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Potential Application of Molecular Markers in Improvement of Vegetable Crops

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ABSTRACT

A molecular markers is basically a DNA sequence that is readily detected and whose inheritance can be easily be monitored. The desirable properties of molecular markers are high polymorphism, co-dominant inheritance, frequent occurrence and even distribution throughout the genome, selectively neutral behavior, open access, easy and fast assay, low cost, high reproducibility and transferability between laboratories, populations or species. There are three different types of markers viz., morphological, biochemical and molecular. Morphological markers have certain constraints i.e. narrow diversity, influenced by environment, problem with epistasis, pleiotropy, incomplete penetrance and variable expressivity. During the last few decades, the use of molecular markers, revealing polymorphism at the DNA level, has been playing a vital part in improvement of vegetable crops and their genetics studies. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics. Molecular markers directly reveal the polymorphism at the level of DNA. Molecular markers are widely utilized in the plant production sector of the developing world even if the present uptake of molecular marker technologies does not reflect their actual potential. The most widespread use of markers has been in germplasm characterization. During the one and half decades, molecular markers have been entered the scene of genetic improvement in a wide range of vegetable crops. Among the major traits targeted for improvement in vegetable breeding programs are disease resistance, fruit yield and quality, tree shape, floral characteristics, cold, hardiness, and dormancy. Although genetic maps have been developed for most of our important vegetable species, and a number of horticulturally important gene loci have been tagged, only a few instances of application of molecular markers for progeny selection have been reported.

Keywords: Potential application, molecular markers, polymorphism, improvement, vegetable crops.

INTRODUCTION

Molecular markers are heritable differences in nucleotide sequences of DNA at the corresponding position on homologous chromosome of two different individuals, which follow a simple Mendelian pattern of inheritance. Over the last two decades, the advent of molecular markers has

revolutionized the entire scenario of biological sciences. DNA-based molecular markers are a versatile tool in the fields of taxonomy, physiology, embryology, genetic engineering, etc. [49]. Molecular markers can be of any kind of marker system that differentiates two individuals at the molecular level. Many types of molecular markers are presently available, but no single marker technique is generally applicable for all applications. As early as the 1970s, protein markers (enzymes) were mainly used as molecular markers. Enzymes (i.e. protein, or direct of gene products) can be visualized using specific stains to get a visible product as a band in an electrophoretic system and different forms of an enzyme (reflected in different colored bands), are called isozymes [55].

Molecular markers more recently, high-throughput genome sequencing efforts, have dramatically increased knowledge of and ability to characterize genetic diversity in the germplasm pool for essentially any crop species. Using maize as one example, surveys of molecular marker alleles and nucleotide sequence variation have provided basic information about genetic diversity before and after domestication from its wild ancestor teosinte, among geographically distributed landraces, and within historically elite germplasm [10,38,8]. This information enriches investigations of plant evolution and comparative genomics, contributes to our understanding of population structure, provides empirical measures of genetic responses to selection, and also serves to identify and maintain reservoirs of genetic variability for future mining of beneficial alleles [32,52]. In addition, knowledge of genetic relationships among germplasm sources may guide choice of parents for production of hybrids or improved populations [11,9].

The advent of DNA recombinant technology opened the area of development and exploitation of DNA-based markers which was further tuned after the development of PCR technology. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective. The first such DNA markers to be utilized were fragments produced by restriction digestion –the restriction fragment length polymorphism (RFLP) based genes marker. Consequently, several markers system has been developed.

Recent advancements in biotechnology have led to the development of more efficient selection tools to substitute phenotype-based selection systems. Molecular markers associated with genes or quantitative trait loci (QTLs) affecting important traits are identified, which could be used as indirect selection criteria to improve breeding efficiency via marker-assisted selection (MAS) in vegetable crops [36].

What is an ideal molecular/DNA marker?

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 homozygous 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.

Regrettably, no single molecular marker meets all these requirements. A wide range of molecular

techniques is available that detect polymorphism at the DNA level. These have been grouped into the following categories based on the basic strategy, and some major ones are described [2].

There are mainly two types of molecular markers i.e. Hybridization based or Non-PCR based marker for example Restriction Fragment Length Polymorphism (RFLP) [5]. Polymerase chain reaction (PCR) based markers example Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990), Sequence characterized amplified regions (SCARs) [34] and Amplified Fragment Length Polymorphism (AFLP) (Vos et al., 1995). Molecular markers are used for development of saturated genetic maps; DNA fingerprinting; Phylogenetic and evolutionary studies; heterotic breeding; gene tagging and marker assisted selection (MAS). Identification of vegetable crop varieties by molecular markers in tomato [39], Potato [4], Onion, garlic and related species [15]. Molecular markers are linked to major disease resistance in tomato like *Meloidogyne incognita* [66]and Tomato mosaic virus [53].

Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers and polymerase chain reaction (PCR)-based markers. In the former, DNA profiles are visualized by hybridizing the restriction enzyme digested DNA, to a labeled probe, which is a DNA fragment of known origin or sequence. PCR based markers involve *in vitro* amplification of particular DNA sequences or loci, with the help of specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermos table DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods such as staining and autoradiography.

PCR BASED APPROACHES:

The main breakthrough of DNA based- a molecular marker was driven by the invention of PCR. PCR markers are based on amplification of sequences using the polymerase chain reaction (PCR). To amplify the targeted sequences, two primers, which should flank the target sequence, are needed. PCR based markers rely on sequence variation in annealing sites or DNA length differences between amplified products obtained from corresponding genotypes.

Random Amplified Polymorphic DNA (RAPD):

Random amplified polymorphic DNA (RAPD) is the simplest example of a PCR marker that involves the use of 10 bp random primers [65]. RAPDs have been widely used for mapping and genetic diversity studies [58,13], but due to their poor reproducibility and lack of locus specificity, scientist tried other possibilities to find reliable and reproducible markers.

Advantages of RAPD

The main advantages of RAPDs are: they are less time consuming, easy to assay, and low quantities of template DNA are required, usually 5-50 ng per reaction. Due to the commercial availability of random primers, no sequence data for primer construction is needed. Moreover, RAPDs have a very high genomic abundance and are randomly distributed throughout the genome.

Disadvantages of RAPD:

The main drawback of RAPDs is low reproducibility hence highly standardized experimental procedures are needed because of its sensitivity to the reaction conditions. RAPD analyses generally require purified, high molecular weight DNA, and precautions are needed to avoid contamination of DNA samples because the short random primers used may amplify DNA fragments in bined advantages of oligonucleotide fingerprinting, RAPD [65]. The technique is successfully employed in the genetic fingerprinting of tomato, kiwi fruit, and closely-related genotypes of *D. bulbifera* [44].

Applications of RAPD

- Measurements of genetic diversity
- Genetic structure of populations

- Germplasm characterization
- Verification of genetic identity
- Genetic mapping
- Development of markers linked to a trait of interest
- Cultivar identification
- Identification of clones (in case of soma-clonal variation)
- Interspecific hybridization
- Verification of cultivar and hybrid purity
- Clarification of parentage

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) [31] 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 [61]. AFLP markers are abundant in nature and have been used for construction of genetic linkage maps [30], high density linkage map of a targeted region [28], identification of QTLs controlling complex traits [70] and studies on genetic diversity [12,50]

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.
6. 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 [23], soybean (Ude et al., 2003).

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 [20,57,42,63. However, they have also been shown to segregate as co-dominant markers in some cases thus enabling distinction between homozygotes and heterozygotes [68,1,63,48]. The microsatellite repeats used as primers can be di-nucleotide, tri-nucleotide, tetra nucleotide or penta-nucleotide. The primers used can be either unanchored [20,33,68] or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences [72]. It is possible to find a large number of these SSRs in an organism for which a great number of ESTs are generated [27].

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.

NON-PCR-BASED APPROACHES:

Restriction Fragment Length Polymorphism (RFLP):

RFLP was the first technology that enabled the detection of polymorphism at the DNA sequence level. Genetic information, which makes up the genes of higher plants, is stored in the DNA sequences. Variation in this DNA sequence is the basis for the genetic diversity within a species.

Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of particular *Restriction Endonucleases*, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another. This technique is mainly based on the special class of enzyme i.e. Restriction Endonucleases. This technique is basically used to identify DNA sequence polymorphisms for genetic mapping of a temperature-sensitive mutation of adenovirus serotypes. They were initially used for human genome mapping (Botstein et al., 1980) but were later adopted for plant genomes. By the use of this technique plants are able to replicate their DNA with high accuracy and rapidity, but many mechanisms causing changes in the DNA are operative. RFLP markers were used for the first time in the construction of genetic maps by [6]. RFLPs, being codominant markers, can detect coupling phase of DNA molecules, as DNA fragments from all homologous chromosomes are detected. They are very reliable markers in linkage analysis and breeding and can easily determine if a linked trait

is present in a homozygous or heterozygous state in individual, information highly desirable for recessive traits [67].

Advantages of RFLP

1. It permits direct identification of a genotype or cultivar in any tissue at any developmental stage in an environment independent manner.
2. RFLPs are codominant markers, enabling heterozygotes to be distinguished from homozygotes.
3. It has a discriminating power that can be at the species / population (single locus probes) or individual level (multi locus probes).
4. The method is simple as no sequence-specific information is required.

Disadvantages of RFLP

The assay is time-consuming and labour intensive and only one out of several markers may be polymorphic, which is highly inconvenient especially for crosses between closely related species. Their inability to detect single base changes restricts their use in detecting point mutations occurring within the regions at which they are detecting polymorphism.

1. Requires relatively large amount of DNA.
2. Conventional RFLP analysis requires relatively large amount of highly pure DNA.
3. A constant good supply of probes that can reliably detect variation are needed.
4. It is laborious and expensive to identify suitable marker / restriction enzyme combinations from genomic or cDNA libraries where no suitable single-locus probes are known to exist.
5. RFLPs are time consuming as they are not amenable to automation.
6. RFLP work is carried out using radioactively labelled probes.

Applications of RFLP

It applied in diversity and phylogenetic studies ranging from individuals within populations or species to closely related species.

It has been used in gene mapping studies.

This technique is useful first time in the construction of genetic maps by Botstein et al.,[6].

Application of Molecular Markers in Improvement of Vegetable Crops

The main applications of molecular markers in improvement of vegetable crops are as follows:

- Assessment of genetic variability and characterization of germplasm.
- Identification and fingerprinting of genotypes.
- Estimation of genetic distances between population, inbreeds and breeding materials.
- Detection of monogenic and quantitative trait loci (QTL).
- Marker-assisted selection.
- Identification of sequences of useful candidate genes.

Development of saturated genetic maps

In the past genetic maps were based mainly on morphological and isozyme markers. But these markers are limited and are influenced by environment and developmental stage. Molecular markers on the other hand are large in number and are not influenced by environment and development stage. Saturated linkage maps are prerequisite for gene tagging, marker assisted selection and map based gene cloning. Yayeh [69] identified first genetic linkages in male fertile garlic accessions based on single nucleotide polymorphism simple sequence repeats and randomly amplified polymorphic DNAs. Thirty seven markers formed nine linkage groups covering 415 centrimorgans (cM) with average distance of 15 cM between loci. A male fertility locus was placed on the map. A 109 point linkage map consisting of three phenotypic loci (P_1, Y_2 and Rs), six restriction fragment length polymorphic DNA (RFLPs), two random amplified polymorphic DNAs (RAPDs), 96 amplified fragment length polymorphism (AFLPs) and two selective amplification of microsatellite polymorphic loci (SAMP) was constructed in carrot by Vivek and Simon [60]. A genetic map of an interspecific cross in Allium based on amplified length polymorphism markers constructed by Van

Heusden *et al* (2000). The map based on *A. cepa* markers consisted of eight linkage groups whereas map based an *A. roylei* markers comprised 15 linkage groups. Zhang *et al* [71] constructed linkage map for watermelon using recombinant inbred lines (RILs) from a cross between the high quality inbred line 97103 and the *Fusarium* wilt resistant plant introduction using RAPD and SCAR markers. This map is useful for further development of quantitative trait loci (QTLs) affecting fruit quality and for identification of genes conferring resistance to *Fusarium* wilt.

Assessment of genetic diversity

Molecular markers have proved to be excellent tools for assessment of genetic diversity in a wide range of plant species. The information is often of direct utility to plant breeders since it is indicative of the performance, adaptation or other agronomic qualities of the germplasm. Molecular markers have provided very useful information about the overall genetic range of crop germplasm. For breeders this information is important to take decisions regarding the utility of germplasm particularly in search for rare and unique genes. Germplasm of narrow genetic base is obviously unlikely to harbour novel genes e.g. those conferring resistance to biotic and abiotic stresses. RAPD analysis of pepper breeding lines [22] revealed very narrow genetic base with more than 50% of the DNA bands being common among all the lines. In an assessment of the world collections of tomato, Villand *et al* [59] found South American accessions to have greater diversity than old world accessions. Shim and Jorgensen [51] carried out AFLP analysis in wild and cultivated carrots and found that the old varieties released between 1974-76 were more heterogeneous than newly developed F_1 hybrids varieties. Archak *et al* [3], using RAPD markers in tomato, found old introductions and locally developed varieties of 1970s exhibiting significantly greater variation than the ones released in 1990s. Ruiz and Martinez [47] studied the genetic variability of some traditional tomato cultivars of Spain using simple sequence repeats (SSR) and sequence related amplified polymorphism (SRMP) markers. They found that Mexican cultivar zapotec, a breeding line and virus resistant commercial hybrid 'Anastasia' were most distant of all the cultivar. Twelve amplified fragment length polymorphism (AFLP) and 10 inter-simple sequence repeat (ISSR) primers were applied to estimate genetic diversity in 68 varieties of cultivated radish by Muminovic *et al* [37]. They detected substantial level of genetic variability in germplasm of cultivated radish and within cultivated material, black radish and French breakfast radish types formed a separate clusters. AFLP marker analysis detected a greater genetic variability among American than among Spanish accessions of *Cucurbita maxima* [14]. Levi and Thomas [29] identified 80.2 - 97.8% genetic similarity among hair loom cultivars of water melon using ISSR and AFLP markers. They also concluded that ISSRs and AFLPs are highly effective in differentiating among water melon cultivars of elite lines with limited genetic diversity than RAPD marks.

Gene tagging

The most interesting application of molecular markers at present time is the ability to facilitate the method of "conventional" gene transfer. Gene tagging refer to mapping of genes of economic importance close to known markers. Thus, a molecular marker very closely linked to gene act as a tag that can be used for indirect selection of gene in breeding programmes with the construction of molecular map, especially the RFLP maps, several genes of economic importance like disease resistance, stress tolerance, insect resistance, fertility restoration genes, yield attributing traits have been tagged. Gene tagging is a pre-requisite for marker assisted selection and map based gene cloning. In case of tomato TMV resistance *Tm-2* locus, nematode resistance, *Mi* gene, *Fusarium oxysporum* resistance gene, powdery mildew resistance gene, has been tagged. Huang *et al* [24] tagged powdery

mildew resistance gene *ol-1* on chromosome 6 of tomato using RAPD and SCAR markers.

DNA fingerprinting for varietal identification

DNA fingerprinting can be used for varietal identification as well as for ascertaining variability in the germplasm. Although any type of marker can be used but RAPDs, microsatellite and RFLPs are marker of choice for the purpose because all these are PCR based and does not require any prior information on nucleotides. The fingerprinting information is useful for quantification of genetic diversity, characterization of accessions in plant germplasm collections and for protection of property of germplasm especially the cms lines. Molecular marker has been used widely for DNA fingerprinting of cultivars and breeding lines in a number of vegetable crops like tomato [26], beans [21], pepper [41], potato [17]. DNA technology has great potential for enhancing purity assessment in hybrids. Genetic purity of three F1 chilli hybrids was determined using two molecular techniques RAPD and ISSR by Mongkolporn *et al* [35]. They found that RAPD analysis successfully detected all three F1 hybridity while ISSR detected only two. This was due to the RAPD marker system producing a greater number of markers than the ISSR system. All the available molecular tools from the simplest and readily useable RAPDs, ISSRs, DNA amplification fingerprinting (DAF) to the more precise, elaborate but robust AFLPs, microsatellites and RFLP based variable number of tandem repeats (VNTR) analyses have been utilized for discrimination of closely related lines as well as high yielding varieties.

Breeding lines and accession identification

Several situations during a breeding programme may require identification of breeding lines and accessions. Mislabelling is a common problem in breeding experiments due to the large number of lines that need to be handled. Breeding lines can get contaminated due to mixing of seed samples and cross contamination in field. Molecular markers are ideal for distinguishing closely related genotypes that differ in few morphological traits. Use of human minisatellite probe 33.15 and the M_{13} repeat sequences for their ability to distinguish sister lines of two F6 backcrosses were demonstrated by Stockton and Gepts [54]. A comparison of the utility of 33.15 and M13 probes with (GACA) and ribosomal DNA sequences with respect to the polymorphism detected was made. The (GACA) repeat was observed to be least efficient in discriminating the closely related lines of beans. Walcott and Fort (1994) could differential nearly identical germplasm lines of bitter head lettuce on the basis of RAPD markers. Kaemmer *et al* [26] fingerprinted tomato accessions using microsatellite probes. The authors reported the utility of the technique in purity testing of breeding lines and in F1 progeny testing. Using RAPD technique, Tivang *et al* [56] revealed variation among and within artichoke breeding populations. Heterogeneity was observed within clonal cultivars. Roose and Stone [46] reported the utility of RAPD and RFLP markers in distinguishing F1 from F2 seeds in asparagus and for evaluation of seed purity. Phippen *et al* [40] used molecular markers to distinguish 14 phenotypically similar accessions of 'Golden Acre' variety of cabbage. Ten pairs of potential duplicate accessions in a total of 134 capsicum accessions were identified by Rodriguez *et al* [45] on the basis of RAPD markers. Further, misclassified and unclassified accessions were placed in the correct groups. Using microsatellite markers, Fisher and Bachmann [16] distinguished 83 accessions of onion.

Sex identification

Early identification of male and female plants can bring considerable efficiency in breeding programmes of dioecious species. Jiang and Sink [25] developed SCAR markers in asparagus which were linked to the sex locus at a distance of 1.6 cm. Codominant STS markers enabling the

differentiation of XY from YY males in asparagus were developed by Reamon Buttner and Jung [43].

Cultivar identification

In crops like tomato, pepper, potato, alliums, cucurbits, lettuce and spinach, microsatellites have been developed to enable highly reliable identification of cultivars. Comparative assessment of different DNA fingerprinting techniques carried out in tetraploid potato revealed 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* [18] found ISSR markers to 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. Broun *et al* [7] identified two telomeric tendemly repeated sequences (7bp) and a closely linked 162 bp subtelomeric repeats in tomato that accounted for 2% of the total chromosomal DNA. These sequences have a very high mutation rate of 2% per generation. They have been shown to be extremely useful for distinguishing otherwise very similar tomato and melon varieties. An important application of molecular identification is the support it is likely to provide to the new plant variety registration system. The Indian Plant Variety Protection and Farmers' Rights Act 2001 grants intellectual property rights to developers of new crop varieties in the form of plant breeders' rights. In order to be eligible for registration and protection under the act, a candidate variety must meet the criteria of distinctness, uniformity and stability (DUS). Morphological data provide the basis for DUS testing. For determining distinctness, the variety is compared for a number of characters with the extent varieties. The PVPFR has also a provision of Essentially Derived Varieties (EDV) wherein the protection benefits are to be shared with breeders of the variety from which the EDV has been derived. For the present, DNA profiles alone as proof of unique identity of a plant variety is not acceptable. None the less, plant breeders may seek to strengthen their claim for protection of new varieties by including molecular profiles as supplementary information to establish the distinctness of their varieties. Molecular profiles may be particularly relevant in cases of biotechnologically developed varieties where only small apparent phenotypic differences exist between new variety and an extinct one.

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 (Table 2), especially in tomato (Table 3). 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.

CONCLUSION

Due to the rapid developments in the field of molecular genetics, a variety of techniques have been emerged to analyze genetic variation in germplasm and gene bank management especially during the last few decades,. The desirable properties of molecular markers are high polymorphism, codominant inheritance, frequent occurrence and even distribution throughout the genome, selectively neutral behavior, open access, easy and fast assay, low cost, high reproducibility and transferability between laboratories, populations, and/or species. Molecular marker maps, the necessary framework for any MAS programme, have been constructed for the majority of important vegetable crop species but the density of the maps varies considerably among species. Currently, MAS does not play a major role in genetic improvement programmes in any of the vegetable crop improvement programme. Enthusiasm and optimism remain concerning the potential contributions that MAS offers for genetic improvement of vegetable crops. Recent advances in molecular biology and biotechnology allow the transfer of specific genes from diverse sources into target crops, thus eliminating the hybridization barrier and time-consuming backcross selection of conventional breeding method. Through transgenic approach, by gene addition, subtraction, and pathway redirection, the genetic constituents of vegetable crops can be modified and broadened, resulting in new and improved traits. The emerging method of QTLs/MAS combines the capabilities of biotechnology and plant breeding technique to tackle and improve multiple gene traits. Its ability to identify and extract genes involving in multiple gene traits, from wild and cultivated germplasm resources, and to integrate them into existing crops, offer a new and potential means to further broadening the genetic diversity for vegetable crops improvement. The major application of markers lies in the strategic research for rapid understanding of basic genetic mechanisms and genome organization at molecular level. The success of DNA/molecular marker technology for bringing genetic improvement in vegetable crops would depend on close interaction between plant breeders and biotechnologists, availability of skilled man power and substantial financial investment on research.

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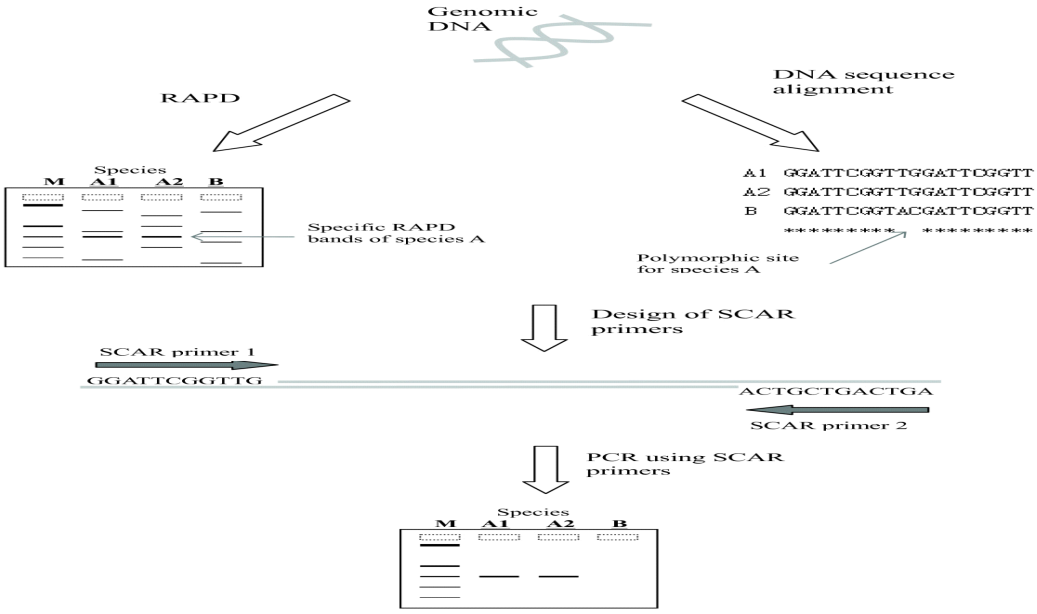

Figure 1: Pictorial illustration of RAPD marker

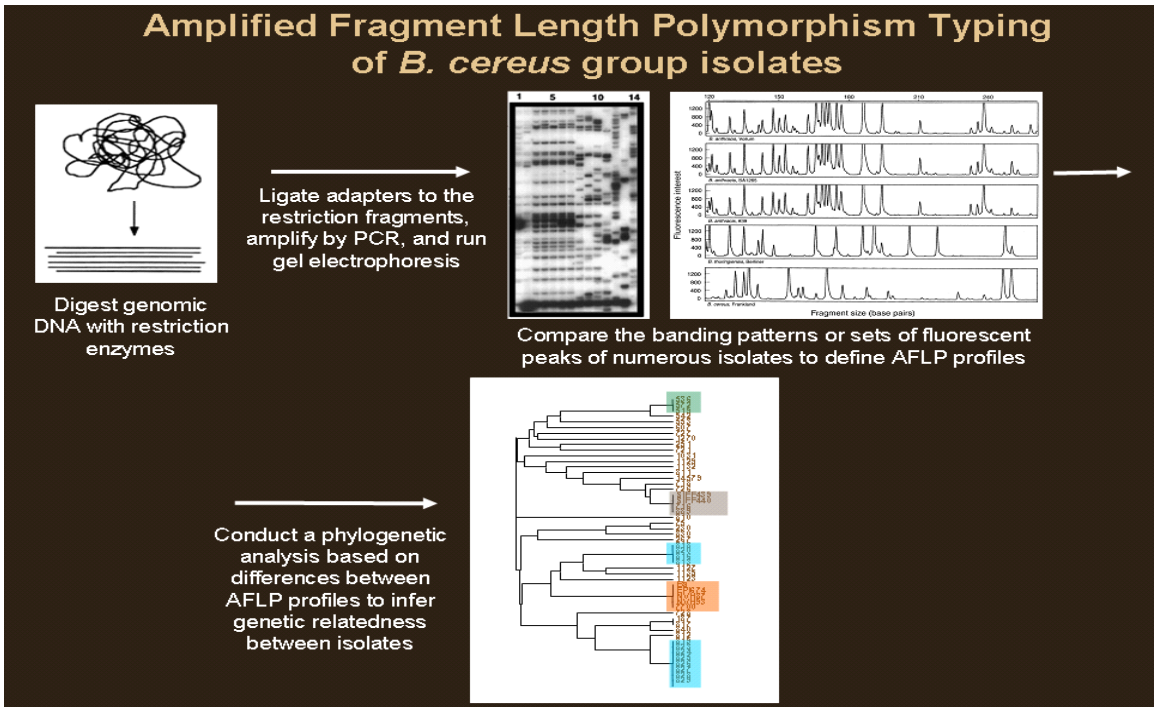

Figure 2: Diagrammatic presentation of AFLP marker technique

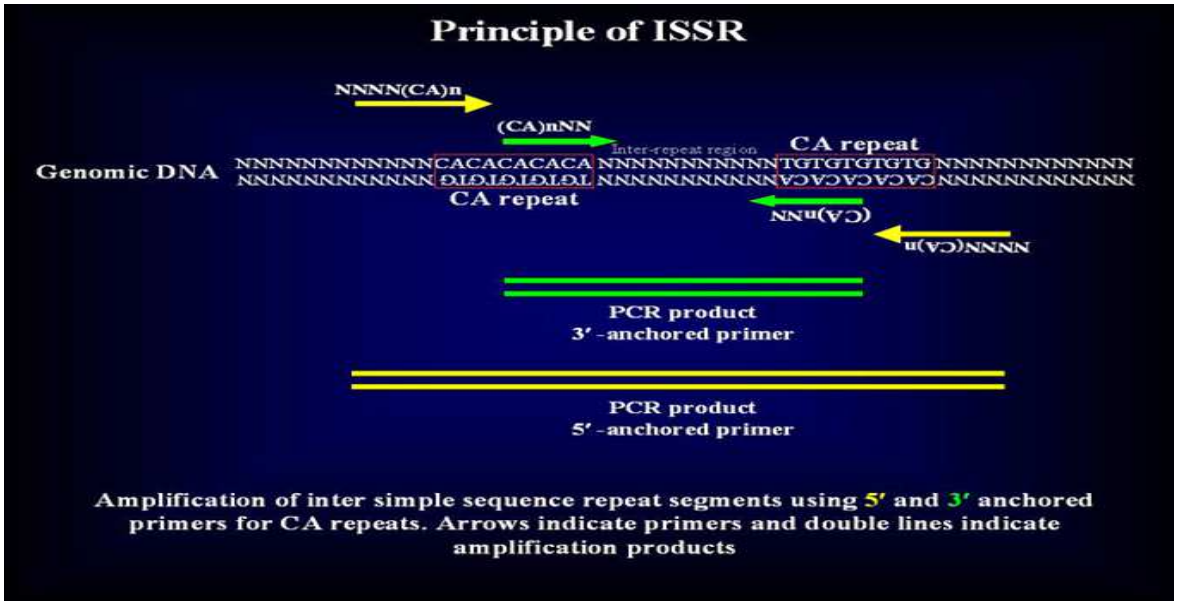

Figure. 3: Pictorial presentation of ISSR technique

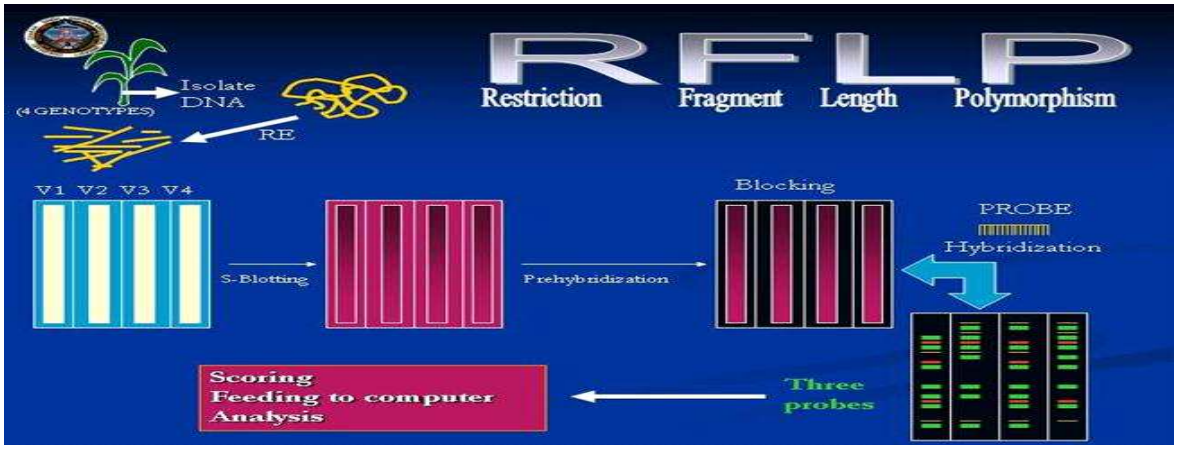

Figure. 4: Illustration of RFLP development

Table 1: Molecular marker technique in Vegetable improvement.

Symbol	Techniques	References
RFLP	Restriction fragment length polymorphism	Botstein *et al* (1980)
SSCP	Single-strand conformation polymorphism	Orita *et al* (1989)
SSLP	Minisatellite simple sequence length polymorphism	Jarmen and Wells (1989)
AP-PCR	Arbitrarily-primed PCR	Welsh and McClelland (1990)
RAPD	Random amplified polymorphic DNA	Williams *et al* (1990)
AS-PCR	Allele specific PCR	Sarkar *et al* (1990)
SAP	Specific amplicon polymorphism	Williams *et al* (1991)

SCAR	Sequence characterized amplified region	Williams *et al* [65]
DAF	DNA amplification finger printing	Caetano-Anolles *et al* (1991)
ISSR	Inter-simple sequence repeat	Zietkiewicz *et al.* [72]; Godwin *et al.*1997
SSR	Simple sequence repeats	Hearne *et al* (1992)
CAPS	Cleaved amplified polymorphism sequence	Lyamichev *et al* (1993)
SSLP	Microsatellite simple sequence length	Saghai *et al* (1994)
STS	Sequence tagged site	Fukuoka *et al* (1994)
ALP	Amplicon length polymorphism	Ghareyazei *et al* (1995)
AFLP	Amplified fragment length polymorphism	Vos *et al* [61] and (Kumar *et al* 2003)

Table 2: Identification of vegetable crop varieties by molecular markers

Sr. No.	Crop	Technique	Reference
1.	Tomato	Microsatellites, RAPD, RFLP	Kaemmer *et al* [26], Bredemeijer *et al* (1998), Noli *et al* (1999)
2.	Potato	AFLP, microsatellites, ISSR, RAPD	McGregor *et al* (2000) Ashkenazi *et al* [4]
3.	Onion, garlic and related species	AFLP, microsatellites, ISSR, RAPD	Arifin *et al* (2000), Fiseher and Bachmann [15]
4.	Pepper	AFLP, RAPD	Prince *et al* [41], Las Heras Vazquez *et al* [22], Paran *et al* (1998)
5.	Brinjal	RAPD	Karihaloo *et al* (1995)
6.	Vegetable brassica	Microsatellites, RAPD	Margale *et al* (1995), Cansian and Echeverrigaray (2000)
7.	Cucurbits	ISSR, microsatellites, RAPD	Gwanama *et al* (2000), Danin *et al* (2001)
8.	Pea	RAPD	Samee and Nasinee (1996)
9.	Beans	RAPD, RFLP	Stockton and Gepts [54]
10.	Spinach	Microsatellites	Groben and Wricke (1998)
11.	Asparagus	RAPD	Khandka *et al* (1996), Roose and Stone [46]
12.	Artichoke	RAPD	Tivang *et al* [56]
13.	Lettuce	AFLP, microsatellites	Hill *et al* (1996)
14.	Carrot & sweet potato	AFLP, RAPD	Shim and Jorgensen [51], He *et al* (1995)

Table. 3. Molecular markers linked to major resistant genes in vegetable crops

Crop	Pathogen	Gene	Marker(s)	Reference
Pepper	Tomato spotted wilt virus	Tsw	RAPD	Jahn *et al* 2000
	Tomato spotted wilt virus	Tsw	CAPS	Moury *et al* 2000
	Xanthomonas	Bs2	AFLP	Tai *et al* 1999

	vesicatoria			
Beans	Common bean mosaic virus	I	RAPD	Melotto *et al* 1996
Pea	Pea common mosaic virus	Mo	RFLP	Dirlewanger *et al* (1994)
	Erysiphe polygone	Er	RAPD	Dirlewanger *et al* (1994)
Cucumber	*F. oxysporum f.* sp. melonis	Fo m2	SSP	Wechter *et al* (1998)
Melons	*F. oxysporum* f. sp. melonis	Fo m2	RAPD	Wechter *et al* (1995)

Table. 4. Molecular markers linked to major resistant genes in tomato

Pathogen	Gene	Marker(s)	Reference
Meloidogyne incognita	Mi	RAPD	Williamson *et al*, [66]
Meloidogyne javanica	Mi3	RAPD	Yaghoobi *et al*, 1995
Cladosporium fulvum	Cf2	RFLP	Dixon *et al*, 1995
Verticillium dahliae	Ve	RFLP	Diwan *et al*, 1999
F. oxysporum f. sp. Radicislycopersici	Fr2	RAPD	Fazio *et al*, 1999
Cucumber mosaic virus	Cmr	RFLP	Stamova and Chetalat, 2000
Yellow leaf curl virus	Ty2	RFLP	Hanson *et al*, 2000
Tomato mosaic virus	*Tm2*	SCAR	Sobir *et al*, [53]