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MOLECULAR SCREENING OF BLAST RESISTANCE RICE LINES FROM INDIGENOUS AND EXOTIC GERMPLASM

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ABSTRACT

Rice is one of the most important crop that helps to alleviate hunger of billions of people and is a staple food in many countries of the World. Rice crop is vulnerable to large number of pathogens responsible for different diseases in rice and among them blast disease is the most threatening disease as it results in enormous yield loss. Blast disease is caused by a fungus named as *Magnaporthe oryzae* (anamorphh: *Pyriculria oryzae*). It affects rice seeds, rice leaves, rice panicles and rice fertility. A positive significant correlation was observed for seed thickness with seed width (r= 0.2818**), length width ratio with seed length (r= 0.8017**) and 1000 grain weight with seed width (r= 0.2766**). Molecular screening of blast resistant gene is done by using 12 SSR markers (simple sequence repeats) and 10 rice lines are selected on the basis of their morphological traits from the field of Faculty of Agricultural Sciences, University of the Punjab, Lahore. SSR markers depicted variations in their results like some SSR markers showed monomorphic results while other showed polymorphic results. The average PIC values (0.54), polymorphism% (87.5), total number of alleles (4.08) and polymorphic alleles (3.58) were determined among indigenous and exotic rice germplasm. Molecular screening is done to detect the resistant gene in selected rice varieties (NSGC 5929, Hansraj 13, 56-5-6) of diverse origin for better production of rice. The main aim of the study is to screen out resistant rice lines on the basis of phenotypic and genotypic traits.

Keywords: Oryza sativa, DNA, SSR, Pathogen, Germplasm, Traits

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the oldest domesticated grains. It is the staple food for more than 3 billion people around the world (Seck *et al*., 2012). It covers 9% of the earth's arable land. It provides almost 15% per capita of protein and 21% of global human energy to each individual (FAO, 2018). Asia accounts for more than 90% of the world's production of rice, along with India, Indonesia and China. Rice basically belongs to genus named *Oryza*. Rice crop has high level of polymorphism, Moreover, rice crop has a small genome size, diploid genetics and useful model experimental plant for the study of other cereal crops (Kurata *et al.,* 1994; Tanksley, 1989; Wang *et al.,* 1992).

There are several diseases of rice and among them blast disease is the most threatening disease.Blast disease cause great economic loss to the rice crop. Blast disease is caused by a fungus named as *Magnaporthe oryzae* (anamorphh: *Pyriculria oryzae*).It belongs to the family *Magnaporthacea*. Class of this fungus is ascomycete as it has the ability to produce sexual spores called as ascospores in a specialized structure called asci. Ascus is produced within a particular structures called as perithecia (Ballini *et al.,* 2008). It infects rice seeds, panicles, collars, neck, leaf and also reduce the leaf area .The most important and diagnostic symptoms are diamond shaped lesions formed on leaves but these symptoms also appear on leaf sheath (Arshad *et al.,* 2008).When conditions like warm temperature and high moisture prevails, infection and disease occurrence is more severe. Rice blast is most likely to occur at moderate temperature. In a case of high moisture which if retain for about 12 hours, it favors the disease to occur and these conditions mostly prevail in flooded rice fields (Arshad *et al.,* 2008).Rice blast disease is designated as a model for study of epidemiology, genetics and molecular pathology (Ashikawa *et al.,* 2008). The entire genome of *M.oryzae* has already been sequenced and published (Valent, 1990; Islam *et al*., 2016). Conventional techniques are not so

cooperative in mass selection of large number of crop species and thus, pathogens evolve to compete with host and changing environmental conditions. Resistance does not remain stable in the host for evolving pathogen; therefore, conventional method is not appreciated by researchers due its numerous drawbacks. So, molecular screening is more readily accepted by the breeders and they give preference to molecular techniques over conventional techniques (Imam *et al.,* 2014). Molecular screening of rice germplasm is done to determine the resistant gene against blast with help of molecular markers such as Simple sequence repeats (SSRs). The purpose of screening is to develop such lines that must possess high commercial value and to obtain high yielding lines for advancement in breeding program. In the process of screening, genetic variability of different germplasm is determined and morphological traits of seeds are studied by using SSR markers (Kim *at el.,* 2010). Molecular screening is the best method and very useful tool for screening and selection of plant material with desirable traits for better yield. Screening is the most important practice to develop commercial hybrids. Simple sequence repeats (SSRs) which are more readily used also referred as microsatellites are 2-5 base set sequence of DNA play an important role in different genetic studies and breeding program (Miah *et al.,* 2013). These markers are of different types that is mononucleotide, di-nucleotide, tri-nucleotide, penta-nucleotide and also hexa-nucleotide. SSR markers are very useful for molecular screening of rice as they possess best characteristics. They are co-dominant, have more reproducibility and assist efficiently in mapping studies of different economically important crop (Seetharam *et al.,* 2009). They have also been extensively used to determine the genetic diversity in other crops like maize, sorghum, cotton and wheat (Edward *et al.,* 2017). They have many desirable features like it contain more information content, greater degree of polymorphism for detection of particular gene, explicit position of alleles and rapid assay of genotyping (Panday *et al*., 2009). SSR markers have wide range of application in different molecular techniques like identification of genotype, protection of variety, evaluation of seeds, check purity of seeds, conservation of germplasm, determination of paternity and identification of QTLs etc. (Nadeem *et al.,* 2018). The objective of the study was to screening of resistant rice lines on the basis of genotypic and phenotypic traits.

MATERIALS AND METHODS

The experiment was performed at the institute of Agricultural sciences, University of the Punjab, Lahore for molecular screening of blast resistance gene and to determine the genetic diversity in Pakistani rice germplasm.

Table 1. Plant material used in the experiment.

Sr. No.	Rice Varieties	Origin	Accession No.	Taxon	Disease Reaction
1.	NSGC 5929	United States, Arkansas	GSOR 311686	*Oryza alta*	Resistant
2.	Basmati-443	Pakistan	0059	*Oryza sativa*	Susceptible
3.	Hansraj 13	Pakistan	0062	*Oryza sativa*	Tolerant
4.	Mushkhan 340 A	Pakistan	0068	*Oryza sativa*	Susceptible
5.	N335	Pakistan	0094	*Oryza sativa*	Susceptible
6.	Super Basmati	Pakistan	Approved variety	*Oryza sativa*	Susceptible
7.	Basmati-370	Pakistan	Approved variety	*Oryza sativa*	Susceptible
8.	Nickerie 19	Suriname	GSOR 310195	*Oryza sativa*	Susceptible
9.	140-4-1-2-5	Suriname	GSOR 310197	*Oryza sativa*	Susceptible
10.	56-5-6	El Salvador	GSOR 310266	*Oryza sativa*	Tolerant

Table 2. Mean values of different morphological traits of rice varieties.

S. No	Variety Name	Mean values of various seed morphological traits				
		Seed length	Seed width	Seed thickness	Seed length/width ratio	1000 grain weight (g)
1.	NSGC 5929	6.5	2.38	2.17	2.73	21
2.	Basmati-443	8.9	2.12	1.90	4.19	22.5
3.	Hansraj 13	7.8	2.30	1.96	3.39	23.5
4.	Mushkhan 340 A	8.2	2.25	1.94	3.64	24
5.	N335	7.9	2.35	2.10	3.36	24.5
6.	Super Basmati	8.9	2.28	1.98	3.90	25
7.	Basmati-370	7.7	2.40	2.16	3.20	23.5
8.	Nickerie 19	7.2	2.45	2.20	2.93	25.5
9.	140-4-1-2-5	7.3	2.47	2.21	2.95	25
10.	56-5-6	7.1	2.48	2.24	2.86	26

Morphological traits of rice varieties

Different morphological characters i.e. seed length, seed width, seed thickness, seed length width ratio and 1000 grain weight of different rice varieties were measured at the time of the maturity of the crop. The experiment was conducted in RCBD design with three replications. The plant material used in the experiment was shown in the Table 1. Different seed morphological traits were shown in the Table 2. Data of each genotype was taken with the help of Vernier Caliper and weighing balance.

Collection of Sample

Sample of leaves were collected in the morning from the backfield of Institute of Agricultural Sciences. Fresh leaves were taken from the field with the help of scissor which were not affected with the disease and leaves were placed in the plastic bag and then for the purpose of identification of samples, tagging was done with name of sample, date, time and place.

Preservation of Sample

Samples were preserved in a cool place to keep the samples fresh for further process of DNA extraction and PCR analysis.

DNA extraction

CTAB method (Muray and Thompson, 1980) was used DNA extraction from fresh leaves of each variety. The extracted DNA samples (with concentration of 40 ng/ul) were diluted using ddH_2O for further PCR analysis. Nano Drop (ND 1000 Spectrophotometer) was used to determine purity, quality and quantity of extracted DNA at 260 and 280 nm.

Polymerase chain reaction (PCR) for amplification of DNA using SSR primers

The basic principal of PCR is to make multiple copies of DNA so we used PCR machine for amplification of DNA with specific required sequence and then multiple copies of DNA was used to study the genetic diversity and then determined resistance gene location with the help of further procedure.

Agrose- gel electrophoresis

After amplification of DNA, the resulted product was analyzed with the help of gel electrophoresis to determine the DNA band presence and position.

Statistical Analysis

Twelve Primers of different polymorphism extent were used for further PCR analysis to determine the band size of each genotype for further screening and selection purpose. PCR techniques were used for genotyping the rice material (Panuad *et al*., 1996) by using different SSRs primers in-vitro conditions for genetic diversity determination of the among the genotypes/germplasm. The analysis of variance and

correlation was carried out through SAS version 9.2 (SAS, 2008). For determination of molecular characteristics the power marker (software package) (Liu and Muse, 2005) was used.

RESULTS

Significance level of seeds of different rice varieties

Significant value i.e. $P<0.01$ and 0.05 depicted the analysis of variance which affect the morphological traits. Almost all the traits showed significant variation among all the genotypes (Table 3). Plant material was collected from different renowned national and international Institutes as given in Table 1. Different rice lines were chosen on the basis of their morphological traits (Table 2). On the basis of SSR markers and different morphological characteristics of seeds, rice varieties were selected to determine resistance gene and genetic diversity (Table 5).

Analysis of variance of different seed morphological traits of various rice genotypes and their mean square values

Analysis of Pearson co-relation manifests significant positive, significant negative and non-significant co-relation. Almost all the traits depicted positive association with other traits and act as an important tool in selecting high yielding, highly resistive and diverse varieties of genotypes. The main purpose of my research work was to select those varieties which show more resistance and to evaluate different morphological traits of rice seeds like seed length, seed width, seed thickness, length/width ratio and 1000 gram seed weight. It proved very helpful in screening and selection of such genotypes of rice that are associated with desirable traits

Seed length shows non-significant negative co-relation with seed width, non-significant co-relation with seed thickness, highly significant co-relation with length/width ratio and non-significant co-relation with 1000 grain weight. Seed width shows non-significant co-relation with seed thickness, highly significant negative co-relation with length/width ratio and highly significant co-relation with 1000 grain weight. Seed thickness shows highly significant negative co-relation with length/width ratio and highly significant co-relation with 1000 grain weight. Length/width ratio shows non-significant negative co-relation with 1000 grain weight (Table 4).

Table 3. Analysis of variance of different seed morphological traits of different rice varieties.

Source of variation	D.F	SL	SW	ST	L/W	1000GW
Genotypes	14	1.43924NS	0.19465 **	0.01364 NS	0.85857**	38.4082**
Replications	2	1.82503NS	0.03747**	0.00207 NS	0.10334**	5.7649**
Error	28	1.01953NS	0.01001**	0.00712NS	0.21321**	4.0189**

Level of significance $P<0.05$=* and $P<0.01$=** SL= seed length, SW = seed width, ST = seed thickness, L/W= length/ width ratio and 1000 grain weight

Correlation among different seed morphological traits of rice

Table 4. Correlation of different seed morphological traits of rice genotypes.

Traits	SL	SW	ST	L/W	1000GW
SL	1.00				
SW	-0.0738NS	1.00			
ST	0.0145NS	0.2818**	1.00		
L/W	0.8017**	-0.6424**	-0.1567**	1.00	
1000GW	0.0623NS	0.2766**	0.2886**	-0.0845NS	1.00

Level of significance $P<0.05$=* and $P<0.01$=** SL= seed length, SW = seed width, ST = seed thickness, L/W= length/ width ratio and 1000 grain weight

Super basmati and basmati 443 had maximum seed length of 8.9cm and NSGC 5929 had minimum seed length of 6.5cm. while, 56-5-6 had maximum seed width of 2.48 cm and Basmati-443 had minimum seed width of 2.12 cm. The variety of 56-5-6 had maximum seed thickness of 2.24 cm and Basmati 443 had minimum seed thickness of 1.90 cm. Basmati-443 has maximum length/width ratio of 4.19cm and NSGC 5929 had minimum l/w ratio of 2.73 cm. While 56-5-6 had maximum 1000 gram seed weight of 26gm and 56-5-6 had minimum 1000 gram seed weight of 21gm (Table 2).

PCR amplification and gel electrophoresis of genomic DNA

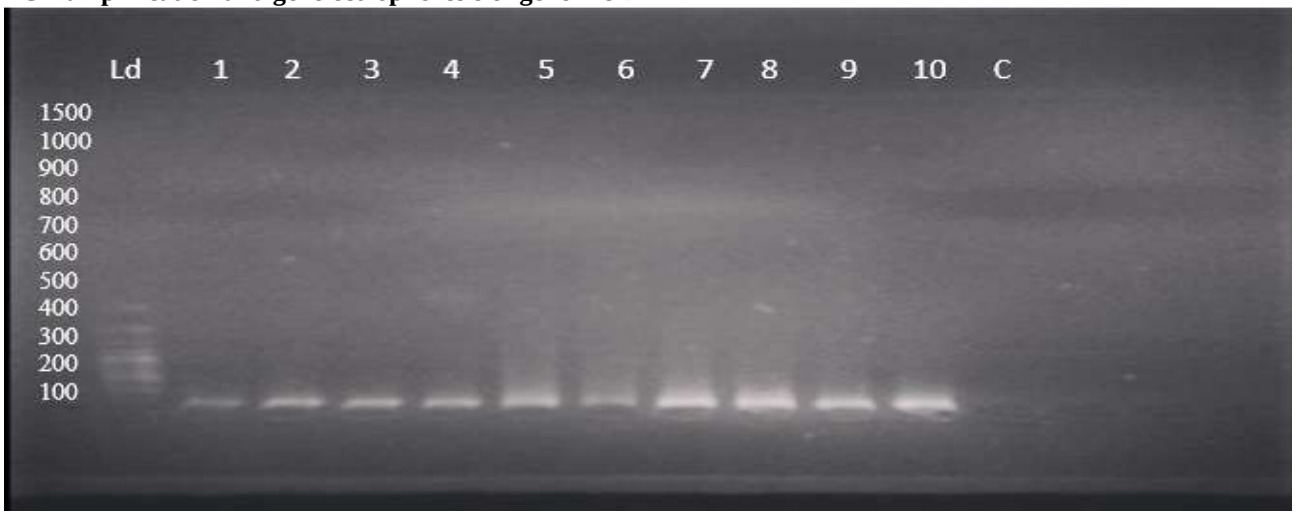

Fig. 1. DNA bands for primer SSR 528 among 10 rice varieties.
Lane 1= NSGC 5929, Lane 2=Basmati 443, Lane3=Hansraj 13, Lane 4= Mushkhan 340 A, Lane 5=N335 Lane 6=Super Basmati, Lane 7=Basmati 370, Lane8= Nickerie 19, Lane 9=140-4-1-2-5, Lane 10=56-5-6 **(Ld-Ladder, C=Negative Control)**

Table 5. Alleles, and PIC values calculated for a set of 10 diverse rice genotypes.

SSR marker	Sequence	Chromosomes location	Product Size(bp)	Total no. of alleles	No. of polymer -phic alleles	% polymer -phism	PIC
RM235	F:AGAAGCTAGGGCTAACGAAC R: TCACCTGGTCAGCCTCTTTC	12	170	3	3	100	0.480
RM224	F: ATCGATCGATCTTCACGAGG R:TGCTATAAAAGGCATTCGGG	11	230	3	2	75	0.540
RM201	F:CTCGTTTATTACCTACAGTACC R:CTACCTCCTTTCTAGACCGATA	9	170	2	2	100	0.460
RM498	F:AATCTGGGCCTGCTCTTTTC R:TCCTAGGGTGAAGAAAGGGG	2	220	4	3	75	0.750
RM528	F:GGCATCCAATTTTACCCCTC R:AAATGGAGCATGGAGGTCAC	6	190	4	4	100	0.380
RM134	F:CTCTGTCTCCTCCCCCGCGTCG R:GCTCTCCGGTGGCTCCGATTGG	7	180	5	4	75	0.590
RM544	F:TGTGAGCCTGAGCAATAACG R:GAAGCGTGTGATATCGCATG	8	126	4	3	75	0.750
RM215	F:CAAAATGGAGCAGCAAGAGC R:TGAGCACCTCCTTCTCTGTAG	9	235	4	4	100	0.570
RM590	F:CATCTCCGCTCTCCATGC R:GGAGTTGGGTCTTGTTCG	10	194	5	4	75	0.430
RM206	F:CCCATGCGTTTAACTATTCT R:CGTTCCATCGATCCGTATGG	11	210	5	4	75	0.460
R4M13	F:TACACGGTAGACATCCAACA R:ATGATTTAACCGTAGATTGG	4	190	6	6	100	0.630
RM208	F:TCTGCAAGCCTTGTCTGATG R:TAAGTCGATCATTGTGTGGACC	2	186	4	4	100	0.440
	Means			4.08	3.58	87.5	0.54

Table 6. Evaluation of varieties based on resistance gene with presence and absence of band.

S.No	Primer	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10
		NSGC 5929	Basmati-443	Hansraj 13	Mushkhan 340 A	N335	Super basmati	Basmati 370	Nickerie 19	140-4-1-2-5	56-5-6
1	**RM235**	BA	BA	BP	BA	BA	BP	BA	BA	BP	BP
2	**RM224**	BP	BA	BA	BA	BP	BP	BP	BP	BP	BP
3	**RM201**	BP	BP	BP	BA	BP	BP	BP	BA	BP	BP
4	**RM498**	BP	BP	BP	BA	BP	BP	BP	BA	BP	BP
5	**RM528**	BP	BP	BP	BP	BP	BP	BP	BP	BP	BP
6	**RM134**	BP	BP	BP	BA	BP	BA	BP	BP	BP	BA
7	**RM544**	BP	BA	BP	BP	BP	BP	BP	BP	BP	BP
8	**RM215**	BP	BA	BA	BA	BA	BP	BA	BP	BA	BP
9	**RM590**	BA	BA	BA	BA	BA	BP	BA	BP	BA	BA
10	**RM206**	BA	BA	BP	BA	BP	BP	BP	BP	BP	BP
11	**R4M13**	BA	BA	BP	BP	BP	BP	BA	BA	BA	BA
12	**RM208**	BP	BA	BP	BP	BP	BA	BP	BP	BP	BP

BP= Band present; BA= Band absent

DISCUSSION

The research work was carried out to screen different genotypes of rice. On the basis of different morphological traits of rice seeds and screening of rice germplasm, various rice genotypes were selected to start breeding program for the development of new germplasm which will show best performance despite of adverse environmental conditions.

Various quantitative and qualitative traits proved very helpful for selection of desirable traits of different rice germplasm. These specific traits determined the rate of growth, germination, vigor and yield of crop etc. These parameters can be used for further classification and breeding research program to develop new plant material. Results indicated that there was a significant correlation among all the traits of rice which contributes in yield potential (Ashfaq *et al.,* 2017).

All the germplasm lines studied showed more variation along with entire set of traits at the level of 1% and 5% significance. Strong association was observed between genotypes and a particular trait that provides the information for the screening and selection good number of genotypes that showed more variability (Kohnaki *et al*., 2013; Konate *et al*., 2016). A strong association is very helpful in selection and identification process for best utilization of genetic material under variable environmental conditions.

A total of 10 rice lines were selected on the basis of their morphological traits for determination of genetic diversity and resistance gene. Further, 12 SSR markers were used for amplification of DNA of selected rice lines. These markers depicted variation in their results as some markers showed monomorphic results while others showed polymorphic results (Seetharam *et al*., 2009). The results were showed in Figure 1, Table 5 and Table 6.

The purpose of using the SSR markers was to determine the genetic diversity and resistance gene in selected rice lines through the amplification of DNA by PCR procedure which helped to develop more resistant rice lines against the blast disease. The average PIC value was observed 0.54 that showed the genetic material has great genetic diversity. The average of total number of alleles (4.08), number of polymorphic alleles (3.58) and polymorphism% 87.5 indicated that the genetic material have potential to produce new plant population with variation that responsible for the evolution of new crop species (Mackay and Powell, 2006; Saeed *et al*., 2014).

Further study and research is required to comprehend the molecular screening at breeding level for better yield production and to stabilize the economic output of major crop like rice.

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CONCLUSION

From the study it was concluded that some rice found to be resistant and tolerant (NSGC 5929, Hansraj 13 and 56-5-6) against blast disease that could be more useful for the production new rice lines with diverse characteristics. These lines could also be very useful in breeding for disease resistance. On the other hand, SSR markers RM528 and RM544 showed more diversity among the rice germplasm lines that could be very fruitful for the evolution of new crop species.

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