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Use of locus-specific AFLP markers to construct a high-density molecular map in barley

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Abstract By using 25 primer combinations, 563 AFLP markers segregating in a recombinant inbred population (103 lines, F_9) derived from L94/Vada were generated. The 38 AFLP markers in common to the existing AFLP/RFLP combined Proctor/Nudinka map, one STS marker, and four phenotypic markers with known map positions, were used to assign present AFLP linkage groups to barley chromosomes. The constructed high-density molecular map contains 561 AFLP markers, three morphological markers, one disease resistance gene and one STS marker, and covers a 1062-cM genetic distance, corresponding to an average of one marker per 1.9 cM. However, extremely uneven distributions of AFLP markers and strong clustering of markers around the centromere were identified in the present AFLP map. Around the centromeric region, 289 markers cover a genetic distance of 155 cM, corresponding to one marker per 0.5 cM; on the distal parts, 906 cM were covered by 277 markers, corresponding to one marker per 3.3 cM. Three gaps larger than 20 cM still exist on chromosomes 1, 3 and 5. A skeletal map with a uniform distribution of markers can be extracted from the high-density map, and can be applied to detect and map loci underlying quantitative traits. However, the application of this map is restricted to barley species since hardly any marker in common to a closely related *Triticum* species could be identified.

Key words *Hordeum vulgare* · AFLP markers · Genetic linkage map · Recombinant inbred lines · Locus specificity

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Introduction

In barley (*Hordeum vulgare* L.), restriction fragment length polymorphism (RFLP) has been extensively used for the construction of genetic linkage maps (Kleinhofs et al. 1988; Shin et al. 1990; Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993 b; Kasha and Kleinhofs 1994). These have enabled the mapping of important agronomic qualitative and quantitative traits, including the *ym*4 virus resistance gene (Graner and Bauer 1993), the *denso* dwarfing genes (Laurie et al. 1993), the liguleless gene (Pratchett and Laurie 1994), a photoperiod-response gene (Laurie et al. 1994), and the quantitative loci for yield, malting quality and disease resistance (Hayes et al. 1994; Han et al. 1995; Kjr et al. 1995; Thomas et al. 1995, 1996). A limitation of the application of RFLPs is the labour and time-consuming technology of Southern hybridisation that has to be repeated for each RFLP marker. Moreover, due to a large genome size ($1C = 5.1 \times 10^9$ bp) (Bennett and Leitch 1995) and relatively lower variation within the barley species, the progress in map construction by RFLP is slow and expensive. Recently, AFLP markers have been developed and their power as genetic markers has been demonstrated (Zabeau and Vos 1993; Vos et al. 1995). A great advantage of the AFLP technique is the simultaneous identification of a large number of marker loci. Moreover, fragments amplified with the same primer combinations and with the same mobility in gels are most likely homologous and hence locus specific (Qi and Lindhout 1997). Becker et al. (1995) has added 116 AFLP markers to the already existing Proctor/Nudinka RFLP map (Heun et al. 1991). Recently, Waugh et al. (1997) increased the marker density in three barley genetic maps by adding 234, 194 and 376 AFLP markers, respectively.

In a project for mapping QTLs for partial resistance to barley leaf rust, we applied the AFLP technique to generate molecular markers. To assign AFLP linkage groups to barley chromosomes, AFLP markers

common to two mapping populations, Nudinka/Proctor and L94/Vada, were identified and subsequently a high-density molecular map was constructed using 103 RILs (F_9) derived from the cross L94 × Vada.

Materials and methods

Plant materials

A population of 103 F_9 recombinant inbred lines (RILs) was obtained from a cross of L94 × Vada by single-seed descent and used as a mapping population. L94 is a line from an Ethiopian landrace, with black and covered seeds; it is extremely susceptible to leaf rust (*Puccinia hordei*). Vada is an obsolete commercial cultivar, with white and naked seeds, bred by the Department of Plant Breeding, Wageningen Agricultural University, and has a high level of partial resistance to *P. hordei* (Niks 1982).

The AFLP protocol

The same AFLP procedure as described by Qi and Lindhout (1997) was used in the present study. Restriction enzymes, adapters and primers were as described in Becker et al. (1995) and Qi and Lindhout (1997). In total, the following 25 primer combinations were employed: E37M32, E37M33, E37M38, E40M32, E40M38, E40M40, E41M32, E41M40, E42M32, E42M40, E32M61, E33M54, E33M55, E33M58, E33M61, E35M48, E35M54, E35M55, E35M61, E38M54, E38M55, E39M61, E42M48, E42M51, and E45M55. The first ten primer combinations have been used before to generate AFLP markers for the construction of the Proctor/Nudinka map (Becker et al. 1995), and the other 15 primer combinations were the most informative ones as indicated in the previous study of Qi and Lindhout (1997).

Data analysis and map construction

Segregating markers in the mapping population were designated according to the AFLP profiles of the parent lines (see GrainGenes WWW page, map data; Qi and Lindhout 1997). Clearly visible markers were scored as dominant. Three morphological markers m*n* (naked seeds), m*B* (black seeds) and m*Pau* (purple auricle), and one disease resistance gene d*ml-o* (resistance to *Erysiphe graminis*), were also scored as qualitative traits. The primer pair KV1 and KV9 derived from the sequence of the *Hor2* gene was used as an STS marker for the *Hor2* locus (for sequences, see Kanazin et al. 1993). The amplified products were digested by *Hae*III to reveal polymorphism. Missing data for any marker were very limited in the present study (< 2%).

A software package, JoinMap 2.0 (Stam 1993; Stam and Van Ooijen 1996) was used for linkage grouping and map construction. Linkage groups were assigned to the corresponding barley chromosomes by using the locus-specific AFLP markers, that had already been mapped on the Proctor/Nudinka map (Becker et al. 1995), morphological markers, and the *Hor2* gene. Kosambi's mapping function was applied for map-distance calculation (Kosambi 1944).

Results

Data scoring

By using 25 primer combinations, 563 easily scored AFLP markers were identified, corresponding to an average of 23 markers per primer combination, ranging from 11 (E40M40) to 33 (E33M61). The number of usable segregating markers was slightly less than observed in a previous study (Qi and Lindhout 1997). This was due to poor separation of amplification products of nearly identical size.

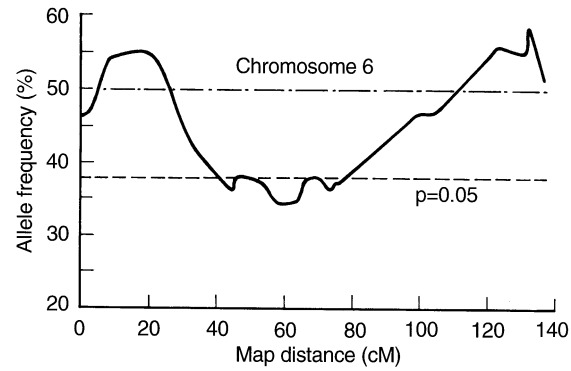


Fig. 1 Frequency distribution of the L94 alleles on chromosome 6 (6H). The fitness test was according to a 1:1 ratio which was approximated in the F_9 RILs population

Among 568 markers, 286 were L94-specific and 281 were Vada-specific; and one STS marker showed co-dominance. The majority of the markers (92%) showed a 1:1 segregation ratio for the two parental alleles ($P \leqslant 0.05$), as was expected for the F_9 recombinant inbred population. Among the 48 markers with distorted segregation, only three were skewed towards L94 alleles and 45 towards Vada alleles; the latter all mapped on chromosome 6 (Fig. 1).

For mapping, groups of markers with identical segregation were regarded as a single marker; the marker with the fewest missing values was chosen as the representative one for this group. In total, 433 markers, of which 61 co-segregated with at least one other marker and 372 of which showed unique segregation, were applied for the construction of linkage groups (Fig. 2).

Map construction

By using ten primer combinations, 38 AFLP markers were identified in our L94/Vada mapping population that were identical in the Proctor/Nudinka population (Becker et al. 1995). Markers in common tightly linked in a single linkage group in our L94/Vada population also showed linkage in the Proctor/Nudinka population. Similar genetic distances and identical orders of the markers shared by the two mapping populations strongly indicated that these AFLP markers are locus specific and hence their map positions can be used as anchor points across populations (Table 1).

The 563 AFLP markers, four phenotypic markers, and one STS marker, were split into 21 groups at

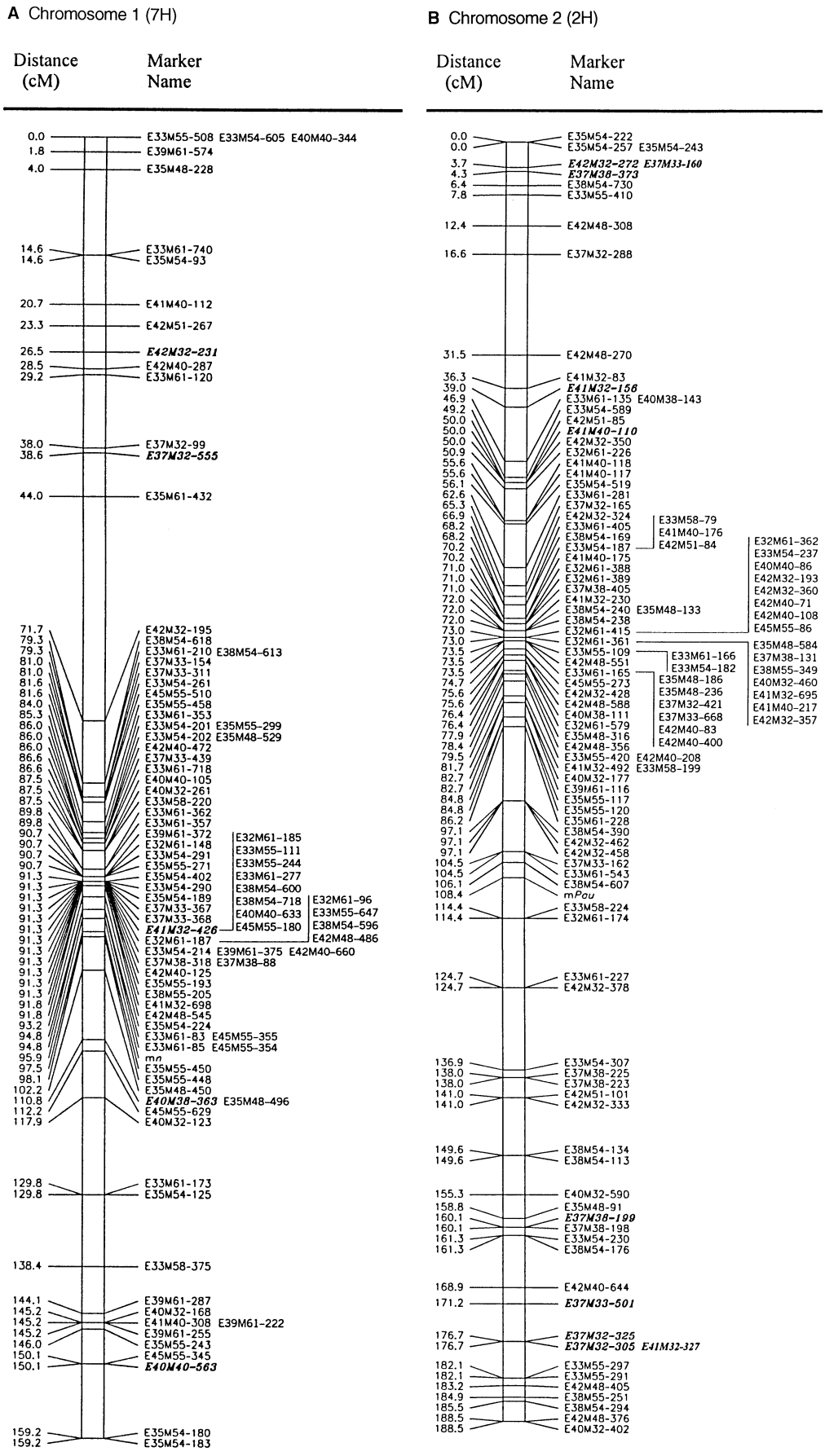


Fig. 2 The barley L94/Vada AFLP map. A–G correspond to barley chromosomes 1 to 7, with the short arm at the top. Markers with a ***bold-italic*** font were common to both the present map and the Proctor × Nudinka map. The markers with identical segregation are aligned with the corresponding representative markers

Fig. 2 See page 378 for legend

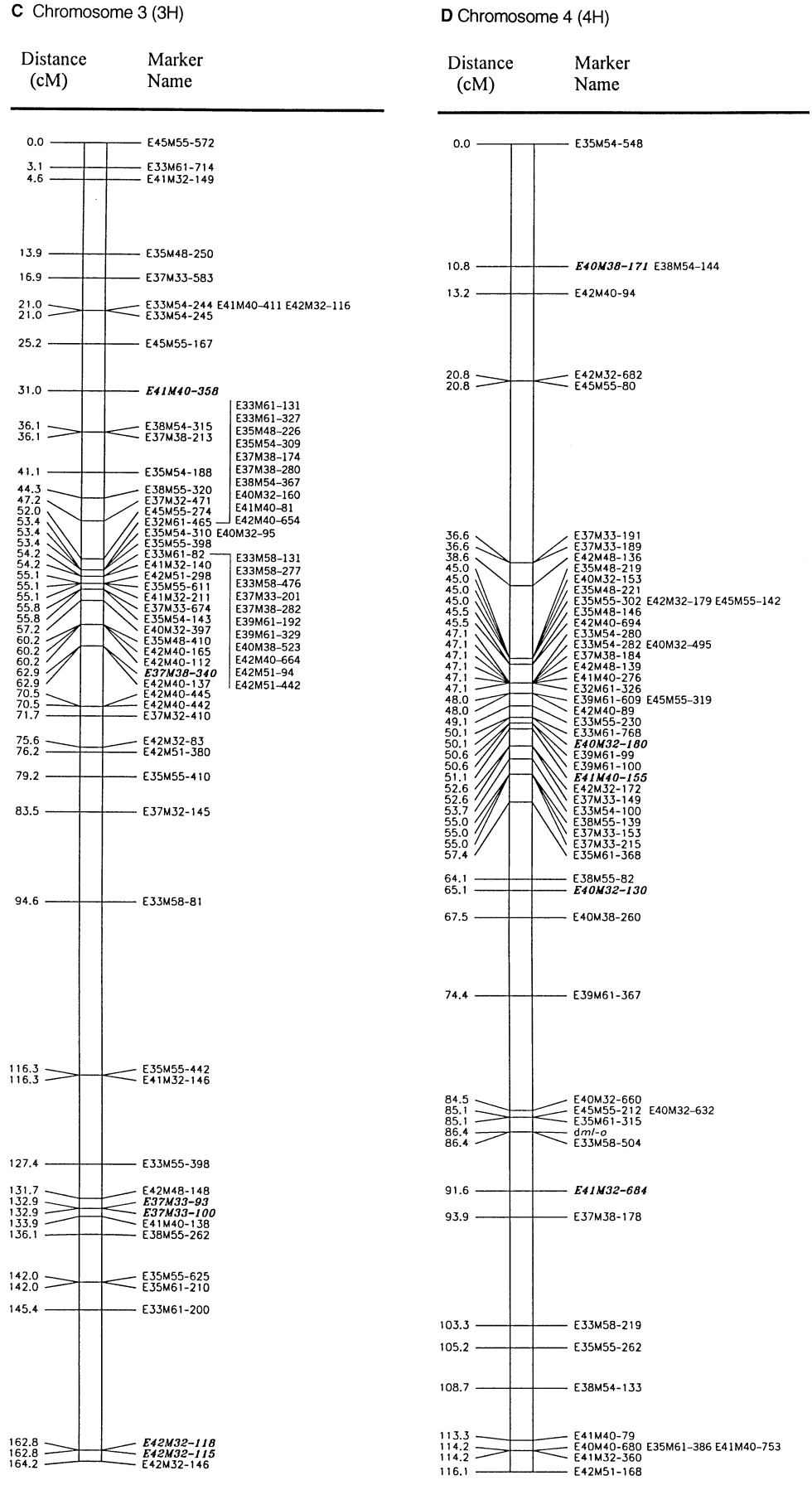
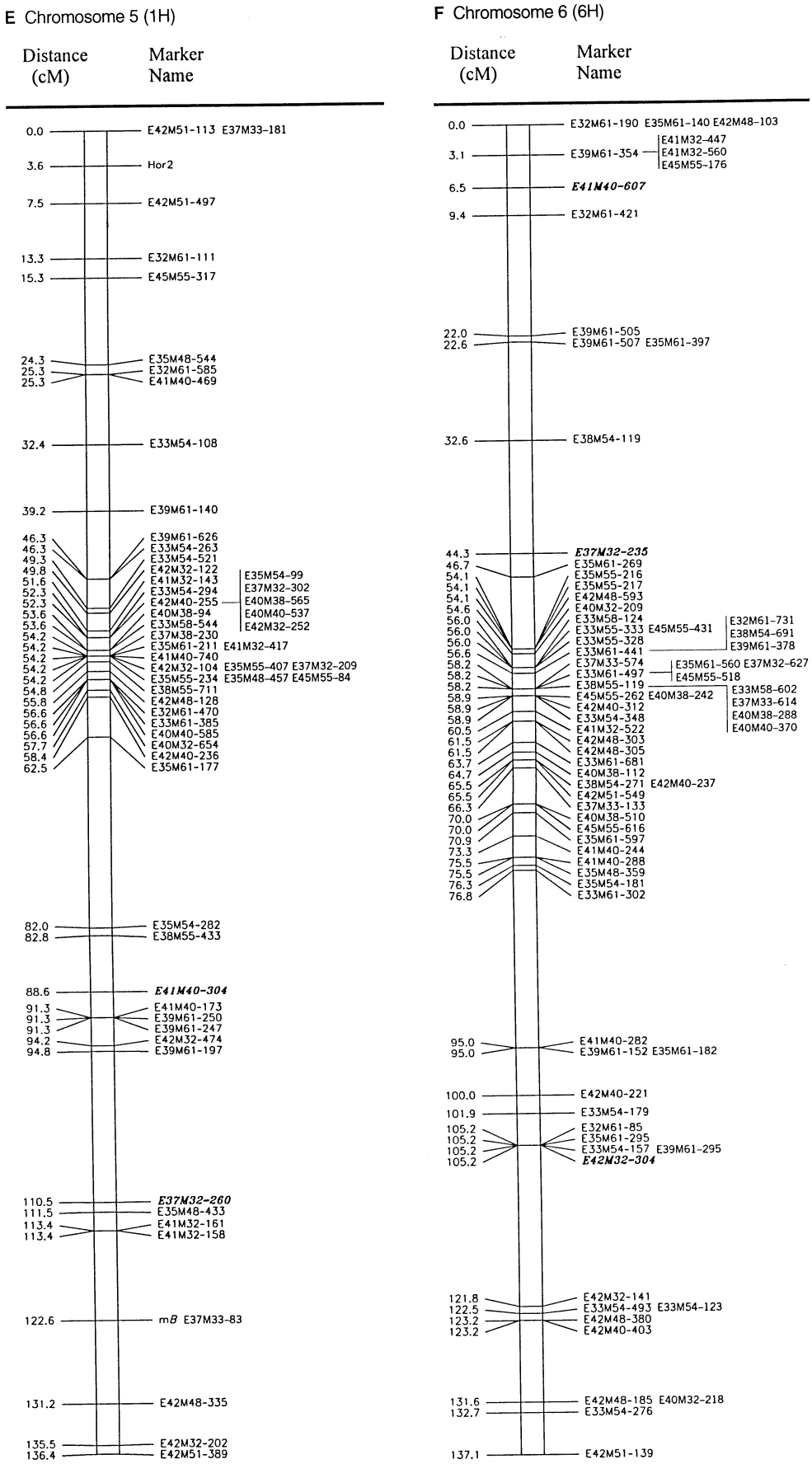
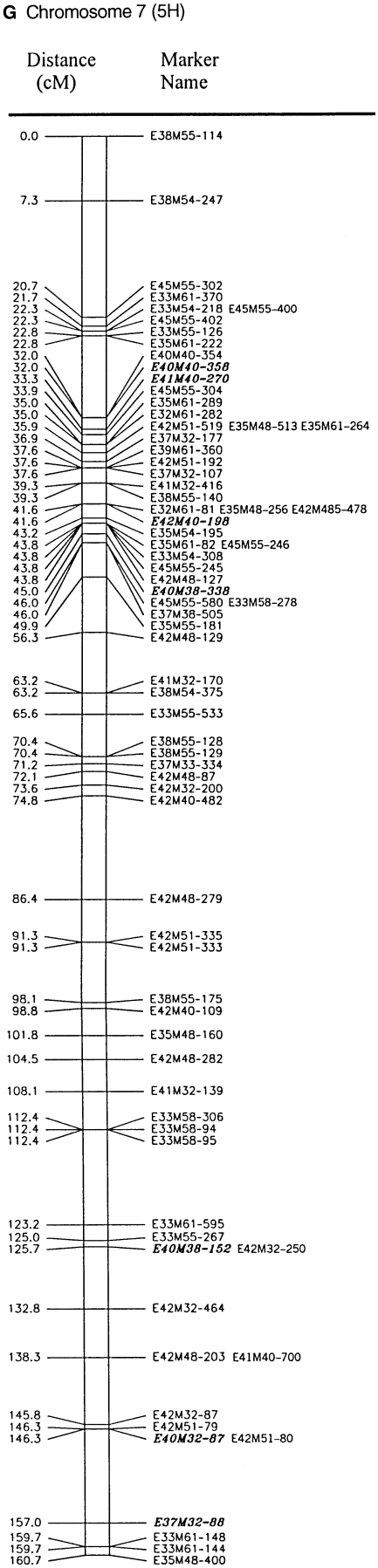


Fig. 2 See page 378 for legend





a LOD threshold grouping value of 7.0. Only two markers, E33M55-191 with 37 missing data and E33M54-310, were not linked to any other marker at a LOD value of 5.0, and one group of three markers remained separated at a LOD threshold value lower than 3.0. The 38 AFLP markers in common, as well as four phenotypic markers (m*n*, m*Pau*, m*B*, & d*ml-o*) and *Hor2*, were used to assign AFLP linkage groups to seven barley chromosomes. Except for the five isolated markers described above, the other 18 groups contained at least one anchor marker and were assigned to the seven barley chromosomes. Chromosomes 1, 2 and 4 were composed of two groups, chromosomes 3, 5, 6 and 7 of three groups. The unassigned group containing three AFLP markers was assigned to chromosome 5 because it showed the tightest linkage (LOD = 2.6 for m*B* and E42M48-335) to the other markers on this chromosome and fitted very well on the map of this chromosome.

The resulting map contains 566 markers covering a total map distance of 1062 cM corresponding to approximately 1.9 cM per marker. Chromosome 2 has the largest number of markers (120) with the longest genetic distance (189 cM), and chromosome 4 is the shortest one. Remarkably, marker clustering was observed on all seven chromosomes (Fig. 2 and Table 2). Using the Proctor/Nudinka AFLP and RFLP combined map (Becker et al. 1995) as a bridge, the present AFLP map was compared with the integrated RFLP map (Qi et al. 1996) which was based on four independent RFLP maps (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993 b; Kasha and Kleinhofs 1994) with known centromere regions (Kleinhofs et al. 1993 a). The clusters of AFLP markers on the present map were very likely also located around centromeric regions. In the putative centromeric regions, jointly spanning 155 cM, 289 markers were mapped, corresponding to 0.5 cM per marker. In contrast, the chromosome arms, spanning 906 cM, were covered by 277 markers, corresponding to 3.3 cM per marker. Despite this small average genetic distance between markers, chromosomes 1, 3 and 5 still contain a gap larger than 20 cM. Several smaller gaps (10–15 cM) are present on the distal parts of the chromosomes (Fig. 2).

There are no clear indications of uncovered regions on the distal parts of each chromosome though some chromosomes were quite short, such as chromosome 7 in the present map (161 cM) compared to the integrated map (195 cM). Conversely, there are also no clear indications of having covered extra distal parts by the AFLP markers, as compared to the integrated RFLP map (with a 1060-cM total length and 880 markers, Qi et al. 1996).

Fig. 2 See page 378 for legend

Table 1 Genetic distances (cM) of tightly linked marker pairs/groups in two mapping populations[a]

Marker pairs/groups	L94/ Vada	Proctor/ Nudinka
E42M32-231/E37M32-555	12.3	16.8 (1)[b]
E41M32-156/E41M40-110	12.8	25.5 (2)
E42M32-272/E37M38-373	0.5	1.0 (2)
E37M38-199/E37M33-501/E37M32-325	10.6/4.9	13.5/7.2 (2)
E41M40-155/E40M32-180/E40M32-130	0.5/15.7	2.1/18.8 (4)
E41M40-270/E40M40-358/E40M38-338	2.5/10.0	3.5/8.2 (7)

[a] As an example, only six pairs and groups are represented in this table
[b] Numbers in parentheses indicated the chromosomes to which these markers were assigned on the Proctor/Nudinka map

In conclusion, despite the non-uniform distribution of markers along chromosomes and the presence of three gaps of more than 20 cM, the present AFLP map most likely covers the entire barley genome, or nearly so. From this high-density map a skeletal map with a fairly uniform distribution of markers can be extracted. Such a skeletal map may serve for the detection and mapping of loci underlying qualitative and quantitative traits.

Discussion

Reliability of the map

Genetic maps are calculated from the recombination rates between loci as a result of chromosome crossovers at meiosis. Recombination rates may be influenced by environmental factors (Allard 1963; Powell and Nilan 1963); hence genetic distances may vary from one mapping population to another. But, in general, recombination rates are under genetic control (Paredes and Gepts 1995) and heavily depend on chromosome structure. Comparison of four independent barley RFLP maps indicated that barley genetic linkage maps are quite stable; marker orders are similar and no obvious rearrangements are detectable (Qi et al. 1996). Comparison of the present map with the Proctor/Nudinka map indicated that the orders of all anchor markers (Fig. 2, markers with ***italic bold*** font) on the seven chromosomes were identical and the distances between tightly linked markers were very similar indeed. Moreover, the positions of four phenotypic markers and *Hor2* were also mapped to their correct positions on the barley genome (Franckowiak 1995; Forster 1996; Jensen 1996; Qi et al. 1996).

Non-systematic changes of marker-allele frequencies along a map are indicative of uncertainties in the order of markers. We did not observe any irregular pattern of segregation distortion in our data (Fig. 1). Altogether, our results indicate that we produced a reliable high-density marker map of the barley genome.

Clustering of markers

A high degree of clustering of markers around the centromere is a notable feature in wheat (Chao et al. 1989; Devos et al. 1992; Hart 1994). The clustering of markers at centromeric, and possibly telomeric, areas was found in the tomato high-density map by Tanksley et al. (1992). Clustering of markers at centromeric regions was also observed on the barley integrated map (Qi et al. 1996). Extreme non-uniform distributions of AFLP markers and strong clustering of markers around the putative centromere were identified in the present AFLP map (Fig. 2 and Table 2). The centromeric suppression of recombination may be the main reason for the clustering of markers (Tanksley et al. 1992; Frary et al. 1996). Surprisingly, clustering is much more pronounced in the present AFLP map than in the RFLP maps. This may be due to differences in the sensitivities of RFLP versus AFLP markers. The AFLP technique is extremely sensitive to polymorphism in the genome, as 1-bp length differences in relatively short DNA fragments (50–1000 bp) are already detectable. In species with a large genome, such

Table 2 Summary of L94/Vada mapping data

Chromosomes	No. of markers	Length (cM)	No. of gaps[a]	Chromosome arms		Centromeric clusters	
				No. of markers	Coverage (cM)	No. of markers	Coverage (cM)
1 (7H)	96	159	1	33	128 (3.9)[b]	63	31 (0.5)[b]
2 (2H)	120	189	0	59	156 (2.6)	61	33 (0.5)
3 (3H)	77	164	1	38	147 (3.9)	39	17 (0.4)
4 (4H)	61	116	0	30	97 (3.2)	31	19 (0.6)
5 (1H)	60	136	1	29	118 (4.1)	31	18 (0.6)
6 (6H)	77	137	0	42	119 (2.8)	35	18 (0.5)
7 (5H)	75	161	0	46	140 (3.0)	29	21 (0.7)
Total	566	1062	3	277	906 (3.3)	289	156 (0.5)

[a] A gap is a distance between two adjacent markers of more than 20 cM
[b] Numbers in parentheses are the average distances per marker interval

as barley, a great portion of repetitive sequences occur in the centromeric regions. Small variations such as 1-bp deletion/insertion in repetitive sequences, and/or variable numbers of short sequence repeats (or simple-sequence length polymorphisms, SSPLs), can be detected by the AFLP technique. However, they will probably not be revealed by Southern hybridization with DNA probes, as the repetitive sequences will usually give multiple signals, and multi-copy probes are generally excluded in RFLP map construction. As the amplification products generated by the AFLP technique may contain repeated sequences, there is a higher chance to identify AFLP markers than RFLPs in highly repetitive regions near the centromere. This may be the most plausible explanation for the stronger clustering of AFLP markers.

Locus specificity

If AFLP products show the same mobility in gels, these are very likely to be homologous and locus specific (Qi and Lindhout 1997). This assumption can be verified by comparing the sequences of co-migrating bands and by genetic linkage analyses, respectively. Roupe van der Voort et al. (1997) sequenced co-migrating amplification products in potato and showed that this assumption is nearly always valid. Waugh et al. (1997) found that 81 co-migrating AFLP markers, segregating in more than one population, mapped to similar loci on the three barley genetic maps and only three markers mapped to different positions. In the present study, all 38 co-migrating bands, segregating in two populations, mapped to the same loci. Altogether, these studies indicate the great probability of the locus specificity of AFLP markers.

To investigate whether less-related populations or species may also show markers in common, the AFLP patterns of barley (*H. vulgare*) were compared with those of three *Triticum* species (data not shown). The lack of co-migrating AFLP products suggests that the genetic distance between these species is too large for markers in common to be identified. Consequently, the use of the locus-specific AFLP markers is limited to populations within species or to very closely related species.

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