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Molecular screening and diversity analysis of rice (*Oryza sativa* L.) genotypes for biotic and abiotic stresses using SSR markers

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Abstract

In this study, rice germplasms were screened for biotic (blast, bacterial blight, brown planthopper, gall midge) and abiotic stress tolerance (drought, salinity) in 50 rice genotypes using 19 trait linked SSR markers.Among the genotypes; B 40 showed amplification of the resistance/tolerance specific alleles for multiple traits, *viz.*, blast, bacterial blight, gall midge, saltol and IRBB 57 showed presence of resistance/tolerance specific alleles for blast, bacterial blight, gall midge and drought. Moreover the genotypes, IR 11 A 546, IR 11 A 581, IR 11 N 169, IRBB 2, IRBB 4, IRBB 5, IRBB 13, IRBB 64, IRBB 12 DS-GMEI-22, IR 64, IRBB 55, IRBB 62, IRBB 14, IRBB 50, IR 11 N 223 showed presence of resistance specific alleles for blast, bacterial blight, gall midge, brown planthopper and drought, Saltol by combination of any of the above three traits. The rice line IRBB 10 showed presence of resistance linked alleles for two gall midge resistance genes,*Gm4* (270bp) and *Gm8* (170bp). Genotypes IRBB 61 and IRBB 62 showed presence of resistance linked alleles for two of the *Saltol* linked loci. The rice genotypes displaying amplification of resistance/tolerance linked alleles for multiple traits can be used as donors for these traits after their validation through phenotypic screening. All the nineteen SSR primers used in this study amplified and showed the polymorphism among the rice genotypes. With an average of 5.42 alleles per locus, a total of 103 alleles were detected. The polymorphism information content (PIC) values ranged from 0.40 to 0.90 with an average PIC value of 0.65 per primer. Rice genotypes were grouped into two main clusters by UPGMA, which were further divided into two sub-clusters. Through Marker Assisted Selection (MAS), this study will assist in selection of parental lines and also for development of new breeding population that will be tolerant to specific biotic and abiotic stresses.

Keywords: SSR, molecular screening, MAS, diversity, biotic and abiotic stress

Introduction

Rice (*Oryza sativa* L.) is one of the most widely cultivated (118 countries) crops in the world. Rice ranks second after wheat in terms of area harvested but in terms of importance as a food crop, rice provides more calories per hectare than any other cereal crop. It's a staple food for more than three billion people in the world (Ma *et al*., 2007).

Exposure of rice crop to various environmental stresses such as abiotic (salinity, heat, drought, cold, submergence, radiation, and heavy metals) and biotic factors (pathogens and herbivores) cause a rigorous yield loss (Gomez, 2013). The emergence of new diseases and insect pests and the changing climate are the major issues that address the requirement for sustainable crop development and resistance to biotic and abiotic stresses. For precise genetic manipulation of complex quantitative traits like yield, tolerance against biotic/abiotic stresses, quality etc., understanding the genetic/molecular basis of target traits needs to be investigated thoroughly.

In the recent times, due to technological innovations and development of DNA based molecular markers it has become possible for the transfer of genes that confer resistance to biotic stresses (bacterial blight, blast, gall midge etc.) and abiotic stresses (submergence, drought, salinity etc.). With the improvements made in the area of molecular markers, the tracking of the genes for resistance has become easier by following the path of markers that are linked/ tagged to each gene for resistance, thus making the identification of plants with two and more genes possible.

Traditional breeding approaches are effective but delay production of climate-resilient variety as they rely on extensive phenotypic screening methods and also are not suitable for making rapid improvement in tolerance to multiple stresses. Hence, molecular breeding can be

preferred as it offers an opportunity to increase the speed and efficiency of plant breeding (Whitford *et al*., 2010). Molecular markers are promising and effective tools for measuring genetic diversity in germplasm collection and elucidating their evolutionary relationships. Using molecular marker technology, it is now feasible to analyze the quantitative traits and identify the chromosomal regions associated with such characters known as quantitative trait loci (QTLs) (Choudhary *et al*., 2008). Identifying such regions will help to increase the selection efficiency in the breeding program.

Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly monolocus, codominant, easily analysed, highly reproducible and cost effective (Gracia *et al*., 2004). Microsatellites or SSR markers are sequences of a few repeated and adjacent base pairs and are abundant throughout the eukaryotic genome (Powell *et al*., 1996). Variations in the number of repeats can be detected by polymerase chain reaction (PCR) with the development of primers (20–30 basepairs) specifically built for amplification and complementary to conserved sequences flanking the microsatellite. SSR markers are able to detect high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies (Ni *et al*., 2002). SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes from germplasm of various sources, even they can detect finer level of variation among closely related breeding lines within a same variety (Lapitan *et al*., 2007).

Different rice varieties of distinct genetic background are a good promise for the future of rice crop improvement programmes as genetic diversity helps in estimating and establishing of genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating progenies with maximum genetic variability and superior recombinations for further selection and introgressing desirable genes from diverse germplasm (Thompson *et al*., 1998 and Islam *et al*., 2012).

The major objective of this study was to screen the genotypes for biotic and abiotic stresses using SSR markers linked with tolerance traits or QTLs and to identify the best genotype to be used as donor for tolerance to multiple stresses in breeding program in the future for development of new rice varieties that are equally beneficial for farmers and the scientific community. In the investigation reported here, 19 SSR markers were used to fingerprint 50 rice accessions. The SSR data was used to evaluate the level of genetic diversity within the rice genotypes and to assess genetic relationship among the accessions.

Materials and Methods

The present study was conducted at the Plant Biotechnology Centre, Dapoli, Ratnagiri district, India in 2017-2018. Fifty rice genotypes obtained from Regional Agricultural Research Station, Karjat, District-Raigad, Maharashtra, India were used for the study (Table 1).

Table 1.Genotypes used in this study

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1	IR 11 A 546	18	IRBB 5	35	IRBB 56
2	IR 11 A 581	19	IRBB 7	36	IRBB 57
3	IR 11 N 121	20	IRBB 8	37	IRBB 58
4	IR 11 N 137	21	IRBB 10	38	IRBB 59
5	IR 11 N 169	22	IRBB 11	39	IRBB 60
6	IR 11 N 239	23	IRBB 13	40	IRRI 123
7	IR 11 N 313	24	IRBB 14	41	IRBB 61
8	IR 12 L 201	25	IRBB 21	42	IRBB 62
9	SAKHA 105	26	IRBB 50	43	IRBB 63
10	B 40	27	IRBB 51	44	IRBB 64
11	IR 552	28	IRBB 52	45	IRBB 65
12	IR 11 A 106	29	IRBB 53	46	IRBB 66
13	IRBB 1	30	IR 11 N 223	47	12 DS-GMEI-22
14	IRBB 2	31	IRBB 54	48	HHZ5-DT20-DT3-Y2
15	IRBB 3	32	IR 09 L 226	49	IR 05 A 272
16	IR 12 L 125	33	IRBB 55	50	IR 64
17	IRBB 4	34	IR 11 N 400		

The DNA was isolated by following the protocol of Edwards *et al*., (1991). For DNA isolation, 10 days old leaves were collected and sterilized with 70% ethanol to avoid the contamination. Leaf tissue (100 mg) was collected and kept in 1.5 ml eppendorf tube which leads to ensure uniform size of sample. Collected tissue was macerated by micro pestle at room temperature without buffer for 15 sec. Extraction buffer (500 µl) was added and leaf tissue macerated gently for few seconds and kept in hot water bath for 45 minutes at 65°C. The sample was cooled down to room temperature and centrifuged at 10000 rpm for 10 minutes. Aqueous layer was transferred to fresh eppendorf tube and 200 µl of chloroform: iso-amyl

alcohol (24:1) was added and mixed by gentle inversion for 5-6 times. The contents were then centrifuged at 8000 rpm for 10 minutes. Supernatant was mixed with double volume of chilled Iso-propanol and incubated at –20°C for overnight. On the next day the solution was centrifuged at 8000 rpm for 10 minutes and pellet was collected. Pellet was washed with 100 µl of 70 per cent ethanol followed by centrifugation at 8000 rpm for 10 minutes. Pellet was dried and re-suspended in 50 µl of 1x TE buffer and incubated at 37°C in a water bath for 30 minutes and stored at -20°C till further use. Quality of the isolated DNA was confirmed by Agarose gel electrophoresis method.

Simple sequence repeat (SSR; *i.e.* microsatellite) marker analysis: For the molecular screening of rice germplasm, nineteen different trait specific SSR markers well distributed on all the 12 chromosomes of rice were used (Table 2). These SSR markers were chosen based on their physical position on the 12 chromosomes of rice genome according to the 'Gramene' database (http//:www.gramene.org) and also based on their linkage to different genes conferring resistance to bacterial blight blast, gall midge and tolerance to salinity and drought. PCR reactions were carried out in Thermal cycler with the total reaction volume of 20µl containing, 10ng of genomic DNA, 10X assay buffer, 10mM of dNTPs, 25mM $MgCl_2$, 10pmol of forward and reverse primers and 3 U Taq polymerase enzyme and Nano pure water. The PCR cycles were programmed as 95°C for 5 min, 94°C for 20 sec, 55°C for 30 sec, 72°C for 45 sec for 35 cycles and an additional temperature of 72°C for 7 min for final extension. The amplified products were separated on 2% agarose gel prepared in 1X TAE buffer and stained with Ethidium bromide.The gel was run in 1X TAE buffer at constant voltage of 80 V for a period of 100 minutes.

Scoring and data analysis: Marker alleles were scored as present (+/1) or absent (-/0). The data was used for similarity based analysis using the programme MVSP-A (Multivariate Statistical Package_5785, Version 3.1).

Table 2. List of SSR primers with their sequences and linked gene

Sr. No	Primer	Sequence Forward primer	Sequence Reverse Primer	Chromosome No.	Linked gene	Reference
1.	RM 140	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	1	*Saltol*	Karmarkar *et al*.,2012
2.	RM 1287	CCATTTGCAGTATGAACCATGC	ATCATGCAATAGCCGGTAGAGG	1	*Saltol*	Ganie *et al*.,2016
3.	RM 562	GGAAAGGAAGAATCAGACACA-GAGC	GTACCGTTCCTTTCGTCACTTCC	1	*Saltol*	Ganie *et al*.,2016
4.	RM 3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC	1	*Saltol*	Islam *et al*.,2015
5.	RM 6775	AATTGATGCAGGTTCAGCAAGC	GGAAATGTGGTTGAGAGTTGAGAGC	6	*Bph25*	Myint *et al*.,2012
6.	RM 309	CACGCACCTTTCTGGCTTTCAGC	AGCAACCTCCGACGGGAGAAGG	12	*Bph26*	Myint *et al*.,2012
7.	RM 5479	CTCACCATAGCAATCTCCTGTGC	ACTTCGTTCACTTGCATCATGG	12	*Bph26*	Myint *et al*.,2012
8.	RM 5926	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC	11	*Pi1*	Thippeswamy *et al*.,2015
9.	RM 8225	GCGTGTTCAGAAATTAGGATACGG	GATCTCGCCACGTAATTGTTGC	6	*Pi-z*	Ashkani *et al*.,2011
10.	RM 206	ATCGATCCGTATGGGTTCTAGC	GTCCATGTAGCCAATCTTATGTGG	11	*Pi-kh*	Kumar *et al*.,2013
11.	RM 212	AAGGTCAAGGAAACAGGGACTGG	AGCCACGAATTCCACTTTCAGC	1	*Dr*	Ashfaq *et al*.,2014
12.	RM 302	TGCAGGTAGAAACTTGAAGC	AGTGGATGTTAGGTGTAACAGG	1	*Dr*	Ashfaq *et al*.,2014
13.	RM 3825	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAGC	1	*Dr*	Kanagraj *et al*.,2010
14.	RM 201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	9	*Dr*	Kanagraj *et al*.,2010
15.	RM 1233	ATGGGCACGTGTAATTCATTCG	ATCCTCGAAAGTAGGAGTAG-GAAAG	11	*Pi-1*	Ramadevi *et al*.,2015
16.	pTA248	AGACGCGGAAGGGTGGTTCCCGGA	AGACCGGGTAAT CGAAAGATGAAA	11	*Xa21*	Sabar *et al*.,2016
17.	RM 122	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC	5	*Xa5*	Sabar *et al*.,2016
18.	RM 22709	CGCGTGGGCGAGACTAATCG	CCTTGACTCCGAGGATTCATTGTCC	8	*Gm8*	Mohapatra *et al*.,2016
19.	RM 547	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC	8	*Gm4*	Kalpana *et al*.,2016

Similarity coefficients were used to construct UPGMA (Unweighted Pair Group Method with Average) to generate dendrogram. Distance matrix and dendrogram was constructed based on diversity coefficient generated from pooled data by using UPGMA, a computer programme for distance estimation. The polymorphism percentage of the obtained bands was calculated by using following formula,

$$\text{Percent Polymorphism} \frac{\text{Total number of Polymorphic alleles}}{\text{Total number of alleles}} \text{ x100}$$

Polymorphism Information Content: Polymorphism Information Content (PIC) value were calculated as per formula developed by Powell *et al.*,(1996).

$$PIC= 1-\sum P_{ij}^{2}$$

Where, P_{ij} is the frequency of i^{th} and j^{th} locus, summed across the entire locus over all lines. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency) were estimated for each profile generated across 50 rice genotypes.

Results and Discussions

Biotic stresses

Blast: Four primers *i.e.* RM8225, RM206, RM5926 and RM1233 were used to identify blast resistance linked alleles in 50 rice genotypes. Marker RM8225 specific to gene *Pi-z* indicated presence of resistance linked alleles (221bp) in the genotypes, IRBB 53, IR 11 N 223, IRBB 57, IRBB 58. Fjellstrom *et al*., (2004), Askani *et al*., (2011) reported resistance linked alleles specific to gene *Pi-z* by using marker RM8225. Marker RM5926 specific to gene *Pi-1* indicated presence of resistance linked alleles (at 176bp) in the genotypes, B 40, IRBB 13, IRBB 65, IRBB 66, 12 DS-GMEI-22, IR 64 (Plate No.1). Thippeswamy*et al*., (2006) and Thippeswamy *et al*., (2015) also evaluated rice genotypes for presence of resistance linked alleles specific to gene *Pi1* by marker RM5926.

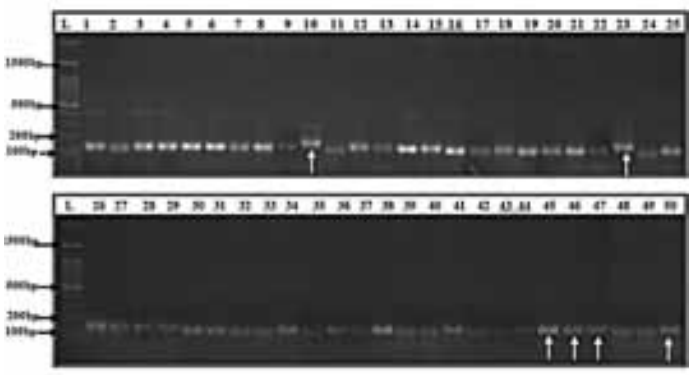

Plate No. 1: Amplified DNA bands of 50 rice genotypes using SSR marker RM5926 linked to Blast resistance trait. L- 100bp ladder. (Arrow indicates presence of the resistance linked allele).

The genotypes were screened for the presence of resistance linked allele for gene *Pi1* by visualization of amplicons of 170 bp fragments using SSR marker, RM1233. The results showed that all the genotypes indicated absence of resistance linked alleles for gene *Pi1* specific marker RM1233. Earlier studies (Ashkani *et al*., 2011; Ramadevi *et al*., 2015; Yadav *et al*., 2017) reported the use of marker RM1233 specific to gene *Pi1* for screening blast resistance in rice genotypes. The genotypes were verified for the presence of blast resistance gene, *Pi-kh* by using the gene specific primer, RM206 which is expected to amplify a 140bp fragment in the genotypes containing the resistance linked allele. No genotype was observed to possess the resistance linked allele for the blast resistance gene, *Pi-kh* for the primer RM206. Kumar *et al*., (2013) screened rice genotypes for blast resistance linked alleles specific to gene *pi-kh* by marker RM206.

Bacterial Blight: In this study, 50 rice accessions were screened to determine resistance status for BLB-resistance genes *viz., Xa5* and *Xa21* by using PCR based microsatellite markers RM122 and pTA248, respectively. Screening for the *Xa5* resistance gene by the amplification of the microsatellite marker RM122, which was employed to track the resistant amplicons of 240-250bp revealed resistance linked alleles in almost all the genotypes in this study. Studies performed by Islam *et al*., (2015), Ullah*et al*., (2012) and Sabar*et al*., (2016) also revealed presence of resistance linked alleles at 240-250bp for *Xa5* gene specific marker RM122. This indicates that bacterial blight gene *Xa5* specific marker RM122 is effective in detecting presence of resistance linked alleles in all the genotypes used in this study. No amplicons (1040bp) specific to resistance linked alleles for *Xa21* gene were detected by marker pTA 248. This indicates that resistance linked alleles for gene *Xa21* were absent in all the genotypes.

Brown Planthopper: The 50 germplasms of rice were evaluated for brown planthopper (BPH) resistance using the SSR markers *viz.,* RM5479, RM6775 and RM309. Among these, RM6775 was most effective in identification of the resistant linked alleles in the genotypes. Marker RM6775 specific to gene *Bph25* indicated presence of resistance linked alleles (192bp) in the genotypes, IRBB 13, IRBB 14, IRBB 64, 12 DS-GMEI-22, HHZ5-DT20-DT3-Y2, IR 05 A 272, IR 64. Marker RM309 and RM5479 specific to gene *Bph26* (at 152bp) indicated absence of resistance linked alleles in all the genotypes. These genotypes may

either have resistance linked alleles for other *Bph* genes which can be detected by using corresponding gene specific markers or these genotypes are devoid of resistance linked alleles for any of the BPH genes. Myint *et al*., (2012) also screened rice genotypes for *Bph25* and *Bph26* genes by the primers RM6775, RM309 and RM5479. Several other workers (Rahman *et al*., 2009; Harini *et al*., 2013; Shabanimofrad *et al*., 2015a and 2015b and Bhogadhi *et al*., 2015) also reported similar marker study for *Bph* genes.

Gall midge: The SSR markers RM547 and RM22709 used in this study reported the presence of resistance linked alleles for biotypes *Gm4* (270bp) and *Gm8* (160 to 170bp), respectively. RM547 at 270bp showed resistance linked alleles in eight genotypes, IR 11 N 169, IR 11 N 239, B 40, IRBB 2, IRBB 4, IRBB 5, IRBB 10, IRBB 50 and marker RM22709 specific to gene Gm8 (at 170bp) indicated presence of resistance linked alleles in the genotypes, IR 11 A 546, IR 11 A 581, IRBB 8, IRBB 10, IRBB 14, IR 11 N 223. Both the markers used in this study were effective in detecting resistance linked alleles. The genotypes showing presence of resistance linked alleles can further be used in various breeding programs. Using flanking SSR markers Sama *et al*., (2012) detected *Gm8* gene in nine rice genotypes. Various previous reports (Kalpana *et al*., 2016 and Mohapatra *et al*., 2016) revealed the strong association of locus specific makers which are in agreement with this study.

Abiotic stresses

Salt Tolerance: Salt tolerance linked markers used in this study were RM140, RM1287, RM562 and RM3412. Saltol QTL detected by the marker RM140 (at 260bp) was observed in the genotypes, IR 11 A 546, IR 11 A 581, IR 11 N 121, IR 11 N 137, IR 11 N 169, B 40, IRBB 7, IRBB 50, IRBB 61, IRBB 62. Saltol QTL detected by the marker RM1287 (at 160bp) was observed in the genotypes, IR 552, IRBB 1, IRBB 2, IRBB 3. Saltol QTL detected by the marker RM3412 (at 211bp) was observed in the genotypes, IR 11 N 137, IR 11 N 169, B 40, IRBB 4, IRBB 5, IRBB 50, IRBB 51, IRBB 53, IRBB 54, IRBB 55, IRBB 56, IRBB 57, IRBB 60, IRBB 61, IRBB 62 (Plate No. 2). Marker RM562 (at 243bp) indicated absence of resistance linked alleles in all the genotypes. Markers used in this study were effective in detecting Saltol QTL in several genotypes screened. Further study may confirm that some of these genotypes might have Saltol QTL and can be used as alternative donors in salt tolerant rice breeding programmes. All the four Saltol linked SSRs used in this study amplified polymorphic bands in the 50 genotypes. Studies performed by Zeng *et al*., (2004), Karmakar *et al*., (2012) Islam *et al*., (2012), Iqbal *et al*., (2015) and Ganie *et al*., (2016) also reported that these markers are highly polymorphic in nature.

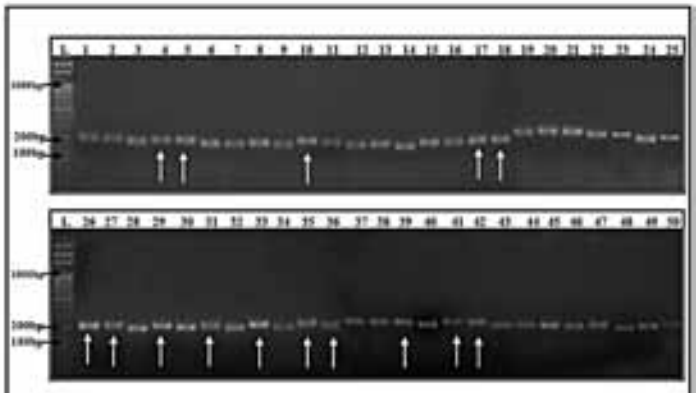

Plate No. 2: Amplified DNA bands of 50 rice genotypes using SSR marker RM3412 linked to salt resistance trait. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele).

Drought Tolerance: Molecular markers linked to drought tolerance in rice are an important tool for screening and selection of drought tolerant genotypes for use in future breeding programs. Drought resistance linked markers used in this study includes RM212, RM302, RM3825 and RM201. Resistance linked alleles Dr (135 bp) detected by the marker RM212 were present in the genotypes, IRBB 55, IR 11 N 400, IRBB 57, IRBB 62, IRBB 63. Markers RM302 (at 140bp), RM3825 (at 147bp) and RM201 (at 220bp) indicated absence of resistance linked alleles in all the genotypes. As these genotypes showed no amplification for these primers, we can suggest the use of other drought related marker combinations to screen the genotypes for drought resistance.

Studies performed by Ashfaq *et al*.,(2014) stated that the markers RM315, RM212 and RM302 on chromosome 1 may be useful for evaluation of diverse germplasm and on the basis of these molecular markers some genotypes of rice were identified as drought tolerant genotypes. This was also linked with the root traits, *i.e.* root length of the genotypes. It is also evident from results given by Kanagaraj *et al*., (2010) that the genomic region RM212–RM302–RM3825 on chromosome 1 is linked to drought resistance traits and may be useful in marker assisted breeding for drought resistance in rice. Various similar reports were observed in the studies done by Kanagaraj *et al*., (2010), Ashfaq *et al*., (2014), Ramadan *et al*., (2015), Freeg *et al*., (2016) and Sindhumole *et al*., (2017).

Genetic Diversity: The polymorphism percentage for each primer was calculated by the ratio of number of

polymorphic bands obtained over the total number of bands produced across the 50 rice germplasms. All the 50 rice accessions were genotyped with 19 trait linked microsatellite markers and were selected for their ability to produce amplified product and detect polymorphism level among the varieties and consistency of the pattern. Total 103 alleles were scored from 19 markers and 100 per cent were found to be polymorphic (Table 4). The overall size of amplified products ranged from 100bp to 1050bp.

Table 3: Genotypes showing amplification of resistance/tolerance specific alleles for multiple resistance / tolerance for different traits

Sr. No	Genotypes	Blast	Bacterial Blight	Brown Plant hopper	Gall Midge	Saltol	Drought
1.	IR 11 A 546	–	+	–	+	+	–
2.	IR 11 A 581	–	+	–	+	+	–
3.	IR 11 N 121	–	+	–	–	+	–
4.	IR 11 N 137	–	+	–	–	+	–
5.	IR 11 N 169	–	+	–	+	+	–
6.	IR 11 N 239	–	+	–	+	–	–
7.	IR 11 N 313	–	+	–	–	–	–
8.	IR 12 L 201	–	+	–	–	–	–
9.	SAKHA 105	–	+	–	–	–	–
10.	B 40	+	+	–	+	+	–
11.	IR 552	–	+	–	–	+	–
12.	IR 11 A 106	–	+	–	–	–	–
13.	IRBB 1	–	+	–	–	+	–
14.	IRBB 2	–	+	–	+	+	–
15.	IRBB 3	–	+	–	–	+	–
16.	IR 12 L 125	–	+	–	–	+	–
17.	IRBB 4	–	+	–	+	+	–
18.	IRBB 5	–	+	–	+	+	–
19.	IRBB 7	–	+	–	–	+	–
20.	IRBB 8	–	+	–	+	–	–
21.	IRBB 10	–	+	–	+	–	–
22.	IRBB 11	–	+	–	–	–	–
23.	IRBB 13	+	+	+	–	–	–
24.	IRBB 14	–	+	+	+	–	–
25.	IRBB 21	–	+	–	–	–	–
26.	IRBB 50	–	+	–	+	+	–
27.	IRBB 51	–	+	–	–	+	–
28.	IRBB 52	–	+	–	–	–	–
29.	IRBB 53	+	+	–	–	+	–
30.	IR 11 N 223	+	+	–	+	–	–
31.	IRBB 54	–	+	–	–	+	–
32.	IR 09 L 226	–	+	–	–	–	–
33.	IRBB 55	–	+	–	–	+	+
34.	IR 11 N 400	–	+	–	–	–	+
35.	IRBB 56	–	+	–	–	+	–
36.	IRBB 57	+	+	–	–	+	+

Sr. No	Genotypes	Blast	Bacterial Blight	Brown Plant hopper	Gall Midge	Saltol	Drought
37.	IRBB 58	+	+	–	–	–	–
38.	IRBB 59	–	+	–	–	–	–
39.	IRBB 60	–	+	–	–	+	–
40.	IRRI 123	–	+	–	–	–	–
41.	IRBB 61	–	+	–	–	+	–
42.	IRBB 62	–	+	–	–	+	+
43.	IRBB 63	–	+	–	–	–	+
44.	IRBB 64	+	+	+	–	–	–
45.	IRBB 65	–	+	–	–	–	–
46.	IRBB 66	+	+	–	–	–	–
47.	12 DS-GMEI-22	+	+	+	–	–	–
48.	HHZ5-DT20-DT3-Y2	–	+	+	–	–	–
49.	IR 05 A 272	–	+	+	–	–	–
50.	IR 64	+	+	+	–	–	–

("+" indicates presence of the resistance linked allele; "-"indicates absence of the resistance linked allele for the marker).

The PIC values were calculated to find out the effectiveness of primers in distinguishing individual accessions (Table 4). The PIC values ranged from 0.40 to 0.90 with an average of 0.65 per primer. Total of 103 alleles were detected with an average of 5.42 alleles per locus. The marker pTA-248 generated a maximum number of alleles (16) while the marker RM5479 produced minimum number of alleles (2). The SSR marker, pTA 248 revealed highest (0.90) PIC value whereas the marker RM22709 revealed the lowest (0.40) PIC value. The higher the PIC value, the more informative is the SSR marker. Hence, primers pTA 248, RM547 and RM302 were found to be highly informative (Table 4).

In this study, a total of 103 alleles were detected with an average number of alleles of 5.42 per locus (ranged from 3 to 16 per locus). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles with higher gene diversity. The average number of alleles (5.42) obtained in this study is higher than the values reported by earlier studies (Islam *et al*., 2012; Gholizadeh *et al*., 2015; Freeg *et al*., 2016; Krupa *et al*., 2017) on smaller germplasm sets and comparable to values reported by Hoque *et al*., (2014). However, lower than the values reported by Jain *et al*., (2004), Giarrocco *et al*., (2007); Thomson *et al*., (2007) and Roy *et al*., (2015) with large germplasms (Table 5). These inconsistencies might be due to the genotypes used and selection of SSR markers.

The markers showed an average PIC value of 0.65 which indicated that SSR markers used in this study were highly informative because only PIC values higher than 0.5 indicate high polymorphism. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Dewoody *et al*., 1995). The overall genetic diversity (PIC=0.65) of the 50 rice germplasm accessions included in this study was comparable to the value reported in previous studies (Jain *et al.,* 2004; Giarrocco *et al*., 2007; Thomson *et al*., 2007 and Roy *et al*., 2015) and higher than the values reported by Hoque *et al*., (2014), Gholizadeh *et al*., (2015), Freeg *et al*., (2016) and Krupa *et al*., (2017) who reported average PIC value equivalent to 0.54, 0.45, 0.52 and 0.49 and smaller than the values reported by Nguyen *et al*., 2012 and Nachimuthu *et al*., (2015) who reported average PIC value of 0.73 and 0.75, respectively. PIC shows how the marker can indicate the population polymorphism depending on the number and frequency of the alleles (Botstein *et al*., 1980). So the PIC reflects a discriminating ability of the marker and, in fact, depends on the number of known alleles and their frequency distribution, thus being equal to genetic diversity.

Genetic distance values between germplasm accessions: On the basis of analysis of SSR scoring, the alleles were converted to binary score based on their presence (1) or absence (0). This data was used for similarity based

analysis using the programme Multivariate Statistical Package(MVSP) to determine the Jaccard's coefficient matrices *i.e.* estimate of similarity among the fifty genotypes.The genetic distances ranged from 0.308 to 1 with an average of 0.77 among these 50 promising genotypes of rice.The lowest GD value (0.308) was found between the genotypes IRBB 59 vs. IRBB 58, IRBB 60 vs. IRBB 59 whereas highest genetic distance value (1) was found between the genotypes IRBB 5 vs. IR 64.Further, the average genetic distance values per genotype from rest of the genotypes based on Jaccard's similarity index of all germplasm lines in rice were also analysed separately.It was revealed that an average genetic distance among the 50 rice accessions ranged from 0.711 (IR 11 N 169) to 0.819 (IR 64)from MVSP analysis (Table 5).

Clustering analysis based on SSR marker analysis: The UPGMA based dendrogram of 50 rice genotypes was generated with Multivariate Statistical Package (MVSP). Clustering pattern of dendrogram generated by using the pooled molecular data of 19 primers of 50 genotypes produced two main clusters namely I and II (Table 6). The major cluster-I comprised of 17 accessions and was further found to be divided into two sub clusters (IA and IB). The major cluster-II comprised of 33 accessions and was further found to be divided into two sub clusters (IIA and IIB). The dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered together (Figure 1).

Table 4: Molecular polymorphism, PIC Values, No. of alleles and size of loci by SSR Primers in rice genotypes

Sr. No.	Primer	Total no. of polymorphic Band	Average No. of bands/ genotype	% Polymorphism	No. of alleles	PIC	Range of amplified products
1	RM 8225	50	1.0	100	6	0.73	180-330
2	RM 206	58	1.16	100	8	0.80	320-530
3	RM 5926	56	1.12	100	5	0.65	120-190
4	RM 122	50	1.0	100	2	0.43	230-270
5	RM 1233	50	1.0	100	5	0.65	150-250
6	pTA248	50	1.0	100	16	0.90	520-1050
7	RM 5479	50	1.0	100	2	0.50	230-340
8	RM 6775	69	1.38	100	7	0.78	120-190
9	RM 309	50	1.0	100	3	0.44	150-220
10	RM22709	52	1.04	100	3	0.40	100-180
11	RM 547	78	1.56	100	9	0.86	190-370
12	RM 140	50	1.0	100	5	0.69	200-320
13	RM 1287	50	1.0	100	5	0.66	150-230
14	RM 562	50	1.0	100	4	0.70	100-200
15	RM 3412	50	1.0	100	4	0.60	180-250
16	RM 212	50	1.0	100	4	0.64	140-220
17	RM 302	83	1.66	100	8	0.83	170-360
18	RM 3825	50	1	100	4	0.61	160-240
19	RM 201	50	1	100	3	0.62	110-160
	Total	**1046**	**20.92**	**-**	**103**		**-**
	Average	**55.05**	**1.10**	**100.00**	**5.42**	**0.65**	

Table 5: Average genetic distance estimates by SSR marker analysis based on Jaccard's dissimilarity coefficient

Sr.No.	Accession	Avg. GD	Sr.No.	Accession	Avg. GD
1.	IR 11 A 546	0.738	26.	IRBB 50	0.786
2.	IR 11 A 581	0.738	27.	IRBB 51	0.765
3.	IR 11 N 121	0.745	28.	IRBB 52	0.751
4.	IR 11 N 137	0.722	29.	IRBB 53	0.740
5.	IR 11 N 169	0.711	30.	IR 11 N 223	0.750
6.	IR 11 N 239	0.715	31.	IRBB 54	0.768
7.	IR 11 N 313	0.762	32.	IR 09 L 226	0.769
8.	IR 12 L 201	0.712	33.	IRBB 55	0.791
9.	SAKHA 105	0.745	34.	IR 11 N 400	0.771
10.	B 40	0.767	35.	IRBB 56	0.754
11.	IR 552	0.766	36.	IRBB 57	0.781
12.	IR 11 A 106	0.794	37.	IRBB 58	0.777
13.	IRBB 1	0.763	38.	IRBB 59	0.773
14.	IRBB 2	0.826	39.	IRBB 60	0.772
15.	IRBB 3	0.778	40.	IRRI 123	0.768
16.	IR 12 L 125	0.773	41.	IRBB 61	0.758
17.	IRBB 4	0.778	42.	IRBB 62	0.825
18.	IRBB 5	0.798	43.	IRBB 63	0.809
19.	IRBB 7	0.786	44.	IRBB 64	0.768
20.	IRBB 8	0.763	45.	IRBB 65	0.767
21.	IRBB 10	0.760	46.	IRBB 66	0.774
22.	IRBB 11	0.764	47.	12 DS-GMEI-22	0.800
23.	IRBB 13	0.767	48.	HHZ5-DT20-DT3-Y2	0.811
24.	IRBB 14	0.779	49.	IR 05 A 272	0.783
25.	IRBB 21	0.815	50.	IR 64	0.819
AVERAGE GENETIC DISTANCE= 0.77					

Table 6: Distribution of 50 rice accessions into different clusters based on SSR analysis

Cluster	Sub cluster	Sub-sub cluster	Number of genotypes	Genotypes
I	IA		2	IRBB 63, IRBB 62.
	IB	IB (i)	5	IRBB 58, IRBB 59, IRBB 60, IRRI 123, IRBB 61.
		IB (ii)	10	IRBB 51, IRBB 53, IR 11 N 223, IRBB 52, IR 09 L 226, IRBB 54, IRBB 55, IR 11 N 400, IRBB 56, IRBB 57.
II	IIA	IIA (i)	3	HHZ5-DT20-DT3-Y2, IR 05 A 272, IR 64.
		IIA (ii)	4	IRBB 64, IRBB 65, IRBB 66, 12 DS-GMEI-22.
	IIB	IIB (i)	12	IR 11 N 313, IR 11 A 106, SAKHA 105, IRBB 1, IR 552, IRBB 3, IR 12 L 125, IRBB , B 40, IRBB 4, IRBB 5, IRBB 7.
		IIB (ii)	14	IR 11 A 546, IR 11 A 581, IR 11 N 121, IR 11 N 137, IR 11 N 169, IR 11 N 239, IR 12 L 201, IRBB 8, IRBB 10, IRBB 11, IRBB 13, IRBB 14, IRBB 21, IRBB 50.

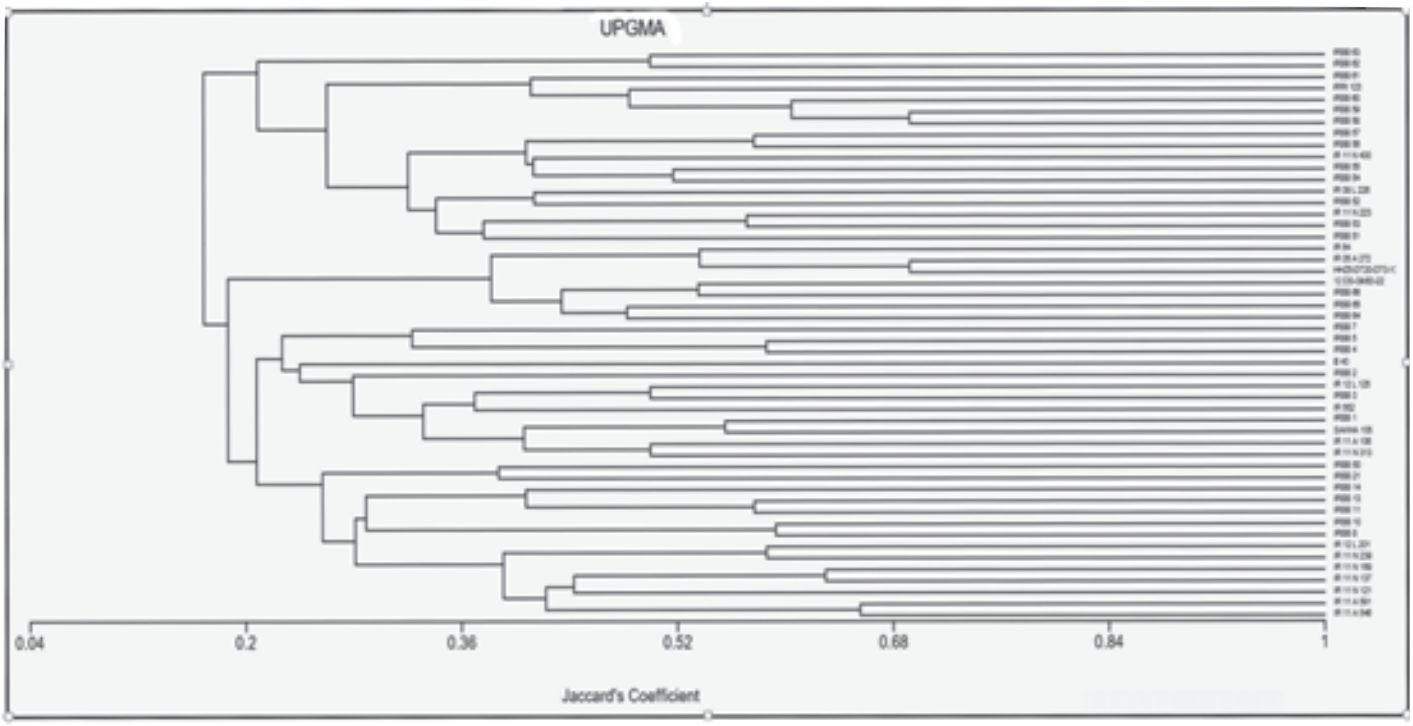

Figure 1: Dendrogram constructed using Jaccard's Similarity Coefficient

Similarly, Choudhary *et al*., (2013) constructed the unweighted neighbour-joining (UNJ) dendrogram on the basis of genetic similarity matrix and grouped 100 genotypes into five clusters *viz.*, landraces, 1970s, 1980s, 1990s, and 2000s. Yadav *et al*., (2013) grouped 88 rice accessions that included landraces, farmer's varieties and popular Basmati lines into two major clusters at the dissimilarity coefficient of 0.55 and further into four clusters at a dissimilarity coefficient of 0.58. Mohiuddin *et al*., (2014) constructed dendrogram based on the Nei's genetic distance calculated from 27 SSR markers generated from the 30 rice accessions. Singh *et al*., (2016) grouped 729 rice varieties into two major clusters, 400 varieties in cluster 1 whereas 329 varieties were grouped into cluster 2. From this study it is revealed that rice varieties are more divergent and the genetic diversity detected using molecular markers in the present investigation indicates the high discrimination capacity of SSR markers precisely due to the multi-allelic nature of SSR markers.

Conclusion

Of all the screened genotypes, B 40 showed presence of multiple resistance traits for the blast, bacterial blight, gall midge and Saltol while IRBB 57 showed presence of multiple resistance traits for blast, bacterial blight, gall midge and drought. Moreover, some genotypes showed presence of three different combinations of resistance traits *i.e.* genotypes IR 11 A 546, IR 11 A 581, IR 11 N 169, IRBB 50, IRBB 2, IRBB 4 and IRBB 5 for bacterial blight, gall midge and Saltol; IRBB 13, IRBB 64, DS-GMEI-22 and IR 64 for blast, bacterial blight and brown planthopper; IRBB 14 for bacterial blight, brown planthopper and gall midge; IRBB 53 for blast, bacterial blight and Saltol; IR 11 N 223 for blast, bacterial blight and gall midge; IRBB 55 and IRBB 62 for blast, Saltol and drought. Many other genotypes also showed amplification of the resistance/ tolerance specific alleles in the present study. These rice genotypes can be exploited for marker-assisted breeding after their validation through phenotype based screening for the target traits. The SSR marker gave more clusters with fewer genotypes in each cluster and therefore, more variation within each cluster. In the present study, the rice varieties are grouped into two major clusters. The genetic diversity and cluster analysis together for stress resistance provides some useful guides for assisting plant breeders in selecting genetically diverse parents for crossing programme and also helps in broadening the genetic base of the rice germplasm.

References

Ashfaq M, Saleem HM, Ali A, Ali M, Hanif S and Mubashar U. 2014. Screening of diverse germplasms for genetic studies of drought tolerance in rice (*Oryza sativa* L.).*Caryologia*, 67(4): 296-304.

Ashkani S, Rafii MY, Sariah M, Akmar AM, Rahim, RA and Latif MA. 2011. Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genetics and Molecular Research*, 10 (3): 1345-1355.

Bhogadhi SC, Bentur JS, Rani CVD, Thappeta G, Yamini KN, Kumar NAP, Jamaloddin M, Swathi G, Lakshmi VJ, Bhanu KV and Satynarayana PV. 2015. Screening of rice genotypes for resistance to brown plant hopper biotype 4 and detection of BPH resistance genes. *Int. J. Life Sci. Biotechnol.Pharma Res*, 4 (2): 90-95.

Botstein D, White RL, Skolnick M and Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*. 32(3):314–31.

Choudhary K, Choudhary OP and Shekhawat NS. 2008. Marker assisted selection: a novel approach for crop improvement. *American-Eurasian Journal of Agriculture*.1:26–30.

Choudhary G, Ranjitkumar N, Surapaneni M, Deborah DA, VipparlaA,Anuradha G and Siddiq EA. 2013. Molecular genetic diversity of major Indian rice cultivars over decadal periods. *PLoS ONE*. 8(6): e66197.

DeWoody JA, Honeycutt RL and Skow LC. 1995. Microsatellite markers in white-tailed deer. *Journal of Heredity*. 86: 317–319.

Edwards K, Johnstone C, and Thompson C. 1991. A Simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research*. 19 (6): 1349.

Fjellstrom R, Mc Clung, AM, Marchetti MA and Shank AR. 2004. Development of DNA Markers Suitable for Marker Assisted Selection of Three Pi Genes Conferring Resistance to Multiple *Pyricularia grisea* Pathotypes. *Crop Science*. 44:1790–1798.

Freeg HA, Anis GB, Abo-Shousha AA, El-BannaAN and El Sabagh A. 2016. Genetic diversity among some rice genotypes with different drought tolerance based on SSR markers.*Cercetări Agronomiceîn Moldova*. 49(3): 39-50.

Ganie SA, Borgohain MJ, Kashyap K, Talukdar A, Pani DR and Mondal TK. 2016. Assessment of genetic diversity of Saltol QTL among the rice (*Oryza sativa* L.) genotypes. *Physiology and Molecular Biology of Plants*. 22 (1):107–114.

Gholizadeh F and Navabpour S. 2014. Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers under salinity stress. *Iranian Journal of Plant Physiology*. 4 (3):1039-1046.

Giarrocco LE, Marassi MA and Salerno L. 2007. Assessment of the genetic diversity in Argentina rice cultivars with SSR markers. *Crop Science*. 47: 853–860.

Gomez SR. 2013. Abiotic and biotic stress in plants. P 1-20. In Molecular stress physiology of plants, Rout, GR., and Das, AB (ed.) Springer, New Delhi.

Gracia AAF, Benchimol LL, Antonica MM, Geraldi IO and Deuza AP. 2004. Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. *Euphytica*. 108: 53-63.

Harini AS, Kumar SS, Balaravi P, Sharma R, Dass MA and Shenoy V. 2013. Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. *African Journal of Biotechnology*.12(19):2515-2525.

Hoque A, Begum S and Hassan L. (2015). Genetic diversity assessment of Rice (*Oryza sativa* L.) germplasm using SSR markers. *Research in Agriculture Livestock and Fisheries*, *1*(1), 37-46.

Iqbal SA, Islam MM, Ahmed Hossain Md and Malaker A. 2015. DNA finger printing of rice lines for salinity tolerance at reproductive stage. *Advances in Crop Science and Technology.* S1: 006.

Islam MR, Gregorio GB, Salam MA, Collard BCY, Singh RK and Hassan L. 2012. Validation of SalTol linked markers and haplotype diversity on chromosome 1 of rice. *Molecular Plant Breeding.* 3:103–114.

Islam MS, Mia M, Khanom SM, Nasiruddin KM, Islam MM. 2015. Screening of aromatic rice cultivars for bacterial leaf blight (BLB) disease resistance Gene Xa5 through molecular marker. *British Biotechnology Journal*. 9(4): 1-13.

Jain S, Jain RK, Mc Couch SR. 2004. Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labelled microsatellite markers. *Theoretical and Applied Genetics*. 109: 965–977.

Kalpana, B. 2016. M.Sc thesis on Morphological and molecular screening of backcross derived lines for gall midge resistance in rice (*Oryza sativa* L.). Professor JayashankarTelangana State Agricultural University.

Kanagaraj P, Prince KS, Sheeba JA, Biji KR, Paul SB, Senthil A and Babu RC. 2010. Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current Science*. 98 (6):836-839.

Karmakar J, Roychowdhury R, Kar RK, Deb D and Dey N. 2012. Profiling of selected indigenous rice (*Oryza sativa* L.) landraces of rarh Bengal in relation to osmotic stress tolerance. *Physiology and Molecular Biology of Plants*.18:125–132.

Krupa KN, Shashidhar HE, Dalawai N, Reddy M and Swamy HVV. 2017. Molecular marker based genetic diversity analysis in rice genotypes (*Oryza sativa* L.) using SSR markers. *International Journal of Pure and Applied Biosciences*. 5(2): 668-674.

Kumar SG, Kumari KA, Durga Rani CV, Sundaram RM, Vanisree S, Jamaloddin M and Swathi G. 2013. Study of simple sequence repeat (SSR) polymorphism for biotic stress resistance in elite rice variety JGL 1798. *African Journal of Biotechnology*. 12 (40): 5833-5838.

Lapitan VC, Brar DS, Abe T and Redofia ED. 2007. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breeding Science*. 57: 263-270.

Ma HL, Zhu JG, Liu G, Xie ZB, Wang Y, Yang L and Zeng Q. 2007. Availability of soil nitrogen and phosphorus in a typical rice–wheat rotation system under elevated atmospheric [CO2]. *Field Crop Research Journal*. 100:44–51.

Mohapatra S, Behera L, Jena MS, Pradhan K, Mohanty SK, and Sahu SC. 2016. Molecular screening of rice genotypes using linked markers for gall midge resistance gene, Gm4. *The Ecoscan*. 10 (1&2): 349-357.

Mohiuddin AKM, Nadia I, Sultana S and Ferdous J. 2014. Diversity analysis of indica rice accessions (*Oryza sativa* L.) using morphological and SSR markers. *Annals of Biological Research*. 5(11):20-31.

Myint KKM, Fujita D, Sonoda T, Yoshimura A and Yasui H. 2012. Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens*) in the rice cultivar ADR52. *Theoretical and Applied Genetics*. 124:495–504.

Nachimuthu VV, Muthurajan R, Duraialaguraja R, Sivakami R, Pandian BA, Ponniah G, Gunasekaran K, Swaminathan M, Suji KK and Sabariappan R. 2015. Analysis of population structure and genetic diversity in rice germplasm using SSR Markers: An initiative towards association mapping of agronomic traits in *Oryza sativa*. *Rice*. 8:30.

Nguyen TTT, Nguyen NMT, Hoang, LH, Furuya N, Tsuchiya K, 2012. Genetic diversity in Vietnamese upland rice germplasm revealed by SSR markers. *Journal of the Faculty of Agriculture Kyushu University*. 57(2): 383-391.

Ni JPM and Mackill DJ. 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science*. 42: 601–607.

PowellW, Marchray GC and Provan J 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*. 1:215–222.

Rahman ML, Jiang W, Chu, SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam, MS, Chin, JH, Jeung JU, Brar DS, Jena KK and Koh H. 2009. High-resolution mapping of two rice brown planthopper resistance genes, Bph20(t) and Bph21(t), originating from *Oryza minuta*. *Theoretical and Applied Genetics*. 119:1237–1246.

Rama Devi SJS, Singh K, Prasad MS, Umakanth B, Ram Kumar G, Viraktamath BC and Madhav MS. 2015. Development and identification of novel rice blast resistant sources and their characterization using molecular markers. *Rice Science*. 22(6): 300–308.

Ramadan EA, Elmoghazy AM and El-Mowafi HF. 2015. Molecular markers based genetic diversity analysis for drought tolerance in rice (*Oryza sativa*. L.) using SSR Markers. *International Journal of Scientific Research in Agricultural Sciences*. 2:137-146.

Roy S, Banerjee A, Mawkhlieng B, Misra AK, Pattanayak A, Harish GD. 2015. Genetic diversity and population

structure in aromatic and quality rice (*Oryza sativa* L.) Landraces from North-Eastern India. *PLoS ONE* 10(10): e0141405. https://doi.org/10.1371/journal.pone.0141405

Sabar M, Bibi T, Farooq HU, Haider Z, Naseem I, Mahmood A and Akhter M. 2016. Molecular screening of rice (*Oryza sativa* L.) germplasm for Xa4, xa5 and Xa21 bacterial leaf blight (BLB) resistant genes using linked marker approach. *African Journal of Biotechnology*. 15(41): 2317-2324.

Sama VK, Himabindu K, Naik SB, Sundaram RM, Viraktamath BC and Bentur JS. 2012. Mapping and marker-assisted breeding of a gene allelic to the major Asian rice gall midge resistance gene Gm8. *Euphytica*. 187(3), 393-400.

Shabanimofrad M, Rafii MY, Ashkani S, Hanafi MM, Adam NA, Latif MA, Rahim HA and Sahebi M. 2015a. Analysis of SSR markers linked with brown planthopper resistance genes ('Bph') using high-resolution melting (HRM) in rice. *Plant Omics*. 8(3): 212.

Shabanimofrad M, Yusop MR, Ashkani S, Musa MH, Adam NA, Haifa I, Harun AR and Latif MA. 2015b. Marker-assisted selection for rice brown plant hopper (*Nilaparvata lugens*) resistance using linked SSR markers. *Turkish Journal of Biology*. 39 (5): 666-673.

Sindhumole P, SalamSA, Sajini S, Waghmare SG. 2017. Molecular characterization of rice (*Oryza sativa* L.) genotypes for drought tolerance using two SSR markers. *Electronic Journal of Plant Breeding*. 8(2): 474-479.

Singh BP, Singh B, Mishra S, Kumar V and Singh NK. 2016. Genetic diversity and population structure in Indian wild rice accessions. *Australian Journal of Crop Science*.10 (2): 144.

Thomson MJ, Septiningsih EM, Suwardjo F, Santoso TJ, Silitonga TS and Mc Couch SR. 2007. Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*. 114: 559–568.

Thompson JA, Nelson R, Land Vodkin LO. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Science*. 38: 1348-1355.

Thippeswamy S, Nagesh K and Reddy CS. 2006. Identification of effective blast resistance genes and validation of linked molecular markers in rice. In proceedings of "International Symposium on Frontiers in Genetics and Biotechnology Retrospect and Prospect, Osmania University, Hyderabad, India.P-III-18.

Thippeswamy SY, Chandramohan SB, Srinivas D, Padmaja and Pravalika K. 2015. Identification of blast resistant rice (*Oryza sativa* L.) genotypes in indigenous and exotic germplasm and validation of pi gene linked molecular markers. *Electronic Journal of Plant Breeding*.10: 5958.

Ullah I, Jamil S, Iqbal MZ, Shaheen HL, Hasni SM, Jabeen S, Mehmood A, and Akhter M. 2012. Detection of bacterial blight resistance genes in basmati rice landraces. *Genetics and Molecular Research*. 11 (3): 1960-1966.

Whitford R, Gilbert M and Langridge P. 2010. Biotechnology in agriculture. P 219-244. In: M.P. Reynolds (ed.) Climate change and crop production, CABI Series in Climate Change, CABI, UK.

Yadav S, Singh A, Singh MR, Goel N, Vinod KK, Mohapatra T and Singh AK. 2013. Assessment of genetic diversity in Indian rice germplasms (*Oryza sativa* L.): use of random versus trait-linked microsatellite markers. *Journal of Genetics.* 92: 545–557.

Yadav MK, Ngangkham U, Subudhi HN and Bag MK. 2017. Use of molecular markers in identification and characterization of resistance to rice blast in India. *PLOS ONE*. 12(6): e0179467.

Zeng L, Kwon TR, Liu X, Wilson C, Grieve CM and Gregorio GB. 2004. Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. *Plant Science*. 166(5): 1275-1285.