



Utility of Molecular markers in Molecular breeding for integrated crop improvement

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ABSTRACT

*Molecular markers are the marvelous assets which are frequently used in the identification of particular genes/QTLs for the trait of interest as well as marker assisted back cross breeding. The utility of molecular markers in crop breeding for introgressions of traits depends on the efficacy of the markers. Molecular marker based foreground selection allows the screening at seedling stage. The background selection is the third and last step of marker assisted back cross breeding which involves the selection of back cross progenies with maximum coverage of genomic region of recurrent parent using chromosome wise maximum molecular marker, for the better recurrent parent genome recovery. There are several markers identified and mapped gene/QTLs and associated DNA markers linked to the gene of interest in rice (*Oryza sativa* L.) for bacterial blight, blast, brown plant hopper, drought, submergence and salinity, in Maize (*Zea mays* L.) for drought tolerance, salinity tolerance, banded leaf sheath blight, polysora rust and leaf blight, in Wheat (*Triticum aestivum* L.)*

Key words: *Molecular markers, foreground selection, background selection, recurrent parent genome recovery, gene based, gene linked markers*

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INTRODUCTION

Molecular breeding is the techniques used for development of resilience for various biotic and abiotic stresses. The term molecular breeding is used for several breeding strategies like marker assisted selection, marker assisted recurrent parent selection, along with marker assisted back cross breeding and genomic selection (1). The molecular breeding is the marvelous application of biotechnological strategies on the basis of genotypic assays used for the trait improvement or to alter plant traits (2). Now days marker assisted back cross breeding is frequently used in various crops for the awareness of the presence of genes/quantitative locus and breeding populations.

Researchers have identified and mapped gene/QTLs and associated DNA markers linked to the gene of interest in rice (*Oryza sativa* L.) for bacterial blight, blast, brown plant hopper, drought, submergence and salinity, in Maize (*Zea mays* L.) for drought tolerance, salinity tolerance, banded leaf sheath blight, polysora rust and leaf blight, in Wheat (*Triticum aestivum* L.) for drought and heat tolerance, rust and pre-harvest sprouting. Marker assisted incorporation of two or more genes (called as gene pyramiding) provide the long durability of resistant power for the disease, insect and pest. However, Molecular breeding offers the opportunity for the plant breeders to develop stress tolerant high yielding cultivars. The molecular breeding would lay the foundation for the modern crop improvement in 12th century (3,4).

MARKER ASSISTED SELECTION

Term marker assisted selection (MAS) was first used by Beckmann and Soller (5). Marker assisted selection is the selection of the allele for trait of interest and marker assisted back cross breeding (MABCB) is the introgression of one or more than one allele from the genetic background of one cultivar to the other cultivar. Recurrent parent genome recovery is the recovery of the original genome of the recurrent parent that can be achieved by the several time back crossing with the recurrent parent (1) and genomic selection is the selection on the basis genome-wide coverage of molecular markers linked to the trait of

interest (6,2). The evolutions of next generation sequencing have also been used by the several workers for detection of genome-wide polymorphism, these tends would accelerate the genomic selection [7].

MECHANISM OF MARKER ASSISTED SELECTION

The marker assisted back cross breeding accomplished in three steps *viz.* fore ground selection, recombinant selection and background selection followed by recurrent parent genome recovery (8). The SSRs, other microsatellites and SNPs markers are frequently used in marker assisted back cross breeding for incorporation of genes or QTLs in most of the cereal crops. The details of the mechanism of marker assisted selection are summarized below:

(i) Foreground Selection

Foreground selection is used for the screening of incorporated allele that is less time consuming than conventional breeding (9). Foreground selection allow the screening at seedling stage. In fore ground selection markers are used for the testing of targeted genes or QTLs (10).

(ii) Recombinant Selection

In recombinant selection the recombinant between flanking markers and loci of interest has to be selected. The size of incorporated chromosome, i.e., the donor chromosome having the target locus, is reduced by this selection. However, in conventional backcross breeding approach, the donor segment of the chromosome remain large even after many back cross generations >10. (11,12). Recombinant selection gives better result in two back cross generations because double recombination events on both sides of target locus are usually rare (13).

(iii) Background Selection and recurrent parent genome recovery

Background selection is the third and last step of marker assisted back cross breeding which involves the selection of back cross progenies with maximum coverage of genomic region of recurrent parent using chromosome wise maximum molecular marker, for the batter recurrent parent genome recovery five or more than five markers per chromosome give robust result (13). Hence, background selection is very useful for the recovery of recurrent parent genome.

MARKERS

Markers are those which mark the traits or features at external and internal level. Genetic markers are categorized in to three group namely morphological or phenotypic marker, biochemical markers and molecular marker.

(i) Phenotypic or Morphological Markers

In terms of crop research, morphological markers are defined as “indicator which indicates the survival of crops in open environment under the adverse climatic and ecological conditions”. In other words morphological markers are those which mark the crops traits for the survival by avoiding particular stress related to traits. Plant height and structural orientation of the up ground and underground organs are the main morphological markers of the crop and tree plant. Morphological markers can easily be characterized phenotypic characters of the plants such as colour of flowers, shape of seeds, growth habits and pigmentation (14).

(ii) Biochemical Markers

Biochemical markers are differences in enzymes that are detected by electrophoresis and specific staining (15). Most of the allozymes or isozymes are used as biochemical markers, they does not require DNA. These are easy, quick and cost efficient markers. Isoenzyme markers are the oldest technique as compared with molecular markers. Isozymes markers have been used in several crop improvement programmes (16, 17, 18). They are codominant markers with the high level of reproducibility. The banding pattern of the Zymograms can easily be interpreted in terms of loci and allele and segregation analysis of the progeny. The lack of their abundance, low level of polymorphism and non differentiative mobility in electrophoresis are the major drawback of the allozymes (19). (Table1)

However, allozymes have been used in studies like out crossing (20), population divergence (21), interspecific relationships (22), genetic inheritance (23), allelic frequency in germplasm (24), hybrid parents 2thidium2lites (25), diversity pattern and finger printing in crops [26, 27, 28, 29] (Table3).

Molecular Markers (Tools of molecular breeding)

Last two decades, DNA-based markers have been used in crop research for genetic diversity (30, 31, 32), sex identification (33) and mapping, tagging of genes (34). According to Stansfield (35) the term MARKER is usually used for “LOCUS MARKERS” and each gene has a specific position along the chromosome called locus.

The utility of the molecular markers are the basis of naturally occurring DNA polymorphism. “Molecular markers are the DNA sequences that are linked with the particular genes/QTLs/traits and whose inheritance would be detected”. Most of the molecular markers are used for the germplasm

characterization and marker assisted indirect selection for the desirable traits (36). An ideal molecular marker should have the following desirable traits- *Viz.*

- (i) Frequently occurrence: Frequently distributed throughout the genome.
- (ii) Molecular markers should must be polymorphic: The polymorphism is measured for the study of genetic diversity.
- (iii) Codominant in nature: For the study of homozygous and heterozygous states of the organism.
- (iv) Reproducibility: It should be highly reproducible.
- (v) Easy asses: It should be easy fast and cheap to detect.
- (vi) Selective neutral behavior and easy exchange of the molecular data between the laboratories.

A wide range of the molecular markers are available for the detection of polymorphism at DNA level (37). Most of the molecular markers are need PCR and some of them have no need of PCR. However, on the basis of utility of PCR, molecular markers are categorized in to two groups i.e. Non-PCR based molecular marker and PCR based marker (36).

(A) Non-PCR based molecular marker

Restriction Fragment Length Polymorphism (RFLP) and Minisatellites/Variable Number of Tandem Repeats(VNTR), are comes under the category of non-PCR based markers. In RFLP, the DNA sequence variation is detected by the digestion of genomic DNA with restriction endonuclease, which cut the DNA at specific sequence, electrophoresed, blotted on the membrane and probed with the labeled clone. These markers are codominant in nature (38, 39). RFLP markers were used for the first time in the construction of genetic maps (40). RFLPs can be used in diversity analysis, phylogenic studies, gene mapping (41), relationship between closely related texta(39, 42), finger printing (43), 3thidium3lite of the genes (44) and construction of genetic map (40).

Minisatellites, Variable Number of Tandem Repeats (VNTRs) can also be used as molecular markers. The 3thidium3lites was introduced (45). These loci contain repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs). Minisatellites are particularly useful in genetic identity and structure analysis, identification of varieties and cultivars (45,46), and population studies (47). (Table3)

(B) PCR based molecular marker

Amplified fragment length polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple sequence Repeat (SSR), Inter Simple Sequence Repeats (ISSR), Single-Strand Conformation Polymorphism(SSCP), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence Characterized Amplified Region(SCAR), and Single Nucleotide Polymorphism (SNP) are PCR based molecular markers. (48).

(i) Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) is an intermediate between RFLPs and PCR. AFLP needs two restriction enzymes. In AFLPs the DNA is digested with restriction enzyme, ligated with oligonucleotide adapters, pre-amplification of the ligated products directed by the primers complementary to the adapters and restriction site sequences, amplification and labelling of the amplified products and finally labelled products are amplified polyacrylamide gel electrophoresis (PAGE) (49, 50). AFLPs are codominant in nature, highly reproducible and sensitive method of polymorphism at DNA level (51). It can be used in genetic diversity, identification of pedigree and fingerprinting of cultivars (52). AFLPS have been used in genetic diversity by several workers in crop plants. (53)used AFLPs in the genetic study of Peanut cultivars, Soybean (54), and Maize (55). (Table 1 & 2)

(ii) Random Amplified Polymorphic DNA

Random Amplified Polymorphic DNA (RAPD) is very quick and easy molecular marker widely distributed in genome of the most of the cereal crops. In RAPD, the polymorphism of DNA is detected by single primer of arbitrary nucleotide sequence which anneals the genomic DNA at two different sites on complementary strands of DNA template. RAPDs are dominant in nature. This is frequently used in the polymorphism studies between the individuals (56). In RAPDs the primers are short synthetic (10bp) of random sequence and their amplified products are amplified by Agarose gel in the presence of carcinogenic agent 3thidium bromide (50). RAPDs have been used for genetic identity and diversity in several crops (57). (58) used RAPD for the distinguish *mugo*and *uncinata* their subspecies. (Table1&2).

(iii) Simple sequence Repeat

Simple sequence Repeat (SSR) is known as microsatellite. They are present in all eukaryotic genome. The term microsatellites were coined by Litt&Lutty (59). In SSRs, ranges of the alleles of different loci do not overlap (60). SSRs are the tandemly repeats of mono, di, tri, tetra and penta nucleotides with different length of the repeating motif. They are widely distributed throughout the genome and display high level of genetic variations based on the differences in the tandemly repeating units at a locus. They are amplified by the PCR using flanking region of the primers where sequences are known. However, SSRs are

the top class of molecular markers associated for the target trait in many crops (61) identified two EST-SSR markers linked to the photoperiod response gene (*ppd*) in wheat. A large number of SSRs found to be associated with the wheat genome (62, 63,64). In general, SSR gives high level of polymorphism and can be used in genetic studied, molecular breeding, (65, 66, 67), germplasm collections (68), phenotypic variations (69) and functional diversity in relation to adaptive variation (70). Simple sequence repeat (SSR) was very useful to identify date palm cultivars, and a high polymorphism has been detected in date palm cultivars (71). (Table 1& 2).

(iv) Inter Simple Sequence Repeats

Inter Simple Sequence Repeats (ISSRs) are DNA fragments of about 100–3000 bp located between adjacent, oppositely oriented microsatellite regions. This technique reported by Zietkiewicz (72). The ISSRs have been involved in the several genetic studies (72, 73). ISSRs have been used in the genetic studies of the tree plants (71,73). Monocotyledon species such as the genus *Poa*(74) and durum wheat (75) have to informative of ISSRs. (Table 1).

(v) Single Strand Conformation Polymorphism

Single Strand Conformation Polymorphism (SSCP), Cleaved Amplified Polymorphic Sequence (CAPS) and Sequence Characterized Amplified Region (SCAR) have also been used several studies like mutation detection (76), gene mapping (77, 78) and marker assisted selection (79). (Table1).

(vi) Single Nucleotide Polymorphism

Another novel type of PCR based molecular marker (Single Nucleotide Polymorphism) has been recently preferred in many genetic studies. SNPs are single base position in genomic DNA where two or more different nucleotide occurs in the different individuals. This type of polymorphism is due to substitution, deletion or insertion. The Single Nucleotide Polymorphism (SNPs) have wide range of linkage map score (80), heterogenetic study, positional cloning of mutant locus and linked gene inheritance in molecular breeding.(Table1&2).

Potential Application and Future prospects of the marker Assisted Selection:

Molecular markers have been used as tools in the various genetic studies in crop plants. Now a day's molecular marker are used in genomic studies for the development of tolerant varieties for biotic and abiotic stresses. A lot genes or QTLs, associated traits have been identified with the involvement of molecular markers for salinity resilient in the major crops like rice, wheat, maize, chickpea, brassica and sorghum. Fruit crops, vegetables and oil yielding crops have also been remarkable using molecular marker. However, in future crop improvement programme molecular markers will prove an asset or marvellous gift for crop the researchers and may play crucial role in national food security as well as worldwide.

Table1: A brief account of the molecular markers and their classification

S.No.	Name of the	Technique Discoverer
1.	Biochemical markers, Allozymes	(81, 82)
2	Molecular markers	
(a)	Non-PCR based techniques	
	Restriction Fragment Length Polymorphisms (RFLP)	(40, 41)
	Minisatellites or Variable Number of Tandem Repeats (VNTRs)	(45)
(b)	PCR-based techniques	
(i)	DNA sequencing, Multi-copy DNA, Internal Transcribed Spacer regions of nuclear ribosomal genes (ITS)	(83)
	Single-copy DNA, including both introns and exons	(84)
(ii)	Sequence-Tagged Sites (STS)	
(iii)	Microsatellites, Simple Sequence Repeat (SSR), Short Tandem Repeat (STR), Sequence Tagged Microsatellite (STMS) or Simple Sequence Length Polymorphism (SSLP)	(59, 65, 66, 67)
(iv)	Amplified Sequence Length Polymorphism (ASLP)	(85)
(v)	Sequence Characterized Amplified Region (SCAR)	(79,86, 87)
(vi)	Cleaved Amplified Polymorphic Sequence (CAPS)	(77, 78)
(vii)	Single-Strand Conformation Polymorphism (SSCP)	(76)
(viii)	Denaturing Gradient Gel Electrophoresis (DGGE)	(88)
(xix)	Thermal Gradient Gel Electrophoresis (TGGE)	(89)
(x)	Heteroduplex Analysis (HAD)	(90)
(xi)	Denaturing High Performance LiquidChromatography (DHPLC)	(91, 92, 93)
(xii)	Multiple Arbitrary Amplicon Profiling(MAAP)	(94, 95)
(xiii)	Random Amplified Polymorphic DNA (RAPD)	(56,57)

(xiv)	DNA Amplification Fingerprinting (DAF)	(94)
(xv)	Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)	(56, 96)
(xvi)	Inter-Simple Sequence Repeat (ISSR)	(72,73)
(xvii)	Single Primer Amplification Reaction (SPAR)	(97)
(xviii)	Directed Amplification of Minisatellites DNA (DAMD)	(98, 99)
(xix)	Amplified Fragment Length Polymorphism (AFLP)	(52)
(xx)	Selectively Amplified Microsatellite Polymorphic Loci (SAMPL)	(51)

Table2: Features of the commonly used molecular markers in crop study

S.N.	Feature	RFLP	RAPD	AFLP	SSRs	SNPs
1	Nature	Codominant	Dominant	Codominant	Codominant	Codominant
2	DNA Require(μ g)	10	.02	.5-1.0	.05	.05
3	DNA quality	High	High	Moderate	Moderate	High
4	PCR based	No	Yes	Yes	Yes	Yes
5	Polymorph loci analysed (No.)	1-3	1.5-50	20-100	1-3	1
6	Ease of use	Not Easy	Easy	Easy	Easy	Easy
7	Amenable to automation	Low	Moderate	Moderate	High	High
8	Reproducibility	High	Unreliable	High	High	High
9	Development Cost	Low	Low	Moderate	High	High
10	Cost per analysis	High	Low	Moderate	Low	Low

Table3: Advantages and disadvantages of Isozyme and molecular markers

Type of markers	Advantages	Disadvantages
ISOZYMES	Evolutionary studies, Isolation easier than DNA, used across species, No radioactive labelling, No need for sequence information	Laborious, less polymorphism, Expensive, Not easily automated
Restriction Fragment Length Polymorphism (RFLP)	High genomic abundance, Co-dominant markers, Highly reproducible, Good genome coverage, map based cloning	Need large amount DNA, Laborious, Need radioactive labelling
Randomly Amplified Polymorphic DNA (RAPD)	High genomic abundance, Good genome coverage, Less amount of DNA, No radioactive labelling, Relatively faster	Dominant markers, Not reproducible, not used across species, Not very well-tested
Simple Sequence Repeat (SSR)	High genomic abundance, highly reproducible, good genome coverage, high polymorphism, Easy to automate, Multiple alleles	Cannot be used across species, Need sequence information, Not well-tested
Amplified Fragment Length Polymorphism (AFLP)	High genomic abundance, polymorphism, No need for sequence information, Can be used across species, Useful in mapping	Very tricky due to changes in patterns with respect to materials used, Not reproducible
Sequence-Tagged Site (STS)	Useful mapping, good genome coverage, highly reproducible	Laborious, Unable to detect mutations, Need sequence information

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