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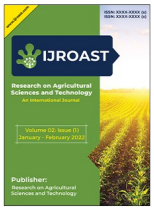

Genetic diversity analysis: Biotic and abiotic stress resistant rice genotypes using Hyper-Variable SSR markers

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ABSTRACT

The objective of present study was to evaluate the genetic polymorphism and identification of diverse parents among the 11 rice genotypes using Hyper-variable Simple Sequence Repeat marker (HvSSR).These microsatellite markers could serve as a powerful tool in selecting genetically diverse germplasms, to execute efficient selection in highly segregating generations. Out of 26 HvSSR markers,22 markers were amplified in all the eleven rice genotypes. A total of 54 alleles were detected with 22 markers with an average of 2 alleles per marker pair. Polymorphic information content (PIC) value of SSR markers ranged from 0.89 (HvSSR2-50) to 0.583 (HvSSR11-21) with an average of 0.73.The UPGMA clustering placed biotic resistant rice genotype namely BM-71 (BPH) and MIL-12 (Blast) in cluster I, while other biotic resistant rice genotypes namely, NLR-145 (Blast) and one abiotic resistant (P use efficiency) rice variety namely Swarna in cluster II. Cluster II also consists of susceptible genotypes namely WGL-44, WGL-14, WGL-962, KNM-118, WGL-32100, Bhadrakali and MTU-1121 with varied level of percent of similarity between the genotypes.Hybridization among these diverse parents may helpful to obtain better genotypes with biotic and abiotic stress resistance. Results obtained from the present investigation would be highly useful in rice breeding programs and may be used for further crop improvement using advance marker systems.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal food crops that feed more than half of the world's population. Worldwide, rice is cultivated in 163 million hectares with an annual production of 741 million tonnes [1]. It is grown under diverse ecological conditions and wide geographical range. Although rice is grown across several countries, more than 90 per cent is produced and consumed in Asia alone. It is the second most consumed cereal food in the world and in India, it ranks first. India is the second largest producer of rice next to China, producing 98.89 million tonnes (mt) with an average productivity of 2276.5 kg ha^{-1} from an area of 43.44 m ha. Genetic diversity analysis and DNA finger printing are very useful in breeding programs, seed conservation and management. The limits of genetic characterization using morphological traits via field evaluation may be overcome by use of DNA markers. Among the DNA markers, Simple Sequence Repeat (SSR) or microsatellite marker is one of the most useful genetic marker systems that use PCR technique to identify differences in microsatellite repeat units. SSR markers are widely used because of its co-dominant, multi allelic, high polymorphism, reproducibility, abundant informativeness, convenience of assay by PCR and distribution throughout the

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genome, independent of environments, independent of tissue effects and providing more precise characterization of genotypes and measurement of genetic relationships than other markers [2], [3] and [4]. Class1 SSRs are perfect repeats >20 nucleotides in length that behave as hyper variable loci, providing a rich source of markers for use in genetics and breeding [5]. Potentiality of evenly distributed hypervariable microsatellite markers in marker-assisted breeding of rice [6]. For similar studies of diversity analysis, SSR markers were used alone by [7], [8], [9] and [10].Considering the importance of fingerprinting, the present study is aimed for identification of Genetic Diversity among various Biotic and Abiotic Stress Resistant Rice Varieties by using SSR Markers.

Materials and Methods

Collection of plant material

A set of 11 rice genotypes (Table 1)were raised during *kharif* -2019 at Regional Agricultural Research Station, Warangal, PJTSAU, Telangana by following standard Agronomic Practices. Leaf samples were collected from all the genotypes at 30 Days after sowing (DAS) for DNA isolation.

Genomic DNA from leaf samples was isolated by following the standard protocol by [11], with few modifications. Final concentration of 30 ng/µl of genomic DNA was used for PCR (Eppendorf) amplification. PCR was performed using 1 U of Taq DNA polymerase (Fermentas, Lithuania) and 1x PCR buffer (Genei, India) in 10-µl reaction volume with a thermal profile of 94 ºC for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, extension at 72 ºC for 1 min and a final extension of 7 min at 72 ºC. The amplified products were electrophoretically resolved on 4% Seakem LE® Agarose (Lonza, USA), containing 0.5 mg/ml of ethidium bromide in 0.5X TBE buffer and visualized under UV.

Data analysis

Allele number was given and scored according to its presence or absence, based on difference in molecular weight. Only the clear and unambiguous bands were scored. 22 polymorphic markers (Table 2) were scored for the presence (1) and absence (0) of the corresponding band among the genotypes. Consequently, a data matrix comprising '1' and '0' was formed and subjected to further analysis. Further processing of data was done by carrying out sequential agglomerative hierarchical non-overlapping clustering (SAHN), on squared Euclidean distance matrix. Similarity matrix was done using Jaccard's coefficient, in which similarity matrices were utilized to construct the UPGMA (Unweighted Pair Group Method with Arithmetic average) dendrogram. Data analysis was done using NTSYs PC [12].

Results and discussion

DNA-based markers are effective and reliable tools for measuring genetic diversity in crop germplasm and studying evolutionary relationship. Molecular genetic techniques using DNA polymorphism is increasingly used to characterize and identify a novel germplasm for uses in the crop breeding process [12] and [13] Parental selection based on the genetic diversity is highly essential to develop a good variety [10].

Table No.1: Details of the rice genotypes used for diversity analysis.

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S. No.	Name of the rice genotype	Characteristic features
1	MIL – 12	An interrogation line of MTU-1010 with 120 days of duration and possessing two major blast resistant genes i.e. *Pi2* and *Pi54* in Homozygous condition.
2	BM – 71	BPH resistant rice variety. MTU IJ 206-7-4-1 (BM-71) has been identified as new resistant donor having strong field resistance to plant hoppers.
3	WGL – 44 (Siddhi)	A high yielding, gall midge and salinity tolerant, Medium Slender MS grain type rice variety with 140 –145 days of duration, it is derived from the cross between BPT5204/IRC5984///KAVYA///KAVYA/BPT5204 released in 2012.
4	WGL – 962	A high yielding, Medium Slender (MS) grain type rice variety with 130 –135 days of duration. It is derived from the cross between BPT 5204/GEB-24//BPT 5204/Shathabdi in 2021.
5	KNM – 118	A high yielding, Long Slender (LS) grain type rice variety with 120 days of duration.It is derived from the cross between JGL 13595 (MTU 4870 / JGL 418) x MTU 1010 released in 2016.
6	NLR – 145	A high yielding blast resistant Long Slender (LS) grain type rice variety with 140 –145 days of duration, released in the year 2013 and it is derived from the cross betweenCICA-4 x IR-625-23-3-1 x Tetep, released in 1993.
7	WGL – 14	A high yielding, Medium Slender (MS) grain type rice variety with 135-140 days of duration, released in the year 2005 and it is derived from the cross between B.P.T5204 and IRC5984/B.P.T.3291 released in 2005.
8	Swarna	A high yielding, Medium Slender (MS) grain type rice variety with150 – 155days of duration, released in the year 2015 and it is derived from the cross between Parentage: Vasista/Mahsuri released 1982.
9.	MTU-1121	A high yielding, Medium Slender (MS) grain type rice variety with 125-130 days of duration, released in the year 2009, and it is derived from cross between BPT 5204/MTU BB 8- 24-1(2015)
10.	WGL-32100	A high yielding, salinity tolerant Medium Slender (MS) grain type rice variety with 135 days of duration, it is derived from cross between Divya/ BPT5204 and suitable for *Kharif* 2006.
11	Bhadrakali	A high yielding gall midge resistant Long Slender (LS) grain type rice variety with 135 days of duration , released in the year 1994 and it is derived from the cross between Palguna and IR36 (1999)

DNA isolation and PCR analysis

Hence, the present investigation envisaged the degree of genetic diversity based on marker data in eleven genotypes of rice (Figure 1A and 1B). Genetic diversity/relatedness among the genotypes was assessed on the basis of Polymorphic information content (PIC) value. Out of 26 HvSSR markers (Table2), four markers (namely HvSSR02-33, HvSSR03-02, HvSSR09-26, HvSSR12-39) were not amplified in all the eleven rice genotypes, while the remaining twenty two markers namely HvSSR01-41, HvSSR01-53,HvSSR02-01, HvSSR02-50, HvSSR03-19, HvSSR03-37, HvSSR04-19, HvSSR04-27, HvSSR05-09, HvSSR05-30, HvSSR06-03, HvSSR06-16, HvSSR06-40, HvSSR07-51, HvSSR08-19, HvSSR09-55, HvSSR10-13, HvSSR10-34, HvSSR11-13, HvSSR11-21, HvSSR11-58, HvSSR1 2-01 were amplified in all the eleven rice genotypes. Polymorphic information content (PIC) value of SSR markers was ranged from 0.89 (HvSSR2-50) to 0.583 (HvSSR11-21) with an average of 0.73. Compared to earlier reports, [15] the PIC values for 26 SSR markers varied from 0.50 (RM6737) to 0.89 (RM3412), with an average PIC of 0.70, we reported higher Polymorphic information content (PIC) value of SSR markers and which was ranged from 0.89 (HvSSR2-50) to 0.583 (HvSSR11-21) with an average of 0.73.

A total of 54 alleles were detected with 22 markers with an average of 2 alleles per marker pair. The number of alleles ranged from 3 (HVSSR01-41 (Figure 1A), HVSSR01-53, HVSSR02-50, HVSSR03-02, HVSSR04-19, HVSSR05-09, HVSSR05-30 (Figure 1B), HVSSR06-03, HVSSR06-16, HVSSR06-40, HVSSR08-19, HVSSR09-55, HVSSR10-13,

HVSSR11-13, HVSSR11-58) to 2 (HVSSR02-33, HVSSR3-37 HVSSR04-27, HVSSR11-21, HVSSR12-01).

Table 2. Details of Markers used for present Study.

S.No	Name of the Marker	Forward Primer Sequence	Reverse Primer Sequence
1	HVSSR 1-41	TGAGTGAGACTTGACAGTGC	AGTTAACACCAATGCTGACC
2	HVSSR 1-53	TGTCGTCCACGTAGTAGGAG	ACACTCCTCCTCTGTTCTCA
3	HVSSR 2-01	AAGAGATGAGAAGAGCAATGA	CAACTTAGAGGAAGAAGGAGG
4	HVSSR2-33	TAATGCACGCACAACTTTAC	TATAGAATGCTGACTGGGCT
5	HVSSR 2-50	TTTCAGGAATCTGATGCTTT	TTAATCAAAGCCCTAACAGC
6	HVSSR 3-02	TAGCGGAGTTGGAATAACAC	CTGCACTGCATACCTCATAA
7	HVSSR 3-37	GGAAATCGTCAAGAACGTC	TAATTGTATACCACTCCGCC
8	HVSSR 4-19	TCGTGGAGTATCCTGTATCC	TTATAACTTGGAGCTCAGGC
9	HVSSR 4-27	ATGGATTTAGGCTTGTTTGA	ATACTGCGAAGGTGAAGAGA
10	HVSSR 5-09	CTCTCCATCTTGCAATCTTC	TGCATGACTCTATCAACCAG
11	HVSSR 5-30	TACGACGGACGATTAAAGTT	GCTAACTCATTCATCTCGCT
12	HVSSR 6-03	CTAGGGAATCAGCGGTTAG	GCTCTCTTGTCCTTCTTCTTC
13	HVSSR 6-16	TCTGAAATGCTGTCATCAAG	GAGCAGAGTAGGACATGAGC
14	HVSSR 6-40	CTCTTCCGTGGTTAAAGAAA	CACTGGTATGATCTCCGACT
15	HvSSR07-18	GGTGTGTTGTCGAATCTCTC	ATGCCATTGTCCTTACATTC
16	HvSSR07-51	CGAGCATGTCTGTCAAGTAA	GTTCGAATGTAATGTTGGCT
17	HvSSR08-14	TCCACTTTACATCGTCACAA	CTACCTCTTAACCGCACATT
18	HVSSR 8-19	CATCTCTTGAGAAATCTGCC	TGTGCATTTCGTCTTTCATA
19	HvSSR09-26	TGGGCATCTGGTACTATCTT	AGCTCATTCCACAGGTTAGA
20	HVSSR 9-55	TTACTCCGCATATATCCATGT	ATTTGACACCAAGTTGATCC
21	HVSSR 10-13	CAGGGAATCAACATCAAAGT	AGCAAGGCAAGTCATCTCTA
22	HVSSR 11-13	TGAAACCACAATGAGTCAAA	GCCCTAAACCCAAATAGAAG
23	HVSSR 11-21	TACGCTATAACCATGAAGCA	CTCCCGTTATTGTCCTTACA
24	HVSSR 11-58	ACTGAATCCTTACTGGAGCA	GGAGATAAGCATTTGGAAGA
25	HVSSR 12-01	GATTTGCAACACGTACGATA	GATCATCCACTCTGAGCAAT
26	HVSSR 12-39	ATCTAACAACAACAATCCCG	CATCTTCATCCCTCGTGTAT

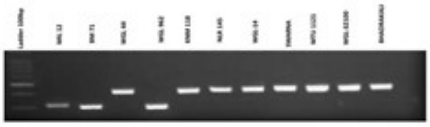


Figure 1A: PCR amplification pattern of 11 rice genotypes using microsatellite marker HvSSR 1-41. The lanes on the top of gel indicates list of rice genotypes and L= 100 bp ladder.

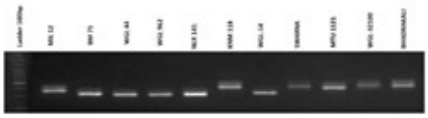
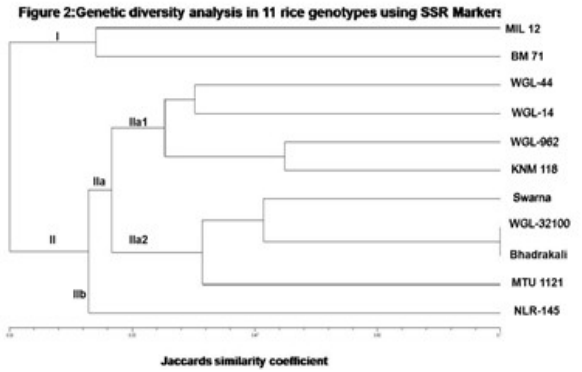


Figure 1B: PCR amplification pattern of 11 rice genotypes using microsatellite marker HvSSR 5-30. The lanes on the top of gel indicates list of rice genotypes and L= 100 bp ladder.

Cluster analysis: The polymorphic bands were scored visually as present (1) or absent (0) on a binary matrix. Genetic similarity between the varieties was estimated using Jaccards Coefficient of similarity index. Dendrogram was performed using the Unweighted Pair Group Method with an Arithmetic mean (UPGMA) algorithm and the NTSYS software (Figure 2). The UPGMA clustering placed BM-71 and MIL-12 in Cluster-I, while remaining 9 genotypes (i.e.WGL-44,WGL-14,WGL-962, KNM-118, Swarna, WGL-32100, Bhadrakali, MTU-1121 and NLR-145) in Cluster-II.

Cluster-II was further divided into two sub clusters i.e. IIa and IIb at 28 % level of similarity. Cluster IIb included only one rice genotype i.e. NL-R145, while Cluster IIa included eight rice genotypes i.e. WGL-44,WGL-14,WGL-962,KNM-118, Swarna, WGL-32100, Bhadrakali and MTU-1121 and which in turned showed 30 % level of similarity. Cluster Iia was further sub divided into Cluster IIa1 and Cluster IIa2 at 31 % level of similarity.Cluster IIaI consists of WGL-44, WGL-14, WGL-962, KNM-118. In Cluster IIa1 WGL-44 and WGL-14 were showed 42 % level of similarity and which interned showed 38 % level of similarity with WGL-962 and KNM- -118.



Cluster IIa2 consist of four rice genotypes i.e.Swarna, WGL-32100, Bhadrakali and MTU-1121 showing similarity 74 % level of similarity. In Cluster I, BM-71 showed 27.4 % level of similarity with MIL-12.

In this dendrogram, two biotic stress resistant rice genotypes namely BM-71 (BPH) and MIL-12 (Blast) formed in cluster I, while other biotic resistant rice genotypes i.e. NLR 145 (Blast) and one abiotic resistant (P use efficiency) rice genotype i.e. Swarna formed in cluster II. In this Dendrogram two blast resistant rice varieties i.e. NLR-145 and MIL-12 were showed 30.2 % level of similarity. Blast resistant donor parent MIL-12 and BPH resistant donor parent BM-71 were showed 41.4 % level of similarity with WGL-44 and 50 % level of similarity with susceptible genotypes WGL-962 and KNM-118, while another blast resistant donor parent NLR-145 showed 27.5 % level of similarity with susceptible genotypes WGL-14. PUP1 donor parent Swarna showed 40 % level of similarity with one susceptible genotype MTU-1121 and 47.8% level of similarity with other two susceptible genotypes WGL-32100 and Bhadrakali. According to our knowledge, this is the first report, where hyper variable SSR markers were used for Genetic diversity analysis among biotic and abiotic stress resistant local rice genotypes. Earlier, simple sequence repeat (SSR) markers were used for assessment of the genetic variation in rice by [16], [9], [17] and [18].

Conclusion

The present investigation, successfully assessed the levels of inter and intraspecific diversity relationships among 11 biotic and abiotic resistant rice genotypes. The UPGMA clustering placed biotic resistant rice genotype BM-71 (BPH) and MIL-12 (Blast) in cluster I, while other biotic resistant rice genotype NLR-145 (Blast) and one abiotic resistant (P use efficiency) rice genotype Swarna in cluster II. In addition to above two rice genotypes, Cluster II also consists of seven susceptible genotypes namely WGL-44, WGL-14, WGL-962, KNM-118, WGL-32100, Bhadrakali and MTU-1121 with

varied level of percent of similarity between the genotypes. Hybridization among these diverse parents may helpful to obtain better genotypes with biotic and abiotic stress resistance. Results obtained from the present investigation would be highly useful in rice breeding programs and may be used for further crop improvement using advance marker systems.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical standard: The authors declare that the studies are done in compliance with ethical standards.

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