European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 25-06-2019; Accepted: 28-07-2019
www.biosciencejournals.com
Volume 7; Issue 5; September 2019; Page No. 68-71

Microsatellite markers and their application in rice breeding for disease resistance: A review

Sonam Mahawar[1], Shobha Kumari[2], Prasenjit Debnath[3]
[1, 2] Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India
[3] Dept. of Plant Pathology, CAT Agartala, Tripura India

Abstract
Microsatellites are also known as simple sequence repeats (SSR), and they are typically composed of 1–6 nucleotide repeats. These markers are abundant, distributed throughout the genome and are highly polymorphic compared with other genetic markers, as well as being species-specific andco-dominant. For these reasons, they have become increasingly important genetic markers in rice breeding programs. The evolution of new biotypes of pests and diseases as well as the pressures of climate change pose serious challenges to rice breeders, who would like to increase rice production by introducing resistance to multiple biotic and abiotic stresses.

Keywords: Molecular markers, rice, disease resistence, microsatellite

Introduction
Marker is a piece of DNA molecule that is associated with a certain trait of an organism OR it is a mark, tag, landmark, benchmark to identify a gene. Genetic markers are used to identify different features in DNA sequence that can be used to differentiate between individuals in a population, or to classify individuals between different varieties or cultivars within a species. The different features in the sequence can be used to identify if that particular region was inherited from the female or male parent.

Genetic markers
Genetic markers are broadly grouped into two categories: classical markers and DNA/molecular markers. Morphological, cytological and biochemical markers are types of classical markers and some examples of DNA markers are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single-nucleotide polymorphism (SNP) and diversity arrays technology (DArT) markers [1]

Classical markers
Morphological markers
Morphological markers can visually distinguish qualities like seed structure, flower colour, growth habit and other important agronomic traits. Morphological markers are easy to use, with no requirement for specific instruments. They do not require any specialized biochemical and molecular technique. Breeders have used such type of markers successfully in the breeding programmes for various crops. Main disadvantages of morphological markers are: they are limited in number, influenced by the plant growth stages and various environmental factors. Since ancient times, humans have successfully used various morphological markers to investigate the variation for utilization in plant breeding.

Cytological markers
Markers that are related with variations present in the numbers, banding patterns, size, shape, order and position of chromosomes are known as cytological markers. These variations reveal differences in the distributions of euchromatin and heterochromatin. For example, G bands are produced by Giemsa stain, Q bands are produced by quinacrine hydrochloride and R bands are the reversed G bands. These chromosome landmarks can be used in the differentiation of normal and mutated chromosomes. Such markers can also be used in the identification of linkage groups and in physical mapping

Biochemical markers
Biochemical markers, or isozymes, are multi-molecular forms of enzymes which are coded by various genes, but have the same functions [2] They are allelic variations of enzymes and thus gene and genotypic frequencies can be estimated with biochemical markers. Biochemical markers have been successfully applied in the detection of genetic diversity, population structure, gene flow and population subdivision [3] They are co-dominant, easy to use and cost effective. However, they are less in number; they detect less polymorphism and they are affected by various extraction methodologies, plant tissues and different plant growth stages [4]

Molecular markers/DNA markers

Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. Insertion, deletion, point mutations duplication and translocation are basis of these polymorphisms; however, they do not necessarily affect the activity of genes. An ideal DNA marker should be co-dominant, evenly distributed throughout genome, highly reproducible and having ability to detect higher level of polymorphism [4]

Classification of molecular markers
Molecular markers are classified into various groups on the basis of:

1. mode of gene action (co-dominant or dominant markers);
2. method of detection (hybridization-based molecular markers or polymerase chain reaction (PCR)-based markers);
3. mode of transmission (paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance or maternal nuclear inheritance)

Different types of DNA molecular markers have been developed and successfully applied in genetics and breeding activities in various agricultural crops. The following section provides some brief information related with molecular markers based on their method of detection. Comparisons of the important characteristics of most commonly used molecular markers are given in Table 1.

Table 1: Different types of molecular markers

Sequence-tagged site (STS):-
1. Helpful in preparing maps
2. High reproducibility
3. Can use filters many times
4. Moderate genome coverage

SSR:-
1. Co-dominant
2. High polymorphism
3. Multiple alleles
4. Highly reproducible

RFLP:-
1. Co-dominant
2. Genomic abundance high
3. Better genome exposure
4. No need for sequence information

RAPD:--
1. Dominant Marker
2. Genomic abundance high
3. Better genome coverage
4. Sequence information unneeded
5. Perfect for automation

AFLP:-
1. High polymorphism
2. Genomic abundance high
3. Can be used across species
4. Not reproducible

Microsatellites

The term microsatellite was first coined by Litt and Luty (1989) [5]. Microsatellites are also known as simple sequence repeats (SSR), and Motifs consisting of 1 to 6 base pairs nucleotide repeats. Microsatellites are simple repeats. The mutation rate of this type of genetic marker has been estimated to be between 10^{-2} and 10^{-4} per generation.These markers are abundant, distributed throughout the genome and are highly polymorphic compared with other genetic markers, as well as being species-specific and co-dominant. For these reasons, they have become increasingly important genetic markers in rice breeding programs. The evolution of new biotypes of pests and diseases as well as the pressures of climate change pose serious challenges to rice breeders, who would like to increase rice production by introducing resistance to multiple biotic and abiotic stresses.

Classification of microsatellites

(A)Based on the arrangement of nucleotides in the repeat motifs

- perfect – CACACACACACACACACACA
- Imperfect-CACACACACA---CACACACA---CACACACA
- Compound- ACACACACACACA CATACATACATA CATACATACATA

(B) Based on the number of nucleotides per repeat

- Mononucleotide- AAAAAAAAA
- Dinucleotide- GTGTGTGTGTGT
- Trinucleotide- CTGCTGCTGCTG
- Tetranucleotide- ACTCACTCACTCACTC
- Pentanucleotide- AAATTAAATTAAATTAAATT
- Hexanucleotide-
- CTTTAACTTTAACTTTAACTTTAA

(**C) Based on location of SSRs in the genome**

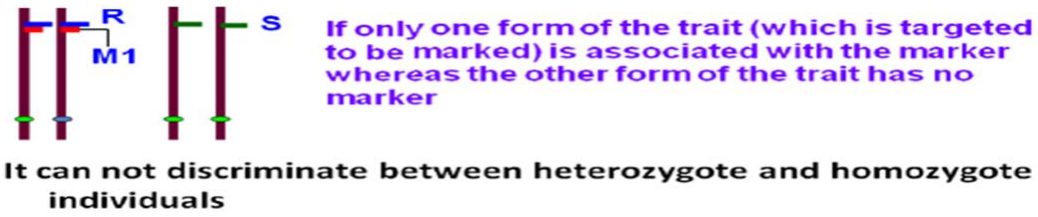
- Nuclear (NUSSRS)
- Chloroplastic (CPSSRS)
- Mitochondrial (MTSSRS)

Advantages of microsatellites as genetic markers: (6)

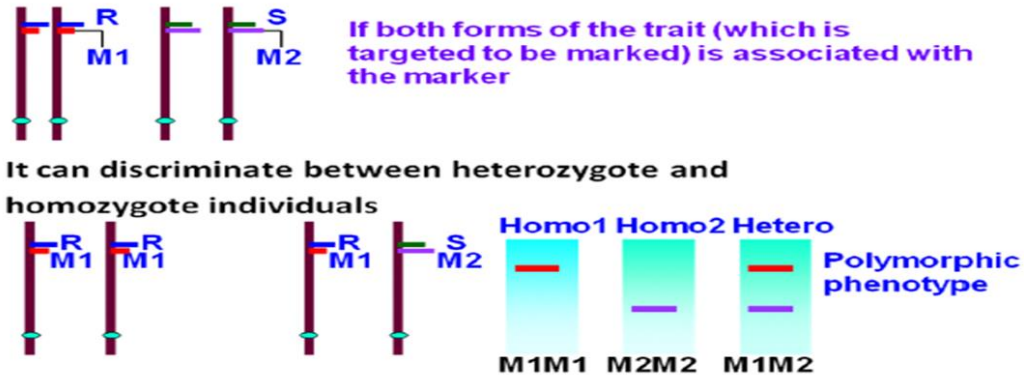
The ideal type of genetic marker should be highly polymorphic, show co-dominant inheritance and be evenly distributed throughout the genome. In addition, particular marker sequences should be easy to access, and analyses should be low cost, high-throughput, reproducible, and transferable between laboratories, populations and/or species. Locus-specific (in contrast to multi-locus markers such as minisatellites or RAPDs).

Method of marker inheritance (e.g., dominant *vs.* codominant) and the type of genetic information needed in the population (7-11).

Dominant Marker



Co dominant Marker



Collard *et al*., 2008 [12].

Microsatellite marker development

Completely sequenced genomes provide the basis upon which to design a large number of gene-based microsatellite markers for example, rice (*Oryza sativa* L.) was the first cereal to have its genome completely sequenced, which has enabled the development of a large number of microsatellite markers [58]**.** Recently**,** Zhang *et al.* [59] developed 52, 485 microsatellite markers that are polymorphic between *indica* and *japonica*. However, the difficulty now lies in choosing the most useful and informative microsatellite markers from such large datasets to use in rice genotyping applications. This problem can be overcome by constructing smaller, informative microsatellite marker databases composed of markers located in potentially functional genic sequences with relatively high polymorphic potential. Considering the excellent genetic attributes and higher predicted informativeness of genic non-coding microsatellite (GNMS) markers, Parida *et al.* [60] identified 19, 555 perfect (GNMS) repeats on chromosomes 1 and 12 in rice. With the entire rice genome now sequenced, microsatellite markers can be developed within a few thousand base pairs of any gene.

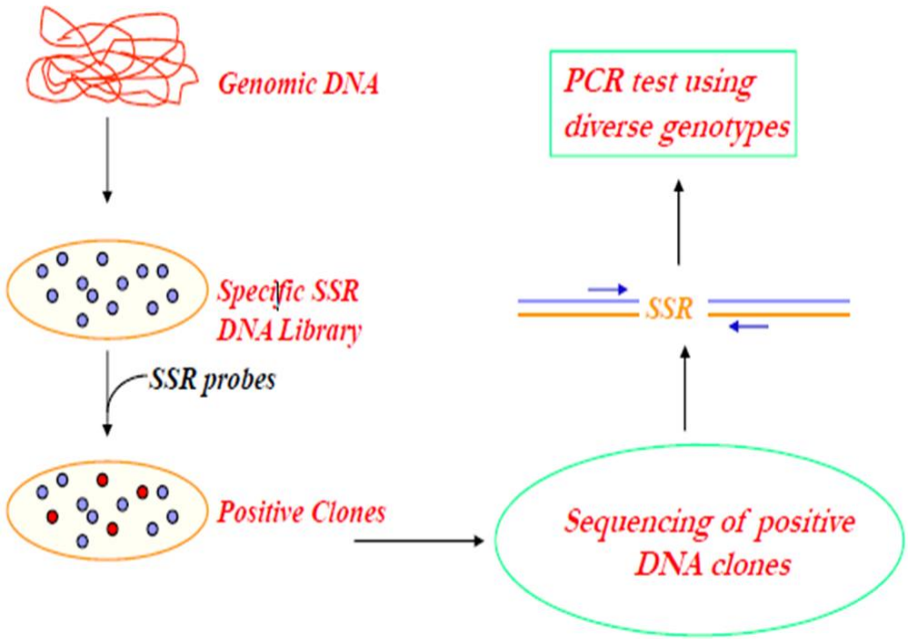


Fig 1: Microsatellite marker development

Identification of microsatellite markers linked to the blast resistance gene Pi-1(t) in rice

Fuentes *et al(* 2008) used a segregating population with identical genetic back-ground (CO39) but with a higher number of segregant lines than the one used by these authors, we have identified two new microsatellite markers (RM1233*I and RM224) highly linked to gene Pi-1(t) (at 0 cm of the gene). Working with several rice populations, Fjellstrom *et al.* (2004) found RM1233*I and RM224 markers highly linked to the resistance genes Pi-khand Pi-Ks. However, it has been shown more recently that Pi-kh mapped at 101.9 cM on Chromosome 11 closely linked to RM206 marker (Sharma *et al*. 2005). The Pi-1(t) gene was previously mapped between markers RG303 and RZ536 (Yu et al. 1996), which are about 24.7 cM apart on chromosome 11 (Chen et al. 1997). In this experiment they found six polymorphic markers in a region of only 13 cM surrounding the blast resistance gene Pi-1(t). Additionally, they found two of these markers (RM1233I and RM224) were closely linked to the gene

Table 1: Quality indicators for six microsatellite markers computed (Fuentes *et al*.,2008)

Probability qualification (%)	Marker analyzed					
	RM 1233 I	**RM 7654 A**	**RM 7654 H**	**RM 7654-2**	**RM 6094**	**RM 224**
False Linkage(x)	0.0	2.9	5.7	2.9	8.0	0.0
False no Linkage	0.8	1.6	2.5	2.4	9.9	0.8
Power	99.2	98.4	97.5	97.6	90.1	99.2
Sensitivity	100.0	99.2	98.3	99.2	91.9	100.0
Specificity	97.2	94.4	91.87	91.8	90.1	97.2
Predictive value Positive (PVP)	100.0	99.2	98.3	99.2	91.9	100.0

Conclusion

Microsatellite markers provide an invaluable tool for plant geneticists and breeders, as detecting polymorphisms which are the limiting factor in many breeding strategies. In the long term, the development of allele-specific markers for genes controlling disease resistance traits (e.g., blast disease resistance) will become increasingly important in the science of rice breeding. Therefore, there exists a great opportunity for more efficient breeding programs and faster development times for new rice varieties resistant to biotic diseases in the future.

References

1. Jiang GL. Molecular markers and marker-assisted breeding in plants. In: Andersen SB, editor. Plant breeding from laboratories to fields. Rijeka: In Tech, 2013, 45-83.
2. Bayley DC. Isozymic variation and plant breeders' rights. In: Tanksley SD, Orton TJ, editors. Isozymes in plant genetics and breeding. Amsterdam: Elsevier; 1983, 425-440.
3. Mateu-Andres I, De Paco L. Allozymic differentiation of the *Antirrhinum majus* and *A. siculum* species groups. Ann Bot. 2005; 95(3):465–473.
4. Mondini L, Noorani A, Pagnotta MA. Assessing plant genetic diversity by molecular tools. Diversity. 2009; 1(1):19–35
5. Litt M, Luty JA. A hypervariable microsatellite revealed by *in vitro* amplication of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. 1989; 44:397–401.
6. Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim H A., Islam Khn, Latif MA. A Review of Microsatellite Markers and Their Applications in Rice Breeding Programs to Improve Blast Disease Resistance. Int. J. Mol. Sci. 2013; 14:22499-22528
7. Staub JE, Serquen FC. Genetic markers, map construction, and their application in plant breeding. Hort Sci. 1996; 31:729–740.
8. Karp A, Kresovich S, Bhat KV, Ayad WG, Hodgkin, T. Molecular Tools in Plant Genetic Resources Conservation: A Guide to the Technologies; IPGRI Technical Bulletin No. 2; International Plant Genetic Resources Institute (IPGRI): Rome, Italy, 1997.
9. Wolfe AD, Liston A. Contribution of PCR-Based Methods to Plant Systematics and Evolutionary Biology. In *Molecular Systematics of Plants II: DNA Sequencing*; Soltis, D.E., Soltis, P.S., Doyle, J.J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1998, 43-86.
10. Mackay TFC. The genetic architecture of quantitative traits. Ann. Rev. Genet. 2001; 35:303-339.
11. Rungis D, Hamberger B, Berube Y, Wilkin J, Bohlmann J, Ritland K. Efficient genetic mapping of single nucleotide polymorphisms based upon DNA mismatch digestion. Mol. Breed. 2005; 16:261–270.
12. Collard C. Y. and David J. Mackill Phil.2008., Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Trans. R. Soc. B. 2005; 363:557–572.