J. Indian Soc. Coastal Agric. Res. 34(2): 7-14 (2016)

Molecular Screening of Rice (*Oryza sativa* L.) Germplasm for Biotic and Abiotic Stresses and Their Diversity Study by Using SSR Markers

A. S. CHUNGADA, N. B. GOKHALE*, D. M. PATIL, S. V. SAWARDEKAR and P. P. PATIL

Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
Dapoli - 415 712, Maharashtra

Received: 05.12.2015 Accepted: 12.07.2016

The present study was carried out to screen the 55 rice germplasm for biotic and abiotic stresses and their genetic diversity analysis by using 18 microsatellite markers pairs distributed throughout the genome. Blast resistance alleles were observed in the genotypes viz., Karjat-184, Karjat-1, IR 65598-112-2, Phule Maval, Bhogavati, Ratnagiri-5, Phule Samruddhi, Ratnagiri-24, Karjat-7, RNT-55-3-2, RNT-1-1-2-1, RNT-66-43-8, Karjat-3, Paras Sona, Panvel-3, Jaya, BR-827 and Karjat-5. Salt tolerant genotypes include; Prabhavati, Ratnagiri-73, Karjat-1, Karjat-4, Paras Sona, RTN Purple, BR 827, IR 65598-112-2, Karjat-5, Ratna, Abhaya, RNT-49-2-3-1-2, IR 68952-5-2-11-8-1, RNT 66-43-8, Jaya, Bhogavati, Phule Samruddhi, IR-46, Indrayani, Saysree, NPT-2, IR-54, RP-4-14, Panvel-1 and IR-44. Bacterial Blight resistant genotypes were; RNT 55-3-2, Ratnagiri-5, Karjat-184, Karjat-1 and KJT-6. Two genotypes namely; Ratnagiri-60 and Ratnagiri-3 were observed tolerant to drought condition since it showed amplification at specific base pair. Gall midge resistant genotypes are Pawana, IR-68, Karjat-4, Ratnagiri-711, Abhaya, Panvel-1, IR-44 and RP-4-14 observed in the present studies. All the eighteen SSR primers used in this study were also subjected to genetic diversity study and primers were amplified, showed the higher level of polymorphism in rice germplasm. A total of 231 loci were generated by 18 primers. Each primer thus produced on an average 12.83 loci in the size ranging from 169.5 bp to 317.22 bp in the 55 rice genotypes in relation to diversity assessment. UPGMA grouped 55 rice genotypes into two main clusters which were further divided into two sub-clusters. This study will be helpful for selection of parental lines and development of new breeding population tolerant to specific traits through Marker Assisted Selection (MAS).

(***Key words**: Biotic and Abiotic stresses, Germplasm, SSR Marker, Polymorphism)*

Biotic and abiotic stresses are still a threat to rice productivity and sustainability. The major challenge is to overcome these constraints and produce high yielding rice varieties with multiple resistances to biotic and abiotic stresses possessing improved grain quality and nutritive value. Developing salt tolerant rice varieties is the most efficient way to stabilize rice production and alleviate food security and poverty in coastal region (Pani *et al*., 2013). Conventional breeding methods of variety development such as selection, back cross breeding etc. are time consuming, laborious and require more years for the development of improved population. DNA markers have enormous potential to improve efficiency and precision of conventional plant breeding via MAS. QTLs mapping studies for diverse crop species have provided an abundance of DNA marker trait associations (Aliyu *et al.,* 2011).

Microsatellite markers have been widely used to screen, characterize and evaluate genetic diversity in cereal species. Microsatellites are simple repeated motifs consisting of 1 to 6 base pairs, and they can be found in both coding and non-coding regions. The primary advantage of microsatellites as genetic markers is that they are inherited in a Mendelian fashion as codominant markers. Furthermore, high polymorphism rates, high abundance and a broad distribution throughout the genome have made microsatellites one of the most popular genetic markers for use in plant breeding programs (Miah *et al*., 2013).

Several rice varieties have been released by Agricultural Research Station, Shirgaon to cater the needs of the farmers. The molecular screening of these genotypes using microsatellite markers were lacking which will provide sufficient knowledge on traits of tolerance among them at molecular level. It will help the breeders to develop strategic breeding programmes in order to produce elite lines. The genotype specific molecular screening will enable to identify and characterize each variety separately.

MATERIALS AND METHODS

Plant material and DNA extraction

The experimental material consisted of 55 rice germplasm that were obtained from Agricultural Research Station Shirgaon, Dist. Ratnagiri, Maharashtra,

**Corresponding Author :* E-mail: nbgokhale@rediffmail.com

India. Details of germplasm are given in Table 1. Genomic DNA was extracted from young leaf tissues using the Doyle and Doyle (1990) method with some modifications. Purification of DNA in some sample was done to remove RNA, proteins and polysaccharides which were the major contaminants. RNA was removed by RNase treatment. RNase was added to the DNA sample @100 µg ml^{-1} and incubated at 37°C for 1 hour. The purity and concentration of DNA in the sample was determined after agarose gel electrophoresis with standard DNA i.e. uncut lamda Hind III DNA on 1% agarose gel and by comparison of the intensity of staining with Ethidium Bromide.

Microsatellite analysis

For the molecular screening of rice germplasm 18 different trait specific simple sequence repeat (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). PCR was performed in 96-well plates containing a total 20 μl volume; 35 ng DNA template, 10X PCR buffer, 15 mM $MgCl_2$, 10 mM dNTPs, 5 PMol forward and reverse primers, 3 U Taq polymerase enzyme and nano-pure water. PCR amplified products were separated by 2.5% Agarose gel electrophoresis at 80V for 2 to 2.5 h in 1X TAE buffer. Gels were stained in ethidium bromide solution and visualized under UV light using the gel documentation system. The gels were photographed under UV light using Pentax K 312 nm camera. The images of gel was taken by the documentation systems

Table 1. *Details of Rice Germplasm used in the study*

Sr. No.	Remark	Name	Sr. No.	Remark	Name
1.	RTNGM-II	Ratnagiri-4	29.	RTNGM-II	ParasSona
2.	RTNGM-II	KJT 14-7	30.	RTNGM-II	Panvel-3
3.	RTNGM-II	Prabhavati	31.	RTNGM-II	Jaya
4.	RTNGM-II	Ratnagiri-73	32.	RTNGM-II	RTN Purple
5.	RTNGM-II	Karjat-184	33.	RTNGM-II	BR 827
6.	RTNGM-II	Karjat-1	34.	RTNGM-II	IR 65598-112-2
7.	RTNGM-II	Pawana	35.	RTNGM-II	Karjat-5
8.	RTNGM-II	IR-68	36.	RTNGM-II	PhuleMaval
9.	RTNGM-II	Karjat-4	37.	RTNGM-II	Bhogavati
10.	RTNGM-II	Ratna	38.	RTNGM-II	Ratnagiri-5
11.	RTNGM-II	Patel-3	39.	RTNGM-II	PhuleSamruddhi
12.	RTNGM-II	PhuleRadha	40.	RTNGM-II	IR-46
13.	RTNGM-II	Ratnagiri-1	41.	RTNGM-II	Indrayani
14.	RTNGM-II	Ratnagiri-24	42.	RTNGM-II	Saysree
15.	RTNGM-II	Karjat-7	43.	RTNGM-II	NPT-2
16.	RTNGM-II	RNT 55-3-2	44.	RTNGM-II	IR-54
17.	RTNGM-II	RNT 1-1-2-1	45.	RTNGM-II	Palghar-1
18.	RTNGM-II	Ratnagiri-711	46.	RTNGM-II	Panvel-1
19.	RTNGM-II	PND-36-2-1-2	47.	RTNGM-II	IR-44
20.	RTNGM-II	IR-72	48.	RTNGM-II	RP-4-14
21.	RTNGM-II	Abhaya	49.	RTNGM-II	KJT-6
22.	RTNGM-II	RNT-49-2-3-1-2	50.	RTNGM-II	Masuri
23.	RTNGM-II	Kasturi	51.	RTNGM-II	Karjat-2
24.	RTNGM-II	IR 68952-5-2-11-8-1	52.	RTNGM-II	Ratnagiri-60
25.	RTNGM-II	RNT 35-1-1-1	53.	RTNGM-II	PKV HMT
26.	RTNGM-II	RNT -8-1-2-2	54.	RTNGM-II	Ratnagiri-3
27.	RTNGM-II	RNT 66-43-8	55.	RTNGM-II	Ratnagiri-2
28.	RTNGM-II	Karjat-3			

(Uvi Tech, Fire reader, Cambridge, England) and saved in computer for further analysis.

Data analysis

The images of gels were carefully studied and amplicons which occurred only once for a particular genotypes were marked which constituted the band for that particular genotype. Additionally, the band fragments present in two genotypes were also marked, which in combination with other bands generated with other primers would constitute the screenings. Molecular size of each amplified fragments were analyzed by using Uvi Tech, Fire reader software programme. Markers were scored for the presence (1) or absence (0) of the corresponding band among the genotypes. UPGMA cluster analysis was performed using Jaccard's similarity coefficient matrices calculated from SSR markers to generate a dendrogram across 55 rice varieties. A pairwise similarity index (SI) was calculated and the UPGMA based dendrogram of 55 rice varieties generated with Multivariate Statistical Package (MVSP).

Table 2. *List of SSR primers and specifications*

Sr. No.	Primer	Sequence Forward and Reverse Primer	Chro-mosome No.	Resistance Size (bp)	Linked gene	Linked Trait
1.	RM 140	F: TGCCTCTTCCCTGGCTCCCCTG R: GGCATGCCGAATGAAATGCATG	1	244-329	*Saltol*	Salt tolerance
2.	RM 1287	F: CCATTTGCAGTATGAACCATGC R: ATCATGCAATAGCCGGTAGAGG	1	158-207	*Saltol*	Salt tolerance
3.	RM 562	F: GGAAAGGAAGAATCAGACACAGAGC R: GTACCGTTCCTTTCGTCACTTCC	1	126	*Saltol*	Salt tolerance
4.	RM-8094	F: AAG TTT GTA CAC ATC GTA TACA R: CGCGACCAGTACTACTACTA	1	171-280	*Saltol*	Salt tolerance
5.	RM 6775	F: AATTGATGCAGGTTCAGCAAGC R: GGAAATGTGGTTGAGAGTTGAGAGC	6	192	*Bph25*	Brown plant hopper
6.	RM 309	F: CACGCACCTTTCTGGCTTTCAGC R: AGCAACCTCCGACGGGAGAAGG	12	152	*Bph26*	Brown plant hopper
7.	RM 5479	F: CTCACCATAGCAATCTCCTGTGC R: ACTTCGTTCACTTGCATCATGG	12	152	*Bph26*	Brown plant hopper
8.	RM-5926	F: ATA TAC TGT AGG TCC ATC CA R: AGA TAG TAT AGC GTA GCA GC	11	135-150	*Pi1*	Blast resistance
9.	RM 8225	F: GCGTGTTCAGAAATTAGGATACGG R: GATCTCGCCACGTAATTGTTGC	6	221	*Pi*	Blast resistance
10.	RM 206	F: ATCGATCCGTATGGGTTCTAGC R: GTCCATGTAGCCAATCTTATGTGG	11	140	*Pi54*	Blast resistance
11.	RM 212	F: AAGGTCAAGGAAACAGGGACTGG R: AGCCACGAATTCCACTTTCAGC	1	135	*Dr*	Drought tolerance
12.	RM 302	F: TGCAGGTAGAAACTTGAAGC R: AGTGGATGTTAGGTGTAACAGG	1	135-140	*Dr*	Drought tolerance
13.	RM 3825	F: CCACTAGCAGATGATCACAGACG R: GAGCACCTCATAAGGGTTTCAGC	1	147	*Dr*	Drought tolerance
14.	RM 1233	F: ATGGGCACGTGTAATTCATTCG R: ATCCTCGAAAGTAGGAGTAGGAAAGC	11	160	*Xa4*	Bacterial leaf blight
15.	pTA248	F: AGACGCGGAAGGGTGGTTCCCGGA R: AGA CCG GGT AAT CGA AAG ATG AAA	11	900-1000	*Xa21*	Bacterial leaf blight
16.	RM 122	F: CTTCTTCCGCTTCCTCCCTTCC R: TGTACCAGTGCACCGAGAGTTGG	5	250	*Xa13*	Bacterial leaf blight
17.	RM 22709	F: CGCGTGGGCGAGACTAATCG R: CCTTGACTCCGAGGATTCATTGTCC	8	170	*Gm8*	Gall midge fly
18.	RM 547	F: TTGTCAAGATCATCCTCGTAGC R: GTCATTCTGCAACCTGAGATCC	8	270	*Gm4*	Gall midge fly

To measure the informativeness of the markers, polymorphism information content (PIC) for each of the SSR markers was also computed.

RESULTS AND DISCUSSIONS

Molecular markers play an important role and are an extremely powerful tool for varietal identification and determining variability between genetic materials of the cultivars. Varietal identification that directly utilizes DNA potentially addresses all the limitations associated with morphological and biochemical data. Characterization of cultivars using DNA profiles with highly polymorphic microsatellite markers has been used successfully for cultivar identification and verification in several crop species (Tautz, 1989). These markers are abundant, distributed throughout the genome and are highly polymorphic compared with other genetic markers, as well as being species-specific and co-dominant. For these reasons, they have become increasingly important genetic markers in rice breeding programs (Miah *et al.,* 2013).

In this study, trait specific 18 SSR markers were used to screen the set of 55 rice genotypes for various biotic and abiotic stresses. The genotype specific molecular screening was enable to identify and characterize each genotype separately. The polymorphic information produced by these markers between different rice genotypes helps us in determining the presence or absence of resistance linked alleles in the given genotypes. The present study entitled "Molecular screening of rice germplasm for biotic and abiotic stresses" was carried out with view to identify trait specific genotypes tolerant to specific stress condition.

Molecular Screening

Blast (*Pyricularia grisea)*

Rice blast is one of the most destructive diseases of rice throughout the world. Resistance linked alleles *pi*

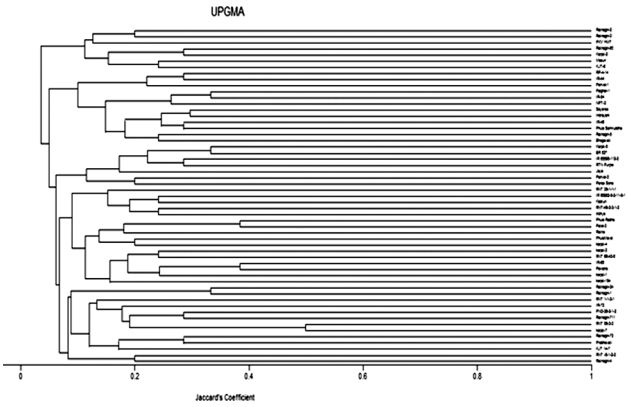

Fig.1. *Dendrogram constructed using Jaccard's Similarity Coefficient*

(221bp) for the marker RM-8225 were present in genotypes; Karjat-184, Karjat-1, IR 65598-112-2, Phule Maval, Bhogavati, Ratnagiri-5 and Phule Samruddhi (Fig. 3). Similarly, resistance linked alleles *pi54* (135bp) for RM-5926 were present in genotypes; Ratnagiri-24, Karjat-7, RNT 55-3-2, RNT 1-1-2-1, RNT 66-43-8, Karjat-3, Paras Sona, Panvel-3, Jaya, BR 827, IR 65598-112-2, Karjat-5 and Phule Maval. Among all the 55 genotypes, no any genotype indicated presence of the resistance linked alleles *pi1* (140bp). Kumar *et al.,* 2013, Ashkani *et al.,* 2011 and Jayawardana *et al.,* 2014 also used microsatellite markers to screen the various genotypes for blast resistance.

Brown plant hopper (*Nilaparvata lugens*)

The brown plant hopper (BPH) is one of the most serious and destructive pests of rice which causes complete drying and plant death, a condition known as "Hopper burn". Resistance alleles *Bph25* (192 bp) for RM 6775, *Bph26* (152bp) for RM 309 and *Bph26* (152bp) for RM 5479 indicated the absence of resistance linked alleles in all the 55 genotypes. Not a single genotype was found carrying a BPH resistance allele. Similar results were also obtained by Myint *et al.,* 2012 while screening an F_2 population of a cross between ADR52 and Taichung 65 for BPH resistance. Yang *et al.,* (2002) and Hong *et al.,* 2006 also used different microsatellite markers to screen the rice populations for BPH resistance.

Gall Midge (*Orseolia oryzae)*

The Asian rice gall midge (*Orseolia oryzae*, Wood-Mason) is a serious pest of rice in India, causing an average annual yield loss of about US $80 million (Bentur *et al.*, 2011). Resistance linked alleles *Gm4* (270 bp) for the marker RM 547 were present in genotypes; Pawana, IR-68, Karjat-4, Ratnagiri-711, Abhaya and alleles *Gm8* (170 bp) for RM 22709 were present in Panvel-1, IR-44, RP-4-14 genotypes which conferred resistance. These results were similar to the findings of Kumar *et al.,* 2013.

Bacterial Blight (*Xanthomonas oryzae* pv. *Oryzae)*

Bacterial blight is one of the most destructive diseases in rice and it is especially prevalent in irrigated and rainfed lowland rice growing areas. Two of the three markers showed polymorphism for BLB in the screened genotypes. Resistance linked alleles *Xa13* (250 bp) for the marker RM-122 were present in two genotypes; RNT 55-3-2 and Ratnagiri-5 (Fig. 2). Blair *et al.,* 1997 found the same results for the genotypes screened against BLB.

Alleles *Xa4* (160bp) for RM-1233 were present in Karjat-184, Karjat-1, KJT-6 genotypes which conferred resistance. The primer pTA-248 indicated absence of the resistance linked alleles *Xa21* (916 bp) in all the 55 genotypes. Xiao-Deng *et al.,* 2012 and Magar *et al.,* 2014 also used pTA- 248 molecular marker to screen the germplasm for BLB for which they observed polymorphism in germplasm.

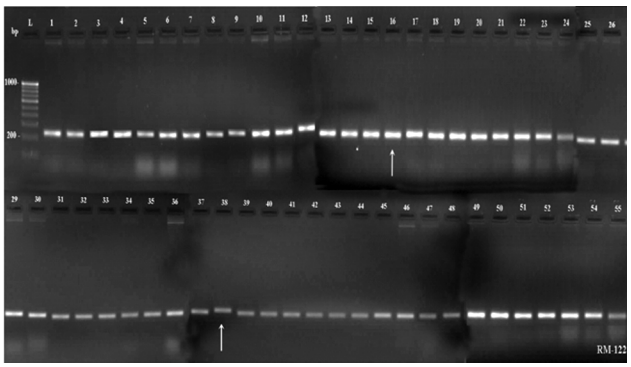

Fig 2. *PCR product for molecular screening with marker RM-122 linked to Bacterial Blight and electrophoresed in a 2% agarose gel. L= 100bp ladder.*

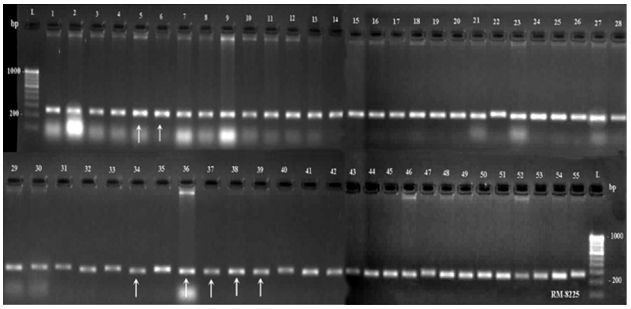

Fig 3. *PCR product obtained from molecular screening with marker RM-8225 linked to Blast genes and electrophoresed in a 2% agarose gel. L= 100bp ladder.*

Salt tolerance

Salt stress is one of the serious abiotic stresses along with other abiotic stresses in rainfed and lowland areas and also in coastal belts of the country. Tolerance linked alleles *saltol* (126 bp) for the marker RM-562 were present in genotypes; Prabhavati, Ratnagiri-73, Karjat-1, Karjat-4, Paras Sona, RTN Purple, BR 827, IR 65598-112-2 and Karjat-5. Similarly, tolerance linked alleles *saltol* (248-264 bp) for RM-140 were present in genotypes; Karjat-1, Karjat-4, Ratna, Abhaya, RNT-49-2-3-1-2, IR 68952-5-2-11-8-1, RNT 66-43-8, Jaya, RTN Purple, BR 827, IR 65598-112-2, Karjat-5, Bhogavati, Phule Samruddhi, IR-46, Indrayani, Saysree, NPT-2, IR-54 and RP-4-14. Alleles *saltol* (162 bp) for RM-1287 were present in genotypes; Panvel-1 and IR-44 varieties which conferred tolerance. Similar results were also obtained by Islam *et al.,* 2012. Alleles *saltol* (173 bp) for RM-8094 indicated absence of the tolerance linked alleles in all the 55 genotypes. Molecular mapping of genes linked to salt tolerance traits was also carried out by Aliyu *et al.,* 2011. Studies at ICAR-CSSRI, Karnal with 21 rice genotypes introgressed with *saltol* QTL developed at IRRI showed that the presence of substantial amount of variability in the *saltol* QTL derived germplasm (Ali *et al.*, 2013).

Drought tolerance

Among the abiotic stresses, drought is a serious limiting factor that reduces rice production and yield stability in rainfed ecosystems. Resistance linked alleles *Dr* (135.8bp) for the marker RM-212 were present in two genotypes; Ratnagiri-60 and Ratnagiri-3 which conferred tolerance. Similar results for drought tolerance were also obtained by Kanagaraj *et al.,* 2010 using RM-212 marker. Tolerance linked alleles *Dr* (135.8 bp) and *Dr* (147 bp) for RM-302 and RM-3825 were absent in all the 55 genotypes. Lin *et al.,* 2006 also used different microsatellite markers to screen the rice populations for drought tolerance.

Genetic diversity

In the present study, 55 rice genotypes were analyzed using 18 SSR primer pairs of which all 18 SSR primers were found to be polymorphic (Table 3). A total of 231 alleles were obtained using 18 SSR primer pairs with an average of 12.83 alleles per primer. The number of alleles amplified for each primer pair ranged from 5 to 20. The markers pTA-248 generated a maximum number of alleles (20). While the primer RM-309 produced minimum number of alleles (5). Similar results were obtained by Singh 2013, A total of 78 alleles were detected across 32 genotypes using 19 SSR markers. Maximum number of polymorphic alleles (9) were obtained with the marker RM 47, while the minimum number of alleles (1) was amplified by RM 264. The average number of polymorphic alleles per marker was 4.41.

The PIC values of primers ranged from 0.52 in SSR primer RM 309 to 0.89 in SSR primer pTA-248 with an average PIC value of 0.76. The high PIC value obtained in the present investigation might be due to high genetic diversity among the rice varieties. 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 (Aliyu *et al.,* 2011).

Table 3. *Molecular Polymorphism, PIC Values, No. of alleles and Size of Loci Revealed by SSR Primers in Rice Genotypes*

Sr. No.	Primer	Total no. of polymorphic	% Polymorphism	No. of alleles	PIC	Range of amplified products
1	RM 140	55	100	15	0.87	31-280
2	RM 1287	55	100	17	0.85	140-340
3	RM 562	55	100	14	0.80	100-250
4	RM-8094	55	100	14	0.78	160-330
5	RM 6775	55	100	7	0.61	120-190
6	RM 309	55	100	5	0.52	150-200
7	RM 5479	55	100	10	0.78	230-340
8	RM-5926	55	100	10	0.76	90-190
9	RM 8225	55	100	11	0.83	200-330
10	RM 206	55	100	15	0.85	320-480
11	RM 212	55	100	15	0.80	20-190
12	RM 302	55	100	19	0.80	240-470
13	RM 3825	55	100	18	0.87	180-360
14	RM 1233	55	100	8	0.65	130-220
15	pTA248	55	100	20	0.89	490-730
16	RM 122	55	100	11	0.70	170-280
17	RM 22709	55	100	9	0.65	90-190
18	RM 547	55	100	13	0.81	190-340
	Total	990	–	231	–	–
	Average	55	100.00	12.83	0.76	169.50-317.22

Table 4. *Clustering Pattern of 55 Rice genotypes*

Cluster		No. of genotypes	Name of the genotypes
I	IA	3	Ratnagiri-2, Ratnagiri-3,PKV HMT
	IB	4	Ratnagiri-60, Karjat-2, Masuri ,KJT-6
	IIA	12	RP-4-14, IR-44, Panvel-1, Palghar-1, IR-54, NPT-2, Saysree, Indrayani, IR-46, Phule Samruddhi, Ratnagiri-5, Bhogavati
II	IIB	36	Phule Maval, Karjat-5, IR 65598-112-2, BR 827, RTN Purple, Jaya, Panvel-3, Paras Sona, karjat-3, RNT 66-43-8, RNT -8-1-2-2, RNT 35-1-1-1, IR 68952-5-2-11-8-1, Kasturi, RNT-49-2-3-1-2,Abhaya, IR-72, PND-36-2-1-2, Ratnagiri-711, RNT 1-1-2-1, RNT 55-3-2, Karjat-7, Ratnagiri-24, Ratnagiri-1, Phule Radha, Patel-3, Ratna, Karjat-4, IR-68, Pawana, Karjat-1, Karjat-184, Ratnagiri-73, Prabhavati, KJT 14-7, Ratnagiri-4.

The Jaccard's similarity coefficient values among these rice genotypes are obtained. The pair wise similarity values ranged from 0 to 0.500. From these studies it is revealed that, rice varieties are more divergent indicating large part of the genome may be dissimilar among themselves. The diversity observed in the sixteen rice landraces four promising genotype mainly attributed to the genetic dissimilarities, the Dice similarity coefficient values among these rice landraces are obtained, the pair wise similarity values ranged from 0.25 to 0.87. Maximum similarity value of 0.87 was noticed between KR 0015 and KR 0029. Minimum similarity value of 0.25 was observed between IR 7199-3R-2-6 and Jhona (Seetharam *et al.,* 2009).

UPGMA cluster analysis was performed using Jaccard's similarity coefficient Matrices calculated from SSR markers to generate a dendrogram for 55 rice genotypes (Fig. 1). The UPGMA based dendrogram of

55 rice genotypes generated with Multivariate Statistical Package (MVSP). It was observed that, two major clusters (Table 4). The first major cluster consists of two sub clusters. Three rice genotypes Ratnagiri-2, Ratnagiri-3 and PKV HMT formed the independent first sub cluster. Second sub cluster consists of four genotypes *viz.,* Ratnagiri-60, Karjat-2, Masuri and KJT-6. The second major cluster consists of two sub cluster. First sub cluster consist of twelve and second sub cluster consist of thirty six rice genotypes. This shows the potentiality of SSR markers for the characterization of germplasm accessions. The clustering pattern revealed that genetic diversity was not associated with geographical diversity.

CONCLUSIONS

A conclusion can be drawn that screening of rice germplasm for biotic and abiotic stresses through microsatellites is very important for identifying parent germplasm with resistant genes that will be exploited for MAS breeding of elite lines that are more stable to stresses and this will lead to increase yields in order to feed the growing population.

REFERENCES

Ali, S. Gautam, R. K., Mahajan, R., Krishnamurthy, S. L. and Sarangi, S. K. (2013). Genetic variability and association analysis for grain yield components among SALTOL QTL introgressed rice genetypes under normal and saline environments. *Journal of the Indian Society of Coastal Agricultural Research*, **31**(1): 34-40.

Aliyu, R., Adamu, A. K., Muazu, S. and Alonge, S. O. (2011). Tagging and validation of SSR markers to salinity tolerance QTLs in Rice (*Oryza spp*). IPCBEE **1**: 328-323.

Ashkani, S., Rafii , M. Y., Sariah, M., Akmar, A. S.,. Rusli, I., Abdul Rahim, H. and Latif M. A. (2011). Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genetics and Molecular Res*earch **10**(3): 1345-1355.

Bentur, J. S., Sinha, D. K., Padmavathy, R. C., Muthulakshmi, M. and Nagaraju, J. (2011). Isolation and characterization of microsatellite loci in the asian rice gall midge (*Orseolia oryzae*). *International Journal of Molecular Sciences* **12**: 755-772.

Blair, M. W. and McCouch, S. R. (1997). Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene *xa-5*. *Theory of Applied Genet*ics **95**: 174-184.

Byerlee, D. (1996). Knowledge intensive crop management technologies, concepts, impacts, and prospects in asian agriculture, *International Rice Research Conference,* 3-5 June, Bangkok, Thailand.

Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus* **12**: 13-15.

Hong, S., Chun-Ming, W., Chang-Chao, S., Yu-Qiang, L., Hu-Qu, Z. and Jian-Min, W. (2006). Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). *Acta Genetica Sinica* **33**(8): 717-723.

Islam, M. R., Gregorio, G. B., Salam, M. A., Collard, B. C., Singh, R. K. and Hassan, L. (2012). Validation of *Saltol* linked markers and heliotype diversity on chromosome 1 of rice. *Molecular Plant Breeding* **3**(10): 103−114.

Jayawardana, W. D., Jayasekera, G. U., Wijesundera, R. C., Dissanayake, D. N., Sooriyapathirana, S. S., Weebadde, C. K., Perera, K. S., Gunapala, K. D. and Hettige, P. (2014). Evaluation of DNA markers linked to blast resistant genes, *Pikh*, *Pit (p)*, and *Pita*, for parental selection in Sri Lankan rice breeding. *Tropical Agricultural Research* **26**(1): 82-93.

Kanagaraj, P., Prince, K. S., Sheeba, J. A., Biji, K. R., Paul, S. B., Senthil, A. and Babu, R. C. (2010). Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current Sci*ence **98**(6): 836-839.

Kumar, S. G., Kumari, K. A., Durga Rani, C. V., Sundaram, R. M., 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.

Lin, M., Lin, C., Chen, J., Lin, J., Cheng, S., Liu , T., Jan, F., Wu, S., Thseng, F. and Ku, H. (2006). Tagging rice drought-related QTL with SSR DNA markers. *Crop Environment & Bioinformatics* **4**: 65-76.

Magar, M. M., Durga Rani, C. V. and Anuradha, G. (2014). Marker assisted selection for bacterial leaf blight resistance in segregating populations of *cottondora sannalu*. *International Journal of Applied Sciences* **2**(3): 229-237.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Nurul Islam, K. and Abdul Latif, M. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences* **14**: 22499-22528.

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

Pani, D.R., Sarangi, S. K., Misra, R.C., Pradhan, S. K., Subudhi, H.N. and Mandal, T.K. (2013). Performance of Rice Germplasm (*Oryza sativa* L.) under Coastal Saline Conditions. *Journal of the Indian Society of Coastal Agricultural Research,* **31**(1): 11-20.

Seetharam, K., Thirumeni S. and Paramasivam K., (2009). Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters**.** *African Journal of Biotechnology* **8**(10): 2050-2059.

Tautz. (1989). Hyper variability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17**: 6463-6471.

Xiao-Deng, Z., Hai-Peng, Z., Rong-Yao, C., Jie-Yun, Z., Shi-Hua, C. and Li-Yong, C. (2012). Breeding of R8012, a rice restorer line resistant to blast and bacterial blight through marker-assisted selection. *Rice Science* **19**(1): 29-35.

Yang, H., Ren, X., Weng, Q., Zhu, L. and He, G. (2002). Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene. *Hereditas* **136**: 39-43.