
Mt-6
LG55 (19.9)
LG54 (19.9)
Lup326 (28.7)
Len-VII
AnMtL6
27.1 ATCP
28.2 AnMtS13
62.2 6DCS
80.2 Lup254
Mt-3
AnMtL6 (38.5)
PTSS4 (40)
ATCP (55)
AnMtS13 (59.3)
Lup254 (68.9)
LG104 (72.5)
LG111 (72.5)
Lup177 ((72.5)
Lup336 (72.5)
Lup66 (88)
Lup210 (88)
Len-VI
PTSS4
22.9 LG111
25.2 Lup299
27.4 LG104
45.4 Lup177
83 SSR212
Len-IV
LSSR3
8.3 EXRN
26.1 Lup183
63.5 SSR302
98.7 SSR59
131.9 SSR204
166 ARG10
185.6 SSR233
211.6 AAS
217.3 SSR317
Mt-2
EXRN (60.6)
LSSR3 (59.9)
AAS (5.9)

Fig. 2 Evidence of macrosynteny between the *L. c.* ssp. *culinaris* and *Medicago truncatula* genomes. Orthologous markers between pairs of LGs are indicated by *broken lines*. Lentil LGs are shown in *black* and the Medicago LGs are shown in *red*. SSR markers are in *red* and *bold*. Marker distances are provided in cM

Identification of corresponding linkage groups between an existing lentil map and the gene-based genetic map

Eighteen SSR anchor markers developed by Hamwieh et al. (2005) were used to connect the two maps. LG-I, II, III, IV, V and VI in the current map correspond to LG3, 2, 5 + 8, 1, 7 and 6, respectively, in the previous map (S2). This designation was based on markers that produced discrete single amplicons of the same size that were used in the construction of both maps. No comparative markers were detected between the LG-VII and LG4 in the earlier map.

Discussion

The first predominantly gene-based consensus map of lentil was established using an F_5 RIL population generated from the cultivars Digger and Northfield. The map is the first to consist of seven linkage groups which corresponds to the cytogenetically determined number of chromosomes in lentil (*L. culinaris*, $n = 7$). The map is also the first linkage map of lentil to be constructed entirely with co-dominant markers. The markers were strategically chosen so that this map can be used to establish syntenic relationships between lentil and the model legume species, *M. truncatula*, and to join this map to the most recent and comprehensive map available for this species (Hamwieh et al. 2005). All 30 SSR primer pairs from that study amplified the parental DNA here as expected. However, the polymorphism detected in this study was much lower (60%) than previously reported (90%). This may be the result of a lower level of diversity existing between Digger and Northfield than that of L 692-16-1(s) × Northfield, or differences in polymorphism detection techniques.

Two characters observed for previous lentil maps were also observed here. These were the clustering of markers in or adjacent to the middle of the linkage groups, and markers with distorted segregation (reviewed by Laucou et al. 1998; Muehlbauer et al. 2006; Winter et al. 2000). In the current study, the clustering of markers may have been due to their gene-based design, as genic markers tend to group together in isolated blocks (King 2002). This may also account for the relatively large distances that were detected between groups of markers on LG-I, LG-IV, LG-VI and LG-VII (Table 3). Twelve percent of the markers in the current map segregated in a distorted fashion, which was comparable to the previous map (9.5% of SSR and 17.8% of AFLPs, Hamwieh et al. 2005). However, since the mapping population used for the previous map was selected for a low level of segregation distortion during early generations (Eujayl et al. 1997), the 12% rate observed in this study was considered relatively low. Much higher levels of marker segregation distortion (38.4%) were reported for a *Cicer* sp. $F_{6:8}$ RIL population (Winter et al. 2000) and in other lentil crosses levels as high as 83.3% have been observed (Eujayl et al. 1997). Marker distortion may result from many factors such as recessive alleles, structural rearrangement or differences in DNA content, abortion of male and female gametes, and the selection of a particular gametic genotype during the construction of a RIL mapping population (Barzen et al. 1995; Berry et al. 1995; Jenczewski et al. 1997; Quillet et al. 1995; Tadmor et al. 1987; Xu et al. 1997). A significant increase in the number of loci that deviated from the expected Mendelian inheritance from F_2 to F_7 generations was observed in tomato (Paran et al. 1995). This increase was suggested to be due to an accumulative effect of selection against alleles of one of the parents during RIL propagation.

Comparative mapping has shown a direct and simple relationship between the *M. truncatula* and *L. culinaris* ssp. *culinaris* chromosomes, with complete homology evident (Fig. 2). High levels of conservation have previously been reported between closely related legumes such as *L. culinaris* ssp. *culinaris* and *P. sativum* (Weeden et al. 1992), *M. sativa* and *P. sativum* (Kalo et al. 2004), *M. truncatula* and *P. sativum* (Aubert et al. 2006), *M. truncatula* and *M. sativa* (Choi et al. 2004a), and at different levels among *M. truncatula*, *M. sativa*, *P. sativum*, *V. radiata*, *G. max*, and *Phaceolus vulgaris* (Choi et al. 2004b). The moderate level of chromosomal rearrangements observed in this study (Fig. 2) may account for the differences in chromosome numbers between the two species (*M. truncatula*: $n = 8$; *L. culinaris* ssp. *culinaris*: $n = 7$). The high level of macrosynteny found in this study will undoubtedly facilitate the identification of markers closely linked to traits of interest in *L. culinaris* ssp. *culinaris*.

Cross-reference to the abundance of genetic information of Medicago from the genome sequence data (http://www.medicargo.org) and extensive EST libraries available for the model legume species, coupled with the current map has opened up a feasible and effective approach through synteny to identify closely

linked markers to traits, candidate genes, and to expedite the isolation of important genes. A similar approach has proved useful in other families such as the Solanaceae (Huang et al. 2005), Poaceae (Armstead et al. 2005; Börner et al. 1998; Mammadov et al. 2005), Rosaceae (Dirlewanger et al. 2004), and Fabaceae (Gualtieri et al. 2002).

Six of the seven LGs in the current map were successfully assigned to seven of the eight main LGs in the map of Hamwieh et al. (2005). By a process of elimination, we can postulate that LG-VII, for which no polymorphic SSR markers were found, is likely to correspond to LG_4 in the previous map. Hamwieh et al. (2005) mapped four morphological traits including pod indehiscence (pi), flower colour (W), seed coat pattern (scp), and fusarium wilt (fw). Comparative mapping in the current study indicated that the flower colour and pod indehiscence genes were located in LG-I of the lentil genome and thus may be present in syntenic regions of either LG-4 or LG-7 of the Medicago genome. Likewise, the genes governing seed coat pattern may be on *M. truncatula* LG-8 and those governing Fusarium wilt may be on *M. truncatula* LG-3.

The parental line Northfield (ILL5588) has been used previously in several lentil genetic mapping projects (Eujayl et al. 1997; Hamwieh et al. 2005; Rubeena et al. 2003). Northfield has several agriculturally important traits including resistance to ascochyta blight caused by *A. lentis* (Ford et al. 1999) and fusarium vascular wilt caused by *Fusarium oxysporum* (Hamwieh et al. 2005). The inclusion of Northfield as one of the parental lines in the present comparative genetic map, therefore, is an advantage since this map can serve as a core map to integrate other maps once anchor markers are mapped. Subsequently, the candidate genes that govern the traits of interest may be sought through collinear syntenic targeting with the ITAPS markers onto the *M. truncatula* genome.

The amplification rate of the ITAPs markers in lentil was dependent on their mode of design. The MP and MLG performed equally well in lentil. The MP markers were based on homology between *M. truncatula* and other legume species, principally soybean, with reference to *Arabidopsis* genomic sequence to infer intron position (Choi et al. 2004a). The MLG markers were designed from *M. truncatula*, lupin and soybean ESTs. The ML markers performed relatively poorly in lentil, perhaps due to being based on only two species and the large phylogenetic distance between lupin and lentil.

A direct comparison between the genomes of lentil and pea has been previously made using 64 morphological, isozyme, and RFLP markers and detected syntenic regions that spanned ~40% of the known genetic map for Lens (Weeden et al. 1992). Unfortunately, there are no shared markers to compare or integrate the data with that presented here. However, comparative mapping between *M. sativa* and *P. sativum* (Kalo et al. 2004), and an investigation of macrosynteny between pea and the model legume *M. truncatula* using common genic markers (Aubert et al. 2006) allows information from those studies to be harnessed to propose a hypothetical relationship between the lentil and pea genomes. *M. truncatula* and *M. sativa* are known to share complete homology at both the macro- and microsyntenic levels (Choi et al. 2004a). Thus, by considering lentil or pea LGs syntenic to the same *M. truncatula* or *M. sativa* LGs to be syntenic to each other, lentil LG-1, 2, 3, 4, 5, 6, and 7 are proposed to be syntenic to pea LG VII + V, IV, II + III + VI, III + IV, I, III and III, respectively.

This study forms a basis for a number of significant outcomes for lentil genomics and legume genomics in general: (1) the genic markers developed here may be used across legume species to determine patterns of chromosomal evolution in the Leguminosae, as argued previously for markers with defined utility (Choi et al. 2006), and to characterize syntenic relationships between *M. truncatula* and cultivated legumes; (2) with the aid of shared anchor markers, the lentil map created may be integrated with all existing lentil maps containing various important domestication traits; (3) the high levels of simple and direct macrosyntenic relationships detected between lentil and *M. truncatula* will enable the future identification of tightly linked markers for direct marker-assisted trait selection and future map-based isolation of candidate genes.

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