5-1995

Identification of DNA Markers in *Triticum aestivum-Aegilops caudata* Additions Lines by Randomly Amplified Polymorphic DNA (RAPD) Technology

Ling Wei
Utah State University

IDENTIFICATION OF DNA MARKERS IN *TRITICUM AESTIVUM-AEGILOPS CAUDATA* ADDITION LINES BY RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNOLOGY

by

Ling Wei

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1995

ACKNOWLEDGMENTS

The Forage and Range Research Laboratory, USDA-ARS, Logan, Utah, provided me financial support, logistical support, use of facilities, and source of plants.

I thank Richard Wang for his time, support, encouragement, and interest in my study. I thank William F. Campbell and Jennifer W. MacAdam for their assistance with the manuscript.

Finally, thanks to my husband, Yiting Zhang, for his support and encouragement during my studies toward the M.S. degree.

Ling Wei

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Identification of DNA Markers in Triticum aestivum-Aegilops caudata Additions Lines by Randomly Amplified Polymorphic DNA (RAPD) Technology

by

Ling Wei, Master of Science

Utah State University, 1995

Major Professor: William F. Campbell
Department: Plants, Soils, and Biometeorology

The objective of this study was to identify DNA markers for each of six added C-genome chromosomes in Triticum aestivum L. cv. 'Alceso'-Aegilops caudata L. addition lines using the randomly amplified polymorphic DNA (RAPD) technique. DNA from Ae. caudata, T. aestivum, amphiploid of T.aestivum X Ae. caudata, and six disomic addition lines of wheat having a pair of Ae. caudata chromosomes was used as the template for the amplification of RAPD markers with a total of 58 random 10-mer oligonucleotide primers. Two primers, OPC-08 and OPJ-16, produced one intense band each

from the amphiploid of T.aestivum X Ae.caudata and Ae. caudata, which was absent in all six addition lines. Each of these two primers produced a chromosome marker that could be tentatively located to the chromosome C^A of Ae. caudata. OPJ-02, OPD-12, OPD-02, OPJ-12, OPD-20, and OPJ-14 produced a marker each for C^B, C^C, C^D, C^E, C^F, and C^G, respectively. OPJ-09 produced C-genome chromosome-specific RAPD markers and OPD-18 and OPD-19 produced wheat-specific RAPD markers. Also, OPC-05 and OPJ-19 produced RAPDs from both wheat and Ae. caudata genomes.

(49 pages)

INTRODUCTION

The use of deoxyribonucleic acid (DNA) markers in genetic mapping, genetic diagnostics, molecular taxonomy, and evolutionary studies has been well established. The most commonly used DNA markers are restriction fragment length polymorphisms (RFLP). But detection of RFLPs by DNA Southern blot hybridization is laborious and incompatible with applications requiring high throughput (Williams et al. 1991). Since the development of the polymerase chain reaction (PCR), this technique has been adapted by many researchers to generate DNA markers.

PCR has revolutionized many standard molecular biological techniques, with modifications of the original procedure designed to suit a range of needs. One such variation generates a specific class of molecular markers termed randomly amplified polymorphic DNA (RAPD) (Waugh and Powell 1992).

In the RAPD assay, a single oligonucleotide of an arbitrary DNA sequence of either 9 or 10 nucleotides (nt) is mixed with genomic DNA in the presence of a thermostable DNA polymerase and a suitable buffer, and then is subjected to temperature cycling conditions typical of the polymerase

chain reaction. The products of the reaction are dependent upon the sequence and length of the oligonucleotide, as well as the reaction conditions. At an appropriate annealing temperature during the thermal cycle, the single primer binds to sites on opposite strands of the genomic DNA that are within an amplifiable distance of each other, and a discrete DNA segment is produced (Williams et al. 1991). The RAPD products are then easily separated by standard electrophoretic techniques and visualized by ultraviolet illumination of ethidium-bromide stained gels.

The RAPD procedure has the advantages of being technically simple and quick to perform, requiring only small amounts of DNA and involving no radioactivity. RAPDs are well suited for use in the large sample-throughput systems required for plant breeding, population genetics, and studies of biodiversity (Waugh and Powell 1992).

For most plants, primers that are 9-10 nts long are predicted to generate, on average, 2-10 amplification products. Polymorphisms result from changes on either the sequence of the primer binding site, which prevent stable association with the primer, or from changes (e.g. insertions, deletions, and inversions, etc.) that alter the

size or prevent the successful amplification of a target DNA. As a rule, size variants are only rarely detected and individual amplification products represent one allele per locus. In inheritance studies, the amplification products are transmitted as dominant markers and segregate in a Mendelian manner (Waugh and Powell 1992).

RAPD is an effective tool in doing research on plant taxonomy. Yang and Quiros (1993) reported a survey of RAPD markers in celery cultivars and their application to cultivar identification and classification. Wilkie et al. (1993) applied RAPD analysis to onion (Allium cepa L.) and other Allium species in order to assess the degree of polymorphism within the genus and to investigate if this approach was suitable for genetic studies of onion. The study demonstrated that an analysis of RAPD markers could be used successfully to study the phylogenetic relationship among species of Allium. Yu and Pauls (1993b) used RAPD markers to estimate genetic relatedness among heterogeneous populations of alfalfa from bulked genomic DNA samples. Their results showed that the RAPD patterns could be used to determine genetic distances among heterogeneous populations and cultivars, which corresponded to the known relatedness.

DNA bulking and methods for comparing RAPD patterns are also very useful for identifying cultivars, for studying phylogenetic relationships among heterogeneous populations, and for selecting parents to maximize heterosis in crosses. In a plant population genetic study, Khush et al. (1992) used RAPD markers to identify seven distinct genotypes among eight heterokaryotic mushroom (Agaricus bisporus (Lange) Imboch) strains; two of the commercial strains were shown to be related to each other through single-spore descent analysis. McCoy and Echt (1993) analyzed RAPD markers in the trispecies hybrids of alfalfa and concluded that RAPD markers offered considerable potential for assaying germplasm introgression.

The RAPD technique has also been used in studying plant genetic variability. Chalmers et al. (1992) used the RAPD method to monitor genetic variability in Gliricidia species. Extensive genetic variability was detected between species and the variability was partitioned between- and within-population components, and population-specific genetic markers were identified. They pointed out that RAPD provides a cost-effective method for the precise and routine evaluation of variability and may be used to identify areas

of maximum diversity. Vierling and Nguyen (1992) used RAPD markers to determine the genetic diversity of diploid wheat genotypes. Their data indicated that RAPD analysis was a powerful tool for determining the extent of genetic diversity among diploid wheat genotypes. Using wheat, barley, rye, and wheat-barley addition lines, Weining and Langridge (1991) detected polymorphism with conserved, semi-random, and random primers. With different combinations of primers, they were able to detect both inter- and intra-specific diversity.

RAPDs are often used in plant breeding. Yu and Pauls (1993a) studied RAPD markers linked to genes controlling somatic embryogenesis in alfalfa. From segregation analyses of the somatic embryogenesis trait and RAPD markers in the F1 population, which was derived from a cross between embryogenic and non-embryogenic alfalfa plants, they identified a polymorphic band that was associated with somatic embryogenesis. Barua et al. (1993) used a series of near-isogeneic lines together with bulked segregant analysis to identify RAPD markers linked to genes determining Rhynchosporium resistance in barley. Lanham et al. (1992) used RAPDs to identify polymorphic molecular markers in a

range of wild and cultivated Arachis species. They found that RAPD markers required small amounts of DNA for screening and constituted an effective tool for early selection of desirable genotypic combinations in breeding programs. In order to facilitate the identification of potato hybrids at an early stage following fusion, Baird et al. (1992) used RAPD markers to molecularly characterize both inter- and intraspecific somatic hybrids of potato.

RAPDs are very useful on plant tissue culture. Brown et al. (1993) used polymerase chain reaction and the associated RAPD technique in the analysis of DNA and specific genes in plant cells at different stages of regeneration in *in vitro* cultures. They showed that both procedures could be used to reproducibly differentiate closely related species as well as to reveal levels of DNA polymorphism in regenerated plants. They also demonstrated that both procedures were applicable at all tissue culture stages, from single isolated protoplasts to regenerated plants.

Echt et al. (1992) analyzed segregation of RAPD fragments to determine if RAPD markers were suitable for use as genetic markers. As a result, RAPD markers appeared to be useful for the rapid development of genetic information in

species like alfalfa where little information currently exists or is difficult to obtain. Waugh et al. (1992) employed RAPDs to demonstrate that potato dihaploids generated after interspecific pollination of a tetraploid Solanum tuberosum L. cultivar by S. phureja Juz. et Buk dihaploid inducer clones could not be of parthenogenetic origin. The molecular evidence emphasizes that RAPD can be used as a general methodology for the detection of alien gene introgression in both natural and cultivated plant populations. Singsit and Ozias-Akins (1993) used RAPD markers to study the genetic variation among androgenetic monoploids of diploid potato species. They demonstrated that it was possible to construct a genetic linkage map, without making crosses, using monoploids derived from single heterozygous diploid clones and RAPD markers. Devos and Gale (1992) used RAPD as a genetic marker system in wheat. Their data showed that reproducible amplification products were obtained from varietal, homozygous single chromosome recombinant line and wheat/alien addition line genomic DNA with selected primers and rigorously optimized reaction conditions. Quiros et al. (1993) evaluated RAPD as a source of markers for use in investigations of potato genetics. As

a result, segregation of 18 loci in diploid S. goniocalyx (Juz. et Buk) Hawkes X S. phureja and 12 loci in tetraploid S. tuberosum ssp. tuberosum X S. tuberosum ssp. andigena families fitted Mendelian and tetrasomic ratios, respectively. Eight loci in the diploid progeny were arranged in three linkage groups. The fact that segregation of these markers fitted the expected ratios indicates that RAPD can be effectively used in potato genetics, breeding, and evolution.

In 1980, T. aestivum cv. 'Alcedo'-Ae. caudata addition lines were established. To date, six wheat disomic addition lines with a pair of added Ae. caudata chromosomes (B, C, D, E, F, and G) have been identified and characterized (Schubert and Junghanns personal communication). Five of these lines, which contain a good resistance against powdery mildew, are located on C-genome chromosomes C, D, E, F, and G. Searching DNA markers of C-genome chromosomes may be useful in linkage studies of disease resistance and alien gene introgression. The purpose of this study is to identify DNA markers for the added C-genome chromosomes into wheat using the RAPD technique.

MATERIALS AND METHODS

Plant materials

Seeds of plant materials for this study were acquired by Dr. Richard Wang from Dr. V. Schubert (Table 1). They were planted in the greenhouse at the Forage and Range research Laboratory, USDA-ARS, Logan, Utah.

DNA extraction

Plant DNA was extracted by the CTAB method. Briefly, 0.1-0.5 g of fresh leaf tissue was ground in liquid nitrogen and mixed with 1 ml of CTAB extraction buffer (0.14 M Sorbitol, 0.22 M Tris-HCl, 0.022 M EDTA, 0.8 M NaCl, 0.8% CTAB, and 1% Sarcosin) and 0.4 ml of chloroform. The sample was incubated at 60°C for 10 min, centrifuged for 5 min, and the supernatant was recovered and mixed with 1.2 volumes of isopropanol. The nucleic acid precipitate was recovered by centrifugation, washed with 1 ml of 70% ethanol, dried, and dissolved in TE (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA).

RAPD procedure

Reactions similar to those described by Williams et al. (1990) were performed with some modifications. RAPD

TABLE 1. Plant materials used in the study

Plants	Species	Genome
1	Aegilops caudata	CC
2	Triticum aestivum	AABBDD
3	Amphiploid of T. aestivum X Ae. caudata	AABBCCDD
4	Disomic addition line of C-genome Ch* B	AABBDD+C^{B}
5	Disomic addition line of C-genome Ch* C	AABBDD+C^{C}
6	Disomic addition line of C-genome Ch* D	AABBDD+C^{D}
7	Disomic addition line of C-genome Ch* F	AABBDD+C^{F}
8	Disomic addition line of C-genome Ch* E	AABBDD+C^{E}
9	Disomic addition line of C-genome Ch* G	AABBDD+C^{G}

*Ch=chromosome

amplification reactions were performed in volumes of 25 ul containing 50 ng of template DNA. The reaction buffer consisted of 200 uM each of dATP, dCTP, dGTP, and dTTP, 500nM oligonucleotide primer (Operon Technologies), 2 units of AmpliTaq DNA polymerase Stoffel fragment (Perkin Elmer) in 10 mM Tris-HCl(pH 8.3), 50 mM KCl, and 50 mM $MgCl_2$. The reaction mixture was overlaid with mineral oil prior to amplification in a Perkin Elmer DNA Thermal Cycler programmed for 40 cycles of 1 min at 93°C (denaturation), 1 min at 35°C (annealing), and 2 min at 71°C (elongation). Aliquots (10 ul) of the amplification products were electrophoresed in a 2% agarose gel and detected by staining with ethidium bromide. One of the two DNA size standards (Bio-Rad's 50-2000 bp Ampli Size and/or pBR322/Hae III 184-587 bp fragments) was used as a size marker (M). The size of a RAPD marker was estimated with the aid of the computer program "fraglength." A total of 58 primers from Operon decamer kits C, D, and J was tested.

RESULTS

Optimization of the DNA concentration

To determine the optimal template concentration, DNA amounts ranging from 25 ng to 200 ng were tested with the constant primer concentration of 500 nM. The results showed that a concentration of 50 ng yielded the greatest number of bands (Fig. 1). Both 25 ng and 200 ng of template DNA produced only one band.

Primer performances

With other amplification conditions being the same, the primer concentration was optimized (Fig. 2). When primer concentrations of 100 nM and 200 nM were used, there was no amplification. As the primer concentration increased, more RAPD bands appeared. The 500 nM concentration gave the effects of primer concentration.

For the 58 tested random 10-mer primers, not all primers performed equally well under the same amplification condition (Fig. 3). Some, presumably due to the lack of suitable priming sites in the genomic DNA, gave poorly amplified banding patterns, while others created discernible bands. Thus, oligonucleotides should be rigorously tested

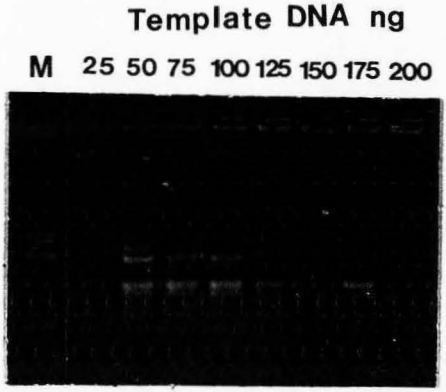

Fig. 1. Effects of template DNA concentrations. From lane 1 to 8, DNA concentration are 25, 50, 75, 100, 125, 150, 175, and 200 ng. Template DNA is amphiploid of Ae. caudata X T. aestivum and primer is OPD-08 500 nM concentration.

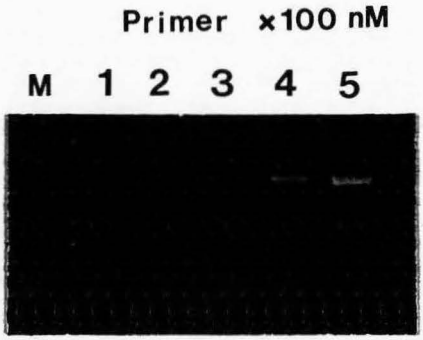

Fig. 2. Effects of primer concentrations. Primer is OPD-08. From lane 1 to lane 5, primer concentrations are 100, 200, 300, 400, 500 nM. Template DNA is Ae. caudata 50 ng concentration. Other reaction mixture concentrations are 200 uM each of dNTP, 2 units of enzyme, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 50 mM $MgCl_2$.

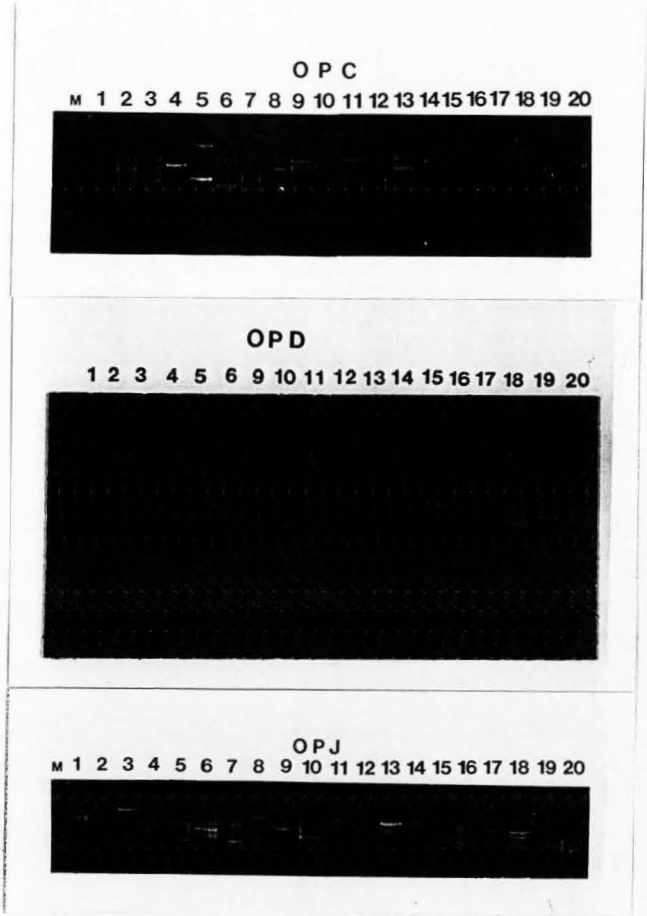

Fig. 3. Results of RAPD reactions with 58 primers. For OPC kits, using disomic addition line of C-genome chromosome G as template DNA. For OPD kits, using Ae. caudata as template DNA. For OPJ kits, using T. aestivum as template DNA. The concentrations of reaction mixture are 200 uM each of dNTP, 500nM primer, 50 ng DNA, 2 units of enzyme, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 50 mM $MgCl_2$.

for priming ability and reproducibility before they can be employed as genetic markers.

C-genome chromosome markers

DNA from Ae. caudata, T. aestivum, amphiploid of T. aestivum X Ae. caudata, and six wheat disomic addition lines having a pair of one Ae. caudata chromosome was used as the templates in RAPD reactions with a total of 58 random 10-mer oligonucleotide primers. Of the primers tested, the most discriminatory primers were OPC-08, OPJ-16, OPJ-02, OPD-12, OPD-02, OPJ-12, OPD-20, and OPJ-14 (Fig. 4-10, Tables 2 and 3), which gave clear differences in banding patterns among the addition lines. Discriminatory bands are indicated by an X in the corresponding figures. For example, using OPJ-02, one additional band of 290 bp was present in Ae. caudata, amphiploid of T. aestivum X Ae. caudata, and disomic addition line of C-genome chromosome B. Therefore, chromosome C^B of Ae. caudata was discriminated from all other addition lines by the presence of one intense band of 290 bp (Fig. 5). Using OPD-12, one additional band of 910 bp was shown in Ae. caudata, amphiploid of T. aestivum X Ae. caudata and disomic addition line of C-genome chromosome C.

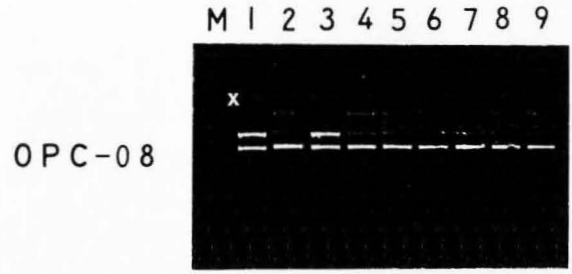

Fig. 4. RAPD marker of C-genome chromosome A. Primer is OPC-08 with 1250 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata. Lane 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

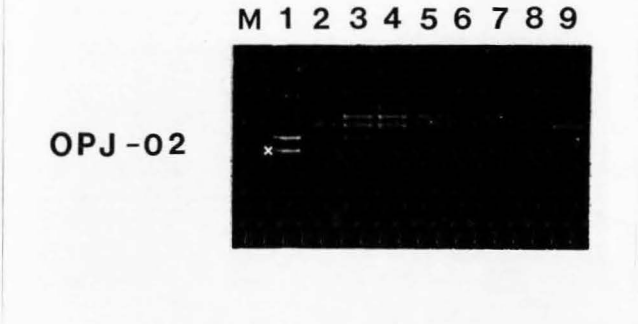

Fig. 5. RAPD marker of C-genome chromosome B. Primer is OPJ-02 with 290 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane. 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

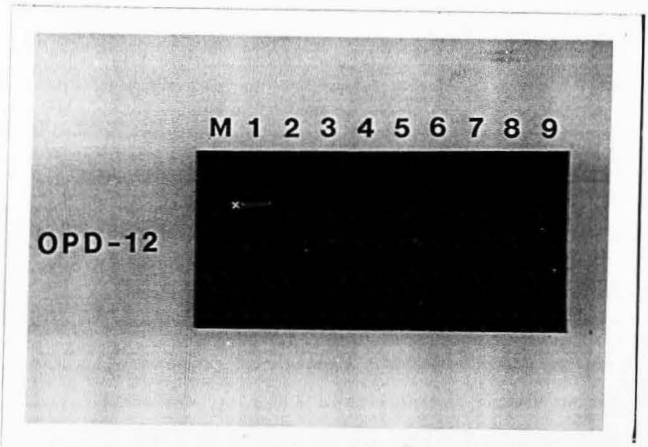

Fig. 6. RAPD marker of C-genome chromosome C. Primer is OPD-12 with 910 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane. 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

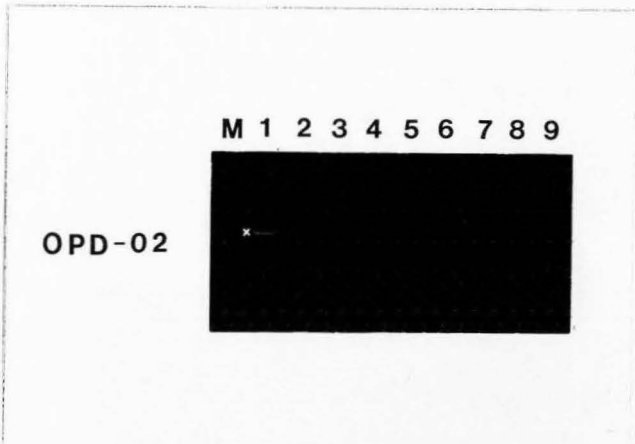

Fig. 7. RAPD marker of C-genome chromosome D. Primer is OPD-02 with 734 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane. 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

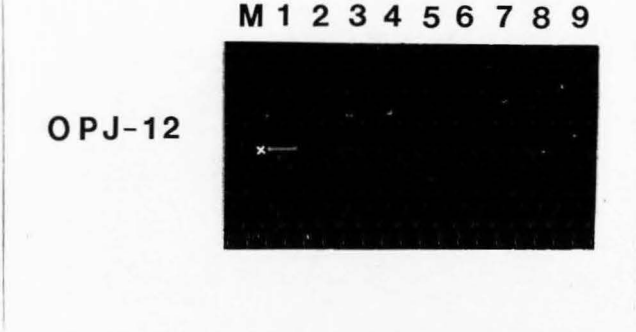

Fig. 8. RAPD marker of C-genome chromosome E. Primer is OPJ-12 with 350 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane. 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

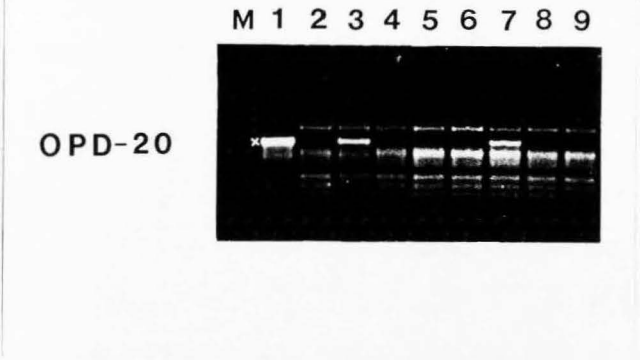

Fig. 9. RAPD marker of C-genome chromosome F. Primer is OPD-20 with 600 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane. 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

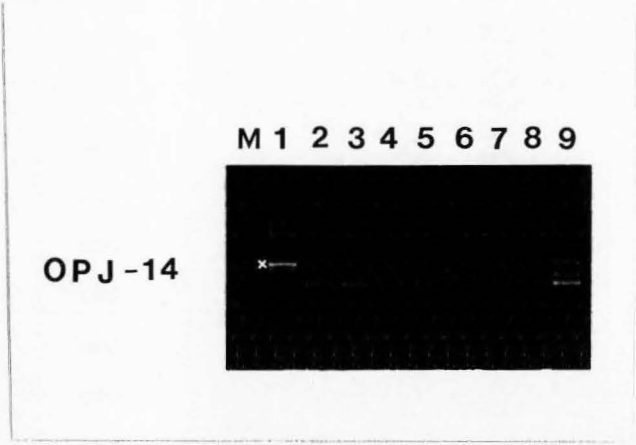

Fig. 10. RAPD marker of C-genome chromosome G. Primer is OPJ-14 with 300 bp. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane. 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

TABLE 2. Chromosome markers of Ae. caudata (C-genome)

Marker location	Primer designation	Primer sequence 5' 3'	Molecular weight of marker (bp)
C^A	OPC-08	TGGACCGGTG	1250
	OPJ-16	CTGCTTAGGG	820
C^B	OPJ-02	CCCGTTGGGA	290
C^C	OPD-12	CACCGTATCC	910
C^D	OPD-02	GGACCCAACC	734
C^E	OPJ-12	GTCCCGTGGT	350
	OPJ-16	CTGCTTAGGG	460
C^F	OPD-20	ACCCGGTCAC	600
C^G	OPJ-14	CACCCGGATG	300

TABLE 3. Presence and absence of RAPD bands in the agarose gel and the interpretations

Primers	Plant materials (Lanes on gel)									Interpretions
	1	2	3	4	5	6	7	8	9	
OPC-08,OPJ-16	+	-	+	-	-	-	-	-	-	RAPD marker on C^A
OPJ-02	+	-	+	+	-	-	-	-	-	RAPD marker on C^B
OPD-12	+	-	+	-	+	-	-	-	-	RAPD marker on C^C
OPD-02	+	-	+	-	-	+	-	-	-	RAPD marker on C^D
OPJ-20	+	-	+	-	-	-	+	-	-	RAPD marker on C^F
OPJ-12,OPJ-16	+	-	+	-	-	-	-	+	-	RAPD marker on C^K
OPJ-14	+	-	+	-	-	-	-	-	+	RAPD marker on C^U
OPJ-08	+	-	+	+	-	-	-	-	+	RAPD marker on C^B+C^U
OPD-15	+	-	+	-	-	-	+	-	+	RAPD marker on C^F+C^U
OPJ-09,OPC-01	+	-	+	+	+	+	+	+	+	RAPD marker for C-genome
OPC-04,OPJ-10	+	-	-	-	-	-	-	-	-	RAPD marker for C is out-competed by wheat DNA
OPC-06,OPD-18 and OPD-19	-	+	+	+	+	+	+	+	+	RAPD marker for wheat
OPC-19	-	+	-	-	-	-	-	-	-	RAPD marker for wheat is out-competed by C-genome DNA
OPC-05,OPJ-19	+	+	+	+	+	+	+	+	+	RAPD marker on both wheat and C-genome DNA
OPJ-17,OPJ-18	+	+	+	+	+	+	+	+	-	A wheat chromosome having a marker that is not on C^U is missing

from all other addition lines by the presence of one intense band of 600 bp (Fig. 9). Using OPJ-14, one additional band of 300 bp was present in Ae. caudata, amphiploid of T. aestivum X Ae. caudata, and the disomic addition line of C-genome chromosome G. Therefore, chromosome C^G of Ae. caudata was discriminated from all other addition lines by the presence of one intense band of 300 bp (Fig. 10). Using OPC-08 and OPJ-16, the amphiploid of T. aestivum X Ae. caudata and Ae. caudata produced one intense band of 680 bp and 820 bp, respectively, which were absent in all six addition lines (Fig. 4 and Fig. 13). Therefore, OPC-08 and OPJ-16 produced one chromosome marker each that could be tentatively located on the chromosome C^A of Ae. caudata.

Some of the primers produced RAPD markers that were located on more than two chromosomes (Table 3). OPD-15 amplified one Ae. caudata chromosome marker of 540 bp that was located on C^F and C^G (Fig. 11). OPJ-08 produced one Ae. caudata chromosome marker with a fragment size of 320 bp, which was located on C^B and C^G (Fig. 12). OPJ-16 produced two Ae. caudata chromosome markers with fragment sizes of 820 bp and 460 bp, respectively, which were located on C^A and C^E (Fig. 13). The 270 bp amplification product with

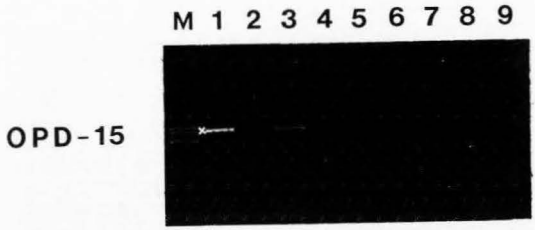

Fig. 11. OPD-15 markers on both C^r and C^g chromosomes. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata. Lane 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

Fig. 12. OPJ-08 markers on both C^s and other chromosomes. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata. Lane 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

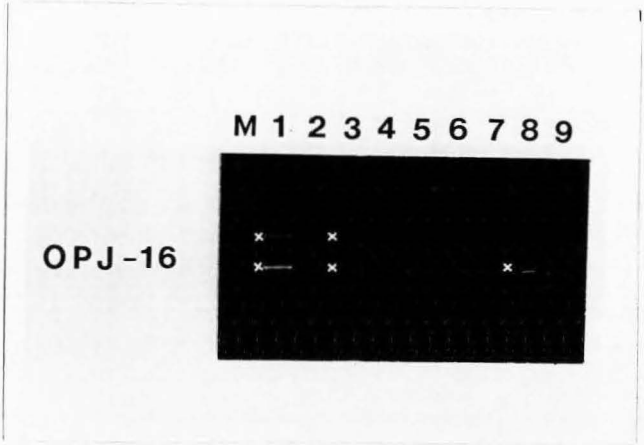

Fig. 13. OPJ-16 markers on both C^A and C^E chromosomes. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

OPJ-09 was present in all tested plants except wheat (Fig. 14), indicating that it was C-genome specific and might represent a family of repetitive DNA sequences dispersed in all C-genome chromosomes. OPC-04 and OPJ-10 amplification products were only present in Ae. caudata, indicating that the template nucleotides in the C-genome were outcompeted by wheat DNA in the RAPD assays. Therefore, these RAPD fragments could not be used as markers in the wheat background.

Wheat-specific RAPD markers

Table 4 list RAPD markers for wheat. The 700 bp and 650 bp amplification products with OPD-18 and OPD-19, respectively, were present in all except Ae. caudata, indicating that OPD-18 and OPD-19 produced T. aestivum specific markers (Fig. 15). OPD-02 produced a 610 bp RAPD marker, which was from wheat genomic DNA (Fig. 7). OPC-19 had one amplification product, which was present in wheat but not in wheat addition lines, indicating that the wheat-specific template nucleotide was outcompeted by C-genome DNA in the RAPD reaction.

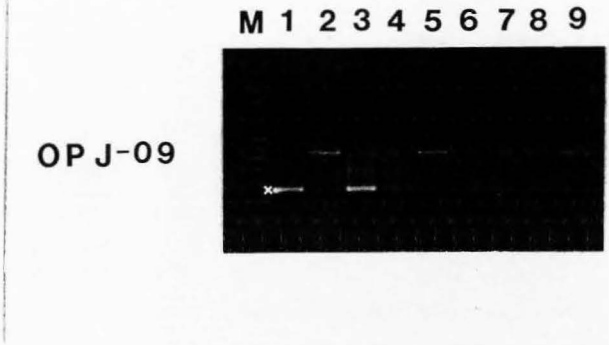

Fig. 14. OPJ-09 produced C-genome chromosome specific markers. Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata. Lane 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

TABLE 4. Primers that produce RAPDs markers from wheat DNA but not from Ae. caudata

Primers	RAPD size (bp)
OPC-04	560
OPC-06	300
OPC-07	270
OPC-09	500
OPC-13	520
OPC-20	900
OPD-02	610
OPD-03	380
OPD-08	480
OPD-09	510
OPD-11	380,310,290,270
OPD-16	270
OPD-18	700
OPD-19	650
OPD-20	800
OPJ-01	280
OPJ-02	600
OPJ-03	1200,600,360
OPJ-06	500
OPJ-07	400
OPJ-08	400,380
OPJ-09	510
OPJ-13	720
OPJ-14	210,190
OPJ-16	300
OPJ-18	320

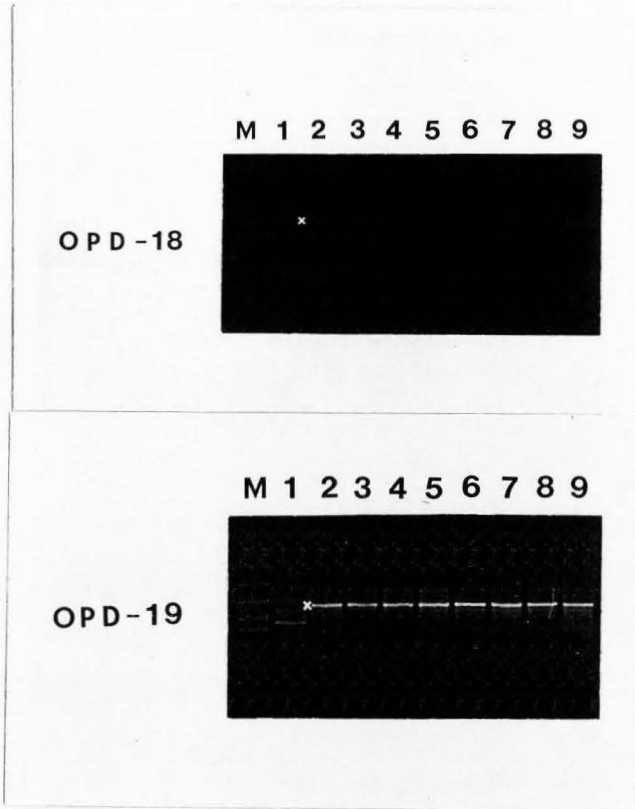

Fig. 15. OPD-18 and OPD-19 produced T. aestivum specific Lane 1. Aegilops caudata. Lane 2. Triticum aestivum. Lane 3. Amphiploid of T. aestivum X Ae. caudata Lane 4. Disomic addition line of C-genome chromosome B. Lane 5. Disomic addition line of C-genome chromosome C. Lane 6. Disomic addition line of C-genome chromosome D. Lane 7. Disomic addition line of C-genome chromosome F. Lane 8. Disomic addition line of C-genome chromosome E. Lane 9. Disomic addition line of C-genome chromosome G.

Other RAPD markers

OPC-05 and OPJ-19 produced RAPD markers that were located on both wheat and C-genome chromosomes. A wheat chromosome might be missing in wheat-C^{a} addition line, as indicated by amplification products from OPJ-17 and OPJ-18 (Table 3). These products were amplified from both C-genome chromosomes other than C^{a} and wheat chromosomes other than the missing one. The remaining primers gave less conclusive results.

DISCUSSION

The goal of this research was to identify chromosome specific RAPD markers that may have potentials in gene mapping and plant breeding. The procedures used in this study were relatively simple, and the polymorphisms detected were repeatable and stably inherited. Furthermore, the genome is most probably randomly sampled without any variation due to ontogenic expression. However, only major amplified fragments should be used as markers because they are reproducible. Minor fragments, which tend to be unstable and therefore not reliable, should not be considered. Variability at low template concentrations may be the result of a reduced probability in initiating amplification reactions and reflect the inefficiency of the priming events (Welsh and McClelland 1990).

Devos and Gale (1992) have discussed the use of RAPD markers in wheat. They found that production of a wheat/alien chromosome addition and substitution lines will benefit from RAPD analysis, as will the introgression by translocation or recombination of alien chromosome segments

into the wheat genome. Similar results were obtained in this study for the segregation of RAPD markers in the parents, amphiploid, and addition lines. By using RAPD analysis, seven addition lines can be discriminated.

Although additional work can be extended from this initial study, results indicate that the RAPD technique can be used to identify all seven C-genome chromosomes. This method should be an effective means to search for the needed C^A addition line of wheat among progenies of the wheat X Ae. caudata amphidiploid. In addition, the RAPD markers may be useful in linkage studies of agronomically important traits, such as disease resistances. The addition line of A. caudata chromosome B contains resistance to brown rust. Disease screening for leaf rust is not feasible in the field and is time-consuming and labor intensive in the greenhouse. Therefore, DNA fingerprinting is an attractive alternative means to identify resistant plants. Segregant analysis can be used to identify whether a RAPD marker is linked to the leaf rust resistance gene. In an F_2 segregation population, RAPD markers of the C-genome chromosome B may be mapped with leaf rust resistance along with other isozyme and RFLP

markers. The RAPD marker most closely linked to brown rust resistance will be useful in selection of resistant progenies. Marker-assisted selection will greatly facilitate gene introgression from the C-genome, or any other alien chromosomes, into wheat.

REFERENCES

Baird, E., Cooper-Bland, S., Waugh, R., DeMaine, M., and Powell, W. 1992. Molecular characterization of inter-and intra-specific somatic hybrids of potato using randomly amplified polymorphic DNA (RAPD) markers. Mol. Gen. Genet. **233**: 469-475.

Barua, U.M., Chalmers, K.J., Hackett, C.A., Thomas, W.T.B., Powell, W., and Waugh, R. 1993. Identification of RAPD markers linked to a Rhynchosporium secalis resistance locus in barley using near-isogeneic lines and bulked segregant analysis. Heredity, **71**: 177-184.

Brown, P.T.H., Lange, F.D., Kranz, E., and Lorz, H. 1993. Analysis of single proptoplasts and regenerated plants by PCR and RAPD technology. Mol. Gen. Genet. **237**: 311-317.

Chalmers, K.J., Waugh, R., Sprent, J.I., Simons, A.J., and Powell, W. 1992. Detection of genetic variation between and within populations of Gliricidia sepium and G. maculata using RAPD markers. Heredity, **69**: 465-472.

Devos, K.M., and Gale, M.D. 1992. The use of random amplified polymorphic DNA markers in wheat. Theor. Appl. Genet. **84**: 576-572.

Echt, C.S., Erdahl, L.A., and McCoy, T.J. 1992. Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome, **35**: 84-87.

Khush, R.S., Becker, E., and Wach, M. 1992. DNA amplification polymorphisms of the cultivated Mushroom Agaricus bisporus. Appl. Environ. Microbiol. **58**: 2971-2977.

Lanham, P.G., Fennell, S., Moss, J.P., and Powell, W. 1992. Detection of polymorphic loci in Archis germplasm using random amplified polymorphic DNAs. Genome, **35**: 885-889.

McCoy, T.J., and Echt, C.S. 1993. Potential of trispecies bridges crosses and random amplified polymorphic DNA markers for introgression of Medicago daghestanica and M. pironae germplasm into alfalfa (M. sativa). Genome, **36**: 594-601.

Quiros, C.F., Ceada, A., Georgescu, A., and Hu, J. 1993. Use of RAPD markers in potato genetics: segregations in diploid and tetraploid families. Am. Potato J. **70**: 35-42.

Singsit, C., and Ozias-Akins, P. 1993. Genetic variation in monoploids of diploid potatoes and detection of clone-specific random amplified polymorphic DNA markers. Plant Cell Reports, **12**: 144-148.

Vierling, R.A., and Nguyen, H.T. 1992. Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes, Theor. Appl. Genet. **84**: 835-838.

Waugh, R., Baird, E., and Powell, W. 1992. The use of RAPD markers for the detection of gene introgression in potato. Plant Cell Reports, **11**: 466-469.

Waugh, R., and Powell, W. 1992. Using RAPD markers for crop improvement. Tibtech, **10**: 186-191.

Weining, S., and Langridge, P. 1991. Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. Theor. Appl. Genet. **82**: 209-216.

Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18**: 7213-7218.

Wilkie, S.E., Isaac, P.G., and Slater, R.J. 1993. Random amplified polymorphic DNA(RAPD) markers for genetic analysis in allium. Theor. Appl. Genet. **86**: 497-504.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531-6535.

Williams, J.G.K., Hanafey, M.K., Rafalski, J.A., and Tingey, S.V. 1993. Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol. **218**: 704-740.

Yang, X., and Quiros, C. 1993. Identification and classification of celery cultivars with RAPD markers. Theor. Appl. Genet. **86**: 205-212.

Yu, K., and Pauls, K.P. 1993a. Identification of a RAPD marker associated with somatic embryogenesis in alfalfa. Plant Mol. Biol. **22**: 269-277.

Yu, K., and Pauls, K.P. 1993b. Rapid estimation of genetic relatedness among heterogeneous populations of alfalfa by random amplification of bulked genomic DNA samples. Theor. Appl. Genet. **86**: 788-794.