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DNA-Based Molecular Markers: Transforming Precision Breeding and Strategic Crop Improvement

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INTRODUCTION

Molecular markers are genomic tools used in the molecular breeding for strategic crop improvement. Most of the molecular markers are used in the markers assisted breeding for introgression of trait to achieve durable resistance. Since last three decades, molecular markers have been used in the improvement of crops for various biotic and abiotic stresses. Molecular markers have also been employed in the genetic diversity analysis in several crops to understand diversity pattern at genomic level. Use of molecular marker system to enhance the efficiency of crop improvement is one of the robust platforms of the durable resistance development by introgressing desirable traits. In the last of 19th century, DNA based molecular markers system came into existence for identification of quantitative trait loci (QTLs) and genes on the chromosomal location of the plant species. However, impact of marker assisted selection employing DNA based marker would be greater in less advanced crop breeding programmes. Most of the molecular markers used in the marker assisted breeding and genetic diversity analysis are closely associated with the nucleotide sequences of DNA at corresponding position of the homologous chromosome of the two different individuals. DNA based molecular markers are versatile tools of genomic study and genomic assisted breeding for introgressing genes to achieve durable resistance (Eagles, et al., 2001). Therefore, DNA based molecular markers system offers several advantages over the traditional phenotypic marker system of crop improvement. The molecular marker assisted selection employing molecular marker would be helpful in the identification of desirable traits and also in the selection of breeding populations and progenies.

2. Molecular marker system

Molecular markers are DNA sequences linked with particular genes and whose inheritance would be detected. Most of the molecular markers are used in the crop germplasm characterization, genetic diversity analysis and marker assisted breeding for the purpose of crop improvement against various biotic and abiotic threats (Jones, et al., 1997). DNA markers in plants have been evolved as first generation molecular markers, completely based on DNA-DNA hybridization techniques and require high quality of DNA in large quantity. In 1980s, this technique was groundbreaking tools for molecular analysis and employed in several plan species for genetic study at DNA-level. This technique was labour extensive, time consuming and has low throughput. Later, DNA-level studies of plants come into existence after the successful demonstration of polymerase chain reaction (PCR) technique in 1983.

Commonly used molecular markers including RFLP, AFLP, RAPD, SSR and ISSRs are widely used in the genetic diversity analysis, germplasm characterization and genomic study (Vos, et al., 1995). Molecular markers help to quantify any DNA sample which we collect from organism and detect it with very low cost. It is also helpful to identifying the differences between any organism in terms of shape, size, structure, property and their nature. In addition, the advancement of molecular marker system shifted towards repetitive DNA system for the development of microsatellites. Most commonly used method for amplification of genomic DNA is the PCR based method in which the primers are labelled using radioactive fluorescence clone to make easy and fast detection (Williams, et al. 1991).

Amplified fragment length polymorphisms (AFLPs) are the second most popular molecular marker system which exists between RFLP and PCR technology. Simultaneously advancement of the molecular maker system reached to satellite system of the DNA detection. The satellites systems of the DNA in the higher organism contain multiple copies of the simple repetitive DNA sequence arranged in the array of vastly differing size (Litt and Luty, 1989). Because of the repetitive nature of the nucleotide sequences, such types of molecular markers are named as simple sequence repeats (SSRs). The simple sequence repeats are the PCR-based molecular markers system in which nucleotide sequences are repeated in terms of di, tri and tetra for construction of marker system. Most of the SSRs are short due to presence of short nucleotide sequence (Temnykh, et al., 2000). They are used in many crops for the genetic diversity analysis and parental polymorphism survey during marker assisted backcross breeding and recurrent parent genome recovery. Another important group of molecular makers are inter-simple sequence repeats (ISSRs) present in the amplifiable distance between two identical microsatellite repeats on the opposite entities of the chromosomal location (Jeffreys, et al. 1985).

Moreover, expressed sequence tag marker (EST) is mRNA based molecular marker system used in the genomic study. ESTs were originally intended as a way to identify gene transcripts, instrumental in gene discovery, for obtaining data on gene expression and regulation, sequence determination and for developing highly valuable molecular markers such as EST-based RFLPs, SSRs, SNPs and CAPS. The cleaved amplified polymorphic sequences (CAPs) are amplified DNA finger print which works as molecular marker followed by digestion of the PCR amplified products. They are more difficult to find due to limited size of the amplified products. Next-generation molecular maker system is one of the major

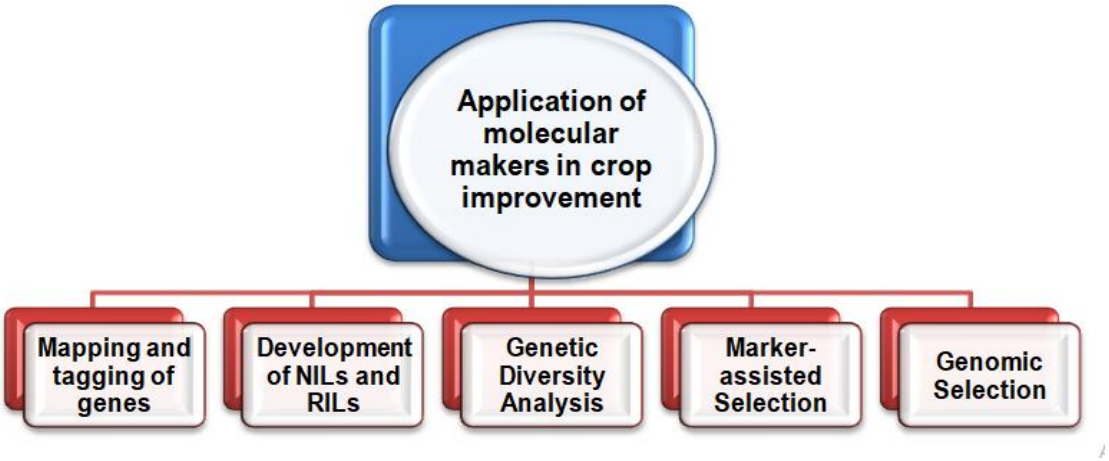
advantageous systems over the molecular marker systems used in 19th century for genetic diversity analysis and marker assisted selection. They are very quick and authentic in the modern era of the genomic studies and provide wonderful platform for the researcher. Genomic assisted breeding, genome-wide association studies and other important features can be easily accessed using next-generation molecular marker system. Recently in 20th century, scientists and researchers developed high density of marker is SNPs (Single base change in DNA sequence) with many advantages over the traditional molecular markers system of the crop improvement (Sobrino, et al., 2005). SNPs are robust molecular marker in the era of molecular breeding. SNPs have broad spectrum of application in crop improvement through genomic assisted breeding. They can also be used in other studies related to molecular mapping and tagging of the genes of interest.

Table-1: Salient features of all generation molecular marker techniques

DNA markers	Heritability	Polymorphism level
RFLP	Co-dominant	Low to Medium
RAPD	Dominant	Medium to High
SCAR	Co-dominant	High
AFLP	Dominant	High
SSR	Co-dominant	High
ISSR	Dominant	High
cpSSR	Co-dominant	Low
SAMPL	Co-dominant	High
SRAP	Dominant	Medium to High
SSCP	Co-dominant	High
CAPS	Co-dominant	High
EST	Dominant	High
STS	Co-dominant	High
RAMP	Co-dominant	High
SNP	Co-dominant	Extremely High
DArT	Dominant	High

3. Application of DNA makers in crop improvement

DNA based molecular marker system is the marvellous gift used as tools for broad range of application ranging from genetic diversity analysis and crop improvement. Numbers of oligogenic and polygenic traits have been identified with desirable features using molecular marker. Most of the molecular makers used in the marker assisted backcross breeding for crop improvement are either gene linked or gene based. Both molecular marker system have capability to depict the position of genes and associated nucleotide sequences on the various chromosomal location of the plant species. Utility of these DNA based markers play vital role in the fore ground selection during the first step of marker assisted breeding (Blair, et al., 1999). Furthermore, they can also employ in the parental polymorphism surveys on the various chromosomal locations with respect to trait of interest and other desirable purposes. Recurrent parent genome recovery can also be achieved using robust molecular makers system. In the modern era of molecular breeding, marker assisted selection is one of the major achievement for the researcher for development of improved crop varieties (Virk, et al., 1997).



3.1. Gene mapping and tagging: Mapping is a technique in which position of genes or molecular markers are depicted on the various chromosomal locations using several categories of the DNA based markers ((Brown and Kresovich, 1996). Molecular mapping through molecular marker can be applicable in terms of genetic mapping, physical mapping and linkage mapping. Genetic mapping is the technique in which position of genes or molecular markers are determined on the basis of recombination frequency. Recombination frequency can be achieved by number of cross over divided by total number of individual involved and then multiplying it by hundred. Physical mapping is another type of mapping techniques employing molecular marker in which position of genes or molecular marker determined in terms of base pair (bp). Third and important type of molecular mapping is the linkage mapping in which suitable mapping populations are created. In context to linkage mapping, the mapping populations are created on the basis of baulk segregation, and F_2 derived population developed through single seed descent method.

3.2. RILs and NILs development: Lines which are identical in all the genes, except one or few gene has considerably known as near isogenic lines. Most of the near isogenic lines are developed through backcrossing with recurrent parent up to 9 to 10 generation can be easily analysed using robust molecular marker system (Wu, et al., 1998). On other hand, recombinant inbreed lines are F_2 derived homozygous lines and are also analysed for desirable traits and gene of interest using molecular marker. Furthermore, the RILs and NILs developed from mapping population may also useful in the breeding programme for strategic crop improvement for various biotic and abiotic threats.

3.3. Exotic crop germplasm characterization: Molecular marker system is one of the robust platform for the genetic diversity analysis of the crop germplasm and also useful in the identification of genes and QTLs on various chromosomal location (Mason-Gamer, et al., 1998). DNA profiling of the crop germplasm for identification of alleles is one of the important practices for the development of platform for marker assisted breeding with aim of introgression in to crop varieties. Numbers of exotic crop germplasm are available and have several desirable traits than can be very useful in the crop improvement. Characterization, identification of the genes and QTLs from exotic crop germplasm and their incorporation into cultivated crop species may provide potential strength to the crop improvement. In context to genetic diversity analysis, it is necessary to understand the genetic variation between existing crop cultivars in comparison to their wild relatives and exotic germplasm. Number of DNA based markers with arbitrary and specific features is available for the DNA fingerprinting of the various class of the crop

germplasm. The molecular marker including AFLP, SSR, STMS, ISSR have potential to access the enriching gene pools and determine how molecular breeder accessing the pre-determine form of the genetic variation.

3.4. Genotyping: The genotypic information available with microsatellite repeats of the nucleotide sequences is useful for quantification of genetic diversity, characterization of crop germplasm and taxonomic study. Microsatellites including SSR and ISSR have been identified as useful marker system for identification STMS system with close proximity of crop cultivar identification. In plant species, the first application of microsatellite marker for identification of crop cultivars was used in the diversity analysis in number of crops like rice, wheat, maize, sugarcane, sorghum, chickpea, etc. One of the most important applications of molecular markers is to identification of sex in the dicotyledonous crops (Ellis, et al., 1998).

3.5. Marker-assisted selection: The diverse class of molecular markers including, RFLP, SSRs, ISSRs, STMSs, CAPSs, RAPDs, SCARs, SNPs have been employed in the stepwise crop improvement for yield, quality, disease resistance and other agronomic traits. The ideal molecular markers frequently distributed throughout the genome have potential to detect the location of gene of interest to be used in marker assisted breeding with purpose of crop improvement. Marker assisted selection for introgression of gene of interest in to crop cultivars have been potentially applied in several crops to achieve durable resistance like, rice, wheat, barley, maize, cotton, tomato, potato, papaya, chickpea etc.. Apart from mapping and tagging of genes, an important utility of RFLP markers has been observed in detecting gene introgression in a backcross breeding and mapping among closely related species. Similar utility of STMS markers has been observed for reliable pre-selection in a marker assisted selection backcross scheme. Besides specific markers, DAMD-based DNA fingerprinting in wheat has also been useful for monitoring backcross-mediated genome introgression in hexaploids wheat (Somers, et al., 1996).

CONCLUSION

Crop improvement with durable resistance potential is one of the major needs of molecular marker and marker assisted backcross breeding. High efficacy and potential of frequent distribution of molecular markers on various chromosomal locations on the crop genotypes fulfil the basic requirement of the crop improvement. Identification of genes and QTLs governing resistance and useful agronomic trait can be easily incorporated from one cultivar to other cultivars through backcross breeding. In the last of 19th century molecular makers have been potentially applied in the crop improvement through marker assisted backcross breeding. Several crops developed through marker assisted breeding are now cultivated and give better performance in term of disease resistance, durable resistance, improved quality, desirable agronomic traits.

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