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Random amplified polymorphic DNA and pedigree relationships in spring barley

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Summary. We investigated random amplified polymorphic DNA (RAPD) in 27 inbred barley lines with varying amounts of common ancestry and in 20 doubled-haploid (DH) lines from a biparental cross. Of 33 arbitrary 10 base primers that were tested, 19 distinguished a total of 31 polymorphisms. All polymorphisms were scored as dominant genetic markers except for 1, where Southern analysis indicated the presence of two codominant amplification products. The inheritance of 19 RAPD polymorphisms and one morphological trait was studied in the DH lines. There was no evidence for segregation distortion, but a group of four tightly linked loci was detected. The frequencies of RAPD polymorphism in pairs of inbred lines were used to compute values of genetic distance (d), which were compared to kinship coefficients (r) between the same pairs of lines. A linear relationship between r and d was evident, but low values of r gave poor predictions of d. Cluster analysis showed that groups of inbred lines based on r were similar to those based on d with some notable exceptions. RAPD markers can be used to gain information about genetic similarities or differences that are not evident from pedigree information.

Key words: Kinship coefficient – Polymerase chain reaction – Genetic markers – *Hordeum vulgare* – Doubled-haploid lines

Introduction

Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) are techniques used to detect polymorphism in DNA sequence. The RFLP approach relies on the cleavage of genomic DNA into different lengths by restriction enzymes; length polymorphism at a given site is then detected by homology to a labelled DNA probe. The RAPD method relies on amplification of polymorphic DNA fragments by the polymerase chain reaction (PCR) using a single oligonucleotide primer of arbitrary sequence. The RAPD method described by Williams et al. (1990), which is similar to the arbitrarily primed PCR (Welsh and McClelland 1990), is a fast and simple alternative to the RFLP approach. A disadvantage of the RAPD method is that markers are usually dominant (Williams et al. 1990).

In barley (*Hordeum vulgare* L.), high-density genetic marker maps are being constructed using both RFLP and RAPD markers (North American Genome Mapping Project 1991; Heun et al. 1991; Graner et al. 1991). In these mapping projects, crosses among parents with common ancestry have been avoided in order to increase the frequency of polymorphism. In contrast, much of the current breeding effort in barley involves crosses among elite lines that share common ancestors. Presumably, there are agronomically useful allelic differences segregating in crosses among related lines, but it is not known whether such crosses will contain sufficient levels of marker polymorphism to permit marker-intensive applications such as marker-assisted selection.

The amount of polymorphism between individuals or groups of individuals can be expressed as genetic distance. Commonly used statistics for genetic distance have been described by Rogers (1972) and Nei (1972). In homozygous individuals with allele frequencies of 1 or 0, Rogers' distance (d) is the ratio of the number of polymorphisms between two lines to the total

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number of polymorphisms that are scored, Nei's identity statistic (I) is equivalent to $1-d$, and Nei's standardized distance (D) is equivalent to $-ln(I)$. Genetic distance has been estimated among inbred lines of soybean (Cox et al. 1985a), wheat (Cox et al. 1985b), common bean (Singh et al. 1991), and maize (Melchinger et al. 1991).

When pedigree information is available, as it often is for cultivars of self-pollinating crops, genetic distance can be estimated using the kinship coefficient (r). This statistic estimates the probability that two alleles at a given locus are identical by descent (Malécot 1948). It can be computed by assuming the diploid inheritance of gametes produced by random Mendelian segregation and by assuming that ancestors with unknown pedigrees are unrelated. Extending this, St. Martin (1982) described a method for computing r among inbred lines of an autogamous species. This method requires the additional assumptions that all lines in a pedigree are selfed to homozygosity and that the allele frequency at most loci is not affected by directional selection during selfing generations. This method has been used to characterize the germ plasm of rapeseed (Lefort-Buson et al. 1987), peanut (Knauft and Gorbet 1989), rice (Dilday 1990), wheat (Cox and Murphy 1990), oat (Souza and Sorrells 1989), and barley (Martin et al. 1991).

Since marker phenotypes and pedigrees both contribute information about genetic distance, Cox et al. (1985a) have suggested combining these sources of information to obtain better predictions of genetic divergence or hybrid performance. Alternatively, it would sometimes be useful for breeders or geneticists to predict the frequency of marker polymorphism between two lines using pedigree information. The expected relationship between r and d is linear (Melchinger et al. 1991), but the degree of collinearity between estimates of these statistics can vary considerably. The Spearman rank correlation coefficient (r_s) between d and $(1-r)$ was 0.74 for a group of inbred maize lines (Melchinger et al. 1991), 0.60 for group of modern soybean cultivars (Cox et al. 1985a), and 0.27 for a group of hard red wheat cultivars (Cox et al. 1985b).

In the present article, we report the use of RAPD markers to detect polymorphism in a population of elite spring barley lines. Our objectives were to determine the frequency of polymorphism among lines with varying amounts of common ancestry and to compare estimates of r with estimates of d. Since RAPD markers are a relatively new tool, we have attempted to characterize their segregation and allelic relationships in a population of doubled-haploid (DH) barley lines, and we discuss their potential for use in barley breeding.

Methods

Materials

Twenty-five inbred lines of barley were chosen to represent elite spring barley germ plasm adapted to eastern Canada. Of these 16 were six-row types ('AC Burman', 'Bruce', 'Cadette', 'Chapais', 'Etienne', 'Joly', 'OAC Kippen', 'Labelle', 'Laurier', 'Leger', 'Loyola', 'Massey', 'Nord', 'OB959-7', 'Perth', 'Sophie') and 9 were two-row types ('Albany', 'Atlanta', 'Birka', 'Craig', 'Helena', 'Micmac', 'Morrison', 'Rodeo', 'Symko'). Two lines from western Canada ('Harrington' and 'TR306'), both two-row types, were also included. Pairwise comparisons among these 27 inbred lines were expected to cover a broad range of genetic distances similar to those encountered in cultivar development. In addition, 20 DH lines produced by the bulbosum method (Kasha and Kao 1970) from the F_1 between 'Chapais' and 'AC Burman' were obtained from L. Shugar (W. G. Thompson and Sons, Blenheim, Canada).

DNA purification

Samples of approximately 100 mg young leaf tissue were macerated in 1.5-ml microtubes using disposable pellet pestle grinders (Kontes). Maceration was continued after adding 0.6 ml CTAB (hexadecyltrimethyl-ammonium bromide) buffer [1.4 *M* NaCl, 20 m*M* EDTA, 100 m*M* TRIS-Cl, 2% (w/v) CTAB pH 8.0], and the samples were incubated at 60 °C for 45 min. The samples were then extracted with 0.6 ml chloroform/isoamyl alcohol (24:1) and centrifuged at 12 000 *g* for 5 min. The aqueous phase was transferred to a 1.5-ml tube where the DNA was precipitated with an equal volume of cold isopropanol. Approximate DNA concentrations for these samples were determined by visual comparison to serial dilutions of a known genomic DNA standard after separation on a 1.2% agarose gel and staining with ethidium bromide.

RAPD analysis

Reactions similar to those described by Williams et al. (1990) were performed with some minor modifications. Reaction volumes of 25 µl were prepared to contain 20 ng genomic DNA, 240 n*M* primer (Operon), 100 µ*M* of each dNTP, and 1.25 units of Taq DNA polymerase (BRL). Reactions were buffered by addition of 1/10 volume of 100 m*M* TRIS-HCl pH 8.8, 500 m*M* KCl, 15 m*M* $MgCl_2$, and 1% Triton X-100, and overlaid with 30 µl heavy mineral oil (Fisher). Reactions were performed in a thermal cycler (Hybaid) operated in a 4 °C refrigerator, where the plate temperature was programmed for 45 cycles of 1 min at 35 °C, 2 min at 72 °C, 1 min at 92 °C, with an initial melt of 94 °C for 5 min, and a final extension of 72 °C for 4 min. Polymorphic markers were scored after separating the reaction products on 1.2% agarose (BRL) gels stained with ethidium bromide.

DNA hybridization and detection

Polymorphic amplification products from the parents were excised from agarose gels and purified using a silica glass suspension (Gene Clean II, Bio 101). Approximately 0.5 µg of each purified amplification product was labelled by random priming with digoxigenin-dUTP (Boehringer Mannheim). DNA from gels containing PCR amplification products was transferred by capillary blotting to nylon membranes (Biotrans, ICN) using a modification of the method of Southern (1975) described by Selden (1991). Transferred DNA was bound to the membranes by baking 1 h at 80 °C. Membranes were prehybridized for 1 h at 68 °C in 50 ml hybridization solution

(5 × SSC (750 m*M* NaCl; 75 m*M* Na_3 citrate pH 7.0); 1% blocking reagent (Boehringer Mannheim); 0.1% *N*-lauryl sarcosine; 0.02% SDS), then hybridized for 10–15 h at 68 °C in 5 ml of the same solution containing approximately 0.5 µg labelled probe. Membranes were then washed twice for 5 min at 25 °C with 2 × SSC; 0.1% SDS and twice for 15 min at 68 °C with 0.1 × SSC; 0.1% SDS. Digoxigenin-labelled DNA was detected using an enzyme-linked colorimetric procedure (Genius, Boehringer Mannheim). Before re-probing, membranes were bleached in dimethyl formamide (60 °C for 15–30 min), rinsed in distilled water, incubated in 0.2 *N* NaOH; 0.1% SDS (30 min at 37 °C), then rinsed in 2 × SSC.

Data analysis

Linkage analysis and χ^2 tests in the DH lines were performed using the computer program Linkage-1, version 3.5 (Suiter et al. 1983). Rogers' *d* was calculated for each pair of inbred lines by dividing the number of polymorphisms between those lines by the total number of polymorphic amplification products that were scored. The inbred lines were clustered on the basis of a distance matrix of *d* using the AVERAGE method in the CLUSTER procedure of SAS (1987). The AVERAGE method initially considers each entry as a separate cluster, then joins clusters sequentially based on increasing average distance between clusters.

Pedigrees for each of the 27 lines and their known ancestors were obtained from cultivar descriptions, breeding records, personal communication with breeders, and from Baum et al. (1985). Kinship coefficients (*r*) were calculated based on the following assumptions: (1) ancestors, breeding lines, and cultivars were homozygous and homogeneous; (2) ancestors without known pedigrees were unrelated ($r = 0$); and (3) a line derived from a cross obtained an equal sample of alleles from each parent. An exception to the first rule was made to account for multiple lines selected from a heterogeneous ancestor. Here it was assumed (as in Cox et al. 1985b; Souza and Sorrells 1989; Knauft and Gorbet 1989; Martin et al. 1991) that the relationship between an ancestor and a line selected from that ancestor was $r = 0.75$. These calculations were performed using a computer program (Tinker and Mather, unpublished) similar to the one described by Delannay et al. (1983). Cluster analysis was performed using a distance matrix of $(1 - r)$ by the same method used for the cluster analysis based on *d*.

Results

RAPD polymorphism between inbred barley lines

A total of 33 10-base primers were tested in each of 27 inbred barley lines to detect and characterize RAPD polymorphism. All 33 primers contained 60% or 70% (G + C), and none of the base sequences had self-complementary ends. Apart from these constraints, the primer sequences were arbitrary and were not pre-selected for use in barley or any other species. Analyses that revealed polymorphic amplification products were repeated using the same DNA from each of 6 or more inbred lines. When possible, these verifications included at least 3 lines where the amplification product was present and 3 where it was absent. Three polymorphic amplification products that were each present in fewer than 3 inbred lines were verified using new DNA extractions from separate plants to establish that these polymorphisms were not artifacts from the DNA extraction or from an accidental seed mixture.

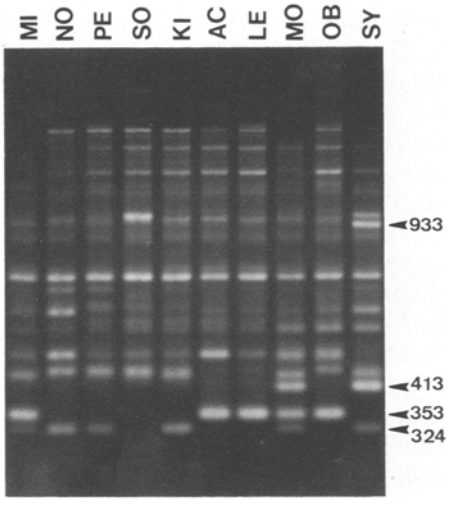

Fig. 1. Polymorphism revealed using primer H8 (5′-GAAACA-CCCC) to amplify genomic DNA purified from barley cultivars (*left to right*) 'Micmac', 'Nord', 'Perth', 'Sophie', 'OAC Kippen', 'AC Burman', 'Leger', 'Morrison', 'OB959-7', 'Symko'. PCR amplification products were separated on a 1.2% agarose gel and stained with ethidium bromide. *Arrows* indicate approximate size (in base pairs) of amplification products scored as markers

Of the primers tested, 14 produced amplification products that were monomorphic across all genotypes, or were polymorphic but not reliable. The remaining 19 primers revealed a total of 31 polymorphisms that could be scored reliably in all 27 barley lines. The number of polymorphic amplification products revealed by each of these primers ranged from 1 to 5 (e.g., Fig. 1). The size of the polymorphic amplification products ranged from 301 to 1881 bp, with an average of 1008 bp. Two polymorphic amplification products (1378 bp and 1448 bp) produced by the same primer (G19) appeared to segregate as codominant alleles and were scored as a single polymorphism.

RAPD polymorphism in a DH population

Primers that revealed polymorphism between 'Chapais' and 'AC Burman' were tested in 20 DH lines produced from the F_1 between these parents. Of the 31 RAPD polymorphisms scored in the inbred lines 14 were expected to segregate in this cross. Of these, 13 polymorphisms (including the codominant polymorphism) segregated as expected. One polymorphism that was expected to segregate was monomorphic, apparently as a result of heterogeneity within 'Chapais'. Six additional RAPD polymorphisms, and one morpho-

logical trait (rough versus smooth awns) were scored in the DH lines. The additional RAPD polymorphisms had been observed between 'Chapais' and 'AC Burman', but they were not scored in the inbred lines because their phenotype was not considered to be reliable in the complete set of inbred lines.

Tests of χ^2 were performed independently for each of the polymorphisms segregating in the DH lines to determine if segregation differed from the expected 1:1 ratio. At a significance level of $P = 0.05$, only 1 of these tests (with an observed ratio of 15:5) was rejected. Since 20 independent χ^2 tests were performed, 1 rejected true hypothesis at $P = 0.05$ would be expected. Considering the 18 dominant RAPD polymorphisms as a group, there were a total of 188 bands scored as negative and 172 bands scored as positive. Based on χ^2, this observation is not different from the expected 1:1 ratio.

To test the genetic independence of these segregating polymorphisms, linkage analysis was performed in the DH lines. Three tight linkage groups were detected among 8 of these polymorphisms. Two groups consisted of pairs of loci linked at 5% recombination. The third group consisted of four loci, three of which did not recombine, and a fourth at a recombination distance of 5%. The polymorphisms that did not recombine were products of separate primers, and they did not contribute identical information in the 27 inbred lines.

Southern analysis

Labelled probes produced from eight different segregating amplification products from 7 different primers were used to detect homologous DNA on Southern transfers from the corresponding reactions. All but one of the Southern analyses supported dominant segregation patterns for the probed fragment (e.g., Fig. 2b and c). The only case where codominance was apparent was when amplification products for primer G19 were probed with the 1378-bp fragment. This fragment contained sequence homology to a 1448-bp product that occurred only in the DH lines where the 1378-bp product was absent (Fig. 3b). These observations are consistent with our treatment of these two amplification products as a single codominant locus.

In five of the Southern analyses (e.g. Figs. 2b and 3b), probes constructed from a single amplification product were homologous to one or more additional amplification products from the same primer in the same reaction. These additional amplification products showed identical segregation patterns to the fragment from which the probe was made, but they were difficult to detect visually on an ethidium bromide stained gel.

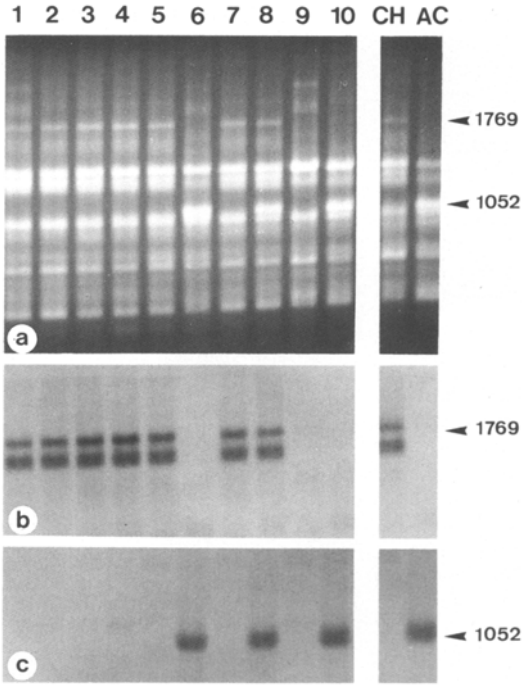

Fig. 2a–c. Dominant polymorphism revealed by primer H5 (5′-AGTCGTCCCC) in 10 doubled-haploid lines produced from the F_1 between 'Chapais' (*CH*) and 'AC Burman' (*AC*). PCR amplification products were separated on a 1.2% agarose gel and stained with ethidium bromide (*panel a*). The amplified fragments from this gel were transferred to a nylon membrane by capillary blotting and hybridized with a digoxigenin-dUTP-labelled, 1769-bp fragment. The membrane was then washed at a high stringency, and the remaining labelled fragments were detected colorimetrically (*panel b*). After bleaching with dimethyl formamide, the membrane was re-probed with a digoxigenin-labelled, 1052-bp fragment (*panel c*)

Values of d and r among 27 inbred barley lines

To compare the amount of polymorphism among different pairs of inbred lines, the 31 polymorphisms scored in the 27 barley lines were used to compute Rogers' d for all 351 pairwise comparisons (Table 1, above diagonal). Values of d were approximately normally distributed, ranging from 0.06 (2 of 31 bands were polymorphic) to 0.61 (19 of 31 bands were polymorphic) with a mean of 0.32. Binomial variance estimates for d can be computed as $V_d = d(d-1)/n$, where n is the number of polymorphisms sampled (31).

The ancestors of the barley lines used in this study included 229 other cultivars and breeding lines with known pedigrees and 70 ancestors for which no further pedigree information could be obtained. A matrix of kinship coefficients was constructed for a total of 326 cultivars, breeding lines, and landraces. Values of r among the 27 lines used in this study (Table 1, below diagonal) ranged from 0 (lines with no shared common ancestors) to 0.92 (lines with similar pedigrees). There were a large number of comparisons for which $r = 0$,

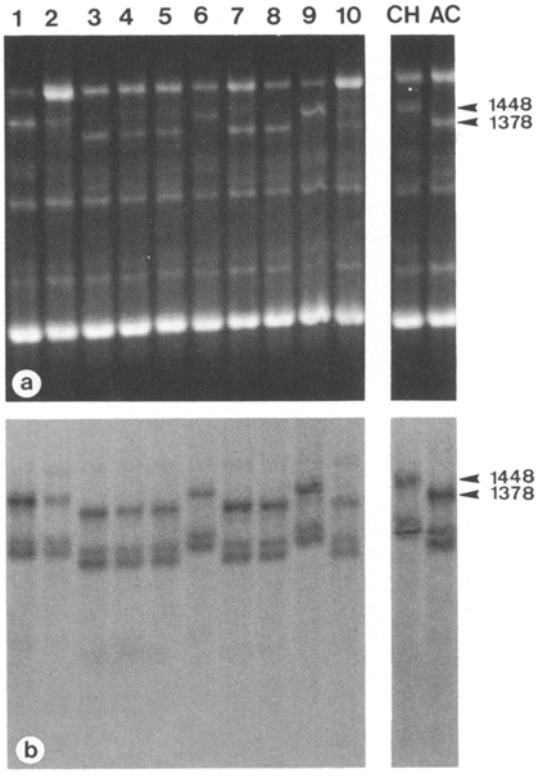

Fig. 3a, b. Codominant polymorphism revealed by primer G19 (5'-GTCAGGGCAA) in 10 doubled-haploid lines produced from the F_1 between 'Chapais' (*CH*) and 'AC Burman' (*AC*). PCR amplification products were separated on a 1.2% agarose gel and stained with ethidium bromide (*panel a*). The amplified fragments from this gel were transferred to a nylon membrane by capillary blotting and hybridized with a digoxigenin-dUTP-labelled, 1378-bp fragment. The membrane was then washed at a high stringency, and the remaining labelled fragments were detected colorimetrically (*panel b*)

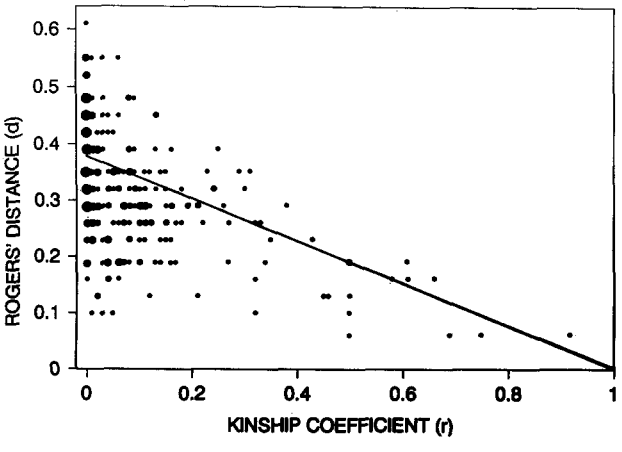

Fig. 4. Relationship between kinship coefficient (r) and Rogers' distance (d) for 351 pairwise comparisons among 27 inbred barley lines. Each *small circle* represents one comparison. *Circles of increasing size* represent 2, 3–8, or 9–29 comparisons, respectively

so the distribution of r was skewed toward lower values, and the average value of r was 0.075.

The strength of the linear relationship between r and d was investigated to determine if r might be a reliable predictor of d for applications requiring estimates of the amount of RAPD polymorphism between two lines. The standardized Mantel statistic (r_M) (Mantel 1967) between the matrices of r and d was estimated as 178.5. This value was highly significant ($P < 0.01$) when tested against the distribution of r_M derived from random permutations of the data in the matricis. The average value of d for unrelated lines ($r = 0$) was 0.38, so predicted values of d can be estimated from Eq. 1 of Melchinger et al. (1991) as $\hat{d} = 0.38(1 - r)$. However, the observed coordinates of r and d deviated considerably from this relationship, especially at low values of r (Fig. 4), where many values of d had 95% confidence intervals that excluded $\hat{d}$. The Spearman rank correlation coefficient (r_s) between $(1 - r)$ and d for all 351 observations was 0.51 ($P < 0.01$); however, when 289 comparisons with $r < 0.125$ were excluded from the data, r_s increased to 0.61 ($P < 0.01$), and when 322 comparisons with $r < 0.25$ were excluded from the data, r_s increased to 0.73 ($P < 0.01$).

Cluster analysis

Cluster analysis of the 27 inbred lines was performed using r and d (Fig. 5) to investigate qualitative similarities and differences between estimates of these statistics. At the highest level of hierarchy both statistics divided the two-row and six-row lines into separate groups, except that in the analysis of r the line 'Birka' formed its own group, and in the analysis of d the line 'Chapais' formed its own group. Within the two-row and six-row groups, other qualitative similarities between the r and d clusters were observed. With r, five clusters containing more than 2 lines were formed at $r = 0.25$. At $d = 0.25$, there were six clusters formed that corresponded approximately to those produced by $r = 0.25$. Similarities between these clusters included the sets {'AC Burman', 'Leger', 'OB959-7'}, {'Etienne', 'Perth'}, {'Cadette', 'Sophie'}, {'Labelle', 'Loyola'}, and {'Craig', 'Rodeo', 'Morrison', 'Symko'}. Some notable similarities seen within the d clusters and not within the r clusters included the placement of 'Nord' and 'Massey' into the {'Etienne', 'Perth'} group, the placement of 'Albany' and 'Helena' into the group containing 'Rodeo', and the clustering together of {'Atlanta', 'Birka', 'Micmac', 'Harrington', 'TR306'}. Notable instances where the d groups were inconsistent with the r groups included the clustering of 'Joly' with the {'Cadette', 'Sophie'} group, and the clustering together of {'Laurier', 'Bruce', 'OAC Kippen'}.

Table 1. Values of $d^a \times 100$ (above the diagonal) and $r^b \times 100$ (below the diagonal) for pairwise comparisons among 27 inbred barley lines

Cultivar name and abbreviation		AC	AL	AT	BI	BR	CA	CH	CR	ET	HA	HE	JO	KI	LA	LU	LE	LO	MA	MI	MO	NO	OB	PE	RO	SO	SY	TR
AC Burman	AC		39	39	35	23	29	48	32	35	29	29	29	35	32	32	6	35	35	35	45	35	6	32	39	29	45	23
Albany	AL	0		26	23	35	42	42	19	35	29	16	35	29	39	26	39	35	29	29	19	42	45	32	19	48	19	29
Atlanta	AT	2	2		10	48	48	55	26	35	16	29	42	35	45	39	39	42	42	10	26	42	39	32	26	55	26	23
Birka	BI	0	1	1		45	45	52	29	39	13	26	39	32	42	35	35	39	39	13	29	45	35	35	29	52	29	26
Bruce	BR	43	0	1	0		19	39	29	32	39	32	19	19	23	10	23	32	32	45	48	19	29	29	42	19	48	32
Cadette	CA	8	0	3	0	7		39	42	19	39	32	19	26	29	23	35	26	32	45	48	26	35	29	48	13	48	39
Chapais	CH	9	0	1	0	16	9		55	45	45	52	26	45	35	29	48	39	39	52	48	39	55	48	48	32	42	52
Craig	CR	0	3	5	1	0	0	0		29	29	10	35	29	39	26	32	35	35	29	26	29	39	26	13	48	19	23
Etienne	ET	15	0	1	0	10	8	4	0		32	32	19	26	35	29	29	19	19	32	35	19	35	10	35	26	35	39
Harrington	HA	1	8	4	2	1	0	1	4	0		26	32	26	35	29	29	32	32	13	29	39	29	29	29	45	29	13
Helena	HE	0	4	7	1	0	0	0	32	0	6		32	26	35	23	35	32	32	32	29	39	35	29	23	39	23	19
Joly	JO	7	0	3	0	6	34	15	0	7	0	0		19	29	16	29	26	26	39	48	26	35	23	42	13	42	39
OAC Kippen	KI	29	0	2	0	17	12	6	0	33	1	0	10		35	16	35	32	26	39	29	26	35	16	35	32	35	26
Labelle	LA	6	0	3	0	4	11	14	0	4	0	0	38	8		19	26	16	29	42	52	23	32	32	45	23	39	42
Laurier	LU	4	0	2	0	3	10	19	0	4	0	0	58	6	61		32	29	29	35	39	23	39	26	39	23	39	29
Leger	LE	92	0	2	0	35	8	8	0	16	1	0	7	31	7	4		29	29	35	45	29	6	26	39	29	45	29
Loyola	LO	9	0	5	0	6	11	8	0	4	0	0	18	9	61	21	9		19	39	48	19	35	23	42	26	35	39
Massey	MA	11	0	4	0	9	18	8	0	16	1	0	17	22	12	10	11	14		39	42	19	35	16	35	32	29	39
Micmac	MI	0	16	5	2	0	0	0	11	0	12	13	0	0	0	0	0	0	0		35	39	42	29	35	52	35	26
Morrison	MO	0	4	1	1	0	0	0	27	0	2	3	0	0	0	0	0	0	0	5		48	45	32	19	61	19	29
Nord	NO	2	0	2	0	0	8	2	0	0	0	0	10	2	11	8	2	13	6	0	0		35	16	35	26	35	39
OB959-7	OB	69	0	2	0	26	6	6	13	12	1	0	5	23	5	3	75	7	8	0	13	2		32	39	29	45	23
Perth	PE	30	0	2	0	19	15	8	0	50	1	0	14	66	8	7	32	8	32	0	0	0	24		32	29	32	35
Rodeo	RO	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	50	0	25	0		55	6	29
Sophie	SO	10	0	3	0	10	45	11	0	11	1	0	46	15	15	16	10	15	24	0	0	12	7	21	0		55	45
Symko	SY	0	4	1	1	0	0	0	27	0	2	3	0	0	0	0	0	0	0	5	50	0	13	0	50	0		35
TR306	TR	1	7	4	1	1	1	0	4	1	21	6	1	1	0	0	1	0	1	10	2	0	1	1	0	1	2	

[a] Genetic distance (d) calculated as the ratio of polymorphic loci to the total number (31) of scored loci

[b] Kinship coefficients (r) calculated by assuming that lines and known ancestors are self pollinated to homozygosity without selection and that ancestors without known pedigrees are unrelated

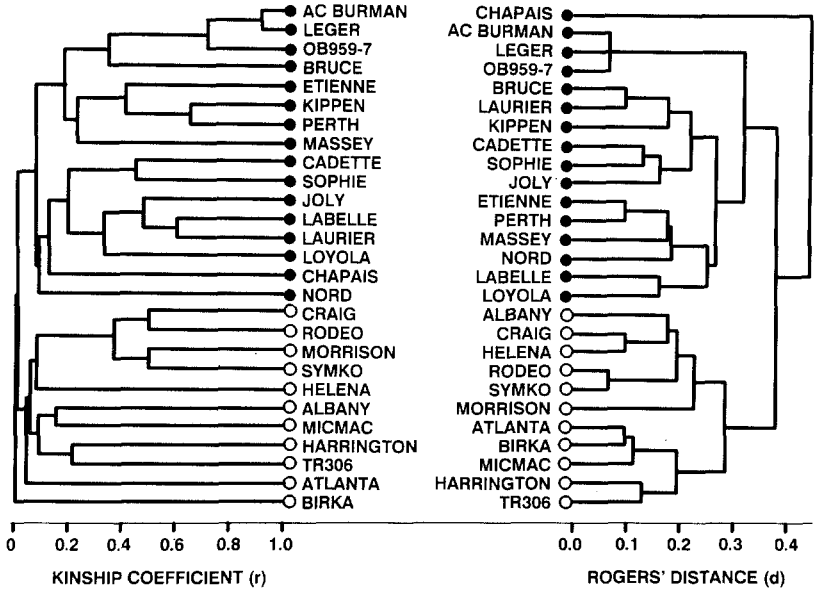

Fig. 5. Clusters based on kinship coefficient (r) or on Rogers' distance (d) for 27 barley lines. Initially, each line was placed in a separate group. Groups were then joined by average distance based on decreasing values of r or increasing values d. *Closed circles* indicate six-row barley lines and *open circles* indicate two-row lines

Discussion

RAPD polymorphism

One of the goals of this research was to investigate the potential of RAPD markers as tools in barley breeding. The procedures used in this study were relatively simple, and the polymorphisms detected were repeatable and stably inherited. Compared with RFLP analysis, a major advantage of the RAPD method may be the reduced effort required for screening. For example, in an RFLP mapping project, 450 genomic and cDNA clones were screened to identify 114 polymorphic RFLP markers between two barley lines with $r = 0.05$ (Graner et al. 1991). In the present study, pairs of lines with a similar value of r required approximately the same proportion of random primers per polymorphic locus. However, since random oligonucleotide primers are available from commercial sources, no preparatory work such as cloning and labelling is required for the screening of RAPD markers.

Hu and Quiros (1991) have discussed the potential of using RAPD markers for cultivar identification in *Brassica* crops. They found that as few as 4 primers could be used to distinguish among 14 broccoli and 12 cauliflower cultivars. While variety identification was not a specific objective of our research, we found that all 27 inbred lines could be distinguished reliably using 9 RAPD polymorphisms revealed by 7 primers. Since polymorphism was found between lines having values of r as high as 0.92, RAPD polymorphism might be useful in distinguishing among barley lines that share a high degree of similarity.

In a sample of 37 RAPD polymorphisms in alfalfa, Echt et al. (1992) found no codominant markers. According to Williams et al. (1990), Kubelik and coworkers found 4 out of 88 RAPD markers in *Neurospora crassa* to be codominant. In the study reported here, we were able to demonstrate only one codominant marker from a sample of 31 RAPD polymorphisms in barley. This scarcity of codominant RAPD markers may be a disadvantage when it is important to distinguish heterozygous loci, but it was not a problem in this study, nor would it be a problem in other studies involving homozygous lines. Perhaps of more concern is the absence of discernable multiple alleles at most RAPD loci.

In this study the RAPD technique was supplemented by Southern blotting and DNA hybridization. This detection method was much more sensitive to the presence of weak amplification products than was ethidium bromide staining. Thus, with Southern analysis, the number of scoreable RAPD markers per primer could probably be increased. However, the extra effort required for this type of analysis may only be justified under circumstances where polymorphism is rare, or where the scoring of a particular polymorphism is critical.

When RAPD reaction products were probed with a single labelled fragment, five of the eight probes revealed second or third fragments that were slightly shorter. In all cases, these additional fragments cosegregated perfectly with the targeted fragments. Because there is sequence homology, it seems likely that shorter fragments are sometimes produced by priming sites that exist within longer amplification products. If so, these internal sites must be relatively

weak, otherwise the shorter fragments would be produced in preference to the longer fragment. Therefore, we speculate that the shorter fragments are produced by one or more weak priming sites within the longer fragments, but that this amplification is rare until the longer fragments become abundant in the reaction mixtures. Most of these shorter fragments were not visible when stained with ethidium bromide, either because they were coincident with a monomorphic fragment or because the they were produced in insufficient quantities.

While the RAPD technique clearly represents a useful method for identifying the presence of genetic polymorphism, more information is required regarding the type of DNA sequence differences represented by RAPD loci. In addition to this, further investigation is needed to address the queston of whether or not RAPD markers are repeatable across a variety of protocols and laboratory conditions.

Segregation and linkage

No segregation distortion was observed for the RAPD markers scored in the DH lines. While the size of this population does not provide a powerful χ^2 test, segregation distortions of the magnitude and frequency observed in the androgenetic DH lines of Thompson et al. (1991) would likely have been detected if they were present. Furthermore, there was no indication that RAPD polymorphisms have been systematically mis-scored as present or absent, since the total number of dominant marker phenotypes was not different from the total number of recessive marker phenotypes.

The haploid barley genome contains seven chromosomes, which together account for at least 1453 cM (Graner et al. 1991). Therefore, the occurrence of four tightly linked loci from a random sample of 20 polymorphisms was unexpected. The probability that these loci are linked is high, but the confidence interval on their actual linkage distance is large due to the small size of the segregating population. Since these linked polymorphisms were produced by unrelated primers and since their pattern of occurrence in the inbred lines was not identical, it is unlikely that they are allelic. The presence of this apparently tight linkage group within a relatively small sample of polymorphisms is an indication that polymorphic RAPD markers may not be distributed randomly throughout the genome.

Comparison of r and d

There was strong evidence for a linear relationship between r and d, but the collinearity between estimates of r and d was low. In particular, low values of r gave estimates of $\hat{d}$ that were significantly different from many observed values of d. This has implications for researchers who are interested in anticipating the degree of genetic similarity among individuals. A geneticist who wants to develop a mapping population may want to choose adapted parents with a high frequency of polymorphism. These results indicate that some choices based on low values of r might fail to show adequate polymorphism. In contrast, some combinations of lines provide a much higher level of polymorphism than would be expected from r. A breeder may wish to combine estimates of genetic similarity with a knowledge of agronomic performance. In some instances the objective may be to select parents that combine an ideal set of agronomic characteristics with a minimum genetic distance so that adapted gene complexes will not be lost. A contrasting breeding strategy would be to choose parents with good adaptation that possess many random genetic differences in the hope of recovering transgressive segregates for a quantitative trait. In either case, a prediction of genetic similarity based on genetic markers provides more information than can gained from pedigree information alone.

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