



## Variability studies of *Magnaporthe oryzae* Using International Rice Blast Differentials Set under Agroclimatic Conditions of Kashmir Valley, India

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

The pathogenic variability of the sixty isolates of *M. oryzae* was confirmed by testing them for virulence and cultivar reaction against International rice blast differentials (IBD) viz., Raminad Str. 3, Zenith, Usen, NP-125, Kanto-51, Dular, Tsia-tiao-sio and Caloro lodging *Piz*, *Pia*, *Pij*, *Pia*, *Pika*, *Pi-k* and *Piks* resistance genes for rice blast either singly or in combination or with an unknown background. The pathotype analysis of the isolates, collected from four surveyed districts of Kashmir valley revealed the presence of four races viz., IC-17, IC-25, ID-1 and II-1 according to the race classification and nomenclature proposed by Ling and Ou (1969). Of the 60 isolates of *M. oryzae*, 25 isolates pertained to race IC-17, 16 and 14 other isolates pertained to race ID-1 and II-1, respectively, whereas four other isolates pertained to race IC-25. The predominant pathotype was IC-17 with a virulence frequency of 60 %, followed by II-1 (46.67%) in district Pulwama, ID-1 (40%) and IC-25 (13.33%) in district Bandipora, respectively. IC-17 was seen to be the predominant race in each location in every district. Race ID-1 has been observed to overcome the maximum number of genes viz., *Pia*, *Pika*+, *Piks* and *Pik*. but none of the isolates was able to overcome Raminad Str 3 and Zenith.

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**Keywords:** Variability; Pathogen; *M. oryzae*; differentials; virulence.

## 1. INTRODUCTION

Among the major constraints in boosting the production and productivity of rice in Jammu and Kashmir state, the onslaught of blast disease is believed to be a major bottleneck and takes a heavy toll of the produce [1]. The disease is caused by a fungus *Magnaporthe oryzae* B. C. Couch and manifests itself at the seedling, tillering and flowering stages of crop growth causing losses on account of leaf-, node- and neck-blast in the state. Frequent epiphytotic of the disease in the state for the last about fifteen years have been inflicting heavy qualitative and quantitative losses to the growers. Cultivation of rice cultivars resistant to blast pathogen (*M. oryzae*) is by far the only viable solution for disease control with no addition to the cost of cultivation and without any deleterious effects on environment [2]. However, resistance in various rice genotypes, exploited for containing blast disease, often breaks down within two or three years [3,4] due to the build-up and appearance of new virulent races of the pathogen [5], thereby indicating the dynamic nature of the pathogen and prevalence of its tremendous pathogenic variability. Discerning the pathogenic variability in *M. oryzae* in the Jammu and Kashmir state is, therefore, pivotal for planning a blast resistance breeding programme aimed at breeding varieties specific to existing pathotypes [6,7].

With this objective, the present study was undertaken to ascertain the pathogenic variability in *M. oryzae* isolates were tested for virulence and disease reaction on eight set of International rice blast differentials.

## 2. MATERIALS AND METHODS

The pathological variability of different isolates of *M. oryzae* was studied by inoculating separately each isolate collected from different rice growing areas (Table 1) on the international blast differentials (Table 2) and their response was examined with regard to the symptoms produced. Seeds of International blast differentials (IBD) viz., Raminad Str. 3, Zenith, Usen, NP-125, Kanto-51, Dular, Tsia-tiao-sio and Caloro were obtained from the Department of Plant Protection, Directorate of Rice Research, Hyderabad, India and Rice Research Station Khudwani. The multiplication of the seeds of these differentials, was done in pots under greenhouse conditions to obtain sufficient seeds

to be used for ascertaining pathological variability of the isolates (Plate 1). For the purpose, seedlings of these differentials were raised in plastic trays (100 x 50 x 15 cm) filled with 10 kg of potting compost and grown under greenhouse conditions at 30±4 °C with a 16-h photoperiod. Plants were fertilized after 8, 15 and 22 days with 5 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> m<sup>2</sup>. Three plants per line of each differential host were maintained in each tray.

### 2.1 Inoculations and Response Assessment

The actively growing *M. oryzae* isolates in Petri plates were exposed to fluorescent light continuously for four days and nights to induce heavy sporulation. The culture was flooded with distilled water mixed with 0.02% Tween-20 and the conidia dislodged by scraping with sterilized rubber spatula. The suspension was filtered through cheese cloth and the concentration of conidia estimated with a hemacytometer and maintained to 5 × 10<sup>4</sup> spores per milliliter (ml) of water. About 30 ml of the conidial suspension of the individual isolates was separately inoculated on seedlings of differential hosts 35 days after sprout sowing, using atomizer. The trays were rotated slowly during the inoculation to ensure uniform distribution of inoculum [6]. The inoculated seedlings were incubated in dew chambers of poly-cages at 24±2°C for 24 hrs, and subsequently transferred into a Hi-tech green house. The responses of the differentials to different *M. oryzae* isolates were assessed 7-11 days after inoculation in terms of expression of blast symptoms. Each seedling of the genotypes was examined and rated using the following classification given by Ou and Ayad [8].

### 2.2 Pathotype Analysis

The extent of pathogenic specialization in rice-infecting isolates of *M. oryzae* was assessed by classifying the isolates into pathotypes based on their virulence spectra on the differential set. The IBD lines, namely, Raminad Str-3, Zenith, NP-125, Usen, Dular, Kanto-51, Tsia-tiao-tsao and Caloro constituted the 'International differential set', Coding of the pathotypes detected by the international differential set was done on the basis of the reaction of the lines as proposed by Ling and Ou [9]. The ultimate data was obtained by repeating the experiment for two consecutive years of 2012 and 2013.

**List 1. Score description**

Score	Score description	Category
0	No evidence of infection	Resistant
1	Brown specks smaller than 0.5 mm in diameter, no sporulation	Resistant
2	Brown specks about 0.5-1 mm in diameter, no sporulation	Resistant
3	Roundish to elliptical lesions about 1-3 mm in diameter with gray center surrounded by brown margins, lesions capable of sporulation	Resistant
4	Typical spindle-shaped blast lesions capable of sporulation, 3 mm or large with gray centers and water soaked or reddish brown margin, little or no coalescing of lesions	Susceptible
5	Lesions as in 4 but the upper portion of one or two leaves killed by coalescing of lesions	Susceptible



**Plate. 1. Different types of blast symptoms observed during race identification**

**3. RESULTS**

**3.1 Reaction of *M. oryzae* Isolates on International Blast Differentials**

Pathological variability of isolates of *M. oryzae* collected from different rice growing areas were

tested for virulence, cultivar reaction and pathotype identification during the years 2012 and 2013 on a set of eight standard international rice blast differentials. None of the isolates exhibited compatible disease reaction on Raminad Str 3 and Zenith, whereas forty-six isolates showed compatible disease reaction on

Kanto, Tsia-tsao-sio and Caloro. Summarized data of reaction on international set of differentials (Table 3) revealed that 25 *M. oryzae* isolates viz., M-1, M-7, M-19, M-53, M-9, M-12, M-18, M-11, M-40, M-42, M-58, M-17, M-37, M-32, M-13, M-38, M-56, M-22, M-28, M-41, M-45, M-47, M-52, M-55 and M-60, exhibited similar pattern of disease reaction, either resistant or susceptible and were categorized to comprise race IC-17. Seventeen isolates viz., M-44, M-59, M-31, M-39, M-15, M-27, M-6, M-34, M-8, M-10, M-51, M-24, M-25, M-50, M-57, M-46, M-48 were categorized to comprise of race ID-1 based on their reaction on the differential set. Fourteen *M. oryzae* isolates namely M-14, M-23, M-43, M-54, M-2, M-49, M-3, M-4, M-20, M-5, M-29, M-36, M-16, M-21 showing an incompatible reaction with all the members of the international differential set were categorized to comprise race II-1 (Table 2). Four other isolates viz., M-26, M-35, M-30, M-33 exhibited a reaction pattern of race IC-25. The results (Table 3) further reveal that most of the rice blast isolates showed virulent reaction on Caloro, Tsia-tia-siao and Kanto-51 followed by Dular (70%). The predominant pathotype was IC-17 with a virulence frequency of 60 % in district Anantnag, 53.33% in district Kulgam, 33.33% in district Bandipora and 20% in district Pulwama. Race ID-1 exhibited the maximum virulence

frequency (40%) in district Bandipora followed by that in district Pulwama and Anantnag 26.67%, each. The virulence frequency of II-1 was the highest in district Pulwama (46.67%) followed by that 20% in district Kulgam, whereas race IC-25 showed the highest virulence frequency of 13.33% in district Bandipora followed by 6.67% each in district Kulgam and Pulwama (Fig. 1). IC-17 was seen to be the predominant race found in every district and each location of the respective district. Race ID-1 was found in every district but not as widespread as the former. IC-25 was observed only in the locations viz., Kakapora, Kulgam, Ajus and Potushai. Race ID-1 has been observed to overcome the maximum number of genes viz., *Pia*, *Pika+*, *Piks* and *Pik* followed by IC-17 which was able to overcome *Pika+*, *Pik* and *Piks*, whereas race IC-25 was able to overcome only *Piks* and *Pik* genes. The isolates from district Pulwama, Bandipora and Kulgam yielded all the four pathotypes viz., IC-17, II-1, ID-1 and IC-25. The virulence frequencies of the four pathotypes for district Kulgam were 53, 20, 20 and 6% and for Pulwama frequencies of virulence were 20, 46, 26 and 6%, respectively (Fig. 1). Similarly, the frequencies of virulence of the four pathotypes in district Anantnag and Bandipora were 60, 13, 26 and 0% and 33, 13, 40 and 13%, respectively (Fig. 1).

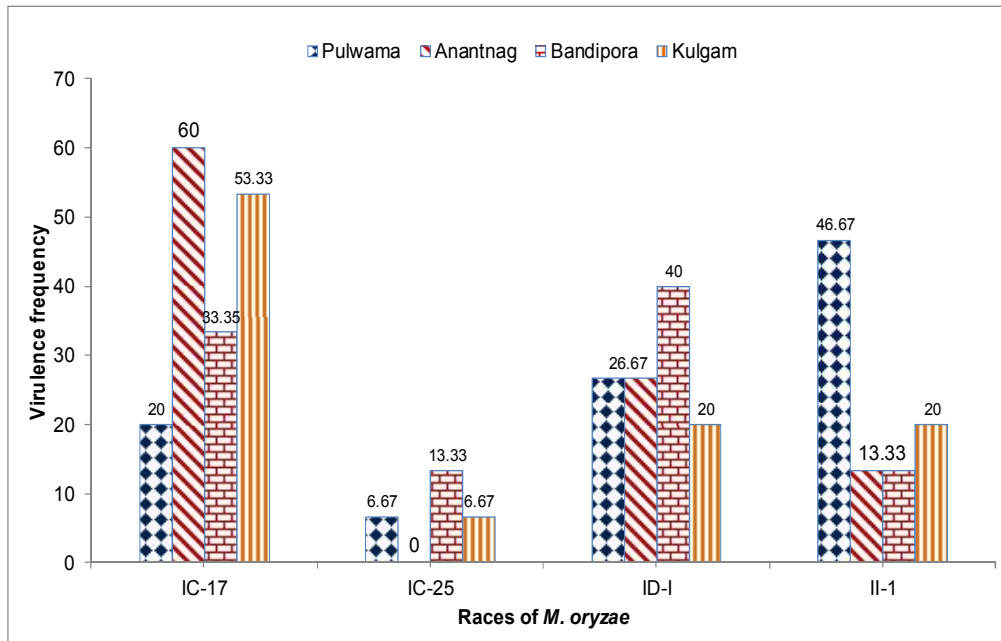
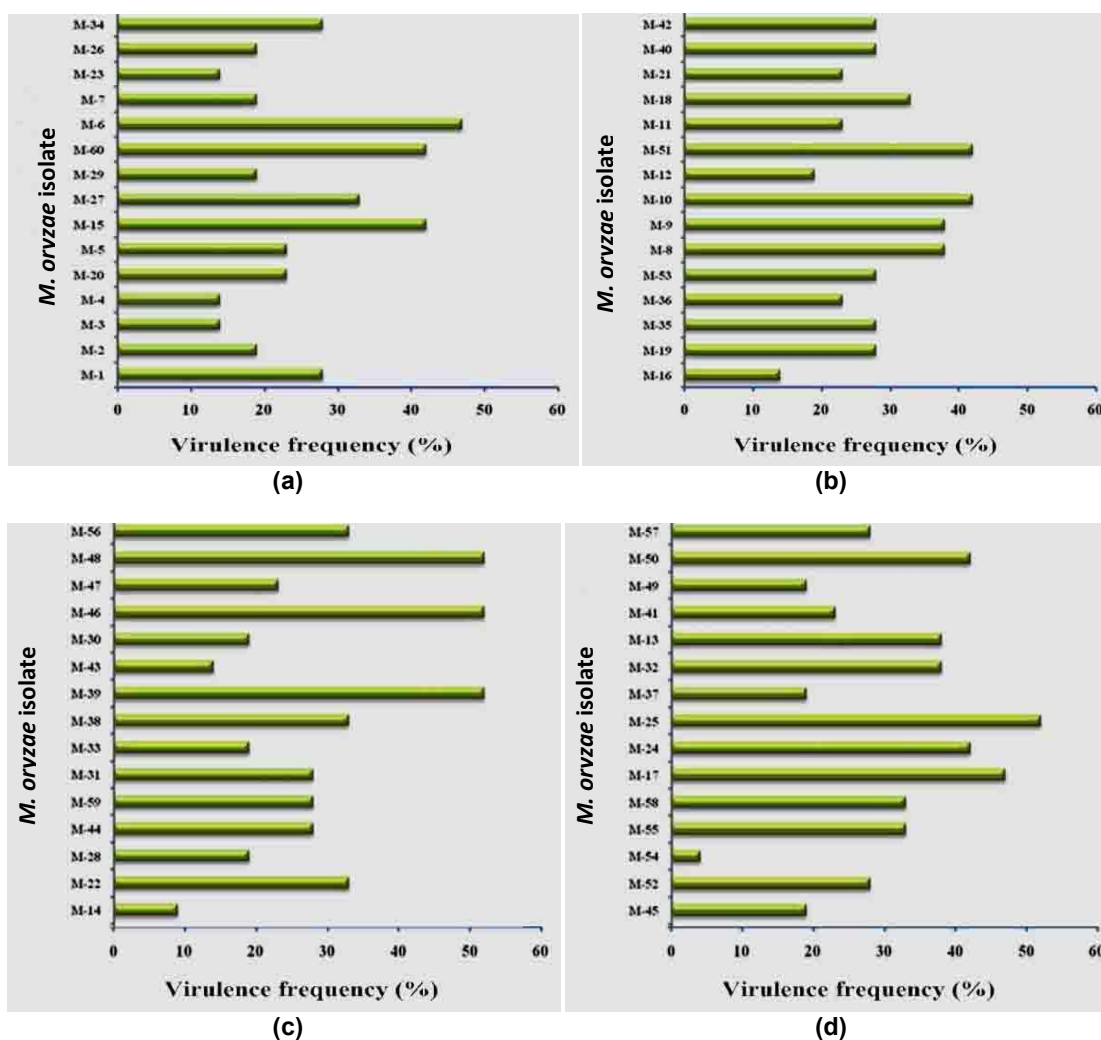


Fig. 1. Virulence frequency of *M. oryzae* races



**Fig. 2.** Frequency of virulence of *Magnaporthe oryzae* isolates on LTH lines  
 (a) *M. oryzae* isolates from district Pulwama; (b) *M. oryzae* isolates from district Anantnag;  
 (c) *M. oryzae* isolates from district Bandipora; (d) *M. oryzae* isolates from district Kulgam

**Table 1a.** Sites of collection and designations of *Magnaporthe oryzae* isolates

Collection site*		Isolate designation
District	Location	
Bandipora	Ajus	M-31, M-33, M-38, M-39, M-43
	Potushai	M-30, M-46, M-47, M-48, M-56
	Bandipora	M-13, M-28, M-32, M-55, M-58
Kulgam	Kulgam	M-16, M-20, M-35, M-36, M-53
	Yaripora	M-11, M-18, M-19, M-40, M-42
	Khudwani	M-8, M-9, M-10, M-12, M-51
Pulwama	Pampore	M-5, M-15, M-27, M-29, M-60
	Kakapora	M-6, M-7, M-23, M-26, M-34
	Pinglin	M-1, M-2, M-3, M-4, M-21
Anantnag	Duroo Shahbad	M-17, M-24, M-25, M-37, M-44
	Larnoo	M-22, M-41, M-49, M-50, M-57
	Anantnag	M-14, M-45, M-52, M-54, M-59

\*Isolations made from leaves of rice plants exhibiting typical symptoms of blast disease

**Table 1b. International rice blast differentials, inoculated for ascertaining pathological variability of *M. oryzae* isolate**

Genotype	Line code	Line name	Resistance gene(s) available
1	2	3	4
<b>International Rice Blast Differential</b>			
1	BL-12	Raminad Str 3	-
2	BL-13	Zenith	<i>Piz + Pia + Pii</i>
3	BL-14	NP 125	-
4	BL-15	Usen	<i>Pia</i>
5	BL-16	Dular	<i>Pika+</i>
6	BL-17	Kanto 51	<i>Pi-k</i>
7	BL-18	Shia tia tsao	<i>Piks</i>
8	BL-19	Caloro	<i>Piks</i>

**Table 2. Disease reaction of *Magnaporthe oryzae* isolates on International rice blast differentials during 2012-13 cropping seasons**

<i>M. oryzae</i> isolate	Rice blast differentials								Race group
	Raminad Str -3	Zenith <i>Piz + Pia + Pii</i>	NP-125	Usen <i>Pia</i>	Dular <i>Pika+</i>	Kanto-51 <i>Pi-k</i>	Tsia-tiao-sio <i>Piks</i>	Caloro <i>Piks</i>	
M-1	-	-	+	-	+	+	+	+	IC-17
M-2	-	-	-	-	-	-	-	-	II-1
M-3	-	-	-	-	-	-	-	-	II-1
M-4	-	-	-	-	-	-	-	-	II-1
M-20	-	-	-	-	-	-	-	-	II-1
M-5	-	-	-	-	-	-	-	-	II-1
M-15	-	-	-	+	+	+	+	+	ID-1
M-27	-	-	-	+	+	+	+	+	ID-1
M-29	-	-	-	-	-	-	-	-	II-1
M-60	-	-	+	-	+	+	+	+	IC-17
M-6	-	-	-	+	+	+	+	+	ID-1
M-7	-	-	+	-	+	+	+	+	IC-17
M-23	-	-	-	-	-	-	-	-	II-1
M-26	-	-	+	-	-	+	+	+	IC-25
M-34	-	-	-	+	+	+	+	+	ID-1
M-16	-	-	-	-	-	-	-	-	II-1
M-19	-	-	+	-	+	+	+	+	IC-17
M-35	-	-	+	-	-	+	+	+	IC-25
M-36	-	-	-	-	-	-	-	-	II-1
M-53	-	-	+	-	+	+	+	+	IC-17

<i>M. oryzae</i> isolate	Rice blast differentials								Race group
	Raminad Str -3	Zenith <i>Piz + Pia + Pii</i>	NP-125	Usen <i>Pia</i>	Dular <i>Pika+</i>	Kanto-51 <i>Pi-k</i>	Tsia-tiao-sio <i>Piks</i>	Caloro <i>Piks</i>	
M-8	-	-	-	+	+	+	+	+	ID-1
M-9	-	-	+	-	+	+	+	+	IC-17
M-10	-	-	-	+	+	+	+	+	ID-1
M-12	-	-	+	-	+	+	+	+	IC-17
M-51	-	-	-	+	+	+	+	+	ID-1
M-11	-	-	+	-	+	+	+	+	IC-17
M-18	-	-	+	-	+	+	+	+	IC-17
M-21	-	-	-	-	-	-	-	-	II-1
M-40	-	-	+	-	+	+	+	+	IC-17

M-42	-	-	+	-	+	+	+	+	IC-17
M-45	-	-	+	-	+	+	+	+	IC-17
M-52	-	-	+	-	+	+	+	+	IC-17
M-54	-	-	-	-	-	-	-	-	II-1
M-55	-	-	+	-	+	+	+	+	IC-17
M-58	-	-	+	-	+	+	+	+	IC-17
M-17	-	-	+	-	+	+	+	+	IC-17
M-24	-	-	-	+	+	+	+	+	ID-1
M-25	-	-	-	+	+	+	+	+	ID-1
M-37	-	-	+	-	+	+	+	+	IC-17
M-32	-	-	+	-	+	+	+	+	IC-17
M-13	-	-	+	-	+	+	+	+	IC-17

Table 2. contd...

<i>M. oryzae</i> isolate	Rice blast differentials								Race group
	Raminad Str -3	Zenith Piz + Pia + Pij	NP-125	Usen Pia	Dular Pika+	Kanto-51 Pi-k	Tsia-tiao-sio Piks	Caloro Piks	
M-41	-	-	+	-	+	+	+	+	IC-17
M-49	-	-	-	-	-	-	-	-	II-1
M-50	-	-	-	+	+	+	+	+	ID-1
M-57	-	-	-	+	+	+	+	+	ID-1
M-14	-	-	-	-	-	-	-	-	II-1
M-22	-	-	+	-	+	+	+	+	IC-17
M-28	-	-	+	-	+	+	+	+	IC-17
M-44	-	-	-	+	+	+	+	+	ID-1
M-59	-	-	-	+	+	+	+	+	ID-1
M-31	-	-	-	+	+	+	+	+	ID-1
M-33	-	-	+	-	-	+	+	+	IC-25
M-38	-	-	+	-	+	+	+	+	IC-17
M-39	-	-	-	+	+	+	+	+	ID-1
M-43	-	-	-	-	-	-	-	-	II-1
M-30	-	-	+	-	-	+	+	+	IC-25
M-46	-	-	-	+	+	+	+	+	ID-1
M-47	-	-	+	-	+	+	+	+	IC-17
M-48	-	-	-	+	+	+	+	+	ID-1
M-56	-	-	+	-	+	+	+	+	IC-17

\*Disease reaction either positive (+)/susceptible (S) or negative(-)/resistant (R)

Table 3. Summarized data on disease reaction of *Magnaporthe oryzae* isolates on International rice blast differential hosts during 2012-13 cropping seasons

<i>M. oryzae</i> isolate	Rice disease reaction* on differential hosts								Race group
	Raminad Str -3	Zenith Piz + Pia + Pij	NP-125	Use Pia	Dular Pika+	Kanto-51 Pi-k	Tsia-tiao-sio Piks	Caloro Piks	
M-1, M-7, M-19, M-53, M-9, M-12, M-18, M-11, M-40, M-42, M-58, M-17, M-37, M-32, M-13, M-38, M-56, M-22, M-28, M-41, M-45, M-47, M-52, M-55, M-60	R	R	S	R	S	S	S	S	IC-17

M-14, M-23, M-43, M-54, M-2, M-49, M-3, M-4, M-20, M-5, M-29, M-36, M-16, M-21	R	R	R	R	R	R	R	R	II-1
M-26, M-35, M-30, M-33	R	R	S	R	R	S	S	S	IC-25
M-44, M-59, M-31, M-39, M-15, M-27, M-6, M-34, M-8, M-10, M-51, M-24, M-25, M-50, M-57, M-46, M-48	R	R	R	S	S	S	S	S	ID-1

\*Disease reaction either susceptible (S) or resistant (R)

#### 4. DISCUSSION AND CONCLUSION

Crop failures due to the sudden out-break of the disease are often due to the breakdown of resistance of the varieties, which indicates the variability in the pathogen, making it, necessary to discern the race spectrum in a given geographical area before breeding attempts for resistance to such races could be initiated. Variability in pathogen is an important to determine the resistance of varieties to one strain and susceptibility to the other. As a result, breeding for durable resistance to rice blast remains a major challenge [10]. In the present investigation, the pathogen virulence study based on disease severity and lesion type indicated tremendous variability of *M. oryzae* in rice-growing regions of Kashmir valley. The pathogenic variability of the sixty isolates was confirmed by testing them for virulence and cultivar reaction against International set of differentials. The pathotype analysis of *M. oryzae* isolates, collected from four surveyed districts of Kashmir valley, revealed the presence of four races viz., IC-17, IC-25, ID-1 and II-1 according to the race classification and nomenclature proposed by Ling and Ou [9]. Of the 60 isolates of *M. oryzae*, 25 isolates pertained to race IC-17, 16 and 14 other isolates pertained to race ID-1 and II-1, respectively, whereas four other isolates pertained to race IC-25. It is interesting to observe that the occurrence of these four pathotypes has been reported in previous studies in India as well [11,1]. Our results further revealed that the race IC-17 was most predominant in every district and at each location of the respective districts followed by ID-1. The predominance of these races and their prevalence in approximately 80 per cent of the rice growing areas of Kashmir [12] are also well documented. Present study is also in conformity with the findings of Veeraghavan and

Premlatha Dath [13] who also infer that the race groups ID-1 and IC-17 are preponderant in North India. During the present study, the race IC-25 was observed only in the locations such as Kakapora, Kulgam, Ajus and Potushai. Also, none of the isolates was able to show a compatible reaction with Raminad Str-3 and Zenith. Zenith has been found to carry resistance genes *Pi-z* and *Pi-a7*. Since *Pi-a* showed a compatible reaction with most of isolates tested, the reaction pattern of Zenith appeared to be due to only *Pi-z*. Most of the rice blast isolates showed a virulent reaction for Caloro, Tsia-tia-siao and Kanto-51 with a frequency of 76.6 per cent followed by Dular with a frequency of 70 per cent, confirming the predominance of IC-17 race found at every location in each district. In addition, a lesser number of isolates exhibited a susceptible reaction on the line NP-125 having an unknown genetic background, thus indicating the presence of race IC-25. The pathogen population forms a 'pathogenic memory' to select races able to infect the cultivars carrying each of the resistance genes. The international differential which characterizes ID-1 race has *Pia* gene which is succumbed by the pathogen isolate belonging to the race. The presence of II-1 race confirms the ambiguity of the International differential set where the entire genetic constitution of the lines is not known. Finally, an incompatible reaction reveals that it is not confirmed which genes are responsible for the resistant reaction in all the lines. Flor [14] also stated and explained that the resistance (R) gene confers specific resistance to a pathogen race that contains a specific avirulence (AVR) gene. In addition, the ability of a plant to express resistance is dependent on the genotype of the pathogen [15]. Therefore, rice cannot be resistant to an isolate/pathotype of *M. oryzae* unless the pathogen has the genes that make it avirulent on the rice plant. An isolate/pathotype

of *M. oryzae* cannot be avirulent on the rice plant unless the rice plant has genes that make it resistant to that isolate.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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