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Molecular profiling of rice for biotic and abiotic stresses

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Abstract
In this study 95 germplasm of rice were evaluated to identify rice cultivars for blast resistance, salinity stress, brown plant hopper resistance, drought resistance and gall midge. DNA was isolated from leaf samples of 95 rice germplasmsby following the protocol of Edwards *et al.*, 1991. Simple sequence repeats (SSR) markers were used for determination of germplasm having characters for biotic and abiotic resistance. Among 17 primers some primers were found which are tightly linked with biotic and abiotic characters. The output of the this experiment is that primer RM8225 linked with blast resistance, RM140 and RM1287 were linked with salinity stress, RM6775 and RM309 linked with brown plant hopper resistance, RM212 and RM201 linked with drought stress and RM22709 linked with gall midge. The output of the study helpful for the breeders to select parents in the breeding programme.

Keywords: Simple sequence repeats, rice germplasm, biotic and abiotic resistance

Introduction
Rice (*Oryza sativa* L.) (2n = 24) is the most important cereal crop which serves as a staple food for over 60 per cent of the world's population (Singh and Singh, 2008) [16]. Global rice demand is estimated to rise from 676 million tons in 2010 to 763 million tons by 2020 and further increase to 852 million tonnes by 2035 (FAOSTAT, 2021) [5]. India is the world's second largest rice producer and consumer next to China, about 90 per cent of all rice grown in the world is produced and consumed in Asian region. In the world, rice is cultivated on about 167.13 million hectares of area with total production of 782.01 million tones and productivity is 3.228 tonnes/ha (Anonymous, 2021) [1]. It is grown in *kharif* and *Rabi* season. The productivity of rice is 2.59 tonnes per hectare and 2.73 tonnes per hectare in *kharif* and *Rabi* season respectively.

Rice has the largest germplasm collections in the world. This accessible collection of diverse cultivated varieties, landraces and related wild species has made great contributions to rice breeding. Landraces harbour a great deal of useful traits with genetic potential for rice improvement and they played a very important role in the local food security and sustainable development of agriculture, in addition to their significance as genetic resource for rice genetic improvement (Tang *et al*., 2021) [19].

Evaluation and characterization of existing landraces of rice is important due to increasing needs of varietal improvement. Land-races are also important genetic resources for resistance to pests and diseases; they provide "adaptability genes" for specific environmental conditions. Incorporation of adaptability genes from landraces could ensure optimum grain yield for the region (Vijayakumar *et al*., 2020) [18].

Microsatellite or Simple sequence repeats (SSR) markers are short tandem repeated motifs that may vary in the number of repeats at a given locus. SSR markers have many advantages over other molecular markers, such as genetic co-dominance. Simple sequence repeats (SSR) markers are better molecular markers to identify genes linked with the biotic and abiotic characters.

Methods and Materials
DNA isolation
The buffers and reagents for DNA extraction were prepared as per Sambrook Maniatis (1989) [15]. Extraction buffer (200 mM Tris-HCl, 25 mM EDTA, 250mM NaCl, 0.5M Glucose, 0.5% SDS, 3% PVP, 0.4% Sodium Bisulphite, 5% Sarcosyl), was used during DNA isolation.

The DNA from 95 rice germplasm (Table No. 1) were isolated by following the protocol of Edwards *et al*., 1991 [4]. Leaf sample 100mg was crushed in mortar and pestle and 1 mL extraction buffer was added and incubated at 65 °C for 45 min. Centrifuged at 10000 rpm for 10 min, supernatant was transfer in another tube and chloroform: isopropanol (24:1) added. Mixed for 5 sec and centrifuged at 10000 rpm for 10 min. Supernatant was taken and Isopropanol was added (1/6th proportion) and store at -20 °C for overnight. Next day centrifugation was done at 10000 rpm for 10 min. and pellet was washed with 70% ethanol. Centrifuged at 10000 rpm for 3 min, and air dried the ethanol, pellet was dissolved in 50 µl of TE buffer.

PCR amplification

Thermalcycler: Eppendorf, Mastercycler gradient supplied by Eppendorf gradient, 2231, Hamburg Germany was used for cyclic amplification of DNA. Taq. Assay buffer, $MgCl_2$, dNTPs, Primers, Taq. DNA polymerase (M/s Bangalore Genei Pvt. Ltd., Bangalore) were used for reaction mixture. Thermal profile was initial denaturation (94 °C for 4 min.), denaturation (94 °C for 30 sec.), annealing (55-60 °C for 30 sec.) extension (72 °C for 01 min.) final extension (72 °C for 07 min.).

Result and Discussion

The standard procedure of DNA isolation along with slight modification of buffer components and their quality were employed (data not shown). The good quality and desired quantity of DNA was obtained. Simple Sequence Repeats (SSR), and they are typically composed of 1–6 nucleotide repeats. 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 Gous *et al*. (2013) [7], Bhargavi *et al*. (2022) [2] and Kushwaha *et al*. (2022) [20].

Blast resistance

Primer RM8225 used to identify blast resistance germplasm namely Try-1, Kochari, LalPatani, Karhad, Ratnagiri 7, PhuleSamruddhi, Ambemohar, RathuHeenati, SinnaSivappu, IRRI 190, IRRI 193, ARC10550, Samba Mahsuri, IRRI 123. (Gentallan *et al*., 2021) [6] and Chakrobarthi *et al*., 2006) [3] also used microsatellite markers to screen the various genotypes for blast resistance.

Salt resistance

Primer RM140 used to identify salt resistance germplasm namely MO-17, MO-19, KJT-2R, RTN 11-2-1-3, Pak Basmati, Super Basmati, Chinoor, Pusa Sugandha-1, Pusa Sugandha-3, Pusa Sugandha-4, Bhogwati, RP Bio 197, RP-BIO-226, Ajaya, Karjat 1, VDN-9-10-1, HRTMS-61. Molecular mapping of genes linked to salt tolerance traits was also carried out by (Krishnendu *et al*., 2021) [9] and (Liu *et al*., 2022) [12].

Brown plant hopper

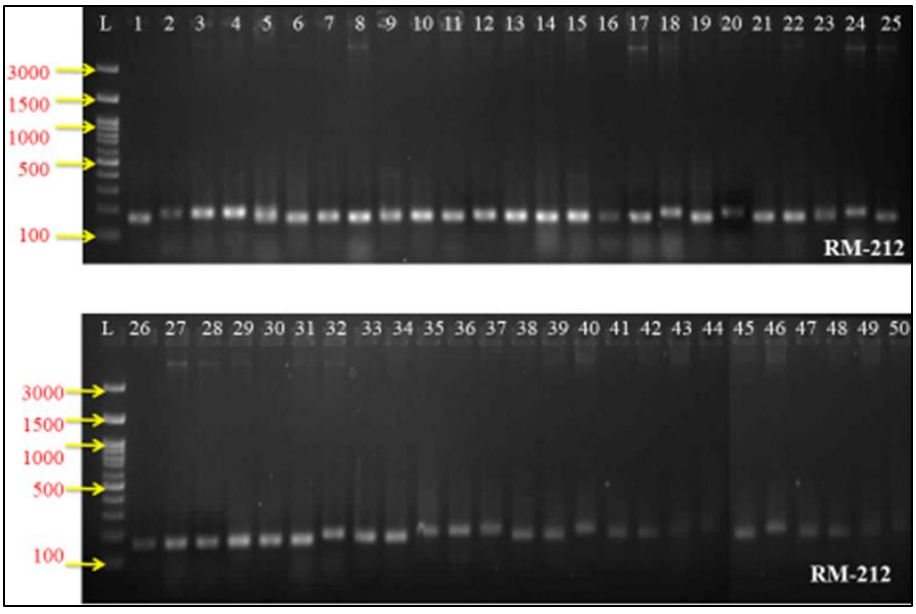
Primer RM6775 and RM309 used to identify brown plant hopper resistance germplasm namely Pusa Sugandha-4, Pusa Sugandha-5, Bhadas bhog, CR2713-180, Kalanamak, NDR6315, Phule Samruddhi, Khara Rata, Try-1, Kochari, BG 367-2, FL 478, MUT NS 1, Rathu Heenati, Sinna Sivappu. Similar results also found by (Li-Hang *et al*., 2020) and (McCouch *et al*., 2018) [14] where they used different microsatellite markers to screen the rice populations for BPH resistance.

Drought resistance

Primer RM212 and RM201 used to identify drought resistance germplasm namely BG 367-2, Milyang 63, FL 478, MUT NS 1, IRRI 192, Dhanesal, Ambemohar, Bhogwati, Sugandha (Figure 1). Similarly (Gregorio *et al*., 2022) [8] and (Mateu-Andres *et al*., 2022) [13] also used different microsatellite markers to screen the rice populations for drought tolerance.

Gall midge resistance

Primer RM22709 used to identify drought resistance germplasm namely MO-6, MO-8, Munga, Patni-6, Pandy, NAUR-1, DRR-50-12, DRR-363-5, DRR-50-10, IR-63879-195-2-2-3-2, RTN-214-1-1-1-2, Chinoor, RP Bio 197, RP-BIO-226, Ajaya, Karjat 2, IR-64.



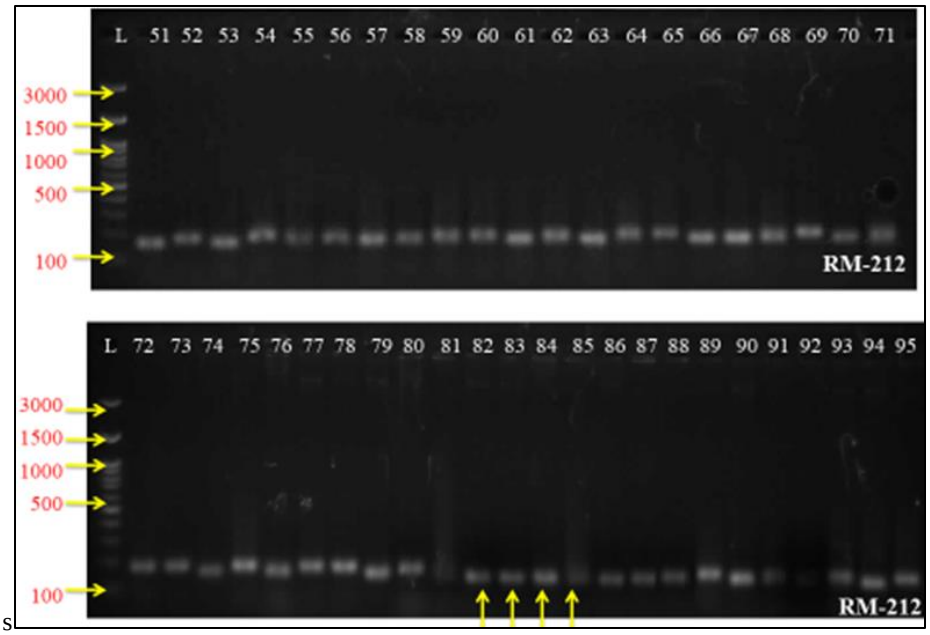


Plate 1: DNA bands amplified from 96 rice genotypes using microsatellite RM-212 marker linked to Drought and electrophoresed in a 1.8% agarose gel. L= 100bp ladder. (Arrow indicates presence of the resistance linked allele)

Table 1: List of rice germplasm

SN	Genotype	Source	SN	Genotype	Source
I	**Red kernel local lines**		**II**	**Restorer lines**	
1	MO-6	ARS, Moncomba	25	KJT-1R	ARS, Karjat
2	MO-8		26	KJT-2R	
3	MO-13		27	KJT-3R	
4	MO-17		28	KJT-4R	
5	MO-19		29	PR-114	IIRR, Hyderabad
6	Khara Rata	KRS,Panvel	30	RTN 11-2-1-3	ARS, Shirgaon
7	Mayekar Bhat	ARS, Karjat	31	PR-118	IIRR, Hyderabad
8	Munga	ARS, Shirgaon	32	DRR-215	
9	Mahsad		33	RTN-69-1-1	ARS, Shirgaon
10	Waksal-207		34	NAUR-1	NAU, Navsari
11	Barmil		35	BL-184AR	ARS, Karjat
12	Jyoti		36	Gurjari	NAU, Navsari
13	Patani-6		37	DRR-50-12	IIRR, Hyderabad
14	Bhadas-79		38	DRR-363-5	
15	Pandy		39	DRR-50-13	
16	Dular		40	DRR-50-10	
17	Try-1		41	DRR-86-8	
18	Kochari		42	IR-63879-195-2-2-3-2	IRRI, Manila,
19	Lal Patani		43	RTN-27-1-1-2	ARS, Shirgaon
20	Dodak		44	RTN-214-1-1-1-2	
21	Karhad		45	VDN-9-10-1	ARS, Vadgaon
22	Ratnagiri 7		46	VDN-10-18	
23	Bela		47	RTN-35-1-1	ARS, Shirgaon
24	Valai		48	Sahyadri 5 R	
			49	HRTMS-61	
			50	CR-3993-2-24-45-2	IIRR, Hyderabad
III	**Aromatic lines**		**IV**	**Lines responsive to biotic and abiotic stresses**	
51	P Basmati	IARI, N. Delhi	70	RS-1113	IIRR, Hyderabad
52	S Basmati		71	RP Bio 197	
53	Kothambir Sal	ARS, Karjat	72	RP-BIO-226	
54	Chinoor	PDKV, Akola	73	Ajaya	
55	P Sugandha-1	IARI, N. Delhi	74	KJT 1	ARS, Karjat
56	P Sugandha-3		75	KJT 2	
57	P Sugandha-4		76	IR-64	IRRI, Manila
58	P Sugandha-5		77	MUDGO	
59	Bhadasbhog	IIRR, Hyderabad	78	MILYANG 46	
60	CR2713-180		79	PTB 33	RARS, Pattambi
61	Kalanamak		80	MTU1010	RARS, Maruteru

62	NDR6315		81	BG 367-2	IRRI, Manila
63	P Samruddhi	MPKV, Rahuri	82	MILYANG 63	
64	Indrayani	ARS, Vadgaon	83	FL 478	
65	Dhanesal	IIRR, Hyderabad	84	MUT NS 1	
66	Ambemohar	ARS, Vadgaon	85	RATHU HEENATI	
67	K Shatabdi	ARS, Karjat	86	SINNA SIVAPPU	
68	Bhogwati	ARS, Radhanagari	87	IRRI 190	
69	Sugandha	VNMKV, Parbhani	88	IRRI 193	
			89	ARC 10550	
			90	S MAHSURI	IIRR, Hyderabad
			91	IRRI 123	IRRI, Manila
			92	IRRI 104	
			93	IRRI 192	
			94	Sonsali	IIRR, Hyderabad
			95	TN-1	

Conclusion

The screening of rice genotype through microsatellites is very important for identifying parental genotype 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.

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