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Screening of rice genotypes against bakanae disease caused by *Fusarium fujikuroi* Nirenberg

ZA Lone, ZA Bhat, S Najeeb*, MA Ahanger, AB Shikari, GA Parray and Sajad Hussain

Sher-e-Kashmir University of Agricultral Sciences and Technology of Kashmir

Email: najeeb_sofi@rediffmail.com

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ABSTRACT

Bakanae is one of the newly emerged diseases of rice and is attaining the status of major disease in Kashmir. Fifty rice genotypes consisting of thirty six germplasm lines and fourteen commercial cultivars were screened under artificially inoculated field and controlled conditions. Of the test lines screened under field conditions, four (GS-88, GS-308, GS-20 and GSL-29) were found highly resistant, five (GS-79, GSL-302, GSL-19, SK-423 and SI-6) as resistant, eighteen moderately resistant and remaining twenty three moderately susceptible. In order to validate the resistance, 27 genotypes, found moderately to highly resistant under field screening, were further screened under artificially inoculated controlled conditions against 20 isolates of F. fujikuroi collected from diverse locations. Seed dip inoculation technique, standardised after evaluating various inoculation techniques, was employed for screening genotypes under controlled conditions. One test line (GS-88) was found resistant with average disease incidence of 18.2 per cent exhibiting resistant response against all the 20 test isolates, 10 genotypes viz., Mushk budgi, China-972, GS-79, GSL-302, GS-27, GSL-19, SK-421, SI-4, GSL-225 and SI-6 with average disease incidence ranging from 31.2 to 39.5 were found moderately resistant with resistant response to 10 to 14 number of isolates and remaining 16 viz., Kamad, China-988, Chenab, SK-408, SK-404, GSL-20, GSL-29, GSL-311, SK-423, SI-V17, SK-292, GS-308, GS-20, GS-82, SK-406 and SK-407 showed moderately susceptible reaction with average disease incidence ranging from 40.5 to 54.2 per cent , having resistant response to less than 11 isolates. None of the lines showed highly resistant, susceptible and highly susceptible response.

Key words: *bakanae, Fusarium fujikuroi, genotypes, rice, screening*

In Jammu and Kashmir state rice is the staple diet for majority of population and is primarily cultivated by small and marginal farmers in an area of 261.25 thousand hectares, whereas in Kashmir valley alone the crop occupies an area of 140.8 thousand hectares with an annual production of 346.8 thousand tones and productivity of 24.79 q ha^{-1} (Governement of J&K, 2013). Though the rice varieties with a genetic yield potential of 7-8 t/ha are available for cultivation, the rice production under Kashmir valley conditions is miserably low. Enhancing rice production is becoming a challenging task especially in the background of continued population growth, depleting land and water resources and yield losses due to biotic and abiotic stresses. Among the biotic stresses, diseases and weeds are of greater economic significance. Bakanae disease caused by *Fusarium fujikuroi* Nirenberg [teleomorph:*Gibberella fujikuroi* (Sawada) Ito] is emerging as a major biotic stress limiting rice production in Kashmir. For the last few years, the disease has been found inflicting heavy economical losses particularly in japonica type of cultivars, grown under high altitude conditions of Kashmir valley (Ahanger *et al*., 2012) which constitute 15-20% rice grown area of the state. The introduction of this disease in Kashmir through seed material is quite possible owing to its seed borne nature.

The disease is widely distributed in rice growing areas including temperate regions of the world (Gupta *et al*., 2015) and responsible for yield losses ranging

from 3.0-95.4 per cent. (Pavgi and Singh, 1964; Ou, 1987; Hajra et al., 1994; Singh and Sunder, 2012). In India, the increasing trend of *bakanae* disease incidence has been observed particularly in basmati type of cultivars (Bashyal *et al*.,2014; Gupta *et al., 2014). The yield losses ranging from 15 to 25 per cent* have been reported from different states of India (Pavgi and Singh, 1964; Rathaiah et al., 1991; Pannu *et al.,* 2012; Sunder *et al.,* 2014).

The disease is reported to be both soil-borne (Nishio *et al*., 1980) as well as seed-borne (Parate and Lanjewar, 1987; Sharma *et al*., 1987; Ahmad and Raza, 1991). Since the disease is mainly seed borne, seed dressing represents the first way to minimize the occurrence of this disease (Ghazanfar *et al*., 2009; Gupta *et al*., 2014). However, use of farmer's own saved seed and non adoption of seed treatment practices by majority of farmers, results in unchecked spread of this disease leading to economic losses. Moreover, seed treatment alone fails to prevent the infection from soil borne inoculum. The most efficient and economical method to mitigate the menance of bakanae disease is therefore, the use of resistant varieties. In order to bread varieties with durable resistance to the bakanae disease, there is need for identification of sources of resistance effective against the range of virulence present in the bakanae pathogen population in the target region. Therefore, the study was conducted to identify resistant rice genotypes.

MATERIALS AND METHODS

Screening under field conditions

Rice genotype set of 50 lines, consisting of 36 germplasm lines and 14 commercial cultivars (Table 2) were screened against bakanae disease under artificially inoculated field conditions. Seeds of test genotypes were sterilized and sown in sterilised soil in plastic trays for raising disease free seedlings. Twenty five days old seedlings of test genotypes were inoculated by dipping their roots in freshly prepared inoculums suspension for 16 hours with mixed culture of isolates representing a Larnoo location of district Anantnag. The inoculated seedlings were transplanted in two rows (50 seedlings/ row) of two meter length under natural field condition at Mountain Crop Research Station, Larnoo, SKUAST-Kashmir (2290 m amsl) which is the hot spot location for bakanae disease. All the recommended agronomic practices were followed. The observation on per cent incidence of bakanae infected plants (which included elongated plants, dwarfed plants and plants with foot rot) was recorded up to 60 days of transplanting to assess the level of resistance and susceptibility of each test genotype according to disease rating scale as adopted by Junaid *et al*. (2000).

Disease incidence (%)	Host response (level of resistance/ susceptibility)
0-10	Highly resistant
11-20	Resistant
21-40	Moderately resistant
41-60	Moderately susceptible
61-80	Susceptible
Above 80	Highly susceptible

Standardization of inoculation techniques

Different inoculation techniques which represented the source of inoculums under natural conditions were evaluated under pot culture using susceptible japonica type paddy cultivar, K-332 to standardise the inoculation technique for screening of rice genotypes under artificially inoculated controlled conditions against the bakanae disease.

Preparation of Inoculum

F. fujikuroi isolate, isolated from the test cultivar K-332 was used for preparation of inoculum. For preparing the inoculums, 15 days old culture of the test isolate was thoroughly meshed in sterilized distilled water and filtered through muslin cloth to obtain spore suspension. The concentration of spores was measured with the help of haemocytometer and maintained at 1.50×10^5 spore ml^{-1} (Ma *et al*., 2008).

Inoculation techniques

Various inoculation techniques *viz*., seed inoculation, soil inoculation before seed sowing, root dip inoculation and soil inoculation at seedling stage were evaluated.

Seed inoculation technique

Sterilized pre-germinated seeds were inoculated with aqueous spore suspension of *F. fujikuroi*. The seeds were soaked in spore suspension for 24 hours and then sown in plastic pots.

Soil inoculation before seed sowing

In this method of inoculation, soil was inoculated prior

to sowing of seeds. *F. fujikuroi* culture was grown in sterilized rice husk for 20 days and then mixed with sterilised potting mixture @ 25 g /kg of soil. The sterilized seeds were then sown after 3 days of inoculation.

Root dip inoculation technique

Twenty days old seedlings grown in sterilized soil were uprooted, washed and inoculated by dipping their roots in freshly prepared inoculums suspension for 16 hours. The seedlings were then planted in pots containing sterilized potting mixture.

Soil inoculation at seedling stage

Soil inoculation at seedling stage was achieved by drenching the sterilized potting mixture with spore suspension @ 250 ml per pot, when seedlings in the pots were twenty days old.

Ten seedlings were maintained for each inoculation technique and replicated thrice. The un-inoculated checks were also maintained for each inoculation technique. The experiment was conducted in CRD and observation on per cent incidence of bakanae disease was recorded for each inoculation

Table 1. Evaluation of different artificial inoculation techniques of *Fuarium fujikuroi*

Inoculation technique	Incubation period (days)	Disease incidence (%)
Seed dip inoculation	14.0	73.33 (4.92)
Soil Inoculation before sowing	20.0	26.66 (2.95)
Seedling dip Inoculation (20 DAS)	16.66	56.66 (4.32)
Soil inoculation at seedling stage (20 DAS)	22.33	16.66 (2.32)
Control	-	0 (0.80)
CD (P<0.05)		0.51

*Figures in the parentheses are arc as in transformed value

Table 2. List of rice genotypes screened against bakane disease

Sl. No	Rice genotype	Type	Sl. No	Rice genotype	Type
Commercial Cultivars					
1	Mushk Budgi	*Japonica*	8	Shalimar Rice-3	*Indica*
2	Kamad	*Japonica*	9	Chenab	*Indica*
3	K-332	*Japonica*	10	China-1007	*Indica*
4	Kohsar	*Japonica*	11	China-972	*Indica*
5	Jehlum	*Indica*	12	China-988	*Indica*
6	Shalimar Rice-1	*Indica*	13	Pusa Basmati-1509	Basmati
7	Shalimar Rice-2	*Indica*	14	Pusa Sugandh-3	Basmati
Rice Germplasm					
1	SKAU-402	*Japonica*	19	GS-82	*Indica*
2	SK-292	*Japonica*	20	GS-88	*Indica*
3	SK-404	*Indica*	21	GS-308	*Indica*
4	SK-405	*Indica*	22	GSL-19	*Japonica*
5	SK-406	*Indica*	23	GSL-20	*Japonica*
6	SK-407	*Indica*	24	GSL-29	*Japonica*
7	SK-408	*Indica*	25	GSL-114	*Japonica*
8	SK-421	*Japonica*	26	GSL-225	*Japonica*
9	SK-422	Basmati	27	GSL-302	*Japonica*
10	SK-423	*Indica*	28	GSL-311	*Japonica*
11	GS-20	*Indica*	29	GSL-312	*Japonica*
12	GS-24	*Indica*	30	GSL-321	*Japonica*
13	GS-27	*Indica*	31	SI-V1	*Indica*
14	GS-31	*Indica*	32	SI-V7	*Indica*
15	GS-38	*Indica*	33	SI-V17	*Indica*
16	GS-43	*Indica*	34	SI-4	*Indica*
17	GS-61	*Indica*	35	SI-5	*Indica*
18	GS-79	*Indica*	36	SI-6	*Indica*

technique after 30 days of inoculation. The inoculation technique with significantly higher number of bakanae infected plants was employed for screening of rice genotypes under artificially inoculated controlled conditions.

Screening under artificially inoculated controlled conditions

Genotypes found moderately to highly resistant under field screening were further screened against twenty test isolates collected from different places (Table 3) using standardised inoculation technique. Seeds of each test genotype were sterilized and inoculated separately with twenty test isolates by soaking the germinated seeds in the concentrated spore suspension of *F. fujikuroi*. Twenty inoculated seeds of each test line were sown in plastic trays, filled with sterilized potting mixture in such a way that the genotype set inoculated with same isolate accommodated in one tray. The trays were incubated in plant growth chamber at 30±2°C day and 25±2°C night temperature, 80±5 per cent relative humidity and alternate periods of 12 hour light and 12 hour darkness and kept under observation for a period of 30 days. The observation on per cent incidence of bakanae infected plants was recorded 30 days after sowing to assess the level of resistance and susceptibility of each test entry according to disease rating scale adopted in case of field screening.

Table 3. Sources of *Fusarium fujikuroi* isolates

Isolate	Location	District	Block	Rice genotype
Ff1	MCRS*, SKUAST-Kashmir, Larnoo	Anatnag	Larnoo	GSL-64
Ff2	MCRS*, SKUAST-Kashmir, Larnoo	Anatnag	Larnoo	GSL-19
Ff3	Khreti	Anantnag	Larnoo	K-332
Ff4	Larnoo	Anantnag	Larnoo	K-332
Ff5	Drawa	Anantnag	Larnoo	K-332
Ff6	Nagam	Kulgam	DH Pora	K-332
Ff7	Khul Ahamdabad	Kulgam	DH Pora	K-332
Ff8	Mandgur	Kulgam	DH Pora	K-332
Ff9	Badoora	Anantnag	Achabal	China-1039
Ff10	Khudwani	Kulgam	Qaimoh	China-1039
Ff11	Thajwara	Anantnag	Achabal	China-1039
Ff12	Qaimoh	Kulgam	Qaimoh	Jehlum
Ff13	Kreeri	Anantnag	Larkipora	Jehlum
Ff14	Chatarpora	Kulgam	Pahloo	Jehlum
Ff15	Nowpora	Kulgam	Larkipora	Jehlum
Ff16	Gasren	Kulgam	Pahloo	K-39
Ff17	Bugam	Kulgam	Qaimoh	K-39
Ff18	Achabal	Anantnag	Achabla	K-78
Ff19	MRCFC** (SKUAST-K), Khudwani	Kulgam	Qaimoh	Pusa Basmati-1509
Ff20	Zangalpora	Anantanag	Pahloo	Local

*MCRS: Mountain Crop Research Station
**MRCFC: Mountain Research Centre of Field Crops

Table 4. Field reaction of rice genotypes to *Fusarium fujikuroi*

Reaction type	Cultivar/germplasm line
Highly Resistant (HR)	GS-88, GS-308, GS-20 and GSL-29
Resistant (R)	GS-79, GSL-302, GSL-19, SK-423 and SI-6
Moderately Resistant (MR)	Mushkbudgi, Kamad, K-332, China-972, China-988, Chenab, SKAU-402, SK-292, SK-404, SK-405, SK-406, SK-407, SK-408, SK-422, GS-24, GS-27, GS-31, GS-38, GS-43, GS-82, GSL-114, GSL-225, GSL-311, GSL-312, GSL-321, SK-421, SI-V1, SI-V7, SI-4, SI-5, SI-V-17
Moderately susceptible (MS)	K-332, Kohsar, Jehlum, Shalimar Rice-1, Shalimar Rice-2, Shalimar Rice-3, China-1007, Pusa Sugandh-3, Pusa Basmati-1509
Susceptible (S)	-
Highly Susceptible (HS)	-

HR= 0–10%, R= 11–20%, MR= 21-40%, MS= 41–60%, S= 61-80%, HS= >80%

Table 5. Response of rice genotypes to different isolates of *Fusarium fujikuroi* under artificially inoculated controlled conditions

Rice genotype	Disease Incidence																				Average Disease Incidence (%)	Reaction Type
	Isolates																					
	Ff1	Ff2	Ff3	Ff4	Ff5	Ff6	Ff7	Ff8	Ff9	Ff10	Ff11	Ff12	Ff13	Ff14	Ff15	Ff16	Ff17	Ff18	Ff19	Ff20		
Mushkbudgi	25.0	65.0	30.0	45.0	35.0	45.0	15.0	25.0	25.0	50.0	30.0	45.0	25.0	30.0	30.0	45.0	30.0	25.0	35.0	25.0	34.0	MR
Kamad	65.0	70.0	85.0	45.0	55.0	50.0	65.0	45.0	45.0	25.0	55.0	5.0	25.0	65.0	15.0	70.0	30.0	45.0	10.0	50.0	46.0	MS
China-972	55.0	15.0	45.0	45.0	45.0	25.0	35.0	15.0	55.0	30.0	70.0	40.0	50.0	20.0	45.0	45.0	25.0	55.0	30.0	30.0	38.7	MR
China-988	45.0	65.0	70.0	75.0	55.0	50.0	65.0	85.0	45.0	25.0	30.0	5.0	60.0	35.0	65.0	20.0	30.0	90.0	15.0	45.0	48.7	MS
Chenab	45.0	45.0	65.0	45.0	55.0	25.0	15.0	15.0	45.0	45.0	45.0	65.0	25.0	75.0	15.0	30.0	50.0	55.0	70.0	45.0	43.7	MS
SK-292	45.0	25.0	65.0	65.0	75.0	25.0	25.0	15.0	45.0	35.0	35.0	25.0	65.0	45.0	55.0	15.0	35.0	85.0	25.0	15.0	41.0	MS
SK-404	45.0	65.0	65.0	70.0	75.0	55.0	75.0	65.0	25.0	35.0	25.0	15.0	10.0	20.0	30.0	70.0	40.0	90.0	5.0	30.0	45.5	MS
SK-406	15.0	25.0	25.0	25.0	25.0	45.0	45.0	65.0	35.0	55.0	65.0	50.0	35.0	45.0	25.0	60.0	60.0	40.0	75.0	45.0	43.0	MS
SK-407	45.0	65.0	85.0	65.0	45.0	35.0	25.0	30.0	65.0	35.0	65.0	35.0	45.0	65.0	45.0	85.0	25.0	85.0	25.0	45.0	50.7	MS
SK-408	65.0	45.0	85.0	65.0	70.0	25.0	15.0	15.0	45.0	15.0	45.0	15.0	50.0	25.0	55.0	55.0	15.0	45.0	65.0	30.0	42.5	MS
SK-421	15.0	25.0	5.0	35.0	15.0	50.0	50.0	45.0	15.0	50.0	35.0	45.0	25.0	45.0	15.0	55.0	60.0	25.0	85.0	85.0	39.0	MR
SK-423	5.0	15.0	25.0	15.0	35.0	45.0	45.0	75.0	45.0	45.0	65.0	85.0	25.0	65.0	25.0	75.0	45.0	55.0	85.0	55.0	46.5	MS
GS-20	65.0	75.0	65.0	85.0	65.0	25.0	25.0	5.0	35.0	35.0	30.0	15.0	75.0	45.0	45.0	75.0	25.0	5.0	45.0	75.0	45.7	MS
GS-27	25.0	25.0	35.0	25.0	30.0	45.0	55.0	65.0	65.0	35.0	25.0	25.0	30.0	55.0	5.0	45.0	25.0	15.0	65.0	45.0	37.0	MR
GS-79	45.0	15.0	5.0	15.0	25.0	15.0	65.0	15.0	35.0	35.0	25.0	15.0	20.0	65.0	25.0	65.0	15.0	85.0	20.0	50.0	32.7	MR
GS-82	45.0	85.0	55.0	85.0	45.0	25.0	25.0	35.0	45.0	35.0	55.0	35.0	45.0	30.0	85.0	30.0	30.0	65.0	20.0	40.0	45.7	MS
GS-88	5.0	25.0	25.0	10.0	15.0	15.0	15.0	15.0	25.0	25.0	5.0	25.0	15.0	15.0	35.0	5.0	25.0	35.0	15.0	15.0	18.2	R
GSL-302	5.0	25.0	5.0	15.0	25.0	65.0	65.0	45.0	45.0	55.0	85.0	85.0	35.0	15.0	35.0	30.0	25.0	45.0	5.0	25.0	36.7	MR
GS-308	25.0	15.0	25.0	25.0	15.0	65.0	65.0	45.0	75.0	75.0	45.0	45.0	15.0	45.0	25.0	45.0	45.0	85.0	75.0	75.0	46.5	MS
GSL-19	25.0	15.0	25.0	25.0	15.0	45.0	65.0	65.0	25.0	15.0	25.0	25.0	35.0	70.0	15.0	45.0	5.0	5.0	15.0	65.0	31.2	MR
GSL-20	25.0	15.0	15.0	15.0	30.0	50.0	65.0	70.0	15.0	65.0	15.0	85.0	25.0	75.0	25.0	65.0	65.0	5.0	70.0	70.0	43.2	MS
GSL-29	45.0	65.0	85.0	75.0	45.0	25.0	25.0	15.0	65.0	30.0	65.0	25.0	65.0	30.0	45.0.	70.0	30.0	30.0	10.0	20.0	43.2	MS
GSL-225	15.0	15.0	5.0	25.0	25.0	45.0	45.0	55.0	35.0	25.0	35.0	15.0	35.0	55.0	25.0	25.0	30.0	75.0	70.0	60.0	35.7	MR
GSL-311	65.0	65.0	75.0	75.0	45.0	55.0	65.0	75.0	20.0	65.0	35.0	65.0	25.0	75.0	25.0	75.0	45.0	25.0	45.0	65.0	54.2	MS
SI-V17	65.0	45.0	45.0	65.0	65.0	75.0	45.0	65.0	15.0	20.0	25.0	35.0	70.0	25.0	45.0	25.0	30.0	20.0	15.0	15.0	40.5	MS
SI-4	45.0	55.0	75.0	85.0	65.0	55.0	55.0	45.0	25.0	25.0	15.0	5.0	55.0	25.0	45.0	35.0	15.0	25.0	25.0	20.0	39.7	MR
SI-6	45.0	65.0	45.0	45.0	55.0	15.0	15.0	25.0	65.0	15.0	45.0	35.0	55.0	25.0	50.0	25.0	30.0	50.0	10.0	25.0	37.0	MR

*R = Resistant (10 – 20 %), MR = Moderately Resistant (20 – 40 %), MS = Moderately Susceptible (40 – 60 %)

Table 6. Categorization of rice genotypes on the basis of their reaction to *Fusarium fujikuroi* under artificially inoculated controlled conditions

Reaction Type	Cultivar/Germplasm line
Highly Resistant (HR)	-
Resistant (R)	GS-88
Moderately Resistant (MR)	Mushkibudgi, China-972, GS-79, GS-27, GSL-19, SK-421, SI-4, GSL-225, GS-302 and SI-6
Moderately susceptible (MS)	Kamad, China-988, Chenab, SK-408, SK-404, GSL-20, GSL-29, GSL-311, SK-423, SI-V17, SK-292, GS-308, GS-20, GS-82, SK-406, SK-407
Susceptible (S)	-
Highly Susceptible (HS)	-

HR= 0–10%, R= 11–20%, MR= 21-40%, MS= 41–60%, S= 61-80%, HS= >80%

RESULTS AND DISCUSSION

Standardization of inoculation technique

Four different artificial inoculation techniques were evaluated to standardize the one, for rapid screening of rice lines against bakanae disease. Of the four techniques, seed dip inoculation technique exhibited significantly highest disease incidence (73.33 %) with least incubation period (14 days) followed by seedling dip inoculation and soil inoculation (before sowing) techniques (Table 1). Soil inoculation (seedling stage) technique was found least effective with 16.66 per cent disease incidence and 22.33 days incubation period. Fiyaz *et al.* (2014) also reported seed dip inoculation technique rapid and more reliable over seedling root dip technique for screening of rice germplasm against bakanae disease. Seed dip inoculation technique has also been employed by various workers for screening of germplasms against bakanae disease (Haque *et al.*, 1979; Wada *et al*., 1990; Ghazanfar *et al.,* 2013).

Screening of rice genotypes

Fifty rice genotypes were screened under field conditions against bakanae disease followed by screening of resistant lines under artificially inoculated controlled conditions against 20 isolates of *F. fujikuroi*. Among the test genotypes screened under field conditions, 4 were categorized as highly resistant, 5 as resistant, 18 as moderately resistant and remaining 23 as moderately susceptible (Table 4). Of the 27 moderately to highly resistant test lines, tested under controlled conditions, only 1 test line was found resistant with resistant response to all the test isolates, 10 moderately resistant with resistant response to 10-14 number of isolates and remaining all as moderately susceptible with resistance response ranging from 5 to 11 isolates (Table 5, 6). Similar efforts have been made earlier to identify rice germplasms with bakanae resistance and rice genotypes with varied levels of resistance have been identified by various workers (Li *et al*., 1993; Zheng *et al*., 1993; Sunder *et al*., 1998; Khokar and Jaffery, 2002; Fiyaz *et al*., 2014).

Bakanae disease is emerging as a potent biotic threat to rice production in northern India particularly under temperate belts. Management of the disease through development of tolerant/resistant cultivars is an economically as well as ecologically a viable option. The *bakanae* resistant rice lines identified in the present study can be utilized in temperate breeding programme for bakanae disease resistance. Further, the identified resistant and susceptible genotypes could be employed for developing mapping population in order to map the QTLs/genes conferring resistance against the disease.

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