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Molecular Markers and a New Vista in Plant Breeding: A Review

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A B S T R A C T

Plant breeding, which set off its journey with human being's shifting from the activities of hunting and gathering towards nurturing seeds under the fixed settlement, is now a well-developed science. The destiny of plant breeding has been transformed since the 1980s, with impressive advancement of the technology called molecular marker. Various categories of molecular markers have been developed and the technologies have boosted crop improvement. RFLP was the first-generation molecular marker system based on the DNA-DNA hybridization method. Then the invention of PCR by Kary Mullis gave birth to the second-generation PCR-based markers. These were not only faster but also less expensive. Now, third-generation markers have taken their entry that is sequence-based. Molecular markers play A large number of important roles ranging from phylogenetic analysis, diversity studies, fingerprinting, construction of genetic maps, heterosis investigation etc. This review may be useful in better understanding the characteristics of various molecular markers.

Introduction

The information concerning the genetic differences available within as well as between a range of populations of plants and, the structure and level of these can take part in a beneficial task in the resourceful exploitation of plants (5). The background of evolution, gene-flow procedure, and system of mating and the density of the population are significant factors employed in the recognition of the structure and the intensity of these types of variations (7). With the aim of investigating the diversity and other vital characteristics, various sorts of morphological and agronomic parameters have victoriously been utilized. Even though the morphological characters, parameters that are ethnological and

cytological have traditionally been employed to characterize intensities and patterns of diversity, these traits single-handedly signify only a little portion of the genome of the plant and are manipulated by various environmental factors (22). These bound their effectiveness in unfolding the potentially multifaceted genetic organizations that may be present within and between the taxa (1).Throughout the last few decades, the progress in molecular biology and biochemistry has assisted to rise above these restrictions by delivering several potent molecular markers to the breeders (8).With the aim of detecting the genetic diversities, a range of molecular strategies has been worked out in several plants, including the vital horticulture crops utilizing DNA based markers. These kinds of markers are in general independent of the environmental influences and are far more abundant than the characters of phenotypic expression supplying obvious information about the variation in the genome which is underlying in an organism. This review aims to illustrate various molecular marker-systems that are engaged in the identification of the plant, improvement of the crop, population, and phylogenetic diversity studies and genome analysis.

Molecular markers/DNA markers

A trait that is polymorphic, effortlessly and reliably recognized, and readily tracked in the segregating generations and signifies the genotype of the individuals that show evidence of the trait is referred to genetic marker. The DNA markers are one type of genetic marker. The DNA-based markers represent the variation present in genomic DNA sequences of different individuals. They are detected as differential mobility of fragments in a gel, hybridization with an array or PCR amplification, or as DNA sequence differences. An ideal molecular marker should be of co-dominant nature, uniformly distributed all through the genome, highly reproducible and should possess the capability to spot a higher intensity of polymorphism (17). The marker system should not require prior information about the genome of an organism. However, none of the marker systems meets all the discussed criteria, but SNPs are the closest to being ideal DNA markers (41, 11) (Table 1).

Classification of molecular markers

The molecular markers are categorized into different groups on the basis of:

Mode of gene action (dominant and co-dominant markers);

Method of detection (hybridization-based and polymerase chain reaction (PCR) based molecular markers);

Mode of transmission (maternal nuclear inheritance, maternal organelle inheritance, paternal organelle inheritance, or the bi-parental nuclear inheritance) (29) (Fig. 1).

Hybridization-based markers

RFLP

Restriction Fragment Length Polymorphism or RFLP was the first molecular marker technique and the only marker system based on hybridization. RFLPs are the first generation of molecular markers. The general procedure for RFLP detection is as follows: (a) To begin with, high molecular-weight genomic DNA is isolated from several individuals/strains of a species or related species, (b) then separately each of the DNA samples is digested with the selected restriction enzyme, (c) the restriction fragments are then separated on the basis of size by gel electrophoresis, (d) next, the fragments are denatured and transferred from

the gel onto a suitable solid support in such a way that the relative positions of the fragments in the gel are preserved (Southern blotting), (e) the fragments are then fixed and exposed to the labeled DNA probe under the favoring DNA-DNA hybridization conditions (Southern hybridization), (f) the probe molecules not involved in hybridization are removed by washing, and (g) the fragments involved in hybridization with the probe are detected as distinct bands by autoradiography ("hot" probes) or by color development ("cold" probes).

DArT

The Diversity arrays technology or DArT offers an immense opportunity to genotype the polymorphic loci, which are all through distributed in the genome. With the aim to rise above some of the limitations conferred by other technologies of molecular markers (for instance RFLP, AFLP, and SSR), this technology was developed. It is a technology of microarray hybridization that is exceedingly reproducible. We do not necessitate prior information about the sequence for the purpose of detecting loci for any trait of our interest (9,38).The advantage of this particular technique which is the most significant is that it is throughput vastly and is exceptionally economical also.

The technology of DArT comprises a number of steps: (a) reduction of the complexity of DNA; (b) creation of the library; (c) the microarray (of libraries) onto glass slides; (d) hybridization onto slides of DNA that are fluoro-labeled; (e) looking at the slides for signal of hybridization and (f) extraction of data and analysis. For a species that is new, DArT markers are found out by screening a library of numerous thousand fragments from a genomic illustration set up from a pool of the samples of DNA taking in the diversity of the species.

PCR based markers

The PCR (Polymerase Chain Reaction) process was discovered in 1983 by Kary Mullis (20). It engages the utilization of Taq DNA polymerase acquired from the *Thermus aquaticus* for the amplification of a very minute quantity of target DNA exponentially. The information about the sequence of the flanking spots of the target DNA is required priorly to plan a suitable primer for the amplification purpose of the selected segment of nucleotide. Along with the DNA polymerase and primers, supplementation of the magnesium ions and the deoxynucleotide triphosphate (dNTPs) together with a proper buffer system are important in the fundamental PCR protocol. The PCR-based markers are far more beneficial over the hybridization-based RFLP due to the necessity of less quantity of DNA, the nonexistence of the radioisotopes, high reproducibility, further reliability, and the higher polymorphism in the petite period.

RAPD

Williams *et al.*, (39) and Welsh and Mcclelland (37) independently developed this technique called Random amplified polymorphic DNA or RAPD. Amplification of the genomic DNA is achieved by the process of PCR using a single, short (10 nucleotide) and random primer. During the PCR, when the two sites of hybridization are in opposite direction and alike to each other, then the amplification occurs. These fragments which are amplified, are dependent totally on the size and length of both the primer and the genome of the target (10). The opted primer should have GC content at least 40%.(39).For the purpose of visualization, the PCR product is next separated in the agarose gel that is stained with the ethidium bromide (12).The presence of polymorphism either between or at the binding sites of primer can be spotted with the

help of electrophoresis by validating the absence or presence of particular bands (10).

AFLP

The development of Amplified fragment length polymorphismor AFLP markers has overcome the inadequacies of the RFLP and RAPD techniques (33).These markers combine PCR technology with the RFLP and, in which the digestion of DNA is carried out and PCR is performed afterward (16). They are cost-effective markers. There is no requirement of prior sequence information to use these AFLP markers. In the case of AFLP, both partly degraded and good-quality DNA can be utilized; though, the DNA should not include any PCR inhibitors or restriction endonucleases. Two restriction enzymes (one rare cutter and one frequent cutter) are employed to make a cut in the DNA.

SSRs or microsatellites

Litt and Luty (1989) introduced the term micro-satellite. SSRs or Simple sequence repeats are the tandem repeat motifs of nucleotides (one to six) that have a presence in large quantities in the genomes of a variety of taxa (2). Microsatellites can be mono, di, tri, tetra, Penta and hexa-nucleotide (35). In the genome, microsatellites are dispersed; though, they have their presence also in the mitochondria (28) and chloroplast (26).Studies also have validated that SSRs have their presence in the expressed sequence tags (ESTs) and in the genes that are employed for protein-coding (18). The existence of SSRs is perhaps due to the slippage of ssDNA, recombination of dsDNA, retrotransposons and the mismatches. The motifs commonly present in SSRs are the Mono (A, T); Di (AT, GA); Tri (AGG); Tetra (AAAC). The sequences which flank the SSRs are mainly conserved and they are exploited to develop the primers. The process of SSR marker development engages the development of an SSR library first and after that revealing of definite microsatellites. In the next step, recognition of the favorable regions for the purpose to design primer is carried out and subsequently PCR is executed. The interpretation and evaluation of the banding-patterns are done and the evaluation of the products obtained from PCR is carried out for the target of investigating polymorphism (27). The SSR markers are believed as a marker of choice, since they are co-dominant, along with high reproducibility and superior genome abundance. They can efficiently be utilized in the mapping studies of the plant (30,13).

ISSR

The system of inter-simple sequence repeat or ISSR marker constructs multiple markers for each assay. This system utilizes primers, based on microsatellites or simple sequence repeats (SSRs) and amplifies the DNA sequences of inter-SSR. Among all of the di-, tri-, tetra-, and pentanucleotide SSRs, the most effective are the repeats of tetra-nucleotide in the purpose to produce polymorphic multiband patterns. The ISSR marker-system employs a17-21 nucleotide length single primer, and the repeats of the selected short sequence make the sequence of primer. To perform the amplification by PCR, of the genomic DNAs from different lines or individuals, a single primer is used. The products of PCR are generally separated by the use of agarose gel electrophoresis. Where the sequence of primer arises two nearby sites in the opposite orientations, there in those genomic regions, the amplification will take place. The system of ISSR spots difference in the length of the section between the two neighboring positions; this variation in length will be generated by comparatively large insertions and/or deletions. The intensity of polymorphism is associated with the genomic diversity within a particular species. Even

though the most ISSR markers are dominant, co-dominant markers can also be spotted. The non-requirement of any sequence data for the function of constructing primer and the necessity of low DNA quantities is the key benefit of the ISSRs.

SAMPL

This SAMPL or Selectively Amplified Microsatellite Polymorphic Locus is a variant of the technique AFLP. It amplifies the loci of a microsatellite by utilizing a single AFLP primer in association with a primer complementary to the compound sequences of the microsatellite. This technique does not necessitate prior to cloning as well as characterization. Due to recurrent null alleles, it is believed to be more relevant to intra-specific than to the inter-specific studies.

SCAR

Paran and Michelmore developed Sequence-characterized amplified region or SCAR markers first in 1993 in lettuce for resistance genes to downy mildew (23).As compared to the RAPD, SCAR markers have more specificity and also hold better reproducibility (42).These are mono-locus and co-dominant markers and are predominantly applied for the purpose of physical mapping (3).The course of action targeted to develop the SCAR markers takes account of purification of the PCR-fragments followed by designing of the SCAR primer (23,42,3,14).The polymorphic bands are identified by employing the agarose gel. The nucleotide sequence of the selected fragment of DNA is then investigated. At National Center for Biotechnology, the information sequence of this polymorphic DNA is examined by carrying out a comparison of known DNA sequences (available in the database) with it for the uniqueness of sequence. After that, this nucleotide sequence of the polymorphic DNA is employed to synthesize precise SCAR primers (3).

SCoT

This Start codon targeted or SCoT is a novel system of the marker. Based on the short conserved regions that flank the start codon ATG in the genome of a plant, this system was developed. SCoT engages 18-mer primer's utilization with a temperature for annealing at 50^{o}C (6).As in ISSR and RAPD markers, here also a single primer is employed in the PCR as the both forward and reverse primer. In general, these markers are reproducible. They are the dominant markers that can be exploited for analysis in plant genetics, analysis of the bulk segregants and also for mapping of quantitative trait loci (QTL) (6).

Sequence-based marker

SNP

The changes in the single base-pair present in the sequence of the genome of any individual are referred to Single-nucleotide polymorphism or SNPs. On the basis of substitution of the nucleotides, SNPs may both be the transitions and transversions. In general, changes of the single base are present in the mRNA, including the SNPs that are insertion or deletions (*i.e.* InDel) in one single base.SNP is able to offer the highest number of markers. In plants, the frequency of SNP, in general, is 1 SNP in each 100–300 bp(41). Usually, in the regions of the genome that are non-coding, the prevalence of SNPs is more. When the presence of an SNP is within regions of coding, either synonymous or non-synonymous mutations can be generated resulting in non-alteration or change of sequence of the amino acid. A huge number of techniques have been developed for the genotyping of SNP based on diverse skills of allelic-discrimination and the platforms for

detection. The easiest and simplest method among these all is the SNP-RFLP. The detection of SNPs is accomplished through analyzing the data of sequences that are stored in the databases. Analyses of SNP genotyping, in the majority, are based on the hybridization (allele-specific), primer extension, oligonucleotide ligation, or the invasive cleavage. SNPs are made the most attractive markers presently for the purpose of genotyping by a range of recent methods for high-throughput genotyping like the GBS, NGS, chip-based NGS etc. (Fig. 2).

Table.1 Comparison of important characteristic features of the molecular markers which are the most commonly used

Characteristics	RFLP	RAPD	AFLP	ISSR	SSR	SNP	DArT
Co-dominant/ Dominant	Co-dominant	Dominant	Dominant	Dominant	Co-dominant	Co-dominant	Dominant
Reproducibility	High	High	Intermediate	Medium-High	High	High	High
Polymorphism level	Medium	very high	High	High	High	High	High
Required DNA quality	High	High	High	Low	Low	High	High
Required DNA quantity	High	Medium	Low	Low	Low	Low	Low
Cost	High	Less	High	High	High	Variable	Cheapest
PCR requirement	No	Yes	Yes	Yes	Yes	Yes	No
Visualization	Radioactive	Agarose gel	Agarose gel	Agarose gel	Agarose gel	SNP-VISTA	Microarray

Fig.1 Classification of marker system

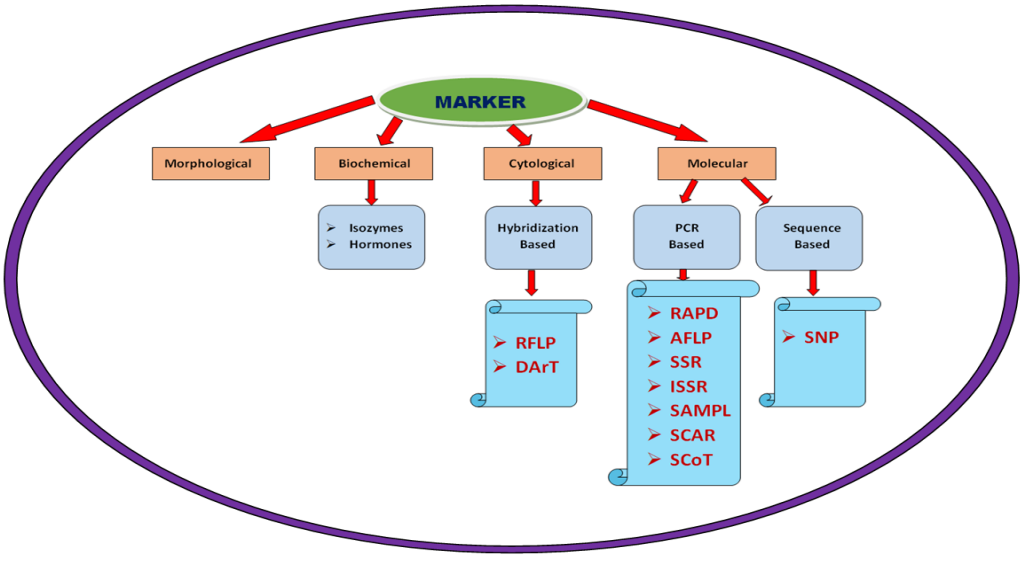
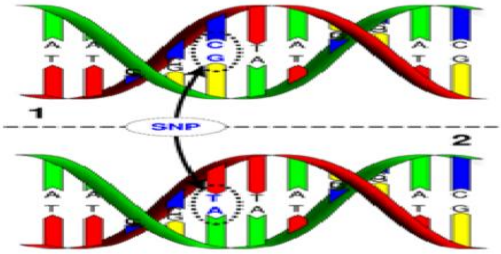


Fig.2 SNPs in DNA



Uses of molecular markers in plant sciences

The molecular markers are very helpful in unequivocal identification of plant varieties, e.g., for the protection of plant varieties. These allow us to construct high-density linkage maps since the number of markers available each cross is greatly huge. They can effectively be used for negative selection, *i.e.*to eliminate the undesirable genes, from the segregating population. Molecular markers help us on a large scale to get entire information regarding the phylogeny as well as evolution (25,31,3436). Genetic diversity assessment and genetic mapping have become easier with the advancement of molecular markers. The molecular markers aid us to carry out heterosis investigation also. In recent times, the SSR markers were applied in rice with the aim of investigating the heterotic groups and also patterns (40).

The wide-ranging molecular markers engaged in different plant researches have been discussed together with their versatile applicability. The hybridization-based DNA markers which are among the markers pioneering for the genetic diversity studies of plant have been outmoded by the development of various more reproducible and reliable markers that are PCR-based. Amongst the all marker technologies, the position of the marker of choice for the earlier 15 years was occupied by the microsatellites (4,19,21,32), due to their capability of being polymorphic highly and so informative character (24). The SNP markers have many promising benefits over those of microsatellite markers, because of high throughput automated analysis characteristic feature, lesser rates of mutation and genotyping costs lower in comparison (19, 15). The most appropriate marker choice will depend eventually on the scrupulous approach of research adopted and the degree of the resolution and polymorphism necessary for the precise study. With swift progress in the field of molecular biology, more efficient and advanced markers may become visible in the near future which can momentously speed up the researches in plant breeding.

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