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Molecular characterisation of crop genetic resources with reference to groundnut and its wild relatives

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Introduction

The wealth of variability available in plants which collectively is termed as plant genetic resources, forms the basic reservoir for the agriculture and hence, the global food security. This variability includes both the wild and cultivated species of plants, which provide both farmers and researchers with the basic material to develop new and productive crops adaptable to the desired environment. However, intensive plant breeding practised over last several years have resulted in considerable loss of genetic variability in cultivated plants due to the replacement of the less preferred plants with profitable ones. Thus the genetic base of the cultivated plants has become narrower and diversity has been replaced by uniformity. This loss of diversity in the crops themselves is compounded by the loss of genes found in wild and weedy species related to the crop plants, because of improved crop husbandry and the destruction of natural ecosystems by man to meet the growing needs of agriculture, unscrupulous grazing, urbanisation etc. However, the future of the plant breeding and crop improvement rests on the skilful exploitation of the wealth of variability available in the germplasm. A considerable amount of concerted efforts are being put on the collection, evaluation, documentation, maintenance and storage of the available germplasm by both national level organisations in most of the nations and the International Plant Genetic Resources Institute (formerly the International Board for Plant Genetic Resources). The term genetic resources can be described as total genetic diversity of the cultivated and their wild relatives which mainly occur in

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their gene centres or outside; weed races occur as weed-crop complexes; land races which are products of traditional agriculture; breeding lines and genetic stocks and obsolete cultivars (Ford-Lloyd, 2001). The utilisation of plant genetic resources in crop improvement programmes rests mainly on identification of promising accessions through evaluation. Characterisation and preliminary evaluation involve the recording of highly heritable (oligogenic) morphological characters, which describe the accession and enable any contamination or mix up at later stage to be identified. Proper characterisation helps in unambiguous discrimination between accessions, detecting redundancies and in monitoring genetic changes during maintenance. The conventional and most used method of characterization of the genetic resources is the morpho-agronomic traits which involves variation in morphological traits like flower colour, testa colour (Plate I-A), seed shape, growth habit and agronomic characters like yield potential, stress tolerance or disease/insect-pest resistance. This still remains the only method used by breeders for some minor crops. These characters suffer from a major disadvantage that these are influenced by environmental factors as well as different stages of the growth of the plant species. The other methods, which found place in the assessment of the genetic variability in plants, were comparative anatomy, physiology, and embryology. Advances in the molecular genetics especially the developments during the last decade have opened up an array of techniques to be used for complementing classical strategies (Weising *et al.*, 1995). These molecular approaches include use of biochemicals (proteins and isoenzymes) and macromolecules like deoxyribonucleic acid (DNA) as markers. Amongst the molecular markers used, DNA markers are more suitable and ubiquitous. Since molecular markers look directly at the genetic material itself, they represent a powerful and potentially rapid method for characterising diversity *per se* within *in situ* and *ex situ* conservation. The molecular markers will add to the information of morphological and agronomic data in fine-tuning of the assessment of genetic diversity, providing more accurate and detailed information than classical phenotypic data. The molecular data would provide the essential information in developing core collection (Hodgkin *et al.*, 1995) and further identification of useful genes in the collection. DNA markers are of immense value in identifying duplicates in collections and genetic distance calculated based on molecular data can be used in the identification of divergent populations. Thus, molecular markers are useful tools in determining the identity of the accessions stored and catalogued; the degree of similarity between and among individuals and accessions in a collection; partitioning of the variation in individuals, accessions, populations and species; detection of specific alleles in the gene bank accessions or populations (Kreseovich *et al.*, 1992); and verification of the integrity of the germplasm and the reliability of its genotypic composition (Ford-Lloyd, 2001). Though the earlier generation markers like proteins and allozyme profiles were found to be efficient in characterisation of plant genotypes, they were influenced by the stage of growth of the plant as well as the physiological stages of the explant used for the assay. In contrast to this the DNA markers are unaffected by these factors. In this chapter, we chose to deal with only the DNA markers which are stable.

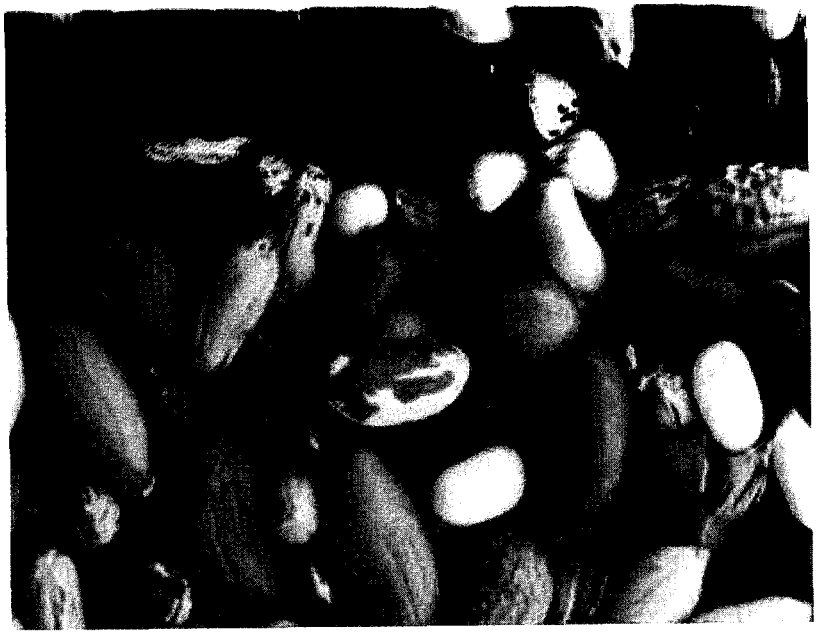

Plate I-A. Genetic variability in the testa colour of cultivated groundnut.

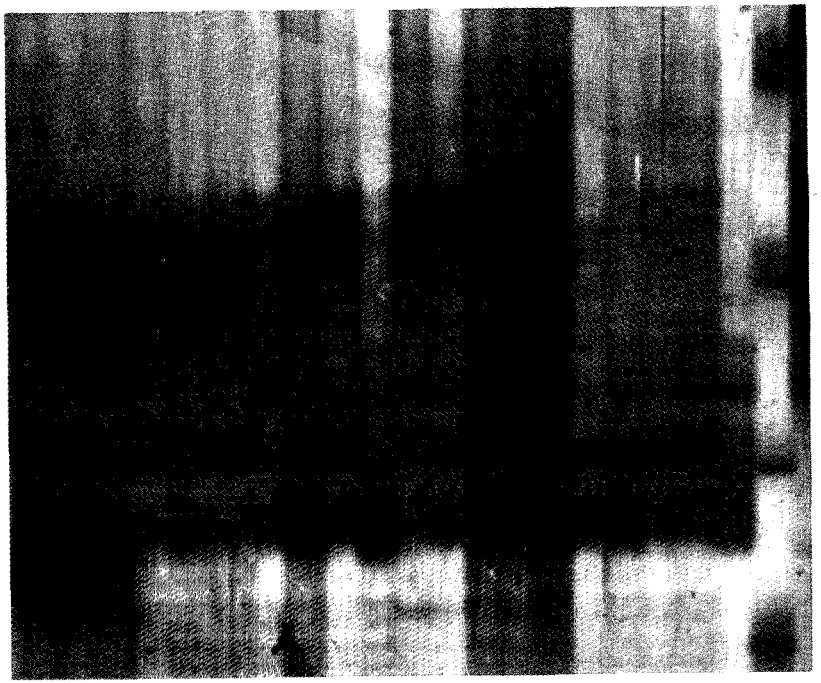

Plate I-B. Sequencing PAGE showing SSR polymorphism in germplasm of cultivated groundnut (silver stained).

The characterisation of genotypes in a population is based on the extent and type of genetic polymorphism apparent in that population. In classical terms, genetic polymorphism is the simultaneous occurrence of a trait in a population in more than one discontinuous genotypes or variants. Though DNA sequencing can bring out the differences in a locus, the approach is very costly, laborious and technically demanding. Thus, the other alternate methods of detecting the difference at DNA level have come into use. Though it is difficult to have an ideal molecular marker, the features like abundance in genome, genome-specificity, high polymorphism, high reproducibility, co-dominance in expression, capability of multiplexing, easy and fast assay, ability to be automated, easy portability between laboratories, low cost of assay and single copy etc. are considered to be qualifying the markers for molecular characterisation.

Molecular markers are increasingly being used for the assessment of phylogenetic relationships and genetic characterisation of plant germplasm. A volume of information is already available in the published literature on various molecular markers (Weising *et al.,* 1995; Staub *et al.,* 1996; Mohan *et al.,* 1997; Karp *et al.,* 1997; Koebner *et al.,* 2001) and new markers are being added to the list very fast. The first generation molecular markers were DNA-DNA hybridisation based and were slow and more technically demanding. The description of restriction fragment length polymorphism (RFLP) as a potential tool in varietal and parental identification marked a new beginning in genetic studies (Botstein *et al.,* 1980; Soller and Beckman, 1983). The invention of the polymerase chain reaction (PCR) had opened up a plethora of relatively simple and inexpensive markers, which are now being used on a large scale. These markers are generally identified as the second generation molecular markers. The third generation markers are based on techniques, which could directly provide sequence information that may use either or both hybridisation and PCR based methods. A very brief account of the most suitable markers and a relative evaluation of their suitability in molecular characterization of plant germplasm is provided below before discussing their exploitation.

Hybridisation based techniques

In these techniques, generally a genomic or cDNA library is prepared and species-specific single locus probes of about 0.5 to 3 kb size will be identified. The probe can be expressed sequence, an unknown fragment of genomic DNA or a part of the sequence of a cloned gene. The DNA profiles are visualised by hybridising the restriction enzyme digested DNA sample, to a labelled probe. The most used markers in this category are RFLP and VNTRs.

Restriction Fragment Length Polymorphism (RFLP)

In RFLP analysis, genomic DNA is restriction enzyme-digested and the resulting fragments are resolved by gel electrophoresis and then transferred on to a nitrocellulose membrane by a process called Southern blotting (Southern, 1975). These blots will then be challenged with specific labelled probes to produce banding

patterns, which can be visualised by autoradiography or fluorescent/chemical methods. The radioactive label-based visualisation methods are robust and allow multiple uses of the DNA separations resulting from a single restriction digest and electrophoresis run and hence, less expensive than the biotin- or deoxygenin-based fluorescent label methods. Specific probe-enzyme combinations give highly reproducible patterns for a given individual whereas the marker will be polymorphic between individuals if any alteration in the restriction site has taken place (Evola *et al.,* 1986; Helentjaris *et al.,* 1986). Genetic maps using RFLP was first constructed by Botstein *et al.* (1980). This marker is co-dominant and can detect heterozygous individuals. RFLP is highly reproducible and portable between laboratories. However, the technique needs more DNA and use of radioactivity, is labour-intensive and not amenable to automation, and low in polymorphism. This marker cannot detect single base changes due to point mutations.

Variable Number of Tandem Repeats (VNTRs)

This technique is a variant of the traditional RFLP. Variable number of tandem repeats can either be microsatellites (2–8bp repeats) or minisatellites (16–100bp repeats). Here synthetic oligonucleotides of simple sequence complexities will be used as probes to detect hyper-variable regions in the genome. This technique is more suitable for identification of genotypes. The simple repeats like (AT)n, (CG)n, (CT)n, (GACA)n and (GATA)n are used in probes. Change in the copy number of these repeats can produce polymorphism, which is generally termed VNTRs. Though hybridisation of these probes produces multi-locus pattern, it has been found useful in generating individual or genotype specific hybridisation patterns.

PCR based techniques

Polymerase chain reaction is a very simple technique, which can be adopted by any modestly equipped laboratory and hence, has become very popular (Jeffreys *et al.,* 1985; Welsh and McClelland, 1990; Williams *et al.,* 1990). In PCR, generally a pair of primers (forward and reverse) is used for the amplification. The primer can be arbitrary or based on the sequence information flanking the DNA to be amplified. In some cases the same primer is used as both forward and reverse, where reverse primer is just the reverse orientation of the forward primer. Since the same primer is used as forward and reverse in multiple loci, the techniques under this category are collectively called as multiple arbitrary amplification profiling (MAAP). Some of the PCR based techniques are briefly described below.

Single primer based PCR techniques

The most popular single primer based PCR techniques are random amplified polymorphic DNA (RAPD), arbitrary primed polymerase chain reaction (AP-PCR) and DNA amplification fingerprinting (DAF). These techniques differ mainly in the length of the primers used for the amplification.

Random Amplified Polymorphic DNA (RAPD)

This is one of the most used techniques owing to its simplicity. In RAPD, arbitrary oligonucleotide primers are used for amplifying genomic DNA. This amplification will result in several discrete amplification products, which can be separated and visualised under UV light by using agarose gel electrophoresis and ethidium bromide staining. The primers amplify the two short DNA segments in the genome with some homology, present on opposite strand of DNA, and close enough to have DNA amplification. Generally several discrete loci in the genome will be amplified and hence the technique is useful for efficient screening of nucleotide sequence polymorphism between individuals (Welsh and McClelland, 1991). RAPD are dominant genetic markers and hence, can not distinguish heterozygote. The technique requires less DNA and very simple. However, it suffers from low reproducibility due to spurious amplification products that lead to inappropriate inferences and less portability between labs.

Arbitrary Primed-Polymerase Chain Reaction (AP-PCR)

The major difference in this method is the length of primer used. The genomic DNA will be amplified using primers of 10–50 bases in length (Welsh and McClelland, 1991). In the first two cycles, annealing is under non-stringent conditions. The amplification products are similar to that of RAPD and can be visualised either by autoradiography or by staining with ethidium bromide in agarose gel.

DNA Amplification Fingerprinting (DAF)

This method was described by Caetano-Anolles *et al.* (1991). PCR reactions with one or more short arbitrary primers of 5–8 bases are used for amplifying DNA. Amplification products can be visualised by using polyacrylamide gel electrophoresis and silver staining. The primers can be labelled by fluorochromes for detection and the process can be automated. Digestion of template DNA with one to three restriction endonucleases enhances amplification of polymorphic DNA, allowing even near isogenic lines to be distinguished. Efforts on optimisation are required for repeatability and portability between labs. This technique has been useful in genetic typing and mapping.

PCR techniques based on a pair of primers

These techniques involve use of forward and reverse primers, which are distinct. In this case the primers used are degenerate or semi arbitrary. There are several marker types, which are based on this approach, and a few of them are described below.

Sequence-Tagged Sites (STS)

The sequence information of RFLP probes, which are capable of detecting polymorphism, can be used for developing PCR primers and then used in RAPD to detect polymorphism. Primers of 18–20 nucleotides are designed to amplify some short, unique fragment of DNA whose sequence is known. This method detects a single, unique, sequence-defined point in the genome. This technique avoids the labelling of the probes, laborious process of hybridisation and use of radioactivity while enjoying all the advantages of RAPD, though design and creation of good primers needs significantly high investment. Polymorphism is generally detected as size difference in the amplified product and if there is no size difference, restriction enzymes can be used to cut the products to identify polymorphism. Since the primers are longer than RAPD primers and based on a specific sequence, this technique detects the same locus reliably and is useful for mapping studies. This approach has been exploited in determining species relationships (Kawase, 1994).

Sequence Characterised Amplified Regions (SCAR)

This technique is useful for converting the RAPD markers to co-dominant marker by specific amplification of a particular locus. Specific RAPD markers are sequenced at their ends and primers of 22–24 nucleotides are designed (Michelmore *et al.*, 1991; Martin *et al.*, 1991). They are similar to STS markers in construction and usage. Sequence characterised regions have better reproducibility than RAPDs and are co-dominant markers. By using longer PCR primers, SCARs do not face the problem of low reproducibility often encountered with RAPDs. However, SCARs may exhibit dominance when one or both primers partially overlap the site of sequence variation. Dominant SCAR markers can often be made co-dominant by digesting the PCR product with restriction enzymes. SCARs have several advantages over arbitrary primers and they can be used for physical and genetic mapping, map-based cloning etc.

Cleaved Amplified Polymorphic Sequences (CAPS)

In this method polymorphic patterns are generated by separating restriction digested RAPD products (Konieczny and Ausubel, 1993) and hence are secondary markers. They are identified using two primers based on the sequence information available in databank of genomic or cDNA sequences or cloned RAPD bands. They specifically amplify single fragments. However, polymorphism of CAPS is revealed by pre-amplification digestion of template DNA with several restriction endonucleases. These markers are co-dominant in nature.

Sequence-Tagged Microsatellite Sites (STMS)

Microsatellites or simple sequence repeats (SSRs) are characterised by the tandem repeats of varying length (Akkaya *et al.*, 1992). This method uses the primers based on the sequence information on the flanking regions of simple sequence repeats in the genomic DNA (Weber and May, 1989). Identification of SSRs requires considerable investment. Polymorphism is resulting from the variations in the motif that is repeated multiple times and flanked by a unique DNA sequence used to develop the SSR primer. The most used repeats are tri- and tetranucleotides (Hearne *et al.*, 1992) though dinucleotides are generally abundant in genomes (Rafalski and Tingey, 1993). The technique of SSR analysis also have all the advantages of RAPD and most robust and highly reproducible. Further, if the primers are designed carefully, it is possible to simultaneously genotype several (3–15) markers associated with amplification products of substantially different sizes (multiplexing). This is especially cost-effective when combined with fluorescent labeling methods. Microsatellites are very useful markers for plant genetic studies due to the very high level of polymorphism detectable by this method. The total number of simple repeats within the targeted microsatellite DNA has a direct relationship with the number of alleles detected and hence, more alleles can be detected in a large population.

Inter Simple Sequence Repeats (ISSR)

The inter simple sequence repeats (ISSR) are amplified using the 3' anchored primers based on the microsatellites (SSR) to reveal the polymorphism (Zietkiewicz *et al.*, 1994). These are predominantly dominant markers. An unlimited number of primers are possible with combinations of di-, tri-, tetra- and pentanucleotides and an anchor of a few bases.

Amplified Fragment Length Polymorphism (AFLP)

This is a combination of RFLP and PCR techniques (Saiki *et al.*, 1988) which does not require sequencing or cloning. This technique is highly reproducible, universally applicable and capable of detecting very high levels of polymorphism (Vos *et al.*, 1995). The procedure essentially involves two steps. In the first step the genomic DNA will be digested with two different restriction enzymes, one a common cutter (eg. *MseI*) and the other a rare cutter (eg. *EcoRI*). Adapters specific to the sticky ends produced by the restriction enzymes will be then ligated and a preselective amplification of the ligation product will be performed. In the second step the preselective amplification products will be again subjected to a selective amplification using primers specific to the adapters and two or three selective nucleotides. One of these selective primer pairs can be labelled either by fluorochromes or by radioactivity. The amplification products can be separated on a denaturing poly acrylamide sequencing gel and then viewed by densitometry (in case of fluorescent labelling) or by autoradiography. The gels can also be silver stained. AFLP

technique can be used in physical mapping as most of the fragments correspond to unique positions in the genome (Hongtrakul *et al.*, 1997). Polymorphic AFLP band can be converted to SCARs as is done in case of RAPDs. Polymorphism is detected as band presence/absence (so it is usually interpreted as dominantly inherited, although claims for co-dominant inheritance are also made based on band intensity). AFLP markers are often inherited as tightly linked clusters in centromeric and telomeric regions of chromosomes, but randomly distributed AFLP markers also occur outside these clusters.

Randomly Amplified Microsatellite Polymorphism (RAMP) and Retroposon Microsatellite Amplified Polymorphism (REMAP)

In randomly amplified microsatellite polymorphism, RAPD is performed according to the standard protocols. The amplification products will then be blotted on to a nylon membrane and then probed using labelled microsatellite oligonucleotide probes. Being a combination of one or more than one marker technique, this has several advantages of RAPD and SSR (Williams *et al.*, 1990; Gupta *et al.*, 1994). This technique has been successfully employed in the genetic fingerprinting of tomato, kiwi fruit and closely related genotypes of *Dioscorea bulbifera* (Richardson *et al.*, 1995).

In retroposon microsatellite amplified polymorphism, RAPD is performed using a pair of primers of which one is an anchored microsatellite and the other is a retroposon long terminal repeat (LTR). In both the techniques, the amplified products resolve the length polymorphism present either at the SSR target site or at the associated sequence between the binding sites of the two primers. The amplified products may further be digested with restriction enzymes to increase the resolution. The advantage of these techniques is that the undigested genomic DNA is used as the template instead of the pre-amplified restriction digested DNA as is done in AFLP.

Expressed Sequence Tags (EST)

Expressed sequence tags were first described by Adams *et al.* (1991). These markers are generated based on the sequence information generated by partial sequencing of random cDNA clones. The partial sequences of cDNA clones, which are generated as part of gene sequencing projects, are used to design 18–20 nucleotide long primers that provide a unique sequence "tagging" the gene. This technique can detect a unique, expressed region of the genome, usually as a size difference in the amplified product, and hence, is inherited in a co-dominant manner. Design and creation of useful primers can be expensive. They are useful in cloning of specific genes and in synteny mapping of functional genes in related organisms. In plants like *Arabidopsis*, rice etc. several EST markers are identified as thousands of functional cDNA clones are already available (Sasaki, 1994; Cooke *et al.*, 1996).

Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms, usually pronounced as 'snips', refers to single base difference between two individuals at a specific locus at a defined position on a chromosome. This might be the result of a purine-purine or pyramidine-pyramidine transition, or a pyramidine-purine, purine-pyramidine transversion. As compared to SSRs, SNPs are more stable, prevalent and occur at high frequencies (Kwok *et al.*, 1996). There are several methods of identifying SNPs within a locus. Sequence analysis is the most direct way of identifying SNPs; however, it is also the most time-consuming and costly approach. The initial step generally is to determine the sequence of the locus for a reference genotype. Once determined, this sequence is used to design oligonucleotide PCR primers, which forms the cornerstone of all subsequent SNP-based technology (Erlich, 1989). After the PCR amplification of the target segment, the alternative amplicon sequences are discriminated by any of the procedures like invasive cleavage by oligonucleotide probes (Lyamichev *et al.*, 1999); PCR-RFLP; TaqMan (Livak *et al.*, 1995) and molecular beacon procedure (Tyagi *et al.*, 1998); oligonucleotide ligation assay (OLA) (Landegren *et al.*, 1988); oligonucleotide microarrays (Sapolsky *et al.*, 1999); dynamic allele specific hybridization (DASH); or other 5' end SNP recognition procedures (Germer and Higuchi, 1999) like pyrosequencing, minisequencing, and matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF) (Haff and Smirnov, 1997).

Comparison of marker types

A comparison of the markers described above reveals that in most of the cases the polymorphism is resulting from the base changes in the target DNA by insertions, deletions or substitutions (RFLP, RAPD, DAF, STS, AFLP). However, the differences in the length of the repeats in the target DNA is the reason for the polymorphism in SSR and ISSR whereas the single base changes are the cause of polymorphism in SNP. The information of DNA sequence is not an absolute necessity in a good number of techniques and so is the requirement of radioactivity for visualisation. The fluorescence and other chemical methods are now equally robust as radioactive methods making the techniques more eco-friendly. Majority of the markers are inherited as dominant markers, which is a limitation in identifying the heterozygous genotypes. Most of the markers described are highly reproducible and are portable between laboratories. A comparison of the markers described is provided in the Table 1. Apart from the characterisation of the germplasm, these markers are useful in other areas of research pertaining to crop improvement also (Table 2).

Selecting the right type of marker

While considering the suitability of the markers, various aspects are to be considered. As mentioned earlier, the factors, which qualify the markers, are the yardsticks for

Table 1. Comparative account of the DNA markers which can be used for characterisation of plant germplasm

Marker	PCR-based	Type of polymorphism	Abundance in Polymorphism	Inheritance	Amount/ quantity of DNA	Sequence information Required	Radioactive detected	Gel system	Development costs	Running costs per data point	Portability
RFLP	No	Base changes	Low-Medium	Co-dominant	High/High	No	Yes/No	Agarose	Medium	High	High
RAPD	Yes	Base changes	Medium-High	Dominant	Low	No	No	Agarose	Low	Low	Low
DAF	Yes	Base changes	High	Dominant	Low	No	Yes/No	Acrylamide	Medium	Medium	Low
SSR	Yes	Variation in length of repeats	High	Co-dominant	Low/ Medium	Yes	No	Acrylamide/ Agarose	High	Medium	High
SCARs/ CAPS	Yes	Base changes	High	Co-dominant	Low/High	Yes	No	None	High	Medium-Low	High
ISSR	Yes	Variation in length of repeats	High	Dominant	Low/ Medium	Yes/No	No	Acrylamide/ Agarose	Low	Low	High
AFLP	Yes	Base changes	High	Dominant	Low/High	No	Yes/No	Acrylamide	Medium-High	Low	High
REMAP	Yes	Variation in length	High	Co-dominant	Low/ Medium	Yes	No	Acrylamide/ Agarose	High	Medium	High
STS / EST	Yes	Base changes	High	Co-dominant/ Dominant	Low/High	Yes	Yes/No	Acrylamide/ Agarose	High	Medium	High
SNP	Yes	Single base changes	Extremely High	Co-dominant	Low/High	Yes	No	Sequencing required	High	Medium	Medium

Table 2. A comparison of applications of the various DNA markers described

Application	SSR	RAPD	RFLP	AFLP	REMAP	STS/ EST	SNP	SCAR/ CAPS
Bulk segregant analysis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Comparative mapping	Yes	Yes	Yes					
Detection of new alleles	Yes	Yes	Yes			Yes	Yes	
Diversity analysis	Yes	Yes		Yes	Yes	Yes	Yes	Yes
Fingerprinting	Yes			Yes	Yes	Yes		
Gene tagging	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Genetic mapping	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Hybrid identification	Yes	Yes	Yes	Yes	Yes	Yes		Yes
Map-based cloning	Yes	Yes	Yes	Yes		Yes		Yes
Marker-assisted selection	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Plant Breeding	Yes	Yes	Yes	Yes			Yes	
Seed testing	Yes	Yes			Yes			
Cultivar identification	Yes	Yes	Yes	Yes	Yes	Yes		

assessing the suitability. The choices of the markers are dependant mainly on the crop species to be assessed and the type of resources available for the work and the time available. Karp *et al.* (1997) has outlined an approach to decision making in selecting the suitable marker (Figure 1). The first aspect in this is whether the selected approach can provide the desired type and amount of data for addressing the problem. For example, if we are looking for the similarities in a collection, the markers of choice should be of highly discriminative type whereas if the objective is only to deduce the evolutionary history, more simple markers may work. However, the level of polymorphism expected or reported earlier in those plant species forms an important factor in selecting the technique. This is specific to plant taxa and the information on the morphological variability, data on isoenzyme polymorphism, geographical distribution, breeding system etc. can be indicative of this. If adequate polymorphism is available, the choice of technique will be very wide and diverse though the data analysis may have difficulties. Low resolution markers like RFLP and CAPS may be easier in data analysis. If low polymorphism is expected, the choice should be of highly discriminative techniques like AFLP or STMS. However, in most of the cases a pre-screening is essential to determine the level of polymorphism likely to be available. The next point to consider is the availability and access to the primers or probes required for the analysis. For techniques like RAPD, AFLP and ISSR, primers are readily available and no development cost is involved. For RFLP, if specific probes are accessible from other workers or of very related taxa some saving on the development cost can be there. However, development of SSR primers etc. will involve huge investment in terms of both time and money. The time period under which the data is to be made available also is a factor in deciding the type of marker approach to be selected. If enough time is at disposal one can choose more accurate and informative methods like STMS or PCR sequencing. In case of a time constraint, simple and faster methods like RAPD, AFLP, ISSR etc. can be chosen and one can go in for even STMS or PCR sequencing if primers are already available and accessible.

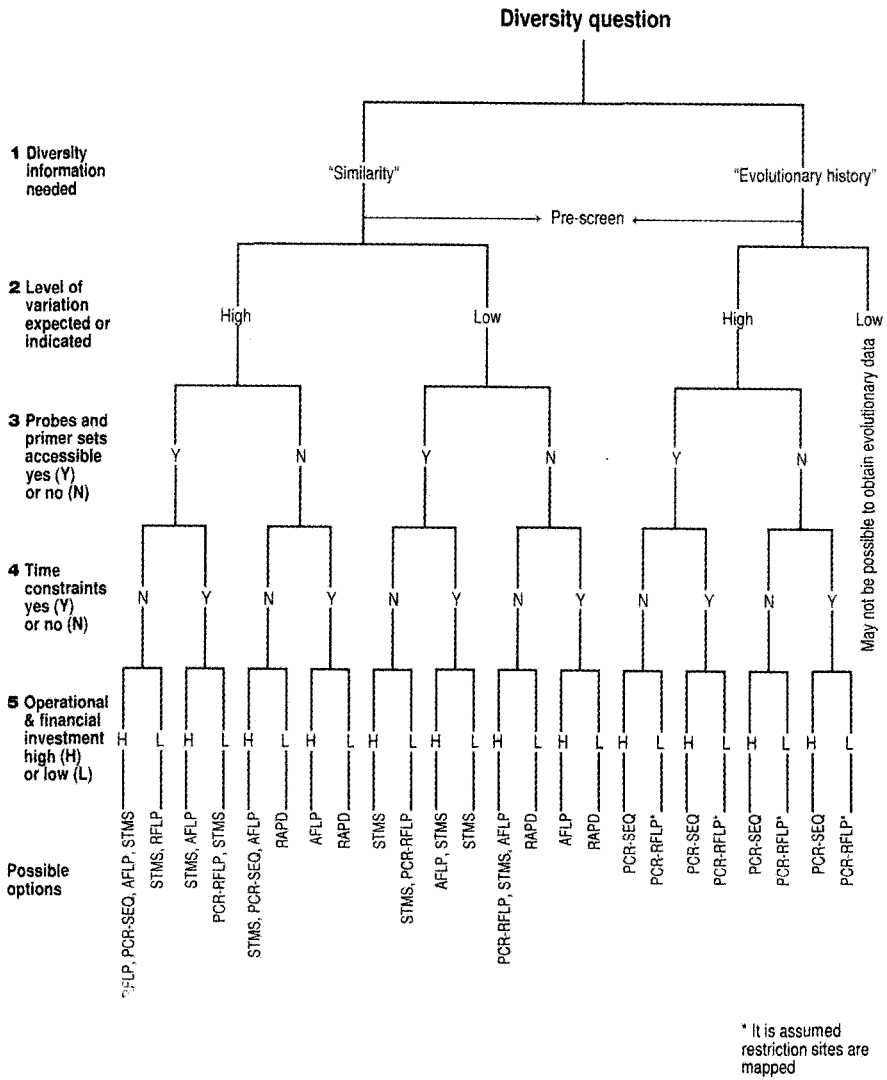

Figure 1. Decision making chart for selection of marker techniques (Karp *et al.*, 1997).

The availability of laboratory facilities, cost of reagents and hence, per assay cost, availability of trained manpower in the working group etc. also are critical in selecting a marker technique of choice. For techniques like RAPD, the level of investment and the technical competence required are very low as compared to other techniques like development and use of SSRs. However, for increased accuracy techniques like CAPS, RFLP or AFLP can be resorted to without much difficulty. Hence, all the aspects like the level of polymorphism, type of inheritance of the marker (dominant or co-dominant), convenience, technical difficulty, availability of

suitable probes/primers, portability, quantity of DNA required and the ease of exchange of data between laboratories are the factors contributing to the choice of marker. It would be difficult to find a marker that meets all the desired criteria, but a marker system can be identified that would fulfill most of the desired qualities.

Applications of molecular markers in plant germplasm characterisation

Molecular methods have started playing an important role in conservation and use of plant genetic resources in the recent years. Specific areas in which molecular marker techniques have been used to support genetic resources activities are: developing sampling strategies and identification of gaps in the collections to plan for future acquisition of germplasm, and managing conserved germplasm — including identification of duplicates, development of core collections, fingerprinting, identification of genetic contamination and quantification of genetic drifts/shifts (Rao, 2004). Ford-Lloyd *et al.* (1997) had outlined four broad areas where molecular markers may be suitably employed to assist gene bank management, organization and access conserved material. They include: 1) the accurate identification of germplasm, 2) the routine maintenance of germplasm, which is a continuous process involving seed testing, rejuvenation and replenishment of stocks, and which will be streamlined by the identification of duplicates and the development of core collections, 3) the selection of germplasm for safe storage at other gene banks, and 4) the choice of germplasm for use by breeders and other researchers involved in making crosses, and mapping, identifying and isolating genes of interest.

Acquisition/Distribution of germplasm

Sampling strategies

Assessments of how completely a germplasm collection represents a particular species's genetic profile or a crop's total gene pool should incorporate a variety of genetic markers. Molecular markers have been applied to study genetic diversity from natural populations and formulate efficient sampling strategies to capture maximum variation for conservation. For example, Miller and Tanksley (1990) recommended predominant sampling of self-incompatible species for germplasm acquisition based on substantially higher level of RFLP variation observed in self-incompatible as compared to self-compatible species of *Lycopersicon*. Genetic variation within and between natural populations of *Digitalis obscura* was quantified using RAPDs and the results were used for optimizing sampling strategies for conservation of genetic resources of the species (Nebauer *et al.*, 1999).

Studies of distribution of genetic diversity using AFLP markers in Sri Lankan coconut populations showed that emphasis should be placed on collecting relatively large numbers of palms from few populations since most of the diversity was within populations rather than between populations (Perera *et al.*, 1998). In another study by Perera *et al.* (1999) using microsatellite loci 14 forms of coconut belonging to

three varieties were characterised. Sri Lankan tall coconuts exhibited higher levels of diversity than the dwarfs and intermediates, and intermediates were more similar to the dwarfs than the talls. A high level of population differentiation was observed between talls and dwarfs, with seven of the eight microsatellites clearly differentiating the two varieties. Some 22 of the 24 talls studied were uniquely genotyped with only two Nawasi individuals being indistinguishable. Only eight of 18 dwarf genotypes could be identified in the study. Molecular characterisation by either ISTR (inverse sequence-tagged repeats) or STMS markers of coconut palms from selected localities in the Southern Tagalog region of Philippines exhibited an exceptionally high proportion of palm diversity (>90%) and high heterozygosity values of palms within sites (Namia, 2002). However, a low proportion of diversity among localities (<6%) was observed indicating that the distribution of variation was quite even among the sites evaluated. The genetic distances were not significantly correlated with geographic distances and euclidean distances of rainfall, temperature and, edaphic characters. Meerow *et al.* (2003) carried out analysis of genetic diversity and population structure within Florida coconut germplasm using microsatellite markers with special emphasis on the Fiji Dwarf cultivar. The highest gene diversity was found in the tall cultivars and the lowest in the Malayan Dwarf. After the tall coconuts, the Fiji Dwarf was most genetically diverse and had the largest number of unique alleles.

De-Oliveira *et al.* (1996) assessed regional and racial specificities in Sorghum germplasm. Three different molecular markers (RFLP, RAPD and ISSR) were used to determine the relatedness of 84 different lines of Sorghum. Both racial characterisation and geographical origin were found to be correlated with relatedness. In some cases, the region of origin was the more significant factor, where samples of different races from the same locality were more closely related than were samples of the same race from different localities. Wild Sorghums were shown to have few novel alleles, suggesting that they would be poor sources of germplasm diversity. The results also indicated that Chinese Sorghums were a narrow and distinctive group that was most closely related to race bicolor.

Jamago (2000) characterised the Philippine mungbean germplasm employing AFLP technique. The analysis for released and local cultivars generated high polymorphism. The local cultivars had the widest range of genetic resemblance (40–92%). Moderate to high phenotypic diversity was estimated for five biographic zones. A subset of 90 mungbean accessions from the sample population used for morpho-agronomic characterisation was evaluated for the AFLP analysis. Molecular assay using four highly discriminatory AFLP primer pairs revealed high polymorphism. UPGMA cluster analyses of the qualitative, quantitative and AFLP data disclosed an absence of duplicate accessions. Each accession was clearly differentiated.

He *et al.* (1995) employed DNA amplification fingerprinting (DAF) approach to develop individual specific profiles and analyse genetic relationships among 72 accessions of sweet potato, including unadapted lines from around the world and a few selected USA cultivars, and one accession of the wild species *Ipomoea triloba*.

Using seven highly informative octamer primers, individual specific DAF profiles were obtained for all accessions tested. The degree of polymorphism in the sweet potato collection was very large, indicating a high level of genetic variation. Several accessions clustered together based on their geographic origin. *Ipomoea triloba* and tetraploid *I. batatas* formed a group distinct from the cultivated sweet potato. They concluded that DAF could be used to sweet potato germplasm characterisation and to identify duplicate accessions or for creation of core subsets, and to facilitate the selection of parents for a breeding programme to ensure a broad genetic base.

Genetic relationships among 125 Spanish melon (*Cucumis melo*) accessions from a Spanish germplasm collection were assessed using 34 RAPD markers and 72 reference accessions drawn from previous studies (Lopez-Sese *et al.*, 2003). Genetic diversity was highest in accessions of African origin and lowest in accessions of Spanish origin. The highest level of polymorphism was detected among melons originating from the central region of Spain. These results indicated that the Spanish melon accessions could be used to broaden the genetic base of local and foreign Casaba germplasm, to enhance the genetic diversity of the US and European commercial melon germplasm, and to delineate collection strategies for acquisition of additional Spanish landraces. Aga *et al.* (2003) used RAPD tool to assess genetic diversity among 144 genotypes representing 16 coffee (*Coffea arabica*) populations. Most of the populations were clustered on the basis of their geographic closeness and an east west differentiation was observed. The results obtained provided information on how to select sites for *in situ* conservation of *C. arabica* germplasm. Similar results were obtained for apricot germplasm collections where SSR markers grouped the genotypes according to their geographic origins and pedigrees (Romero *et al.*, 2003). Another study on assessment of genetic variability in 74 apricot germplasm accessions by Zhebentyayeva *et al.* (2003) using SSR markers showed that genetic differentiation of native germplasm into traditional ecogeographical groups was low, with a high level of genetic identity between the groups. However, the groups were not evidently based on the geographical origin of the cultivars.

A selected set of accessions of components of the azuki bean (*Vigna angularis*) complex comprising 123 cultivated accessions and 23 wild or weedy accessions from Bhutan, China (including Taiwan), India, Japan, Korea, and Nepal was analyzed by Zong *et al.* (2003) using AFLP methodology. The results indicated five major groups of azuki bean germplasm primarily associated with geographic origin of accessions and their status: wild, weedy, or cultivated. The results suggested that domestication of azuki bean occurred at least twice, once in the Himalayan region of southern Asia and once in northeast Asia, and there were important gaps in the germplasm collections of azuki bean and its close relatives from various parts of Asia and that specific collecting missions for *Vigna* germplasm related to azuki bean in the highlands of subtropical Asia were needed.

Assessing redundancies

The molecular data on genetic diversity provides information on gaps in terms of

or duplication wastes resources through increased cost of conservation and management. RAPD analysis in *Brassica oleracea* revealed that 14 phenotypically uniform accessions could be reduced to four groups with minimal loss of genetic variation (Phippen *et al.*, 1997). In case of cocoa (*Theobroma cacao*), AFLP and SSR analysis were performed at the United States Department of Agriculture on germplasm accessions maintained in several international collections to evaluate the utility of these procedures for DNA fingerprinting of this tree crop. Based on this study, 15 primers for SSR markers have been selected as an international standard technique for *T. cacao* molecular characterisation. Preliminary molecular studies on a large international collection in Trinidad and Tobago indicated that as much as 20–30% of the collection may be mislabelled or labelled with different names (Saunders *et al.*, 2001). Benson *et al.* (2001) used SSR markers to characterise 65 accessions of tea crabapple (*Malus hupehensis*) contained in the US National Plant Germplasm System (NPGS). The SSR phenotypes and ploidy information obtained through flow cytometry revealed the Yichang clone of the crabapple under various accession names in arboreta. Hence, the study helped to identify duplicate accessions of the Yichang clone held in the NPGS.

AFLP technique was used by van Treuren *et al.* (2001) to characterise 29 flax accessions of material derived from research activities (breeding lines). Based on similar accession names, the breeding lines could be classified into three series that were expected to contain redundancies. In addition, 12 reference cultivars were also analysed. A total number of 144 polymorphic bands (59.8%) were scored among the 164 individuals investigated. In general, relatively high levels of intra-accession variation were found, even for the cultivars examined. This finding was not in line with the low out-crossing rates reported for flax. For the cultivars 40.5% of the variation was distributed among accessions within groups and all pair wise comparisons were significantly different, except for one case. Both for the series of breeding lines and the cultivars, the major part of the variation was distributed among individuals within accessions. Pair wise comparisons of accessions were performed by analysis of molecular variance in order to identify redundant germplasm. Stepwise bulking of accessions until all remaining accessions were significantly different showed that the 29 accessions of breeding lines could be reduced to 14.

Intra-accession variation in barley was determined by AFLP markers and results were used to evaluate the efficacy of splitting heterogeneous accessions into distinct lines in order to avoid the negative effects of selection and genetic drift during regeneration (van Treuren and van Hintum, 2001). Khadari *et al.* (2003) detected several cases of mislabeling, synonymy and homonymy in more than 100 accessions of French olive collection using RAPDs, mtDNA RFLPs and SSR markers. From the results they constructed a molecular database for the reference collection and to analyse genetic diversity for further prospecting, and for introducing new olive accessions. Some of the other studies where molecular markers have been used to identify redundancies in collections include perennial kales (Zeven *et al.*, 1998), wheat (Cao *et al.*, 1998), grapevine (Cervera *et al.*, 1998), Sorghum (Dean *et al.*, 1999), cassava (Chavarriage *et al.*, 1999), and barley (Lund *et al.*, 2003).

Formation of core subsets

According to its original definition given by Frankel (1984), a core subset of a germplasm collection contains, with minimal redundancy, most of the entire collection's genetic diversity. Defining core subsets and their integration into germplasm management strategies are two of the most complex issues to be addressed by the germplasm managers (Bretting and Widrlechner, 1995). Presently, molecular markers have been used to identify groups from which core collection accessions can be selected or to monitor the effectiveness of one or the other strategy in capturing genetic diversity found in the whole collection (Rao, 2004).

Skroch *et al.* (1998) compared the common bean (*Phaseolus vulgaris*) core collection established from the nearly 24000 accessions held in the germplasm collection at CIAT, Columbia and reserve germplasm accessions sampled from this collection using RAPD technique. RAPD markers were used to compare core and reserve samples based on marker frequencies, marker diversity and nearest neighbour and multidimensional scaling analyses of marker-derived genetic distance matrices. No significant differences were found between core and reserve samples based on marker data. Thus, enrichment of diversity was not detected in the core sample indicating that the method used to develop the core collection had not been very efficient in capturing diversity. RAPD markers were also used to assist the assembly of a core collection for cultivated Andean potato species (*Solanum phureja*) by Ghislain *et al.* (1999). Tohme *et al.* (1999) reported use of RAPD and AFLP markers to characterise the wild and cultivated bean core collections, and microsatellite and AFLP markers to characterise the cassava core collection held at CIAT. Assessing diversity at the molecular level provided valuable information on the genetic structure of each core collection and new insights on diversity within and between gene pools for bean and cassava.

Grenier *et al.* (2000) evaluated three different sampling methods to constitute a core collection of Sorghum landraces maintained by the ICRISAT for the extent of genetic diversity captured based on microsatellites analysis. Three subsets of around 200 accessions were established following the three sampling strategies. An assessment was done of the genetic diversity retained by each sampling strategy using the polymorphisms at 15 microsatellite loci. The average allelic richness and average genetic diversity for the three subsets were comparable. A high percentage of rare alleles was maintained in the three subsets. The global molecular diversity retained in each subset was not affected by the sampling procedure based upon phenotypic characters.

Systematic and eco-geographic relationships

One of the most important roles of genetic markers in plant germplasm management is elucidating the systematic relationships and characteristic genetic profiles of germplasm. Such studies include analyses of comparative degrees of evolutionary genetic divergence; amounts, patterns, and apportionment of genetic diversity; and the evolutionary and/or human selective forces moulding the preceding

characteristics (Bretting and Widrlechner, 1995). Molecular markers are being increasingly used to resolve problems of taxonomy and phylogenetic relationships, as a good knowledge of genomic homologies helps in devising appropriate breeding strategies for introgression of genes from one species to another. Ramanatha Rao and Riley (1994) reviewed some of the earlier studies in this area. Variation within species has also been studied to explore geographic or ecological patterns of distribution of diversity in many crops and their wild relatives.

Bhat *et al.* (1995) employed RAPD and RFLP techniques to profile the banana and plantain cultivars. The diploid ancestral species of cultivated banana and plantains, namely *Musa acuminata* subsp. *malaccensis*, an A-genome donor, and *M. balbisiana*, a B-genome donor, were farthest apart from each other in the phenogram. The edible fruit yielding cultivars with the genomic constitutions AA, AAA, AB, AAB, ABB and ABBB grouped in different clusters according to overall genetic homologies. Cluster analysis of the RFLP data also resulted in a phenogram comparable to the one obtained with RAPD analysis. Sanchez *et al.* (2000) employed AFLP technique to characterise around 130 entries of Colombian collection of Musaceae. The AFLP analysis grouped accessions containing genomes AA and AAA. The highest similarity index was found within the Cavendish group. Accessions containing the ABB genome showed different degrees of similarity, allowing discrimination between Colombian accessions and those obtained from West Africa.

Molecular characterisation and chromosome location of repeated DNA sequences in *Hordeum* species and in the amphiploid tritordeum (*Tritordeum ascherson*) was carried out by Ferrer *et al.* (1995) using RFLP technique. The study demonstrated that species that shared a basic genome showed more similar hybridisation fragment patterns than species with different genomes. No interspecific differences were found between the diploid species. The taxonomic characterisation of annual Beta germplasm in a genetic resources collection using RAPD markers was reported by Shen *et al.* (1996) and Mandolino *et al.* (1996). Rossetto *et al.* (2002) examined the taxonomic relationship between *Vitis vinifera* and *Cayratia saponaria* using microsatellites and found sufficient inter-specific variation to distinguish the two vary closely related species. Xu *et al.* (2002) surveyed the variation in chloroplast DNA SSRs in wild and cultivated soybean accessions collected from various Asian countries. Analyses suggested that cultivated soybeans originated independently in different regions from different wild gene pools and/or hybrid swarm between cultivated and wild forms. Isshiki *et al.* (2003) used RFLP analysis of mitochondrial DNA in potato and six related *Solanum* species, for assessing phylogenetic relationships.

Genetic relationships among *Carica papaya* cultivars, breeding lines, unimproved germplasm, and related species were established using AFLP markers (Kim *et al.*, 2002). Cluster analysis of 71 papaya accessions and related species suggested limited genetic variation in papaya. *Carica papaya* shared the least genetic similarity with six other *Carica* species. The results from AFLP markers provided detailed estimates of the genetic variation within and among papaya cultivars, and supported the notion that *C. papaya* diverged from the rest of *Carica* species early in the evolution of this genus. Tang and Knapp (2003) carried out phylogenetic analysis

in sunflower accessions using microsatellite loci. The study revealed the possibility of multiple domestication origin of sunflower.

Microsatellite markers were used to detect polymorphism among different accession of *Oryza glaberrima* and *O. sativa* by Talag and Li (2001). The polymorphic markers were used to characterise the introgression lines generated from crosses of elite breeding lines of *O. sativa* with *O. glaberrima*. Thirty-one markers detected the introgression from *O. glaberrima* to *O. sativa*. The introgressed segments of *O. glaberrima* were found in homozygous as well as in heterozygous forms in these lines. To study genetic diversity and relationships of wild relatives of rice, 58 accessions of *Oryza rufipogon*, *O. nivara*, *O. sativa* f. *spontanea* and the cultivated *O. sativa*, representing a wide range of their distribution, were analyzed using the RFLP technique (Lu *et al.*, 2002). Considerable genetic diversity was scored among the *Oryza* accessions but the cluster analysis of the accessions did not show an apparent grouping based on the species classification, instead they were scattered randomly in different groups. The *Oryza* accessions from the same geographic region, or near-by geographic regions, tended to be clustered in the same groups. The *indica* rice varieties showed relatively high genetic diversity and were scattered in different groups of their wild relatives, but the *japonica* varieties showed a relatively low variation and formed an independent group. It was concluded that geographic isolation has played a significant role in the differentiation of the *Oryza* accessions; therefore, a wide geographic range needs to be covered in collecting wild rice germplasm for *ex situ* conservation.

Zeid *et al.* (2003) used AFLP markers to study the genetic diversity among a large set of inbred lines of recent elite faba bean (*Vicia faba*) cultivars with Asian, European (Northern and Southern) and North African origin. Based on clustering, only the Asian lines were distinct as a group, the other lines showed no marked further grouping. To evaluate the genetic structure of the Asian soybean population, Abe *et al.* (2003) analyzed SSR loci of 131 accessions introduced from 14 Asian countries. Cluster analysis clearly separated the Japanese from the Chinese accessions, suggesting that the Japanese and Chinese populations formed different germplasm pools. Relatively high genetic diversity and the absence of region-specific clusters in the southeast and south/central Asian populations suggested that soybean in these areas had been introduced repeatedly and independently from the diverse Chinese germplasm pool.

A total of 94 *Solanum* accessions, including eggplants and related species, were characterized by AFLP technique (Furini and Wunder, 2004). The analysis was efficient in the assignment of a species name for eight out of nine accessions that were not previously classified, and revealed that 14 further accessions were misnamed in the collection originally received. Offei *et al.* (2004) studied 70 cocoyam accessions collected from the eastern and Volta regions of Ghana using RAPD technique. The accessions did not cluster into their distinct geographical regions suggesting that there may have been movement of germplasm across the two regions. AFLP was used to investigate the genetic relationships among 96 tropical maize inbred lines from two different origins (Miranda Oliveira *et al.*, 2004). The

polymorphism level among the genotypes and the possibility of their allocation in heterotic groups were evaluated. The analysis showed AFLP to be a robust assay, revealing a great power of detection of genetic variability in the tropical germplasm, and also demonstrated to be very useful for guiding breeding programs.

The genetic relatedness among 118 globe artichoke accessions, including clones belonging to the same varietal type, two accessions of cultivated cardoon (*Cynara cardunculus* var. *altilis*) and four accessions of wild cardoon (*C. cardunculus* var. *sylvestris*) was measured using AFLP by Lanteri *et al.* (2004). They concluded that AFLP markers could be useful in evaluating *Cynara cardunculus* genetic diversity and in classifying accessions to phylogenetic groups based on their genetic similarity values. Genetic variation among artichoke clones belonging to the same varietal type was in some cases higher than that found among accessions differently named and coming from different areas. Awasthi *et al.* (2004) employed RAPD and ISSR techniques to study the genetic diversity and interrelationships among 12 domesticated and three wild mulberry species. Cluster analysis of RAPD and ISSR data resulted into two clusters, one comprising polyploid wild species and the other with domesticated (mostly diploid) species. The results suggested that RAPD and ISSR markers were useful for mulberry genetic diversity analysis and germplasm characterization, and that putative species-specific markers may be obtained which can be converted to SCARs after further studies. Thirty-four sequence-related amplified polymorphism (SRAP) primer combinations were used to differentiate diploid, tetraploid, pentaploid and hexaploid buffalograss (*Buchloe dactyloides*) genotypes, representing diverse locations of origin (Budak *et al.*, 2004). Cluster analysis indicated that there were eight clusters and the genotypes with potential traits for turfgrass improvement could readily be distinguished, based on SRAP.

Though earlier studies on the genus *Arachis* using RFLP and RAPD have revealed very little demonstrable polymorphism in the cultivated groundnut, Halward *et al.* (1992), Raina *et al.* (2001), Subramanian *et al.* (2000), and Bhagawat *et al.* (1997) reported polymorphic RAPD patterns in cultivated groundnut by using refined protocols (Figure 2). However, due to the inherent problems in the RAPD technique, these reports remained inconclusive. He and Prakash (1997), Radhakrishnan *et al.* (2002), and Dwivedi *et al.* (2001) have revealed some amount of polymorphism in cultivated groundnut using AFLP (Figure 3) and DAF. Later on the simple sequence repeat (SSR) primers, though a few, were employed in the detection of DNA polymorphism in cultivated groundnut (Plate I-B). However, the available information still remains to be insufficient for the cultivar identification and fingerprinting. Among the wild relatives of *Arachis* abundant polymorphism has been observed by RFLP (Kochert *et al.*,1991; Paik-Ro *et al.*, 1992) RAPD (Raina *et al.*, 2001; Dwivedi *et al.*, 2001; Lanham *et al.*, 1992; Mallikarjuna *et al.*, 2003a); AFLP (He and Prakash, 1997; Radhakrishnan *et al.*, 2002) and SSR (Hopkins *et al.*, 1999; He *et al., 2003*) (Figure 4). The DNA polymorphism apparent in the wild species of groundnut has been used by several authors to work out the species relationships. Many of the diploid species of *Arachis* has A-genome while *A. batizocoi* (Singh and Moss, 1982), *A. ipaensis, A. hoehnii, A. valida* and *A. magna* (Milla *et al.*, 2003)

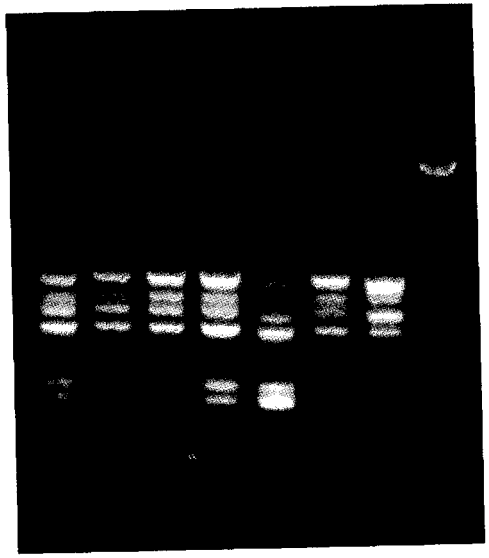

Figure 2. Agarose gel showing RAPD polymorphism in cultivated groundnut.

have B-genome and *A. glandulifera* has D-genome (Stalker and Moss, 1987). At least six diploid species (*A. villosa, A. cardenasii, A. batizocoi, A. ipaensis, A. duranensis* and *A. correntina*) have been proposed as putative genome donors to cultivated groundnut. Kochert *et al.* (1991, 1996) based on the evidence from the RFLP and cytogenetics had proposed *A. duranensis* as one of the progenitors of the cultivated groundnut. Using RFLP data, Galgaro *et al.* (1998) have shown that sections *Arachis* and *Extranervosae* form two clearly defined groups, and sections namely, *Heteranthae, Caulorrhizae* and *Triseminatae* form the third group. The results for RFLP analysis done by Burow *et al.* (1999) suggested that *A. batizocoi* is less closely-related to *A. hypogaea* than are the A-genome accessions. While the lower similarity suggests that it is unlikely that *A. batizocoi* is the B-genome donor to *A. hypogaea.* Based on comparative studies on distribution of two ribosomal gene families and occurrence of centromeric bands using fluorescent *in situ* hybridisation (FISH) and genomic *in situ* hybridisation (GISH), Raina and Mukai (1999a,b) proposed that *A. ipaensis* and *A. villosa* are the wild progenitors of *A. hypogaea* and *A. monticola.* From the data on RAPD, and ISSR, Raina *et al.* (2001) found that *A. villosa, A. ipaensis, A. hypogaea,* and *A. monticola* clustered together, and *A. duranensis* which is considered to be one of the potential A-genome donors clustered together with *A. cardenasii, A. valida, A. khulmanii* and *A. correntina* supporting their earlier observations. In AFLP analysis, species from section *Arachis* were grouped together with *A. glandulifera* showing distant relationship between *A. hypogaea* and the A- and B-genome species (Gimenes *et al.*, 2002). Species from section *Erectoides* grouped with *A. glabrata* (section *Rhizomatosae*) and *A. rigonii* (section *Procumbentes*) showed close relationship with *A. dardani* (section *Heteranthae*). Mallikarjuna *et al.* (2003a) studied 32 accessions of *Arachis*

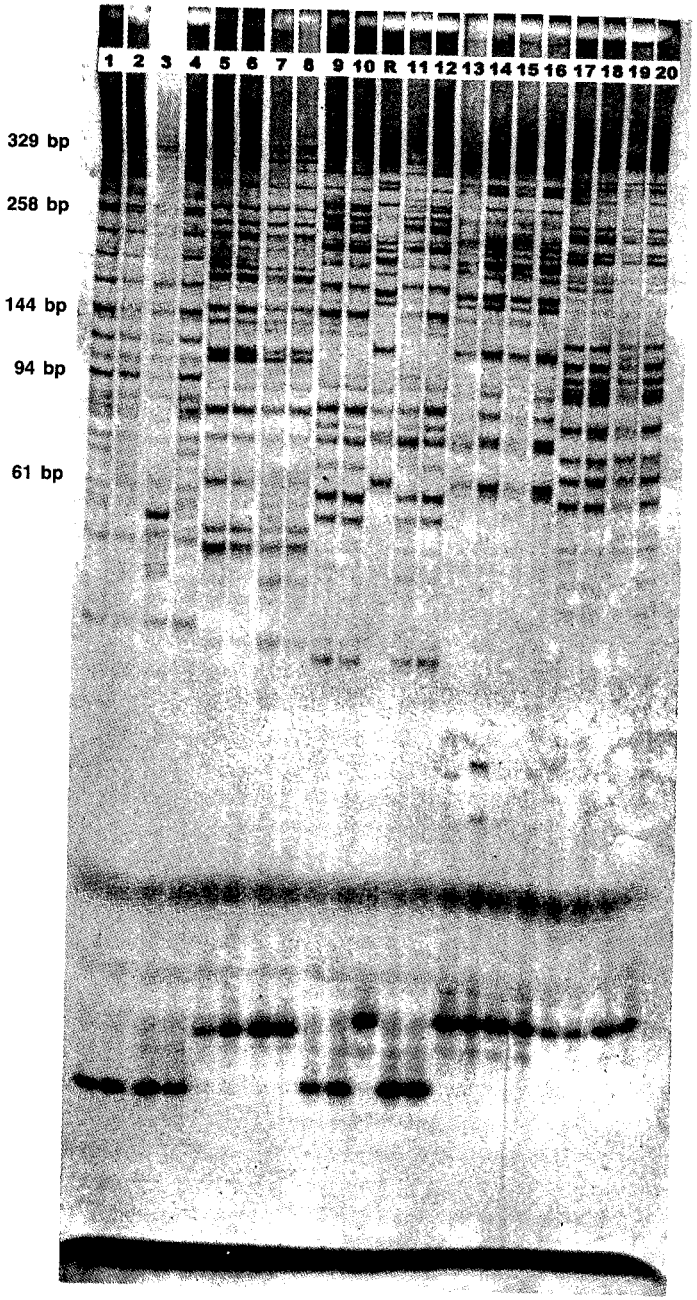

Figure 3. AFLP Gel (silver stained) showing DNA polymorphism in cultivated groundnut. 1=ICGS1 P3, 2=Kadiri3 P3, 3=ICGS 44 P3, 4=ICGS11 P3, 5= ICGS1 P50, 6= Kadiri3 P50, 7= ICGS44 P50, 8= ICGS11 P50, 9= ICGS1 P51, 10= Kadiri3 P51, R= Reference, 11= ICGS44 P51, 12= ICGS11 P51, 13= ICGS1 P52, 14= Kadiri3 P52, 15= ICGS44 P52, 16= ICGS11 P52, 17= ICGS1 P63, 18= Kadiri3 P63, 19= ICGS44 P63, 20= ICGS1 P63.

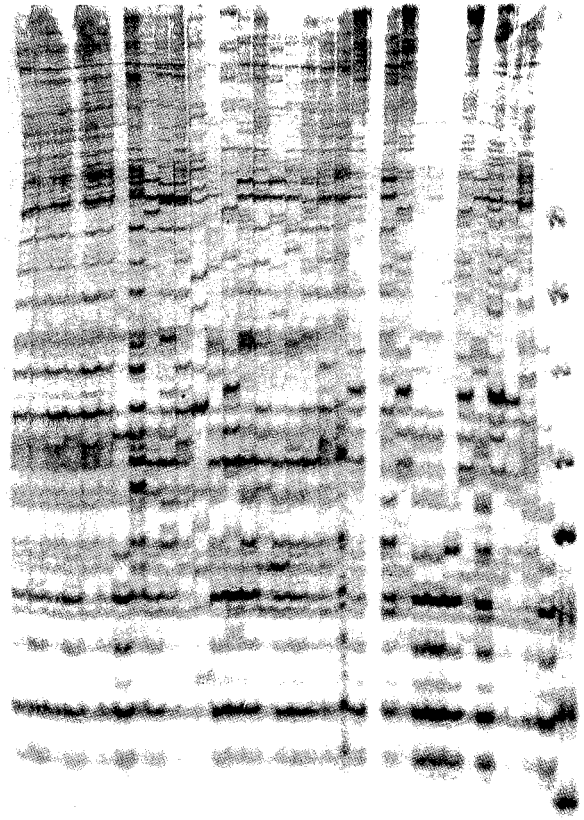

Figure 4. AFLP profile of cultivated and wild relatives of groundnut (Silver stained). *Arachis hypogaea, A. hypogaea* var. *hirsuta, A. hypogaea* var. *fastigiata, A. hypogaea* var. *peruviana, A. hypogaea* var. *aequatoriana, A. benensis, A. khulmanii, A. Stenosperma, A. batizocoi, A .batizogaea, A. cardenasii, A. Correntina, A. Cruziana, A. diogoi, A. duranensis, A. helodes, A. kempff-mercadoi, A. magna, A. monticola, A. villosa, A. cryptopotamica, A. hermanii, A. oteroi, A. paraguariensis, A. stenophylla, A. dardani, A. pusilla, A. appressipila, A. kretschmeri, A. rigonii, A. glabrata, A. pintoi.*

belonging to 25 wild species, and grouped under six sections including *A. hypogaea* using RAPDs. *Arachis hypogaea* grouped with *A. monticola,* a tetraploid wild species from section *Arachis*. *Arachis stenosperma* accessions were grouped together. Wild species from section *Arachis* with B-genome formed two clusters, with one cluster having *A. batizocoi* showing distant relationship and the other cluster with *A. hoehnii* showing close relationship. The D-genome accession, *A. glandulifera* remained apart. Though the grouping of most of the wild species were based on crossability (Mallikarjuna and Bramel, 2001; Mallikarjuna *et al.*, 2003b) and morphological characters (Krapovickas and Gregory, 1994) some of the accessions of

A. cardenasii (ICGS 11558 and 11559) from section *Arachis* did not group with any of the A-, B- and D-genome species of section *Arachis* and with each other. Hence, the conclusions based on these types of data on polymorphic DNA patterns alone may not be sufficient to make valid conclusions on phylogenetic relationships of groundnut species.

Some other examples where molecular markers have been used to work out taxonomic relationships include Brassica (Lazaro and Aguinagalde, 1996), taro (Irwin *et al.*, 1998), tomato (Noli *et al.*, 1999), *Malus sieversii* (Geibel *et al.*, 2000), banana (Pillay *et al.*, 2001), mango (Kashkush *et al.*, 2001), bambara groundnut (Amadou *et al.*, 2001; Massawe *et al.*, 2002), *Cicer* sp. (Iruela *et al.*, 2002), Sorghum (Nkongolo and Nsapato, 2003), sweet potato (Gichuki *et al.*, 2003), tea (Balasaravanan *et al.*, 2003) and chicory (van Cutsem *et al.*, 2003).

Fingerprinting of germplasm

Properly chosen DNA marker system could be individual specific, largely uniform within a variety and stable over environments. Molecular markers have been employed for fingerprinting, verification of accession identity and genetic contamination. Many DNA markers both specific as well as arbitrary have been used so far, for DNA fingerprinting of various classes of germplasm. For example, microsatellites were used to distinguish different cultivars of grapevine (Thomas *et al.*, 1994) and to compare landraces and develop unique DNA profiles of soybean genotypes (Rongwen *et al.*, 1995). Hongtrakul *et al.* (1997) and Fang *et al.* (1997) used molecular markers to fingerprint sunflower and trifoliate orange germplasm, respectively. Del Rio *et al.* (1997) and Wu *et al.* (1998) using RAPD technique studied the changes in genetic diversity following regeneration of potato and rapeseed accessions, respectively. The genetic identity of eight bread wheat accessions maintained in the Gatersleben gene bank and regenerated up to 24 times was studied for the verification of the integrity and genetic stability of gene bank accessions using wheat microsatellite markers (Borner *et al.*, 2000). It was demonstrated that microsatellite markers could be used to analyse bulks of seeds stored for more than 50 years in a seed reference collection at room temperature. No contamination due to foreign pollen or incorrect handling during the multiplication cycles was discovered.

Characterisation of plant varieties and germplasm was initiated at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi in 1986 under National Facility for Plant Tissue Culture Repository (NFPTCR). Later in 1996, National Research Centre on DNA Fingerprinting (NRC DNAF) was established and started working at the NBPGR. The NRC DNAF has been successful in developing molecular markers for cultivar identification in a broad spectrum of crops encompassing cereals, millets, pulses, oilseeds, fibre crops, vegetables and fruits. RAPD, AFLP, ISSR, STMS and anchored PCR are being used to detect variation, develop molecular fingerprints and analyse genetic diversity (Karihaloo *et al.*, 2001). Several varieties in important crops have been fingerprinted at the NRC DNAF (Table 3). Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis was also carried out at the NRC DNAF. The AFLP, with its superior marker utility, was concluded to be the marker of choice for cashew genetic analysis (Archak *et al.*, 2003).

Table 3. Varieties fingerprinted at NRC DNAF, New Delhi

Crop	*Varieties Fingerprinted*	*Techniques*
Cereals		
Rice	274	STMS, AFLP, RAPD
Wheat	153	STMS, AFLP, RAPD
Sorghum	65	STMS, AFLP, RAPD
Barley	54	STMS, RAPD
Millets		
Pearl millet	17	AFLP, RAPD
Pulses		
Chickpea	72	STMS, AFLP, ISSR, RAPD
Mungbean	96	AFLP, RAPD
Pigeon pea	60	AFLP, RAPD
Black gram	52	AFLP
Peas	35	AFLP
Lentil	65	AFLP
Oilseeds		
Niger	30	RAPD
Brassica	42	AFLP, ISSR, RAPD
Soybean	72	AFLP, RAPD
Safflower	14	AFLP
Sesame	67	AFLP, RAPD
Fiber		
Cotton	94	AFLP, RAPD
Vegetables		
Tomato	27	RAPD
Chillies	38	AFLP, ISSR, STMS, RAPD
Brinjal	19	ISSR, RAPD
Fruit and Nuts		
Banana	243	STMS, AFLP, RFLP, RAPD
Mango	23	AFLP, ISSR, RAPD
Citrus	34	AFLP, ISSR
Cashew	140	AFLP, ISSR, RAPD
Medicinal Plants		
Neem	69	AFLP, ISSR, RAPD
Vetiver	22	AFLP, RAPD
Saffron	13	AFLP
Total	1890	

(*Source*: http://nbpgr.delhi.nic.in)

Future prospects

The value of molecular biology for monitoring the genetic status of germplasm collections is increasingly felt in the recent years. The greater availability of the

molecular markers and its refinements offer greater prospects for accurately defining genetic resources in terms of genetic diversity. The large number and variability of accessions held usually determines the approach that can be employed in the plant genetic resources management. Further advances involving the characterisation of germplasm and the molecular markers are likely to arise from the new marker technologies. The development of EST libraries together with the most robust technologies like DNA chips and micro arrays can definitely take the characterisation of genetic recourses to new era. The potential for evaluating huge number of germplasm accurately with minimum efforts with these highly specific techniques can definitely make the germplasm conservation and management more easy, effective and economic. The use of techniques like SNPs will improve the efficiency of germplasm management by making the identification of duplicates in collections easy and increase the accuracy of taxonomic classifications of the accessions conserved.

A quick, simple but reliable molecular protocol must be combined with an appropriate strategy for handling large sample sizes. By facilitating better understanding of diversity, molecular marker techniques are proving extremely useful in identification of redundancies in germplasm collections, in testing accession stability and integrity, and in supporting the development of effective management strategies both for *ex situ* and *in situ* conservation. Molecular markers are also being increasingly used to support the crop improvement initiatives involving incorporation of useful and novel genes from the land races and wild relatives. With the advent of new IPR regime, molecular markers may be instrumental for addressing controversial issues faced by germplasm managers. Molecular characterisation of plant genetic resources does have enormous potential for optimising germplasm conservation and utilization, especially by providing the precise details of the genetic architecture of plant germplasm.

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