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Comparative AFLP mapping in two hexaploid oat populations

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Abstract Amplified fragment length polymorphisms (AFLPs) can be used to quickly develop linkage maps in plant species and are especially useful for crops with large genomes like oat (*Avena sativa* L., 2n=6x=42). High reproducibility and consistency are crucial if AFLP linkage maps are employed for comparative mapping. We mapped AFLP markers in combination with restriction fragment length polymorphism (RFLP) markers in two recombinant inbred populations of hexaploid oat in two laboratories to test the consistency of AFLP markers in a polyploid crop. Eight primer combinations produced 102 and 121 scoreable AFLP markers in the respective populations. In a population from the cross Kanota×Ogle, AFLP markers were placed onto a RFLP reference map consisting of 32 linkage groups. Nineteen linkage groups from another population from the cross Kanota×Marion were assigned to the reference map using AFLP and RFLP markers homologous to those used in the Kanota×Ogle cross. Reproducibility of AFLP assays was high in both laboratories and between laboratories. The AFLP markers were well-distributed across the genome in both populations. Many AFLP markers tended to extend the distance between adjacent RFLP markers in linkage analysis. Of the 27 polymorphic AFLPs common in both populations, 20 mapped to homologous linkage groups, 4 were unlinked in at least one population, and 3 mapped to different linkage groups in the two crosses. We believe that 1 of the 3 markers that mapped to a different linkage group in the two populations mapped to homoeologous linkage groups. The linkage map of hexaploid oat is not yet complete, and genomic rearrangements such as translocations exist among cultivars and are likely to account for the remaining two non-syntenous mapping results. AFLPs provide not only a fast and powerful tool for mapping but could be useful in characterizing genomic structural variations among germplasms in hexaploid oat.

Keywords *Avena sativa* · AFLP · Comparative mapping · Homology · Homoeology · RFLP

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Introduction

The identification and characterization of quantitative trait loci (QTLs) for important traits has been widely performed to facilitate marker-assisted selection in crop improvement. Consistency of QTLs across germplasm is required when markers are to be used for selection in multiple populations. Comparative QTL mapping across different populations of a specific crop is a valuable method to identify QTLs that are expressed consistently and to validate their effects. Comparative QTL analysis across populations depends on a molecular marker system that can be applied reliably and that detects homologous loci independent of the population used for QTL detection. A large number of linkage maps in many plant species have been generated using restriction fragment length polymorphisms (RFLPs) (for review,

see Phillips and Vasil 2000). The advantage of using RFLPs in comparative mapping is their relatively high reproducibility and reliability for detecting shared polymorphisms in different populations, despite low levels of polymorphism in some species.

The evaluation of RFLPs is time-consuming, labor-intensive and requires large amounts of DNA. Other marker types based on polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSRs), have been used in mapping studies. RAPDs are understood to be less reliable than RFLPs (Jones et al. 1997), while SSRs are not available in many crops. The method of amplified fragment length polymorphism (AFLP) (Vos et al. 1995; Zabeau and Vos 1993) combines the reliability of RFLPs and the power and sensitivity of PCR-based methods. AFLPs have been used for QTL mapping studies in many crops, including rice (Nandi et al. 1997), barley (Powell et al. 1997), and oat (Jin et al. 1998). The AFLP method requires restriction enzyme digestion of genomic DNA followed by two steps of PCR amplification. The reproducibility of AFLPs was high when the same sugarbeet DNA template was analyzed in a network of different laboratories (Jones et al. 1997) or when different DNA samples of the same genotypes were repeated in the same laboratory (Becker et al. 1995). The sizes of the AFLP products are used to establish shared polymorphisms across populations. Waugh et al. (1997) showed that AFLP product size could be successfully applied to detect homology in three mapping populations of barley. Jin et al. (2000) used AFLP markers to assign linkage groups between two hexaploid oat populations. In their study, 11% of the AFLP fragments with common product sizes mapped to different linkage groups, consequently questioning the reliability of AFLP marker use for comparative mapping.

In the past, linkage mapping and QTL detection in hexaploid oat (*Avena sativa* L.) relied mostly on RFLP markers. To date, the genetic linkage map of hexaploid oat consists of 32 linkage groups which are not all yet assigned to the 21 chromosomes (Kianian et al. 2000a). Several QTL mapping studies have been reported for oat (Beer et al. 1997; Holland et al. 1997; Kianian et al. 1999, 2000b; Siripoonwiwat et al. 1996). The correct identification of common linkage groups for QTL validation across populations can be problematic due to the hexaploid nature of the oat genome. Theoretically, each RFLP probe can identify three different loci in homoeologous regions. It is only possible to identify homologous markers if the same restriction enzymes were used and the same RFLP pattern was detected across populations. Jin et al. (1998) performed QTL mapping using an AFLP linkage map that employed AFLP product size to assign linkage groups in a hexaploid oat population to a reference RFLP/AFLP linkage map in another population. In some cases the assignment was based on a single AFLP, requiring 100% homology to ensure correct identification of the genomic location of an AFLP marker.

In the study reported here, we employed AFLP markers generated in two different laboratories in combination with RFLP markers for linkage mapping in two hexaploid oat populations. Our main objective was to assess the homology of AFLP markers in a comparative linkage map between the two populations. Anchor RFLP markers mapped in both populations provided a high degree of confidence for determining whether AFLPs can consistently detect homologous loci in hexaploid oat. Secondary objectives were to determine the reproducibility of AFLP markers generated in two laboratories and two populations and to evaluate whether AFLP markers can be used to improve the linkage map of hexaploid oat.

Materials and methods

Mapping populations

Two populations of recombinant inbred lines (RILs) were used in this study. The population Kanota×Ogle (K/O) originated from 71 F_6-derived lines developed from a cross between the facultative winter-type cultivar Kanota and the spring cultivar Ogle (O'Donoughue et al. 1995). Progenies of those lines were advanced by single-seed descent to produce 71 F_{10}-derived lines, which were used in the AFLP analyses. The population Kanota×Marion' (K/M) used for AFLP analyses consisted of 137 F_6-derived RILs developed from a cross between Kanota and the spring cultivar Marion (Kianian et al. 1999). RFLP assays were performed on F_6-derived RILs in both populations and have been described previously (Kianian et al. 2000a; O'Donoughue et al. 1995).

AFLP assays

AFLP assays were performed for K/O on the 71 F_{10}-derived lines at Agriculture and Agri-Food Canada in Winnipeg, Manitoba, Canada, and for K/M on 137 F_6-derived lines at the Department of Agronomy and Plant Genetics, University of Minnesota, USA, according to the method of Zabeau and Vos (1993) with modifications. For the K/O population AFLP analyses, dried DNA was provided to the Winnipeg laboratory by Dr. N. Tinker, Agriculture and Agri-Food Canada, Ottawa, Ontario. The RILs of both populations were grown in 1998 in growth chambers: K/O at Ottawa and K/M at Minnesota. Tissue was collected from 2-week-old plants and lyophilized. DNA was extracted using the method of Saghai-Maroof et al. (1984). Template DNA (1 µg) was digested with 5 U of the restriction enzymes *Mse*I and *Pst*I (Gibco/BRL, Rockville, Md.) and ligated to 25 pmol of *Mse*I and 2.5 pmol *Pst*I adaptors (adaptors and primers were supplied by Integrated DNA Technologies, Coralville, Iowa) in a volume of 50 µl. The pre-amplification was performed using 1 µl ligated DNA and 37.5 ng pre-amplification primers (*Mse*I+1 and *Pst*I+1). The pre-amplification reaction was diluted 1:5 with ddH_2O; 2 µl of this dilution and 30 ng of selective amplification primers (*Mse*I+3 and *Pst*I+3) were used for the selective amplification with eight primer combinations in both populations. Denatured PCR reactions were loaded on 5% denaturing polyacrylamide gels and electrophoresed at 80–85 W for 2.5–3 h. Silver staining using the SILVER SEQUENCE staining procedure (Promega) was performed to visualize DNA. The gels were dried, and a permanent image of the gel was captured with a flat bed scanner (Epson ES-300C) in Winnipeg or using Automatic Processor Compatible (APC) film (Promega) in Minnesota. The original gels were scored by two independent readers; the primarily dominant and some codominant scores were entered into a database. A χ^2 analysis was performed for each AFLP marker locus to test for deviations from the expected allele frequencies of 0.5 at a significance level of 0.05. Because multiple tests were performed for each population, appropriate Type-I error rates were determined by the sequentially rejective Bonferroni procedure (Holm 1979).

A comparison of AFLP products from the same primer combination in both populations was performed using gel scans in K/O

and APC film in K/M. All three parents (Kanota, Ogle, and Marion) were included in the gels in Minnesota to facilitate the comparison. Common polymorphisms were identified when fragments had the same size and pattern in both populations.

The AFLP assay was repeated for the parental lines and 10 random RILs from each population with three primer combinations. The bands for marker aaccac376 were excised separately in K/O and K/M from the silver-stained gel, eluted in 20 µl TE buffer and reamplified using the same primer combination and the same conditions as for the selective amplification. The amplification product was purified using Wizard PCR Preps DNA Purification System (Promega) and sent to a service lab for sequence analysis.

Linkage mapping

The K/O population had been used previously to generate a genetic linkage map that serves as a base map for genetic studies in hexaploid oat. This map is being updated continually as new marker data are generated (Kianian et al. 2000a; O'Donoughue et al. 1995). The current map consists of a framework with 221 mapped anchor RFLP loci forming 32 linkage groups. An additional 439 markers, most of which were RFLPs, were mapped relative to this framework. AFLP markers were mapped with the anchor RFLPs to test whether the linkage groups of the framework map could be collapsed by AFLPs covering previously undetected portions of the genome. Because the framework map represents the best order of all markers, with approximately equal spacing between them, the AFLP markers were assigned relative to the anchor markers for the comparative mapping instead of re-mapping the framework. Assignment of AFLP loci to the K/O map was performed using the software package MAP5 (Tinker 1999).

In population K/M, a new framework map was constructed using 60 previously mapped RFLP markers which had been chosen to represent specific regions associated with groat quality traits (Kianian et al. 2000a, b) and 118 AFLP markers. The software package MAPMAKER (Lander et al. 1987) was used to assign markers to linkage groups at LOD 6.0. Ordering within linkage groups was performed at LOD 3.0. Markers that extended the distance between 2 loci were excluded from the framework map and assigned relative to the remaining anchor markers using the program MAP5. Linkage groups in K/M were assigned to linkage groups in K/O using homologous RFLP and AFLP markers.

Results and discussion

Reproducibility of AFLPs

The reproducibility of AFLPs was high across the two populations in both laboratories (data not shown). Almost all fragments produced in Winnipeg for population K/O could also be identified on gels in Minnesota for Kanota and Ogle. However, major differences occurred in the intensity of the bands for some primer combinations on different gels. This resulted in a reduction of cross-mappable AFLP markers because some polymorphisms could only be scored in one population due to faint bands in the other one. Similar differences in the intensity of AFLP bands were observed within the K/M population, for which two parts of the population had to be amplified in two separate PCR reactions and products electrophoresed in two separate gels. In some cases, gel electrophoresis had to be repeated for one part of the population to obtain the same quality as in the other part. Differences in band intensity between the two populations appear to result from methodology rather than differences between germplasm. Since bands could be reproduced consistently in the PCR reaction and only intensity differences were observed across gels, differences were assumed to be a result of the silver-staining procedure.

Segregation and linkage of AFLPs

In the K/O population, eight primer combinations produced 102 polymorphic AFLP fragments. The χ^2 analysis revealed that 16 of these showed significant distortion from the expected allele frequency of 0.5; all but one of these were extremely skewed toward Kanota. These 16 markers remained unlinked in a preliminary analysis and were subsequently excluded from the data set. Of the remaining 86 markers, 71 mapped to 21 of the 32 linkage groups in the K/O base map, but 15 remained unlinked. This relatively high proportion (17%) of unlinked AFLP markers was surprising because the linkage map in this population contains over 700 markers. The AFLP markers were mapped with the complete data set of 700 RFLP markers to determine whether they would form linkage groups with previously unlinked markers. Three AFLP markers could be associated with unlinked RFLP markers by forming two short new linkage groups. These may represent regions of the genome which could not be linked to other linkage groups due to the lack of bridging markers.

In the K/M population, 121 AFLP markers were generated by eight primer combinations. Only 2 markers showed significant distortion from the expected gene frequency of 0.5. A new framework map was developed consisting of 103 anchor markers mapped to 27 linkage groups, spanning a total distance of 736 cM. Eight linkage groups contained only AFLP loci, and each linkage group contained at least 1 AFLP locus. Twenty-one AFLP markers and 11 RFLP markers remained unlinked, indicating that higher map coverage is needed in this population.

Comparative mapping

Six common primer combinations produced about 80 scoreable polymorphisms in each population of which 27 (33%) were polymorphic in both populations and were used for comparative mapping (Table 1). In the comparative map, 19 linkage groups in K/O also could be identified in K/M. Eight of the common linkage groups could be assigned using cross-mapped AFLP and RFLP loci; 5 were assigned based only on AFLP loci; 6 were assigned based only on RFLP loci (Fig. 1). The relative order of loci was conserved with some minor inversions of adjacent markers except on linkage group 3–38, where a major rearrangement occurred (Fig. 1). This rearrangement could be explained by the fact that linkage group 3 in K/O is assumed to involve a translocation between chromosomes 7 C and 17 (Kianian et al. 1997). Linkage groups joined by mapping or aneuploid analysis (Kianian et al. 2000a) after the first version of the K/O map had

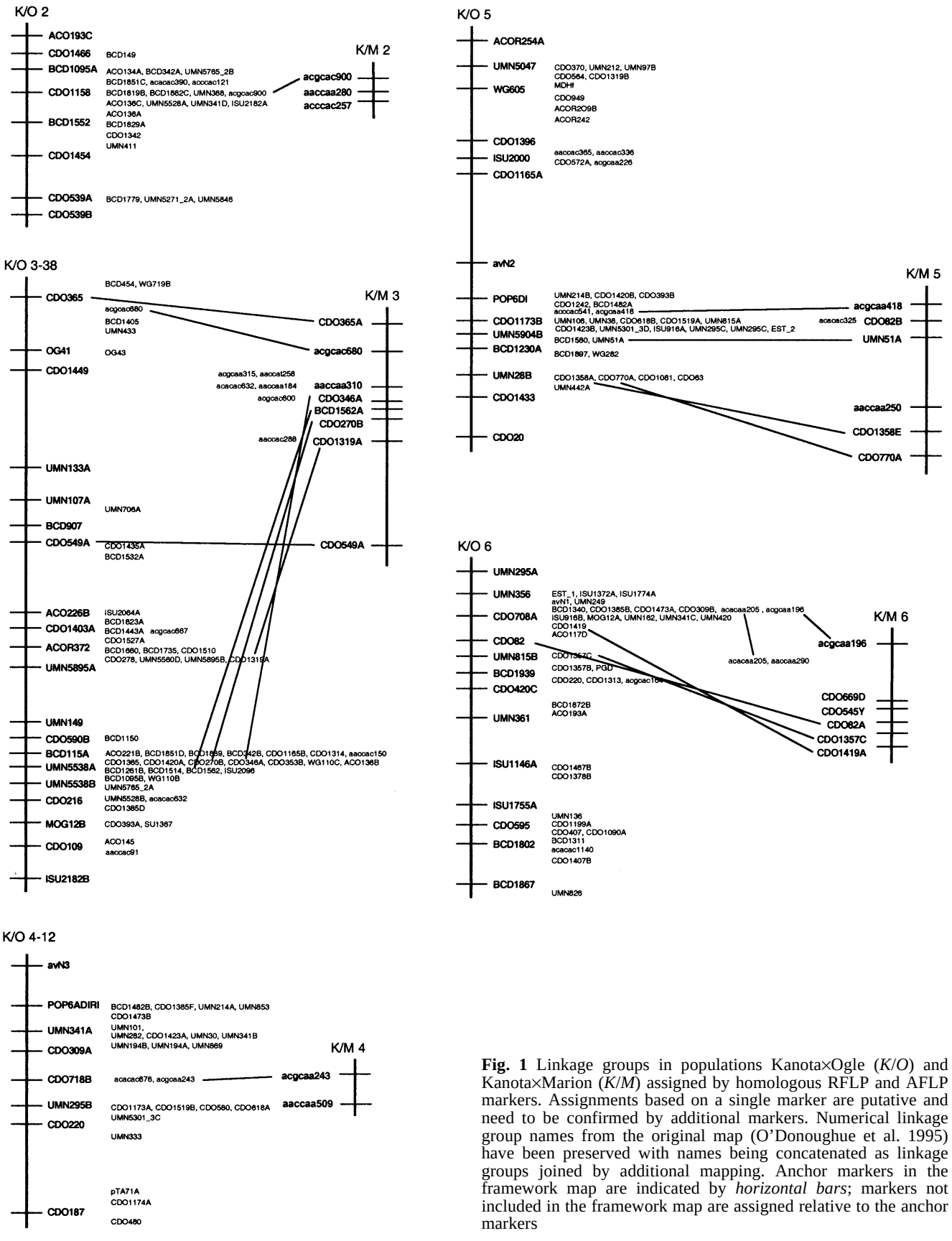

Fig. 1 Linkage groups in populations Kanota×Ogle (*K/O*) and Kanota×Marion (*K/M*) assigned by homologous RFLP and AFLP markers. Assignments based on a single marker are putative and need to be confirmed by additional markers. Numerical linkage group names from the original map (O'Donoughue et al. 1995) have been preserved with names being concatenated as linkage groups joined by additional mapping. Anchor markers in the framework map are indicated by *horizontal bars*; markers not included in the framework map are assigned relative to the anchor markers

Linkage groups in populations KxO and KxM assigned by homologous RFLP and AFLP markers

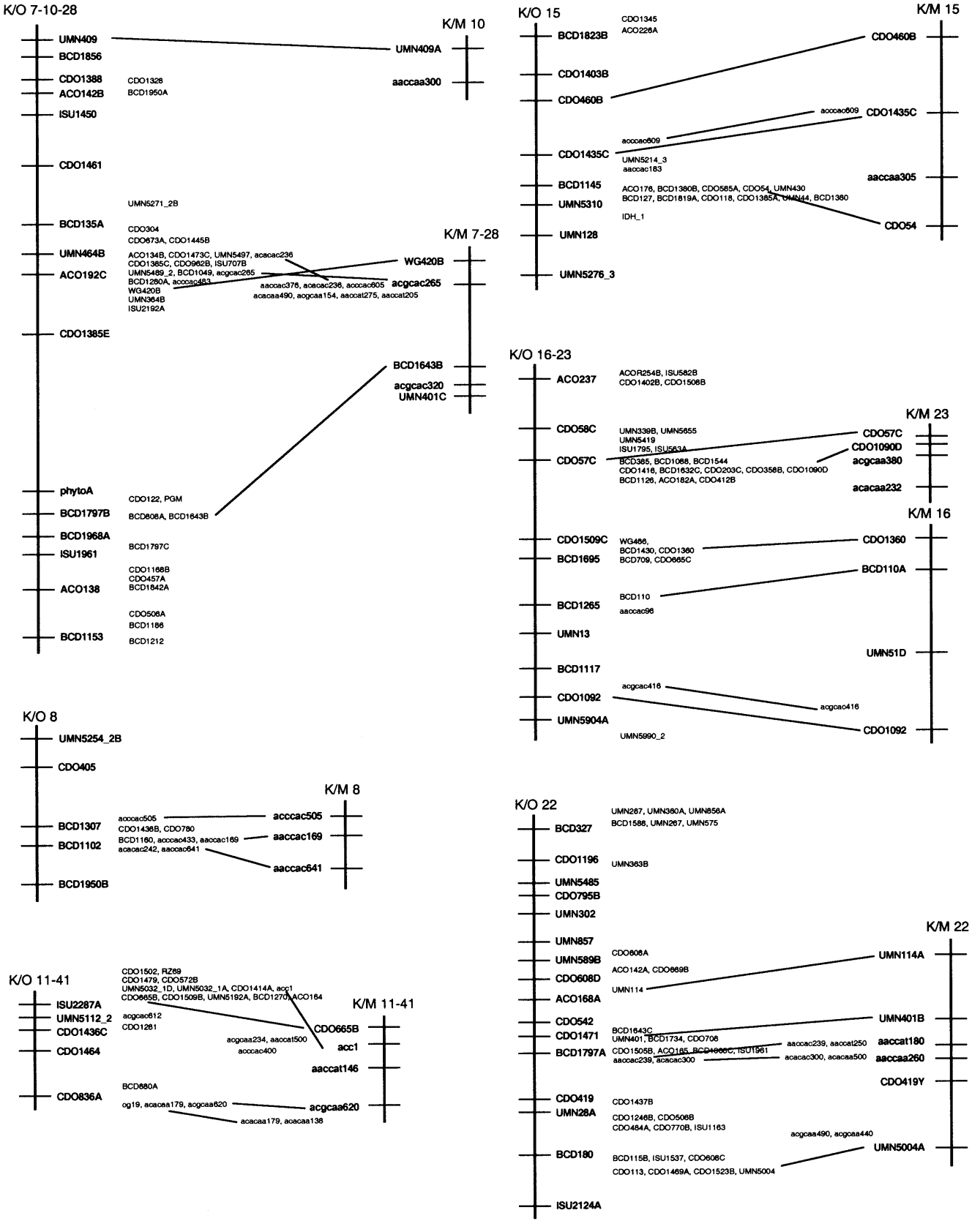

Fig. 1 continued

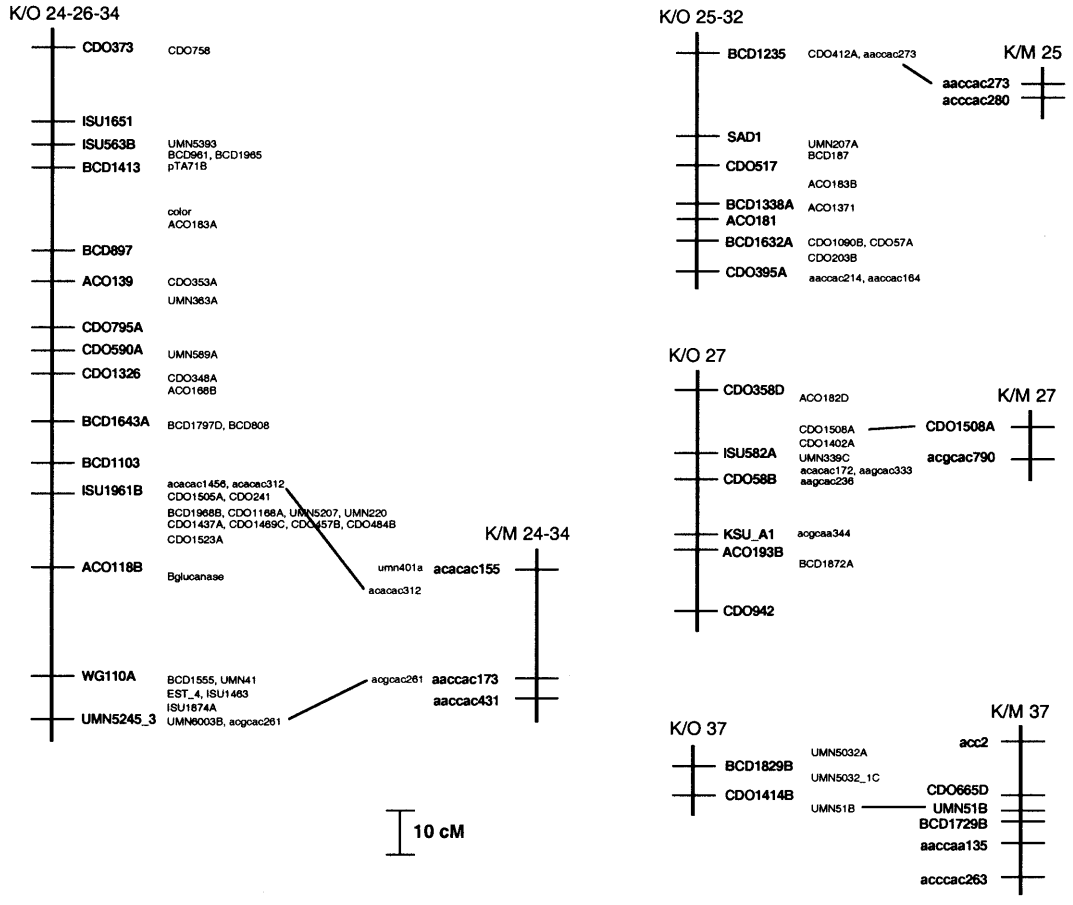

Fig. 1 continued

been published were also joined in K/M; for example, linkage groups 7–28, 11–41, and 24–34. In contrast, linkage analysis in K/M could not confirm that linkage groups 16 and 23 are linked (Fig. 1). Linkage analysis in K/M supported the joining of linkage groups 7–28 and 11–41 into a single linkage group (LOD >3.0) by an AFLP marker (data not shown). However, there was no indication of linkage between these groups in K/O. This assignment in K/M needs to be tested using additional markers or aneuploid analysis. Seven small linkage groups containing 2–4 loci that were identified in K/M could not be assigned to linkage groups in K/O; 6 of these contained only AFLP loci that were not polymorphic in K/O, and 1 contained only RFLP loci that could not be scored in K/O. Three small linkage groups in K/M were assigned to linkage groups in K/O based on a single AFLP marker and 3 were assigned based on a single RFLP marker; these assignments need to be verified by additional markers.

Of the 27 cross-mapped AFLP markers, 17 were associated with the same anchor markers or mapped in the same order in K/O and K/M and 3 were assigned putatively to common linkage groups. Two AFLP markers remained unlinked in both populations, and 2 markers were assigned to linkage groups in K/O but were unlinked in K/M. The latter 2 markers map to regions where the coverage was not high enough in K/M to link them to other loci. Three markers (aaccac376, acccac263, and acgcaa234) were assigned to different linkage groups in K/O and K/M. The parent lines Kanota, Ogle, and Marion plus a subsample of 10 RILs from each population were used to repeat the selective amplification with these three respective primer combinations and were electrophoresed on a single gel to confirm that the bands had the same size in both populations. The acccac263 and acgcaa234 markers amplified a band in Kanota but not in Ogle or Marion (Fig. 2). Therefore, it can be ruled out that these 2 markers identify different loci in K/O versus K/M. The aaccac376 marker amplified a band in Ogle and Marion but not in Kanota. The bands from Ogle and Marion appeared to have exactly the same size when analyzed in a single gel. Sequence analysis was performed on the aaccac376 fragment to determine whether it was identical in both populations.

The sequence data revealed that the segments in Ogle and Marion were mostly identical, although some single nucleotide polymorphisms were detected. Marker aaccac376 was assigned to linkage group 7 in K/M and linkage group 17 in K/O. Two RFLP probes (wg420,

Table 1 Number of total scoreable polymorphisms per primer combination in populations Kanota×Ogle and Kanota×Marion, number of polymorphisms scored as dominant markers for Kanota, Ogle, and Marion, codominant markers in Kanota×Marion, and number of common polymorphisms across populations

Primer combination	Kanota×Ogle[a]			Kanota×Marion				Common polymorphisms		
	Total polymorphisms	Source of fragment		Total polymorphisms	Source of fragment			Total polymorphisms	Source of fragment	
		Kanota	Ogle		Kanota	Marion	Codominant		Kanota	Ogle or Marion
P-ACA/M-CAC	14	8	6	11	3	5	3	3	2	1
P-AAC/M-CAC	21	10	11	12	7	5	0	5	3	2
P-ACG/M-CAC	17	2	15	15	7	6	2	5	2	3
P-ACC/M-CAC	8	5	3	9	3	5	1	4	3	1
P-ACG/M-CAA	12	8	4	15	5	8	2	5	4	1
P-ACA/M-CAA	9	5	4	16	8	8	0	5	4	1
Total	81	38	43	78	33	37	8	27	18	9

[a] All polymorphisms were scored as dominant markers in Kanota×Ogle

isu707) detected homoeologous loci on linkage groups 7 and 17 in K/O (Kianian et al. 2000a) and were linked to aaccac376. Common AFLP fragments may represent homoeologous segments in polyploids. Another possibility is that a translocation occurred between homoeologous segments. Acccac263 mapped to a small, unnamed linkage group (Fig. 1) containing only 2 loci in K/O and was assigned to linkage group 37 in K/M. It is possible that these 2 groups are linked, but distances between markers in K/O may be too large to confirm the association by linkage analysis. Acgcaa234 mapped to linkage group 11 in K/M and linkage group 30 in K/O. There was no indication of homoeology between these groups and no association could be found by linkage analysis. It seems that common polymorphisms can detect different linkage associations in different hexaploid oat lines. Genomic rearrangements are common in the oat genome (Kianian et al. 1997) and provide a likely explanation for the observed differences between populations. Similar to our results, Jin et al. (2000) found that 11% of 97 co-migrating AFLP markers mapped to different linkage groups in the two oat populations Kanota×Ogle and Clintland 64×IL86–5698. In their study, however, it remained unclear whether these fragments were allelic or identified different polymorphisms.

The use of AFLP maps for comparative QTL studies could lead to incorrect conclusions if QTLs are cross-mapped between populations based on a single AFLP marker. On the other hand, comparative AFLP studies may help to identify structural variations of the oat genome in different populations, thereby providing additional information to complete the oat reference map.

Jin et al. (2000) detected 52% polymorphisms in common between two oat populations with shared ancestors. Of the 33% polymorphisms in common between the populations in our study, 18 (22%) were scored as dominant markers in Kanota and nine (11%) were scored as dominant in Ogle or Marion (Table 1). Given the large number of AFLPs that can be generated in each cross, it should be feasible to detect a sufficient number of common polymorphisms even for large genomes like

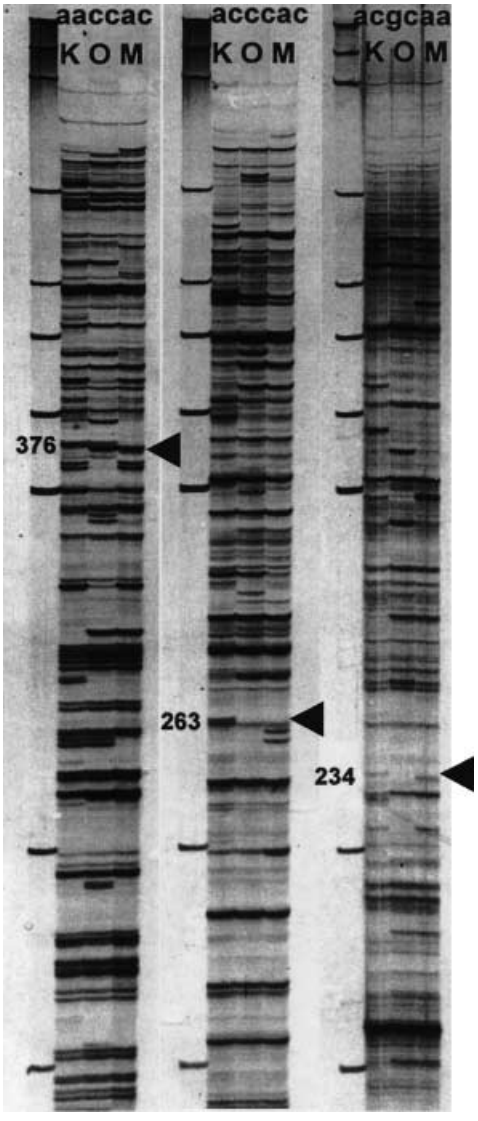

Fig. 2 AFLP pattern of Kanota (*K*), Ogle (*O*), and Marion (*M*) on silver-stained polyacrylamide gels for primer combinations P-AAC/M-CAC, P-ACC/M-CAC, and P-ACG/M-CAA. Markers aaccac376, acccac263, and acgcaa243 are indicated by *arrows*

oats, at least when shared ancestors exist. In barley, the amount of common polymorphisms in two populations not sharing a common parent was 10.2%, and only 1% common polymorphisms were detected across three populations (Waugh et al. 1997).

Feasibility of AFLP linkage mapping

AFLP markers provided considerable coverage of the oat genome in both populations. All linkage groups in the K/M map contained at least 1 AFLP marker. Most of the linkage groups, except for some of the smaller ones, contained at least 1 AFLP marker in K/O. A few clusters of AFLP markers were identified on linkage groups 17 and 30 in population K/O and on linkage groups 7–28, 3, and 22 in population K/M. These regions also had high coverage with RFLP markers and may represent areas of reduced recombination due to proximity to centromere or translocation breakpoints (Kianian et al. 2000a). There was no evidence that AFLP markers have a higher tendency to be clustered in certain regions of the genome than the RFLPs. In contrast, Becker et al. (1995) found that AFLP markers did not map to regions with high RFLP marker density and tended to flank RFLP marker clusters rather than interrupt them. In contrast to our study, these authors employed the endonuclease *Eco*RI instead of the methylation-sensitive enzyme *Pst*I. Using *Pst*I, highly methylated repetitive DNA is avoided, and the distribution of the AFLPs may be similar to the RFLP loci in oat, represented mainly by cDNAs. Castiglioni et al. (1999) reported in comparisons of AFLP marker distributions in maize that *Eco*RI markers clustered mainly at centromeric regions whereas the *Pst*I markers localized more in the hypomethylated telomeric regions. Waugh et al. (1997) reported that more clusters were found in AFLP than in RFLP linkage maps. This assumption could not be tested in our study because the number of RFLPs in K/O was higher than the number of AFLPs; therefore, more RFLP clusters were detected than AFLP clusters.

In both populations, AFLP markers assigned to a specific region in the linkage map often expanded the distance between 2 adjacent markers. They were placed relative to an anchor marker rather than including them in the framework map. In K/O, RFLP and AFLP assays were performed using RILs from two different generations, which could explain some mismatches between the two marker types. In K/M, the same generation of RILs was used for RFLPs and AFLPs, and mismatches should not occur. Becker et al. (1995) reported that 118 AFLP markers placed onto a RFLP map of barley double haploid (DH) lines (Heun et al. 1991) resulted in an increase of 777 cM, 375 cM of which was caused by singletons (a single locus scored in opposite phase than the adjacent markers) and doubletons (2 loci scored in opposite phase than the adjacent markers). The authors did not detect many scoring errors and concluded that the singletons represented real double crossovers. Considering the low probability of a high number of double crossovers in DH lines, it is more likely that they are due to other reasons. Jones et al. (1997) reported that up to 50% of the AFLP fragments produced in one laboratory did not amplify initially when the same experiment was repeated in other laboratories. After they became more experienced with the technique, the reproducibility increased to almost 100%. Similar amplification problems may have occurred for some RILs in our study and could explain why AFLPs tended to extend the distance between 2 RFLP markers.

An additional observation in our study was the high proportion (16%) of AFLP markers showing a significant distortion from the expected allele frequency in K/O. These markers remained unlinked and could not be used in subsequent analyses. Most of the distorted markers were dominant for Ogle and showed a clear polymorphism between the parents (a band was present in Ogle and absent in Kanota in the Winnipeg gels), yet only a small proportion of the RILs showed the presence of the band. In the case of partial digestion of the template DNA (Vos et al. 1995), partial AFLP products should be visible on those gels. For all distorted markers across primer combinations, a small subset of RILs showed additional bands. It is possible that these bands represent additional fragments due to partial digestion, which, by chance, also occurred in Ogle. Ogle did not show the respective bands in the gels analyzed at Minnesota, further suggesting that those fragments are due to partial digestion. However, it is surprising that the same additional fragments were amplified in the subset of RILs, which would mean that specific restriction sites were involved.

Distortion was not often observed in the K/M population, and only 2 markers (2%) were significantly distorted from the expected segregation ratio. Here, the distortion of a few AFLPs was most likely a genetic segregation distortion, which was also reinforced by the distorted segregation ratio of flanking RFLP markers. In a mapping study using 113 DH lines in barley, Becker et al. (1995) found that 6% of the AFLP markers showed a distortion from the expected segregation ratio of 1:1. Their results confirmed that in most cases AFLP markers segregate as expected.

Errors in AFLP scores caused by partial digestion or amplification would have a confounding effect on QTL analyses. It is important to verify the AFLP marker scores, especially when they are associated with a QTL. RFLPs or SSRs in close linkage with an AFLP marker could be used for verification, and the presence of singletons in the tested regions may be an indicator of an error. If other markers are not available, AFLP markers can be converted to PCR-based markers (Cho et al. 1996) to verify the initial AFLP segregation.

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