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DNA BASED MOLECULAR MARKERS IN PLANT VARIETAL IDENTIFICATION: A REVIEW

Manisha Sharma and *Apekshita Singh
Amity Institute of Biotechnology
Amity University Uttar Pradesh, Sector-125, Noida, UP, India
*Corresponding Author
Email:asingh20@amity.edu

Introduction

Recently, there has been a dramatic increase in crop yields leading to the development of new cultivars and varieties. A variety, of a cultivated crop species, differs from other varieties of the same species by one or more specific characteristics such as disease resistance, plant height, yield, maturity etc. Several molecular marker systems have been developed for distinguishing varieties or species of agricultural and horticultural importance. It is very important to define identity, purity and stability of varieties for breeders' rights protection institutions as well as for an effective seed quality control programme. Identification of varieties is indeed important as it not only helps in assessing purity, in measuring genetic diversity, improving germplasm for better agronomic performance and in delimiting germplasm duplicates. This can be achieved by simple morphological characters to biochemical identification and molecular markers.

Although majority of varieties are routinely described on the basis of morphological features of seed, seedling and mature plant. But now due to advancement made in the breeding programmes it is becoming difficult to distinguish genotypes solely on the basis of morphological features. Thus, there is need to look for other markers which can supplement the morphology based approach. DNA based molecular markers proves to be better approach as they detect high level of polymorphism. There is no dearth of molecular markers and they are being redesigned and modified to suit the rapid identification and detection of polymorphic content. Also they are unlimited in number, rapid and not influenced by tissue specificity and development stage. The following gives a general approach about the commonly used nucleotide markers for varietal identification.

RFLP: Restriction fragment length polymorphism refers to the occurrence of variation in length of DNA fragments that are produced after cleavage with type II restriction endonucleases. Differences in DNA lengths observed is due to the presence or absence of recognition site for that particular restriction enzyme. It shows codominant inheritance. RFLP markers were used to construct first molecular map of human genome (Botstein *et al*. 1980). Six years later, the first linkage map in plants were developed for maize and tomato (Hlentjaris *et al* 1986). Mc Couch *et al*. (1998) constructed RFLP genetic map of rice which covered 1.389 cM of rice genome. RFLP technique also helps in detecting naturally occurring variations between plants and uses them as genetic markers which can be followed during progeny analysis in the same manner as conventional markers. Dallas (1988) was able to distinguish among different rice cultivars, *Oryza sativa*, by hybridizing restriction-digested rice DNA with the human 33.6 minisatellite probe. In plants, RFLP will remain as most widely accepted technique and has been used for making detailed map of major crops like potao, wheat and maize. But its two limitations has motivated the development of several alternative technologies which are mostly PCR based. The first major limitation is large amount of DNA(50-200ug) of highly purified DNA of individuals is necessary to generate fingerprint. The second limitation is that closely related species usually contain same alleles. Other limitations include prior knowledge of DNA sequence, cloned and characterised probes and involves use of labeling method as radioactive or fluorescent.

The probes could be low copy sequences derived from the genomic DNA clones, specific gene probes of nuclear, mitochondrial, and chloroplast genomeorigin, and characterized or uncharacterized cDNAs. The usage of microsatellites (oligofinger printing) and multigene families as probes ismost common in deciphering polymorphism in nuclear genomes. CodominantRFLP markers are highly reproducible.

PCR -RFLP or CAPs (Cleaved amplified polymorphic sequence): The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method is based on the digestion of PCR amplicons with appropriate restriction enzymes to produce distinct polymorphic fragments used as markers. These are co-dominant in nature. It is highly advantageous as a number of universal primers such as ITS (internal transcribed region in rDNA) region, choloroplast gene probes or any intron region probe can be used. Gheriyaze *et al*. (1995) used in identifying Iranian rice varieties, Biswas (2013) used this technique in medicinal plants.

RLGS: Restriction Landmark Genomic Scanning (RLGS)- Developed by Hayashizaki *et al*. (1993). It can be

used in two dimensional electrophoresis It employs two-dimensional electrophoresis (2DE) of genomic DNA, which allows visualization of thousands of loci.Noguchi *et al*. (2017) used this technique to distinguish 'Juncus' cultivars. Takamiya *et al*. (2009) in rice cultivars and hybrids**.**

RAPD: Random amplified polymorphic DNA is a technique which utilizes single, short symthetic oligonucleotide primers for Polymerase chain reaction (PCR). The prime whose sequence has been chosen at random initiates replication at its complementary sites on the DNA producing fragments upto about 2kb long which can be separated by markers and thus polymorphism between individuals is defined as the presence or absence of a particular band. The widely used RAPD analysis (Williams *et al.,* 1990, Welsch and Mc Clelland 1990) relies on a single 10bp primer of largely arbitrary sequence except that primers are selected to have 60% or more G+C content to obtain stronger binding to the template. This technique was widely used to fingerprint a wide range of species from microorganisms (Welsch and Mc Clelland 1900). In plants, it was used to identify wheat, corn and soyabean(Williams *et al*., 1990) cultivars. RAPD marker system has found widespread use and posseses a no. of very positive features being multiplex and universal system. RAPD was found to have same resolving power as RFLP while determining genetic relationships between lines of oilseed rape (Halden *et al*., 1994). RAPD has thus alleviated some problems associated with RFLP and has been widely used in screening DNA sequence based polymorphisms at a large no. of loci, because it requires small amount of DNA (15-25 ng), non radioactive assay, can be performed in several hours.

The further development of RAPD methodology has produced other PCR based markers: SCARs (sequence characterised amplified regions) which involves sequencing its termini and designing longer primers (24 nucleotides) for specific amplification of markers (Paran and Michelmore 1993), ASAPs (allele specific associated primers which involve amplification of DNA template in microtitter plates generating only a single DNA fragment at stringent annealing temperatures (Gu *et al*., 1995). Report the application of RAPD markers for the identification of broccoli and cauliflower cultivars (Hu and Quiros, 1991).

RAPD technique has been used successfully for genotyped identification in ornamental (Torres *et al*., 1993; Wolff *et al*., 1995). Genotyping of cowpea varieties by RAPD markers revealed some DNA bands specific to higher or lower nitrogen-fixing varieties in this crop (Fall *et al*., 2003).

An analogue of RAPD, UP-PCR has been widely used to reveal polymorphism in fungi and bacterial species (Bulat *et al*., 1996). The technique consists of DNA amplification with a single universal random primer that is16 to 20 nucleotides long. In comparison to RAPD (34°-38°C), the annealing temperature is higher (55°C) in this marker system. The universal primers that have been utilized are 45, 3-2, 0.3.1, and AS15, whose sequences are described elsewhere (Bulat *et al*., 1996). Random primers 10 to 15 nucleotides in length are used to amplify target DNA under low stringency conditions for two amplification cycles (Welsh and McClelland 1990). Increased stringency of annealing at later cycles of amplification generates reproducible products, which are resolvable by PAGE and detected by autoradiography. Numerous amplicons are generated using arbitrary primers merely five nucleotides in length (Caetano-Anollés *et al*., 1991). The amplified fragments are resolved with PAGE only.

Using arbitrary Primers RAF is the most effective technique developed by Waldron *et al*. (2002). RAF (randomly amplified DNA Fingerprinting) is a modification of DAF (DNA Amplification fingerprinting), which utilizes 10mer primers in the hot start (85°C) PCR followed by touchdown PCR insubsequent cycles. RAF was used in wheat, tomato, soyabean cultivars studies. RAF is more robust. The radiolabeled PCR products are separated on polyacrylamide gel and detected. Use of fluorescein-tagged oligonucleotide (fluorescent RAF) can be an alternative to radiolabeling. The technique has the highest marker index, equivalent to that of the AFLP marker system. RAF successfully used in Macademia (Peace *et al*., 2003).

AFLP: Amplified fragment length polymorphism was designed (Zabeau and Vos, 1993; Vos *et al*., 1995) as anucleic acid fingerprinting method to exploit molecular genetic variations existing between closely related genomes in the form of RFLPs. It represented an ingenious combination of RFLP and PCR methodology. AFLP analyses became soon very popular, mainly because of the large numbers of polymorphic bands obtained in a single experiment. The number of fingerprints observed is 10-100 fold greater with AFLP than with other commonly employed methods. This technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. It involves three steps: i) restriction of DNA and ligation of oligonucleotide adaptors ii) selective amplification of sets of restriction fragments, iii) gel analysis of the amplified fragments. Powell *et al*. (1996) showed AFLP technique as a better approach for cultivar identification. AFLP provides large number of markers in a single analysis without requiring sequence information for their development (Vos *et al*., 1996).

Although reagents and equipments are expensive initially, but when brought upto scale in large population studies they are lower in costs to RFLP analysis. AFLP has been employed in genetic analysis of many ornamental crops like rose (Hubbard *et al*., 1992) and chrysanthemum (Wolff *et al*., 1994).

Selective Amplification of Microsatellite Polymorphic Loci (SAMPL)-The SAMPL technique, introduced by Morgante and Vogel (1994), is a microsatellite-based modification of AFLP. It involves construction of a preamplified library in the same way as in AFLP. Selective amplification, however, utilizes an AFLP primer in combination with amicrosatellite-based SAMPL primer. Since one of the primers utilized in this technique involves amicrosatellite primer and the steps involved are the same as in AFLP, the technique is also referred to as microsatellite fragment length polymorphism (MFLP). The SAMPL primer commonly utilized is a compound SSR, 18 to20 nucleotides in length. Primers with a single SSR anchored at the 5' end with a nonmicrosatellite sequence have allowed the amplification of different kinds of tri-, tetra-, and pentanucleotide repeats in addition to compound microsatellites (Rakoczy-Trojanowska and Bolibok, 2004).

Use of SAMPL has been done in identification and genetic analyses of Lactuca sativa cultivars (Witsenboe *et al*.,

1997). Sarwat *et al*. (2007) used SAMPL in Tribulus, a medicinal herb for identifying geographically distinct samples.

DAMD: The direct amplification of minisatellite DNA (DAMD) utilizes primers that are specific for minisatellites rather than microsatellites (Zhou *et al*., 1997). Minisatellites are VNTRs (variable number of tandem repeats) in genome 16-64 bp in length. DAMD was used in rice cultivars. Metais *et al*. (2000) used the technique in *Phaseolus vulgaris* commercial lines.

DALP: Direct amplification of length polymorphism

The technique developed by Desmarais *et al*. (1998) has the advantage of a high-resolution fingerprint technique with the possibility of characterizing the polymorphism more effectively. The technique uses modified Arbitrary Primed PCR to produce genomic fingerprints and enables sequencing of DNA polymorphisms in virtually any species. Two primers are utilized; the selective primer is composed of universal M13 sequencing primer as the core sequence followed by an arbitrary sequence; the reverse primer is universalM13 sequencing primer alone. Such pairs are efficient in producing multiband patterns where inter individual length variations, including microsatellite polymorphism, can be detected. The amplification products are detected on denaturing polyacrylamide gels. Because of the special design of the selective primers, each band of different patterns can be sequenced with the universal sequencing primers, no matter which selective primer was used in AP-PCR. Cui *et al*. (2014) used in *Panax t*o distinguish populations.

Micro-satellites: Simple sequence repeats (SSRs), Simple sequence length polymorphism (SSLPs), short tandem repeats (STRs), sequence tagged microsatellites (STMs) is a class of repetitive sequences which are widely distributed in all eukaryotic genomes. They comprise of 2-5 bp monomeric repeat units. Variable number of repeats between individuals is a result of slippage of the DNA Polymerase during DNA replication (Tautz *et al.,* 1989).This length variation is a source of polymorphism even between closely related individuals. Such microsatellites sequences can be easily amplified by PCR using a pair of flanking locus specific oligonucleotides as primers and detect DNA length polymorphism (Litt and Luty, 1989, Weber and May, 1989). These are long PCR primers which are specific to single genetic locus they are codominant and most importantly they are multiallelic and detect a much higher level of DNA polymorphism than any other marker system. Microsatellites were used as genetic markers for genetic mapping of eukaryotes in early 90's (Beckmann and Soller). In plants, Akkaya *et al*. (1992) reported the use of SSR technique fingerprinting species. Conditt and Hubell (1991) were the first one to publish the study of microsatellite and their abundance in plant species. It is considered to be the most powerful technique for identification and variety protection. There is a large cost involved in primer identification of SSRs and still they have been used in major crops like corn, soybean, wheat and rice. Twenty-one random and 29 SSR primers were used to assess genetic variation and interrelationships among subspecies and botanical varieties of cultivated peanut, *Arachis hypogaea* ($2n = 4x = 40$) (Raina *et al*. 2001).

Inter simple sequence repeats (ISSR): The technique is similar to MP-PCR but utilizes anchored (5' or 3' anchored) SSR primers (Zietkiewicz *et al*., 1994). Different scientists have variously called this technique anchored microsatellite primed PCR (AMPPCR), inter-microsatellite PCR (IM-PCR), inter-SSR amplification (ISA), or anchored simple sequence repeats (ASSR) (Gupta and Varshney, 2000).The inter-simple sequence repeat (ISSR) method developed by Gupta *et al*. (1994) and Zietkiewicz *et al*. 1994) relied on microsatellite-complementary PCR primers that could be used in an anchored or unanchored version. It amplifies the region between two SSRs.

ISSRs have been used for cultivar identification in a number of species such as in 34 potato cultivars (Prevost *et al*., 1999), in commercial cultivars of bean (Metais *et al*., 2000). Brick and Sivolap,(2001) used ISSR in soyabean cultivar distinction.

Twenty-four commercial of *Phaseolus vulgaris* have been evaluated by RFLP, directed amplification of minisatellite DNA- PCR (DAMD-PCR), ISSR, and RAPD marker analysis (Métais *et al*.2000).

Sequence-Related Amplification Polymorphism (SRAP): Li and Quiros (2001) developed this technique for amplification of open reading frames (ORFs) using pairs of primers with AT- and GC-rich cores. The forward and reverse primers are 17 or 18 nucleotides long. The core sequence or filler sequence is 13 to 14 bases long in both the primers, followedby sequence CCGG in the forward primer and AATT in the reverse primer. The core is followed by three selective nucleotides at the 3' end. The purpose of using CCGG and AATT sequences in the core sequence of forward and reverse primer, respectively, is to target exons and introns (exonsare normally rich in GC-rich regions while introns and promoters are rich inAT-rich regions). The amplified DNA fragments are separated on denaturing acrylamide gels and detected by autoradiography. It was successfully used in varietal identification in Brassica oleracea. SRAPs were also easily amplified in other crops such as potato, rice, lettuce, Chinese cabbage (*Brassica rapa* L.), rapeseed (*Brassica napus* L.) etc.

Target Region Amplification Polymorphism (TRAP):- The technique developed by Hu and Vick (2003) uses two primers of 18 nucleotides in length to generate polymorphic markers around targeted genes. One of the primers, the fixed primer, is designed from the targeted EST sequence in the database (partial sequence of a candidate gene); the second primer is an arbitrary sequence with either an AT- or GC-rich core to anneal with an intron or exon, respectively. For different plant species, each PCR reaction can generate as many as 50 scorable fragments on a polyacrylamide sequencing gel. The technique is useful in tagging genes governing desirable agronomic traits of crop plants. Like RAPDs, TRAP markers are quick and easy to set up, and each reaction can produce a profile comparable to AFLP or RAF marker systems.

Common features of SRAP and TRAP (target region amplification polymorphism (TRAP) method) include the use of two primers of about 18 nucleotides length (one of which targets a protein-coding region), and non-stringent PCR conditions during the first five cycles.

Random Amplified Microsatellite Polymorphisms (RAMPs)-Amplification is performed using two primers, a 5'-anchored SSR primer and a RAPD primer (Wu *et al*., 1994). Amplified products resolve length differences due to the SSR target itself or the sequence between two primers. The digestion of amplification products with restriction enzymes produces digested RAMPs (dRAMPs; Becker and Heun, 1995).RAMPs used to distinguish closed lines of capsicum cultivars (Ki Min *et al*., 1998).

Random amplified hybridization microsatellites (RAHM): Genomic DNA is amplified with a single arbitrary primer (usually 10-mer as in RAPD). The gel containing separated products is blotted. The membrane is subsequently hybridized with microsatellite probes to produce fingerprints that are microsatellite based (Richardson *et al*., 1995).Cifarelli *et al*. (1995) called this technique random amplified hybridization microsatellites (RAHM) while Ender *et al*. (1996) termed it randomly amplified microsatellite sequences (RAMS).

EST: Expressed Sequence Tags. ESTs are typically unedited, single-read sequences produced from cDNAs. Currently, they are the most widely sequenced nucleotide commodity from plant genomes in terms of the number of sequences and the totalnucleotide count. Swelling EST databases archive all the available ESTs and provide methods to search for individual sequences on the basis of species, clone, or homology attributes. The molecular markers that have been developed by exploiting EST databases include EST-SSRs, EST-SNPs, ESTP, COS, and TRAP.

EST-SSRs: Amodest 1 to 5 percent of the ESTs in various plant species have been found to have SSRs of suitable length for marker development (Eujayl *et al*., 2004). EST-SSRs also have a high probability of being associated with gene expression and gene function and thus are capable of assaying polymorphism carried by coding regions of the genome. EST- and cDNA-derived SSRs have several important advantages over anonymous markers (Varshney *et al*. 2005). First, developing markers from already existing sequences is easy, fast and economical. Any type of microsatellite will be detected, whereas only SSRs with predefined motifs are captured by enrichment strategies. Also, EST-SSRs are physically linked to an expressed gene, which may encodea trait of interest. They can be transferred across taxa as primer target sequences that reside in transcribed DNA regions that are expected to be relatively conserved. Sometimes, there may be limited polymorphism by EST SSRs due to its association with coding regions, resulting in fewer alleles and/or lower observed heterozygosity (Thiel *et al*. 2003), but not always.

Expressed Sequence Tag Polymorphism (ESTP): The technique involves designing primers separated by an amplifiable EST segment, and using these primers for PCR amplification of genomic DNA.

Single Nucleotide Polymorphism (SNP): Polymorphism corresponding to differences at a single nucleotide position (substitution, deletion or insertion-indels) occur approximately every 1.3kb pairs (Cooper *et al*., 1985; Kwok *et al*., 1996) and are referred to as SNPs. Within the coding regions, an SNP is either nonsynonymous and results in an amino acid sequence change, or it is synonymous and does not alter the amino acid sequence (neutral SNP; Soleimani *et al*., 2003).

They are the most common class for detection of the smallest unit of genetic variation among individuals within a species and are usually bi-allelic variations between individuals that occur in genes (promoters, exons, or introns) or between genes (intergenic) (Bhattramakki *et al*., 2000; Rafalski, 2002; Vignal *et al*., 2002). Various gel and non gel based method are used for detection of already characterized SNPs. This will be facilitated due to automation and high throughput approach which are already available for work on SNP. Matrix assisted Laser Desorption/Ionisation Time-of-flight (MALDI TOF) mass spectroscopy is becoming popular for SNP genotyping.

Single nucleotide polymorphisms (SNPs)are the most abundant sequence variations found in plant genomes and are widely used as molecular genetic markers in genetic linkage mapping (Troggio *et al*., 2007), diversity analysis (Ravel *et al*., 2006), cultivar identification (Dong *et al*., 2010; Jiang *et al*., 2010; Li *et al*., 2010) and marker assisted selection (Bang *et al*., 2007).

Development of SNP markers from EST databases relies upon the underlying redundancy within EST collections. SNPs can be searched, either in the individual specific ESTs from different accessions of the same species or in the contigs. In the former case, EST sequences derived from different genotypes can be aligned for the identification of SNPs. To date, a large number of SNP mining tools are available to automate the process of SNP discovery.

SNPs in single copy number regions, not part of genes may also be detected. In general, primers are equipped at their 5′-ends with non-templated extensions designed to bind sequencing primers. This approach is quite straightforward, if the individuals to be genotyped by sequencing are homozygous. In that case individual polymorphisms are easy to detect, and haplotypes could be unambiguously determined. SNP detection is linked to haplotype, which is defined here as a combination of alleles at closely linked loci that tend to be inherited together. In the case of heterozygous individuals, individual SNPs can still be detected, although indels cause difficulties. Also, haplotypes could not be unambiguously determined except by cloning the PCR products to isolate individual haplotypes. Fang *et al*. (2014) used 60 SNP markers from EST database in 40 varieties *Camilla sinensis*. See *et al*. (1998) used in barley cultivar identification. Zhang *et al*. (2020) used SNP approach in soyabean cultivars.

Cleaved amplified polymorphic sequence (CAPS) analysis is the most widely used approach for the detection of single nucleotide polymorphisms. However, this technique is limited to mutations, which create or disrupt a restriction enzyme recognition site. A modification of CAPS, termed derived CAPS (dCAPS), eliminates the need for SNP to fall within a restriction site. Rather, a restriction enzyme recognition site, which includes the SNP, is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The PCR product thus modified is subjected to restriction digestion, and the presence or absence of SNP is determined by the restriction pattern (Neff *et al*., 1998). A host of restriction enzyme-independent SNP assays have been developed such as allele-specific PCR, SSCP, primer-directed nucleotide incorporation assays, dideoxy fingerprinting, and oligonucleotide fluorescence quenching assays.

Retrotransposon based markers

Sequence Specific Amplified Polymorphism (SSAP)

The first retrotransposon-based method to be developed (Waugh *et al*., 1997) is a modification of AFLP. The amplified products are between a retrotransposon integration site and a restriction site. Restriction digestion of genomic DNA, adaptor ligation, and preamplification PCR are steps similar to AFLP. Two primers are used in selective amplification; one of them is a normal AFLP primer and the other is an LTR primer based on the sequence of LTR or sequence of polypurine tract (if LTR is very small; Schulman *et al*., 2004) region adjacent to LTR.

S-SAP often produces highly variable fingerprints that are frequently more informative than AFLP. The technique detects two retrotransposons or LTRs sufficiently close to one another in the genome to permit PCR amplification of the intervening region utilizing outward-facing LTR primers (Kalendar *et al*., 1999). The amplification products are resolved generally by agarose gel electrophoresis. In case of labeled primers, a sequencing gel system may be employed.Ellis *et al*. (1998) studied SSAP in 15 cultivars of *Pisum.* Similarly, Syed *et al*. (2005) used SSAP among other markers in population analysis in *Lectuca* cultivar interspecific crosses.

Inter-Retrotransposon Amplified Polymorphism (IRAP): In the inter retrotransposon amplified polymorphism (IRAP) approach developed by Kalendar *et al*. 1999, primers aredirected towards the LTRs of BARE-1, a retrotransposon of barley. These authors also introduced retrotransposo nmicrosatellite amplified polymorphism (REMAP) which combines outward-facing LTR-specific primers with anchored microsatellite primers. The same strategy, known as copia-SSR, was simultaneously developed by Provan *et al*. (1999).

Retrotransposon Microsatellite Amplified Polymorphism (REMAP) and Exon Retrotransposon Amplification Polymorphism (ERAP):

REMAP uses an outward-facing LTR primer in conjunction with an SSR primer containing a set of repeats and one or more non repeat bases atthe 3' end to serve as an anchor. The primer allows amplification if retrotransposonor LTR derivatives are sufficiently close to SSR (Kalendar *et al*., 1999). ERAP utilizes an LTR primer in conjunction with a gene specific primer. The retrotransposons sufficiently close to gene regions are thus amplified.

Retrotransposon Based Insertion Polymorphism (RBIP)

This is a single-locus, codominant marker technique, that detects retrotransposon insertions using primers flanking the insertion site and primers from the insertion (LTR) itself (Flavell *et al*., 1998). The size of the PCR product indicates which allele (occupied by retrotransposon or unoccupied) has been amplified. Because retrotransposon insertions are thousands of bases in length, the unoccupied-site PCR (complementary PCR reaction using host-specific primers) produces no product from an occupied site.

RBIP has been used in rice (Vitte *et al*., 2004) to analyse subspecies and varieties.

Inter-MITE Polymorphism (IMP)

The technique developed by Chang *et al*. (2001) involves amplification between two adjacent MITEs. To generate IMPs, primers are designed from consensus sequences of terminal inverted repeats of MITEs. Many families of MITEs such as *Stowaway, Tourist,* and *Barfly* have generally been utilized to design outwardly directed MITE primers. These retrotransposon based methods have been exploited in pea germplasm collection containing thousands of samples (Jing *et al*.2010).

Conclusion

The DNA based markers have now become an important component in resolving issues of variety identity. These have the ability to provide an improved method to identify and chracterise the products of plant breeding. The DNA based data can also help in providing intellectual property protection. Earlier genetic maps consisted of only phenotypic markers and/or isoenzymes and these maps consisted of no more than few markers per chromosome. With the advancement of DNA marker technologies, unlimited markers were available for construction of genetic maps. Most traits of agronomic and economic importance are classified as multigenic or quantitative. Molecular markers have been use to identify quantitative trait loci (QTL) which enhance the efficiency of selecting complex trait in plant breeding. Molecular markers can provide information that can help define the distinctiveness of species and their ranking according to the number of close relatives and their ranking according to the phylogenetic positions. Now, majority of assays involves non gel based systems. Molecular beacons (Tyagi *et al.,* 1998, Coy *et al*., 1999) which are hair pin shaped hybridization probes that can be used to monitor PCR product formation with fluorescence, either during or after the amplification process. In near future, the microchip based hybridization systems will become a common place.

The cultivars and varieties have less genetic variation detectable using conserved genetic markers. So new and varied techniques keep on developing. They also have much to offer to the resolution of problems concerning hybridization and polyploidy also. Thus, DNA marker field will continue to be exploited in near future.

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