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An integrated interspecific AFLP map of lettuce (*Lactuca*) based on two *L. sativa* × *L. saligna* F_2 populations

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Abstract AFLP markers were obtained with 12 *Eco*RI/*Mse*I primer combinations on two independent F_2 populations of *Lactuca sativa* × *Lactuca saligna*. The polymorphism rates of the AFLP products between the two different *L. saligna* lines was 39%, between the two different *L. sativa* cultivars 13% and between the *L. sativa* and *L. saligna* parents on average 81%. In both F_2 populations segregation distortion was found, but only Chromosome 5 showed skewness that was similar for both populations. Two independent genetic maps of the two F_2 populations were constructed that could be integrated due to the high similarity in marker order and map distances of 124 markers common to both populations. The integrated map consisted of 476 AFLP markers and 12 SSRs on nine linkage groups spanning 854 cM. The AFLP markers on the integrated map were randomly distributed with an average spacing between markers of 1.8 cM and a maximal distance of 16 cM. Furthermore, the AFLP markers did not show severe clustering. This AFLP map provides good opportunities for use in QTL mapping and marker-assisted selection.

Keywords Genetic linkage map · Lettuce · *Lactuca saligna* · AFLP markers · Interspecific cross

Introduction

Our knowledge on the structure and function of plant genomes is rapidly expanding by the fast development of techniques in molecular biology like automated sequencing, DNA library construction and screening, and DNA marker technologies. The new research field about maintaining, ordering and using all this genome information is designated as "Bioinformatics". This covers fundamental research topics like gene organisation and synteny among genomes. A more-applied field is plant breeding where bioinformatics will facilitate marker-assisted selection programs with most emphasis on quantitative traits.

The molecular information of a plant genome is usually presented in the framework of a genetic linkage map. To create such a genetic map informative markers need to be developed and screened on a segregating population. To this end, markers of several types are available. Former genetic maps of many plant species are mainly constructed with RFLPs as markers. The advantages of RFLPs are the locus-specificity and codominant inheritance. The disadvantage is that the technology is time-consuming, laborious and costly. Nowadays, new DNA marker technologies are available, which are PCR-based, need less template DNA and are less laborious. Examples of commonly used PCR-based marker technologies are CAPS (Konieczyn and Ausubel 1993), SSRs (Van de Wiel et al. 1999) and AFLPs (Vos et al. 1995). CAPS and SSRs are reliable markers with potentially many alleles and hence a codominant inheritance. These markers are mainly used as easy applicable markers for specific loci. Their disadvantage is the *a priori* sequence information that is required to design the locus-specific primers. In contrast, the AFLP technique does not require *a priori* sequence information and combines the advantages of RFLP markers with the advantages of PCR. AFLP markers are efficient and reliable and can be used across species like is shown for tomato, potato, barley and maize (Van Eck et al. 1995; Qi et al. 1998; Haanstra et al. 1999; Vuylsteke et al.1999).

These new marker technologies allow the efficient construction of high-density maps, which have several applications in genetics and breeding; for instance, comparison of the synteny among genomes of related species or genera as shown for *Solanaceae*, cereals and *Brassica* species (Gale and Devos 1998; Hu et al 1998;

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Livingstone et al. 1999). This allows the construction of integrated genetic maps among species or within genera and to make comparisons between related genera (Qi et al. 1996; Sebastian et al. 2000).

Furthermore, genetic maps are essential to locate the genes that are involved in the expression of traits. This can easily be done for simple heritable traits based on one gene, but is also possible for complex traits which are based on more genes (QTLs). In the latter case, large segregating populations (n>100) are required to unravel the number of loci involved in the trait.

When the map positions of important genes are known, indirect selection of plants bearing the useful genes can take place at the DNA level on the basis of flanking markers linked to the genes of interest. This, so called "marker-assisted selection", has high potentials in plant breeding (Bernatsky and Tanksley 1989; Lande and Thompson, 1990; Knapp 1998).

In lettuce, a genetic map is available, which is based on the intraspecific cross "Calmar×Kordaat" and consists of 13 major and four minor linkage groups spanning a total length of 1,950 cM (Kesseli et al. 1994). It consists mainly of RFLP and RAPD markers with an average spacing of 6.1 cM and major gaps of up to 28 cM. It has been used to map *Dm* genes and other disease resistance genes (Maisonneuve et al. 1994; Okubara et al. 1994; Witsenboer et al. 1995).

We are interested in *Lactuca saligna* (wild lettuce) as a source for resistance to downy mildew (*Bremia lactucae*). The resistance from *L. saligna* is probably not race-specific and therefore probably controlled by a different resistance mechanism than the gene-for-gene resistance mechanism of introgressed race-specific resistance genes (*Dm* genes) in *Lactuca sativa* (Bonnier et al. 1992; Lebeda and Reinink 1994). *L. saligna* and lettuce (*L. sativa*) are crossable but due to their genetic distance the success of crosses is low, which results in reduced germination, vigour and fertility of the progenies (de Vries 1990; Koopman et al. 1998). To map the downy mildew resistance in *L. saligna* we aimed at constructing a genetic map based on a *L. sativa* × *L. saligna* cross.

In the present study two different independent F_2 populations of *L. sativa* × *L. saligna* crosses were generated from which a dense integrated genetic linkage map was constructed mainly based on AFLP markers.

Materials and methods

Plant material

Two F_2 mapping populations were generated for this study. The parents of Population A were *L. saligna* CGN 5271 as female parent, and *L. sativa* cv "Olof", a butterhead cultivar, as male parent. The parents of Population B were *L. saligna* CGN 11341 as female parent and *L. sativa* cv "Norden", a butterhead cultivar as male parent. The two *L. saligna* parents had a very distinct morphology. There is no information available on their geographical origin.

The F_2 populations consisted of 126 plants for Population A and 54 plants for Population B. Each F_2 population was derived from a randomly chosen single F_1 plant. Populations A and B were supplied by the breeding companies Nickerson-Zwaan and Rijk Zwaan, respectively.

Table 1 List of primer combinations used for AFLP analyses. The names and the last three selective nucleotides of the primers are shown. For pre-amplification, the same primers were used without the last two selective nucleotides

Primer	M48 CAC	M49 CAG	M54 CCT	M58 CGT	M59 CTA	M60 CTC
E35 ACA	×	×			×	×
E38 ACT			×			
E44 ATC	×	×				
E45 ATG	×	×				
E49 CAG				×		
E51 CCA		×				
E54 CCT	×					

DNA isolation

Leaf material was collected from 8-week old F_2 plants that were grown in the greenhouse. Genomic DNA was extracted from frozen leaves according to the procedure described by Van der Beek et al. (1992) with some minor modifications: after hooking the DNA out of the isopropanol mixture, the DNA was washed overnight in 76% ethanol and 10 mM NH_4Ac, dried and dissolved in 200 µl of sterile TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA).

AFLP analysis

The AFLP procedure was performed according to the two step amplification as described by Vos et al. (1995) using the enzyme combination *Eco*RI/*Mse*I. A total of 12 primer combinations, selected from a study on informative primer combinations in lettuce (Van Wijk, personal communication), were employed. The following seven primer combinations, E44M48, E35M48, E49M58, E54M48, E45M49, E51M49 and E38M54, were applied to all F_2 plants of both populations, while five other primer combinations, i.e. E45M48, E35M60, E44M49, E35M49 and E35M59, were only applied to 90 F_2 plants of Population A (Table 1).

AFLP marker nomenclature and analysis of gel images

AFLP markers were designated with the name of the two primers (e.g. E35M48) used to amplify the DNA, followed by the molecular size as the number of nucleotides of the amplification product estimated from the mobility in the gel compared to a size standard. In case two different bands from the same primer combination were almost, but not exactly, identical in size, their marker names were extended with "a" for the larger fragment and "b" for the smaller one. The other extensions in the marker names refer to the specific parent that showed this amplification product (see legend of Fig. 1).

The scoring of the AFLP markers produced with primer combinations E44M48, E35M48, E49M58, E54M48, E45M49, E51M49 and E38M54 were mainly based upon the presence or absence of the amplification product (e.g. dominant scoring). Only when intensity differences of the amplification products allowed distinguishing between homozygotes and heterozygotes, were the markers scored codominantly. All markers generated with these seven primer combinations were scored twice, and discrepancies were resolved. The AFLP markers in Population A produced with primer combinations E45M48, E35M60, E44M49, E35M49 and E35M59 were predominantly scored codominantly using proprietary software (developed at Keygene).

Calculation of polymorphism rates based on AFLP data

All amplification products obtained by using the 12 primer combinations on all four parents were counted. The polymorphism rate was defined as the number of segregating amplification products divided by the total number of amplification products within the size range of 60–590 basepairs.

SSR primers

The following SSR primer pairs obtained from Van de Wiel and developed on *L. sativa* were tested on the four parent lines: LsA001, LsA002a, LsA003, LsA004a, LsA006, LsB101, LsB102, LsB104, LsB105, LsB106, LsB107, LsB108, LsB110, LsB111a, LsB71f6r, LsB8, LsD035, LsD046, LsD101, LsD103a, LsD106G, LsD107G, LsD108, LsD109, LsD110a, LsE003a, LsE006, LsE009, LsE011, LsE018, LsF018, LsG001G and LsH001 (Van de Wiel et al. 1999). Only when both parents showed unique alleles were the F_2 populations screened for segregation of such SSR markers.

The following additional SSR primer pairs were tested in collaboration with Michelmore (Davis, Calif., USA) and showed polymorphism among the four parental lines and in the two F_2 populations: L1722, L1723, L222, L2211, L2278, L2524#2 and L317. More SSRs obtained from Michelmore were tested on the parental lines, but did not show unique alleles for each parent and were not tested on the F_2 populations (data not shown).

SSR analysis

Amplification of SSRs was performed in 20-µl PCR reactions containing 20 ng of template DNA, 0.4 U of *Taq* polymerase, 40 ng of both primers, 2 µl of 10× reaction buffer (the same as employed in AFLP analysis) and 0.1 mM of all four dNTPs. The following PCR program was used: 1 min at 94°C, 40 cycles of 45 s for the annealing temperature, 1 min 45 s for extension at 72°C, 45 s of denaturation at 94°C with a final step of 3 min at 72°C. PCR products were run on 3% agarose gels to separate amplification products with larger size differences. Otherwise, they were separated on denaturing polyacrylamide gels with conditions similar to AFLP analysis in order to separate amplification products with lengths between 80 and 500 nucleotides.

In cases where more amplification products were obtained (the SSR was multilocus), an extension to the original name was given with first the specification of the parent and than the estimated fragment size.

The SSRs were scored based upon the presence or absence of the amplification products of the parents. SSRs were scored codominantly in case were both parents showed unique alleles.

Linkage analysis and map integration

To analyse the scored markers, segregation distortion tests and linkage analyses were performed by using JoinMap 2.0 (Stam and Van Ooijen 1995) on each mapping population.

For the F_2 segregation ratios a χ^2 test for skewness was performed with a threshold level for significance of 0.5%. For Population A, markers codominantly scored were tested against the 1:2:1 ratio, referring to homozygous *L. sativa*: heterozygous: homozygous *L. saligna*. Markers dominantly scored were tested against the 3:1 ratio, representing homozygous *L. sativa* plus heterozygous: homozygous *L. saligna* or homozygous *L. saligna* plus heterozygotes: homozygous *L. sativa*.

For linkage analysis, markers were assigned to linkage groups by increasing the LOD score for grouping with steps of one LOD unit. The calculations of the linkage maps were done by using all pairwise recombination estimates smaller than 0.45, LOD scores higher than 0.01, and Kosambi's mapping function.

After the calculation of a map for each population the two maps were integrated by using JoinMap 2.0 after merging the pairwise recombination frequencies and the corresponding LOD scores of both populations. Again, linkage groups were assigned by increasing the LOD score for grouping with steps of one LOD unit. Map distances were calculated using Kosambi's mapping function, pairwise recombination estimates smaller than 0.45, and LOD scores higher than 0.5, to save calculation time.

Markers, that could not reliably be fitted by JoinMap due to conflicting recombination estimates, but which had a LOD score for linkage with another marker higher than or equal to 10 or 5 combined with a recombination frequency smaller than or equal to 5 or 10%, were manually placed on the map at the most-likely position and given an extension "!".

Results

Plant material

To establish a reliable map it was aimed that the population size was more than 100 F_2 individuals. Population A consisted of 162 seeds, which germinated well and resulted in 126 full-grown F_2 plants. Population B had a much lower germination rate of 42%, resulting in only 54 F_2 plants out of 130 seeds.

The variation in the morphology of the F_2 plants of both populations was very high. The fertility of the F_2 plants was very low compared to the parent plants. In both populations 37% of the F_2 plants were sterile. The rest of the F_2 plants varied in seed set, ranging from a few to more than 100 seeds per plant.

AFLP analysis and polymorphism rates

By analysing 12 primer combinations on all four parents 1,317 different amplification products were generated. From these AFLP amplification products 1,096 were segregating in the F_2 populations and were ascribed to one of the parents as they showed to be parent-specific (Table 2). The polymorphism rate between *L. sativa* and *L. saligna* in Populations A and B was 81.4% and 80.9% respec-

Table 2 Specificity and number of AFLP amplification products generated with 12 primer combinations. *L. sativa* specific means that the amplification product is found in *L. sativa* cv "Olof" and in *L. sativa* cv "Norden", while Olof specific means that the amplification product is found in *L. sativa* cv "Olof" only and not in Norden. Similarly for the *L. saligna* specific, *L. saligna* A specific and *L. saligna* B specific amplification products. Constant bands are amplification products found in all four parents

12 primer-combinations	*L. sativa* specific	Olof specific	Norden specific	*L. saligna* specific	*L. saligna* A specific	*L. saligna* B specific	Constant bands	Total # of bands
Average	39	3	3	28	10	8	18	109
Total	473	40	33	338	119	93	221	1317

tively, the polymorphism rate between *L. sativa* cv "Olof" and *L. sativa* cv "Norden" was 13.4%, and between *L. saligna* A and *L. saligna* B was 38.5%. Twenty-nine amplification products were excluded from the analyses, because they could not be ascribed to only one parent.

On average, with each primer combination 109 amplification products were produced of which 45 (=39+3+3) were detected only in *L. sativa* and 46 (=28+10+8) were detected only in *L. saligna* (Table 2).

In Population A, screened with all 12 primer combinations, 482 polymorphisms were scored. Fifty percent of the segregating amplification products showed a nearly identical mobility on the gel. Therefore they could not be scored reliably and were not included in the analyses. The other 50% of the segregating amplification products were scored unambiguously. Population B was analysed with seven primer combinations and yielded 294 scorable polymorphisms.

SSR analysis

From the 76 SSR primer pairs tested, only four of them, i.e. L317, L222, L2211 and LsB104, were scored codominantly. Most of the other SSR primer pairs yielded an amplification product in the *L. sativa* parent only, which resulted in a dominant scoring. The rest did not show any polymorphism between the parents.

Genetic linkage map and segregation distortion of Population A

In Population A 482 AFLP markers and 12 SSR markers were scored and used for map calculation. These markers were assigned to linkage groups at a LOD threshold of 6.0. The genetic map derived from Population A contained 412 markers (83% of the total number of markers) on ten linkage groups, covering a total map length of 895 cM (data not shown).

In this F_2 population 25% of the loci showed segregation distortion. Linkage Group 7 showed an average skewed ratio of 37 : 44 : 8 instead of 1:2:1 over its entire length, severely favouring *L. sativa* alleles. Furthermore, skewness of a similar severity was observed at one of the ends of Linkage Groups 4, 6 and 9, all in favour of *L. sativa* alleles (Table 3). An average segregation distortion of 3:43:39 favouring *L. saligna* alleles was found distal on Linkage Group 4 and a similar severe skewness was found on Linkage Group 5 (Table 3). Besides skewness an excess of heterozygotes was also found with an average ratio of 20:62: 4 on Linkage Group 8 at 21–45 cM.

Genetic linkage map and segregation distortion of Population B

In the smaller F_2 Population B, 294 AFLP markers and eight SSRs were used for map calculation. The markers were assigned to linkage groups at a LOD threshold of 4.0 resulting in a map of 13 linkage groups (data not shown). The alignment of the maps of both populations revealed that the common markers fell in the same linkage groups. Based on the alignment, six groups in Population B corresponded with three groups of Population A, as Population A contained several bridging markers that were not scored in Population B. Consequently, the six groups in Population B were merged into three groups.

Fixed-order files from Population A with common markers at ≥15-cM intervals were used to generate a genetic map of Population B. This resulted in a map of 223 markers (74% of the total number of markers) on ten linkage groups covering a total map length of 627 cM.

Two regions on Linkage Groups 5 and 6 in population B showed severe skewness favouring both *L. saligna* alleles (Table 3).

Table 3 Observed segregation distortion, per population and per linkage group

Linkage group	Region in cM	Favouring alleles of
Population A		
4	0–9	*L. saligna*
4	119–148	*L. sativa*
5	0–41	*L. saligna*
6	78–88	*L. sativa*
7	0–73	*L. sativa*
9	0–31	*L. sativa*
9	85–112	*L. sativa*
Population B		
5	0–37	*L. saligna*
6	0–9	*L. saligna*

Integrated map

The two linkage maps, generated from the two F_2 populations were very similar with respect to marker order and distance for each linkage group. Consequently, an integrated map, comprising markers of both populations, was constructed. The markers were assigned to nine linkage groups at a LOD threshold of 6.0. This corresponds with the chromosomal number of lettuce. The numbers given to the linkage groups correspond with the group numbering used for the "Calmar×Kordaat" map (Kesseli et al. 1994) with the exception of Group 6 in this map that corresponds with Group 12 in the "Calmar×Kordaat" map. We follow the nomenclature for chromosomal numbers as proposed by Michelmore and Van Wijk for the "Calmar×Kordaat" map, which allows the alignment of both maps with other maps of lettuce having markers in common (Michelmore and Van Wijk, in preparation).

Over the two populations 533 different markers were scored, of which 488 (=92%) were mapped covering a total map length of 854 cM (Fig. 1). From these mapped markers, 124 (25%) were scored in both populations

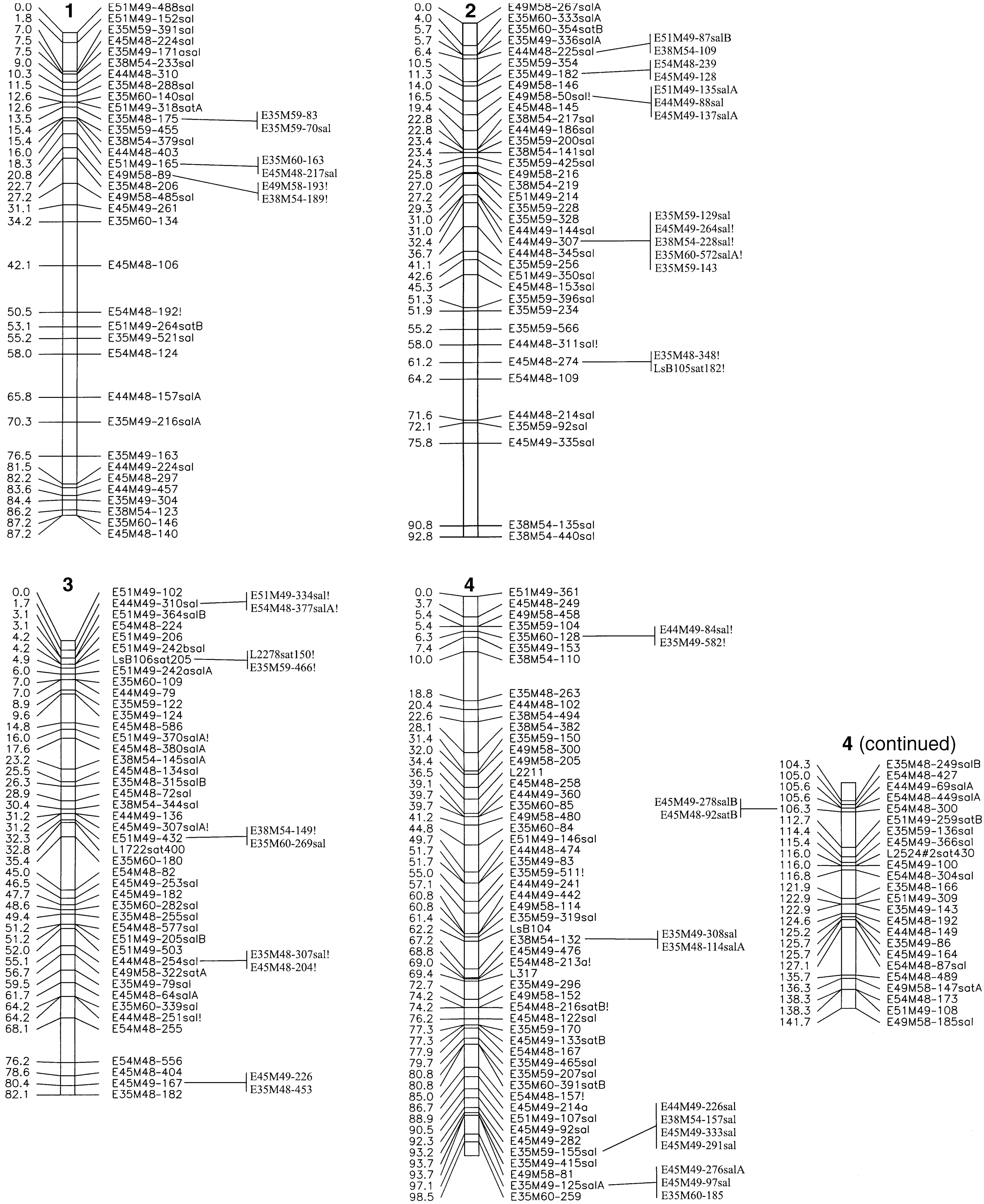
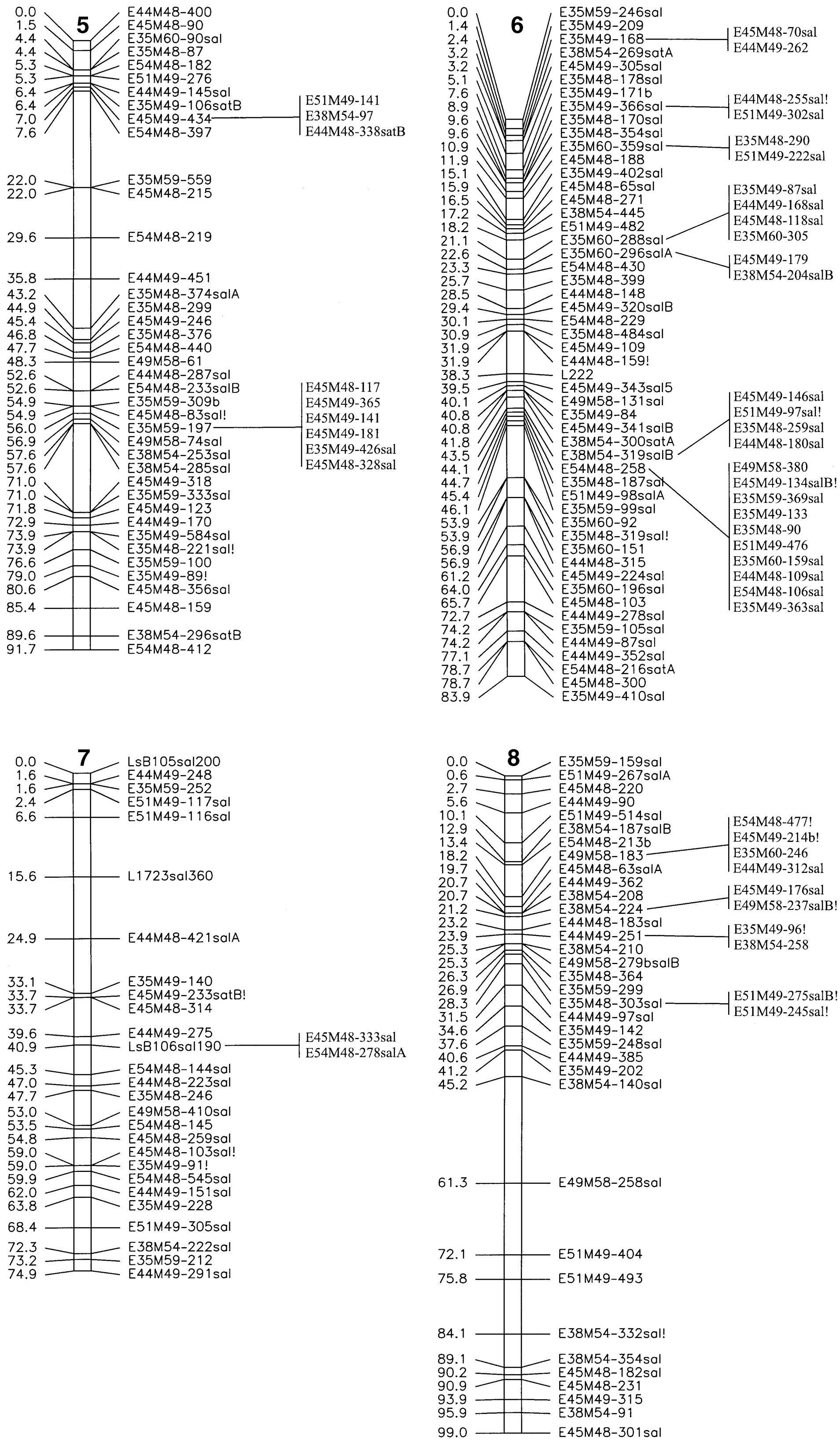


Fig. 1 An integrated map based on two interspecific F_2 populations between *L. sativa* and *L. saligna*. Chromosome 4 is split up because of its length. Markers with no extension only give an amplification product in *L. sativa*. The extensions satA, satB, sal, salA and salB represent markers that only give amplification products in respectively *L. sativa* Olof, *L. sativa* Norden, *L. saligna*, *L. saligna* A and *L. saligna* B. The extension ! means that a marker is placed there manually at the most-likely position with restrictions to the recombination frequency and the LOD score (see Results). When three or more markers mapped on the same position they were put aside

Fig. 1 (continued)



and were located at similar map positions. Therefore, they were considered as common markers. Out of 488 mapped markers 12 were SSR markers of which four were scored codominantly.

The distribution of markers over the map was random and no clear clustering of markers was observed except for a small cluster in the centre of Chromosome 6 where 17 markers were present in an interval of 0.6 cM.

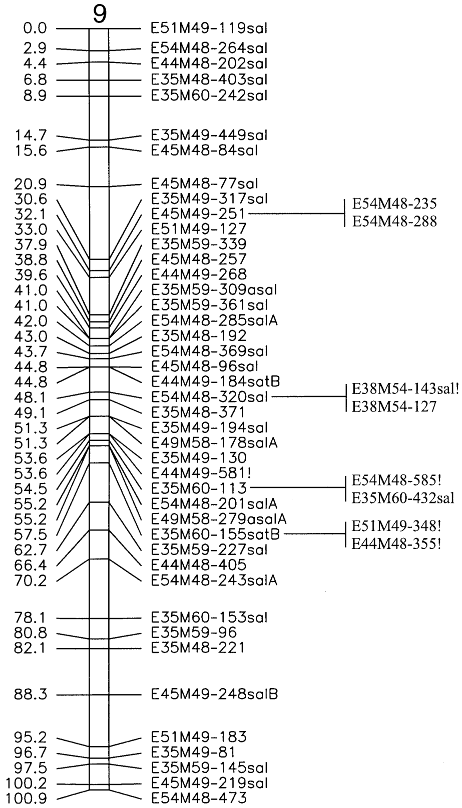


Fig. 1 (continued)

The average spacing between markers (including markers at the same position) was 1.8 cM and the largest gap between two markers was 16 cM.

Co-linearity between the three maps

Both individual maps had ten linkage groups, whereas the integrated map had nine linkage groups corresponding to the nine chromosomes of lettuce (Table 4). The two linkage groups representing Chromosome 8 in both individual maps were not joined because the linkage between the distal markers E49M58–258sal, E38M54–140sal and E51M49–245sal was lower than the LOD threshold for grouping (LOD 6.0 and 4.0 in Populations A and B, respectively). In the integrated map the two groups were joined because the linkage between the distal markers of the two groups was above the LOD threshold for grouping (LOD 6.0). This was due to the summed number of genotypes from both populations, which increases the LOD score for linkage between these markers (Fig. 2). The other eight linkage groups were similar in marker order and distance among the maps. The only exception was marker E54M48–216, which was mapped in Population A on Chromosome 6 and in Population B on Chromosome 4. Apparently, this is not a common marker. On the integrated map their parent-specific extensions "satA" and "satB" distinguish these markers.

Table 4 Comparison of maps of population A, B and the integrated map

Item	Map of population A	Map of population B	Integrated map
# Of linkage groups	10	10	9
Total map length (cM)	895	627	854
# Of common markers[a]	124	124	124
# Of specific markers[b]	288	98	364
Total # of markers	412	223	488

[a] Common markers are scored and mapped in both populations
[b] Specific markers are scored and mapped in just one of two populations.

Furthermore, through integration of the maps the number of population-specific markers dropped from 385 to 363. These lost specific markers were "Population B"-specific markers that had a LOD score higher than 4.0 but lower than 6.0, and therefore could not meet the criteria for the integrated map.

The marker order between all three maps was highly similar with some minor rearrangements of marker orders at small map intervals of less than 5 cM (for example, in Chromosome 8 in Fig. 2). As the accuracy of the location of the markers in the maps is about 5 cM, these smaller differences are probably due to errors in the data set.

The genetic distances between the maps were similar, although the length of the map of Population B is 30% smaller than the length of the map of Population A. By counting the map distances from the most-distal common markers to the end of the chromosome in Population A minus the map distances from the most-distal common markers to the end of the chromosome in Population B, it was estimated that one-third of the 30% difference in map lengths between the populations was due to an extension of the chromosome lengths by distal markers only scored in Population A.

Discussion

Polymorphism rates

As expected, the polymorphism rate between the two species *L. sativa* and *L. saligna* was very high (81%). The polymorphism rate between the two *L. saligna* parents was also quite high (38.5%). This was not really surprising because morphologically they were also quite different. For instance, line A had pinnatifid, deeply lobed leaves and line B did not have lobed leaves. The polymorphism rate between the two *L. sativa* parents was 13.4%, which is similar to that in the "Calmar×Kordaat" map (Kesseli et al. 1994). In consequence, our integrated map consists predominantly of markers that discriminate between *L. sativa* and *L. saligna*. In addition, it provides several markers that can be used to distinguish between *L. saligna* lines and between *L. sativa* cultivars, although for the latter to a lesser extent.

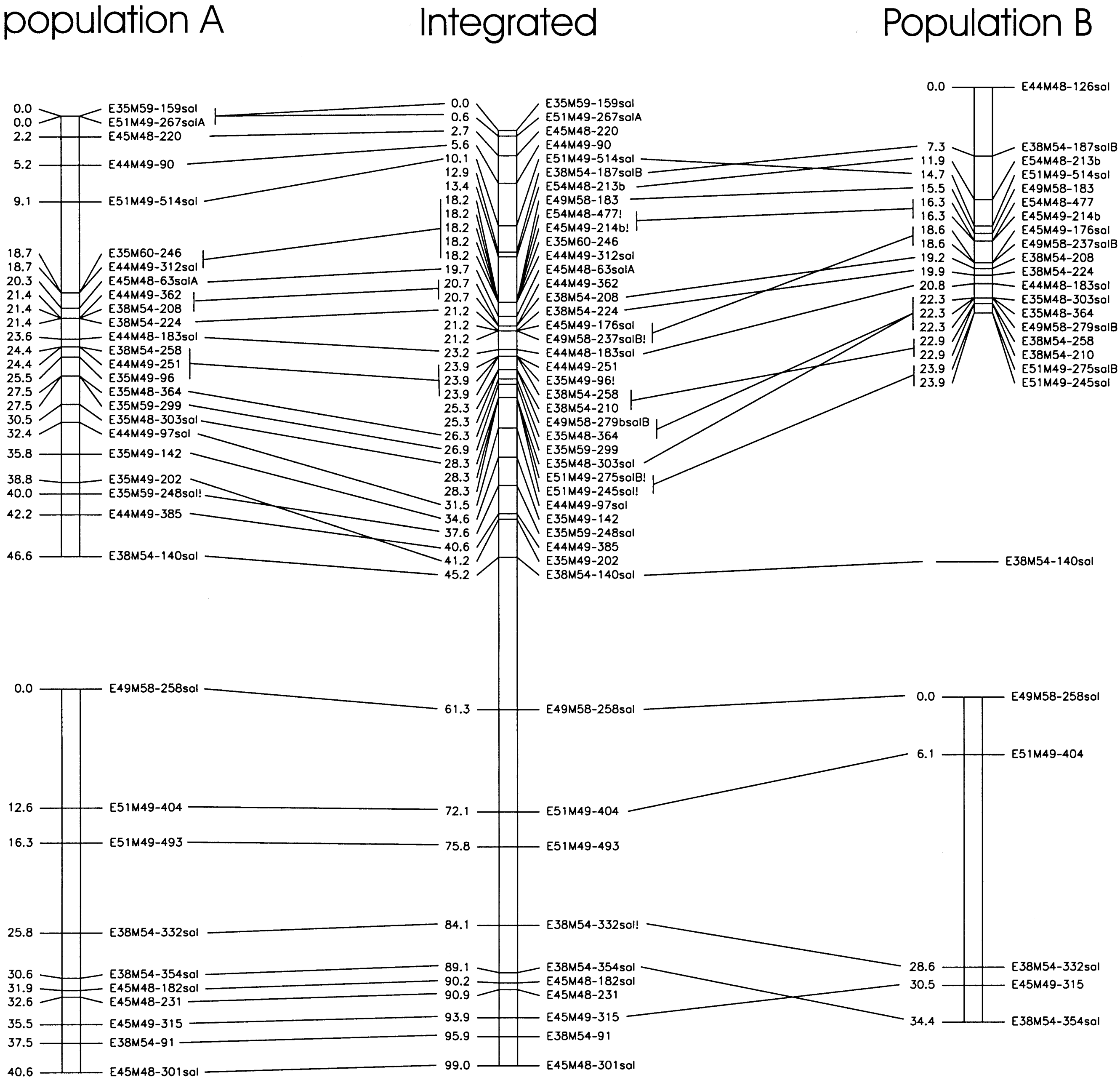


Fig. 2 Comparison of Chromosome 8 of the integrated map and the corresponding linkage groups of Populations A and B. For the nomenclature of markers see legends of Fig. 1. Common markers between maps are connected by lines

Segregation distortion

The observed distorted segregation ratios calculated from the AFLP markers in the populations were only similar between the populations for the top of Chromosome 5, favouring *L. saligna* alleles. This may mean that gametes with one or more *L. saligna* alleles on the top of Chromosome 5 have a much higher fitness than those genotypes with the corresponding *L. sativa* alleles. The observed selection for heterozygotes on Chromosome 8 of Population A can be due to a locus with a high overdominance effect.

The amount and severity of observed skewness in the F_2 populations was similar to other reported skewnesses in F_2 populations, like tomato (Haanstra et al. 1999), onion (Van Heusden et al. 2000) and maize (Vuylsteke et al. 1999)

Map construction

The high level of polymorphism between *L. sativa* and *L. saligna*, and the high number of loci simultaneously analysed per experiment by the AFLP technique, facilitated the efficient construction of genetic linkage maps of the two interspecific populations.

When the individual maps of the populations were compared, both were highly similar in marker order and distances. The 30% difference in map length between the populations can be explained by two causes. First, map inflation is known to result from scoring errors, even if these occur at a rate below 2%. This is because errors induce an increase of recombinants. This relative map inflation becomes more severe as the average marker distance gets smaller (Lincoln and Lander 1992). So Population A, having more markers than Population B, will for this reason result in a longer map distance than Population B.

Secondly, one-third of the 30% difference in map length between the populations can be explained by the presence of more distal markers in Population A compared to Population B.

The high similarity in marker order and in marker distance among the two maps facilitated the integration of the maps. The integrated map consists of nine linkage groups, has 488 markers and is 854-cM long. Compared with the "Calmar×Kordaat" map of more than 13 groups, 319 markers and 1,950 cM, our map shows the expected number of chromosomes and is considerably shorter. Striking differences between the construction of the maps are: (1) our integrated map used 180 (126 +54) instead of 66 F_2 plants as mapping population, (2) the "Calmar×Kordaat" map contains 41% RAPD markers which are now considered as poorly reproducible, and (3) different mapping software, with different mapping functions, was used. For our integrated map JoinMap 2.0 (Stam et al. 1995) was used instead of Linkage 1 (Suiter et al. 1983) and Mapmaker 2.0 (Lander et al.1987) for the "Calmar × Kordaat" map.

In the present study AFLP markers have shown to be reliable, efficient and locus specific markers. This latter is shown by the fact that out of 125 previously considered common markers 124 were mapped to the same locus.

Codominant and monolocus SSRs are also reliable and very informative, but are less efficient than AFLP markers and therefore are not recommended for generating a map. Moreover, in the present study only four SSRs could be scored codominantly. This reflects the fact that SSRs are more informative for closely related genetic populations in lettuce.

Random distribution of markers

Several publications on genetic linkage maps with AFLP markers, based on the *Eco*RI/*Mse*I restriction enzyme combination, report that these markers tend to cluster around centromeric regions (Qi et al. 1998; Haanstra et al. 1999; Vuylsteke et al 1999; Young et al. 1999). An excess of repeats in the centromere may explain this phenomenon, observed in other crops. These repeats may have relatively more one-base pair mutations detected by AFLPs and less recombination than other regions of the genome, which results in the AFLP clusters on the map.

Severe clustering of markers was not manifest in the present genetic linkage map of lettuce. If the above-mentioned theory holds true, the centromeric regions of lettuce will have relatively fewer repeats compared to the rest of the genome and compared to other crops like tomato, barley, maize and soybean. Alternatively, the centromere in lettuce could be much smaller compared to the other crops. In this case the regions with suppressed recombination are much smaller.

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