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RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) IN CEREAL IMPROVEMENT[1,2]

T. Demeke[3,5], B. Sasikumar[4], P. Hucl[3], R.N. Chibbar[5,*]

[3] *University of Saskatchewan, Crop Development Centre, 51 Campus Drive, Saskatoon, SK, S7N 5B1, Canada*
[4] *National Research Centre for Spices, ICAR, Marikunnu, Calicut, Kerala, India*
[5] *National Research Council, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N OW9*

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ABSTRACT - DNA based molecular markers have become important tools for developing improved cultivars and for studying phylogenetic relationships. The most commonly used molecular markers are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites. The RAPD technique is a variation of the polymerase chain reaction (PCR) that has been widely used as a molecular marker since 1990. The RAPD technique is easier, faster and requires a smaller amount of DNA than RFLP, AFLP or microsatellites. The successful use of the RAPD technique in marker identification, genetic mapping, cultivar identification and phylogenetic analysis in cereal crops is discussed in this paper. The reproducibility problem of the RAPD technique and ways to improve the assay are also discussed.

KEY WORDS: RAPD; Primers; Molecular markers; Polymorphism; Cereals; DNA; AFLP; RFLP; Microsatellities.

INTRODUCTION

Variability is the basis of all plant breeding. Conventional plant breeding focuses on selection of superior progeny from segregating populations, and selection is mostly based on phenotypic characteristics. Despite the use of many statistical and genetic tools to reduce the environmental effects on the selection of appropriate genotype (s), there is a confounding effect of environmental factors on phenotype. Molecular techniques are increasingly being used by plant breeders to develop improved cultivars. A new class of DNA-based genetic diagnostic tools that are independent of environmental factors are available to plant breeders to select desired genotypes. Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites are examples of molecular markers currently in use. These molecular techniques have been used for marker identification, DNA fingerprinting, phylogenetic analysis and genetic mapping. This review focuses on the use of RAPD markers for cereal improvement.

BACKGROUND AND MOLECULAR BASIS

The RAPD assay was developed by two groups. A group at DuPont Co. (Wilmington, USA) called the new method RAPD (random amplified polymorphic DNA) (Williams *et al.*, 1990) and described its genetic mapping applications. Another group at the California Institute of Biological Research, USA (Welsh and Mccelland, 1990) focused on genome fingerprinting and called their assay arbitrarily primed polymerase chain reaction (AP-PCR). Both these assays are based on the observation that a single short oligodeoxynucleotide of a randomly chosen sequence, when mixed with genomic DNA, dNTPs, buffer, and thermostable DNA- polymerase, and subjected to temperature cycling amplified several DNA fragments (Innis *et al.*, 1990).

RAPD assay is a modification of the basic polymerase chain reaction (PCR) technique (Mullis *et al.*, 1986). This assay, unlike the PCR, does not require knowledge of the target DNA sequence, and a single arbitrary primer will support DNA amplification from a genomic template if binding sites on opposite strands of the template exist within a distance that can be traversed by the thermostable *Taq* DNA polymerase. Usually, random oligonucleotides (9 or 10 bases) are used as a primer to amplify discrete fragments of ge-

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* For correspondence (fax +1 306 975 4839).

nomic DNA. The complementarity between the primer and the template need not be perfect (WILLIAMS *et al.*, 1991). However, the distance between the primer binding sites should be no smaller than the lower detection limit of the separation medium used to visualize the amplified DNA products (usually several nucleotides) and no longer than the maximum length of the DNA efficiently amplified by *Taq* polymerase (about 2000 nucleotides) (INNIS *et al.*, 1990). The primers are generally of random sequence, contain at least 50% G and C and without internal inverted repeats. The products are easily separated by standard electrophoretic technique and visualized under ultraviolet (UV) illumination of ethidium bromide stained agarose gels. Polymorphism results from changes in either the sequence of the primer binding site (e.g., point mutation) which prevent stable association with the primer or from changes which alter the size or prevent amplification of target DNA (e.g., insertions, deletions, inversions).

MODIFICATIONS OF RAPD AND ASSAY IMPROVEMENTS

1. DNA amplification fingerprinting (DAF)

A modification of the RAPD assay, named DNA amplification fingerprinting (DAF), has been described by CAETANO-ANNOLES *et al.*, 1991. The difference from the other procedures is that the PCR products are separated on polyacrylamide urea gels and visualized by silver stain. In the DAF procedure, primers as short as five nucleotides, produce complex banding patterns ideally suited for genome fingerprinting applications. Depending on the primer and template combination used, from ten to one hundred DNA fragments can be amplified.

2. Denaturing gradient gel-electrophoresis (DGGE)

Denaturing polyacrylamide gradient gel-electrophoresis (DGGE) has been used to resolve DNA sequence differences among fragments of similar or identical size (FISCHER and LERMAN, 1983, MYERS *et al.*, 1987). Using the DGGE procedure, single base differences result in altered migration of DNA fragments and thus produce polymorphic DNA fragments. Because of this, the DGGE procedure is considered to be highly suitable for self pollinating species. DWEIKAT *et al.* (1993) have described the advantages of DGGE-RAPD over RFLP and agarose-RAPD to distinguish wheat and barley lines with just one or two primers. A high frequency of polymorphism in amplified DNA has also been reported in maize with the DGGE procedure (RIEDEL *et al.*, 1990). This procedure will also be useful for phylogenetic analysis and cultivar identification as the technique results in the production of several amplified DNA fragments per primer.

3. Directed search (amplification of low copy DNA)

In general, cereals have a high level of repetitive DNA sequences (e.g., about 70% of the DNA sequences in wheat are repetitive). The PCR favors amplification of sequences that are highly abundant and less informative. Removal of repetitive DNA sequences before PCR has been reported to produce polymorphic and reproducible DNA fragments (EASTWOOD *et al.*, 1994). Hydroxylapatite column chromatography is used to enrich low copy DNA sequences and to remove the repetitive DNA sequences (CLARKE *et al.*, 1992). Careful preparation of the hydroxylapatite columns is required to obtain low copy DNA that is suitable for PCR. Once the procedure is standardized, it could be useful to screen markers linked to disease resistance and other traits. The polymorphic DNA fragments could also be used directly as RFLP probes.

4. Temperature sweep gel-electrophoresis (TSGE)

Temperature sweep gel-electrophoresis (TSGE) of RAPD products, has been reported to detect more polymorphic DNA than the agarose gel and DGGE methods (PENNER and BEZTE, 1994). In this procedure, denaturing polyacrylamide gel is used to run the PCR products, and during electrophoresis the separating gel is exposed to temperature gradients from 25 to 45°C over a 4 h period. If the procedure is well optimized it could be very useful for phylogenetic analysis, cultivar identification, etc.

5. Sequence characterized amplified regions (SCARs)

The PCR protocols vary among laboratories, and RAPD markers may not work in all situations. To circumvent this problem, PARAN and MICHELMORE (1993) developed a dependable PCR-based technique called sequence characterized amplified regions (SCARs). In this procedure, the polymorphic DNA fragment is cloned and sequenced. Forward and reverse primers are designed based on the sequence information for stable amplification of DNA. Polymorphism may not be achieved if there is a point mutation, small deletion or addition of a DNA fragment in the PCR product amplified by SCAR primers. In this case, the PCR amplified DNA products have to be restricted with several enzymes in order to generate polymorphism.

TABLE 1 - *Comparison of the most commonly used molecular markers.*

Features	RFLP	RAPD	Microsatellites	AFLP
Distribution	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous
Inheritance	Codominant	Mostly dominant	Codominant	Dominant
Polymorphism	Medium	High	High	Very high
Detection of alleles	Yes	No	Yes	No
No. loci detected	1-3	1-10	1-5	20-100
Sample throughput	Low-medium	High	high	Very high
Part of genome surveyed	Low copy coding regions	Whole genome	Whole genome	Whole genome
Technical difficulty	Intermediate	Simple	Simple	Intermediate
Reproducibility	High	Intermediate	High	High
Amount of DNA required	2-30 µg	1-100 ng	50-100 ng	100 ng
Radioisotope	Usu. yes	No	No	Yes
Types of probes	Low copy genomic DNA or cDNA clones	Usu. 9-10-mer oligonucleotides	Specific repeat DNA sequence	Specific DNA sequence
Recurring cost	High	High	Intermediate-High	High
Time factor	Slow (labor intensive)	Fast	Fast	Intermediate
Reliability	High	Intermediate	High	High

COMPARISON OF RAPD MARKERS WITH OTHER DNA BASED GENETIC MARKERS

A comparison of the most commonly used molecular markers is presented in Table 1. Each molecular marker has its advantages and disadvantages. Compared with RFLP based genetic markers, RAPD markers offer a number of advantages in a plant breeding program. The RAPD procedure is easy to work with, does not require radioactivity, requires the least amount of DNA, and does not need DNA sequence information. Simulating a maize genotyping experiment using RFLP (chemiluminescent RFLP and radioactivity based RFLP) and RAPD markers, RAGOT and HOISINGTON (1993) could not find any absolute advantage of one method over the other. However, RAPD markers were generally found to be more cost and time effective for studies involving small sample sizes, while RFLP markers were found to be more useful for large sample sizes. RFLP mapping in wheat has been hindered by a low level of inter-varietal polymorphisms (GALE *et al.*, 1990). However, modifications of the RAPD procedure such as DGGE should help to generate polymorphisms at the inter-varietal level in cereals.

Eukaryotic genomes are interspersed with tandem repeats of DNA, called microsatellites (simple sequence repeats, SSR). The microsatellite primers consist of repeats of one to five nucleotides. Microsatellites are analyzed by PCR amplification of a short genomic region containing the repeated sequence and the amplified products analyzed by gel electrophoresis. Microsatellites show a high frequency of variation in the number of repeats in different individuals or accessions. These differences are revealed on agarose or polyacrylamide gels as size variations of the amplified products. Genetic diversity has been detected in closely related bread wheat lines using microsatellite markers (PLASCHKE *et al.*, 1995). Microsatellites are codominant markers and are thus more informative than the RAPD technique. Microsatellites also detect more polymorphism than RFLP (RUSSEL, *et al.*, 1996).

Amplification fragment length polymorphism (AFLP™) is another molecular marker that has shown great potential in fingerprinting and genetic mapping (THOMAS *et al.*, 1995, VOS *et al.*, 1995, ZABEAU *et al.*, 1993). AFLP™ is a PCR-based technique for detecting DNA polymorphism in which small genomic restriction fragments are selectively amplified. The technique involves digestion of genomic DNA with a pair of rare and frequent cutter restriction enzymes. The restriction fragments are ligated to two different adapters to the respective ends and these subsets are amplified. PCR primers are designed in such a way that 3' nucleotides of the primers extend to the unique sequences of each restriction fragment. These nucleotides serve as selective bases to amplify only those restriction fragments with complementary sequences using radioactive labeling of one primer. Up to 100 - 150 single stranded amplification products can be detected on denaturing polyacrylamide gels. The AFLP technique has been used to identify markers linked to disease resistance (THOMAS *et al.*, 1995) and to fingerprint

TABLE 2 - *Use of RAPD to identify markers linked to disease and pest resistance and other qualities in cereals.*

Crop	Character (s)	References
Wheat	Cadmium uptake - durum	PENNER *et al.*, 1995
	Common bunt	DEMEKE *et al.*, 1996
	Leaf rust (*Lr9*)	SCHACHERMAYR *et al.*, 1994
	Leaf rust (*Lr24*)	SCHACHERMAYR *et al.*, 1995
	Leaf rust (*Lr24*)	DEDRYVER *et al.*, 1996
	Powdery mildew	HARTL *et al.*, 1995
	Powdery mildew (*Pm21*)	QI *et al.*, 1996
	Hessian fly	DWEIKAT *et al.*, 1994
	Streak mosaic virus	TALBERT *et al.*, 1996
	Cyst nematode	EASTWOOD *et al.*, 1994
Barley	"gs4 & o" traits with root rot (associations)	KUTCHER and BAILEY, 1993
	Leaf rust (*Puccinia hordei*)	POULSEN *et al.*, 1995
	Mild mosaic virus and yellow mosaic virus	ORDON *et al.*, 1994
	Rhynchosporium secalis	BARUA *et al.*, 1993a
	Root rot and spot blotch	KUTCHER *et al.*, 1996
	Milling energy requirements	CHALMERS *et al.*, 1993
	stem rust resistance (Rpg1)	HORVATH, *et al.*, 1995
Rice	Bacterial blight (*Xa 21*)	RONALD *et al.*, 1992
	Bacterial blight (*xa 13*)	ZHANG *et al.*, 1996
	Blast (*Pi-10*)	NAQVI and CHATTOO, 1996
	Gall midge (*Gm2*)	NAIR *et al.*, 1995
	Gall midge (*Gm4t*)	NAIR *et al.*, 1996
Oat	Crown rust (*Pc68*)	PENNER *et al.*, 1993c
	Day length insensitivity	WIGHT *et al.*, 1994
	Stem rust gene (*Pg3*)	PENNER *et al.*, 1993b
Sorghum	Head smut	OH *et al.*, 1994
	Downy mildew	GOWDA *et al.*, 1995

DNA (VOS *et al.*, 1995). With barley genotypes, the highest diversity index was obtained for AFLP as compared to RFLP, SSR and RAPD (RUSSEL *et al.*, 1996). RFLP gave the lowest diversity index, while microsatellites and RAPD gave intermediate levels of diversity. The disadvantage of the AFLP technique is the requirement of radioactivity and the inability to distinguish homozygotes from heterozygotes. The latter is a limiting factor for genetic mapping applications.

USE OF RAPD IN MARKER IDENTIFICATION AND GENETIC MAPPING

RAPD markers have been used to identify markers linked to disease and pest resistance, and other desirable traits in cereals (Table 2). In most cases nearly-isogenic lines and bulked segregant samples are used to screen several primers in order to identify one or more primers that show polymorphism between the two DNA samples (MARTIN *et al.*, 1991, MICHELMORE *et al.*, 1993, TANKSLEY *et al.*, 1995). For the production of near-isogenic lines, a recurrent parent and a donor parent are crossed. Selected offspring are then backcrossed to the recurrent parent for five or six cycles so that the recurrent parent and the new homozygous line will have a similar genetic composition, with the exception of the introduced trait from the donor parent. DNA from the two near-isogenic lines is used to screen random primers to generate polymorphism. In bulked segregant analysis, a single cross is made between two lines, and DNA from F_2 plant samples is used for analysis. In the case of disease resistance, DNA from several F_2 resistant plants is bulked, and similarly DNA from several F_2 susceptible plants is bulked. The DNA from the two bulks are used to screen primers. Polymorphism in DNA fragments will be observed between the two lines if several primers are screened. The number of primers to be screened depends on the plant species used and the frequency of polymorphism achieved. For cereals, hundreds of primers have to be screened to get one or more polymorphic fragments. SCAR primers can be developed by cloning and sequencing the polymorphic DNA fragment for stable PCR amplification as described above. Once a primer that shows polymorphism is found, it has to be checked against other genotypes carrying or missing the trait of interest. In addition, linkage and recombination values have to be calculated using segregation values obtained from F_2 plant samples. This will indicate the closeness of the identified marker to the gene of interest. Very tight linkage facilitates the identification of the gene of interest.

RAPD markers have also been used in genetic mapping of cereals (Table 3). Identification of molecular markers linked to traits of interest is facilitated by the availability of genetic maps. RAPD markers are dominant, showing the presence or absence of a single allele, and this has to be taken into account for mapping studies. Markers residing on a single chromatid as can be found in a backcross or recombinant inbred population, in haploid or gametophytic tissue, or an F_2 population where RAPD markers are amplified from a single parent are mapped (RAFALSKI and TINGEY, 1993). Some of the SCAR primers developed from RAPD have turned out to be codominant (PARAN and MICHELMORE, 1993) and thus more informative. The combined use of different molecular markers will be beneficial for mapping studies in cereals.

TABLE 3 - *Use of RAPD markers for genetic mapping of cereals.*

Crop	Character (s)	References
Barley	Mapping of genes for height, heading and growth habit	Barua *et al.*, 1993b
	Molecular, isozyme, and morphological map	Kleinhofs *et al.*, 1993
	Distribution of RAPD markers on a linkage map	Giese *et al.*, 1994
	Combined mapping of AFLP and RFLP markers	Becker *et al.*, 1995
Wheat and barley	Comparison of physical and linkage maps for group 7 chromosomes	Hohmann *et al.*, 1995b
Rice	RFLP and RAPD mapping of rice *Gm2*	Mohan *et al.*, 1994
	Tagging and mapping	Wang *et al.*, 1995
Cereals	Identification and mapping of polymorphisms	Weining and Langridge, 1991

USE OF RAPD IN CULTIVAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS

RAPD markers have been used extensively for cultivar identification and phylogenetic analysis (Demeke and Adams, 1994, Tingey and Del Tufo, 1993, Williams *et al.*, 1993). The applicability of RAPD markers in quantifying genetic diversity, in germplasm screening and in pedigree assessment in cereals is summarized in Table 4.

Germplasm characterization is often based on morphological and agronomic traits, which are usually affected by environment. RAPD analysis is a simple, fast, and relatively inexpensive tool to characterize germplasm collections and to estimate their level of diversity. One of the problems in germplasm repositories is the accumulation of duplicate samples. Sorting out duplicate samples will save time, money and storage space. RAPD analysis has been used successfully to identify duplicate accessions in rice (Fukuoka *et al.*, 1992, Virk *et al.*, 1995). RAPD markers can also be used for cultivar identification. It is difficult to distinguish among closely related cultivars of cereals using morphological analysis, but molecular markers such as RAPD can accurately distinguish closely related cultivars. Inter- and intra-specific diversity has been reported in wheat, barley, and wheat-barley addition lines using conserved, semi-random, and random primers (Weining and Langridge, 1991).

RAPD markers have potential to analyze genetic relationships from sub-generic to clonal levels. RAPD markers were found to be useful in classification of *Juniperus* species at the subgeneric level (Adams and Demeke, 1993). Potato clonal variants which were inseparable by morphological analysis were distinguished by RAPD analysis (Demeke *et al.*, 1993). In a similar study, the sequence homology of co-migrating RAPD markers was similar within a genus, across a species, and among populations of a species in *Brassica* (Lanner *et al.*, 1996). The RAPD markers were found to be reliable from genus to population level. A RAPD fragment used as a probe seldom hybridized to unexpected DNA fragments in different samples. Therefore, amplified DNA fragments with similar molecular weight generally exhibit genetic similarity.

TABLE 4 - *Use of RAPDs in phylogenetic analysis, cultivar identification, and germplasm screening in cereals.*

Crop	Character (s)	References
Wheat	Genetic diversity	Vierling and Nguyen, 1992
	Intervarietal relationships	Joshi and Nguyen, 1993a; Joshi and Nguyen, 1993b
	Genetic diversity	Dhaliwal *et al.*, 1993
	Segregation in diploid wheat	Echt *et al.*, 1992
Rice	Germplasm screening	Virk *et al.*, 1996
	Identification & parentage determination	Wang *et al.*, 1994
	Identification (accessions)	Fukuoka *et al.*, 1992
	Sorting duplicate accessions	Virk *et al.*, 1995
Barley	Analysis of species	Gonzalez and Ferrer, 1993
	Pedigree relationship	Tinker *et al.*, 1993
	Phylogeny	Marillia and Scoles, 1996
Oat	Genetic relationships	Heun *et al.*, 1994
Triticeae	Genome and species specific	Wei and Wang, 1995
Cereal	Pedigree assessment	Dweikat *et al.*, 1993
Sorghum	Genotype characterization	Pammi *et al.*, 1994
	Polymorphism	Tao *et al.*, 1993
Maize	Polymorphism in diallel crosses and inheritance	Heun and Helentjaris, 1993

However, QUIROS *et al.* (1995) have cast doubt about homology of amplified DNA fragments of same size in a *Brassica* study based on sequence analysis. Detailed DNA sequence analysis of RAPD fragments of similar size is required to confirm this. However, studies to date generally support the morphological, chemical, isozyme and RFLP data previously established, thus confirming the reliability of RAPD markers for phylogenetic analysis. Some primers amplify DNA that is highly conserved and will help to generate DNA polymorphism at high levels of classifications, whereas other primers amplify DNA that is highly variable and useful for classification at the varietal and clonal levels (ADAMS and DEMEKE, 1993).

The number of primers needed to assess genetic relationships or for cultivar identification varies according to species and type of primer used. In our *Brassica* study, the use of 11 primers gave the expected relationships, whereas the use of 17 primers did not show an improvement (DEMEKE *et al.*, 1992). JOSHI and NGUYEN (1993b) used 40 primers to assess genetic relationships among wild and cultivated tetraploid wheats. Since the average of data generated by all primers is statistically analyzed, the more primers used, the better the assessment will be.

OTHER USES OF RAPD MARKERS

1. Detection of gene introgression

To transfer disease resistance genes from wild relatives, DNA markers can speed up the selection of resistant offsprings that contain the lowest amounts of the donor genome at every generation (TANKSLEY *et al.*, 1989). RAPD markers have been used to assess gene introgression from other species to wheat. Examples include 1RS rye chromosome segment (IQBAL and RAYBURN, 1995), rye chromatin (FRANCIS *et al.*, 1995), *Thinopyrum bessarabicum* chromosomes (KING, *et al.*, 1993; WILLIAM *et al.*, 1993), and deletions induced by an *Aegilops cylindrica* chromosome in common wheat (HOHMANN *et al.*, 1995a). In barley, transfer of a dominant gene for powdery mildew resistance has been assessed by RAPD markers (XU and KASHA, 1992).

2. Detection of quantitative trait loci (QTL)

Most agronomically important traits are multigenic. Molecular markers are being used for QTL analysis (TANKSLEY, 1993). In maize, molecular markers have been used to map and to engineer quantitative traits (STUBER, 1995). QTLs for resistance to *Gibberella zeae* have been mapped using RAPD markers (PE *et al.*, 1993). A linkage map has been constructed and QTL analysis performed using RAPD markers for a genetically narrow cross in cucumber (SERQUEN *et al.*, 1996). To date, limited QTL analysis of cereals has been conducted using RAPD markers.

REPRODUCIBILITY OF RAPD MARKERS

The disadvantage of RAPD markers is that they behave as dominant markers and have a problem of reproducibility (KLEINHOFS, 1992, DEVOS and GALE, 1992). Failure of RAPD bands to follow expected inheritance patterns as well as an excess of nonparental RAPD bands in offspring of known pedigree has also been reported (HEUN and HELENTJARIS, 1996, POOLER and SCROZA, 1993, RIEDY *et al.*, 1992). HALWARD *et al.* (1992) reported the lack of polymorphism in 25 germplasm lines of cultivated peanut using 10 RAPD primers, and the authors assumed this to be due to the narrow genetic base of cultivated peanut. HALLDEN, *et al.* (1996) have studied genetic variability of RAPD markers using doubled haploids and heterozygous *Brassica napus* DNA samples. The heterozygous DNA samples were formed by mixing equal amounts of DNA from different plant samples. They focused on lack of reproducibility of an expected DNA fragment in heterozygous samples. The expected DNA fragments disappeared in 14% of the heterozygous DNA samples. They suggested competition among RAPD fragments to be a major source of genotyping error in RAPD analysis. On the other hand, heteroduplex formation (occurrence of DNA fragments in progenies that are not in parents) was reported to be a rare occurrence.

To obtain reproducible results with RAPD, a range of experimental parameters such as the amount of template DNA, pH of the buffer, level of magnesium, type and concentration of primers, concentration of *Taq* polymerase, number of reaction cycles, annealing temperature, etc., have to be optimized. A specific combination of plant genotype and primer can result in a different banding pattern if the experimental conditions are altered (MUNTHALI *et al.*, 1992). The level of reproducibility of random amplified polymorphic DNA analysis among different laboratories has been reported (PENNER *et al.*, 1993a). The failure of RAPD reproducibility among laboratories was reported to be due to variation in the overall temperature profiles (especially the annealing temperature) inside the tube. Furthermore, the use of the same set of conditions, optimized specifically for a cultivar or species, could produce the same pattern of amplification

(MUNTHALI *et al.*, 1992). However, it is not uncommon to see some DNA fragments that are not reproducible for unknown reasons. For phylogenetic analysis, the PCR amplification has to be repeated, and only those DNA fragments that are reproducible should be used for analysis. It is worthwhile to prescreen primers on two to three cultivars/lines to select the ones that produce many, reproducible fragments, and use the selected primers for phylogenetic analysis. In addition, developing SCAR primers as explained above will assure reproducibility of PCR product for marker identification. However, not all primers produce detectable DNA fragments. FRITSCH *et al.* (1993) screened 480 UBC and Operon primers and listed oligonucleotide primers that consistently amplified DNA in three plant genera. These data can be used as a reference when using oliogonucleotide primers for RAPD analysis for the first time.

SUMMARY AND CONCLUSIONS

The RAPD procedure is simple, rapid, requires a small amount of DNA, and the resulting amplified DNA fragments can be easily visualized. In cereals, the RAPD procedure has been widely used for identification of markers linked to disease and pest resistance. This has been especially beneficial in cases where phenotypic analyses do not give a clear indication of pest resistance. The DNA analysis could be done on half-seeds to select suitable progeny before planting. RAPD analysis will also facilitate mapping of simple and quantitative traits. Identifying molecular markers linked to quantitative traits such as yield and drought resistance will be very useful for cereal improvement.

One of the most important uses of RAPD markers will be in crop germplasm characterization and evaluation. Thousands of accessions are stored in different germplasm centers around the world. Some of the germplasm accessions are duplicates and eliminating these could save time, space and money, thus increasing storage efficiency. Genetic variability is fundamental to crop improvement, and RAPD analysis could be especially useful in quantifying the diversity of accessions. This could become a reality for large numbers of accessions as the DNA extraction and RAPD analysis procedures become automated. Computer technologies will have to be developed in tandem with the DNA analysis technologies to analyze the generated data.

RAPD markers have also proved valuable in phylogenetic analysis and cultivar identification. They have been used to analyze relationships ranging from subgeneric to cultivar level. Since phylogenetic analysis is based on the use of several primers the cumulative data will be more informative and dependable. Even though cereals exhibit low levels of polymorphism, variations in RAPD procedure such as DGGE and TSGE will help to detect diversity in DNA patterns.

Many of the important agronomic traits such as disease resistance are transferred from wild relatives to well adapted crop cultivars. RAPD analysis can be used to assess the introgression of these gene (s). RAPD markers have also the potential to detect somaclonal variation in tissue culture derived plants and will find application in assessing genetic changes in transgenes. This will be important as the work on plant transformation expands.

The concern about reproducibility of RAPD markers can be overcome by optimizing the parameters used, by developing SCAR primers, by selecting suitable oligonucleotide primers, and by using several primers and scoring only the reproducible DNA fragments in the case of phylogenetic analysis.

As outlined above, RAPD markers can play an important role in cereal improvement programs. However, the combined use of different molecular markers such as RFLP, RAPD, AFLP, and microsatellites will likely be more useful than using only one type of marker. Some of the pitfalls shown by one marker could be overcome by using the other markers.

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