Theor Appl Genet (1998) 97: 83–89

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A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines

Received: 6 November 1997 / Accepted: 10 February 1998

Abstract A genetic linkage map of *Lens* sp. was constructed with 177 markers (89 RAPD, 79 AFLP, six RFLP and three morphological markers) using 86 recombinant inbred lines ($F_{6:8}$) obtained from a partially interspecific cross. The map covered 1073 cM of the lentil genome with an average distance of 6.0 cM between adjacent markers. Previously mapped RFLP markers were used as anchor probes. The morphological markers, pod indehiscence, seed-coat pattern and flower-color loci were mapped. Out of the total linked loci, 8.4% showed segregation distortion. More than one-fourth of the distorted loci were clustered in one linkage group. AFLP markers showed more segregation distortion than the RAPD markers. The AFLP and RAPD markers were intermingled and clustering of AFLPs was seldom observed. This is the most extensive genetic linkage map of lentil to-date. The marker density of this map could be used for the identification of markers linked to quantitative trait loci in this population.

Key words *Lens culinaris* subsp. *orientalis* · Recombinant inbred lines · AFLP · RAPD · Genetic map

Communicated by J. W. Snape

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Introduction

In lentil (*Lens* sp.) sources of resistance to key biotic and abiotic stresses have been identified and are being introduced into adapted lines (Erskine et al. 1994). The construction of a detailed genetic linkage map is becoming of crucial importance for identifying the location of such traits to facilitate their directed manipulation in crop-improvement programs. Recombinant inbred lines (RILs) represent an important genetic resource for creating and exploiting linkage maps (Burr et al. 1988). RILs, as well as doubled-haploid lines, have been used to construct linkage maps in various important crops, and are ideal for collaborative mapping projects. Additionally, RIL mapping populations provide sufficient seed for replicated field-testing and facilitate the localization of quantitative characters.

The current lentil maps (Havey and Muehlbauer 1989a; Weeden et al. 1992; Tahir et al. 1993) consist of a small number of markers, mainly isozymes and RFLPs, covering a relatively small portion of the lentil genome. However, other DNA molecular markers such as RAPDs and AFLPs have been used to study biodiversity and genetic relationships in *Lens* species (Ahmed et al. 1996; Sharma et al. 1996). AFLPs provide a robust method for producing linkage maps in breeding populations that have a relatively narrow genetic base. As described in many comparative studies, AFLP technology is considered to have a high degree of marker utility arising from its high multiplex ratio (Powell et al. 1997). Previously, we studied the usefulness of RAPD markers for lentil genetic mapping and for evaluating populations in the F_2 generation for the production of RILs (Eujayl et al. 1997). From this information, we identified a population that exhibited a relatively high level of polymorphism and little segregation distortion. This population was used to construct an extensive genetic linkage map in lentil using different types of DNA markers.

Materials and methods

Plant material

Seeds of an F_2 population were kindly provided by Prof. F. J. Muehlbauer, Washington State University, Pullman, USA. The parents were selected because they exhibited contrasting morphological characters and different responses to biotic and abiotic stresses. The female parent is a single-plant selection from the line ILL5588 (Jordanian land race from the ICARDA collection). The male parent [L692-16-1(s)] was produced as described by Eujayl et al. (1997). The F_2 population was advanced to F_6 using random single-seed descent. Three generations were produced per year under controlled environmental conditions. Sixteen seeds of 86 F_6 plants were grown as one family; thereafter, each family was advanced to the F_8 to obtain the RIL population.

DNA extraction

Total genomic DNA was extracted following the procedure described by Saghai-Maroof et al. (1984). Fresh above-ground parts from seedlings (4–6 weeks old) of the parents and a bulk of 16 F_8 individuals from each of the 86 RILs ($F_{6:8}$) were collected for DNA isolation. The DNA was RNAase-treated and quantified in agarose gels by comparison with standard lambda DNA.

RFLP analysis

Parental DNA (3–5 μg) was digested with restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII, Boehringer Mannheim) for 4 h at 37°C. The digested DNA fragments were electrophoresed in 1% agarose gels. Inserts of 17 clones from lentil cDNA and genomic libraries (Havey and Muehlbauer 1989a; Table 1) were digoxigenin-labelled by PCR under conditions recommended by the manufacturer (Boehringer Mannheim). Southern blots were obtained on uncharged nylon membranes. Hybridization and band detection were carried out as described by Hoisington et al. (1994). Probe/enzyme combinations that detected polymorphic fragments between the parents were used for the segregation analysis of the RILs using the same procedure.

RAPD analysis

The protocol of Williams et al. (1991) was employed with minor modifications for RAPD analysis. A total of 390 decamer primers obtained from Operon Technologies, Alameda, Calif. USA (Kits A–X), were used to screen the parents for polymorphism. The reaction conditions have been described elsewhere (Eujayl et al. 1997) except that the volume was reduced to 25 μl. RAPD products were electrophoresed in 1.6% agarose gels. The parents were screened for polymorphism (presence/absence of bands) with the primers mentioned above and polymorphic primers were used for segregation analysis.

AFLP analysis

The protocol for the AFLP assay was carried out as described by Zabeau and Vos (1993) with minor modifications according to Powell et al. (1997). Aliquots of 1.5–2 μg of parental and RIL DNA were digested with a combination of *Pst*I and *Mse*I or *Eco*RI and *Mse*I restriction enzymes. The linker-adapter and primer sequences were as described in the double-digestion patent for the above enzymes. Pre-amplification was carried out with 1-bp extension primers (+A). Selective amplification of restriction fragments was conducted using primers with two, three or four selective nucleotides. Seventeen and 33 primer combinations for the *Pst*I/*Mse*I and *Eco*RI/*Mse*I digests, respectively, were used to screen for polymorphism between the parents (Table 1). The *Pst*I and the *Eco*RI primers were end-labelled with γ^{33}P-ATP and T4 polynucleotide kinase for the selective amplification of the restricted fragments. The restriction fragments were electrophoresed in 6% polyacrylamide gels as described by Vos et al. (1995). The dried gels were exposed to X-ray films and the autoradiographs were obtained after 4–7 days. The results were visually scored for the presence or absence of a given band.

Morphological markers

The population was grown in the field at the Tel Hadya Research Station of ICARDA, Syria, for two seasons and was evaluated for the morphological characters flower color (*W*), pod dehiscence (*Pi*) and seed coat pattern (*Scp*) (Tahir et al. 1993). The RILs ($F_{6:8}$) were scored for bearing flowers with complete blue standard petals or white petals with blue veins (Wilson and Hudson 1978), pod dehiscence, and the production of seeds with a marbled-1 seed-coat pattern (Vandenberg and Slinkard 1990).

Linkage analysis

Linkage analysis was assessed with MAPMAKER/EXP version 3.0 (Lincoln et al. 1992). To identify linkage groups, pairwise comparisons

Table 1 The 50 AFLP primer combinations and 17 RFLP clones used to screen the parents for polymorphism

1. P14/M36	18. E16/M18	35. E36/M67	1. CMH-25
2. P16/M23	19. E16/M55	36. E42/M18	2. CMH-32
3. P16/M42	20. E16/M88	37. E42/M32	3. CMH-33
4. P16/M43	21. E16/M181	38. E42/M48	4. CMH-34
5. P16/M81	22. E26/M18	39. E42/M43	5. CMH-45
6. P16/M88	23. E26/M32	40. E42/M67	6. CMH-49
7. P17/M37	24. E26/M42	41. E43/M18	7. CMH-52
8. P17/M44	25. E26/M43	42. E43/M32	8. CMH-65
9. P17/M181	26. E26/M48	43. E43/M48	9. CMH-69
10. P17/M238	27. E26/M62	44. E43/M62	10. CMH-71
11. P17/307	28. E26/M67	45. E43/M67	11. CMH-93
12. P18/M40	29. E36/M18	46. E48/M18	12. CMH-95
13. P22/M41	30. E36/M32	47. E48/M42	13. EMH-1
14. P24/M18	31. E36/M42	48. E48/M43	14. EMH-8
15. P34/M13	32. E36/M43	49. E48/M48	15. PMH-14
16. P42/M48	33. E36/M48	50. E48/M62	16. PMH-79
17. P46/M47	34. E36/M62		17. PMH-119

and grouping of markers was carried out at a LOD score 4.0 and a maximum distance of 25 cM (Haldane function). The marker order was confirmed with the ripple command.

Results

The screening of the parents for polymorphism by RAPD primers revealed 116 polymorphic primers (29.7%). Seventy primers were selected, on the basis of their reproducibility, for segregation analysis. This produced 104 polymorphic fragments, of which 89 were assigned to linkage groups and 15 remained unlinked. Of the total number of AFLP primers tested, 70% of the *Pst*I/*Mse*I and 15% of the *Eco*RI/*Mse*I combinations were polymorphic. The polymorphic AFLP primers are listed in Table 2. A *Taq*I/*Hin*dIII digest produced smeary amplifications and extremely low polymorphism (data not shown). Only the prominent and easily scored bands were considered for analysis. A total of 121 polymorphic AFLP fragments was obtained. To avoid identifying spurious linkages and incorrect conclusions about the level of distortion, 21 AFLPs and 11 RAPDs were excluded from the segregation analysis because they contained $> 5\%$ missing data. In total 79 AFLP loci were assigned to linkage groups while 21 remained unlinked. Examples of segregating RAPD and AFLP markers are shown in Figs. 1 and 2 respectively.

The cDNA clones detected one to two polymorphic alleles, segregated co-dominantly and showed no biased segregation. Seven previously mapped polymorphic RFLP probes were selected to anchor this map to the current lentil, pea and chickpea maps. The RFLP marker CMH93 was mapped in LG3, CMH34 and CMH71 in LG4, and CMH49b in LG6, as shown in Fig. 3, while EMH8 remained unlinked.

A chi-square analysis of 214 loci revealed that 22 of the RAPD and AFLP loci deviated from the expected Mendelian segregation ratio of 1:1 at $P < 0.5$ (Table 3). Clustering of distorted loci was observed in LG7 accounting for 26.6% of the total distorted loci. The remaining distorted loci were largely found at, or near, the (presumed) ends of the linkage groups. The three morphological loci each showed a monogenic mode of inheritance without distortion.

Table 2 AFLP primer combinations that generated polymorphism products in *Pst*I/*Mse*I and *Eco*RI/*Mse*I enzyme digests

Primer combination	3′ Selective nucleotides[a]		No. polymorphic products
	P/E	M	
P14/M36	AT	ACC	11
P16/M42	CC	AGT	15
P16/M43	CC	ATA	10
P16/M88	CT	TGC	8
P17/M37	CG	ACG	9
P17/M44	CG	ATC	11
P17/M181	CG	CCCC	9
P17/M238	CG	GATC	6
P17/M307	CG	TCAG	2
P18/M46	CT	ATT	8
P22/M41	GT	AGG	13
P34/M13	AAT	AG	8
E16/M18	CC	CT	2
E16/M43	CC	ATA	2
E42/M32	AGT	AGT	2
E42/M48	AGT	CAC	1
E42/M67	AGT	GCA	2

[a]P = *Pst*I, *E* = *Eco*RI, M = *Mse*I

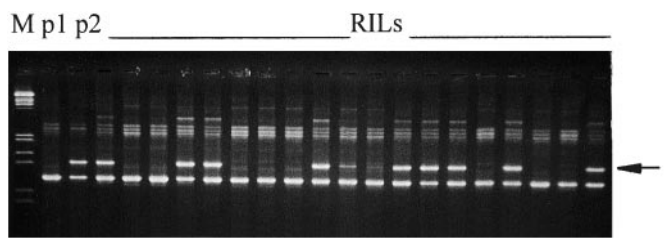

Fig. 1 Polymorphism of OPn19a in the parents and its segregation in 19 RILs. The first lane (*M*) from the left is λ-DNA digested with *Hin*dIII. The *arrow* indicates the type of fragment that is considered for analysis. This locus is mapped in LG2

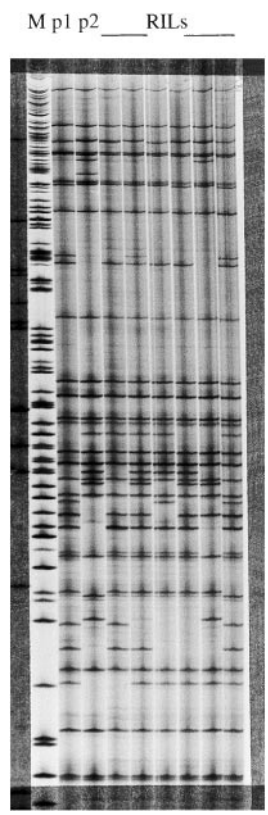

Fig. 2 Segregation of AFLPs detected in a *Pst*I/*Mse*I digest using the P16/M43 primer combination in the parents and six RILs. A sequencing ladder (G) denoted by *M* in the first lane from the left is used as a size marker

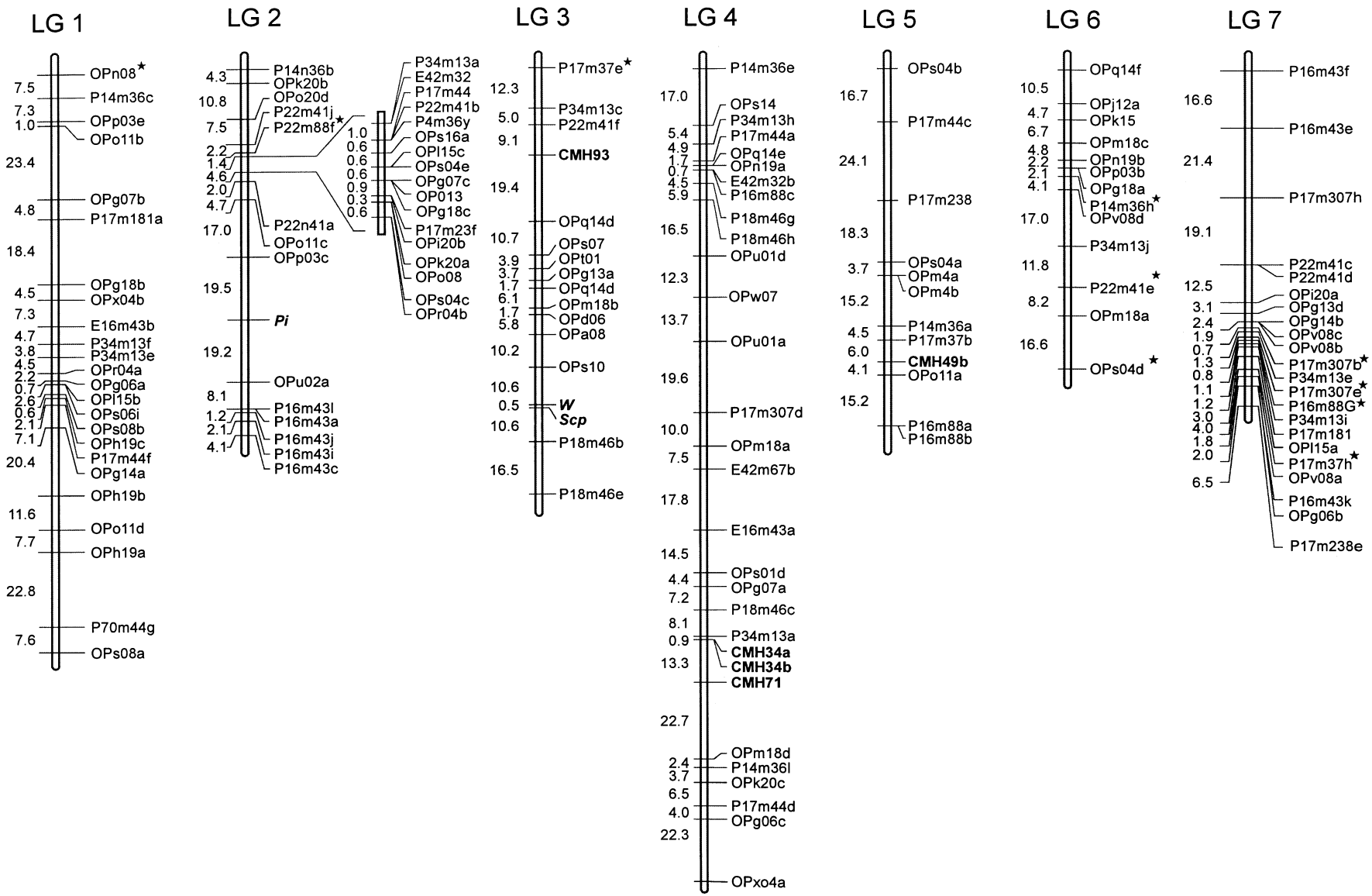
LG 1
OPn08★
7.5
P14m36c
7.3
OPp03e
1.0
OPo11b
23.4
OPg07b
4.8
P17m181a
18.4
OPg18b
4.5
OPx04b
7.3
E16m43b
4.7
P34m13f
3.8
P34m13e
4.5
OPr04a
2.2
OPg06a
0.7
OPl15b
2.6
OPs06i
0.6
OPs08b
2.1
OPh19c
7.1
P17m44f
20.4
OPg14a
OPh19b
11.6
OPo11d
7.7
OPh19a
22.8
P70m44g
7.6
OPs08a
LG 2
4.3
P14n36b
OPk20b
10.8
OPo20d
P22m41j
7.5
P22m88f★
2.2
1.4
4.6
2.0
4.7
P22n41a
17.0
OPo11c
OPp03c
19.5
Pi
19.2
OPu02a
8.1
P16m43l
1.2
P16m43a
2.1
P16m43j
4.1
P16m43i
P16m43c
1.0
0.6
0.6
0.6
0.9
0.3
0.6
P34m13a
E42m32
P17m44
P22m41b
P4m36y
OPs16a
OPl15c
OPs04e
OPg07c
OP013
OPg18c
P17m23f
OPi20b
OPk20a
OPo08
OPs04c
OPr04b
LG 3
12.3
P17m37e★
P34m13c
5.0
P22m41f
9.1
CMH93
19.4
OPq14d
10.7
OPs07
3.9
OPt01
3.7
OPg13a
1.7
OPq14d
6.1
OPm18b
1.7
OPd06
5.8
OPa08
10.2
OPs10
10.6
0.5
W
Scp
10.6
P18m46b
16.5
P18m46e
LG 4
17.0
P14m36e
OPs14
5.4
P34m13h
4.9
P17m44a
1.7
OPq14e
0.7
OPn19a
4.5
E42m32b
5.9
P16m88c
P18m46g
16.5
P18m46h
OPu01d
12.3
OPw07
13.7
OPu01a
19.6
P17m307d
10.0
OPm18a
7.5
E42m67b
17.8
E16m43a
14.5
OPs01d
4.4
OPg07a
7.2
P18m46c
8.1
P34m13a
0.9
CMH34a
CMH34b
13.3
CMH71
22.7
OPm18d
2.4
P14m36l
3.7
OPk20c
6.5
P17m44d
4.0
OPg06c
22.3
OPxo4a
LG 5
16.7
OPs04b
P17m44c
24.1
P17m238
18.3
OPs04a
3.7
OPm4a
OPm4b
15.2
P14m36a
4.5
P17m37b
6.0
CMH49b
4.1
OPo11a
15.2
P16m88a
P16m88b
LG 6
10.5
OPq14f
OPj12a
4.7
OPk15
6.7
OPm18c
4.8
OPn19b
2.2
OPp03b
2.1
OPg18a
4.1
P14m36h★
OPv08d
17.0
P34m13j
11.8
P22m41e★
8.2
OPm18a
16.6
OPs04d★
LG 7
16.6
P16m43f
P16m43e
21.4
P17m307h
19.1
P22m41c
P22m41d
12.5
OPi20a
3.1
OPg13d
2.4
OPg14b
1.9
OPv08c
0.7
OPv08b
1.3
P17m307b★
0.8
P34m13e
1.1
P17m307e★
1.2
P16m88G★
3.0
P34m13i
4.0
P17m181
1.8
OPl15a
2.0
P17m37h★
OPv08a
6.5
P16m43k
OPg06b
P17m238e

Table 3 RAPD and AFLP loci displaying significant deviation from a 1 : 1 segregation ratio

Locus	P_1 alleles	P_2 alleles	Chi^{2a}
OPno8	33	53	4.20
OPs04e	50	31	4.00
OPs04d	31	50	4.00
OPg13c	55	31	6.15
P17m307d	39	46	5.51
P14m36h	55	30	4.10
P14m36j	53	32	4.71
P18m46h	56	28	9.35
P17m37g	29	56	7.95
P17m37i	69	15	33.44
P17m37j	36	48	17.44
P18m88f	51	33	4.87
P18m88g	30	54	6.30
P17m307a	29	55	6.35
P17m307b	33	51	4.87

[a] $P < 0.05$

Table 4 Unassigned segments of linked markers and the distances in cM between adjacent markers (locus 1 and locus 2). Where three or more markers are linked on one segment, the middle ones (in the locus-2 column) are repeated in the locus-1 column

Segment	Locus 1	Locus 2	Distance (cM)
1	P16m88j	P17m307a	0.6
2	P14m36I	P14m36j	0.0
3	P23m41g	P17m7i	19.1
4	P18m46h	P17m37f	18.7
5	E42m48a	CMH49a	11.7
6	OPg13c	OPm4a	14.5
	OPm4a	P16m88d	19.5
7	OPu01c	OP01b	16.1
	OPs01b	OPi18b	2.4
	OPi18b	P17m37c	7.0
8	OPo15b	OPo15a	2.0
	OP15a	OPs01c	3.3

Linkage analysis

One hundred and four RAPDs, 100 AFLPs, seven RFLPs and three morphological loci were analyzed for genetic linkage. The analysis revealed seven linkage groups comprising 12 or more markers (Fig. 3). This equals the haploid number of chromosomes in the lentil genome. Additionally, eight unassigned segments (Table 4), with doublets and up to four markers per segment, were detected. A partial genetic linkage map was constructed with 177 markers spanning 1073 cM with an average distance of 6.0 cM between markers. Thirty seven markers remained unlinked. The RAPD markers were more regularly spaced along the linkage groups than the AFLP markers, which showed some level of clustering particularly in LG2. Ten and five pairs of RAPD and AFLP markers, respectively, showed no recombination. The pod indehiscence marker was assigned to LG 2. Flower color (*W*) and seed-color pattern (*Scp*) were linked at a distance of 0.5 cM and were assigned to LG 3. This is the first mapping of the flower-color marker in the lentil genome. The two loci, *W* and *Scp*, were flanked by OPs10 and P18m46b markers at 10.6 cM, respectively. The linkage analysis revealed gaps of more than 20 cM within some of the major linkage groups. The distribution of linked, unlinked and distorted markers is shown in Table 5.

Fig. 3 Genetic linkage map of lentil showing the distribution of different markers at a LOD score of 4.0. The linkage groups are named *LG1 through LG7*. Loci names, on the right side of the bars, preceded by *P* or *E* are AFLP markers and those preceded by *OP* are RAPD markers. The *last letter* of the locus name indicates the molecular weight of the AFLP and RAPD fragments, from *a* (highest) to *z* (lowest). The RFLP and morphological markers are in *bold type*. *Asterisks* denote the distorted loci. Genetic distances, on the left side of the bars, are in cM (Haldane function)

Discussion

A genetic linkage map of *Lens* sp. was constructed with 174 DNA markers (89 RAPDs, 79 AFLPs, and six RFLPs) and three morphological markers, spanning 1073 cM of the genome. Previously reported maps (Havey and Muehlbauer 1989a; Weeden et al. 1992) were only sparsely covered with markers, spanning 330 and 560 cM of the genome with average marker distances of 12.8 ± 3.2 cM and 8.75 cM respectively. In our map, the average distance between markers was 6.0 cM and the estimated amount of DNA covered per map unit was 3786 kbp/cM, thus providing a better genome coverage. So far, this is the most extensive genetic linkage map of the genus *Lens* constructed from a single cross with various types of molecular markers. The use of a RIL population ($F_{6:8}$) allowed us to overcome the limitations of the RAPD and AFLP markers for genetic mapping due to their largely dominant mode of inheritance. The segregation of all types of markers used in the population was 1 : 1, except for that of four RAPD and 11 AFLP markers. Previously, we used RAPD markers to evaluate two F_2 populations for segregation distortion and their usefulness for developing mapping populations in later generations (Eujayl et al. 1997). The RILs used in this study were developed from an F_2 population that exhibited relatively low segregation distortion (10%). This was confirmed in the F_6-derived RIL population used in this study, which exhibited 8.4% distorted loci. The evaluation of segregation distortion at the F_2 was therefore useful for testing the utility of later mapping populations.

Table 5 Comparison of the distribution and level of distorted loci detected by different types of markers

Marker type	Total no. analysed	Total no. linked	Major linkage groups		Unassigned segments		Total unlinked	
			Normal	Distorted	Normal	Distorted	Normal	Distorted
AFLP	100	79	56	7	12	4	16	5
RAPD	104	89	75	3	10	1	13	2
RFLP	7	6	5	0	1	0	1	0
Morph.	3	3	3	0	0	0	0	0
Total	214	177	139	10	23	5	30	7

The AFLP technique has been reported as a reliable and reproducible assay in many crops; furthermore, a large number of loci can be detected in a single assay (Powell et al. 1997). The application of the AFLP technique has a major advantage in crops with a large genome and the unavailability of genomic cDNA libraries, or other DNA sequence-based markers. In our analysis, 70% of the *Pst*I/*Mse*I primers detected polymorphic loci compared with only 15% of the *Eco*RI/*Mse*I primers, indicating that the former are more efficient in detecting polymorphism than the *Eco*RI/*Mse*I primers in lentil. This supports the findings of Sharma et al. (1996) who used the same enzyme combination for a genetic diversity study in lentil that generated a high level of polymorphism of 23–52 AFLP markers/primer combination. The mapping of AFLPs increased the number of detectable polymorphic fragments and strengthened the reliability of the framework of this map, and has major advantages over RAPD markers due to its robustness and transferability. This is the first time that AFLPs have been used for genetic mapping in lentil.

RFLP markers are known to be efficient for mapping and for detecting polymorphic loci in the genomes of many important crops. However, in the cultivated lentil, Havey and Muehlbauer (1989a,b) found insufficient polymorphism for RFLPs to generate a detailed genetic linkage map. Nevertheless, we have confirmed the mapping of the RFLP clones CMH34, CMH49, CMH71 and CMH93 and the morphological markers *Scp* and *Pi*, which were mapped in lentil, pea (Havey and Muehlbauer 1989a; Weeden et al. 1992) and chickpea (Simon and Muehlbauer 1997). Previously, CMH34 and CMH71 were mapped in linkage groups 4 and 2 respectively (Weeden et al. 1992) while in our study they were linked at 13.1 cM on one linkage group (LG4). CMH93 was mapped on linkage group 1 of the chickpea map (Simon and Muehlbauer 1997) and on LG3 in our lentil map. This marker had not been mapped before in lentil. As we have not mapped any of the adjacent markers to CMH34, CMH71 or CMH93 we cannot resolve whether the discrepancy in positions is due to locus duplication or to the rearrangement of linkage relationships. As a result, the mapping of these five anchor loci does not elaborate the conservation of linkage relationships between pea, chickpea and lentil.

Comparing all distorted markers, neither of the parents showed a significant excess of the distorted alleles. The AFLP loci detected more distorted loci than the RAPD loci. Cloutier et al. (1997), comparing maps developed in an F_2 and two microspore-derived populations of *Brassica napus* L., conclude that small, but significant, segregation distortion results in reduced estimates of the recombination fraction and only extreme segregation distortion may lead to spurious linkage. We observed a relatively small amount of segregation distortion with a clustering of distorted loci in LG7 and with the remaining distorted loci at the presumed ends of the linkage groups. A clustering of distorted loci has often been reported within the linkage groups constructed in several species (Graner et al. 1991) and might be explained by linkages to incompatibility loci (Wricke and Wehling 1985) or else linkage to a lethal allele in gametes (Pillen et al. 1992). Even though segregation distortion can be seen in intraspecific crosses we also have to consider that part of the wild *Lens culinaris* subsp. *orientalis* genome is in the male parent of this cross, and might have elevated the probability of reduced recombination and hence segregation distortion.

The large number of unlinked markers (37) reflects the need for a large number of markers to evenly cover the lentil genome. Compared with most food legumes, lentil has a relatively large genome (C value = 4.21 pg, Arumuganathan et al. 1991). Vallejos et al. (1992) analyzed 224 RFLPs in a mapping population of *Phaseolus vulgaris* L. and conclude that the small number of loci analyzed was responsible for the insufficient genome coverage and uneven distribution of markers. Similarly, it is likely that the relatively low number of DNA markers mapped in this study was responsible for the disagreement in the total number of linkage groups and the haploid number of chromosomes in lentil ($2n = 14$). This situation suggests that more informative markers should be used and that the number of markers for segregation analysis should be increased. With the use of the simple-sequence-repeat (SSR) markers, which have a high information content

compared with other markers, a larger proportion of polymorphic markers might be generated in a single cross. A high-density genetic linkage map in crosses where few polymorphic RFLP markers are detectable could be generated (Rafalski et al. 1996). We consider that SSRs are markers that have a high potential to enrich the lentil map. The map density obtained in this study provides the basis for developing a high-density linkage map for lentil.

Distances of more than 20 cM between markers in the present map are common in many maps of the crops that have recently received attention in molecular-marker research. Many attempts to generate high-density maps have revealed that there are some regions with a high density of markers, while other regions are sparsely covered. It has been proposed that the generation of chromosome-specific libraries is a way to improve the efficiency of adding markers to the sparsely mapped regions (Chen and Armstrong 1995).

In summary, we have developed a partial genetic linkage map for *Lens* sp. based on morphological, RFLP, RAPD, and AFLP markers. We found the RAPD and AFLP markers are useful for constructing a genetic linkage map of lentil. The population of recombinant inbred lines produced for this mapping effort exhibited a high level of polymorphism for DNA markers, high variation to biotic (e.g. *Fusarium* vascular wilt) and abiotic stresses (e.g. frost susceptibility), quantitative traits, and a relatively low level of segregation distortion. Therefore, we recommend the exploitation of this population for collaborative mapping to develop a saturated genetic linkage map of lentil.

Acknowledgements The authors acknowledge Dr. J. Russell and Ms. P. Lawrence for their assistance during the AFLP analysis at the CMG, Scottish Crop Research Institute, UK. This project was funded by BMZ, Germany, Project No. 95.7860.0-001-01, and UNDP/French Government, Project No. RAB/87/025.

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