Utility of DNA Markers in rice breeding

Dr. Nisha Sulari Kottearachchi
Senior Lecturer
Department of Biotechnology
Faculty of Agriculture and Plantation Management
Wayamba University of Sri Lanka,
Makandura, Gonawila, Sri Lanka
T:P: 94-071-8122228
Fax: 94-031-2299248
E-mail: kottearachchins@yahoo.com

Abstract
This review indicates applied uses of DNA markers in rice breeding and provides the updated information on different types of DNA markers with sequence information. Markers such as SSR, InDel and Perfect markers or functional markers are the main choices of rice breeders nowadays as they are convenient to use and easily discriminable between parents. The limitations of conventional breeding such as linkage drag and lengthy time consumption can be overcome by utilizing DNA markers in breeding. The major applications of DNA markers such as marker assisted selection and Quantitative Trait Loci (QTL) mapping etc have been illustrated with successful examples. Most of practically used markers that are linked with agronomically important traits in rice have been exemplified with primer information and related citations. Finally direct economic benefits given by molecular markers and the significance of establishing database on rice cultivars along with DNA marker profiles have been discussed.

Key words: DNA markers, Marker assisted selection, Quantitative trait loci

Introduction

The phenotypic differences that distinguish one plant from another are encoded in the plant's DNA, the hereditary material. In conventional plant breeding, the phenotype of a plant is visually assessed by measurable traits and the best-performing plants are chosen within populations by phenotype details. This process can be difficult, slow, need expertise labour, influenced by the environment, and costly. Moreover, many important agricultural traits, including yield, disease resistance, abiotic stress tolerance etc. so-called quantitative traits, are the most difficult to breed due to environmental influences and genotype-environment interaction on trait expression. To get rid of these limitations, plant breeders use DNA markers that represent the gene responsible for trait and selection based on makers linked to gene is called Marker Assisted Selection (MAS). As the marker is located near the DNA sequence of the desired gene and as it is transmitted from one generation to the next by the standard laws of inheritance, varietal breeding followed by MAS has been a reliable technology to expedite breeding process.

Commonly used DNA markers

DNA markers are basically classified as hybridization based markers and polymerase chain reaction (PCR) based markers. Hybridization based markers refer to the Restriction Fragment Length Polymorphic (RFLP) markers which are visualized by hybridization of restriction enzyme digested DNA to a labelled probe of known sequence. However, RFLP markers are not in used nowadays due to complications in protocols. The other type of markers which is called PCR based markers, involve *in-vitro* amplification of specific DNA sequence by using either specifically designed or arbitrarily chosen random primers. Among the PCR based DNA markers, randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), inter-simple sequence repeats (ISSRs), simple sequence repeats (SSRs), sequence tagged sites (STS), Sequence characterized amplified regions (SCARs) and Single Nucleotide Polymorphism (SNP) based markers are commonly used markers in tagging and mapping genes in rice. SNPs are usually covert into cleaved amplified polymorphism (CAPs) markers. Among all markers microsatellites or simple sequence repeats (SSRs) have been the major choice in rice breeders, specially due to the abundance of SSR makers. A total of 18,828 Class 1 di, tri and tetra-nucleotide SSRs, were identified on the rice genome (IRGSP, 2005) and, using those repeated sequences, countless amount of SSR markers have been developed in rice and their chromosomal location and polymorphism levels have been determined (http://www.gramene.org). Novel PCR-based marker called insertion/deletion (InDel) markers that shows polymorphisms mainly between *Indica* cultivars and *Japonica* cultivars (Shen et al., 2004) have been popular among breeders nowadays. Due to their simplicity in design and sharp discrimination in genotyping, InDel markers have routinely been used in MAS and mapping studies in the combining instances of *Japonica* and *Indica* genomes.

Functional markers or perfect markers that are developed by referring the gene sequence obtained by map based cloning are generally considered as more precise markers. This is achieved by fine mapping the gene to the closest possible level followed by cloning to confirm the function. Then the sequence of the cloned gene or the allele is analyzed for the presence of functional nucleotide polymorphism and marker can be developed if the functional polymorphism exist corresponding to the presence and the absence of the particular allele-sequence. The function of the protein encoded by the cloned gene is determined by comparing with the amino acid sequence of known proteins. The table 1 shows some of such proteins revealed by the cloned genes of agronomically important in rice, and the details of PCR based gene specific markers are indicated in Table 2.

Table 2: Proteins encoded by some agronomically important genes identified by map based cloning

Name of the gene*	Encoded protein	Reference.
Bacterial blight resisatnt-*Xa21*	NBS-LRR type receptor kinase that recognize bacterial exudates and induce defence signalling pathway.	Song et al. (1995)
Blast resistance -*Pi9*	Nucleotide-Binding Site–Leucine-Rich Repeat Protein *(Nbs2-Pi9)* that contribute for broader spectrum resistance	Qu et al. (2006)
Semi- dwarf - *Sd1*	Defective gibberellin-20-oxidase contributing to	Spielmeyer et al. (2002)

	semidwarf phenotype.	
Grain length/shape-*GS3*	Transmembrane protein that function as a negative regulator to prevent the growth of the grain size	Fan et al. (2006)
Fragrance -*Badh2.1*	Mutated Betaine aldehyde dehydrogenase enzyme that initiate the fragrant compound, 2-Acytle Pyroline, synthesis	Bradbury et al. (2005)
Submergence tollernce-*Sub1A*	Ethylene response factor-like gene that confers submergence tolerance to rice	Xue et al. (2006)
Brown plant hopper resistance-*Bph14*	Coiled nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) protein that activate salicylic acid signalling pathway for the defence	Du et al. (2009)

*Primer sequence information of the markers developed to identify the gene, is indicated in Table 2,

Genes tagged with DNA markers in rice

In rice many DNA markers closely linked to the genes have been mapped during past two decades and now they are used to detect the presence of gene without waiting to observe its phenotypic appearance. This has been a great advantage to breeders as the selection can be carried out even at seedling stage without following morphological traits. Also screening can be done even without having the incidence of pests and disease if the markers are associated with pest and disease tolerance. Several genes for bacterial blight (BB) resistance have been mapped namely, *Xa1*, *Xa5*, *Xa13*, *Xa21*, *Xa26*, *Xa27* etc. By pyramiding several of these genes into one line, plants have expressed strong resistance to BB (Kottapalli et al. 2010, Shanti et al. 2010). A high yielding, fine-grain type rice variety called Samba Mahsuri has been bred by introgression of *Xa21, Xa13* and *Xa5 into a one line* through marker-assisted breeding (Sundaram et al. 2010). The *Xa21* inherited in *Oryza longistaminata*, was cloned and identified as the allele that encodes a receptor with leucine rich repeats (LRRs) in the extra-cellular domain in the cell membrane and it is a key recognition molecule of *Xanthomonas oryzae* pv oryzae for the induction of plant's innate immunity (Ronald et al. 1992, Song et al. 1995). A perfect marker, *pTA248*, has been developed to discriminate BB resistance and BB susceptibility based on the functional polymorphism of *Xa21*. Table 2 indicates various markers linked with BB resistant genes that have been reported in various mapping studies.

The Fragrant gene markers, ESP, IFAP, INSP, EAP developed from the cloned mutated allele of the *badh2* gene in rice, is one of the most frequently used perfect markers in discriminating Basmati type aromatic rice (Bradbury et al 2005). *Badh2* gene encodes the enzyme, batain aldehyde dehydrogenase 2 whose loss of function leads to the accumulation of 2AP, the major fragrant volatile compound in rice. *ESP*, *IFAP*, *INSP*, *EAP* markers have been developed based on DNA sequence of fragrant allele that contains 8bp deletion in 7th exon of *badh2*. Although Basmati fragrant allele contain 8bp deletion, most of other aromatic rice contain different other mutations that leads to fragrance. For instance, aromatic rice originated in Myanmar do not contain Basmati type 8bp deletion, instead they contain 3bp insertion in 13th exon of *badh2* gene that is named as *badh2.8* allele. Based on the functional polymorphism at 13th exon of *badh2,* Myint et

al. (2012) have developed a perfect marker named as *3In2AP* to discriminate aromatic rice habituated in Myanmar region.

Although many functional markers have not been developed due to the fact that many of the mapped genes have not been cloned, researchers have used other markers such as SSR, SNP etc. that are tightly linked with genes in Marker Assisted Breeding (MAB) in rice. If the marker co segregate with the gene of interest and if polymorphism appear in the desired cross, it is convenient and economical to carry out the selection with the assistance of the particular marker. Table 2 indicates most of closely linked markers to many of agronomically important traits in rice that are practiced in rice breeding.

Table 2: Closely linked markers to various agronomically important traits in rice

Character	Gene	Ch	Name of the marker and sequence	Reference
Bacterial blight resistance	*Xa1*	4	16PFXa1/*EcoR*V -SNP (F:ACGGTTCTGAAGGTCGTCAT R:TGCAAGAGCTCCGGTTTAGG)	Shin et al. (2006), Shin et al. (2007)
Bacterial blight Resistance	*Xa4*	4	MP-STS (MP1 : ATCGATCGATCTTCACGAGG MP2 : TCGTATAAAAGGCATTCGGG)	Ma et al. (1999)
Bacterial blight resistance	*xa5*	5	RG556/*Dra*I -SNP (F:TAGCTGCTGCCGTGCTGCGC, R:AATATTTCAGTGTGCATCTC)	Huang et al. (1997), (Yoshimura et al. (1995)
			RM122 - SSR (F:GAGTCGATGTAATGTCATCAGTGC, R:GAAGGAGGTATCGCTTTGTTGGAC)	Chen et al. (1997) Ullah et al. (2012)
Bacterial blight resistance	*Xa7*	6	M5- STS (F:CGATCTTACTGGCTCTGCAACTCTGT, R:GCATGTCTGTGTCGATTCGTCCGTACGA)	Porter et al. (2003) Zhang et al. (2009)
Bacterial blight Resistance	*Xa13*	8	RG136 / *Hinf* I - SNP (F:CCCAGAAAGCTACTACAGC, R:GCAGACTCCAGTTTGACTTC)	Zhang et al. (1996), Huang et al. (1997)
Bacterial blight resistance	*Xa21*	11	pTA24 - STS (F:AGACGCGGAAGGGTGGTTTCCCGGA, R:AGACGCGGTAATCGAAAGATGAAA)	Ronald et al. (1992), Huang et al. (1997)
Blast resistance	*Pi1*	2	MRG4766 -SSR (F:ATTGCTGCAAAGTGGGAGAC, R:AAGTGGAGGCAGTTCACCAC)	Chen et al. (2007) Li et al. (2012)
Blast resistance	*Pi9*	6	pBA14 -SCAR (F:TGGTGCACTCAGAAAGAA, R: GCAGTGTCATCTTGTCTCC)	Liu et al. (2002)
			pB8 -SCAR (P1:CCGGACTAAGTACTGGCTTCGATA, P2:CCCAATCTCCAATGACCCATAAC)	Liu et al. (2002), Wen and Gao (2011)

Blast resistance	*Pia*	11	YCA72/ *Hinf* I -SNP (F:AGGAGAAGAAGCCACCAAGG, R:GAGCTGCCACATCTTCCTT)	Cho et al. (2007)
Blast resistance	*Pi36 (t)*	8	RM8018-SSR (F:AATTCATACACACTTGTGCC, R:ATTTGCTTGAGCAAGCTTAT) RM5647-SSR (F:ACTCCGACTGCAGTTTTTGC, R:AACTTGGTCGTGGACAGTGC)	Liu et al. (2005)
Blast resistance	*Pi39*	4	RM3843-SSR (F:ACCCTACTCCCAACAGTCCC, R:GGGGTCGTACGCTCATGTC) RM5473-SSR (F:ACCACAAACGATCGCGTC, R:GAGATTAACGTCGTCCTCCG)	Terashima et al. (2008)
Blast resistance	*Pi40*	6	9871.T7E2b-GS (F:CAACAAACGGGTCGACAAAGG, R:CCCCCAGGTCGTGATACCTTC)	Jeung et al. (2007)
Plant height	*Sd1*	1	Sd1- GS (F: CACGCACGGGTTCTT CCAGGTG, R: AGGAGAATAGGAGATGGTTTACC)	Ellis and Spielmeyer (2002)
Fragrance in rice	*Badh2.1*	8	Badh2.1- *GS* (ESP: TTGTTGGAGCTTGCTGATG, IFAP: CATAGGAGCAGCTGAAATAATACC, INSP: CTGGTAAAAAGATTATGGCTTCA, EAP: AGTGCTTTACAA GTCCCGC)	Bradbury et al. (2005)
Fragrance in rice	*Badh2.8*	8	3In2AP- GS (F: GTCCTGTTCAATCTTGCAGC, R: CTTGATGCAACCATGTCATA)	Myint et al. (2012)
Root length	*Root QTL*	9	RM242-SSR (F:GGCCAACGTGTGTATGTCTC, R:TATATGCCAAGACGGATGGG) RM201-SSR (F:CTCGTTTATTACCTACAGTACC R:CTACCTCCTTTCTAGACCGATA)	Steele et al. (2006)
Grain length/shape	*GS3*	3	GS3-GS (EFP: AGGCTAAACACATGCCCATCTC ERP: CCCAACGTTCAGAAATTAAATGTG CTG) IRSP: AACAGCAGGCTGGCTTACTCTCTG IFLP: ACGCTGCCTCCAGATGCTGA	Ramkumar et al. (2010)

Submergence tolerance	*Sub1*	9	RM219-SSR (F:CGTCGGATGATGTAAAGCCT, R:CATATCGGCATTCGCCTG) RM464A-SSR (F:AACGGGCACATTCTGTCTTC, R: TGGAAGACCTGATCGTTTCC) ART5 -SSR (F:CAGGGAAAGAGATGGTGGA, R:TTGGCCCTAGGTTGTTTCAG) SC3-SSR (F:GCTAGTGCAGGGTTGACACA, R:CTCTGGCCGTTTCATGGTAT)	Xu et al. (2004), Cuc et al. (2012)
Brown plant hoper resistance	Bph1	12	BpE18-3- STS (F:CGCTGCGAGAGTGTGACACT, R:TTGGGTTACACGGGTTTGAC)	Kim et al. (2005)
Brown plant hoper resistance	*Bph14*	3	Bph14-GS M1 - (F:ATGGCGGAGCTAATGGCCACCA, R:AGAGTTCTTTATATCATGGAATCA) M2 (F:GATCATGAGATTGACGTGGAAA, R:AAGTCACTTAGCTTTGGTG) M3 (F:AGTCGATGGAACTCCAAGGG, R: GATGAGTATGCTTGAGGCCC) M4 (F:AATCTTGCTTAGGAGAGCTCGC, R: CTACTTCAAGCACATCAGC)	Mai and Hong (2012)
Brown plant hoper resistance	*Bph18(t),*	12	7312.T4A -STS (F:ACGGCGGTGAGCATTGG, R:TACAGCGAAAAGCATAAAGAGTC)	Jena et al.(2006), Suh et al. (2011)
Gall midge resistance	*GM1*	9	RM444-SSR (F: GCTCCACCTGCTTAAGCATC R: TGAAGACCATGTTCTGCAGG) RM219-SSR (F: CGTCGGATGATGTAAAGCCT R: CATATCGGCATTCGCCTG)	Biradar et al. (2004)
Gall midge resistance	*Gm7*	4	SA598-SCAR (F: GATCATTGGAGCAACATTCTG, R: CATTTCTAATTCTTTCTTCAA)	Sardesai et al. (2002)

Tolerance to low phosphorus levels in the soil	*Pup1*	12	Pup1-GS Pup1-K29 - F:CCATAGTAGCACAAGAAACCGACA, R: GCTTCAATGAGCCCAGATTACGAA) Pup1-K42 -(F:CCCGAGAGTTCATCAGAAGGA, R:AGTGAGTGGCGTTTGCGAT) Pup1-K46 -(F:TGAGATAGCCGTCAAGATGCT, R:AAGGACCACCATTCCATAGC) Pup1-K59 - (F:GGACACGGATTCAAGGAGGA, R:TGCTTTCCATTTGCGGCTC)	Chin et al. (2010)

Abbreviations:
Ch: chromosome
Marker type; STS: **s**equence tagged sites, SCAR: sequence characterized amplified regions**,** SNP: single nucleotide polymorphism, SSR: simple sequence repeats, GS: gene specific markers

Applications of DNA markers in QTL mapping and backcross breeding

QTL is statistically significant ***locus*** (may include one gene or cluster of genes) that quantitatively affects a phenotype of interest with physical boundaries defined by linked molecular markers. Molecular markers are usually applied in mapping of QTLs which is called polygenic traits, because mapping of QTLs depends on the crossover between linked markers in biparental populations. Once the linkage map is constructed, QTLs can be identified by dissecting the association between marker and the variation of phenotype through statistical analyses. Many major QTLs have been identified by flanking markers that are tightly linked with the QTL of many traits, such as drought, submergence tolerance, etc in rice. Best performing varieties have been developed by introgressing several agronomically favoured QTLs into a one line. QTL mapping thus represents the foundation of the development of markers for MAS.

Marker Assisted Backcrossing (MAB) is practiced to introgress genes from a donor into the background of an elite variety (recurrent parent) and to recover the recurrent parent genome as rapidly as possible by back cross breeding. In other words, markers can be used to select against the donor genome, which may accelerate the recovery of the recurrent parent genome. With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent. If polymorphism exists between parents, these markers can be used in foreground selection (plants having the marker allele of the donor parent at the target locus) and background selection (recurrent parent marker alleles in all genomic regions except the target locus).

A study on MAB breeding programme has improved the root morphological traits of Indian upland rice variety, Kalinga III, leading to drought tolerance, from the donor parent, Azucena, an upland Japonica variety from Philippines (Steele et al. 20006). Accordingly, the target segment on chromosome 9 of Azucena, flanked by RM242-RM201, significantly increased root length under both irrigated and drought stress treatments, confirming the root -length -QTL from Azucena functions in a novel genetic background of Kalinga III. Although the exact gene has not been cloned and identified, the simple flanking markers helped for the confirmation of the introgression of the QTL of interest.

Another study on QTL mapping showed that the major QTL, named *Sub1*, (Xu and Mackill, 1996), provides tolerance to complete submergence of rice for up to 2 weeks. RM464A was shown to be tightly linked marker to the gene and flanking markers RM219 and RM316 ensured efficient foreground and recombinant selection as polimorphism appeared in the reported cross (Xu et al 2004, Septiningsih et al. 2009). According to their results, the marker, RM219 has been highly polymorphic among diverse cultivars and hence, it would be a potential marker to be used in breeding studies related to *Sub1* QTL. The *Sub1* gene has been successfully introgressed through marker-assisted backcrossing into a popular high-yielding variety from India, Swarna, producing Swarna-Sub1 (Neeraja *et al.*, 2007). Following similar MAB procedure, Samba Mahsuri-Sub1, a tolerant version of the popular Indian cultivar Samba Mahsuri, has been developed within two rounds of backcrossing and one generation of self-pollination (Septiningsih et al. 2009). These case studies prove the usefulness of DNA markers in discovering genes and the potential of conventional breeding to be successful with the aid of molecular markers.

Future perspective of MAS in rice breeding

By adopting MAS minimum of major two barriers can be overcome. One major drawback of conventional backcrossing is the lengthy time to develop a new variety. Six to eight backcrosses are usually necessary with approximately 3-4 years of breeding work. Another barrier is occurrence of "linkage drag" which refers to presence of undesirable genes in the chromosomal region of the target gene thereby difficulty of avoiding such traits from the conventional breeding. Economic analysis undertaken by Alpuerto (2008) has shown potential impacts of utilizing MAS by overcoming drawbacks of conventional breeding, in rice, that ultimately reduce the cost of production and promote economic growth. Alpuerto (2008) used the economic surplus approach to measure the benefits of MAB for salinity tolerance in rice for Bangladesh, India, Indonesia, and Philippines, and for rice with tolerance to P-deficient soils in Indonesia. Results indicated that the benefits over 15 years of planting salt-tolerant varieties were $226.9 million in the Philippines, $3.666 billion in Bangladesh, $4.848 billion in India, and $895.7 million in Indonesia. Therefore, it is evident that use of molecular markers gives directs benefits to the economy and hence, it is necessary to establish facilities to adopt MAS and training breeders on handling of molecular markers within rice breeding institutes. Also it is necessary to focus on researches that deal with QTLs or gene mapping studies as they provides foundation steps to investigate DNA markers that are tightly linked with traits. Also it is necessary to develop database on rice varieties across the world along with the information on prevalence of genes/QTLs so that it would enhance the possibility of acquiring QTLs/genes into varieties and increase the varietal choice for breeders.

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