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SSR BASED SCREENING OF RICE GERMPLASM AGAINST BLAST DISEASE AND ITS GIS MONITORING IN PUNJAB, PAKISTAN

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ABSTRACT

Rice blast disease has a potential threat to rice crop and causes huge yield losses in Pakistan and other regions of the World. Field trials were conducted and fifty diverse genotypes were screened under normal field conditions. The crop was subjected to artificial inoculation of *Magnaporthe oryzae* 0.5mL of each plant under field condition for assessment of varying degree of resistance or susceptibility. Phenotypic data was also recorded for seed phenotypic traits. To validate phenotypic screening molecular markers were used to check the presence of blast resistance gene. Map constructed using ArcGIS software exhibits the features of disease and its progress. During the year 2013 maximum disease incidence (40%) was found in Mandi Bahaudin. While during the year 2014 Kamalia was the most affected area with an incidence of 24 %. Visual scoring in field revealed that one out of 50 varieties was highly resistant and one was resistant. Similarly disease resistance markers were used to screen out the resistant genotypes i.e. Rondo and Bomba against blast disease. These two resistant genotypes may be very useful for the development of new plant population to minimize the severity of the disease and helpful for strengthen the economy of the country.

Keywords: Rice, GIS, SSR, Genotypic, Phenotypic

INTRODUCTION

Rice blast is a major global rice disease. Its causal agent is plant pathogenic fungus *Magnaporthe oryzae* (Telomorph) (Herbert) Barr (Anamorph: *Pyricularia oryzae*) Couch and Kohn (2002). It is the most alarming disease among all rice diseases and occurs in rice growing areas irrespective of climate, geographical and environmental conditions. It is constant threat to the crop and has never been eliminated from a region in which rice is grown. All crop stages and all parts of the plant are susceptible. This disease is reported to occur in more than 85 countries resulting in financial loss up to 65% in vulnerable cultivars (Jayawardana *et al*., 2014).

In recent years, evidences suggest that the pathogen has its prevalence in the entire Punjab province of Pakistan. Because of its wind borne nature it often occurs in the form of patches and sometimes spread even more and turn into epidemics. In Pakistan, during the last two decades rice blast is mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and Gaggoo Mandi (Mustafa *et al*., 2018). Current surveys (2014-15) indicate Kamalia and Mandi Bahaudin as adversely affected areas. Study of pathogen is very much essential to combat the disease. Pathogen was purified from infected samples. Morphological and genetic study of *Pyricularia oryzae* was conducted to identify the actual casual pathogen that spreading this threatening disease. On the other hand, the pathogenicity test lies on inoculation of genetically pure strains of pathogen (Liu *et al*., 2015).

Geographic Information System (GIS) is computer based software that is used to interpret, manipulate and manage geographical data into a required presentable format. By the help of this software, information or data is geographically referenced, which means that data is displayed according to its location. The data is displayed spatially or geographically, in the form of figures and maps, in a way that best exhibits the information of that particular region (Seyedeh and Ham, 2019). Behind this software works a whole science discipline called Geographic Information Science and this software is small demonstration of this science. It is now vastly being used in agricultural sciences in developed countries. It can perform innumerable functions including recording fluctuations in precipitation, temperature, crop output and input, mapping disease incidence and progress, thus by doing so, helping in predicting disease path and its distribution and informing about epidemics. The recognition and

solitude of extra variety boost level of resistance (R) genetics and virus a virulence gene are now needed to expand knowing of molecular systems in the host-pathogen connections (Hubert *et al*., 2015).

Microsatellites are also known as the Simple Sequence Repeat (SSRs) or Short Tandem Repeat (STRs). Microsatellites are very simple recurring elements which include of 1-6 base pairs, and they can be found in both programming and non-coding areas of DNA. These are very numerous throughout the genome and are extremely polymorphic and varieties particular. Along with many other purposes, these are used to detect the presence or absence of DNA stretch/gene linked to specific attributes e.g. resistance against disease. They are extensively being utilized because they can be readily amplified by simple conventional polymerase chain reaction (Miah *et al*., 2013a; Miah *et al*., 2013b).

Rice blast resistance genes are tightly linked to molecular markers. Therefore, marker assisted selection is the best tool to screen resistant rice germplasm. Also, this technique does have an advantage over conventional screening because of its overall easiness, effectiveness and accuracy. In present study, we performed phenotypic screening, utilized different SSR markers to detect resistance genes in the selected germplasm, and monitored the occurrence of rice blast disease in Punjab province using GIS.

MATERIALS AND METHODS

Plant Material: The experiment was conducted at experimental fields of Institute of Agricultural Sciences, University of the Punjab, Pakistan in the year 2013-14. Screening test included 50 rice (*Oryza sativa* L.) varieties from different origins (Table 1). Seeds were collected from four different rice germplasm collection centers including United States Department of Agriculture (USDA), International Rice Research Institute, (IRRI) Philippines, National Agricultural Research Centre, (NARC) Islamabad and Rice Research Institute (RRI) Kala Shah Kaku.

Nursery Preparation: Seeds were sown in blocks of 1.5 x1.5 feet for nursery preparation. Each variety was sown in separate block. Nursery was sown on 24 May 2015. It was ready for transplanting 35 to 40 days after sowing.

Transplanting: The plants were transplanted into separate blocks following randomized complete block design with three replications. Each block contained 15 plants. Plant to plant distance was 8 inches and row to row spacing was 16 inches.

Seed Morphological Traits: Morphological data was recorded as described by Ashfaq *et al*. (2013) and Ashraf *et al*. (2012). Seeds (with bran) morphological traits viz. seed length, width, thickness, length/ width (L/W) ratio were recorded with the help of Vernier calipers and thousand seed weight with the aid of digital weighing balance for 50 genotypes. Three replicates each having 5 seeds were randomly selected. A total of 15 seeds of each genotype were selected to record measurements .

Field Trial

Transplantation: After 40 days plants were transplanted. Disease Rating Scale (DRS) as recommended by IRRI was followed for disease scoring.

Collection, Isolation and Purification of Blast Pathogen: Blast infected samples were obtained from Rice Research Institute, Kala Shah Kaku. After collection they were brought into plant pathology lab for isolation of *P. oryzae*. For the purpose of isolation leaves were cut into small 1-3 cm pieces and surface was sterilized with 2.5 % sodium hypochlorite solution. Then they were placed on water agar to eliminate the growth of secondary pathogens. Once pathogen started emerging from infected leaf tissue, the pathogen mass was transferred to 2 % Potato Dextrose Agar media. Culture plates were incubated at 25 $^{\circ}$C for 7 days and were constantly kept in check. After culture development, pathogen was studied microscopically and shape of conidia and hyphae were recorded, Colony characteristics were also observed.

Preparation of Inoculum: Preparation of inoculum was carried out three weeks before the regular screening of the germplasm. Seeds of a susceptible cultivar Kashmir basmati were sown in several earthen pots placed in greenhouse. Aqueous spore suspension of concentration 1×10^6 spores/mL was prepared. After the development of rice blast disease, diseased seedlings were cut and chopped into small pieces and kept for the disease nursery as a source of inoculum.

Application of Inoculum: At two weeks stage, the seedlings were inoculated with aqueous suspension of 1×10^6 spores/ml of *P. oryzae*. Spore suspension of fungus was sprayed on the crop in the form of fine mist. Disease leaves were also spread in the field.

Microsatellite Based Screening of Rice Germplasm against Blast Disease: Extraction of plant genomic DNA is the first step towards microsatellite based screening. At seedling stage DNA was extracted from fresh leaves of each rice genotype using high throughput DNA extraction method (Liang *et al*., 2016). To determine purity, quality and quantity of extracted DNA at 260 and 280 nm Nano Drop (ND 1000 Spectrophotometer) was used. The extracted DNA samples (with concentration of 40 ng/µL) were diluted using ddH_2O for further PCR analysis. Polymerase Chain Reaction to Check the Presence of Blast Resistant Gene in Rice*:* PCR conditions were followed according to the protocol. The annealing temperature was adjusted as per mentioned in the primers. Six SSR markers on the whole with two markers specially designed for screening for blast were used. Rice markers RM166 and RM208 were specifically used to check the presence of Pi-b gene that is responsible of resistance in rice against blast disease (Table 4).

Construction of GIS Map Revealing % Disease Incidence: ArcGIS 10.2.2 software was accessed from Department of Geographic Information Sciences, Punjab University Lahore. Thematic data on Tehsil level regarding losses due to blast disease was mapped using this software. Special help was sought from the software expert.

Disease Incidence Percentage: By definition it is the" percentage of diseased plants in population of plants". Also, "Incidence is the rate of new (or newly diagnosed) cases of the disease. It is generally reported as the number of new cases occurring within a period of time."
It is calculated by:

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} x100$$

Statistical Analysis: 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 (Ishihara *et al*., 2014) by using different SSRs primers in-vitro conditions for genetic diversity determination of the among the genotypes/germplasm. The power marker (software package) Zhang and Xie (2014) was used to determine the molecular characteristics of genotypes. The analysis of variance and correlation was carried out through SAS version 9.2.

RESULTS AND DISCUSSION

Field screening of rice varieties: Visual scoring in field revealed that one out of 50 varieties was highly resistant and one was resistant. Out of all basmati varieties Shaheen basmati was found moderately resistant. Two varieties Erythrocerosramsz and Sanakevelle were kept in grade 3 of moderately resistant varieties (Table 3).

GIS based mapping of blast disease: Geographically referenced map constructed using ArcGIS10.2.2 software (Fig. 1) represented distribution of the disease in Punjab province. Forty-two tehsils of Punjab were reported where disease incidence was recorded. During the year 2013 maximum disease incidence (40%) was found in Mandi Bahaudin (Fig. 1). While during the year 2014 Kamalia was the most affected area with an incidence of 24 % (Fig. 1). The percentage on which map was based was disease incidence percentage. Disease prevalence was different from disease incidence. Prevalence was the number of cases of a disease that are present in a specific plant population at a given time, whereas incidence referred to the number of new cases that develop in a given period of time.

Genotype Clustering on the basis of genotypic and phenotypic traits: The 50 rice genotypes on the basis of various morphological traits were representing the variation among rice varieties. On the other hand, all the genotypes showed genetic variation regarding all the seed morphological traits (Table 2). After screening on the basis of phenotypic traits and properties of the genotypes i.e. resistant, tolerance, susceptible, clustering and grouping of genotypes were studied on the basis of band size of the PCR product of each genotype along with the specific type of primer (Fig. 2, 3). In the dendrogram there were 11 clusters that were further grouped in little chunks. Each chunk differed from the other. First chunk from the top contains 7 varieties (Gui 99, L-203, Shaheen Basmati, Dokri Basmati, Pk 786, Basmati 370 and Kasalath) which were much closer to the next group containing two varieties Bomba and Rondo. Grouping together of these two varieties showed that both were resistant and gave band with the related primers along with the entire set of genotypes. Other remaining clusters were closely related with each other

and genotypes showed variation on the basis genetic characteristics. The genotypes within the cluster showed similarity and among the cluster showed variability (Blue Nile, 7845, E425 (Cluster III) 8900, Sanakevelle (Cluster IV), Pimple marker, Embpara, 7813, Shali-i- Mahin, Kashmir Basmati, 7229, 7597 (Cluster V), RZ. No.III, Mayhiya, UVS SEL (Cluster VI), Sathi Basmati (Cluster VII), Jasmine 85, 7214, 7870 (Cluster VIII), Khusab-95 (IX), Yang Ku Tsi, 7239, 7651 (Cluster X), 7835, Chini Sakkor, Erythrocers Ramsz, Sada Hayat (Cluster XI).

Table 1. Plant material used in the experiment.

S.No.	Variety Name	Origin	Accession No	Taxon
1	Bomba	Spain	GSOR 310110	*Oryza sativa* L.
2	Yang Ku Tsi	China	IRRI-IRGC-1198	*Oryza sativa* L.
3	Acc.7813	Pakistan	GPNO.7813	*Oryza sativa* L.
4	Shali-I-Mahin	Afghanistan	IRRI-IRGC-3455	*Oryza sativa* L.
5	UvsSel	Switzerland	GSOR 310606	*Oryza sativa* L.
6	Kashmir Basmati	Aj.Kashmir	GPNO. 22231	*Oryza sativa* L.
7	Pimplemarker	Pakistan	-	*Oryza sativa* L.
8	Acc.7597	Pakistan	ACC.7597	*Oryza sativa* L.
9	Rz.No.111	Zaire	IRRI-IRGC-3435	*Oryza sativa* L.
10	Blue Nile	South Africa	IRGC-3435	*Oryza sativa* L.
11	Rondo	America	RU0603075	*Oryza sativa* L.
12	Erythrocerosramsz	Poland	GPNO.18791	*Oryza sativa* L.
13	Sanakevelle	Liberia	IRRI-IRGC-4129	*Oryza sativa* L.
14	Sada Hayat	Pakistan	-	*Oryza sativa* L.
15	Pk-786	Pakistan	-	*Oryza sativa* L.
16	Shaheen Basmati	Pakistan	-	*Oryza sativa* L.
17	Kasalath	India	IRGC 117617	*Oryza sativa* L.
18	Dokari Basmati	Pakistan	-	*Oryza sativa* L.
19	Khusab 95	Pakistan	-	*Oryza sativa* L.
20	Basmati 370	Pakistan	IRGC-9026	*Oryza sativa* L.
21	ChiniSakkor	Pakistan	-	*Oryza sativa* L.
22	Gui 99	China	NSGC 6153	*Oryza sativa* L.
23	L-203	United States	88-Y-774	*Oryza sativa* L.
24	Mayhiya	Fiji	IRRI-IRGC-9771	*Oryza sativa* L.
25	Embpara 1200	Brazil	F 737	*Oryza sativa* L.
26	Jasmine 85	United States	GSOR 102174	*Oryza sativa* L.
27	Sathi Basmati	Indo- Pak	-	*Oryza sativa* L.
28	Acc.7645	Pakistan	ACC.7645	*Oryza sativa* L.
29	Acc 7835	Pakistan	ACC 7835	*Oryza sativa* L.
30	Acc 7214	Pakistan	ACC 7214	*Oryza sativa* L.
31	Acc 8900	Pakistan	ACC 8900	*Oryza sativa* L.
32	Acc 7670	Pakistan	ACC 7670	*Oryza sativa* L.
33	E425	Senegal	310328	*Oryza sativa* L.
34	Acc 7239	Pakistan	ACC 7239	*Oryza sativa* L.
35	Acc 7651	Pakistan	ACC 7651	*Oryza sativa* L.
36	Acc 7229	Pakistan	ACC 7229	*Oryza sativa* L.
37	Np-125	India	IRRI-IRGC-3701	*Oryza sativa* L.
38	Bombilla	Spain	GSOR 301285	*Oryza sativa* L.
39	Sonharisagadasi	Pakistan		*Oryza sativa* L.
40	SecanoDobrazil	El Salvador	IRRI-IRGC-3375	*Oryza sativa* L.
41	Taino 33	Taiwan	IRRI-IRGC-7333	*Oryza sativa* L.
42	Nsgc-5945	Sierra Leone	NSGC 5945	*Oryza sativa* L.
43	TaichuMochi	Taiwan	GPNO 2606	*Oryza sativa* L.
44	AllorioLamba	France	GPNO 13818	*Oryza sativa* L.
45	Basmati 2000	Pakistan	-	*Oryza sativa* L.
46	Kerangserang	Indonesia, Java	GPNO. B051	*Oryza sativa* L.
47	Arabi	Egypt	GPNO. 24234	*Oryza sativa* L.
48	Cypress	America	GSOR 101299	*Oryza sativa* L.
49	IR- 64	Philippines	IR 52297-64-3-2-2	*Oryza sativa* L.
50	Xiangzhaoxian No. 15	China	NSGC 6161	*Oryza sativa* L.

Table 2. Analysis of variance of different seed morphological traits of various rice genotypes and their mean square values.

Source of variation	DF	SL	SW	ST	L/W	1000GW
Genotypes	49	3.196**	0.966**	1.689**	0.049**	0.301**
Replications	2	0.003	1.265	0.028	0.0001	0.0018
Error	98	0.060	0.470	0.020	0.0051	0.1550

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

Table 3. Screening and classification of rice varieties against blast disease identification of *Pyricularia oryzae.*

GRADE	HOST RESPONSE	VARIETY
0	Highly Resistant	Rondo
1	Resistant	Bomba,Kasalath
2	Modrately Resistant	Shaheen Basmati
3	Moderately Resistant	Erythrocerosramsz, Sanakevelle
4	Moderately Susceptible	Shali-I-Mahin, Rz.No.111, Acc.7597
5	Moderately Susceptible	Yang Ku Tsi, UvsSel
6	Susceptible	Sada Hayat, Blue Nile, Pimple marker,
7	Susceptible	Gui 99,Jasmine 85, Khusab 95, Kashmir Basmati
8	Highly Susceptible	Acc.7813, E425, Sathi Basmati, Embpara 1200, Mayhiya, L-203, ChiniSakkor, Basmati 370, Dokari Basmati, Pk-386,
9	Highly Susceptible	Xiangzhaoxian No. 15, IR 64, Cypress, Arabi, Kerangserang, Basmati 2000, AllorioLamba, TaichuMochi, Nsgc-5945, Taino 33, SecanoDobrazil, Sonharisagadasi, Bombilla, Np-125, Acc. 7229, Acc. 7651, Acc. 723, Acc. 7670, Acc. 8900, Acc. 7214, Acc. 7835, Acc.7645,

Microsatellite based screening of rice: The PCR product of these two specific primers RM-166 and RM-208 was 327bp and 173bp, respectively (Fig. 4, 5). Overall these SSR primers were used for the determination of genetic diversity of rice lines for further screening against disease resistance and high yield requirements. The highest gene diversity and PIC value indicated by RM 252 and lowest indicated by RM 166 against the entire set of all genotypes. The results were shown in the Fig. 4 and Table 5.

In this study, the PCR based screening and its GIS monitoring of different rice lines were tested against blast disease to determine the resistance level for their effective use in plant genetics. For this purpose different molecular markers and diverse germplasm material were used for the identification of resistivity against this threatening disease. Plant breeders often use cultivars designed in other countries to extend the inherited background of the raised cultivars being designed such as the major infection illnesses of boost, but most reproduction programs of grain have a filter inherited variety of reproduction resources. Many grain types have been designed as completely immune to *M. oryzae* strains, but soon malfunction of grain happened because of the appearance of more powerful controversial isolates of grain fungus (Nasruddin *and* Amin, 2013). Two specific primers viz. RM 166 and RM 208 were related to *Pi-b* gene. This gene was mapped on chromosome number 2 in rice genome and was found to be responsible for resistance in rice against blast disease (Scheuermann *et al*., 2012).

Table 4. Sequence of Gel Samples banding pattern information of entire set of rice germplasm lines along with various rice markers.

NAME OF VARIETY	MARKERS					
	RM 166	RM250	RM224	RM208	RM30	RM252
BOMBA	1	1	1	1	1	1
YANG KU TSI	0	1	1	0	1	1
ACC.7813	0	1	0	0	1	0
SHALI-I-MAHIN	0	1	0	0	1	0
UVS SEL	0	1	1	0	1	1
KASHMIR BASMATI	0	1	1	0	1	0
PIMPLE MARKER	0	1	1	0	1	0
ACC, 7597	0	1	1	0	1	0
RZ.NO.111	0	1	1	0	0	1
BLUE NILE	0	0	1	0	1	1
RONDO	1	1	1	1	1	0
ERYTHROCEROS RAMSZ	0	1	1	0	1	1
SANAKEVELLE	0	1	1	0	1	0
SADA HAYAT	0	1	1	0	1	1
PK-386	0	1	1	0	0	1
SHAHEEN BASMATI	0	1	0	0	0	1
KASALATH	0	1	1	0	0	1
DOKARI BASMATI	0	1	1	0	0	1
KHUSAB 95	0	1	1	0	0	1
BASMATI 370	0	1	1	0	0	1
CHINI SAKKOR	0	1	1	0	1	1
GUI 99	0	1	0	0	0	1
L-203	0	1	1	0	0	1
MAYHIYA	0	1	1	0	1	1
EMBPARA	0	1	1	0	1	0
JASMINE 85	0	1	1	0	1	0
SATHI BASMATI	0	1	1	0	1	1
ACC.7645	0	0	1	0	1	1
ACC.7835	0	1	1	0	1	1
ACC.7214	0	1	1	0	1	0
ACC.8900	0	0	1	0	1	0
ACC.7670	0	1	1	0	1	0
E425	0	0	1	0	1	1
ACC.7239	0	1	1	0	1	1
ACC.7651	0	1	1	0	1	1
ACC.7229	0	1	1	0	1	0

The primer RM 166 had lowest PIC value of 0.0994 and primer RM 252 had highest PIC value of 0.3550 (Table 4). Gene diversity was also highest for RM 252 which is 0.4614. Lowest gene diversity was of marker RM 166 with an overall mean of 0.2834. Similarly, Singh *et al*. (2015) evaluated a core subset of the USDA 1790 rice germplasms and they found some accessions contained *Piz-5* gene with additional R genes. In addition we verified

accuracy of our results, a total of 13 alleles were detected at the loci of six microsatellite markers across fifty rice germplasm lines (Fig. 3, table 5). The results revealed that diversity related primers showed distinct polymorphisms among the cultivars studied. Among the 4 polymorphic markers each of them produced 2 alleles while disease related markers RM 166 produced 2 alleles while RM 208 produced 3 alleles. The amplification size of all 12 genotypes for each marker allele varied from 105-321bp. Identical outcome was revealed by Kim *et al*., 2010 in 84 accessions of grain germplasms owned and operated more than three beneficial groups of the eight grain resistant level of resistance genetics, and Imam *et al*., 2014 revealed the inherited regularity of the nine significant grain blast level of resistance genetics *Piz, Piz-t, Pik, Pik-p, Pik-h, Pita/Pita-2, pita, Pi9* and *Pib*, varied from 6 to 97% in the choose set of grain germplasms. Accessibility of allele is one for every for beginners which signifies that there is only one locus for every for beginners. This research showed the application of SSR indicators to recognize grain types likely taken the same R genetics with possibly novel level of resistance. Rice types with a number of alleles in normal with any particular level of resistance might have the same blast R gene, and knowing the organic variety at the particular gene is very important to development of particular R gene using DNA marking into grain reproduction system (Taguch *et al*., 2014; Ashkani *et al*., 2015; Hasan *et al*., 2017).

Table 5. Characteristics of the SSR used and their frequency allele, gene diversity and PIC values calculated for a set of 50 diverse rice genotypes.

MARKER	MAJOR ALLELE FREQUENCY	ALLELE NO	AVAILABILITY	GENE DIVERSITY	PIC
RM 166	0.9444	2.0000	1.0000	0.1049	0.0994
RM250	0.8889	2.0000	1.0000	0.1975	0.1780
RM224	0.8889	2.0000	1.0000	0.1975	0.1780
RM208	0.7778	3.0000	1.0000	0.3642	0.3267
RM30	0.7500	2.0000	1.0000	0.3750	0.3047
RM252	0.6389	2.0000	1.0000	0.4614	0.3550
Mean	0.814817	2.166667	1.0000	0.283417	0.2403

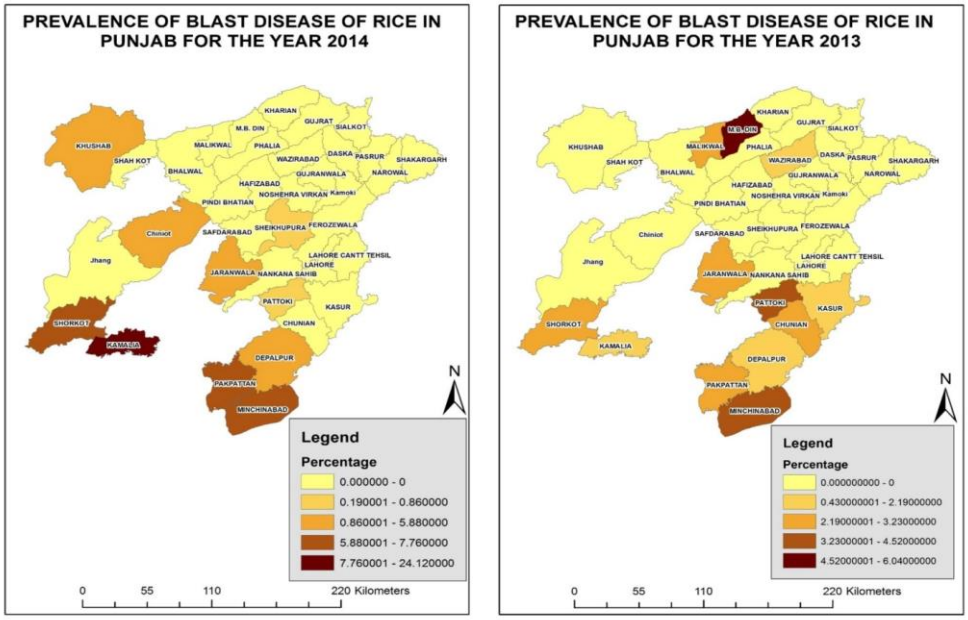

Fig.1. Prevalence of blast disease of rice in Punkab, Pakistan for the year 2013-14.

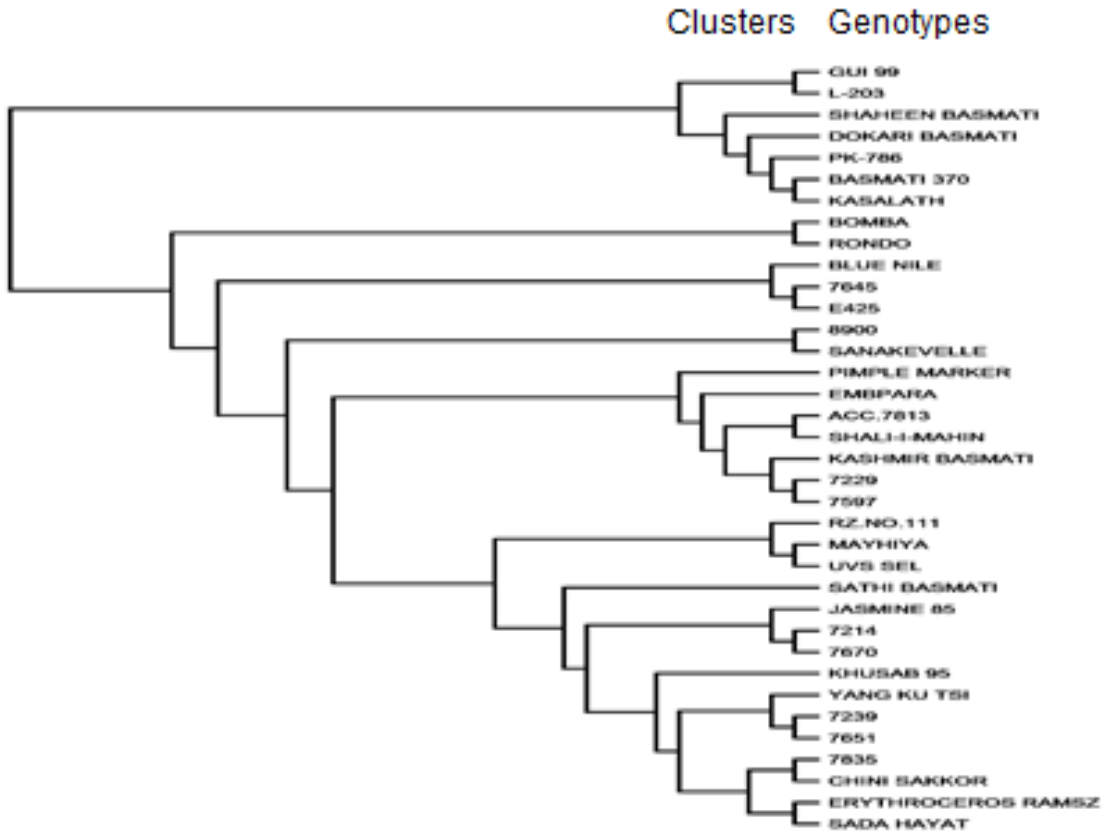

Fig.2. Cluster analysis of local and exotic rice germplasm lines for screening.

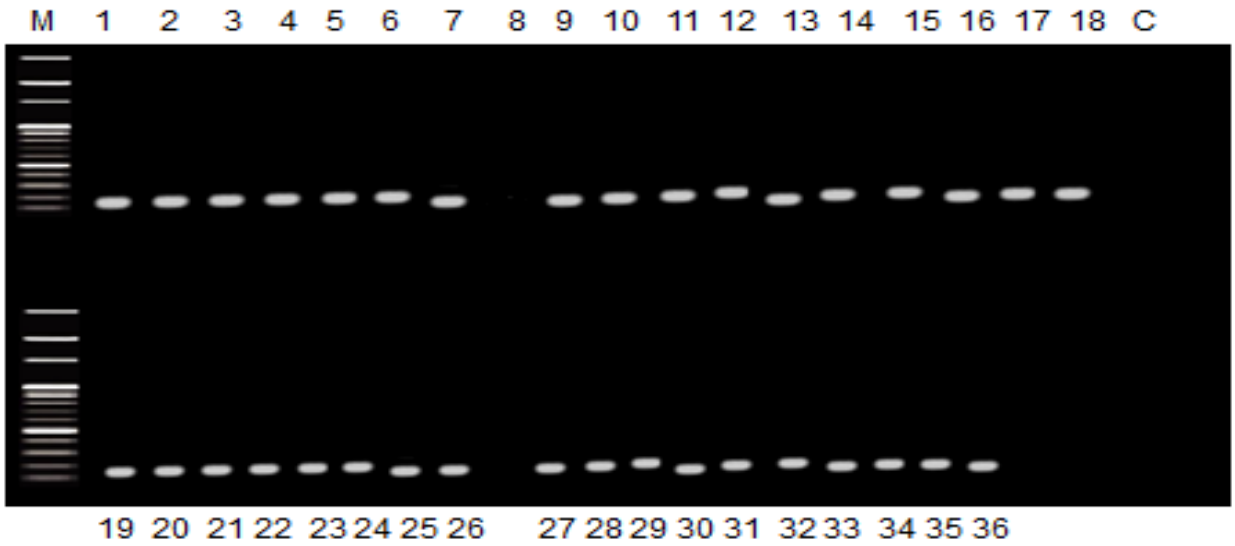

Fig.3. Genetic diversity and screening of diverse rice germplasms. PCR profile of RM 250 showing the range of alleles in diverse rice genotype. "M" represents the DNA Marker, samples 1 to 36 represent different rice genotypes; "C" of H_2O used as a negative control.

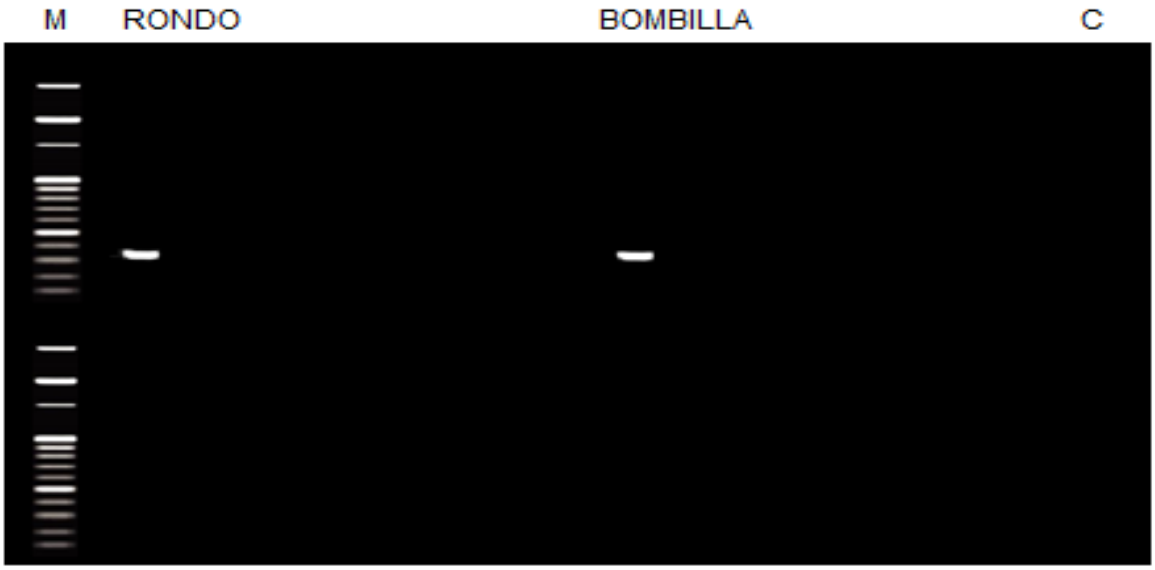

Fig.4. PCR profile of primer RM 166 along with entire set genotypes 1 to 36 for disease resistance.

Fig.5. PCR profile of primer RM 208 along with entire set genotypes 1 to 36 for disease resistance.

Clustering and grouping of genotypes on the basis of various morphological traits gave the opportunity to breeders and scientific community for early screening and selection of the potential rice genotypes. Cluster analyses provide the information about the similarity and dissimilarity of the genotypes and also provide the information regarding within group and between group genotypes (Fig. 2). Likewise, next coming groups show greater differences with respect to earlier coming groups. Similar report also made earlier by Roy *et al*. (2012b). The plot was a clear representation of seed morphological traits. It started declining by reaching maximum at 10 mm. Width and thickness was in range of 2-3mm as shown by the plot. Length/ width ratio was slightly higher and finally thousand grain weight which was in grams rises till 25 grams form 17 grams.

Genetic diversity among the germplasm of rice and within the pathogen often brings to unreliable marking and phenotype research. GIS have the advantage in determining R genetics, but its power can be found in the sturdiness of the indicators used. The recognition and research of grain boost level of resistance genetics indicates that DNA primers produced from the gene is an important device for boost gene recognition and testing among the grain germplasm (Roy *et al*., 2012a *and* Ghazanfar *et al*., 2009). In this study, the PCR based indicators employed for testing of different boost level of resistance genetics are well recognized and effective (Joshi *et al*., 2009). The efficient results revealed with the chosen SSR indicators for specific genetics was highly efficient and make them

the marking of choice for molecular testing of grain boost level of resistance genetics among the grain germplasm. Place collie breeders often use cultivars designed in our nation to extend the genetic qualifications of the enhanced cultivars being designed such as the major fungal diseases of blast, but most breeding programs of rice have a narrow genetic diversity of breeding resources (Fukuoka *et al*., 2015).

From the map it can be concluded that the path of disease progress was in the direction of wind from North to South (Fig. 1). Map illustrated that it is typically showing characteristics of wind born disease. In 2014 tehsil Kamalia was the source point of the disease. Then it moved to the neighboring areas along the wind also, moved in peripheries and upward direction. As the distance from source point increases the incidence of disease decreases. For fungal identification morphological species concept is very important. Based on morphology, fungus can be identified. On plants, elliptical diamond shape lesions were formed. The spots were initially white to light brown in color upon ageing they turned into dark brown to grey. Ghazanfar *et al*. (2009) reported the screening of more than one hundred lines and classified them in accordance with the disease rating scale of IRRI.

CONCLUSION

Both microsatellite based screening and GIS based monitoring is more reliable and efficient way of screening plants against diseases. Using this simple PCR based technique we have screen varieties that are resistant against blast i.e. Rondo and Bomba. Geographic Information System is a very important tool to predict disease progress and taking preventive measures. The Information obtained from data analysis may prevent great losses or epidemics. In addition, a higher degree of genetic variability and the localization of more markers on the rice linkage map will provide additional resources for genomic analysis and rice breeding. Therefore, there exist great opportunities for more efficient breeding programs and faster development times for new rice varieties resistant to biotic diseases in the future. These genotypes could be used for breeding purposes for the development of new resistant plant populations against new pathogen races which cause severe diseases in rice crop. The information is equally beneficial both for the scientific and farmers community for the enhancement and high yield production of rice crop.

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