Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; SP1: 456-460

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Molecular screening of blast resistant gene in thirty rice (*Oryza sativa*) genotypes through Blast specific primers

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Abstract
Rice is the first cereal genome with a complete sequence and a model crop that has important relationships with other cereal species. It has a small genome size of 430 Mb (IRGSP, 2005) which is one-tenth the size of the human genome. The complete sequence is available for both *japonica* and *Indica* genomes. Among the thirty rice genotypes, PB-2 showed the minimum disease severity at 7 DAI (1.40%) and at 14 DAI (1.50%) followed by Tetep showed the minimum disease severity at 7 DAI (1.40%) and at 14 DAI (1.50%) from tillering stages whereas, Basmati-370 showed the maximum disease severity at 7 DAI (3.90%) and at 14 DAI (3.93%) from tillering stages. The five genotypes (Ranbeer Basmati, PB-1, VB-23, BPT-5204 and B-370) which showed the absence of Blast resistance genes indicated the highest AUDPC (65-84), therefore regarded as BLAST susceptible genotypes. The five genotypes (HB-1, N-22, PR-106, Tetep and PB-2) which showed the absence of Blast resistance genes indicated the lowest AUDPC (6-12), therefore regarded as Blast resistant genotypes.

Keywords: Rice, Blast, molecular Markers

Introduction
Rice is the first cereal genome with a complete sequence and a model crop that has important relationships with other cereal species. It has a small genome size of 430 Mb (IRGSP, 2005) which is one-tenth the size of the human genome. The complete sequence is available for both *japonica* (Goff *et al.*, 2002) [1] and *Indica* (Yu *et al.*, 2002) [2] genomes. According to some earlier estimates, if the number of genes in each of the cereal genomes is considered to be about 30,000, rice will have an average of approximately one gene every 15-kilo base pairs (kbp). However, recent data place the number of genes about 50,000 (Goff *et al.,* 2002 and Yu *et al.,* 2002) [1, 2], reflecting even higher gene density. The International Rice Genome Sequencing Project (IRGSP) reported a total of 37,544 non-transposable element protein-coding genes in the whole rice genome (International Rice Genome Sequencing Project 2005). These included disease-resistance (R) genes, which are also called resistance gene analogs (RGAs) due to their sequence homology to cloned resistance genes or to those involved in defense-related mechanisms.

Yield loss due to Blast can be as high as 50% when the disease occurs in epidemic proportions. *Magnaporthe grisea* (Hebert) Barr (syn: *Pyricularia grisea* Sacc.), a filamentous heterothallic ascomycetous fungus is the causal organism of the Blast. The genus *Magnaporthe* collectively parasitizes more than 50 hosts, individual isolates have limited host range and cross-infectivity is relatively rare. The ability of this fungus to quickly overcome resistance within a short time after the release of a new cultivar has made breeding for resistance a constant challenge. An understanding of the structure and dynamics of pathogen population is essential for the prudent implementation of strategies for management of the disease. Marker-assisted backcrossing has enormous potential to introduce the Blast resistance genes into diverse rice cultivars (Collard *et al.*, 2008) [3]. Introgression of Blast resistant genes into advanced improved rice lines is a cost-effective and environmentally friendly approach to combat yield losses (Wen and Gao, 2012) [4]. The main advantage of marker-assisted selection is the accuracy of selection of the true plant within the short breeding cycle to produce Blast resistant rice varieties. Currently, the Blast resistant breeding program has achieved greater success with the advent of marker-assisted selection (Ragimekula *et al.*, 2013) [5].

Material and Methods Plant Materials
In the present study, a total of thirty rice genotypes collected from various parts of India

(Table 1) were screened for the identification of Blast resistance genes (*Pi-1, Pi-2, Pik, Pi-5, Pi-9 and Pi-b*). The leaf sample of all the thirty rice genotypes were collected separately from the Crop Research Centre, of Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh (North West Plains Zone, India, 28.99ºN and 77.70ºE) in the year 2013. 100-120 plants from each rice genotype were planted in separate plots with three replications.

Table 1: List of thirty rice genotypes used for screening.

S.N.	Varieties	Sources	Characteristics of the phenotypes
1	Improved Samba Mahsuri	Hyderabad	Semi dwarf, resistant to BLB, coarse grain
2	Improved PB 1	New Delhi	Super fine, semi dwarf, BLB resistant
3	Samba Mahsuri	Hyderabad	Semi dwarf, coarse grain
4	IR 29	Philippines	Mid-duration, aerobic, non-scented. IR29 is resistant to certain bacterial groups.
5	VLD-85	Almora	Semi dwarf, short duration, coarse grain
6	HB-1	HAU, Haryana	Basmati. Super fine, long duration, semi dwarf
7	Vallabh Basmati-21	Meerut	Super fine, long grain, semi dwarf, short duration
8	CSR-23	Karnal	Semi dwarf, salt tolerant, coarse grain
9	IRBB-16	New Delhi	Semi dwarf
10	NDR-118	Faizabad	dwarf, non scented, medium duration
11	Pusa Basmati-1509	New Delhi	Long grain, semi dwarf, short duration
12	PB-1	New Delhi	Super fine, long grain, semi dwarf, short duration
13	Basmati-370	Punjab	Poor plant type, grains long slender type
14	Basmati-386	Punjab	Traditional basmati rice variety
15	N-22	Nagina, U.P.	Semi dwarf, non scented, salinity resistant
16	CSR-10	Karnal	Semi dwarf, salt tolerant, coarse grain
17	PR-106	Punjab	Dwarf, short duration, coarse grain,
18	Vallabh Bangni	Meerut	Dwarf, medium duration, violet color foliage
19	Vallabh Basmati-22	Meerut	Super fine, semi dwarf, photosensitive
20	CSR-30	Karnal	Semi dwarf, salt tolerant, coarse grain
21	Vallabh Basmati-23	Meerut	Super fine, long grain, semi dwarf, medium duration
22	Vallabh Basmati-24	Meerut	Super fine, long grain, semi dwarf, medium duration
23	IR-64	Philippines	Medium bold grain
24	Ranbir Basmati	J&K	Basmati, tall, short duration
25	Tetep	Vietnam	Blast resistance, tall, coarse grain
26	PB-2	New Delhi.	Super fine, semi dwarf, export quality
27	Taroari Basmati	HAU, Haryana	Basmati, tall, long duration, export quality
28	Type-3	Nagina, U.P	Semi dwarf, basmati
29	CSR-27	Karnal	Semi dwarf, salt tolerant, coarse grain
30	Pusa-1401	New Delhi	Basmati aromatic semi dwarf, medium duration

Multiplication and maintaining of Blast culture

Isolation and multiplication of inoculums

Inoculums were prepared as described by Pan *et al.,* (1996) [6]. Blast infected leaf samples were collected from Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U.P. (India). Infected leaf samples having spores and mycelium of *Pyricularia oryzae* in abundance were surface sterilized and thin leaf sections from these leaves were placed on Potato Dextrose Agar (PDA) in the Petri plates and incubated at 28ºC for proper growth.

Inoculums having a concentration of 10×10^4 to 50×10^4 conidia per ml were used for the inoculation of plants. The inoculums suspension of the Blast was prepared in Molecular Biology Laboratory, of the Department of Genetics and Plant Breeding.

Creation of artificial epiphytotic conditions

Blast disease was induced by inoculating of Blast pathogen in the field. A pure culture of most aggressive isolate of *Pyricularia oryzae* characterized and multiplied at our Center, was uniformly applied on all crosses by hand sprayer at three growth stages *viz*., crown root initiation, seedling emergence, and tillering stages during the evening hours following the method of Chaurasia *et al.,* (1999) [7]. Plots were irrigated immediately after inoculation to maintain high relative humidity, which facilitates spore germination and disease development. Fields were frequently irrigated to induce environmental conditions conducive to Blast pathogen.

Total genomic DNA isolation

Genomic DNA of all the thirty rice genotypes was isolated from fresh, healthy and young leaf tissues from twenty days-old seedlings using CTAB (Cetyl- Tri Methyl Ammonium Bromide) method (Murray and Thompson, 1980). The DNA was purified by adding RNase (10µg/100mL) to the samples at the rate of 1.0µL/100 ml of crude DNA. DNA quantification was done using 0.8% Agarose gel. The uncut DNA was used as standard and the final concentration was adjusted to 25µg/µL and stored at -20°C for further use. In the present study, previously reported gene specific markers were used for the detection of the presence of BLAST resistance genes the primers were synthesized from Bangalore Genei, Bangalore, India.

Field based disease assessment and sampling

The PCR products were measured as polymorphic bands pattern. The data was scored using "+" sign for presence of resistant genes and "–" sign for those having no resistant genes in (Table 2). Disease evaluation was started 30 days after seeding and continued for two observations at 7 days after inoculation and 14 days after inoculation for leaf Blast and infection type (qualitative resistant to Blast) was measured based on a scale of 0.0 to 5.0 that, resistant (R) = 0.0 to 2.0; intermediate resistant (MR) = 2.1 to 3.0 and susceptible (S) = 3.1 to 5.0. The severity of disease in a field

was recorded as a percentage of tissue area infected out of total leaf area examined. Percentage average lesion area of 15 leaves collected was measured for disease severity in the field. Following scale was used for scoring of Blast severity in the field (Pasha, *et al.* 2013) [8].

Results and Discussion

Blast is a most devastating rice disease, and the current rice cultivars including basmati have narrow genetic base excluding Blast resistance genes. Therefore, rice growers experience significant yield losses every year. This genetic bottleneck can be efficiently resolved by incorporating and pyramiding multiple Blast resistance genes through marker-assisted breeding methods. Till date, Several Blast resistance genes have been identified and characterized in rice and incorporated and pyramided through MAS to develop resistant cultivars (Babujee and Gnanamanickam, 2000, Barman (2004) [9, 10]. Kumar *et al*., 2010 and Padmavathi *et al*., 2004) [11, 12]. The identification and utilization of genes conferring Blast resistance from rice landraces to high-yielding susceptible, commercial rice cultivars is a more efficient way to combat Blast disease without compromising with the grain quality and will also help broaden the genetic base of rice genotypes. The knowledge of the effective resistance genes and the pathogen population structure would be helpful in deploying the suitable resistance genes in different rice growing areas (Jeger 2004) [13]. Therefore, in the present study, we have reported the gene linked marker based identification of thirty rice genotypes for Blast resistance and these results were also validated with disease indicating parameters.

Identification, characterization and validation of (*Pi-1, Pi-2, Pik, Pi-5, Pi-9 and Pi-b*) genes with morphological data

Amplification of *Pi1* gene carrying of rice germplasm by SSR marker showed the Blast resistant (R) fragment as 480 bp. Attempts have been made to explore Indian rice germplasm for bacterial blight resistance genes viz. (*Pi-1, Pi-2, Pik, Pi-5, Pi-9 and Pi-b*). Molecular and conventional approaches were used for confirming the presence of these genes. Out of thirty rice genotypes, eighteen genotypes viz., HB-1, CSR-23, IRBB-16, NDR-118, Basmati-370, PB-1, PR-106, Vallabh Bangni, Vallabh Basmati-22, CSR-30, Vallabh Basmati-23, Vallabh Basmati-24, IR-64, Taroari Basmati, PB-2, Tetep, Type-3 and CSR-27 showed the appropriate amplification for Blast resistant fragments of *Pi1* gene, The existence of *Pi2* gene in rice germplasm was amplified through SSR marker and showed the 1100 bp positive fragment. Out of thirty, nine genotypes viz., Samba Mahsuri, IR 29, HB-1, Vallabh Basmati-21, Basmati-370, Basmati-386, N-22, CSR-10 and Taroari Basmati showed the appropriate amplification of 1100 bp for Blast resistant fragments of *Pi2* gene, Examination of Pik^h gene carrying rice germplasm by SSR marker showed the Blast resistant (R) fragment as 190 bp (Fig. 4.4.3). Out of thirty genotypes, twenty two rice genotypes *viz.,* Improved Samba Mahsuri, Improved Pusa Basmati 1, Samba Mahsuri, IR 29, VLD-85, HB-1, Vallabh Basmati-21, CSR-23, IRBB-16, Basmati-370, PB-1, Basmati-386, N-22, Vallabh Bangni, Vallabh Basmati-22, Vallabh Basmati-23, Vallabh Basmati - 24, Ranbir Basmati, PB-2, Type-3, Tetep and Pusa-1401 showed the appropriate amplification for Blast resistant fragments of *pikh* gene, Amplification of *Pi5* gene carrying of rice germplasm by SSR marker showed the Blast resistant (R) fragment as 750 bp. Out of thirty rice genotypes, fourteen viz., Improved Samba Mahsuri, Improved Pusa Basmati 1, Samba Mahsuri, IR 29, IRBB-16, NDR-118, Pusa Basmati-1509, Basmati-386, PR-106, Vallabh Bangni, Vallabh Basmati-22, CSR-30, Ranbir Basmati and PB-2) showed the appropriate amplification for Blast resistant fragments of *Pi5* gene, The existence of *Pi9* gene in rice germplasm was amplified through SSR marker and showed the 1500 bp positive fragment. Out of thirty rice genotypes, twenty four genotypes viz., Improved Samba Mahsuri, Improved Pusa Basmati 1, IR 29, VLD-85, HB-1, Vallabh Basmati-21, CSR-23, IRBB-16, NDR-118, Basmati-370, Basmati-386, N-22, CSR-10, PR-106, Vallabh Bangni, Vallabh Basmati-22, CSR-30, Vallabh Basmati-23, IR-64, Ranbir Basmati, Taroari Basmati, Tetep, Type-3 and CSR-27 showed appropriate amplification of 1500 bp for Blast resistant fragments of *Pi9* gene and Examination of *Pib* gene carrying rice germplasm by SSR marker showed the Blast resistant (R) fragment as 380 bp (Fig. 4.4.6). Out of thirty rice genotypes, nineteen genotypes namely, Improved Samba Mahsuri, Improved Pusa Basmati 1, VLD-85, Vallabh Basmati-21, CSR-23, NDR-118, Basmati-370, PB-1, Pusa Basmati-1509, Basmati-386, N-22, CSR-10, PR-106, CSR-30, Vallabh Basmati-24, IR-64, Ranbir Basmati, Tetep and PB-2 showed the appropriate amplification for Blast resistant fragments of *Pib* gene. After screening, five genotypes viz., HB-1, N-22, PR-106, Tetep and PB-2 were selected as resistant and five genotypes i.e. Basmati-370, CSR-10, Vallabh Basmati-24, CSR-27 and Pusa-1401 were selected as susceptible the similar result were found (Kumar *et al.*, 2010) [11]. Blast disease of rice, caused by the ascomycete fungus *Magnaporthe oryzae* (formerly known as *Magnaporthe grisea*, (Padmavathi 2004) [12], is one of the most devastating diseases of rice (*Oryzae sativa* L.) worldwide and its frequent appearance during all stages of plant growth greatly decreases yield and grain quality due to Blast disease (Kumar *et al.,* 2010) [11]. However, AUDPC is considered as the best parameter to declare a variety resistant or susceptible (Jeger, 2004) [13]. AUDPC provide more precise and pragmatic classification of resistance and susceptible genotypes (Chaurasia *et al*., 1999 and Jager, 2004) [7, 13] than that based on the % disease score of each genotype. The segregating populations thus obtained should be handled as per pedigree method (Allard, 1960). Tightly linked DNA markers may facilitate early selection for Blast resistance genes in breeding programs. These markers may also be useful to map new genes for resistance to Blast isolates. Closely linked molecular markers are likely to enhance (Mehla *et al*., 2011) [14] the efficiency of selection of resistant genotypes in rice breeding programs.

Morphological data also validated with the molecular data and confirmed the pattern of Blast resistance in rice genotypes on the basis of lesion length on leaf surfaces. Among the thirty rice genotypes, PB-2 showed the minimum disease severity at 7 DAI (1.40%) and at 14 DAI (1.50%) followed by Tetep showed the minimum disease severity at 7 DAI (1.40%) and at 14 DAI (1.50%) from tillering stages whereas, Basmati-370 showed the maximum disease severity at 7 DAI (3.90%) and at 14 DAI (3.93%) from tillering stages. The five genotypes (Basmati-370, CSR-10, VB-24, CSR-27 and Pusa Basmati-1401) which showed the indicated the highest AUDPC (65-84), therefore regarded as BLAST susceptible genotypes. The five genotypes (HB-1, N-22, PR-106, Tetep and PB-2) which showed the Blast resistance genes indicated the lowest AUDPC (6-12), therefore regarded as Blast resistant genotypes in (Table 3). The present investigation demonstrates the potential of molecular markers in identification of genes governing agronomically desirable traits.

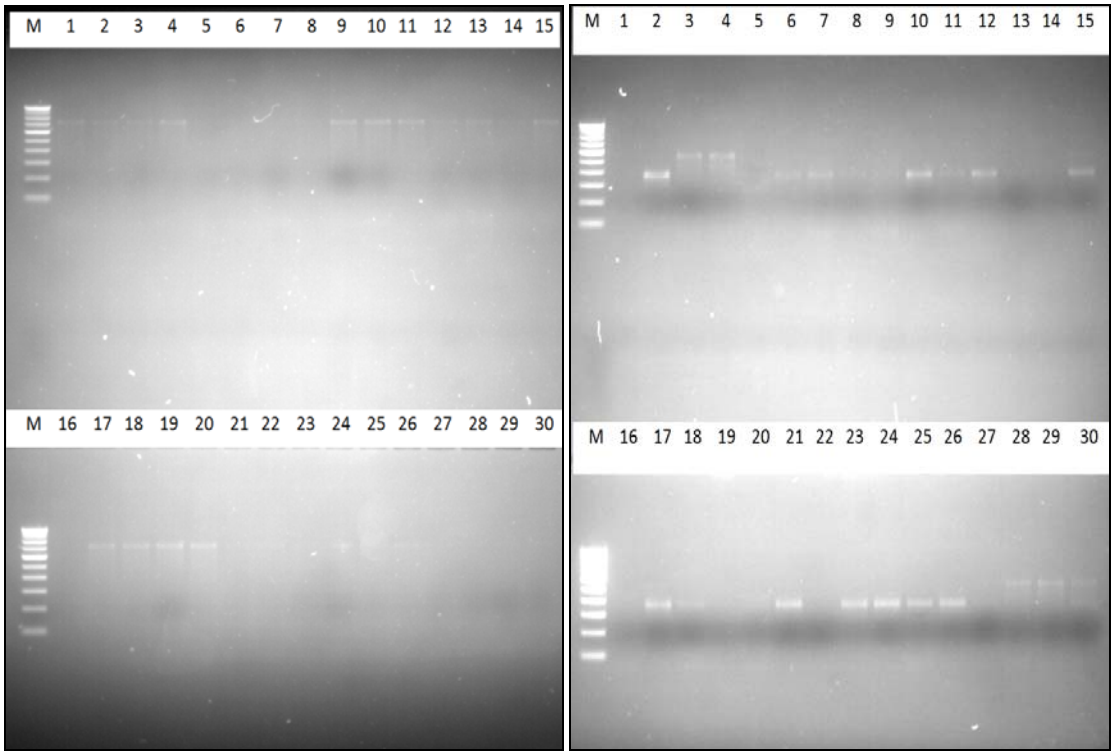

Fig 1: The amplification product of *Pi5 and Pib* gene specific primer in 30 rice genotypes Approx.750 and 380 bp respectively amplification product of *Pi5* gene. M represents 100 bp ladder.

Table 2: Blast resistance genes present in thirty rice genotypes.

S. No.	Genotype	Amplification of Blast resistance gene					
		Pi1	***Pi2***	***Pik^h***	***Pi5***	***Pi9***	***Pib***
1	Improved Samba Mahsuri	-	-	+	+	+	-
2	Improved PB1	-	-	+	+	+	+
3	Samba Mahsuri	-	+	+	+	-	-
4	IR 29	-	+	+	+	+	-
5	VLD-85	-	-	+	-	+	+
6	HB-1	+	+	+	-	+	-
7	Vallabh Basmati -21	-	+	+	-	+	+
8	CSR-23	+	-	+	-	+	-
9	IRBB-16	+	-	+	+	+	-
10	NDR-118	+	-	-	+	+	+
11	Pusa Basmati-1509	+	+	+	+	-	-
12	PB-1	+	-	+	-	-	+
13	Basmati-370	-	-	-	+	+	-
14	Basmati-386	-	+	+	+	+	-
15	N-22	-	+	+	-	+	+
16	CSR-10	-	+	-	-	+	-
17	PR-106	+	-	-	+	+	+
18	Vallabh Bangni	+	-	+	+	+	-
19	Vallabh Basmati-22	+	-	+	+	+	-
20	CSR-30	+	-	-	+	+	-
21	Vallabh Basmati-23	+	-	+	-	+	+
22	Vallabh Basmati-24	+	-	+	-	-	-
23	IR-64	+	-	-	-	+	+
24	Ranbir Basmati	-	-	+	+	+	+
25	Tetep	+	-	+	+	+	-
26	PB-2	+	-	+	+	-	+
27	Taroari Basmati	+	+	-	-	+	-
28	Type-3	+	-	+	-	+	-
29	CSR-27	+	-	-	-	+	-
30	Pusa-1401	-	-	+	-	-	-

(+) indicate present of band and (–) indicate absent of band.

Table 3: Mean performance of Disease severity and AUDPC 30 rice genotypes characters

S. No	Genotypes	Disease severity (%)		AUDPC
		7DAI	14DAI	
1	Improved Samba Mahsuri	2.47	2.73	20
2	IPB1	2.03	2.21	11
3	Samba Mahsuri	1.80	2.20	13
4	IR 29	2.73	3.00	36
5	VLD-85	1.73	2.27	12
6	HB-1	1.70	2.00	8.0
7	Vallabh Basmati - 21	3.80	3.60	16
8	CSR-23	3.70	3.20	45
9	IRBB-16	2.60	3.30	36
10	NDR-118	3.73	3.33	56
11	Basmati-370	3.90	3.93	85
12	Pusa Basmati-1	2.33	2.27	48
13	Pusa Basmati-1509	2.70	2.00	31
14	Basmati-386	2.40	3.10	16
15	Nagina-22	1.82	1.93	7.0
16	CSR-10	3.80	4.00	65
17	PR -106	1.90	2.00	12
18	Vallabh Bangani	2.80	2.31	34
19	VB-22	1.20	1.20	14
20	CSR -30	3.60	3.10	18
21	Vallabh Basmati - 23	1.70	2.50	38
22	Vallabh Basmati -24	3.00	2.53	74
23	IR-64	2.80	2.90	26
24	Ranvir basmati	2.40	2.50	18
25	Tarori Basmati	3.33	4.00	78
26	Pusa Basmati-2	1.40	1.50	9.0
27	Tetep	1.70	1.80	6.0
28	Type-3	3.00	3.12	62
29	CSR -27	3.60	2.87	72
30	Pusa -1401	3.00	3.27	68

Reference

1. Goff SA, Ricke D, Lan TH, Presting G, Wang R Dunn M. A draft sequence of the rice genome (*Oryza sativa L. ssp. japonica*). Science. 2002; 296:92-100.
2. Yu J, Hu S, Wang J, Wong GK, Li S, Liu B *et al*. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science, 2002; 296:79-92.
3. Collard BCY, Mackill DJ. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B363. 2008; (1491):557-572.
4. Wen S, Gao B. Introgressing Blast Resistant Gene *Pi-9 (t)* into Elite Rice Restorer Luhui17by Marker-Assisted Selection, Rice Genomics Genet, 2012; 2(4):31-36, 4: 31-36.
5. Ragimekula N, Varadarajula NN, Mallapuram SP, Gangimeni G, Reddy RK, Kondreddy HR. Marker assisted selection in disease resistance breeding. J. Plant Breed. Genet. 2013; 1:90-109.
6. Pan QH, Wang L, Ikehashi H, Tanisaka T. Identification of a new Blast resistance gene in Kasalath using Japanese differential cultivars and isozyme markers. Phytopathology. 1996; 86:1071.
7. Chaurasia S, Joshi AK, Dhari R, Chand R. Resistance of foliar blight of wheat. Genetic Resourcess and Crop Evaluation. 1999; 46: 469-474.
8. Pasha Aram, Babaeian-Jelodar Nadali, Bagheri Nadali, Nematzadeh Ghorbanali, Khosravi Vahid. A Field Evaluation of Resistance to Pyricularia oryzae in Rice Genotypes, Intl J Agri Crop Sci. 2013; 5(4):390-394.
9. Babujee Lavanya, Gnanamanickam SS. Molecular tools for characterization of rice Blast pathogen (*Magnaporthe grisea*) population and molecular marker-assisted breeding for disease resistance. Current Science, 2000; 78(3):10.
10. Barman SR. Identification of a major Blast resistance gene in the rice cultivar 'Tetep'. Plant Breeding. 2004; 123(3):300-302.
11. Kumar N, Singh D, Sachin, Sirohi P, Kumar A, Sirohi A *et al*. Identification of Genes for Resistance to Blast (*Magnaporthe oryzae)*: Molecular Breeding Strategies for Improvement of Rice (*Oryza sativa* L.) Vegetos, 2010; 23(1):151-155.
12. Padmavathi G, Ram T, Satyanarayana K, Mishra B. Identification of Blast (*Magnaporthe grisea*) resistance genes in rice. Curr Sci, 2004; 88(4):628-630.
13. Jeger MJ. Analysis of disease progress as a basis for evaluating disease management practices. Annu. Rev. Phytopathology. 2004; 42:61-82.
14. Mehla K, Chaudhary S, Kumar A, Kumar V, Chauhan P, Gupta S *et al*. Advances in DNA sequencing: Challenges and limitations of personal sequencing. African Journal of Agricultural Research. 2011; 6(6):1277-1280.