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# Study of Linkage Relationships of Randomly Amplified Polymorphic DNA (RAPD) Markers in a Doubled Haploid Population of Barley

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## ABSTRACT

The segregation of alleles at RAPD loci was evaluated in 33 doubled haploids progeny of barley obtained from anther culture of $F_1$ plants derived from crosses between Blenheim and TS264. A total of 165 primers were used to generate amplification products using PCR and 22 of them detected polymorphism at 27 loci between the parents and were also scored for the 33 doubled haploids progeny. MAPMAKER programme was used to estimate linkage relationships between segregating loci and four linkage groups were established.

**Key Words**: Doubled haploid; Barley; PCR; RAPD marker; Linkage

## INTRODUCTION

The detection and exploitation of naturally occurring DNA sequence polymorphism represents one of the most significant developments in molecular biology (Waugh & Powell, 1992). Restriction Fragment Length Polymorphism (RFLP) has been used extensively for genetic studies (Devarumath *et al*., 2002). However, the technical complexity of performing RFLP analysis has promoted a debate on whether the routine application of RFLPs in large-scale crop improvement programme is feasible. An alternative method to RFLP is based on the Polymerase Chain Reaction (PCR). The PCR technique is based on the *in vitro* amplification of specific DNA sequences by the simultaneous primer extension of complimentary strands of DNA. This method was described by Mullis and Faloona (1987). Since its development, PCR has revolutionized many standard molecular techniques, with modifications of the original procedure to suit a range of needs. One such variation generates a specific class of molecular marker termed Randomly Amplified Polymorphic DNAs (RAPDs) developed by Williams *et al*. (1990) and Welsh and McClelland (1990). Allelic variations detected by RAPDs tend to be inherited in a dominant manner and hence heterozygote can not be usually distinguished from homozygous dominant individuals. Creation of doubled haploids from heterozygous plants give completely homozygous plants in a single generation and this offer the quickest possible approach to homozygosity. The absence of within family segregation results in doubled haploids being a valuable source both for cultivar production and the analysis of polygenic sources of variation. Doubled haploids can be derived from plants which have undergone just one round of recombination and therefore, give maximal expression of linkage relationships. Doubled haploid families can be extensively replicated and this facilitates the partitioning of the total phenotypic variation into heritable and non-heritable components. This approach (called localize mapping) has already been used to map quantitative traits of *Arabidopsis thaliana* (Reiter *et al*., 1992).

## MATERIALS AND METHODS

**Plant material**. A commercial spring cultivar Blenheim, an advanced SCRI line TS264 and 33 doubled haploid lines obtained from anther culture of $F_1$ hybrid derived from a cross between Blenheim x TS264 were used.

**Plant DNA isolation**. Total cellular DNA was isolated from seedlings by a modified version of the CTAB (hexadecyltrimethyl ammonium bromide) method (Saghai-Maroof *et al*., 1984).

**Primer and product indexing.** The following convention was adhered throughout. For example, SC10-65-400. SC stands for Scottish Crop as it was synthesized at the Scottish Crop Research Institute and 10 nucleotide (nt) in length and number 65 in primer collection list. The product of interest 400, generated with this primer is 400 nt in length. Similar is the case for OP (Operon – manufacturer) primer. For example, OPX17-700. OP stands for Operon and X stands

**Table I. Primers used and the amplification products generated from PCR carried out on Blenheim, TS264 and their 33 doubled haploids progeny by different primers**

| Parameter | Number |
|---|---|
| Primers screened | 165 |
| Polymorphic primers | 22 |
| Per cent of polymorphic primers | 13 |
| Total amplification products | 984 |
| Amplification products per primer | 5.96 |
| Segregating loci | 27 |

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for letter index and under each letter there are 1 - 20 different primers of 10 nt length and 700 is the amplification product generated by this primer.

**PCR.** The starting mixture of PCR was made in a 50 µL volume containing 20 ng of genomic DNA, 0.2 µM of primer, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.4 unit of *Taq polymerase* (Perkin Emler Cetus), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ and 0.001% gelatin. Amplifications were performed in a Perkin Elmer Cetus DNA Thermal Cycler programmed for 45 cycles and each cycle comprised 1 minute at 92°C, 3 minutes at 35°C and 2 minutes at 72°C using the fastest available transitions between temperatures. Reaction products were resolved by electrophoresis (1.2 V/cm) in an agarose gels and detected by staining with ethidium bromide (0.5 µg/mL).

**Linkage mapping.** For each segregating locus, a goodness of fit to a ratio 1:1 was determined by *Chi*-square analysis. Recombination fractions between all pairs of markers and map distances were all determined by the computer programme MAPMAKER (Lander *et al*., 1987). The map was developed using a LOD (logarithm of odd ratio) score of 4.0 and a recombination fraction at which linkage can be detected between markers utilizing 56 backcross progeny (Tanksley *et al*., 1988). The map of each ordered linkage group was then generated by the MAP function of MAPMAKER.

## RESULTS AND DISCUSSION

The two parents Blenheim and TS264 were screened by random primers to detect polymorphism between them. A total of 165 different primers of arbitrary nucleotide sequence were used to generate amplification products.

**Fig. 2. Linkage relationships of eight RAPD markers forming four linkage groups**

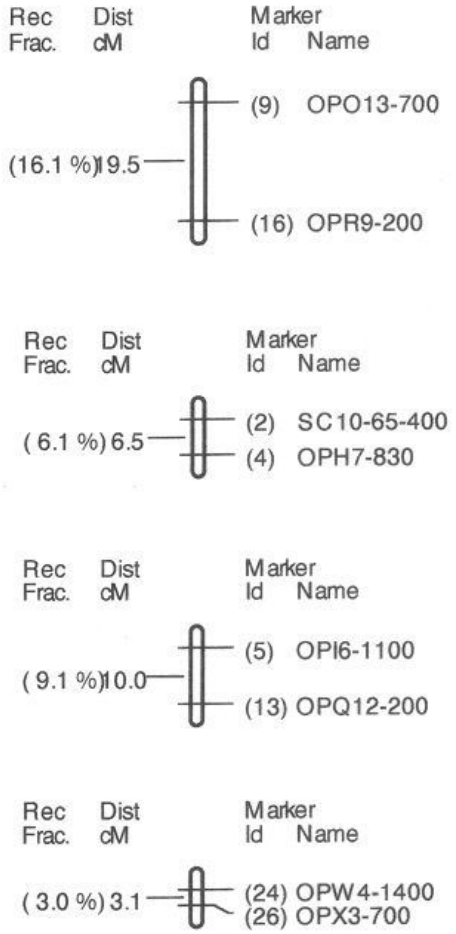


**Fig. 1. Amplification products resulting from PCR by the primer OPQ12 (5′AGTAGGGCAC 3′) carried out on Blenheim, TS264 and their 33 doubled haploids progeny. The arrows show polymorphic band between the parents and these bands also showed segregation in 33 doubled haploids progeny. Marker lane (λ DNA digested with *Eco*RI and *Hind*III) gives molecular size of the product in base pair**

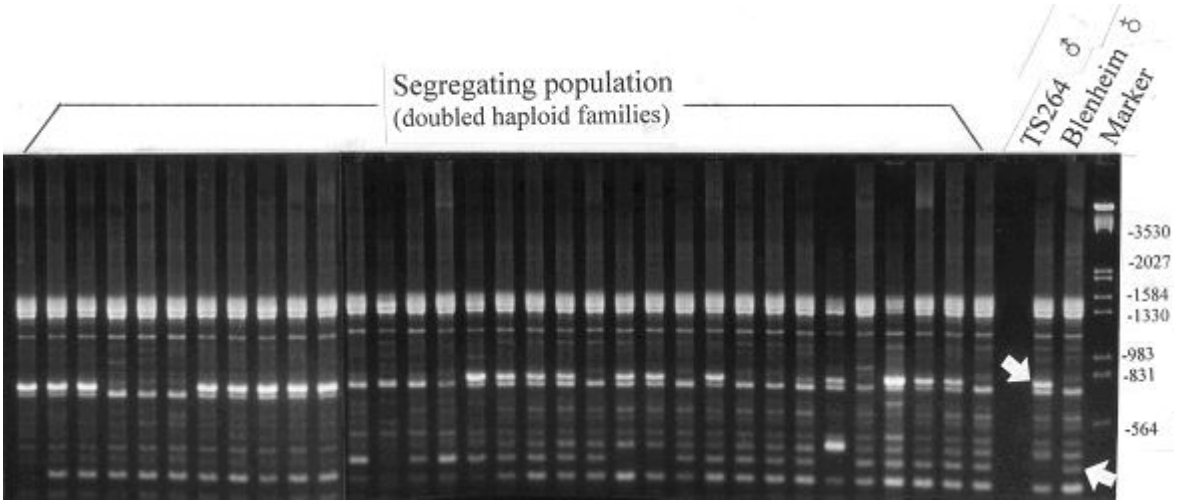

**Table II. Chi-square ($\chi^2$) analysis of segregating RAPD markers in a doubled haploid population of barley derived from the cross between Blenheim and TS264**

| Markers identified | Base sequence 5′ to 3′ | Blenheim Type | TS264 Type | $\chi^2$ (1:1) |
|---|---|---|---|---|
| OPX17-700 | GACACGGACC | 11 | 22 | 3.667* |
| OPX3-700 | TGGCGCAGTG | 18 | 15 | 0.273 |
| OPT4-2500 | CACAGAGGGA | 23 | 10 | 5.121* |
| OPT4-500 | CACAGAGGGA | 16 | 17 | 0.030 |
| OPT7-700 | GGCAGGCTGT | 17 | 16 | 0.030 |
| OPQ12-200 | AGTAGGGCAC | 28 | 05 | 16.030* |
| OPQ12-700 | AGTAGGGCAC | 14 | 19 | 0.758 |
| OPR19-1400 | CCTCCTCATC | 06 | 24 | 10.800* |
| OPR15-2000 | GGACAACGAG | 15 | 17 | 0.125 |
| OPR15-700 | GGACAACGAG | 14 | 19 | 0.758 |
| OPR9-200 | TGAGCACGAG | 17 | 14 | 0.290 |
| OPR7-400 | ACTGGCCTGA | 16 | 17 | 0.030 |
| OPI18-980 | TGCCCAGCCT | 17 | 14 | 0.290 |
| OPI6-1100 | AAGGCGGCAG | 29 | 04 | 18.939* |
| OPH7-830 | CTGCATCGTG | 21 | 12 | 2.455 |
| SC10-65-400 | CAGGGGTGAT | 21 | 12 | 2.455 |
| SC10-65-200 | CAGGGGTGAT | 20 | 13 | 1.485 |
| OPO13-700 | GTCAGAGTCC | 21 | 12 | 2.455 |
| OPO13-400 | GTCAGAGTCC | 16 | 17 | 0.030 |
| OPW4-1400 | CAGAAGCGGA | 19 | 14 | 0.758 |
| OPW5-2000 | GGCGGATAAG | 22 | 11 | 3.667* |
| OPQ8-2400 | CTCCATGGGA | 13 | 20 | 1.485 |
| OPB4-700 | GGACTGGAGT | 21 | 10 | 3.903* |
| OPJ5-980 | CTCCATGGGG | 13 | 20 | 1.485 |
| OPP8-700 | ACATCGCCCA | 18 | 14 | 0.500 |
| OPP4-2000 | GTGTCTCAGG | 18 | 15 | 0.273 |
| OPU12-1580 | TCACCAGCCA | 17 | 16 | 0.030 |

*$P < 0.05$

While most of the products were common to both individuals, one product was amplified from one individual but not from the other. In some cases, a primer detected two polymorphic loci between the parents and also segregated in the doubled haploid population. This is illustrated in Fig. 1 where a 0.7 kb fragment was generated in TS264 but not from Blenheim while a 0.20 kb fragment was generated in Blenheim but not from TS264. A total of 984 amplification products were detected with 165 primers. Twenty two primers were found polymorphic and 27 loci were shown to segregate in the doubled haploid population. An average 13% of the primers were found polymorphic and the number of products per primer was 5.96. Data on the segregation of amplification products (loci) are presented in Table II. *Chi*-square tests were performed to test for significant ($P<0.05$) departures from expected 1:1 ratio. The majority of the loci did not deviate from the expected ratio but seven loci namely, OPX17-700, OPT4-2500, OPQ12-200, OPR9-1400, OPI6-1100, OPW5-2000 and OPB4-700 exhibited segregation distortion (Table II). Of these seven loci, five exhibited an excess of Blenheim alleles while loci OPX17-700 and OPR19-1400 showed an excess of TS264 alleles.

The RAPD loci segregating in this cross were analyzed using MAPMAKER programme for co-segregation analysis and logarithm of odds ratio (LOD) score of 4 was established for linkage and Haldane's mapping function was used to convert recombination frequencies to map distances in centimorgan (cM). One cM is the distance that separates two genes between which there is a 1% chance of recombination. Eight loci were shown to be genetically linked and four linkage groups were established (Fig. 2).

The use of RAPD markers provides an efficient, reproducible and relatively inexpensive approach to comparative genome mapping studies (Rival *et al*., 1998; Brunner *et al*., 2000). In particular, the level of variation for RAPDs appears to be higher than comparable classes of molecular markers (Wachira *et al*. 1995; Raina *et al*., 2001). RAPDs can be exploited as markers that segregate in a Mendelian manner (Reiseberg *et al*., 1993; Rani *et al*., 1995). Doubled haploid families extracted from $F_1$ hybrids heterozygous for marker genes are expected to segregate in a 1:1 ratio. Classification of doubled haploids families into two groups carrying the alternative allele allows the transmission of marker genes to be evaluated and linkage relationship established. Pairs of linked gene do not segregate according to Mendel's Law of Independent Assortment. The parental combinations are strongly favoured. Non-parental combinations arise as a result of recombination. The frequency of recombination between two loci is the important index in linkage mapping because it is this frequency that is taken as a measure of their genetic distance apart. The greater the distance between two loci the more likely it is that there will events in the intervening area and *vice versa*. By comparing the recombination frequencies

of numerous loci pairs, a picture can be gradually built up of which loci are linked. In plant breeding, the usefulness of genetic markers is based on finding tight linkages between the markers and the genes of interest (Pillay *et al*., 2000). Such linkage permits one to infer the presence of a desirable gene by assaying for the marker. There are a number of single gene traits that are frequently transferred from one genetic background to another by breeders. One example is genes conferring resistance to pathogens. Traditionally, progenies are screened for the presence of disease resistance genes by inoculations with a pathogen. However, simultaneous or even sequential screening of plant with several pathogens can be difficult or impractical. In contrast, detecting disease resistance genes by their linkage to RAPD markers makes it practical to screen for many different disease resistance genes simultaneously without the need to inoculate the population (Tanksley *et al*., 1989).

Doubled haploids in conjunction with RAPDs provide an efficient way of developing linkage maps. Since doubled haploids represent fixed inbred lines which can be extensively replicated, they provide a means of obtaining estimates of the environmental and genetic factors controlling quantitative traits.

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