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Molecular markers: Role in plant sciences

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Abstract
Recently, considerable emphasis has been placed on the development of molecular marker technology to be used for a variety of applications including in both basic plant research and plant breeding programs. Various types of techniques are used to estimate genetic diversity such as dominant markers (Random Amplified Polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), Arbitrarily primed polymerase chain reaction (APPCR), Inter-simple sequence repeat (ISSR) and Amplified Fragment Length Polymorphism (AFLP), and co-dominant markers (Restriction Fragment Length Polymorphism (RFLP), Simple sequence repeats (SSRs), Sequence characterized amplified regions (SCARs), Cleaved amplified polymorphic sequence (CAPS), Expressed sequence tags (ESTs), Single Nucleotide Polymorphisms (SNPs) and sequence tagged sites (STSs). These markers can be used to study the evolutionary relationships among individuals. In this article we attempt to review most of the available DNA markers that can be routinely employed in various aspects of plant genome analysis such as characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding, and diagnostics.

Keywords: markers, DNA, RAPD, AFLP, RFLP

Introduction
Triclosan (TCS) [5-chloro-2-(2,4-dichlorophenoxy) phenol], is a typical chemical in Genetic markers are variants in the DNA that are associated with a specific disease phenotype revealing variations. DNA Marker technology has revolutionized the world of genetic research. These markers can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a gene pool. Markers whose presence confers a high level of probability of disease would be most useful as diagnostic tools. A marker may have functional consequences, such as altering the expression or function of a gene that directly contributes to development of disease. Alternatively, a marker may have no functional consequences but may be located near a functional variant such that both the marker and variant tend to be inherited together in the population at large. This review article deals on the basic principles, requirements, advantages, and disadvantages of the most widely used molecular markers developed during the last two decades of molecular biology research and utilized for various applications in the field of biological science especially in crop improvement.

Genetic markers
A genetic marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like mini satellites.

Types of Genetic markers
1. Morphological markers
2. Protein (biochemical) markers
3. DNA (molecular) markers

1. Morphological markers
The use of morphological marker techniques as an important tool to select the plants with desired traits had started in breeding long time ago. During the evolution of plant breeding, the markers used mainly included visible traits, such as leaf shape, flower proposed uses of molecular markers into crop color, pubescence color, pod color, seed color, seed shape, awn

Type and length, fruit shape, flesh color, stem length etc. However, morphological determinations need to be taken by an expert in the species, they are subject to changes due to environmental factors and may vary at different developmental stages and their number is limited.

2. Protein (biochemical) markers

Protein markers may also be categorized into molecular markers though the latter are more referred to DNA markers. Isozymes are alternative forms or structural variants of an enzyme that have different molecular weights and electrophoretic mobility but have the same catalytic activity or function. Isozymes reflect the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution (Xu, 2010) [41]. Therefore, isozyme markers can be genetically mapped onto chromosomes and then used as genetic markers to map other genes. They are also used in seed purity test and occasionally in plant breeding. There are only a small number of isozymes in most crop species and some of them can be identified only with a specific strain. Therefore, the use of enzyme markers is limited.

3. Molecular markers

DNA markers are defined as a fragment of DNA revealing mutations/variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a par- ticular sequence of DNA in a population or gene pool. Such fragments are associated with a certain location within the genome and may be detected by means of certain molecular tech- nology. Simply speaking, DNA marker is a small region of DNA sequence showing poly-morphism (base deletion, insertion and substitution) between different individuals. There are two basic methods to detect the polymorphism: Southern blotting, a nuclear acid hybridization technique (Southern 1975) [37], and PCR, a polymerase chain reaction technique (Mullis, 1990) [26]. Using PCR and/or molecular hybridization followed by electrophoresis (e.g. PAGE – polyacrylamide gel electrophoresis, AGE – agarose gel electrophoresis, CE – capillary electrophoresis), the variation in DNA samples or polymorphism for a specific region of DNA sequence can be identified based on the product features, such as band size and mobility. DNA markers are also called molecular markers in many cases and play a major role in molecular breeding. Therefore, molecular markers in this article are mainly referred to as DNA markers except specific definitions are given, although isozymes and protein markers are also molecular markers.

An ideal molecular marker must have some desirable properties.

1. Highly polymorphic nature: It must be polymorphic as it is polymorphism that is measured for genetic diversity studies.
2. Codominant inheritance: determination of homo-zygous and heterozygous states of diploid organisms.
3. Frequent occurrence in genome: A marker should be evenly and frequently distributed throughout the genome.
4. Selective neutral behaviours: The DNA sequences of any organism are neutral to environmental conditions or management practices.
5. Easy access (availability): It should be easy, fast and cheap to detect.
6. Easy and fast assay
7. High reproducibility
8. Easy exchange of data between laboratories.

It is extremely difficult for a single genetic marker to possess all properties above. Depending on the type of study to be undertaken a marker system can be identified that would fulfill at least a few of the above characteristics.

a) Types and description of DNA markers

i. Non - PCR based genetic markers

a. RFLP(Restriction fragment length polymorphism)

ii. PCR based genetic markers

a. RAPD (Random Amplified Polymorphic DNA)
b. SSR (Simple Sequence Repeat)
c. SCAR (Sequence characterized amplified regions)
d. CAPS (Cleaved amplified polymorphic sequence)
e. ISSR (Inter-simple sequence repeats)
f. AFLP (Amplified fragment length polymorphism)

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) analysis was one of the first techniques to be widely used for detecting variation at the DNA sequence level. RFLPs are inherited naturally occurring mendelian characters. The principle behind the technology rests on the possibility of comparing band profiles generated after restriction enzyme digestion in DNA molecules of different individuals. Diverse mutations that might have occurred affect DNA molecules in different ways, producing fragments of variable lengths. These differences in fragment lengths can be seen after gel electrophoresis, hybridization (Southern, 1975) [37] and visualization.

Advantages of RFLP markers

1. RFLP markers were used for constructing genetic maps.
2. RFLPs are codominant and reliable markers in linkage analysis, breeding.
3. Can be easily determined in homozygous or heterozygous state of an individual.

Disadvantages of RFLP markers

1. The large amount of DNA required for restriction digestion and Southern blotting.
2. Expensive, time-consuming and hazardous.
3. Only one marker may be polymorphic, which is highly inconvenient especially for crosses between closely-related species and their inability to detect point mutations and polymorphism (Botstein *et al*. 1980; Winter and Kahl 1995) [4, 40].

Random amplified polymorphic DNA (RAPD) technique

A Random Amplified Polymorphic DNA (RAPD) technique is based on the polymerase chain reaction and has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary (10 bases) oligonucleotide primers. With this technique, there is no specific target DNA, so each particular primer will adhere to the template DNA randomly. As a result, the nature of the obtained products will be unknown. The DNA fragments generated are then separated and detected by gel electrophoresis. RAPDs can be detected by running PCR products through electrophoresis on an agarose or acrylamide

gel. In both cases, the gel is stained with ethidium bromide. The difference obtained by running RAPD products in acrylamide versus agarose lies only in the degree of resolution of bands. In most cases, agarose gel electrophoresis gives sufficient resolution.

Advantages of RAPDs

1. High number of fragments are generated.
2. It is Simple.
3. Arbitrary primers are easily purchased, with no need for initial genetic or genomic information. Only tiny quantities of target DNA are required.
4. Unit costs per assay are low.

Disadvantages of RAPDs

1. RAPD markers are dominant. Amplification either occurs at a locus or it does not, leading to scores based on band presence or absence. This means that homozygotes and heterozygotes cannot be distinguished. In addition, the absence of a band through lack of a target sequence cannot be distinguished from that occurring through the lack of amplification for other reasons (e.g. poor quality DNA), contributing to ambiguity in the interpretation of results.
2. Nothing is known about the identity of the amplification products unless the studies are supported by pedigree analysis.
3. Problems with reproducibility result as RAPD suffers from sensitivity to changes in the quality of DNA, PCR components and PCR conditions, resulting in changes of the amplified fragments. Reproducible results may be obtained if care is taken to standardize the conditions used.
4. The presence of a band of identical molecular weight in different individuals is not evidence per se that the individuals share the same (homologous) DNA fragment.
5. A band detected on a gel as being single can comprise different amplification products. This is because the type of gel electrophoresis used, while able to separate DNA quantitatively (i.e. according to size), cannot separate equal-sized fragments qualitatively (i.e. according to base sequence).

Inter simple sequence repeat (ISSR)

Inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. These are ideal DNA markers for genetic mapping and population studies because of their abundance. ISSRs have high reproducibility possibly due to the use of longer primers (16–25 mers) as compared to RAPD primers (10 mers) which permits the subsequent use of high annealing temperature (45– 60 ◦ C) leading to higher stringency. ISSRs segregate mostly as dominant markers following simple Mendelian inheritance. However, they have also been shown to segregate as co-dominant markers in some cases thus enabling distinction between homozygotes and heterozygotes. The microsatellite repeats used as primers can be dinucleotide, trinucleotide, tetra nucleotide or penta-nucleotide. The primers used can be either unanchored or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences. It is possible to find a large number of these SSRs in an organism for which a great number of ESTs are generated.

Advantages of ISSR

1. The main advantage of ISSRs is that no sequence data for primer construction are needed.
2. ISSR segregates mostly as codominant markers.
3. They are highly polymorphic.
4. The reproducibility of microsatellites is high.

Disadvantages of ISSR

1. The main drawbacks of microsatellites are that high development costs.
2. ISSR is a multilocus technique; disadvantages include the possible non-homology of similar sized fragments.

Application of ISSR

Because of the multilocus fingerprinting profiles obtained, ISSR applied in

1. Genomic fingerprinting.
2. Genome mapping.
3. Genetic diversity and phylogenetic analysis.
4. Determining SSR motif frequency.
5. Gene tagging and use in marker assisted selection.
6. Clone and strain identification

Sequence characterized amplified regions (SCARs)

SCARs take advantage of a band generated through a RAPD experiment. They use 16-24 bp primers designed from the ends of cloned RAPD markers. This technique converts a band prone to difficulties in interpretation and/or reproducibility into being a very reliable marker (Paran and Michelmore 1993) [30].

Advantages

1. The main advantage of SCARs is that they are quick and easy to use. In addition, SCARs have a high reproducibility and are locus-specific. Due to the use of PCR, only low quantities of template DNA are required (10–100 ng per reaction).

Disadvantages

1. Disadvantages include the need for sequence data to design the PCR primers.

Applications

1. SCARs are locus specific and have been applied in gene mapping studies and marker assisted selection (Paran & Michelmore 1993) [30].

Simple sequence repeats (SSRs)

SSRs, also called microsatellites, short tandem repeats (STRs) or sequence-tag- ged microsatellite sites (STMS), are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di-, tri- and tetra-nucleotide repeats, e.g. (GT) n, (AAT) n and (GATA)n, are widely distributed throughout the genomes of plants and animals. The copy number of these repeats varies among individuals and is a source of polymorphism in plants. Because the DNA sequences flanking microsatellite regions are usually conserved, primers specific for these regions are designed for use in the PCR reaction. One of the most important attributes of microsatellite loci is their high level of allelic variation, thus making them valuable genetic markers.

The unique sequences bordering the SSR motifs provide templates for specific primers to amplify the SSR alleles via PCR. SSR loci are individually amplified by PCR using pairs of oligonucleotide primers specific to unique DNA sequences flanking the SSR sequence. The PCR-amplified products can be separated in high-resolution electrophoresis systems (e.g. AGE and PAGE) and the bands can be visually recorded by fluorescent labeling or silver-staining.

Advantages
1. Require very little and not necessarily high quality DNA.
2. Highly polymorphic.
3. The loci identified are usually multi-allelic and codominant.
4. Bands can be scored either in a codominant manner, or as present or absent.
5. Evenly distributed throughout the genome.
6. Interpretation of result is simple.
7. Easily automated, allowing multiplexing.
8. Good analytical resolution and high reproducibility.

Disadvantages
1. Complex discovery procedure.
2. Costly.

Amplified fragment length polymorphism (AFLP)
AFLP is a technique based on the detection of genomic restriction fragments by PCR amplification and can be used for DNAs of any origin or complexity. Amplified fragment length polymorphism (AFLP), which is essentially intermediate between RFLPs and PCR. AFLP is based on a selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) or by capillary electrophoresis. The key feature of AFLP is its capacity for "genome representation" and the simultaneous screening of representative DNA regions distributed randomly throughout the genome. AFLPs are DNA fragments (80-500 bp) obtained from digestion with restriction enzymes, followed by ligation of oligonucleotide adapters to the digestion products and selective amplification by the PCR. This is a highly sensitive method for detecting polymorphism throughout the genome, and it is becoming increasingly popular. It is essentially a combination of RFLP and RAPD methods, and it is applicable universally and is highly reproducible. It is based on PCR amplification of genomic restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of few nucleotide bases. AFLP markers are abundant in nature and have been used for construction of genetic linkage maps, high density linkage map of a targeted region, identification of QTLs controlling complex traits and studies on genetic diversity

Advantages of AFLP
1. This technique is extremely sensitive.
2. It has high reproducibility, rendering it superior to RAPD.
3. It has wide scale applicability, proving extremely proficient in revealing diversity.
4. It discriminates heterozygotes from homozygotes when a gel scanner is used.
5. It is not only a simple fingerprinting technique, but can also be used for mapping.

Disadvantages
1. High molecular weight of DNA.
2. It is highly expensive and requires more DNA than is needed in RAPD (1 mg per reaction).
3. It is technically more demanding than RAPDs, as it requires experience of sequencing gels.
4. AFLPs are expensive to generate as silver staining, fluorescent dye, or radioactivity detect the bands.
5. Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence.
6. The major disadvantage of AFLP markers is that these are dominant markers.

Applications of AFLP
1. It applied in studies genetic identity of cultivar.
2. It determines phylogenetic studies of closely related species.
3. AFLP markers have successfully been used for analyzing genetic diversity in some other plant species such as peanut and soybean.

Cleaved amplified polymorphic sequence (CAPS)
CAPS are a combination of the RFLP and PCR and it was originally named PCR RFLP. The technique involves amplification of a target DNA through PCR, followed by digesting with restriction enzymes (Michaels and Amasino 1998) [24]. Hence, CAPS markers rely on differences in restriction enzyme digestion patterns of PCR fragments caused by nucleotide polymorphism between samples. Critical steps in the CAPS marker approach include DNA extraction, and the number or distribution of polymorphic sites, and PCR conditions.

Advantage of CAPS markers
1. Since analysis of restriction fragment length polymorphisms is based on PCR amplification, it is much easier and less time-consuming than analyzing alternative types of markers that require southern hybridizations.
2. CAPS primers developed from ESTs are more useful as genetic markers for comparative mapping study than those markers derived from non-functional sequences such as genomic microsatellite markers. CAPS markers are inherited mainly in a co-dominant manner (Matsumoto and Tsumura 2004) [21].

Disadvantage of CAPS markers
Show only low level of polymorphism that is more difficult to find because of the limited size of amplified fragment.

Sequence tagged site (STS)
STS was first developed by Olsen *et al*. (1989) [29] as DNA landmarks in the physical mapping of the human genome, and later adopted in plants. STS is a short, unique sequence whose exact sequence is found nowhere else in the genome. Two or more clones containing the same STS must overlap and the overlap must include STS. Any clone that can be sequenced may be used as STS provided it contains a unique sequence. In plants, STS is characterized by a pair of PCR primers that are designed by sequencing either an RFLP probe representing a mapped low copy number sequence (Blake *et al*. 1996) [3] or AFLP fragments. STS markers are codominant, highly reproducible, suitable for high throughput and

automation, and technically simple for use (Reamon-Buttner and Jung 2000) [32].

Expressed Sequence Tags (ESTs)

An Expressed Sequence Tag or EST is a short (300–500 bp) sub-sequence of a cDNA sequence. The cDNAs used for EST generation are individual clones from a cDNA library which are complemen- tary to mRNA, so the ESTs represent portions of expressed genes. ESTs are used to identify gene transcripts, and play an important role in gene discovery and gene sequence. Since the ESTs are often partial sequences that correspond to the same mRNA of an organism, they are assembled into contigs so as to reduce the number of expressed sequence tags for downstream gene discovery analysis.

Advantages of STS and ESTs

1. Fast
2. cDNA sequences
3. non- radioactive

Disadvantages of STS and ESTs

1. substantially decreased level of polymorphism
2. sequence information required

The features of the widely used DNA markers discussed above are compared in Table 1. The advantages or disadvantages of a marker system are relevant largely to the purposes of re- search, available genetic resources or databases, equipment and facilities, funding and per- sonnel resources, etc. The choice and use of DNA markers in research and breeding is still a challenge for plant breeders. A number of factors need to be considered when a breeder chooses one or more molecular marker types (Semagn *et al*., 2006a) [35]. A breeder should make an appropriate choice that best meets the requirements according to the conditions and re- sources available for the breeding program.

Table 1: Comparison of most widely used DNA marker systems in plants; Adapted from Collard *et al*. (2005) [7], Semagn *et al*. (2006a) [35], Xu (2010) [41] and others.

Feature and description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	Moderate to high	Very high
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Whole genome	Whole genome
Expression/inheritance	Co-dominant	Dominant	Dominant/ codominant	Co-dominant	Co-dominant
Number of loci	Small (<1,000)	Small (<1,000)	Moderate (1,000s)	High (1,000s – 10,000s)	Very high (>100,000)
Level of polymorphism	Moderate	High	High	High	High
Type of polymorphism	Single base changes, indels	Single base changes, indels	Single base changes, indels	Changes in length of repeats	Single base changes, indels
Type of probes/primers	Low copy DNA or cDNA clones	10 bp random nucleotides	Specific sequence	Specific sequence	Allele-specific PCR primers
Cloning and/or sequencing	Yes	No	No	Yes	Yes
PCR-based	Usually no	Yes	Yes	Yes	Yes
Radioactive detection	Usually yes	No	Yes or no	Usually no	No
Reproducibility/ reliability	High	Low	High	High	High
Effective multiplex ratio	Low	Moderate	High	High	Moderate to high
Marker index	Low	Moderate	Moderate to high	High	Moderate
Amount of DNA required	Large (5–50 μg)	Small (0.01–0.1 μg)	Moderate (0.5–1.0 μg)	Small (0.05–0.12 μg)	Small (≥ 0.05 μg)
Quality of DNA required	High	Moderate	High	Moderate to high	High
Technically demanding	Moderate	Low	Moderate	Low	High
Ease of use	Not easy	Easy	Moderate	Easy	Easy
Time demanding	High	Low	Moderate	Low	Low
Ease of automation	Low	Moderate	Moderate to high	High	High
Development/start-up cost	Moderate to high	Low	Moderate	Moderate to high	High
Cost per analysis	High	Low	Moderate	Low	Low
Number of polymorphic loci per analysis	1.0–3.0	1.5–5.0	20–100	1.0–3.0	1.0
Primary application	Genetics	Diversity	Diversity and genetics	All purposes	All purposes
Proprietary rights required	No	Yes and licensed	Yes and licensed	Yes and some licensed	Yes and some licensed

Application of molecular markers

The advancement of the molecular biotechnology opens new vistas to fastened the breeding program of vegetable crops by using a various molecular marker in the different methods of breeding and their steps for enhancing of the improvement program.

1. Genetic Linkage Maps

It is a graphical representation of an array of loci segregating populations, including F_2 generations, backcross progeny, recombinant inbred lines, etc. to be used to study recombination between markers (Lefort- Buson *et al*., 1990) [18]. Using the molecular technique, the selection of plant material depends on the biology of the species and the objectives of the study. Various working on different vegetable crops and other crops also, they developed molecular maps for several crops, including tomato (Tanksley *et al*., 1992) [5] and rice (Causse *et al*., 1994) [6].

2. Assessment of Genetic Diversity

Diversity studies using molecular markers are now common practice, several workers using this technique in different vegetable crops. Dominant markers like RAPD used for the analysis of pepper breeding lines (Heras *et al*., 1996) [13] revealed very narrow genetic base with more than 50% of the DNA bands being common among all the lines. Villand *et al*. (1998) [39] reported that in an assessment of the world collections of tomato and found South American accessions to

have greater diversity than old world accessions. Shim and Jorgensen (2000) [36] also carried out AFLP analysis in diversity studies between wild and cultivated carrots and found that the old varieties released during 1974-1976 and newly developed F1 hybrid varieties. Archak *et al*. (2002) [2] using RAPD markers in tomato for the same purpose and, found old introductions and locally developed varieties of 1970s exhibiting genetically differed with those who released in 1990s.

Simple sequence repeats (SSR) and sequence related amplified polymorphism markers were used by Ruiz and Martinez (2005) [34] to study the genetic variability of some traditional tomato cultivars of Spain. In this study, it was revealed that the Mexican cultivar Zapotec, a breeding line and virus resistant commercial hybrid "Anastasia" were found genetically most distant of all the cultivar. Muminoric *et al*. (2005) [27] used 12 AFLP and 10 inter- simple sequence repeat (ISSR) primers to estimate genetic diversity in 68 varieties of cultivated radish. According to him substantial level of genetic variability in germplasm of cultivated radish and within cultivated material, black radish and French breakfast radish types formed a separate cluster. In another study, AFLP marker analysis detected a greater genetic variability among American than among Spanish accessions of Cucurbita maxima (Ferriot *et al*., 2004) [9]. Levi and Thomas (2004) [19] identified 80.2-97.8% genetic similarity among hair loom cultivars of watermelon using ISSR and AFLP markers and they concluded that ISSRs and AFLPs are highly effective in differentiating among watermelon cultivars of elite lines with limited genetic diversity revealed by RAPD markers. Ansari and Singh (2013; 2014) [1] also reported that RAPD and SSR markers are effective in differentiating among the genotypes of *Solanum aethiopicum* and *Solanum melongena.*

3. Gene Tagging

Gene tagging is a pre-requisite for MAS and map based gene cloning. Tagging of gene in important vegetable crops has been made viz., in tomato TMV resistance Tm-2 locus, nematode resistance, Mi gene, Fusarium oxysporum resistance gene, and powdery mildew resistance gene, etc. Huang *et al*. (2000) [14] also make possible to tag powdery mildew resistance gene ol-1 on chromosome 6 of tomato using RAPD and SCAR markers.

4. DNA Fingerprinting for Varietals Identification

This is an important tool to detect and identify any genotype of crop plants as well as whole living organism. A large number of molecular marker has been used today for DNA fingerprinting of cultivars and breeding lines in a number of vegetable crops viz., tomato (Kaemmer *et al*., 1995) [15], beans (Hamann *et al*., 1995) [12] pepper (Prince *et al*., 1995) [35], and potato (McGregor *et al*., 2000) [22]. This technology has a great potential for enhancing purity assessment in hybrids also. Genetic purity can also be detected using this technique as reported by Mongkolporn *et al*. (2004) [24] F_1 Chilli hybrids was determined using two molecular techniques RAPD and ISSR. They found that RAPD analysis successfully detected all three F_1 hybridity whereas; ISSR detected only two due to the RAPD marker system producing a greater number of markers than the ISSR system.

5. Identification of Cultivar

Identification of cultivars is essential today to prevent infringement and duplication of the same genotype in the germplasm conservation and registration. Now in several vegetable crops microsatellites have been developed to enable highly reliable identification of cultivars like tomato, pepper, potato, alliums, cucurbits, lettuce, and spinach. Comparative assessment of five different DNA fingerprinting techniques carried out in tetraploid potato genotype revealed by AFLP to have the highest discrimination power followed in decreasing order by multilocus SSR, RAPD, ASSR, and single locus SSR. In pepper, Gaikwad *et al*. (2001) [10] also found ISSR markers was the most efficient in detecting polymorphism. However, due to very high number of markers generated per assay by AFLP, the marker index of AFLP markers was prominently higher than that of ISSR and RAPD. In another study, Broun *et al*. (1992) [5] identified two telomeric tendemly repeated sequences (7bp) and a closely linked 162 bp subtelomeric repeats in tomato and they accounted for 2% of the total chromosomal DNA. These sequences have a very high mutation rate of 2% in each generation. They have been shown to be extremely useful for distinguishing and very similar to tomato and melon varieties.

6. Monogenic disease resistance

Development of resistant varieties against important insect-pest and diseases of vegetable crops is the first step to fight against infestation of insect-pest and diseases, which may reduce the use of chemical pesticide and beneficial for the eco-friendly management of insect-pest and diseases of vegetable crops. Use of resistance varieties are also helps in the organic farming or cultivation of vegetable crops. Molecular markers are now using in the resistant breeding program. MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker if a narrow linkage has been established between them. This technique has been utilizing in the breeding program to enhance and rapid selection in the early generation. The likelihood of detecting a marker linked to a disease resistance gene is inversely proportional to the genetic distance between the marker and the gene. For a better estimation, the genetic distance between the marker and the gene must be calculated from a large population or better from several crosses, in light of this concept, the genetic distances may greatly vary between crosses (Messeguer *et al*., 1991) [23]. Linkages have been frequently observed between markers and monogenic disease resistance by mapping on a genetic linkage map. RFLP or RAPD are the most wide used marker techniques for this strategy.

7. Polygenic disease resistance

Almost all complex disease resistances (i.e., quantitatively expressed) are assumed to be under oligogenic or polygenic control (Mather and Jink, 1971) and/or influenced by the environmental factors too. To solve this problem quantitative trait loci, or quantitative trait locus (QTLs) (Geldermann, 1975) [11] are considered to identify chromosome sites at which genes that have effect on quantitative traits can be located. The quantitative nature of resistance against certain biotic and abiotic stresses would result from the simultaneous and independent allelic variation of such genes involved are influenced by the effect of environment (East, 1916; Yule, 1906) [8, 43]. The search for linkages between molecular markers and QTLs of particular quantitative trait is based on this hypothesis.

8. Detection of QTLs

The detection of linkages between markers and QTLs are the

important objective of the breeders engaged in the resistance breeding of crop plants; it can be performed using various statistical methods. The statistical approach using the analysis of variance estimates to fulfill this goal and the degree of association between a genotypic marker (an allelic form) and a phenotypic trait which may also be influenced by various environmental factors. Resulting this, the phonotypical values are the dependent variables and the genotypic markers correspond to the treatment or the factor (source of variation). Analysis of variance models of increasing complexity provide accurate information on the genetic basis of the resistance in the crop plants, for instance, the effect of individual markers (one-way ANOVA), the effect of pairs of markers in the factors of two-way analysis (epistasis by two-way ANOVA) (Lefebvre, 1993) [17]. The interval mapping approach (Lander and Botstein, 1989) [29] helps to consider linkages between markers. Using the maximum likelihood equation, the method provides an estimate and also to expressed as limit of detection (LOD) score of the likelihood of the presence of a QTL for regular intervals throughout the genome based on flanking marker information which useful in the genomic study as well as for the sequencing technique of the genome of an organism. The LOD scores actually depend on the localization of the QTL with respect to the flanking markers and the magnitude of its effect; it is also on the probability that there is a QTL present in the chromosome. When examining the curves representing LOD, this method would be very powerful because it accounts for recombination rates of different markers. To use this method, it requires the markers to have been mapped and the trait that have to a Gaussian distribution, a condition although which is not always satisfied in the study of disease resistance genes (semi-quantitative data), ANOVA and interval mapping are the most currently used methods for this technique. Since disease resistance is to be assessed with ordinal scales and data do not always show a normal distribution, concern researchers have been testing putative QTLs with non-parametric statistical tests here (Young and Tanksley, 1989) [42]. In another way using maximum likelihood, mean squares, linear, and multiple regressions, have been described for another method (Rodolphe and Lefort, 1993) [33]. With the help of different molecular markers, polygenic disease resistance can be partitioned, and individual effects can be examined (components of resistance). Results of genetic studies of complex interactions have been reported and first report on insect resistance in tomato (Nienhuis *et al*., 1987) [28] and then quantitative resistance to pathogenic fungi and bacteria and nematodes also. In addition, QTL mapping could be useful for identifying loci involved in quantitative components of resistance to viral infections in crop plants and rate of its multiplication as well as its movement in the host and disease progression. New genes for partial resistance against this problem might be identified by using the technique.

9. Strategies for targeted mapping

Now today it is possible to identify markers for disease resistance genes directly without drawing a genetic linkage map, as drawing of genetic linkage map is a time-consuming procedure. The direct use of molecular markers is essentially limited to monogenic traits only since it consists of identifying a particular genomic region coding for the trait. Studies using aneuploid lines to identify the chromosomes or chromosome arms that carry disease resistance genes and near isogenic lines or bulk segregant analysis to identify markers located near disease resistance genes are the suitable examples for this aspect (Lefebvre, 1993) [17].

10. MAP based gene cloning

One of the most serious limitations to the advance of plant molecular biology and biotechnology is the difficulty in isolating genes responsible for specific characters, yield, disease resistance, insect resistance and quality are just few of the important characters for which genetic variation exists within crop species, but for which the corresponding genes have not yet been cloned. The advent of genome mapping at the DNA level (especially RFLPs) has provided a method for localizing genes of economic importance to specific chromosomal positions. The ability to map any gene of economic importance to a defined chromosomal site opens the possibility of isolating genes via chromosome walking. This method is called map based gene cloning.

Marker assisted selection (MAS)

In this technique, linkages are sought between DNA markers and agronomically important traits such as resistance to pathogens, insects and nematodes, tolerance to abiotic stresses, quality parameters, and quantitative traits. Instead of selecting for a trait, the breeder can select for a marker that can be detected very easily in the selection scheme. The essential requirements for marker assisted selection in a plant breeding program are as follows:

DNA marker based selection for disease resistant trait essentially requires following conditions:

- The identified DNA marker(s) should co- segregate or closely linked (1 cM or less) with the resistant trait. Alternatively, less tightly linked flanking markers should be available for the resistant gene(s).
- The availability of an efficient screening technique(s) for DNA markers, which can be practically feasible to handle large populations.
- The screening technique should have high reproducibility across laboratories.

A number of markers linked with monogenic disease resistance are available in vegetable crops especially in tomato. Such mapping has been facilitated by the use of different kind of mapping populations like near isogenic lines (NILs) developed by repeated back crossing, recombinant inbred lines (RILs) developed by single seed decent or double haploid (DH) methods. Now a days, bulk segregants analysis (BSA) is increasingly being used to map monogenic resistance, because it allows rapid mapping of genes.

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