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SCREENING OF DIFFERENT RICE (*ORYZA SATIVA* L.) VARIETIES FOR GENETIC DIVERSITY AND BACTERIAL BLIGHT RESISTANCE GENE

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Abstract

Rice (*Oryza sativa* L.) is one of the most important crop feeding about 2.5 billion people around the world and it is a major source of nutrition. Asian countries are the main producers as well as consumers of the rice. *Oryza sativa* and *Oryza glaberrima* are the two common cultivated rice species. *Oryza sativa* is cultivated in Asian regions, while *Oryza glaberrima* is cultivated in African region. Rice is the second most important food crop in Pakistan. More than 40% of the world's rice crop is lost annually due to biotic stress such as pests, insects, weeds and pathogens. This study was carried out to identify bacterial blight resistance genes (*Xa13* and *Xa21*) in Pakistani, Indian, Japanese, Taiwanese and Philippine's rice varieties. Investigation of genetic diversity among rice varieties was also carried out by total seed proteins profiling using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein bands of size ranging from 10 kDa to 125.8 kDa were observed. Genetic similarity values ranged from 0.22 to 1.00 and cluster analysis divided all the varieties into five groups. For molecular identification of *Xa13* and *Xa21* genes fragment, sequence-tagged-site (STS) markers were used. *Xa13* gene fragment was present in 30 varieties and *Xa21* gene fragment was present in 43 varieties. Genetic diversity was present among rice varieties. The information about the genetic diversity of studied rice varieties will be very useful in identification and selection of suitable parents for use in breeding programs to develop unique germplasm that complement existing varieties regarding high yield and resistance to bacterial leaf blight disease.

Key words: *Oryza sativa*, Genetic diversity, Bacterial blight, Resistance genes, Molecular identification.

Introduction

Genetic diversity is the foundation of the genetic improvement of crops. International Rice Research Institute (IRRI) seeks to understand rice genetic diversity. IRRI also uncover new genes and traits in rice that will help rice producers face challenges brought about by climate change, pests, diseases and other unfavorable conditions. The knowledge of the extent and pattern of diversity in the crop species is a prerequisite for any crop improvement as it helps breeders in deciding suitable breeding strategies for their future improvement (Sano, 2000). Assessment of genetic diversity through SDS-PAGE is easy and cheap. Protein markers have emerged as a possible tool for studies on genetic variability and have effectively been employed for identification of varieties in a number of crop plants. Seed storage protein profiling can be used for different purposes like varietal identification, germplasm characterization, determination of phylogenetic relationship among different species and biosystematics analysis (Sammour, 1991). Asian cultivated rice (Oryza sativa L.) has been suggested to have a polyphyletic origin (Agrawal *et al.*, 2003) in which two distinct groups, Indica and Japonica, were domesticated in the southeast part of South Asia and southern China, respectively (Huang *et al*., 1997). In a recent study it has been reported that the modern japonica was independent of the historical japonica and exotic japonica groups as determined by population structure and phylogeny analysis (Hong *et al.,* 2019).

Pakistan is among the few countries that is producing and exporting very good quality rice. In Pakistan rice is the second most important food crop, not only in view of local consumption but also in view of large exports. Globally rice is presently grown on about 167.25 million hectares, with an entire production of 495.9 million metrictons in the 2018/2019 crop year (www.Statistica.com). Like many different food crops, rice is the most important food crop serving half of the world population. Genetic diversity of rice germplasm compared with other crops is quite large. Rice subspecies like indica, japonica and javanica consists of huge reservoir of rice germplasm produced by intermingling of landraces and cultivars (Garris *et al*., 2005). Landraces are important genetic sources because they possess large genetic variability which may be utilized to develop rice genotypes having broad gene pool (Kobayashi *et al*., 2006). A large number of varieties and advanced cultivars had been released for cultivation in many different regions, however have a narrow genetic base (Rabbani *et al*., 2008).

Different research activities in Pakistan on rice are focused for increasing the yield and resistance for diseases and pests. For this, mechanization for cultivation of rice, adaptation of the advanced and improved varieties and most importantly, use of biotechnological techniques for the incorporation of genes for disease resistance have arisen in Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan.(Anon., 2000a). Salt tolerance researches are also in progress at PARC, but no research were made for grain quality evaluation of local rice genetic assets; however grain quality of some better

varieties was reinforced (Ahmad & Akram, 2005). Though, recently it was found in Pakistan that rice with better grain quality must be produced at national level (Shamim *et al*., 2017; Fatima *et al*., 2018).

The maximum loss in seed yield is 37.02% caused by weeds followed by insect-pests 27.9% and diseases 15.6% (Mondal *et al.*, 2017). Bacterial leaf blight (BLB), blast (*Magnaporthe grisea*), sheath blight (*Rhizoctonia solani*) and Tungro virus are the most common diseases caused by bacterial, fungal and viral pathogens (Velusamy *et al*., 2006). In Asia the oldest known bacterial disease is bacterial leaf blight which is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and it is the most serious bacterial disease in many rice growing regions of the world (Xu *et al*., 2010).

Approximately 34 genes in which 23 are dominant and 11 are recessive have been identified that confer resistance towards numerous strains of *X. oryzae* (Chen *et al*., 2011), mostly in non-basmati rice. This study was aimed to check the genetic diversity at seed storage protein level and bacterial blight resistance genes in local Pakistani and exotic varieties.

Materials and Methods

Plant materials: All the plant materials used in this study were obtained from Plant Genetic Resource Program (PGRP), National Agricultural Research Centre (NARC). Total 74 different varieties mostly Pakistani but also varieties from other countries like India, Taiwan, Philippine and Japan were used.

Electrophoresis: SDS-PAGE analysis of total seed storage protein was carried out by using standard method of Laemmli *et al*., (1970). Molecular protein marker of size 10-180 kDa (Bench-Mark™ Prestained Protein Ladder, Cat.No.10748-010, and Lot No.1046147), Invitrogen Life Technologies was used as standard. The reference value (Rf) for each known and unknown protein was determined for calculation of molecular weight of seed storage proteins.

Statistical analysis

Scoring of clear bands was performed for statistical analysis on the basis of polypeptide bands presence (1) or absence (0) for the binary data matrix. For all likely pairs of protein band types the similarity index (s) was determined by using the formula (S = w / (a + b – w). For Dendrogram construction similarity index was converted into a dissimilarity matrix through the method of Un-weighted Pair-group by mean of Arithmetic Averages by following method of Sneath & Sokal (1973). All the statistical analyses were performed using statistical package NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

Molecular identification of bacterial blight resistance genes by STS markers: DNA was extracted for all used varieties and amplification of *Xa13* and *Xa21* genes was carried out by PCR. STS marker of *Xa21* was used according to method of Ronald *et al*., (1992). STS marker for *Xa13* was used as mentioned by Huang *et al*., (1997). Through electrophoresis the amplified products were resolved on 1.5% of agarose gel, stained with ethidium bromide and observed under Ultra Trans Illuminator and scored for the presence and absence of *Xa21* and *Xa13* gene fragment. DNA marker of size 50-1000bp (Gene Ruler 50 bp DNA Ladder; ready-to-use; Catalog No. SM0373, Thermo Fisher Scientific) was used as standard for estimation of band size of bacterial blight resistance gene.

Results and Discussion

In order to enhance the export and local consumption potential of rice, there is a crucial requirement to improve the quality of Pakistani rice grain and to develop varieties resistant to various diseases. These aims may be accomplished by continuous research and development programs.

Biochemical (SDS-PAGE) analysis of total seed storage proteins: The electrophoretic pattern of proteins of studied varieties (61) showed significant diversity of rice genotypes as presented in Fig. 1A, B & C. Through SDS-PAGE, 67 different protein bands of size ranging from 10 kDa to 125.8 kDa were observed (Table 2). Some bands with lower molecular weight were also observed but they were not recorded due to their inconsistency and variation in sharpness or density. Out of 67 bands, 10 bands were monomorphic (more common), 42 were polymorphic bands (less common) and 15 were unique bands. Maximum numbers (9) of bands were present in Lateefy and minimum numbers (2) of bands were present in Mahlar-346, Shua-92, Khushboo-95, Dokri-Basmati and Shakar. Band of 39.8 kDa was quite frequent occurring as it was present in 25 varieties. According to Ali *et al*., (2007), the equivalence in the major protein polypeptides bands among a number of genotypes states that the genes coding these proteins are conserved. Band of 112.2, 100, 97.7, 85.1, 83.1, 77.6, 70.7, 69.1, 57.5, 51.2, 46.7, 45.6, 37.7, 25.7 and 10 kDa were least occurring as they were present in Sonehri-Sugdasi, Sathra, Basmati-2000, Sathra, Basmati-370, Dehraduni, Basmati-198, Sugdasi-Ratria, Basmati-370b, Pusa-1121, Basmati-C622, Basmati-370a, DR82, Pusa-Basmati, and Lateefy, respectively (Table 1). Arun *et al*., (2010) carried out similar research on 48 rice varieties, and found proteins of band of 70, 65, 60, 57, 37, 38, 39, 22, 23, 13 and 10kDa.

Similarity matrix and cluster analysis: Genetic similarity matrix was calculated by using software NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA). Similarity indices were calculated for all possible pairs among 61 rice varieties. Genetic similarity ranged from 0.22 to 1.00. Many combinations showed the highest similarity matrix of 1.00. The lowest similarity matrix of 0.22 was observed in two combinations which were Shua-92 and Dehraduni and Mahlar-346 and Purple-Marker (Fig. 2).

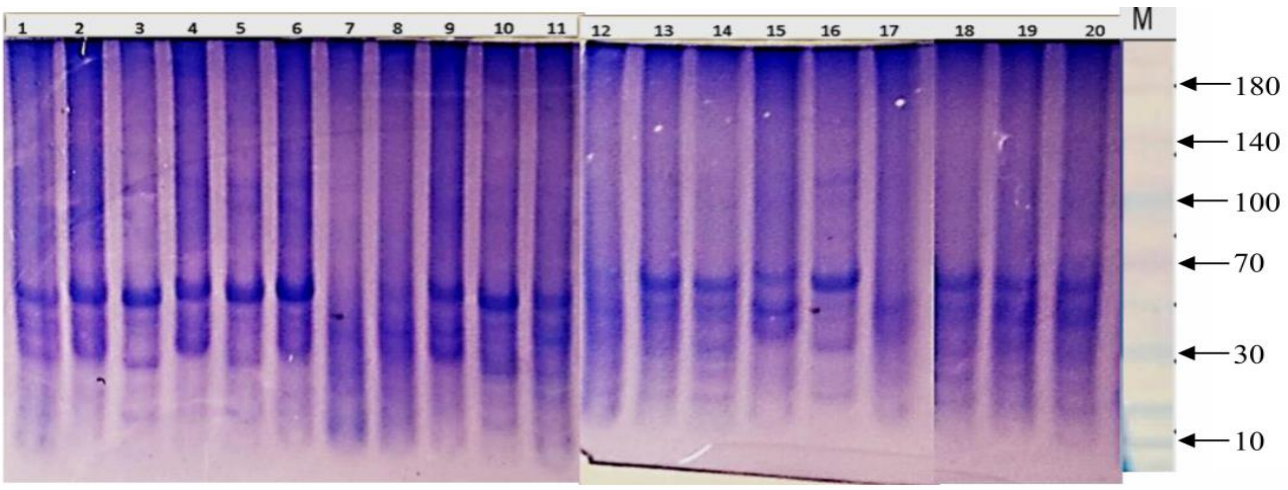

Fig. 1A. SDS-PAGE showing electrophoretic banding pattern of different rice varieties. Lane 1-20 represent varieties IR6, Basmati-Pak, Basmati-198, PK177, Basmati-370, Sathra, Mahlar-346, Palman-Sufaid, KS282, Super-Basmati, Kangni-27, Pakhal, Shaheen-Basmati, Kashmir-Basmati, KSK133, Basmati-2000, Shua-92, Jajai-77, KangnixTorh and Sugdasi-Sadagulab respectively. Lane M indicates molecular weight markers.

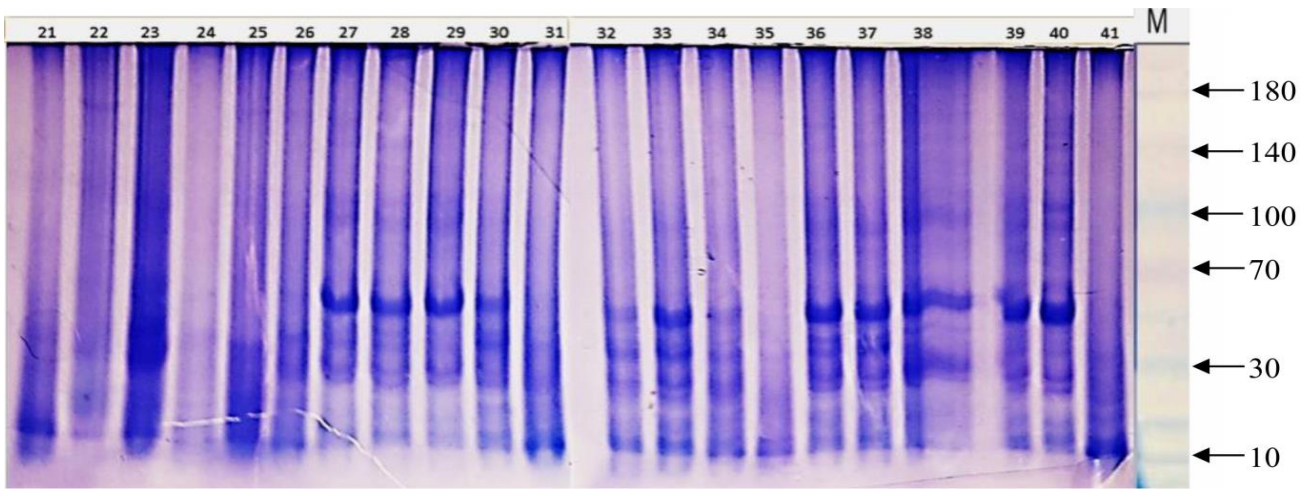

Fig. 1B. SDS-PAGE showing electrophoretic banding pattern of different rice varieties. Lane 21-41 represent varieties DR83, Khushboo-95, Kanwal-95, Dokri-Basmati, Shakar, IR6(C), Sugdasi-Ratria, Kasalath, IR9, Kharai-Ganja, Basmati-C622, Dehradune-Basmati2, Azucena, DR92, Shadab, Basmati-370a, DR82, Dehradun-Basmati1, Basmati-217, Sonehri-Sugdasi, and Nipponbare. Lane M represents the molecular weight markers.

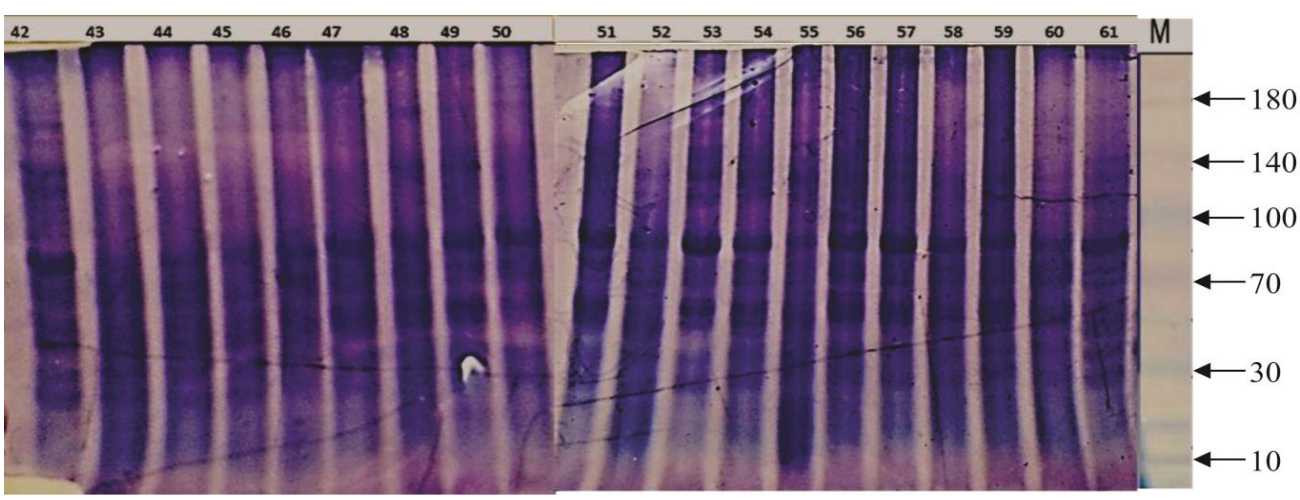

Fig. 1C. SDS-PAGE showing electrophoretic banding pattern of different rice varieties.Lane-42-61 represent varieties Dehraduni, Dehradune, Ranbir-Basmati, Pusa-1121, Pusa-Basmati, TN1, PAU-201, Karnal-Basmati, Basmati-370b, Mushkan-Rice, JP5, Lateefy, Punjab-Basmati, Early-Basmati, Purple-Marker, PK386, Type-3, Mutant-370, IR36, and Dehradun-Basmati3. Lane M represents the molecular weight markers.

Table 1. List of varieties and their country of origin.

S. No.	Variety name	Country of origin	S. No.	Variety name	Country of Origin
1.	Basmati-370	Pakistan	38.	TN1	Taiwan
2.	Sathra	Pakistan	39.	Basmati-370a	India
3.	Mahlar-346	Pakistan	40.	Dehradun-Basmati1	Nepal
4.	Palman-Sufaid	Pakistan	41.	Dehradun-Basmati2	India
5.	Basmati-C622	Pakistan	42.	Basmati-217	India
6.	Basmati-Pak	Pakistan	43.	Punjab-Basmati	India
7.	Basmati-198	Pakistan	44.	Pusa-Basmati	India
8.	PK177	Pakistan	45.	Ranbir-Basmati	India
9.	KS282	Pakistan	46.	PK386	Pakistan
10.	Basmati-2000	Pakistan	47.	Pusa-1121	India
11.	KSK133	Pakistan	48.	PAU-201	India
12.	Shaheen-Basmati	Pakistan	49.	Mutant-370	India
13.	Kashmir-Basmati	Pakistan	50.	Mushkan-Rice	India
14.	Pakhal	Pakistan	51.	Purple-Marker	Pakistan
15.	Jajai-77	Pakistan	52.	Basmati-370b	India
16.	Kangni-27	Pakistan	53.	Dehraduni	India
17.	KangnixTorh	Pakistan	54.	Dehradune	India
18.	Sugdasi-Sadagulab	Pakistan	55.	Dehradun-Basmati3	India
19.	Sonehri-Sugdasi	Pakistan	56.	Early-Basmati	India
20.	Sugdasi-Ratria	Pakistan	57.	Karnal-Basmati	India
21.	Dokri-Basmati	Pakistan	58.	Type-3	India
22.	Kharai-Ganja	Pakistan	59.	ARC10025	India
23.	IR6	Pakistan	60.	Lashmi-Digha	Bangladesh
24.	DR82	Pakistan	61.	Bhaturi	Bangladesh
25.	DR83	Pakistan	62.	Pankiraj	Bangladesh
26.	Lateefy	Pakistan	63.	Aus-133	Bangladesh
27.	IR9	Pakistan	64.	Aus-176	Bangladesh
28.	DR92	Pakistan	65.	Aus-190	Bangladesh
29.	Kanwal-95	Pakistan	66.	Aus-346	Bangladesh
30.	Shakar	Pakistan	67.	Korchampuri	Bangladesh
31.	Shua-92	Pakistan	68.	Naroi	Bangladesh
32.	Khushboo-95	Pakistan	69.	Saita	Bangladesh
33.	Shadab	Pakistan	70.	Balla-Bokri	Bangladesh
34.	IR36	IRRI Philippines	71.	Baturi	Bangladesh
35.	Nipponbare	Japan	72.	IR6	IRRI Philippines
36.	Azucena	Philippines	73.	JP5	Pakistan
37.	Kasalath	India	74.	Super-Basmati	Pakistan

The Dendrogram was constructed by using Unweighted Pair Group Method of Arithmetic Mean (UPGMA) averages that divided 61 varieties into five groups (Fig. 2). Group I consisted of four varieties in which IR6, Palman-Sufaid and KS282 have 100% similarity. Within group 1 PK177 was grouped with these three varieties having 86% similarity. Group II consisted of 34 varieties which have 100% similarity. Group III consisted of 10 varieties, Basmati-198, Basmati-370 and PK386 have 100% similarity and Sathra, Basmati-2000 and Sonehri-Sugdasi also have 100% similarity. Group IV consisted of 12 varieties, within this group Khushboo-95, Dokri-Basmati and Shakar have 100% similarity and Kanwal-95 and Shadab have 100% similarity and IR6(c) and DR92 have 100% similarity. Group V comprised of only 1 variety which was Mahlar-346 and this was the only variety which was separate from all other varieties and also this variety have minimum similarity of 0.22 with Purple-Marker. This variety was found to be the most diverse from all other varieties (Fig. 2). As a whole, the Dendrogram revealed low genetic diversity at protein level. This result was supported by Sultana *et al*., (2005), who reported a low to medium level of intra–specific variation for seed protein among rice (*Oryza sativa* L.) genotypes. Arun *et al*., (2010) demonstrated in a similar study, that their cluster analysis of 48 rice varieties revealed that 75% of total tested varieties (36 varieties) fell in the same group, indicating low genetic diversity at protein level. This is true in case of rice because the seed storage proteins are the major determinant of end use quality, which is a highly selected trait (Fufa *et al*., 2005) and therefore this could be a reason for low genetic diversity. Seed storage proteins from five different types of colored rice grains were analyzed using SDS-PAGE. Several proteins bands were detected only on specific rice and it could be as biochemical markers for further research (Sari *et al*., 2019). Out of 117 tested rice genotypes, 114 genotypes were in one cluster which also indicates low level genetic diversity (Shende *et al*., 2019). A total of thirty two rice varieties including both traditional and newly improved varieties were subjected to seed storage protein analysis using SDS-PAGE and silver staining. Resultant gel showed a total of 12 bands consisting of 4 monomorphic and 8 polymorphic bands (Vithyashini & Wickramasinghe1, 2015). So the SDS-PAGE offers a means for solid genotypes discrimination on the basis of genetic variation in seed protein/ polypeptides.

Table 2. Molecular weights of different bands found in 61 rice varieties using SDS-PAGE.

S. No.	Varieties	Band-1 (kDa)	Band-2 (kDa)	Band-3 (kDa)	Band-4 (kDa)	Band-5 (kDa)	Band-6 (kDa)	Band-7 (kDa)	Band-8 (kDa)	Band-9 (kDa)
1.	IR6	0	0	52.4	34.6	28.1	24.5	0	0	0
2.	Basmati-Pak	0	0	39.8	34.6	28.1	24.5	16.9	0	0
3.	Basmati-198	89.1	70.7	37.1	30.9	26.9	21.3	0	13.8	0
4.	PK177	0	0	52.4	31.6	26.9	0	0	0	0
5.	Basmati-370	83.1	79.4	39.8	31.6	28.1	22.3	0	14.1	0
6.	Sathra	100	85.1	41.6	35.4	30.9	24.5	18.1	14.4	0
7.	Mahlar-346	0	0	0	28.1	0	21.3	0	0	0
8.	Palman-Sufaid	0	0	39.8	34.6	30.9	23.4	0	0	0
9.	KS282	0	0	41.6	35.4	30.9	26.9	0	0	0
10.	Super-Basmati	0	0	39.8	34.6	30.9	23.4	18.1	14.4	0
11.	Kangni-27	0	0	39.8	30.9	27.5	21.3	16.9	13.8	0
12.	Pakhal	0	0	0	31.6	22.9	0	15.8	13.4	0
13.	Shaheen-Basmati	0	0	41.6	27.5	24.5	19.9	17.3	13.4	0
14.	Kashmir-Basmati	0	0	39.8	29.5	21.8	19.9	17.3	14.1	0
15.	KSK133	0	0	50.1	31.6	26.9	0	17.3	0	0
16.	Basmati-2000	125.8	97.7	45.7	31.6	29.5	21.8	15.8	14.1	0
17.	Shua-92	0	0	0	27.5	26.9	0	0	0	0
18.	Jajai-77	0	0	50.1	35.4	31.6	21.8	17.3	13.4	0
19.	KangnixTorh	0	0	45.7	35.4	27.5	26.9	17.3	13.4	0
20.	Sugdasi-Sadagulab	0	0	47.8	31.6	21.8	20.4	17.7	13.4	0
21.	DR83	0	0	0	44.6	0	0	19.9	18.1	0
22.	Khushboo-95	0	0	0	44.6	0	0	0	18.1	0
23.	Kanwal-95	0	0	0	38.9	0	28.1	0	17.3	0
24.	Dokri-Basmati	0	0	0	38.9	0	0	0	17.3	0
25.	Shakar	0	0	0	38.9	0	0	0	17.3	0
26.	IR 6(C)	0	0	0	50.1	36.3	0	0	19.9	0
27.	Sugdasi-Ratria	0	0	69.1	45.7	36	29.5	0	17.3	0
28.	Kasalath	0	0	66	44.6	36	29.5	0	16.9	0
29.	IR9	0	0	66	42.6	33.8	28.1	20.8	17.3	0
30.	Kharai-Ganja	0	0	66	44.6	31.6	28.1	21.8	19.9	0
31.	Basmati-C622	0	0	0	46.7	42.6	28.1	0	19.9	0
32.	Dehradune-Basmati2	0	0	63	47.8	35.4	31.6	0	22.3	0
33.	Azucena	0	0	63	50.1	38.9	35.4	33.8	22.3	0
34.	DR92	0	0	0	45.7	35.4	0	0	19.9	0
35.	Shadab	0	0	0	39.8	0	31.6	0	19.9	0
36.	Basmati-370a	0	0	63	50.1	45.6	31.6	0	22.3	0
37.	DR82	0	0	63	50.1	37.7	35.4	28.1	25.1	0
38.	Dehradun-Basmati1	0	0	63	50.1	39.8	35.4	0	22.3	0
39.	Basmati-217	0	0	79.4	44.6	39.8	35.4	28.1	22.3	0
40.	Sonehri-Sugdasi	125.8	112.2	56.2	44.6	35.4	31.6	25.1	19.9	0
41.	Nipponbare	0	0	0	42.6	0	28.1	25.1	19.9	0
42.	Dehraduni	89.1	77.6	39.8	30.9	0	20.4	15.8	12.3	0
43.	Dehradune	0	0	0	39.8	30.9	22.9	15.4	12.3	0
44.	Ranbir-Basmati	0	0	56.2	39.8	36.3	27.5	20.4	15.4	0
45.	Pusa-1121	0	0	51.2	39.8	34.6	27.5	20.4	15.4	0
46.	Pusa-Basmati	0	0	56.2	39.8	36.3	25.7	20.4	16.9	0
47.	TN1	0	0	56.2	45.7	38.9	29.5	27.5	16.9	0
48.	PAU-201	0	0	56.2	45.7	39.8	30.9	20.4	16.9	0
49.	Karnal-Basmati	0	0	56.2	44.6	38.9	29.5	20.4	16.9	0
50.	Basmati-370b	0	0	57.5	39.8	0	30.9	20.4	15.8	0
51.	Mushkan-Rice	0	0	50.1	39.8	34.6	25.1	17.7	14.7	0
52.	JP5	0	0	50.1	39.8	34.6	22.9	17.7	0	12.5
53.	Lateefy	95.4	72.4	50.1	37.1	29.5	20.8	15.4	14.7	10
54.	Punjab-Basmati	0	0	50.1	39.8	34.6	25.1	17.7	15.8	12.5
55.	Early-Basmati	95.4	0	50.1	39.8	0	25.1	15.4	14.1	0
56.	Purple-Marker	95.4	79.4	50.1	37.1	25.1	0	17.7	15.8	0
57.	PK386	95.4	72.4	50.1	39.8	34.6	25.1	0	13.4	0
58.	Type-3	0	0	44.6	39.8	34.6	25.1	0	14.1	0
59.	Mutant-370	0	0	56.2	39.8	37.1	28.1	15.8	14.1	0
60.	IR36	0	0	56.2	44.6	39.8	29.5	20.8	14.1	0
61.	Dehradun-Basmati3	0	0	63	44.6	39.8	28.1	19.9	15.8	0

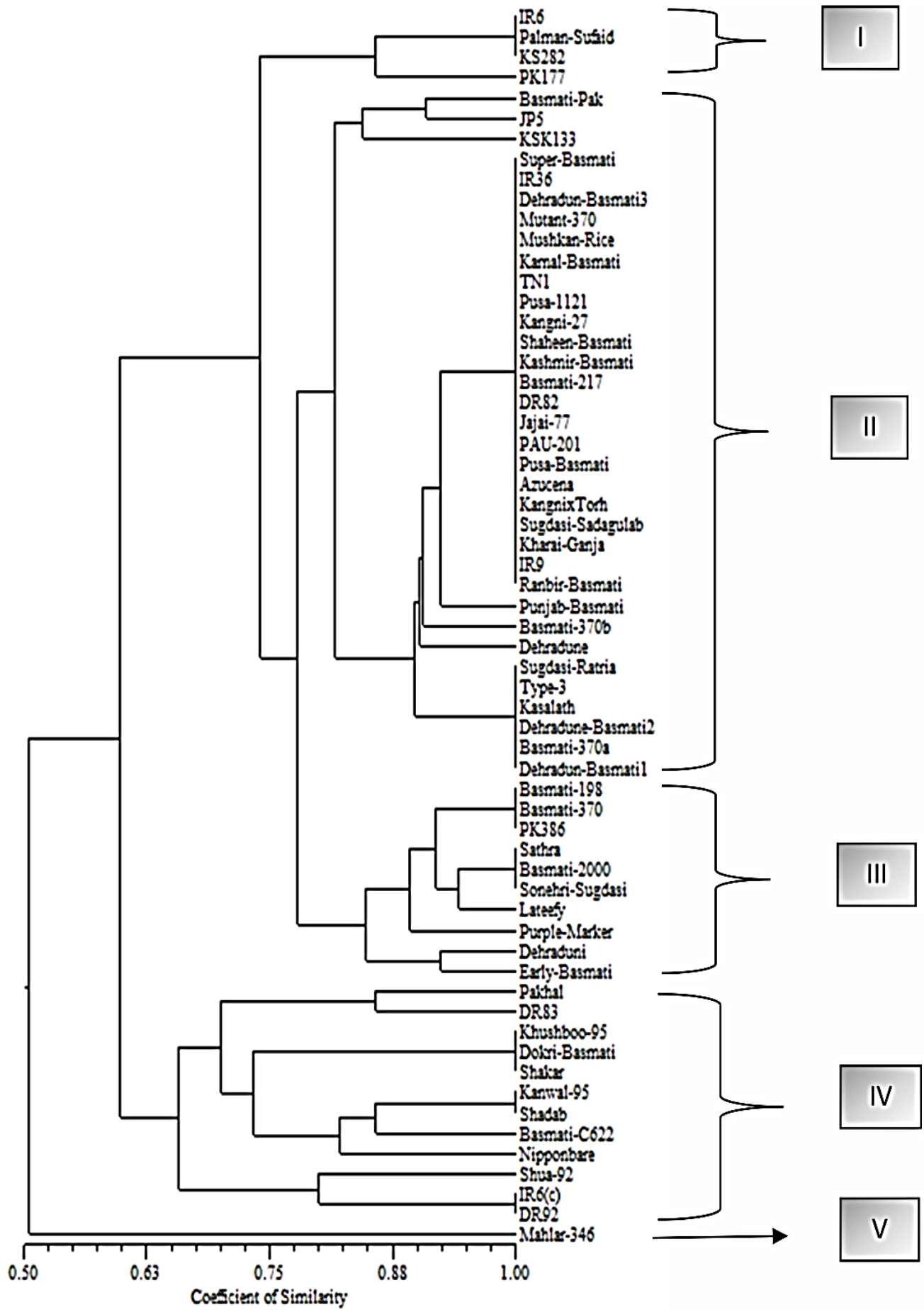

Fig. 2. Dendrogram showing banding pattern associated with polymorphism in the seed storage proteins of rice using (SDS-PAGE) data.

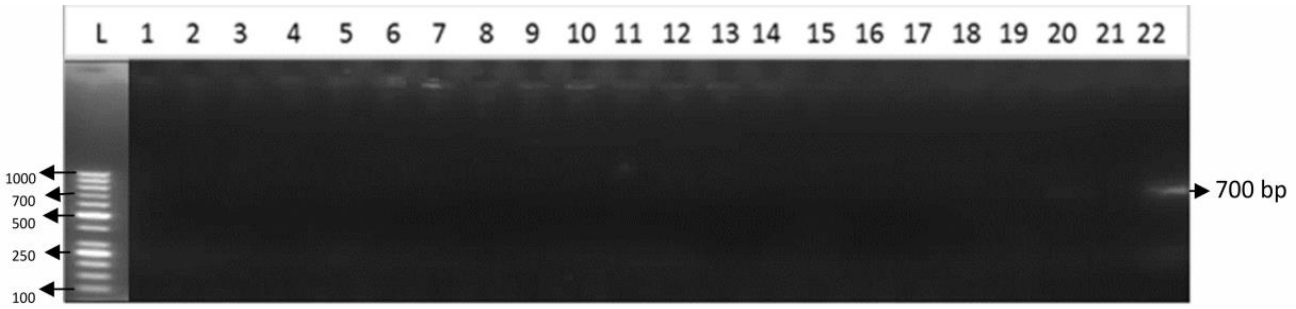

Fig. 3A. DNA banding pattern of 22 rice varieties for identification of Bacterial Blight *Xa21* gene Lane L= 50bp gene ruler, Lane1= Basmati-370, Lane 2= Sathra, Lane 3= Mahlar-346, Lane 4= Palmansufaid, Lane 5= Basmati-C622, Lane 6=Basmati-Pak, Lane 7= PK-177, Lane 8= KS-282, Lane 9= Shaheen-Basmati, Lane 10= Kashmir-Basmati, Lane 11= Kangni-27, Lane 12= Sugdasi-Sadagulab, Lane 13= Sonahri-Sugdasi, Lane 14= Dokri-Basmati, Lane 15= Kharai-ganja, Lane 16= IR6, Lane 17= Lateefy, Lane 18= Shua-92, Lane 19= Shadab, Lane 20= IR-36, Lane 21= Kasalath, Lane 22= TN1.

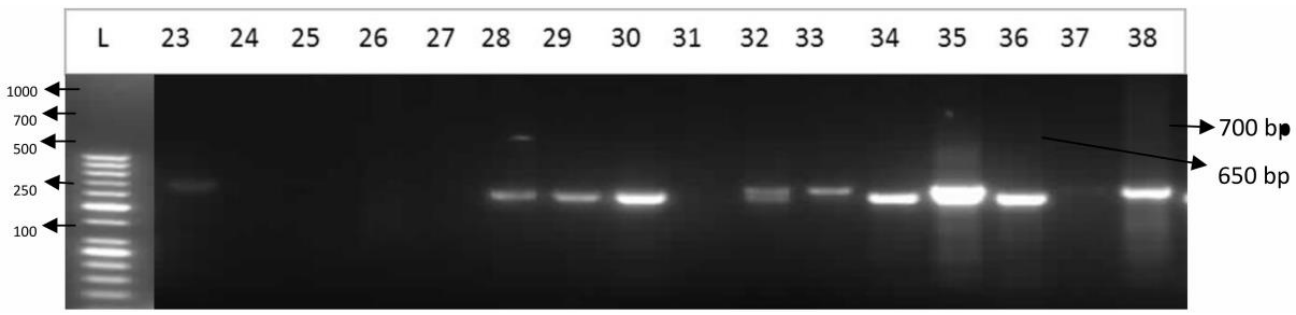

Fig. 3B. DNA banding pattern of various rice varieties for identification of Bacterial Blight *Xa21* gene. Lane L= 50bp gene ruler, L 23= Dehradun-Basmati, L 24= Basmati-217, L 25= Punjab-Basmati1, L 26= Pusa-Basmati1, L 27= Ranbir-Basmati, L 28= PK-386, L 29= Pusa-1121, L 30= PAU-201, L 31= Mutant-370, L 32= Early-Basmati, L 33= Pank-Iraj, L 34= Karnal-Basmati, L 35= ARC10025, L 36= Aus-133, L 37= Aus-176, L 38= Lashmi-Digha.

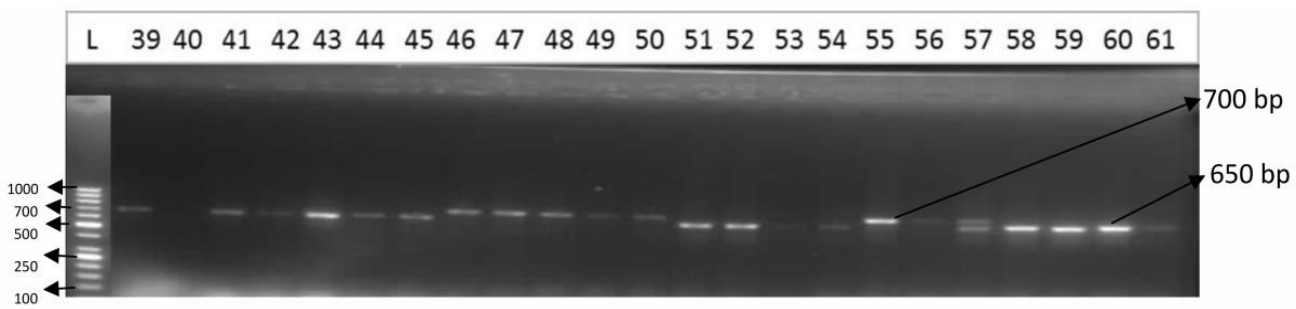

Fig. 3C. DNA banding pattern of various rice varieties for identification of Bacterial Blight *Xa21* gene. Lane L= 50bp gene ruler, L 39= Bhaturi, L 40= Aus-190, L 41= Aus-346, L 42= Basmati-198, L 43= Korchampoori, L 44= Basmati-2000, L 45= KSK-133, L 46= Naroi, L 47= Pakhal, L 48= Jajai-77, L 49= Kangnix-Torh, L 50= DR-83, L 51= Sugdasi-Ratri, L 52= Saita, L 53= DR-82, L 54= IR-9, L 55= Balla-bokri, L 56= DR-92. L 57= Kanwal-95, L 58= Shakar, L 59= Khushboo-95, L 60= Nipponbare, L 61= Azucena.

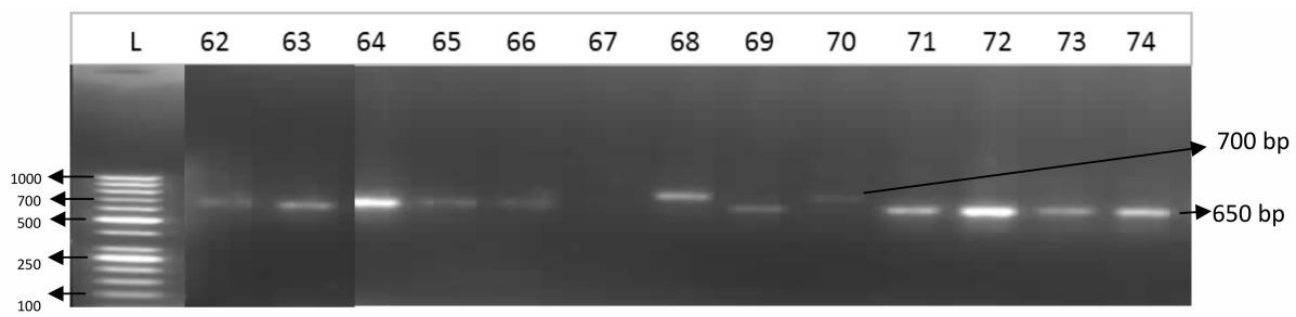

Fig. 3D. DNA banding pattern of various rice varieties for identification of Bacterial Blight *Xa21* gene. Lane L= 50bp gene ruler, L 62= Dehradun Basmatia, L 63= Basmati-370a, L 64= Dehradun-Basmatib, L 65= Bas.370b, L 66= Mushkan-Rice, L 67= Purple-Marker, L 68= IR6, L 69= Dehraduni, L 70= Dehradune, L 71= Baturi, L 72= Type-3, L 73= JP-5, L 74= Super-Basmati.

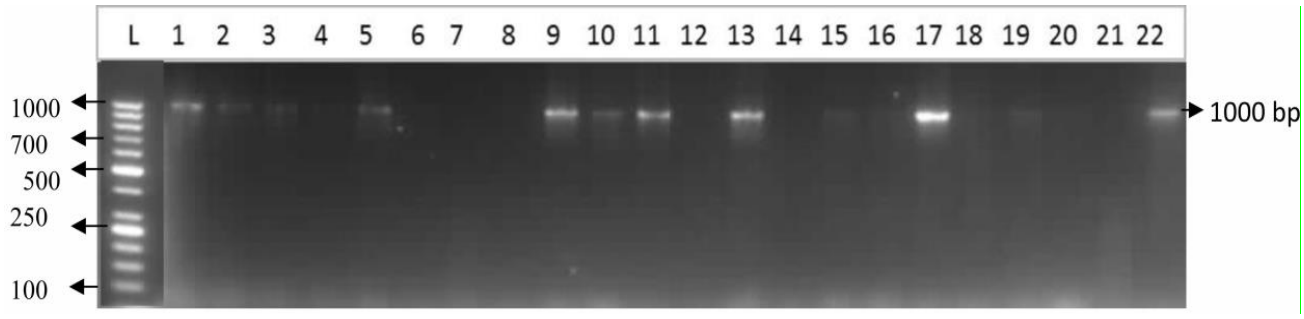

Fig.4A. DNA banding pattern of various rice varieties for identification of Bacterial Blight *Xa13* gene. Lane L= 50bp gene ruler, Lane1= Basmati-370, Lane 2= Sathra, Lane 3= Mahlar-346, Lane 4= Palmansufaid, Lane 5= Basmati-C622, Lane 6=Basmati-Pak, Lane 7= PK-177, Lane 8= KS-282, Lane 9= Shaheen-Basmati, Lane 10= Kashmir-Basmati, Lane 11= Kangni-27, Lane 12= Sugdasi-Sadagulab, Lane 13= Sonahri-Sugdasi, Lane 14= Dokri-Basmati, Lane 15= Kharai-ganja, Lane 16= IR6, Lane 17= Lateefy, Lane 18= Shua-92, Lane 19= Shadab, Lane 20= IR-36, Lane 21= Kasalath, Lane 22= TN1.

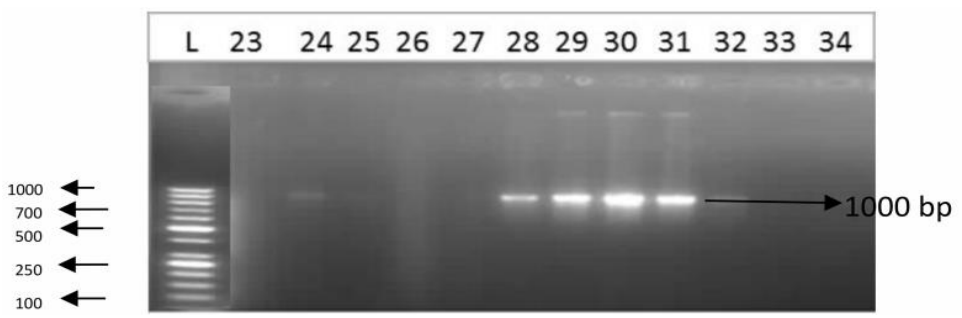

Fig. 4B. DNA banding pattern of rice varieties for identification of Bacterial Blight *Xa13* gene. Lane L= 50bp gene ruler, L 23= Dehradun-Basmati, L 24= Basmati-217, L 25= Punjab-Basmati1, L 26= Pusa-Basmati1, L 27= Ranbir-Basmati, L 28= PK-386, L 29= Pusa-1121, L 30= PAU-201, L 31= Mutant-370, L 32= Early-Basmati, L 33= Pank-Iraj, L 34= Karnal-Basmati.

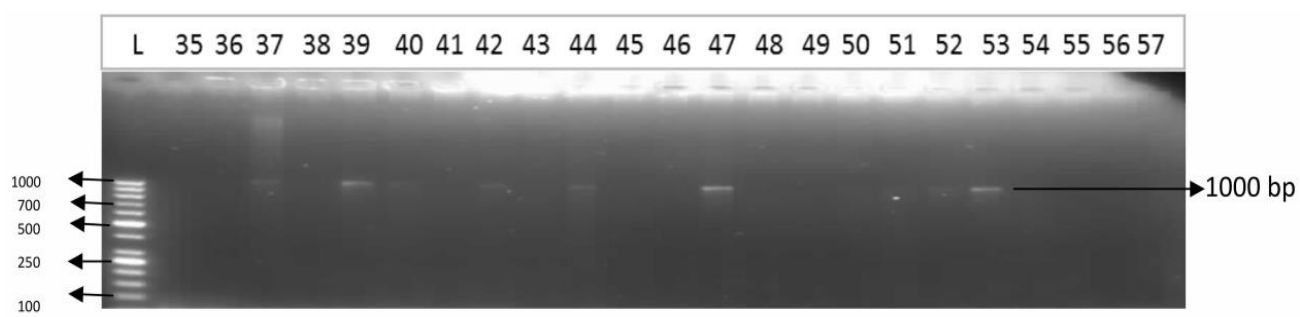

Fig. 4C. DNA banding pattern of various rice varieties for identification of Bacterial Blight *Xa13* gene. Lane L= 50bp gene ruler, L 35= ARC10025, L 36= Aus-133, L 37= Aus-176, L 38= Lashmi-Digha, L 39= Bhaturi, L 40= Aus-190, L 41= Aus-346, L 42= Basmati-198, L 43= Korchampoori, L 44= Basmati-2000, L 45= KSK-133, L 46= Naroi, L 47= Pakhal, L 48= Jajai-77, L 49= Kangnix-Torh, L 50= DR-83, L 51= Sugdasi-Ratri, L 52= Saita, L 53= DR-82, L 54= IR-9, L 55= Balla-bokri, L 56= DR-92. L 57= Kanwal-95.

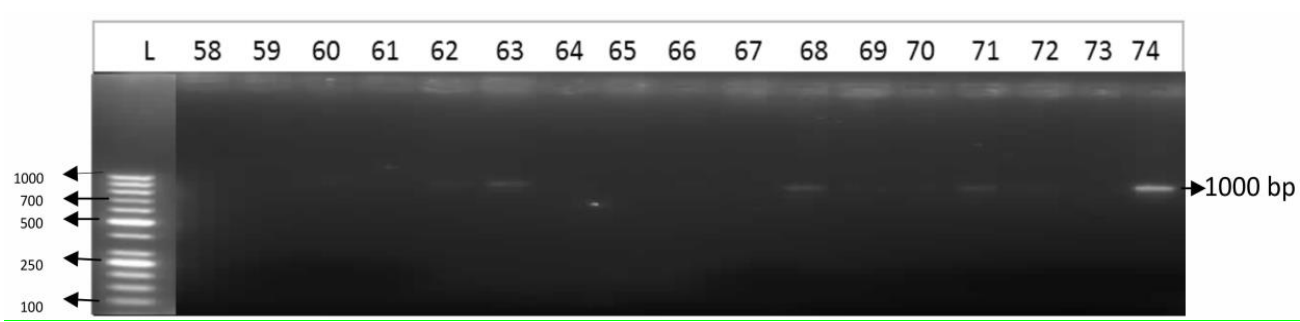

Fig. 4D. DNA banding pattern of various rice varieties for identification of Bacterial Blight *Xa13* gene. Lane L= 50bp gene ruler, L 58= Shakar, L 59= Khushboo-95, L 60= Nipponbare, L 61= Azucena, L 62= Dehradun Basmatia, L 63= Basmati-370a, L 64= Dehradun-Basmatib, L 65= Bas.370b, L 66= Mushkan-Rice, L 67= Purple-Marker, L 68= IR6, L 69= Dehraduni, L 70= Dehradune, L 71= Baturi, L 72= Type-3, L 73= JP-5, L 74= Super-Basmati.

Molecular identification of *Xa21* and *Xa13* bacterial blight resistance gene fragment: The primer amplified fragment of 700bp and 650bp in rice varieties. *Xa21* gene fragment was present (+) in 43 varieties and absent (-) in 31 varieties (Fig. 3A, B, C, D). The varieties in which *Xa21* gene was present are TN1, Dehradun-Basmati, PK-386, Pusa-1121, PAU-201, Early Basmati, Pank-Iraj, Karnal-Basmati, ARC10025, Aus-133, Lashmi-Digha, Bhaturi, Aus-346, Basmati-198, Korchampoori, Basmati-2000, KSK-133, Naroi, Pakhal, Jajai-77, Kangnix-Torh, DR-83, Sugdasi-Ratri, Saita, IR-9, Balla-Bokri, DR-92, Kanwal-95, Shakar, Khushboo-95, Nipponbare, Azucena, Dehradun-Basmatia, Basmati-370a, Dehradun-Basmatib, Basmati-370b, Mushkan-Rice, IR6, Dehraduni, Dehradune, Baturi, Type-3, JP5, and Super-Basmati. DNA fragment (pTA248) of 700bp linked to Xa21 gene has been reported by Abbasi *et al*., (2011). The primer amplified fragment of 1000 bp in rice varieties. Out of 74 rice varieties *Xa13* gene fragment was present (+) in 30 varieties and absent (-) in 44 varieties (Fig. 4A, B, C, D). The varieties in which *Xa13* gene was present are Basmati-370, Sathra, Mahlar-346, Basmati-C622, Shaheen-Basmati, Kashmir-Basmati, Kangni-27, Sonehri-Sugdasi, Kharai-Ganga, Lateefy, Shadab, TN1, Bas.217, PK-386, Pusa-1121, PAU-201, Mutant-370, Early Basmati, Aus-176, Bhaturi, Basmati-198, Basmati-2000, Pakhal, Saita, DR-82, Dehradun-Basmatia, Basmati-370a, IR6, Baturi, and Super-Basmati (Fig. 4A, B, C, D). Xa13 linked gene fragment of 1000 bp has been reported by Abbasi *et al*., (2011).

Molecular evaluation of 74 rice varieties for Bacterial blight resistance genes *Xa21* and *Xa13* was performed. These genes were present in some varieties and absent in others. Kadu *et al*., (2015) did similar research in which they employed molecular analysis in the F2 population of cross Dubraj and RP-Bio 226 for presence of Bacterial Blight resistance genes (*Xa5, Xa13* and *Xa21)* and identified 120 plants with single resistance gene and with combinations of two and three resistance genes. Similar results were reported by Basavaraj *et al*., (2010) introgressed two BB resistance genes *Xa13* and *Xa21* into the restorer line PRR78 and maintainer line Pusa 6B of the Basmati quality hybrid Pusa RH10. Fourteen plants identified with only *Xa21* gene, among them eight plants (32, 34, 41, 43, 45, 47, 48 & 49) were found to be heterozygous and six (10, 18 31, 33, 42 & 44) plants were homozygous. Shalini *et al*., (2016) used STS marker pTA248 for *Xa21* gene and functional markers for genes *Xa13* and *Xa5.* They identified thirteen plants containing *Xa13* and *Xa21* and fourteen with *Xa21* gene out of fifty plants screened. Since the most effective 'R' genes are *Xa21* and *Xa13*, a combination of these two genes will be a natural choice.

Conclusion

Pakistan and genotypics this study. The information about the genetic diversity found in rice varieties used in present study will be useful in identification and selection of suitable parents for future breeding programs to develop unique germplasm that complement existing varieties. The varieties having resistance genes *Xa21* and *Xa13* will be used for bacterial blight resistant breeding program. It is suggested to do further research work using SNP markers to carry out fingerprinting study as SNPs are highly abundant and less susceptible to mutations.

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