Combined AFLP and RFLP mapping in two hexaploid oat recombinant inbred populations

Hua Jin, Leslie L. Domier, Xuejen Shen, and Frederic L. Kolb

Abstract: A combined AFLP and RFLP map was constructed for hexaploid oat (Avena spp.). The segregation of AFLP markers was scored in two hexaploid oat recombinant inbred line (RIL) populations, the ‘Kanota’ × ‘Ogle’ RFLP population, and a population derived from ‘Clintland64’ and ‘IL86-5698’, barley yellow dwarf virus (BYDV)-sensitive and BYDV-tolerant lines, respectively. More than 300 AFLP markers were scored in each population, of which 97 could be scored in both populations. AFLP markers were linked to RFLP markers in 32 of 36 ‘Kanota’ × ‘Ogle’ RFLP linkage groups. The addition of the AFLP markers to the ‘Kanota’ × ‘Ogle’ RFLP data set combined markers from four pairs of linkage groups and increased the size of the map from 1402 cM to 2351 cM. Thirty linkage groups were observed in the ‘Clintland64’ × ‘IL86-5698’ population, two of which could be consolidated by comparing the maps from both populations. The AFLP and RFLP markers showed very similar distributions in the ‘Kanota’ × ‘Ogle’ population with a tendency of each type of marker to cluster with markers of the same type. The placement of a set of AFLP markers on the ‘Kanota’ × ‘Ogle’ linkage map will enrich the RFLP map and allow others to relate AFLP markers for agronomically important genes to the reference ‘Kanota’ × ‘Ogle’ linkage map.

Key words: amplified fragment length polymorphism, Avena, comparative mapping.


Mots clés : polymorphisme de longueur des fragments amplifiés, Avena, cartographie comparée.

Introduction

Oat (Avena spp.) is a significant cereal crop (Murphy and Hoffman 1992). Cultivated oat is an allohexaploid with 21 haploid chromosomes and a large genome size (Bennett and Smith 1976). To aid in the understanding and manipulation of the oat genome, molecular linkage maps have been con-
AFLP analysis

Genomic DNA was extracted from 10 to 15 plants of each RIL with CTAB (cetyltrimethylammonium bromide) (Doyle et al. 1990). AFLP protocols and the sequences of the selective nucleotides of primers used for AFLP analysis are as previously described (Jin et al. 1998). A set of 80 primer combinations was evaluated for the ability to produce informative banding patterns between the two pairs of parental lines. Based on this information, the ‘Kanota’ × ‘Ogle’ RIL population was evaluated with 19 and the ‘Clintland64’ × ‘IL86-5698’ RIL population with 26 primer combinations. Eighteen primer combinations were used in both populations. Polymorphic bands were numbered consecutively from the bottom of the films using the primer abbreviations in Table 1. All AFLP reactions were performed at least twice.

Map construction

Linkage analysis and map construction were performed using MAPMAKER version 3.0 (Lander et al. 1987). The ‘Kanota’ × ‘Ogle’ data set from Siripoonwiwat et al. (1996) was used to place markers on the hexaploid RFLP map. This data set consists of 252 RFLP markers relatively evenly spaced across 36 linkage groups. Because of the presence of heterozygous markers in the ‘Kanota’ × ‘Ogle’ data set, markers in both populations were evaluated as ‘F2 intercrosses’. Markers were grouped in both populations with a log-likelihood of the odds (LOD) score of 8.0 and maximum recombination level of 0.30. Once a map order had been established, linked markers were assigned to intervals with a LOD score of 2.0. Recombination frequencies (r) for the RIL populations were calculated from the F2 recombination fractions (R) reported by MAPMAKER using the equation $r = R/2(1 - R)$ (Haldane and Waddington 1931) and converted to cM using the Kosambi mapping function (Kosambi 1944).

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Results

AFLP analysis in RIL populations

AFLP fingerprints were generated for the parents and the members of the mapping populations. Each AFLP primer combination produced an average of about 100 bands. Approximately 10% of the bands were polymorphic between ‘Clintland64’ and ‘IL86-5698’. Up to 30% of the AFLP bands were polymorphic in the ‘Kanota’ × ‘Ogle’ population. In the ‘Kanota’ × ‘Ogle’ and ‘Clintland64’ × ‘IL86-5698’ populations, 354 and 317 AFLP markers were scored, respectively. When bands that were polymorphic between ‘Kanota’ and ‘Ogle’ were compared to those generated from ‘Clintland64’ and ‘IL86-5698’, about 52%, 185 markers, co-migrated. Ninety-seven of these co-migrating polymorphic bands were scored in both RIL populations and evaluated for linkage. Most of the markers that showed distorted segregation in one population were more normally distributed in the other.

Linkage analysis of AFLP markers

Analysis of the segregation of the AFLP markers in the ‘Clintland64’ × ‘IL86-5698’ (C × I) RIL population identified 30 linkage groups, which were comprised of 265 markers and represented 1363 cM (Fig. 1). About two-thirds of the AFLP markers (193) were assigned to intervals with a LOD score of 2.0. Recombination frequencies (r) for the RIL populations were calculated from the F2 recombination fractions (R) reported by MAPMAKER using the equation $r = R/2(1 - R)$ (Haldane and Waddington 1931) and converted to cM using the Kosambi mapping function (Kosambi 1944).

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In the ‘Kanota’ × ‘Ogle’ population, the AFLP markers were linked to RFLP markers in 32 of 36 RFLP linkage groups (Fig. 2). AFLP markers were linked to RFLP markers as close as 0.8 cm. The four linkage groups that did not contain AFLP markers were small, containing only 2 to 5 loci and represented just 70 cm. At a LOD score of 8.0 and a recombination fraction of 0.3, most of the linkage groups were the same as those reported by O’Donoughue et al. (1995). However, the addition of the AFLP markers to the ‘Kanota’ × ‘Ogle’ RFLP data set combined markers from groups 5 and 30, 6 and 37, 24 and 26, and 25 and 32. The combined map contained 139 AFLP markers and 193 RFLP markers that could be assigned to unique positions. Additionally, 124 AFLP and 35 RFLP markers were placed relative to the mapped markers. The remaining markers could not be assigned to unique intervals. Before adding the AFLP markers the map was 1402 cm. The addition of the markers increased the size of the map to 2351 cm, of which 481 cm were from extension and 382 cm were from expansion.

When AFLP markers that could be scored in both populations were linked in one population, they were found to be linked in the second population 89% of the time (Table 2). Ten of the ‘Clintland64’ × ‘IL86-5698’ linkage groups contained at least two AFLP markers that were mapped in both populations, which made it possible to assign these AFLP linkage groups to their corresponding linkage groups on the ‘Kanota’ × ‘Ogle’ RFLP map. Within these paired groups, many of the markers were linked in similar orders in both populations (Fig. 3). The relative positions of markers that could only be mapped to an interval were less conserved than those of markers that could be assigned to unique positions. Two linkage groups from the ‘Clintland64’ × ‘IL86-5698’ population (C × II and C × I7) each shared two or more AFLP markers with ‘Kanota’ × ‘Ogle’ linkage group 36 (Fig. 3). The association of the two groups was supported by the observation that the markers in linkage groups C × II and C × I7 formed a single linkage group at a LOD of 6.0 and a recombination fraction of 0.3.

Table 1. Sequences of AFLP primers used for selective amplifications.

<table>
<thead>
<tr>
<th>Primers</th>
<th>EcoRI core sequence</th>
<th>Primers</th>
<th>MseI core sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI11 (e1)</td>
<td>-AAC</td>
<td>MseI11 (m1)</td>
<td>-CAA</td>
</tr>
<tr>
<td>EcoRI12 (e2)</td>
<td>-AAG</td>
<td>MseI12 (m2)</td>
<td>-CAC</td>
</tr>
<tr>
<td>EcoRI13 (e3)</td>
<td>-ACA</td>
<td>MseI13 (m3)</td>
<td>-CAG</td>
</tr>
<tr>
<td>EcoRI14 (e4)</td>
<td>-ACC</td>
<td>MseI14 (m4)</td>
<td>-CAT</td>
</tr>
<tr>
<td>EcoRI15 (e5)</td>
<td>-ACG</td>
<td>MseI15 (m5)</td>
<td>-CTA</td>
</tr>
<tr>
<td>EcoRI16 (e6)</td>
<td>-ACT</td>
<td>MseI16 (m6)</td>
<td>-CTC</td>
</tr>
<tr>
<td>EcoRI17 (e7)</td>
<td>-AGC</td>
<td>MseI17 (m7)</td>
<td>-CTG</td>
</tr>
<tr>
<td>EcoRI18 (e8)</td>
<td>-AGG</td>
<td>MseI18 (m8)</td>
<td>-CTT</td>
</tr>
<tr>
<td>EcoRI19 (e9)</td>
<td>-AAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EcoRI10 (e10)</td>
<td>-ATC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Primer abbreviations used to identify AFLP bands in parentheses.

*EcoRI primer core sequence: 5'-GACTGCGTACCAATTC-3'.
*MseI primer core sequence: 5'-GATGAGTCCTGAGTAA-3'.

Distribution of AFLP markers

RFLP and AFLP markers showed very similar distributions in the ‘Kanota’ × ‘Ogle’ RIL population. However, RFLP and AFLP markers usually formed separate groups of linked markers (Fig. 2). Less frequently, individual AFLP markers were inserted between RFLP markers. In the ‘Kanota’ × ‘Ogle’ population no linkage groups were identified that contained only AFLP markers.

Discussion

In this study, we analyzed the segregation of AFLP markers in two hexaploid oat RIL populations and showed that the AFLP markers could be transferred between oat mapping populations. Because of the need to derive plant populations that segregate loci controlling particular traits, it is often necessary to establish a basic molecular map for future mapping studies. AFLP analysis provides a method to quickly apply a set of informative markers to the population and construct a basic framework from which information can be immediately drawn about the genetic location of agronomically important genes. The polymorphisms observed in one population often were detected in the second and frequently were linked with other markers in the same group. These observations suggest that most of the co-migrating bands are homologous and can be used to transfer linkage information from one oat mapping population to another. However, about 11% of the co-migrating markers mapped to different linkage groups, which underscores the importance in bridging maps of using multiple markers and (or) confirming the identity of bands through nucleotide sequence analysis. The number of AFLP markers that can be mapped in two or more populations likely will be associated with the relatedness of the crosses analyzed. The parental lines ‘Ogle’ and ‘IL86-5698’ shared ‘Egdolon’ and ‘Tyler’ oat lines in their pedigrees, which may have contributed to the number of bands that could be scored in both populations. These results are similar to those of Waugh et al. (1997) and
**Table 2.** Linkage groups containing AFLP bands that co-migrate in both populations.

<table>
<thead>
<tr>
<th>Linkage Group</th>
<th>'Kanota' × 'Ogle'</th>
<th>'Clintland64' × 'IL86-5698'</th>
<th>Common*</th>
<th>Linked†</th>
</tr>
</thead>
<tbody>
<tr>
<td>K × 02</td>
<td>C × I13</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>K × 03</td>
<td>C × I2</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K × 04</td>
<td>C × I4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>K × 05</td>
<td>C × I12</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>K × 08</td>
<td>C × I15</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K × 014</td>
<td>C × I22</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>K × 015</td>
<td>C × I12</td>
<td>13</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>K × 016</td>
<td>C × I11</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>K × 017</td>
<td>C × I6</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>K × 023</td>
<td>C × I15</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>K × 024</td>
<td>C × I17</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K × 030</td>
<td>C × I10</td>
<td>14</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>K × 036</td>
<td>C × I11 and C × I7</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

*Total number of co-migrating markers linked to at least one of the indicated linkage groups.

†Number of markers linked to the indicated linkage groups.

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**Fig. 2.** Association of AFLP markers and RFLP markers in the ‘Kanota’ × ‘Ogle’ (K × O) RIL population. Map distances are given in centiMorgans (Kosambi function). Markers in parentheses have been assigned to intervals only. AFLP markers are shown in bold. The names of markers that segregated in the ‘Kanota’ × ‘Ogle’ population, but not in the ‘Clintland64’ × ‘IL86-5698’ population are appended with an x.

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**Fig. 3.** Comparison of AFLP marker orders in the ‘Clintland64’ × ‘IL86-5698’ (C × I) and ‘Kanota’ × ‘Ogle’ (K × O) RIL populations. Map distances are given in centiMorgans (Kosambi function). Markers in parentheses have been assigned to intervals only. Solid lines between maps indicate the positions of co-migrating markers that could be mapped to unique locations in both populations. Dashed lines connect markers that could only be assigned to an interval in at least one of the populations.
Becker et al. (1995), which showed that many AFLP markers mapped to the same genetic loci in three different barley populations and that AFLP and RFLP markers tend to form distinct groups. The linkage information also can be used to form bridges to RFLP maps, which can in turn be used to identify other potentially informative markers linked to the loci of interest.

RFLP analysis will remain the method of choice for cross-species comparative genetic studies because of the conservation of gene sequences that hybridize to cDNA probes among grass species (Devos and Gale 1997; Gale and Devos 1998a; Gale and Devos 1998b; O’Donoughue et al. 1995; Van Deynze et al. 1995). The combination of AFLP and RFLP data allowed us to identify agronomically important chromosomal regions in other cereal genomes (Jin et al. 1998). Indirectly, the combined data could be useful for the identification of AFLP markers that are even closer to target loci, which could be used for marker assisted selection or the eventual cloning of agronomically important genes.

While some researchers have reported relatively uniform distributions of AFLP markers (Becker et al. 1995; Keim et al. 1997), others have observed strong clustering of AFLP markers, sometimes in association with telomeric or centromeric regions (Alonso-Blanco et al. 1998; Qi et al. 1998). The combination of AFLP and RFLP mapping data also allowed us to identify agronomically important chromosomal regions in other cereal genomes (Jin et al. 1998). Indirectly, the combined data could be useful for the identification of AFLP markers that are even closer to target loci, which could be used for marker assisted selection or the eventual cloning of agronomically important genes.

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