Theor Appl Genet (1996) 93: 606–612

V. H. Beaumont · J. Mantet · T. R. Rocheford
J. M. Widholm

Comparison of RAPD and RFLP markers for mapping F_2 generations in maize (*Zea mays* L.)

Received: 19 October 1995 / Accepted: 19 January 1996

Abstract The F_2 generations from two maize crosses were used to compare the ability of RAPD and RFLP marker systems to create a genetic linkage map. Both RFLPs and RAPDs were shown to provide Mendelian-type markers. Most of the RFLPs (80%) could be placed with a good level of certainty (LOD>4) on the genetic linkage map. However, because of their dominant nature, only between 37% and 59% of the RAPDs could be placed with such a LOD score. The use of combined data from RFLPs and RAPDs increases the level of information provided by RAPDs and allows the creation of a combined RFLP/RAPD genetic linkage map. Thus, the RAPD technique was found to be a powerful method to provide improved probes coverage on a previously created RFLP map and to locate markers linked to chromosomal regions of interest.

Key words Molecular markers · RFLP · RAPD · Genetic linkage map

Introduction

The development of nuclear restriction fragment length polymorphism (RFLP) has been useful for developing linkage maps in many species, for example, tomato (Bernatzky and Tanksley 1986), maize (Helentjaris 1987), lettuce (Landry et al. 1987), potato (Bonierbale et al. 1988), rice (McCouch et al. 1988) and barley (Heun et al. 1991). However, RFLP analysis is an expensive and time-consuming technology. Also, RFLPs may not provide detailed coverage throughout the genome. More recently, another type of molecular marker, RAPDs (random amplified polymorphic DNAs), was described simultaneously by Welsh and McClelland (1990) and Williams et al. (1990). This technique uses the polymerase chain (PCR) reaction to generate random amplified fragments of DNA with 10-mer primers. The advantages of RAPD markers include the ease and rapidity of analysis, the availability of a large number of primers and the very small amount of DNA required for analysis (Welsh and McClelland 1990; Williams et al. 1990). The first reports showed that RAPDs can efficiently generate markers that are randomly distributed throughout the genome or were linked to specific genes (Martin et al. 1991; Michelmore et al. 1991; Quiros and Morgan 1991). However, RAPDs do not always segregate in a Mendelian fashion (Echt et al. 1992, Reiter et al. 1992), and the genetic background seems to influence amplification of the target fragments (Heun and Helentjaris 1993). The cost of a data-point obtained with RAPDs seems to be similar to that found with RFLPs (Ragot and Hoisington 1993) for a low number of datapoints. However, because of the dominant nature of RAPDs, these markers provide much less information on the recombination frequency. The purposes of our experiments were to (1) evaluate the development of a genetic map using RAPD markers only, (2) compare the information provided by RAPDs and RFLPs and (3) compare the locations of RAPD marker loci in two closely related crosses.

Communicated by G. E. Hart

V. H. Beaumont
Südwestdeutsche Saatzucht. Im Rheinfeld 1–13,
76437 Rastatt, Germany

J. Mantet
Semences Cargill/Ets Lesgourgues, Boissay BP17,
28310 Toury, France

T. R. Rocheford · J. M. Widholm (✉)
Department of Crop Sciences,
University of Illinois at Urbana-Champaign, 1201 W. Gregory,
Urbana, IL 61801, USA

Materials and Methods

Genetic material

The F_2 generations from two crosses, H99 × Pa91 (HP) and Pa91 × FR16 (PF), were studied. The F_2 seeds were produced at Urbana, Illinois, in 1992, taking care that no selection occurred during

or after the pollinations. A total of 55 plants for HP and 57 plants for PF were used in this study.

DNA extraction

Leaf samples from F_2 plants grown in the field were harvested and lyophilized. DNA was extracted using the CTAB method described by Saghai-Maroof et al. (1984).

PCR reaction

The PCR reaction was performed in 10-µl aliquots according to Williams et al. (1990) with minor modifications. All components were pipetted using a Biomek 1000 automate. The samples were subjected to 1 min at 94°C, then to 45 repeats of the cycle 10 s at 94°C, 20 s at 35°C and 45 s at 72°C and finally incubated for 10 min at 72°C. The amplification was performed in a Perkin Elmer DNA thermocycler. Once the PCR was completed, the samples were electrophoresed at 100 V for 2 h in a 0.8% agarose gel prepared with TAE buffer (0.04 *M* TRIS-acetate, 0.002 *M* EDTA). Ten-mer primers (Operon) were chosen for the polymorphism they revealed between the parents. A first screening was done using DNA extracts from the three parents FR16, H99 and Pa91. For all primers showing at least one unambiguous and qualitative (presence or absence) polymorphic band, a second PCR reaction was run with independent DNA extracts of the parents. Thus, we could assess the repeatability of the RAPD patterns obtained. For mapping, the Operon name of the primer is followed by the approximate molecular weight of the fragment, as determined by the software MW-RFLP v1.2 (DNA ProScan, Nashville, Tenn.).

Southern blots and hybridizations

Ten micrograms of DNA was digested with the restriction endonucleases *Eco*RI, *Eco*RV or *Hin*dIII. Digested DNA was fractioned by gel electrophoresis on 0.8% agarose gels in TAE buffer (0.04 *M* TRIS-acetate, 0.002 *M* EDTA) and Southern transferred (Southern 1975) to Magnagraph nylon membranes (MSI) using 25 m*M* Na_2HPO_4/NaH_2PO_4, pH 6.5, as a transfer buffer. The DNA was immobilized on the membrane with a UV Stratalinker (Stratagene). For hybridizations, bnl (Brookhaven National Laboratory, Long Island, N.Y., USA), umc (University of Missouri, Colombia, Mo., USA), npi and php (Pioneer Inc.) maize RFLP probes were used for this study. The probes were labeled by random-primed DNA labeling according to the manufacturer's instructions (United States Biochemical Corp.). The blots were prehybridized for at least 4 h, using a hybridization oven and bottles in the hybridization solution (6× SSPE, 5× Denhardt's solution, 1%SDS, 50 µg/ml denatured salmon sperm DNA). The heat-denatured radiolabeled probe was then poured in the bottle, and hybridization was performed overnight at 65°C. The blots were then washed in 2× SSC, 0.5% SDS for 15 mn at room temperature and twice in 0.1× SSC, 0.1% SDS at 65°C for 30 mn. X-ray films were exposed to the blots for at least 48 h at –70°C.

Segregation and linkage analysis

The observed segregations were tested against a 1:2:1 (RFLPs) or 3:1 (RAPDs) Mendelian segregation model using a χ^2 test with 2 and 1 degrees of freedom, respectively for $\alpha = 5\%$. To compare the LOD scores of the two types of markers, we ran a two-point analysis on RAPD data only, RFLP data only and combined RAPD and RFLP data using the "lod" command of MAPMAKER v3.0 (Lander et al. 1987). For each cross (HP and PF), one genetic map was built with both RFLP and RAPD data, using the multipoint capabilities of the software MAPMAKER. Linkage groups were first established at a LOD score of 3 and a maximum distance of 50 cM (Haldane function). With the groups defined, we used the "order" command of MAPMAKER with a LOD score of 3, then 2. This means MAPMAKER ordered the markers having a LOD score higher than 3 and places the remaining markers if their LOD score was higher than 2. Markers with a LOD score lower than 2 were placed in multiple intervals. Because of the small amount of informative meiosis in our subset of plants, some markers close to each other (<5 cM) could not be mapped in a single position at a high LOD score. Thus, for each marker of the "multiple position markers" class, the closest probable marker already ordered was removed from the analysis, and the "order" command was run again. If the marker could then be placed at a LOD score higher than 2, we integrated both markers in the map. All map distances were calculated using the Haldane function.

Results and discussion

Polymorphism

Polymorphism was found between the parents for 54% of the RFLP probes screened when three restriction enzymes were used (Table 1). This percentage does not include the dominant markers or markers with multiband patterns. For the choice of RAPD primers, we considered only unambiguous and qualitative (present or absent) fragments that gave repeatable patterns when tested twice with parental DNA. Depending upon the cross, 34–37% of the primers revealed polymorphism (Table 1). Heun and Helentjaris (1993) found 37.1% polymorphic primers in a study of maize inbreds, which is similar to our results. Although the percentage of polymorphic primers was lower for RAPDs than for RFLPs, an average of 1.5 polymorphic scorable fragments per RAPD primer was observed. Thus, compared to RFLPs, RAPDs appear to possess good power to detect sequence polymorphism among maize lines.

Segregation of the markers

Of the 161 RFLPs scored, only 5 (3%) deviated from the expected 1:2:1 Mendelian segregation at a probability level

Table 1 RFLP probes and RAPD primers screened for polymorphism with the parents H99 versus Pa91 (HP) and Pa91 versus FR16 (PF)

RFLP	HP	PF
Probes screened for polymorphism between the parents	182	169
Probes revealing polymorphism	101	92
Percentage polymorphic probes	55%	54%
Probes selected for mapping	84	81
RAPD	**HP**	**PF**
Primers screened for polymorphism between the parents	250	250
Primers revealing polymorphism	86	93
Percentage polymorphic primers	34%	37%
Number of polymorphic fragments	126	150
Primers selected for mapping	48	45
Fragments selected for mapping	67	73

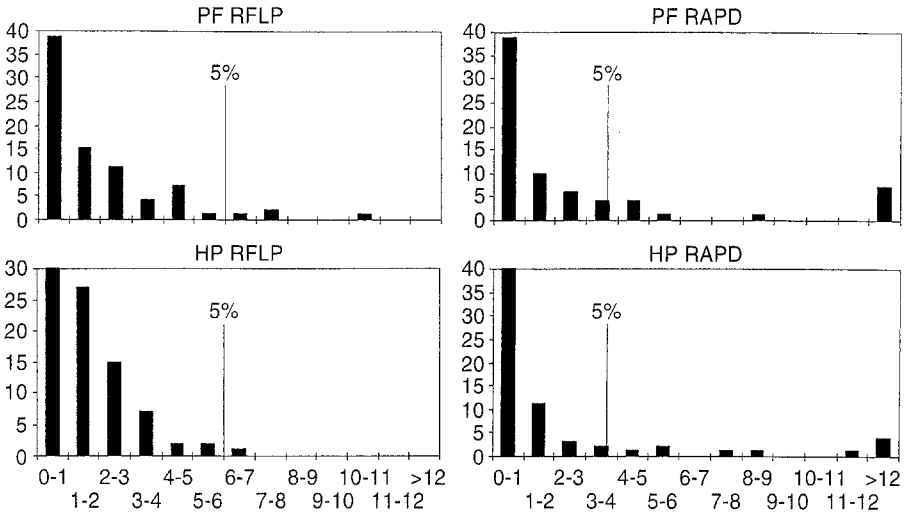

Fig. 1 Distribution of χ^2 values for segregation ratios of RFLPs and RAPDs for the F_2 generations PF and HP. The *X-axis* indicates the range of the χ^2 values, the *Y-axis* gives the number of markers reaching this range

higher than 0.05 (Fig. 1). These results indicate there was little segregation distortion in these F_2 populations since the actual number of deviating probes is close to that expected by chance. However, of the 140 RAPD markers scored, 24 (17%) deviated from the 3:1 Mendelian segregation for $\alpha = 0.05$. Since the RFLPs did not show any distortion, the deviations observed with RAPDs may be explained by the possibility that a percentage of the RAPD fragments scored did not segregate in a Mendelian fashion. We did not find any correlation between the molecular weight of the fragment scored (from 400 to 2000 bp) and the χ^2 value for it (data not shown). Heun and Helentjaris (1993) found that only 10% of the products they scored did not behave in a Mendelian fashion when analyzing F_1 generations and considered this result to be a low estimate. It is apparent that some precautions must be taken to accurately identify Mendelian markers among RAPD products.

Informativeness

The level of information provided by a pair of markers in order to evaluate the recombination fraction depends on the type of markers used (dominant or codominant) and their repulsion or coupling phase (Allard 1956). From Allard (1956), we can easily deduce the levels of information obtained from a pair of RFLP markers, a pair of RAPD markers and a combination of RFLP and RAPD markers when an F_2 generation is studied, as shown in Fig. 2. These data demonstrate clearly that RAPDs are not very powerful in evaluating the recombination frequency, especially when they are in repulsion phase and tightly linked. The use of a combination of RFLP and RAPD markers permits the production of more information (Fig. 2).

Another way to increase the level of information obtained from RAPD primers is to study specific $F_{2:3}$ families, which would allow the discrimination, within the dominant parent RAPD class, of the heterozygous F_2 plants from the homozygous F_2 plants. The number of F_3 plants to analyse for each progeny depends upon the risk of error chosen for the discrimination. For instance, the analysis of 16 F_3 plants from each F_2 plant would allow discrimination of the heterozygous F_2 plants with a risk of error of $\alpha = 0.01$ (Coe 1994). In order to get the same level of information as obtained with RFLPs, one should then increase the number of data points 16-fold, which is not a desired solution. For these reasons, we tried to find good criteria to identify codominant RAPDs.

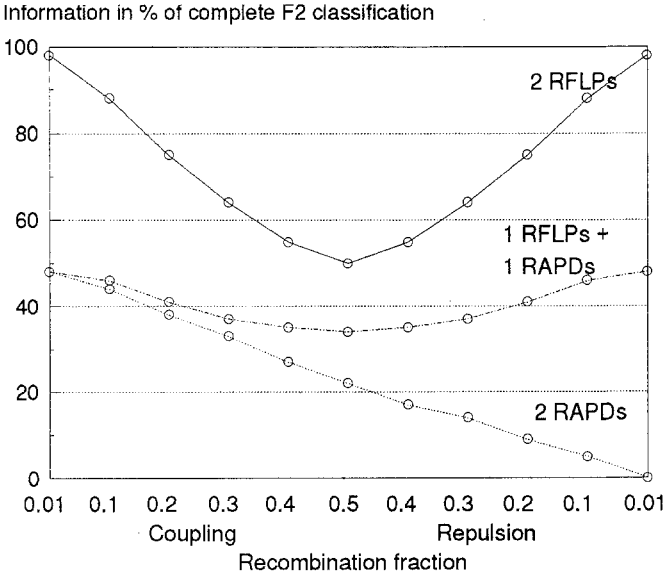

Fig. 2 Level of information from various pairs of molecular markers when an F_2 generation is analysed (from Allard 1956), assuming RFLPs to be codominant and RAPDs to be dominant

Table 2 Pairs of PCR fragments selected from criteria 1 and 2 as candidates for codominant markers (see discussion). For each pair of fragments, the cross where it was found, the group and LOD score and the value of the χ^2 against a 1:2:1 segregation are also given

PCR fragments	Cross	Group (LOD 3, 50 cM)	LOD	χ^2
H13-640/H13-1050	PF	Chromosome 5/chromosome 5	2.56	3.26
N09-850/N09-960	PF	Chromosome 2/chromosome 1	1.34	3.98
R05-1220/R05-1430	PF	Chromosome 4/chromosome 4	1.84	2.32
X04-1230/X04-1420	PF	Chromosome 9/chromosome 2	0.91	2.57
X09-1100/X09-1230	PF	unlinked/unlinked	0.34	9.09*
Y10-1100/Y10-1110	PF	Chromosome 10/chromosome 7	2.03	0.23
Y20-550/Y20-1550	PF	Chromosome 1/chromosome 1	1.21	0.96
R05-1180/R05-1390	HP	Chromosome 4/chromosome 4	3.66	12.72**

*,** Deviation from a 1:2:1 distribution significant at the 0.05 and 0.005 level, respectively

Table 3 Distribution of the LOD obtained from a two-point analysis of RFLPs, RAPDs and the combination of both, for the crosses PF and HP (F_2 generation)

Analysis	HP generation F_2				
	LOD>4	4>LOD>3	3>LOD>2	2>LOD	Total
RFLPs alone	68	5	2	9	84
RAPDs alone	25	11	8	23	67
RFLPs+RAPDs combined	117	19	9	6	151
RFLPs in combination	75	6	2	1	84
RAPDs in combination	42	13	7	5	67
Analysis	PF generation F_2				
	LOD>4	4>LOD>3	3>LOD>2	2>LOD	Total
RFLPs alone	67	1	2	11	81
RAPDs alone	43	3	10	17	73
RFLPs+RAPDs combined	124	9	8	13	154
RFLPs in combination	70	1	2	8	81
RAPDs in combination	54	8	6	5	73

RAPD codominant markers

The criteria used to identify eventual RAPD codominant markers were, in order:

(1) The presence of two polymorphic fragments amplified with the same primer, one fragment being recessive for one parent, the other recessive for the other parent (markers in repulsion).
(2) No recombinant individual should be present in the genotypes studied. The only recombinant class which can be distinguished with dominant markers such as RAPDs is the double recessive genotype class. Because this class is represented with the RAPD markers by the absence of both fragments, it can easily be subject to error when scoring the data. Thus, the number of eventual codominant markers might be underestimated.

The pairs of fragments which fit these criteria are listed in Table 2. Then the following additional points were considered:

(3) The pairs of fragments which fit both criteria given above will be mapped close to each other. However, one cannot expect very high LOD scores since the level of information provided by two dominant markers in repulsion decreases dramatically with the percentage of recombination (see previous discussion). Thus, the LOD score of a pair of fragments is not very useful for discriminating codominant RAPD markers. To confirm the linkage, we considered that both fragments should be linked to a third marker or group of markers with a good level of certainty: this information is given by the "group" order of MAPMAKER.
(4) One can create a codominant marker using the data from the two fragments and test its segregation against a Mendelian 1:2:1 segregation using a χ^2 with two degrees of freedom.

From the data, three pairs of RAPD fragments, R05-1220/1430, H13-640/H1050 and Y20-550/1550 (PF cross), were retained as putative codominant markers since they fit the four criteria described. However, none of these criteria is sufficient to conclude the presence of RAPD codominant primers.

Mapping

To compare the level of informativeness brought by both types of markers, we computed the LOD scores obtained by a two-point analysis. The results from a two-point analysis do not depend upon the number and the closeness of

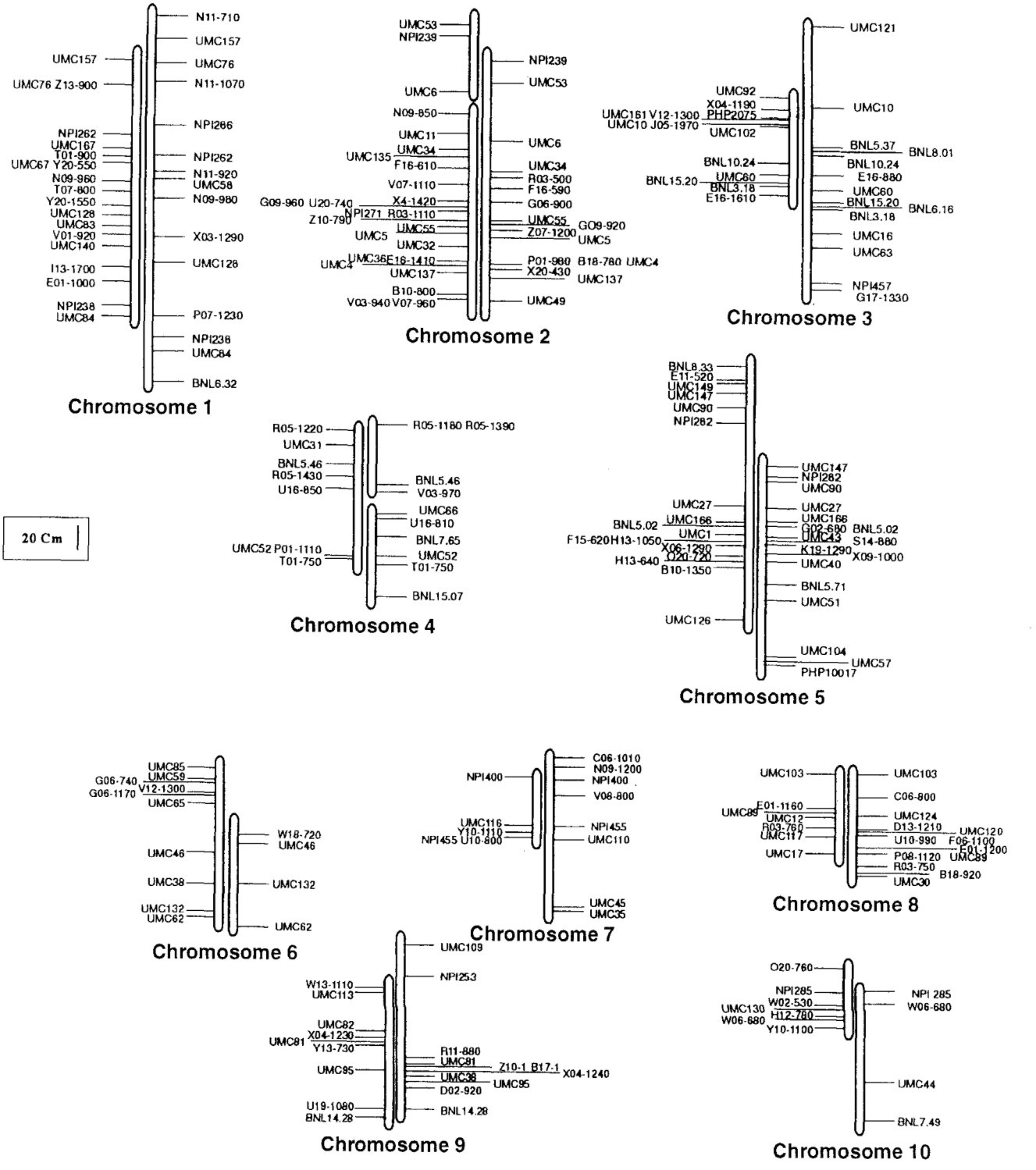

Fig. 3 RFLP and RAPD genetic maps from the crosses PF (*left*) and HP (*right*). The RFLPs are designated *BNL*, *NPI*, *PHP* or *UMC* and the RAPDs by the Operon primer designation followed by the approximate molecular weight fragment seen on the gel

the markers in one given linkage group. Thus, we could compare the results obtained with RFLPs and RAPDs even when the number of markers (and perhaps the distribution over the genome) is different in the two classes of markers. When analysed separately, more than 80% of the RFLPs obtained a LOD score higher than 4 (Table 3) while only 37 to 59% of the RAPDs obtained such LOD scores. However, the number of RAPDs having a LOD score smaller than 2 can be greatly reduced by combining the data with RFLPs: from 23 to 5 for the cross HP and from 17 to 5 for the cross PF. This situation was previously described in *Vicia faba*, where Torres et al. (1993) could not map RAPDs in repulsion phase. The position of the RFLPs and RAPDs when analysed in combination is given in Fig. 3. Two independent linkage groups were found for

Table 4 Primers producing PCR fragments of same approximate size in both crosses HP and PF (F_2 generation) and chromosome location of these fragments

Primer	HP generation F_2		PF generation F_2	
	PCR product (bp)	Chromosome	PCR product (bp)	Chromosome
E01	1200	8	1160	8
F15	710	Unlinked	620	5
F16	590	2	610	2
G09	920	2	960	2
N09	980	1	960	1
R03	750	8	760	8
R05	1180	4	1220	4
R05	1390	4	1430	4
T01	750	4	750	4
U16	810	4	850	4
U19	990	Unlinked	1080	9
V03	970	4	940	2
V12	1300	Unlinked	1300	3
W06	650	10	650	10
X04	1200	Unlinked	1190	3
X04	1240	9	1230	9
Z07	1650	Unlinked	1650	Unlinked

chromosome 2 with the cross PF and for chromosome 4 with the cross HP.

The orders given by MAPMAKER (using the command "order") for the RFLPs match a previously published map (Gardiner et al. 1993) in most cases, although some differences in the linkage distances can be found. The orders and linkage distances are also very similar for both crosses (concerning the 56 RFLP probes common to both crosses).

The distribution of the RAPDs seems to be quite uniform over the ten chromosomes, which supports the hypothesis that RAPDs provide good genome coverage. Twenty-four primers revealed polymorphism in both the HP and PF crosses. Of these, 15 gave 17 PCR fragments with approximatly the same-sized fragments (within 100 bp) in both crosses (Table 4). Only 12 of these 17 PCR fragments could be mapped in both the HP and PF crosses. Notably, each of the 12 fragments was mapped to the same location in both crosses, except for the 970-bp fragment generated by the primer V03 (Table 4, Fig. 3). Thus, in the case of crosses having one common parent, the creation of a common linkage map containing RAPDs appears to be possible.

Although 17% of the RAPDs in our study did not segregate in a 3:1 fashion, the RAPD technique proved to be a good way to produce polymorphic Mendelian-type markers. However, the dominant behavior of RAPDs dramatically decreases the amount of information produced by each datapoint in situations where heterozygous genotypes are found. Thus, RAPDs should be most useful in mapping studies for situations where heterozygous genotypes are not present (haploid tissue or haplodiploid lines, recombinant inbred lines). However, once the genetic map is created, the use of the RAPD technique seems to be a powerful way to generate markers for any region of interest.

Acknowledgements The authors are grateful to Fabienne Monory, Sandrine Gonzalez, Helène Guillot for their technical support with the RAPD analysis. This work was supported by funds from the Illinois Agricultural Experiment Station, the Consortium for Plant Biotechnology Research, The North Central Biotechnical Program and Semences Cargill/Ets Lesgourgues.

References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Coe EH (1994) Genetic experiments and mapping. In: Freeling M, Walbot V (eds) The maize handbook. Springer, Berlin Heidelberg New York, pp 190–197

Echt CS, Erdahl LA, McCoy TJ (1992) Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Theor Appl Genet 86:329–332

Gardiner JM, Coe EH, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized-F_2 population. Genetics 134:917–930

Helentjaris T (1987) A genetic linkage map for maize based on RFLPs. Trends Genet 3:217–221

Heun M, Helentjaris T (1993) Inheritance of RAPDs in F_1 hybrids of corn. Theor Appl Genet 85:961–969

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozymes, disease resistance and morphological markers. Genetics 116:331–337

Martin GB, Williams JG, Tanksley SD (1991) Rapid identification of markers linked to *Pseudomonas* resistance gene in tomato by using random primers and near isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Michelmore RW, Paran I, Kessli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Quiros CF, Morgan K (1991) Peroxidase and leucine aminopeptidase in diploid Medicago species closely related to alfalfa: multiple gene loci, multiple allelism and linkage. Theor Appl Genet 60:221–228

Ragot M, Hoisington DA (1993) Molecular markers for plant breeding: comparisons of RFLP and RAPD genotyping costs. Theor Appl Genet 85:975–984

Reiter SR, Williams JGK Feldman KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8019

Snedecor GW, Cochran WG (1980) Statistical methods, 7th edn. The Iowa State University Press, Ames, Iowa

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Torres AM, Weeded NF, Martin A (1993) Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. Theor Appl Genet 85:937–945

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535