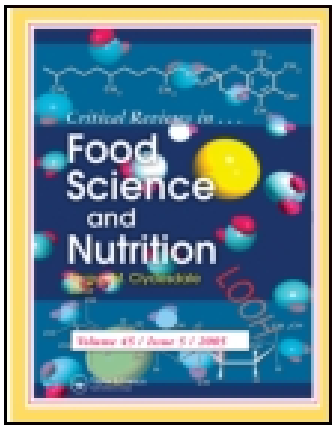

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Simple Sequence Repeat Markers in Genetic Divergence and Marker-Assisted Selection of Rice Cultivars: A Review

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Simple Sequence Repeat Markers in Genetic Divergence and Marker-Assisted Selection of Rice Cultivars: A Review

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Sequencing of rice genome has facilitated the understanding of rice evolution and has been utilized extensively for mining of DNA markers to facilitate marker-assisted breeding. Simple sequence repeat (SSR) markers that are tandemly repeated nucleotide sequence motifs flanked by unique sequences are presently the maker of choice in rice improvement due to their abundance, co-dominant inheritance, high levels of allelic diversity, and simple reproducible assay. The current level of genome coverage by SSR markers in rice is sufficient to employ them for genotype identification and marker-assisted selection in breeding for mapping of genes and quantitative trait loci analysis. This review provides comprehensive information on the mapping and applications of SSR markers in investigation of rice cultivars to study their genetic divergence and marker-assisted selection of important agronomic traits.

Keywords Rice cultivars, simple sequence repeat, markers, quantitative trait loci, diversity

INTRODUCTION

Rice (*Oryza sativa*) belongs to family Graminae and subfamily Oryzoidea, is the major staple food for one-third of the world population, and occupies one-fifth of the total land covered under cereals (Chakravarthi and Naravaneni, 2006). It is the most diversified crop species due to its adaptations to wide range of geographical, ecological, and climatic regions. It holds a unique position among domesticated crop species as it is a staple food and first fully sequenced crop genome (Garris et al., 2004). As a model crop with fully sequenced genome, rice gives unique opportunities to researchers for using different genomic approaches to study its varietal divergence and history of crop improvement. The other important factors that make the rice as a model crop are its diploid genetics, relatively small genome size (Kurata et al., 1994; Xu et al., 2005), significant level of polymorphism (McCouch et al., 1997), and large amount of well-conserved genetically diverse material.

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Characterization of rice varieties by traditional morphological, physiochemical, and enzymatic methods proved unsuccessful in differentiating very closely related groups and need long time for characterization. Molecular markers-based characterization seems to be the most suitable, reliable, and effective in finding out uniformity, distinctness, and stability in different varieties (Sarao et al., 2010). DNA-based markers are neutral and have no effect on phenotype, no epistatic effect, and are not influenced by the environment (Ye-yun et al., 2005), which are the critical factors in case of morphological-based characterization. DNA markers are simple, quick, less environmentally conditioned, experimentally reproducible, and can be applied in the identification, registration of plant variety, in monitoring of seed purity, and its authenticity with high accuracy, high reliability, and low cost (Cirillo et al., 2009). Such markers are useful in food traceability to certify the origin and quality of product to prevent the fraudulent approaches and a given item can be monitored at any stage of food chain.

Plant genome analysis in a number of plant species including rice have shown that by using different markers, genetic relationship between different varieties can vary significantly (Parson et al., 1997; Virk et al., 2000). The most widely adopted markers

Table 1 Different molecular markers used for genetic divergence in rice

Marker name	Abbreviation	Nature	Remarks	Varieties tested	References
Amplified fragment length Polymorphism	AFLP	Dominant	Detection of genomic restriction fragment by PCR amplification	49 33	Prashanth et al. (2002) Aggarwal et al. (2002)
Inter simple sequence repeats	ISSR	Co-dominant	Single primer based on SSR motif	42 84	Joshi et al. (2000) Cao et al. (2006)
Random amplified polymorphic DNA	RAPD	Dominant	Random primers for PCR amplification	18 75 8	Patra and Chawla (2010) Parvaiz et al. (2010) Skaria et al. (2011)
Restriction fragment length polymorphism	RFLP	Co-dominant	Based on restriction digestion and hybridization with probe	71	Olufowote et al. (1997) Kojima et al. (2005)
Sequence characterized amplified regions	SCAR	Co-dominant	RAPD marker termini sequenced for designing longer primer	26	Singh et al. (2011)
Simple sequence repeats	SSR	Co-dominant	Based on tandem repeat flanking sequence	11 21	Herrera et al. (2008) Rahman et al. (2011)
Single nucleotide polymorphism	SNP	Co-dominant	DNA sequence differs by single base	6	Nasu et al. (2002)

to study the genetic relationship in different crops including rice (Table 1) are amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), and restriction fragment length polymorphism (RFLP), having their own advantages and disadvantages and requiring careful evaluation before being effectively used for genetic analysis (Saini et al., 2004). SSR markers showed much more polymorphism than most of the other DNA-based markers as they are co-dominant in nature and are available in large quantity, thus making them most popular and ideal marker in identification of plant varieties (Ye-yun et al., 2005; Sarao et al., 2010).

SIMPLE SEQUENCE REPEAT MARKERS

SSR markers are actually noncoding regions that remained conserved during the course of evolution and are ideal for DNA fingerprinting and varietal identification. These are also known as the microsatellites (Litt and Lutty, 1989) and short tandem repeat (STR) that contain tandem repeats (di, tri, tetra, or penta) that vary in the number of repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs) or hypervariable regions (Mittal and Dubey, 2009). Since these are abundant, hypervariable, multiallelic, and evenly distributed throughout the nuclear genome, they provide a valuable source of polymorphism, making them important class of genetic markers (Varshney et al., 2005). In addition, the highly polymorphic nature of many SSR markers is of particular value when analyzing closely related varieties (McCouch et al., 2002). SSRs were first studied in humans and have now been found in a wide array of other eukaryotes, including mammals, birds, fish, insects, yeast, and plant species (McCouch et al., 1997). Microsatellite markers can also be utilized as different forms to find out the polymorphisms, and some of the important molecular markers commonly derived from the SSR markers are given in Table 2.

Table 2 Use of SSR markers in different forms to detect polymorphism in rice cultivars

S. no.	Marker	Abbreviation	Remarks	Reference
1	Sequence tagged microsatellite markers	STMS	Primers opposite to the flanking region of the SSR markers are developed to amplify the specific locus, and the variation in the length of the repeating units is found to detect the polymorphism in the different individuals.	Davierwala et al. (2000)
2	Expressed sequence tagged microsatellite markers	EST-SSRs	Microsatellite primers of the EST-SSRs are mainly designed to amplify the coding region of a specific gene locus and these will be utilized to detect the polymorphism at the level of gene expression region.	Dracatos et al. (2006)
3	Sequence characterized amplified regions markers	SCAR	RAPD markers are used for amplification and amplified regions are first sequenced and then utilized to develop the microsatellite primer pairs for the amplification of a specific gene locus.	Khan et al. (2007)
4	Inter simple sequence repeats	ISSR	In this marker system, primers based on SSR markers are utilized to amplify ISSR DNA sequences. ISSR assay can be undertaken for any species that contains a sufficient number and distribution of SSR motifs and has the advantage that genomic sequence data is not required.	Youssef et al. (2010)

MAPPING OF SIMPLE SEQUENCE REPEATS IN RICE

The current level of genome coverage provided by SSR markers in rice is sufficient for genotype identification, gene and quantitative trait locus (QTL) analysis, screening of large insert libraries, and marker-assisted selection in breeding (McCouch et al., 1997). SSRs were characterized and mapped in rice genome from partial sequencing of cDNA and genomic DNA clones and it was observed that the most frequently repeated SSR motif was d (CCG/CGG)n (Miyao et al., 1996). Mapping of 121 SSR markers having genome-wide coverage of the 12 chromosomes, with an average distance of one SSLP (SSR polymorphism) per 16–20 cM has been done (Chen et al., 1997). These SSLP loci included 86 poly (GA), six poly (TCT), one poly (ATT), and one poly (AATT) motifs. Similarly, mapping of 312 microsatellite markers on the basis of partial cDNA sequences available in GenBank and from Tsp509-digested small-insert genomic library has been developed (Temnykh et al., 2000). A total of 2414 nonredundant SSR primer pairs, representing 2240 unique marker loci, have been developed and experimentally validated for *O. sativa* L. (McCouch et al., 2002). The sequence of rice chromosome 4 was screened for the chromosomal positions, and composition of SSR, to produce a detailed map displaying all possible SSR motifs and to use them for comparative genome analysis of the *indica* and *japonica* subspecies (Li et al., 2004a, 2004b). A total of 1844 SSR markers with SSR motifs of 20 bp and repeated unit length of 1–6 base pairs were mapped and these SSRs were found to be occurred once in every 18.8 kb, with one SSR per 23.8 kb and 16 kb on the short and long arms, respectively, while no SSR was detected in the core region of the centromere. Sequence variations in SSRs were observed between two cultivars that can provide a great opportunity for SSR-based marker development and comparative genome analysis of the subspecies.

MAP-BASED SEQUENCING OF RICE GENOME

International Rice Genome Sequencing Project (IRGSP) has developed and analyzed a complete, highly precise sequence of the rice genome that is anchored to the genetic map (IRGSP, 2005). The project came out with many prominent attributes of rice genome like the genome size of 389 Mb, which was found to be 260 Mb larger than the fully sequenced dicot model plant *Arabidopsis thaliana*. Their sequencing covered 95% of the total genome including all of the euchromatin and two complete centromeres thus having 370 Mb of finished sequence. A total of 37,544 nontransposable element-related protein-coding sequences were detected and also identified 11,487 Tos17 retro transposon insertion sites, of which 3243 are in genes. A total of 2859 genes were found to be unique to rice and the other cereals, and have recognized 80,127 polymorphic sites that can distinguish between two cultivated rice subspecies, *japonica* and *indica*. Single-nucleotide polymorphism (SNP) frequency was observed to be varied from 0.53% to 0.78%, which is 20 times the frequency observed between the Columbia and *Landsberg erecta* ecotypes of *Arabidopsis*. This map-based sequencing of rice genome has proven useful for the identification of many genes that are fundamentals of many agronomic traits or rice. Along with the identification of genes, single-nucleotide polymorphisms (SNP) and SSRs were additionally identified that in future pick up the pace in improvements of rice production.

GENETIC DIVERGENCE IN RICE

There are 21 wild species in genus *Oryza* in which nine are tetraploid and remaining are diploid. There are two cultivated rice species, *O. sativa*, the Asian rice (grown worldwide), and *O. glaberrima*, the African rice (grown on a limited scale in West Africa). The varieties of *O. sativa* are classified into six groups on the basis of genetic affinity in which the *indica* belongs to Group I and *japonicas* to Group VI. Cultivated *indica* varieties have dispersed throughout the tropics and subtropics from Eastern India, whereas *japonica* varieties moved northward from Southern China and developed into temperate ecotypes (Khush, 1997).

Cultivation and selection by farmers and researchers for centuries under varied growing and cultural conditions have resulted in the development of abundant of rice varieties. About 120,000 distinct rice varieties exist in the world, which are grown in more than 100 countries (Singh, 2011). Different centers, such as the International Rice Research Institute (IRRI) in the Philippines, the West Africa Rice Development Association (WARDA) in Cote d'Ivoire, the International Institute for Tropical Agriculture (IITA) in Nigeria (on behalf of WARDA), and the International Centre for Tropical Agriculture (CIAT) in Colombia, have been developed to maintain rice germplasm collections (www.irri.org). IRRI holds the largest germplasm collection having more than 112,000 rice accessions and is known for the most genetically diverse and complete rice collection in the world (www.fao.org). China has about 40,000 and India about 25,000 accessions in the gene banks while the other countries have smaller collections.

Rice varieties differ from each other in growth duration, photoperiod sensitivity, grain size, shape, color, and endosperm properties. Varieties also differ in the level of tolerance/resistance to abiotic and biotic stresses (Khush, 1997). Regarding quality, aroma holds prominent place in rice diversity that forms basis of aromatic and nonaromatic rice varieties. Aromatic, highly valued rice are collectively called "basmati," occupies a prime position in Asia, Europe, and the USA, and are characterized by extra-long superfine slender grains and pleasant aroma after cooking (Bhattacharjee et al., 2002). The extent of genetic diversity in a crop population also depends on recombination, mutation, selection, and random genetic drift (Pervaiz et al., 2010).

APPLICATIONS OF SIMPLE SEQUENCE REPEAT MARKERS

SSR markers have a large number of applications in rice cultivar identification, genetic relationships, marker-assisted selections, detection of genes, and quantitative trait loci analysis. These are ideal markers that would be able to reveal multiple alleles, as they are evenly distributed in rice genome and are easy to score.

DNA FINGERPRINTING AND GENETIC VARIABILITY IN RICE CULTIVARS

Thousands of new accessions of rice are introducing into germplasm every year, thereby necessitating assessment of their molecular diversity before elimination of the redundant genotypes. The knowledge regarding the amount of genetic variation in germplasm accessions and genetic relationships between genotypes is an important consideration for designing effective breeding programs, an essential prerequisite for the preservation of endangered species. SSR markers are efficient in detecting genetic polymorphisms among rice genotypes.

Estimation of Genetic Diversity in Basmati and Non-Basmati Rice Cultivars

DNA fingerprint database of 24 rice genotypes including three traditional basmati, nine cross-bred basmati, eight *indica*, and three *japonica* rice varieties was developed on the basis of allelic diversity using 50 microsatellite markers (Siwach et al., 2004). Similarly, genetic diversity and pattern of relationships were studied among the 18 rice genotypes representative of the traditional basmati, cross-bred basmati, and non-basmati (*indica* and *japonica*) rice varieties using AFLP, ISSR, and SSR markers (Navinder et al., 2004), and it was revealed that all the three marker systems would be able to distinguish between all the 18 rice cultivars.

Genetic diversity was measured using 35 microsatellite markers in 75 genotypes of rice grown in Pakistan (Perviaz et al., 2010). A total of 142 alleles were detected at 32 polymorphic SSR loci. A dendrogram based on total microsatellite polymorphism grouped 75 genotypes into four major clusters at 0.40 similarity coefficient, differentiating aromatic from nonaromatic ones. Similar study was carried out to evaluate the genetic relationship among 41 traditional and improved cultivars of Pakistani rice by means of 30 microsatellite markers distributed over the whole rice genome. It has been observed that all the tested markers were polymorphic and a total of 104 alleles were detected (Rabbini et al., 2010).

The analysis of genetic diversity among 16 aromatic short grain local land races collected from different parts of India and 30 basmati cultivars collected from the traditional basmati growing areas has been done using 26 SSR markers (Sivaranjani et al., 2010). Single markers (RM28102) or combination of two (RM577, RM30) markers were found to be effective in discrimination between both the aromatic groups. Based on the similarity coefficient values, genotypes were classified into two major clusters with 70% dissimilarity revealing presence of high diversity.

Estimation of Genetic Diversity (Other than Basmati Varieties) in Rice

T38 rice cultivars of particular interest to US breeding programs and two wild species accessions were evaluated by means of 111 microsatellite markers distributed over the whole rice genome and a total of 753 alleles were detected (Ni et al., 2002). Genetic diversity of 11 Venezuelan rice cultivars by 48 SSR markers has been done and a total of 203 alleles were detected (Herrera et al., 2008). Similarly, 12 SSR markers were used to characterized 417 landraces collected in 1986, 1987, and 2003, in the state of Goias (Brazil), to study the impact of rural exodus or replacement of rural varieties by commercial cultivars (de Oliveira Borba et al., 2009).

Identification and differentiation of 17 HYVs and 17 local rice cultivars including two wild rice cultivars have been done by using SSR and it was observed that all the analyzed microsatellite markers were polymorphic in nature with an average number of 6.33 alleles per locus (Rahman et al., 2009). A total of three variety-specific alleles (RM-11/147, RM-151/289, and RM-153/178) were identified for BR-11, Badshabhog, and BR-19 cultivars. Genetic variability in Spanish rice cultivars was evaluated by fluorescently labeled primer pairs (Wankhade et al., 2010). In the same regard, 54 microsatellite markers were used to estimate genetic diversity of 53 Sarawak rice cultivars in which a total of 43 alleles were detected with an average of 3.58 alleles per locus. The unweighted pair group method (UPGMA) with arithmetic mean dendrogram ($r = 0.789$) revealed two major groups with six sub-clusters and the wide range of similarity values (0.24–1.0) showed a high degree of diversity among the cultivars (Lee et al., 2011). Some of the popular Indian rice varieties were analyzed by SSR markers for diversity analysis and it was observed that all of the SSR markers were polymorphic in nature (Upadhyay et al., 2011).

DETECTION OF ADULTERATION OF BASMATI WITH NON-BASMATI RICE

Basmati rice has both aroma and post-elongation properties, mainly preferred by the consumers and no other variety in the world have both these characteristics. But since the yield of basmati rice, per acre of land, is less than half of that of non-basmati rice and because of higher inputs, basmati rice has become unaffordable for most of the people. Basmati associates have made basmati rice affordable for people of various income brackets—with its varietal difference (Lopez, 2008).

Many methods have been developed to detect adulteration in rice, like smelling of grains after boiling in water or treated with potassium hydroxide and chromatographic analysis of aromatic compounds (Lorieux et al., 1996). It is very difficult on morphological or chemical basis to identify a true basmati variety, since many evolved cross-bred non-basmati varieties also resemble the basmati on morphological basis. So techniques based on molecular basis proved effective in characterizing basmati and non-basmati varieties (Lopez, 2008). Two classes of DNA markers, i.e., fluorescence-based ISSR markers and SSR markers were developed for the purpose of carrying out the genetic analysis of basmati rice varieties (Nagaraju et al., 2002). They have found such markers efficient, cost-effective, and helpful in detecting a higher degree of polymorphism in rice. Traditional basmati was differentiated from cheap cross-bred and long rice varieties by using 35 SSR markers (Pal et al., 2004). Out of 123 alleles, 25 alleles were found to be present only in basmati varieties. Basmati, *indica*, and *japonica* rice genotypes were analyzed for genetic diversity and a total of 235 alleles were detected by 30 SSR loci out of which 62 (26.4%) of them were present only in basmati (Jain et al., 2004).

Capillary electrophoresis-based multiplex microsatellite marker assay was reported first time for detection as well as quantification of adulteration in basmati rice samples by Archak et al. (2007). They used single-tube multiplex assay for eight microsatellite loci to generate variety-specific allele profiles that can detect adulteration from 1% upwards.

MARKER-ASSISTED BREEDING

Traditionally, plant breeding is based on phenotypic selection of superior individuals for crop improvement, yet many limitations encountered during this process viz. effect of environment, genotype, unreliability, and being expensive in terms of time and cost. But in recent times, the development of molecular marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers (Babu et al., 2004). With the development and availability of an array of molecular markers and genetic maps in crop plants, MAS has become possible for traits both governed by major genes and quantitative trait loci (QTLs). Many reports have been published about the linkage between SSR and agronomically important QTLs and genes that are discussed below.

Mapping and Detection of Quantitative Trait Loci

Currently, many SSR markers are available for detection of QTL in rice, linked to important quality and agronomic trait that aid in breeding superior lines that can grow well in local climates and have good yield under stress conditions.

Mapping of Quantitative Trait Loci Related to Quality Traits

The gelatinization temperature (GT), gel consistency (GC), and amylose content (AC) are the three major rice traits that are directly related to cooking and eating quality (Little et al., 1958). GT is the temperature at which starch irreversibly loses its crystalline order during cooking that is mainly responsible for cooking time and the capacity to absorb water during the cooking process, whereas GC and AC are responsible for softness and for texture and appearance in rice. Hence, regulation of these three traits in rice has been a major concern of rice breeders. To facilitate the development of new varieties with high cooking and eating qualities, it is necessary to understand the genetic and quantitative inheritance of such traits. QTLs that accounted for 80% and 57% phenotypic variation for AC and GC, respectively, on chromosomes 3, 4, 6, and 7 were detected (Lancreas et al., 2000). Similarly, four QTLs for AC, three for GT, and five for GC were found using backcross-inbred lines (Li et al., 2004a, 2004b). The QTL on chromosome 6 covered the *wx* gene region and mainly contributed to the variance between *japonica* and *indica* varieties. Effect of 12 QTLs for three traits, with a QTL corresponding to the *wx* locus showing a major effect on AC and GC, and a QTL corresponding to the *alk* locus having a major effect on GT, has been observed (Fan et al., 2005). One QTL for texture on chromosome 3 and four QTL for AC on chromosomes 3, 7, 9, and 12 were mapped (Wada et al., 2006). A genetic linkage map was developed by using SSR and STS markers for detection of QTLs related to different grain characteristics, i.e., 1000 grain weight (qgw9.1 and qgw11.1), grain width (qgw2.2), length-to-width ratio (qlw2.1), and grain thickness (qgt2.1) were detected for two years to study the interactions between QTL and the environment, and it was concluded that the main-effect QTLs of grain width and length-to-width ratio were easily influenced by the environment (Qin et al., 2008). Using 110 polymorphic SSR markers and 209 recombinant inbred lines, QTLs related to AC, GC, and GT were mapped on seven different chromosomes of rice cultivars (Amarawathi et al., 2008).

Mapping of Quantitative Trait Loci Related to Agronomic Traits

A total of 11 QTLs located at chromosomes 2, 3, 4, 6, 11, and 12 were detected in eight traits, i.e., heading date, flag leaf length, plant height, panicle length, panicle weight, seed set, weight of 100 grains, and grain weight by using 93 SSR markers (Susanto et al., 2008). Similarly, the parental screening of IR64 and *Tarome molaei* was done with 235 SSR markers, out of which 114 markers gave clear polymorphic bands that were used to construct a linkage map to search for QTLs associated with panicle length, number of grain per panicle, and panicle grain sterility (Ahamadi et al., 2008).

Drought is one of the major limited factors that affect the growth of rice in many countries. A total of 525 SSR markers were chosen to screen a F2 mapping population derived from a cross between Taichung 189, a susceptible *japonica* line, and Milyang 23, a tolerant *indica* line, to find out the chromosome region associated with drought tolerance (Lin et al., 2007). Further, 121 SSR markers were performed to search potential QTL regions and a total of four QTLs associating to drought sensitivity index were detected. Applying these SSR markers that are closely linked with drought tolerance, it will be possible to facilitate early selection of drought tolerant lines and shorten breeding period. Similarly, 90 polymorphic SSR makers were used for QTL mapping for salinity tolerance at seedling stage of a F2 breeding population derived from the cross between BRRI dhan40, a moderately tolerant female parent, and IR61920-3B-22-2-1, a highly tolerant male parent (Islam et al., 2011).

Bulked segregant analysis (BSA) serves as an alternative approach for rapid identification of markers associated with drought resistance traits (Kanagaraj et al., 2010). BSA was carried out to identify markers linked to drought resistance using 23 recombinant inbred (RI) lines of IR20/Nootripathu, two *indica* ecotypes with extreme drought response. The parents were screened for polymorphism using 1206 rice microsatellite primer pairs. Out of 134 SSR polymorphic primers between parents, three primers showed polymorphism between bulks. It was found that three primers co-segregated among the individual RI lines constituting the respective bulks. The genomic regions flanked by SSR markers have been reported to be associated with several drought resistance component traits that will aid in marker-assisted breeding for drought resistance in rice.

MAPPING OF GENES

Genetic improvement of important quality and agronomic traits are of major concern for rice breeders to attain high yields and better quality characteristics. The development of molecular marker techniques has enabled researchers to map important traits in the rice genome and can be used in marker-aided selection of these traits. SSR markers are particularly useful for gene mapping and marker-based selection, since these markers are amenable to high-throughput analysis and are informative in many types of genetic crosses.

Cytoplasmic male sterility (CMS) is a common plant reproductive feature that has been extensively used as an important tool to exploit heterosis and to develop hybrids in many crops. *Rf* genes are needed for restoring fertility to CMS lines. Searching for and molecular tagging of restorer genes are of high importance where phenotyping is very time consuming and requires the determination of spikelet sterility in testcross progeny. A fertility restorer gene was mapped by using SSR and CAPS markers in rice line IR36 in a F2 population developed from the cross Neda-A×IR36 (Alavi et al., 2009). The genetic linkage analysis indicated that three SSR markers (RM1, RM3233, RM3873) and one CAPS marker (RG140/EcoRI) on the short arm of chromosome 1 were linked to *Rf*3. Seven SSR markers linked with *eui* locus at the genetic distance from 1.0 to 7.1 cM were identified (Khera et al., 2009). Furthermore, these markers were validated in F2 (IR58025A/IR91-1591-3) and backcross (IR58025B/IR91-1591-2/IR58025B) population segregating for *eui* gene. The SSR markers reported in this study might be useful to the rice breeders interested in transferring *eui* gene into their promising parental lines of hybrid rice. Some of the other

Table 3 Mapping of important genes in rice with SSR markers

S. no.	Trait	Gene	Marker	Chromosome	References
1	Amylose content	*Wx*	RM190	6	Chen et al. (2008)
2	Aroma	*Fgr*	RM223, RM342, RM515	8	Kibria et al. (2008)
			RM23120, RM3459	8	Sun et al. (2008)
3	Blast resistance	*Pi36(t)*	RM5647, RM8018	8	Liu et al. (2005)
		Pi37(t)	RM128, RM486	1	Chen et al. (2005)
		Xa33(t)	RM30, RM7243, RM5509, RM400	6	Korinsak et al. (2009)
4	Brown plant hopper resistance	*Bph18*	RM463	12	Jena et al. (2006)
5	Cytoplasmic male sterility	*eui-1*	RM6054, RM3870, RM3476, RM5970, RM7801, RM7446, RM3620	5	Khera et al. (2009)
		eui-2		10	
6	Dwarfness	*H*	RM302	1	Wang et al. (2009)
7	Elongated uppermost internode	*Eui1*	RM164, AC9	5	Hong-Li et al. (2004)
8	Erect panicle	*EP*	RM1189, RM257, RM242, RM3787, RM1013	9	Kong et al. (2007)
9	Gall midge resistance	*Gm1*	RM219, RM316, RM444	9	Biradar et al. (2004)
10	Gel consistency	*Wx*	RM540, RM8200	6	Su et al. (2011)
11	Gelatinization temperature	*ALK*	M5522, M4785	6	Zhenyu et al. (2003)
12	Grain length	*Lk-4(t)*	RM16, RM282	3	Liang-Qiang et al. (2006)
13	Grain length/width ratio	*gw-5*	RM3328, RM3322, RM5874, RM5994	5	Wan et al. (2008)
14	Grain width	*gw-5*	RM3328, RM3322, RM5874, RM5994	5	Wan et al. (2008)
15	Low gluten content	*lgc1*	SSR2-004, RM1358	2	Wang et al. (2006)
16	Panicle characters	*Ssi1*	S13623, S05756, RM3475, RM237	1	Sunohara et al. (2006)
17	Salt tolerance	*Salt*	RM223	8	Lang et al. (2008)

important genes that are mapped with the help of SSR markers are shown in Table 3.

SCREENING OF BIOTYPES

Brown planthopper is one of the most serious rice insect pests all over the world. Exploiting new resistance genes and breeding advanced genetic stocks are important for breeding resistance varieties. More than 1200 accessions of common wild rice (*Oryza rufipogon*) were evaluated for the resistance to several biotypes of BPH. Thirty resistant accessions were obtained and six of them showed broad spectrum resistance to five or all of the six BPH biotypes, i.e., biotypes 1 and 2, Bangladesh, Mekong (Vietnam), Cuulong (Vietnam), and Pantnagar (India), which are spreading most rice growing regions in the world. A total of 257 extreme resistant and susceptible plants were mapped with the marker for the resistance genes. The mapping showed three SSR markers (RM273, RM6506, and RM252) located in the middle of long arm of the chromosome 4, cosegregated with one of the resistance genes (Li et al., 2010).

Three biotypes of gall midge were screened by using SSR and it was revealed that 15 loci were hyper variable and showed polymorphism among different biotypes of this pest (Bentur et al., 2011). Inheritance studies with three markers revealed sex linked inheritance of two SSRs (Oosat55 and Oosat59) and autosomal inheritance of one marker (Oosat43). Screening of biotypes by SSR markers will prove to be a useful tool to devise strategies for integrated pest management and in the study of biotype evolution in different pests of rice.

CONCLUSIONS AND FUTURE PROSPECTS

SSR markers offer many advantages such as reliability, reproducibility, discrimination, standardization, and cost effectiveness over other markers in improvement of rice. They have shown potential for large-scale DNA fingerprinting and mapping of agronomically important QTLs/genes due to their high level of polymorphism, abundance, accuracy, and repeatability. But still, improvement in rice will face formidable challenges to provide mankind with an appropriate level of food security while enhancing the sustainability of agricultural practices, lowering their environmental impact, and preserving the remaining biodiversity. There is need to genetically tailor the developed and newly developing rice accessions for maximizing yield, yield stability, and quality improvement by using a combination of efficient molecular markers. SSR markers should, therefore, be possible to exploit the flow of genes or quantitative trait loci of interest in rice and make prediction about crossing and selection that will increase the efficiency of variety development. In addition, microsatellite marker analysis can be automated and this feature is attractive for marker-assisted selection program. Thus, SSR markers can be a useful tool to increase the effectiveness in selection of new high yielding hybrids and can facilitate the study on the complex traits of rice to greater extent in the future.

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