Use of Random Amplified Polymorphic DNA Analysis for Economically Important Food Crops

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Abstract

The objective of this review is to summarize numerous studies on the use of the random amplified polymorphic DNA (RAPD) technique on rice, corn, wheat, sorghum, barley, rye, and oats to examine its feasibility and validity for assessment of genetic variation, population genetics, mapping, linkage and marker assisted selection, phylogenetic analysis, and the detection of somaclonal variation. Also we discuss the advantages and limitations of RAPD. Molecular markers have entered the scene of genetic improvement in different fields of agricultural research. The simplicity of the RAPD technique made it ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics.

Key words: random amplified polymorphic DNA; food crops; poaceae.


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The grass family, Gramineae (Poaceae), is a diverse, widely distributed group of plants. Its wide distribution and large number of economically important food crops (corn, wheat, rice, and sorghum) make the grass family one of the most globally important plant families. Rangelands, principally dominated by grasses, represent nearly one half of the earth’s surface. As rangeland species, they provide tremendous grazing resources, shaped by evolution to withstand grazing, fire, disease, and periodic droughts. They represent the best use for lands that are typically unsuited for tillage agriculture, since most rangelands are too arid, rocky, or rough to be cultivated. Domesticated grasses on the other hand provide the bulk of the basic food resources, and world politics are influenced by production and distribution of corn, wheat, or rice (http://spuds.agron.ksu.edu/Grassint.htm). Given their ecological dominance, it is not surprising that grasses play a central role in the human endeavor. Grasses are a major food source for humans (Gaut 2002). It is a very important family for people and herbivorous animals. Food for people and livestock is rice (Oryza sativa), corn or maize (Zea mays), wheat (Triticum aestivum), rye (Secale cereale), sorghum (Sorghum bicolor), barley (Hordeum vulgare), teosinte (Z. mexicana), oats (Avena sativa); and many other species; also bamboo shoots used in Chinese and Cantonese foods. Three grain crops, wheat, rice and maize are predominant food sources, but the grasses also include several additional and perhaps unappreciated crops.

Much of our most basic understanding of genetics has its roots in plant genetics and crop breeding. The study of plant has led to important insights into highly conserved biological process and a wealth of knowledge about development. Agriculture is now well positioned to take its share of benefits from genomics (Aljanabi 2001). In the 20 years since that discovery, many different types of DNA-based genetic markers have been used for the construction of genetic maps, for the analysis of genetic diversity, trait mapping, as well as for applied diagnostic purposes. A bewildering array of acronyms, such as restriction fragment
length polymorphism (RFLP); simple sequence repeats (SSR); amplified fragments length polymorphism ( AFLP) and random amplified polymorphic DNA (RAPD) and many others describe these methodologies (Reiter et al. 2001; Antoni 2002). Use of DNA based genetic markers has forever changed the practice of genetics. Molecular techniques proved useful in a number of ways to improve the conservation and management of plant genetic resources. Markers are of interest to plant breeders as a source of genetic information on crops and for use in indirect selection of traits to which the markers are linked. Genetic diversity provides farmers and plant breeders with options to develop, through selection and breeding, new and more productive crops, that are resistant to virulent pests and diseases and adapted to changing environments (Ranade et al. 2001; Rao 2004).

The potential of molecular systems for producing robust and reliable cultivars descriptions, suitable for database use and largely unaffected by environment, has become increasingly attractive. Their potential for application in cultivars registration is therefore under careful and active consideration within the international union for the protection of new varieties of plants (Camlin 2003).

Over the last several years, there has thus been marked increase in the application of molecular markers in the breeding programs of various crop plants. Molecular markers not only facilitate the development of new varieties by reducing the time required for the detection of specific traits in progeny plants, but also fasten the identification of resistance genes and their corresponding molecular markers, thus accelerating efficient breeding of resistance traits into wheat cultivars by marker assisted selection (Debas and Paramjit 2001).

The RAPD method appeared a decade ago as an alternative in genetic relationship studies. The technique generates polymorphic band patterns, produced by polymerase chain reaction (PCR) using arbitrary DNA sequence primers (Xena de Enrech 2000). RAPD markers are polymorphic DNA separated by gel electrophoresis after PCR using short random oligonucleotide primers. It has been particularly used for genetic and molecular studies, as it is a simple and rapid method for determining genetic diversity and similarity in various organisms. RAPD also has the advantage that no prior knowledge of the genome under research is necessary (Fischer et al. 2000; Kleinbunga et al. 2000). It has been concluded that although AFLP analysis is superior in terms of efficiency, RAPDs may still be used as reliable markers in small low-tech laboratories (Kjolner et al. 2004). RAPD markers can be used in various types of plant genetic studies: (i) genetic diversity, variations and relationships; (ii) detection, identification and/or localization of molecular markers linked to a specific trait; (iii) hybrids detection; (iv) genetic maps; and (v) somaclonal variation analysis. During the last few years, we used RAPD for a wide range of applications in different organisms (Ali and Ahmed 2001; Ali et al. 2002; Ali 2003a, 2003b; Ali et al. 2003; Soliman et al. 2003; Ahmed et al. 2004; Ali et al. 2004a, 2004b; El-Manhaly et al. 2004; Salem et al. 2005; El-Zaeeem et al. 2006).

This review attempts to summarize different efforts in direction of using RAPD markers in economically important food crops of the grass family (Poaceae) such as wheat, rice, corn, sorghum and other species. This review summarizes numerous studies on the use of the RAPDs technique on rice, corn, wheat, sorghum, barley, rye, and oats to examine its feasibility and validity for: (i) assessment of genetic variation; (ii) population genetics; (iii) mapping, linkage and marker assisted selection; (iv) phylogenetic analysis; and (v) detection of somaclonal variation. We also discuss detection of hybrids for some of the crops. The advantages and major limitations of RAPDs will be discussed here.

Rice

To identify the genetic basis of the weedy rice distributed in various countries of the world, 152 strains of weedy rice collected from Bangladesh, Brazil, Bhutan, China, India, Japan, Korea, Nepal, Thailand and the USA were tested for variations in six morpho-physiological characteristics and in 14 isozyme loci (Suh et al.1997). In this study, four groups designated as I, II, III and IV were identified. In group I, classified as indica, several strains showed japonica-specific RAPD markers, while some others had japonica cytoplasm with indica-specific RAPD markers in a heterozygous state at several loci. One weedy strain belonging to group II showed a wild rice-specific allele at the Est-10 locus. However, in groups III and IV, no variation was found either for the markers on Est-10 or for the RAPD loci tested. Genetic variation within and between five populations of O. granulata from two regions of China was investigated using RAPD and inter simple sequence repeats (ISSR) markers (Qian et al. 2001). Twenty RAPD primers used in this study amplified 199 reproducible bands with 61 (30.65%) polymorphic; and 12 ISSR primers amplified 113 bands with 52 (46.02%) polymorphic. Both RAPD and ISSR analyses revealed a low level of genetic diversity in wild populations of O. granulata. Genetic diversity and population genetic structure of natural O. rufipogon populations in China were studied based on ten microsatellite loci (Zhou et al. 2003). For 12 populations collected from four regions, a moderate to high level of genetic diversity was observed at population levels. These authors stated that the results are in good agreement with previous allozyme and RAPD studies. In India genetic variation among the seventeen basmati rice accessions collected from the farmers’ fields located at different places was analyzed using ten arbitrary ten-mer oligonucleotide primers by RAPD method (Verma et al. 1999). The number of polymorphic/monomorphic bands among the pairwise combinations of the accessions and the total number of bands were determined to categorize all of the accessions in different groups of similarity and dissimilarity at the genetic
level. The Oceanian and African forms of *O. perennis* were substantially different from all other groups. Thomas et al. (2001) assessed the nature and distribution of genetic variation among 11 populations of *O. malampuzhaensis* using RAPD markers. The results revealed low genetic variation in *O. malampuzhaensis*. Cluster analysis of pairwise genetic distances of populations revealed three distinct clusters. Genetic variation within and between eight natural populations of *O. rufipogon* from China and Brazil was investigated at the DNA level by analysis of RAPD fragments (Ge et al.1999). Out of 60 random primers, which were initially screened against DNA from four individuals, 20 generated highly reproducible RAPD fragments, which were then used for further population analysis. With these primers, 95 discernible DNA fragments were produced and 78 (82.1%) were polymorphic, which indicated that high levels of genetic variation existed in these natural populations. In addition, the Chinese populations showed greater polymorphism than those from Brazil at both the population and regional levels.

To study the usefulness of the RAPD technique as a powerful tool for distinguishing different cytoplasms by other techniques appear to be similar. RAPD profiles were generated using mitochondrial DNA (mtDNA) isolated from two cytoplasmic male-sterile lines, two restorer lines and four maintainer lines of rice (Sane et al. 1997). The different lines of rice were distinguishable on the basis of RAPD profiles. DNA polymorphism between a cytoplasmic male-sterile rice line II-32A, the male-fertile maintainer counterpart II-32B, a fertile revertant (T24), as well as two commercial *indica* restorers, was analyzed with RAPD (Shen et al. 1998). A very low degree of polymorphism was found between the revertant T24 and II-32A compared with that of *indica* rice varieties.

Bulked segregant analysis was carried out on progeny of a cross between the Chinese rice cultivar Duokang and the gall midge-susceptible cultivar Feng Yin Zhan using the RAPD method (Katiyar et al. 2001). One RAPD marker amplified a locus linked to Gm-6 (t). This locus was subsequently mapped to rice chromosome 4 in a region flanked by two cloned RFLP markers. Recently, a RIL (recombinant inbred line) population from the cross between AnnongS-1 and Nanjing 11 was developed and used for the fine mapping of the *tms5* gene using molecular marker techniques combined with bulked segregant analysis (BSA) were used (Wang et al. 2003). As a result, two AFLP markers, one RAPD marker, one STS marker, one CAPs marker and four SSR markers were found linked to *tms5* gene. Sheath blight is one of the most severe rice diseases worldwide. An *F₂* population from a cross between transgenic rice cultivars carrying a resistant gene to sheath blight and a highly susceptible one is used to identify molecular markers linked with the resistant trait. Five molecular markers, including three RFLP markers converted from RAPD and AFLP markers, and two SSR markers were identified to link with the resistant gene (Che et al. 2003). To better understand the molecular basis underlying cell death and disease resistance in rice, a map-based cloning strategy has been used to isolate Spl11. Five Spl11-linked RAPD markers were developed and four of them were mapped to rice chromosome 12 (Zeng et al. 2002). Three molecular markers tightly linked to Spl11 were identified in this study. With respect to the *Pi9(t)* and *Pi2(t)* genes were introgressed from different resistance donors. To further test their resistance spectrum, 43 blast isolates collected from 13 countries were used to inoculate the *Pi2(t)* and *Pi9(t)* plants (Liu et al. 2002). Three RAPD markers tightly linked to *Pi9(t)* were identified using the bulk segregant analysis technique. The *Pi2(t)* gene was tightly linked to all of the *Pi9(t)* markers in 450 *F₂* plants. In a mutation-breeding program initiated in 1999 at the Instituto Agronomico de Campinas, SP, Brazil, a rice line, IAC103, was selected for mutational studies with gamma radiation and ethyl methyl sulfonate mutagenesis, with the aim of developing a herbicide-resistant crop (Sandhu et al. 2002). Resistant and susceptible lines produced variation in the RAPD patterns.

**Maize**

Yuan et al. (2000) used RFLPs, SSRs, AFLPs and RAPDs to detect the genetic diversity among 15 maize inbred lines. A total of 56 probe enzyme combinations, 66 SSR primers, 20 RAPD primers and nine AFLP primer combinations were identified with polymorphism among the entries, which produced 167, 201, 180 and 87 alleles, respectively. Furthermore, a comparison of genetic similarity matrices revealed that the estimates of correlation coefficients based on these markers were significantly correlated, but the correlation of RAPD maker data with other markers was lower. The inheritance of shoot regeneration through shoot-tip meristem culture derived from maize seedling was evaluated, and the markers (RAPD and SSR) associated with this regeneration character were identified both in a group of North American maize inbreds and a cross population (Li et al. 2004). Five RAPD markers were identified to be relevant to percentage of regeneration in a maize shoot-tip culture system.

Guo et al. (2001a) studied the genetic variation in the maize population GT-MAS: gk, and correlated the RAPD marker association with the resistance to *A. flavus* and aflatoxin production. Of 40 RAPD primers, only 15 gave sufficient numbers of reproducible and readily scored polymorphic bands suggesting that this population was highly homogeneous. The results showed that this population is heterogeneous and individuals are different in resistance to *A. flavus* and aflatoxin production.

Intergeneric somatic hybridization was carried out between albino maize (*Z. mays*) protoplasts and mesophyll protoplasts of wheat (*T. aestivum*) by polyethylene glycol (PEG) treatments (Szarka et al. 2002). Using total DNA from hybrid plants, three RAPD primer combinations produced bands resembling the wheat profile.
**Wheat**

Among the food crops, wheat is one of the most abundant sources of energy and proteins for the world population. Ninety-five percent of wheat grown today is of the hexaploid type, used for the preparation of bread and other baked products. Nearly all of the remaining 5% is durum (tetraploid) wheat, which is mainly used for making pasta, macaroni and biscuits. Wheat is characterized by a large genome size (approximately 17 000 Mb), thus making the improvement process by any method genetically challenging (Debasis and Paramjit 2001). In wheat, the RAPD is mainly used to mark and locate target genes; identify and mark fragments of (alien) chromosomes; determine the genetic relationships between *Triticum* sp. and related species; and analyze the genetic diversity of wheat varieties (Yu et al. 2004).

Several studies have been carried out to determine genetic variability and/or similarity using RAPD molecular markers. Gerashchenkov et al. (2000) determined the level of genetic polymorphism in the collection of spring common wheat genotypes based on RAPD-PCR analysis. Intraspecific polymorphism of the original wheat forms was 20%. These authors added that the technology of direct *in vitro* androgenesis does not lead to genome rearrangements and may be used for rapid production of pure lines of such a complex allopolyploid as spring common wheat. To determine RAPD-based genetic similarity between accessions and to derive associations between *Fusarium* head blight (FHB) and RAPD markers, RAPD markers have been used to characterize the genetic diversity among 35 spring wheat cultivars and lines with different levels of *Fusarium* resistance (Sun et al. 2003). Genetic similarity calculated from the RAPD data ranged from 0.64 to 0.98. These results suggested that a collection of unrelated genotypes can be used to identify markers linked to an agronomically important quantitative trait, hence these markers will be useful for marker assisted breeding and can be used as candidate markers for further gene mapping and cloning.

Recently, Fahima et al. (2002) examined the diversity in 20 microsatellite loci of wild emmer wheat, *T. dicoccoides*. These authors stated that the microsatellite results are non-random and in agreement with the previously obtained allozyme and RAPD patterns, although the genetic-diversity values obtained with microsatellites are much higher. They suggested that RAPD markers are useful for the estimation of genetic diversity in wild material of *T. dicoccoides* and the identification of suitable parents for the development of mapping populations for the tagging of agronomically important traits derived from *T. dicoccoides*.

Sometimes crop germplasm collections contain a considerable percentage of misclassified accessions, which may affect the use of germplasm for agricultural crop improvement. Numerous studies were carried out to detect, identify and/or localize molecular markers linked to quantitative trait loci (QTL) using RAPDs. In recent years, RAPD and other PCR based markers like sequence characterized amplified regions (SCAR), sequence tagged sites (STS) and differential display reverse transcriptase PCR (DDRT-PCR) are increasingly being used for the identification of desirable traits in wheat and related genera. These markers have been used in particular for disease resistance against viral and fungal pathogens and also for insect and nematode pests and have the potential of pyramiding of resistance genes for effective breeding programs. PCR based markers have been extensively characterized for genes of resistance against Hessian fly, and Russian wheat aphid, *D. noxia* (Venter and Botha 2000).

Wheat head blight caused mainly by *Fusarium graminearum*, is an important wheat disease, causing yield and quality losses. The breeding of resistant varieties is the key measure to control this disease, but the conventional breeding method is of low efficiency. The marker-assisted selection can significantly improve the breeding efficiency (Yu et al. 2004). They used 520 random primers to analyze DNA from resistant and susceptible parents. Four markers linked to FHB resistance were obtained. RAPD analysis showed that the inserts of the recombinant plasmids were DNA of S1384–640. The sequencing result showed that the cloned fragment was 648 bp. The RAPD analysis was made in Pm2 near-isogenic lines (NILs) with 265 random primers (Liu et al. 2000). Seventeen out of the 256 tested primers amplified the polymorphic DNA in the NILs. Five of them showed the same discriminating results in more than four replications. Hessian fly is one of the world’s most destructive insect pests of wheat (*T. aestivum*).

To identify DNA markers closely linked to genes in wheat (*T. aestivum*) that condition resistance to Hessian fly, Dweikat et al. (2002) used the combination of near-isogenic lines (NIL) and RAPD analysis to screen up to 2 000 primers to identify DNA markers that are linked to gene H6 that confers resistance to biotype B of the insect. Six primers that show polymorphic fragments associated with resistance by H6 have been identified. Three of the six markers were successfully converted to sequence tagged site (STS) markers.

Venter and Botha (2000) identified a putative marker linked to the Dn5 resistance gene using RAPD analysis. This marker was tested in a segregating F2 population carrying the Dn5 resistance gene and proved able to differentiate between the segregating individuals. RAPD markers linked to the Yr5 gene between the near-isogenic line using 520 10-mer random primers have been identified (Zhong et al. 2002). Three polymorphic DNA fragments were found linked to the Yr5 gene.

The development of *Septoria nodorum* blotch-resistant cultivars has become a high priority objective for durum wheat breeding programs. Marker-assisted selection enables breeders to improve selection efficiency. In order to develop markers for resistance to *S. nodorum* blotch, two RAPD markers, were detected by bulked segregant analysis (Cao et al. 2001). This was the first report of DNA-based markers linked to resistance for Septoria nodorum blotch in durum wheat. Wheat
streak mosaic virus (WSMV), vectored by the wheat curl mite (Acer tulipae), is an important disease of wheat (T. aestivum) in the North American Great Plains. Resistant varieties have not been developed as field screening for virus resistance is laborious and beyond the scope of most breeding programs.

Near isogenic lines with the dwarf gene (Rht3) and their segregating population were analyzed by PCR and RFLP technology (Wan et al. 2001). In RAPD analysis, out of 310 random primers (10 bp) screened, only three primers revealed polymorphisms in NIL from 310 random primers. Furthermore, the linkage analysis indicated that Xpsr584 was linked to Rht3 with a genetic distance 8.0 cM. In an attempt to identify DNA markers associated with kernel hardness trait in wheat, 100 recombinant inbred lines (RILs) derived from a cross between a hard grain land race, NP4, and a soft grain variety, HB 208, were screened with 100 ISSR and 360 RAPD primers (Galande et al. 2001). Eighteen markers were assigned to seven linkage groups covering 223.6 cM. These results indicated that phenotypic expression of kernel hardness is controlled by many QTLs and is interdependent on various related traits.

In order to search for evidence of the introgression of wheat traits into these wild relatives, Guadagnuolo et al. (2001a) collected seeds of English and Austrian populations of bearded wheatgrass (Elymus caninus L.) and sea barley (Hordeum marinum Huds.) growing in the vicinity of wheat (T. aestivum). Seeds were sown and plants grown for subsequent analyses using morphological and genetic (isozymes, RAPD and wheat microsatellites) markers. Numerous species-specific DNA markers of wheat were amplified. Intergeneric somatic hybridization was carried out between albino maize (Z. mays) protoplasts and mesophyll protoplasts of wheat (T. aestivum) by polyethylene glycol (PEG) treatments. Using total DNA from hybrid plants, three RAPD primer combinations produced bands resembling the wheat profile (Szarka et al. 2002). Bil’Danova et al. (2002) analyzed the genomes of alloplasmic wheat lines using RAPD and random amplified microsatellite polymorphism. The RAPD patterns of two lines contained fragments present only in the patterns of the parental wheat varieties and fragments absent from the latter. Protoplasts of Avena sativa L. (A) were fused by the PEG method with protoplasts of two cultivars of wheat (Fengning et al. 2003). Regenerated clones were produced in all fusion combinations and recognized as somatic hybrids in nature by cytological, isozyme, RAPD and 5S rDNA spacer sequence analysis. Protoplasts of wheat cv. Jinan 177 were fused by PEG method with the UV irradiated protoplasts of A. littoralis – a salt-tolerant plant intertribal to wheat (Wei et al. 2001). The early-formed regenerated clones were identified as hybrids by chromosome, isozyme and RAPD analysis. To confirm the identity of added N-genome chromosomes, RFLP analyses were carried out on wheat-Aegilops uniaristata Vis. by addition and translocation lines (Iqbal et al. 2000). Chromosome-specific RAPD and microsatellite markers were identified for all of the added Ae. uniaristata chromosomes available in this set of addition lines. Guadagnuolo et al. (2001b) estimated the potential of gene flow between wheat (T. aestivum) and jointed goatgrass (A. cylindrica Host.), after mixing pollinations in experimental and natural conditions. A set of species-specific RAPD and microsatellite markers were used to detect the presence of parental markers in the progeny of the plants used in these experiments. About 25% of the wheat specific RAPD markers were found in the BC1 plants.

Since saturated molecular maps became available, it has been possible to localize genes within genomes more precisely. For mapping or tagging the genes of interest, different approaches such as RFLP, RAPD, STS or SSR analysis can be applied (Khlebstkina et al. 2002). During the last decade, various genes were mapped in wheat using molecular markers. Updated reviews are frequently summarized in the Catalogue of gene symbols for wheat and its supplements (McIntosh et al. 2000; McIntosh et al. 2001).

**Sorghum**

Sorghum (Sorghum bicolor L.) is an important crop in the semiarid tropics that also receives growing attention in genetic research. A comprehensive reference map of the sorghum genome would be an essential research tool. The extent and distribution of genetic variation in wild sorghum collected from five different geographical regions in Ethiopia were analyzed using RAPD markers for 93 individuals representing 11 populations (Ayana et al. 2000). Nine primers generated a total of 83 polymorphic bands with 8–12 bands per primer and a mean of nine bands across the 93 individuals. Recently, Uptmoo et al. (2003) evaluated 46 sorghum accessions derived from Southern Africa were evaluated on the basis of AFLPs, RAPDs and SSRs. Mean genetic similarity was estimated at 0.88 based on RAPDs, 0.85 using AFLPs and 0.31 based on SSRs. Agrama and Tuinstra (2003) applied SSR and RAPD analyses in sorghum germplasm analysis to compare suitability for quantifying genetic diversity. The RAPD primers were less polymorphic with nearly 40% of the fragments being monomorphic. Based on the results of these studies, SSR markers appeared to be particularly useful for the estimation of genetic similarity among diverse genotypes of sorghum.

Panday et al. (2002) identified molecular markers linked to the locus for disease resistance using RAPD analysis coupled with bulk segregant analysis. Two primer sequences were found linked to the locus for disease resistance.

A combined sorghum linkage map from two recombinant inbred populations was constructed using AFLP, SSR, RFLP and RAPD markers (Haussmann et al. 2002). It was in good agreement with other published sorghum linkage maps, from which it deviated by a few apparent inversions, deletions, and additional distal regions. Agrama et al. (2002) used ninety-three recombinant inbreds of Sorghum that were derived from a cross...
between two sorghum lines Greenburg [GB] Biotypes I and K (GBIK) and Redian to identify QTLs for resistance and tolerance to greenbug biotypes I and K. Seventy five RAPDs were mapped in 12 linkage groups covering 1.530 cM. The RAPD markers were associated with the expression of all resistance and tolerance traits.

**Barley**

The phylogenetic relationships of 16 barley cultivars from different countries, and all having a known pedigree, were analyzed using 353 PCR markers (125 RAPDs and 228 ISSRs) (Fernandez et al. 2002). Kochieva et al. (2001) used RAPD to analyze six species, three populations, and seven regional cultivars of barley. A unique pattern of amplified DNA products was obtained for each species of the genus Hordeum and high polymorphism of barley species was revealed. Thirty doubled haploid lines of barley derived from F1 of a cross between the six-rowed cultivar Pomo and two-rowed cultivar Maresi were examined for susceptibility to Fusarium seedling blight and head blight, measured by mycotoxin (nivalenol) content of kernels (Bocianowski et al. 2003). RAPD polymorphism was analyzed by using 53 decamer primers. Amplification products were 200 bp up to 2000 bp in size on average 5.7 per primer and the total number of APs was 284; 51.06% of which were polymorphic.

Inheritance of resistance to covered smut in the barley line Q21861 was studied using a doubled-haploid population produced by crossing Q21861 with the line SM89010 (Ardiel et al. 2002). Of 440 random 10-mer primers tested using bulked segregant analysis, one primer resulted in a reproducible polymorphic band. This marker was converted to a sequence-characterized amplified region marker linked in coupling (5.5 cM).

The first genetic map of the wild South American barley species *Hordeum chilense* is presented. The map, based on an F2 population of 114 plants, contains 123 markers, including 82 RAPDs, 13 SSRs, 16 RFLPs, four SCARs, two seed storage proteins and two STS markers. The map spans 694 cM with an average distance of 5.7 cM between markers (Hernandez et al. 2001). Six additional SSRs and seven additional SCARs that were not polymorphic were assigned to chromosomes using wheat*H. chilense* addition lines. The Rfm1a gene restores the fertility of msm1 cytoplasmic male-sterile lines in barley. Matsui et al. (2001) identified three RAPD markers linked to the *Rfm1* locus using isogenic lines and segregating BC1F1 and F2 populations. *Pyrenophora graminea* is the seed-borne pathogen causal agent of barley leaf stripe disease. Near-isogenic lines carrying resistance of the cv. “Thibaut” against the highly virulent isolate Dg2 were obtained by introgressing the resistance into the genetic background of the susceptible cv. “Mirco” (Taconi et al. 2001). By using NILs, a RAPD marker associated with the resistance gene was identified. Model population of spring barley Oregon Wolfe Pack dihaploid lines, derived from F1 of a cross between dominant marker stock and recessive line, was studied for the presence of morphological markers. For detection of DNA variability RAPD analysis of these genotypes was carried out for mapping the barley genome. Polymorphic fragments correlating with morphological traits were detected using primer P89 (Bal’vinskaia and Sivolap 2000). Hoffman and Dahleen (2002) characterized detectable genetic variability present within a select set of four closely related malting barley cultivars using RFLP, PCR-RAPD and AFLP molecular markers. The markers that identified polymorphism among the select malting cultivars tended to link with each other and to map in chromosomal regions associated with quantitative trait loci for agronomic and malting quality traits that differed among the four cultivars. Osmotic adjustment (OA) was previously demonstrated to be an important adaptive mechanism of drought tolerance in cereals.

**Rye**

Little is known about the extent and patterns of distribution of RAPD diversity in outcrossing species. This study was the first step in using RAPD markers to quantify the amount and distribution of genetic variation within and between accessions of nine landraces and three cultivars of cultivated rye from Northern Europe (Persson et al. 2001). A high level of RAPD variation was detected, demonstrating the usefulness of RAPDs for genetic characterization in rye.

Restriction fragment length polymorphism-based genetic map of rye, developed previously using a cross of lines DS22RXL10 (F2 generation), was extended with 69 RAPD and 12 isozyme markers (Masojc et al. 2001). The actual map contains 282 markers dispersed on all seven chromosomes and spans a distance of 1.140 cM. The efficiency of mapping RAPD markers was close to 10 loci per 100-screened arbitrary primers.

Myskow et al. (2001) presented an attempt to supply breeders of hybrid rye with more genetic information on inbred lines, using molecular markers. Eighteen polymorphic loci detected by means of the RAPD technique and mapped on 2R-7R rye chromosomes, were applied to study genetic similarities among forty inbred lines of rye. RAPD analysis was carried out to assess DNA variation among rye plants regenerated from immature embryos and inflorescences (Linacero et al. 2000). From the studied plants, 40% showed at least one variation, and the number of mutations per plant was quite high, ranging from one up to 12.

**Oats**

Benchacho et al. (2002) compared data of isozyme and RAPD markers in the populations of two tetraploid species of wild oats: *A. barbata* populations collected in Argentina, and
Advantages of RAPDs

Variation in genome size among grasses probably reflects fluctuations in the amount of repetitive DNA per genome, but the history and causes of chromosome number changes remain unclear. Despite substantial variation among genomes, comparative maps suggest that grass genomes retain extensive regions of colinearity (Gaut 2002). In the classic breeding approach, the markers were invariably the visible morphological and other phenotypic characters, and the breeders expended considerable effort and time in refining the crosses as the tight linkage or association of the desired characters with the obvious phenotypic characters was never unequivocally established. Furthermore, indirect selection for a trait using such morphological markers was not practical due to: (i) a paucity of suitable markers; (ii) the undesirable pleiotropic effects of many morphological markers on plant phenotype; and (iii) the inability to score multiple morphological mutant traits in a single segregating population (Ranade et al. 2001). In recent years, marker systems based on hybridization techniques such as RFLP, have been replaced by faster and cheaper PCR-based methods. The ability to identify genetic variation is indispensable to effective management and use of genetic resources. Traditionally, diversity is assessed by measuring variation in phenotypic traits such as flower color, growth habit or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. This approach has certain limitations: genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence (Rao 2004).

The RAPD technique has further advantages over other systems of genetic documentation because it has a universal set of primers; no preliminary work such as probe isolation, filter preparation, or nucleotide sequencing is necessary. These primers adhere to a specific nucleotide segment of the genomic DNA. The DNA is cut into many segments of a specific length, which can be measured using gel electrophoresis. For a mutation to change the RAPD pattern, it must occur in the priming region or must change the length of the DNA between priming regions. In this way the RAPD analysis can provide a simple and reliable method for measuring genomic variation. Because it is a relatively straightforward technique to apply, and the number of loci that can be examined is unlimited, RAPD analysis is viewed as having a number of advantages over RFLPs and other techniques. In many instances, only a small number of primers are necessary to identify polymorphisms within a species. The ease of the RAPD technique could lead to the automation of genetic mapping and to the extension of genetic analysis to cover organisms that lack an ample number of phenotypic markers to completely describe their genome (Williams et al. 1990). For any population a selective process can produce change only if there are some variations to select among. No amount of reproduction can affect a population’s genetic composition if all individuals are identical. From an evolutionary standpoint the progressive accumulation of genetic variation is thought to have given rise, beginning with common ancestors, to the diversity of life. The process of continued evolution is critically dependent on renewed variation. Thus, genetic variation can be thought of as the “fuel” for evolution. The genetic differences in the populations were easily detectable using the RAPD analysis with single-primer DNA amplifications the RAPD method showed a more pronounced effect of isolation-by-distance in comparison with allozymes (Mamuris et al. 1999). This supported the use of RAPD analysis as an effective tool in species identification and cross-contamination tests among different cell lines (Guo et al. 2001b). The RAPD-PCR method can be applied to detect genetic diversity and similarity in numerous organisms using the various primers (Bernardi and Talley 2000). For all of these reasons the RAPD assay has been used to construct phylogenetic trees for resolving taxonomic problems in many organisms. It has been concluded that although AFLP analysis is superior in terms of efficiency, RAPDs may still be used as reliable markers in small low-tech laboratories (Kjolner et al. 2004). On the other hand, Sun et al. (2000) reported that the RAPD and AFLP techniques are powerful DNA fingerprinting methods for classification of Artemia species and strains. However, Yue et al. (2002) indicated that the microsatellite system detected population subdivision more efficiently than either RAPD or AFLP.

Limitations of RAPDs

Some limitations restrict the practical application of RAPD analysis (e.g. dominance, reproducibility, homology inferences and artifact fragments). Dominance is a major limitation of RAPD. These markers are thought to be dominant, with polymorphisms detected as either band presence or absence. Dominant
markers are not as efficient as co-dominant markers for population genetics studies. Zhou et al. (2001) reported that gynogenetic silver crucian carp is very sensitive to reaction conditions, but cloning and sequencing can overcome this problem. Several studies have reported poor reproducibility for RAPD markers. On the other hand, the major problem associated with the RAPD method is the reproducibility of the profiles. Many studies confirmed that with the optimized conditions and protocols, the consistent and reproducible RAPD results were achieved. Yu et al. (2004) used the optimized RAPD conditions and protocols that had been approved in their lab. The results of four RAPD markers associated to the FHB resistance were obtained consistently and repetitively by multiple replication examinations. However, there are some requisites for its correct application. A strict control of working conditions is demanded. Furthermore, due to the anonymous character of polymorphic bands and the difficulties for establishing homologies, it is also recommended to confine RAPD uses to the specific or infra specific levels. Comparisons based on genetic distance calculations are accepted provided they do not require parsimony analysis methods (Xena de Enrech 2000). Genomic DNA enriched for low-copy sequences was used for RAPD analysis to overcome the lack of reproducibility due to the highly repetitive DNA sequences present in wheat. Furthermore, some approaches aimed at improving the RAPD analysis, such as the selection of a large number of primers or changes in detection techniques, markedly increase the time and cost of PCR analysis. Therefore, the development of alternative PCR procedures that use single primers would seem to be an important step (Gawer et al. 2002). Furthermore, Bagley, Anderson and May (2001) assessed polymorphism and reproducibility of the two common fingerprinting techniques, RAPD and AFLP in pedigreed populations of rainbow trout (Oncorhyncus mykiss) to derive general rules for selective removal of problematic fingerprint bands. They found that by excluding bands that comprised less than 1% of total intensity, and by excluding the largest and smallest 10% of the bands, they could achieve nearly 100% reproducibility of AFLP fingerprints. Similar application of band exclusion criteria to RAPD fingerprints did not significantly enhance their reproducibility, and at least 15% of RAPD bands were not fully repeatable, heritable, or transmittable.

More generally, the use of RAPDs as systematic characters has several limitations, and the relevance and taxonomic meaning of RAPD groupings always need careful comparison with that of other sources of data. Usefulness of RAPDs as systematic characters is limited because of difficulties in assessing character homologies. However, RAPD-PCR has the potential to survey entire genomes, and RAPDs may provide insights into organismal evolutions that are overlooked by single-gene comparisons. Combining RAPD-PCR and sequencing methods to produce phylogenetic characters thus still may hold some promise in evolutionary genetics and systematics; by determining the nucleotide sequences of randomly amplified products, homologies between RAPDs could be inferred with greater confidence and nucleotide sequences that are variable in homologous RAPD fragments could be used as phylogenetic characters.

In the case of investigation of polymorphism in closely related strains, the highest possible complexity of the patterns obtained by RAPD-PCR is required to assure revealing of limited polymorphisms. Most parameters (reaction components concentration, additives, different polymerases, and thermal profiles) affecting RAPD-PCR should be examined, in an effort to increase pattern complexity (Diakou and Dovas 2001). Fraga et al. (2002) analyzed the effect of changing concentrations of the primer, template DNA and Taq DNA polymerase with the goal of determining their optimum concentration for the standardization of the RAPD technique for genetic studies of Trichomonas vaginalis. To ensure that the amplified DNA bands originated from genomic DNA, and not primer artifacts, negative controls should be carried out for each primer/breed combination (Ali 2003a). No amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions.

Conclusion and Future Prospects

The effectiveness of RAPD in detecting polymorphism between and among different populations, their applicability in population studies, and the establishment of genetic relationships were demonstrated in this review. In particular, genetic diversity data provide information on gaps in terms of coverage in gene pools as well as redundancies; in effect, material with similar characteristics that wastes resources through increased cost of management. Reproducible fingerprints can be produced from replicate DNA preparations by different operators in independent experiments over time and using thermal block-based cyclers. An exchange of RAPD data between laboratories should take into consideration the sources of variations and standardized methodology should be devised.

It is important to mention the fact that data results from RAPD assays can be extended to further dissect traits in a more refined way to exact knowledge on specific genes and genetic pathways using other molecular methodologies. There is also the opportunity and need to study sequences of specific polymorphic bands, to determine the genes detected by RAPD experiments. Further studies with other molecular methodologies are essential to clarify and confirm genetic relationships among different species depicted using RAPDs.

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