



Identification of resistant sources against bakanae disease in short grained aromatic rice (*Oryza sativa*)

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ABSTRACT

An experiment was conducted during rainy (*kharif*) seasons of 2019 and 2020 at the research farm of ICAR Indian Agricultural Research Institute, New Delhi to screen 90 genotypes of scented short grained rice (*Oryza sativa* L.) for *Fusarium fujikuroi* resistance. Genotypes Kankjeer A, Lectimanachi-A, Sumati, Pankhali-203, GR-102, NWGR-3042, Geetanjali, R 1432-261-105-2-1-2, Khaskani, C-4-63-G, Calrose 76, JJ 92, Koliha, Hari Shankar, Kusuma, IR 74717-3-3-1-3, IR 74725-115-3-3-3, IR 74728-134-1-1-3, Hansraj, Anterved and GAR-1 were identified as moderately resistant with the disease rating of 3 in both the years of evaluation. Further, a set of genotypes with different disease response was evaluated for different parameters including root length, shoot length, number of fibres/threads in roots, days of symptom appearance and area under disease progress curve (AUDPC). Disease severity was observed to be significantly correlated ($r = 1.0$) with AUDPC and days required for the symptom appearance ($r = 0.95$). Results indicated that apart from the disease severity, AUDPC could also be considered as a one of the component for the bakanae disease resistance. The resistant sources identified in the present study can be utilized in rice breeding programme against bakanae disease.

Keywords: Aromatic rice, AUDPC, Bakanae, *Fusarium fujikuroi*, Resistance

Rice (*Oryza sativa* L.) is a major source of food for a larger part of the world population. Rice production is affected by different diseases of fungal, bacterial, viral or nematode origin. Bakanae disease caused by *Fusarium fujikuroi* Nirenberg (teleomorph *Gibberella fujikuroi* (Sawada) Ito & K. Kimura) is an emerging disease of the rice. This disease was first identified in 1828 in Japan, and at present it is emerging as a potential threat in Japan, Taiwan, Thailand and India (Gupta *et al.* 2015, Bashyal *et al.* 2016a, 2016b). In India, high incidence of bakanae disease has been observed in Pusa Basmati-1121, Pusa Basmati-1509, Pusa-2511, CSR-30, Dehradun Basmati and Pakistani Basmati and yield losses of 15–25% has been reported (Gupta *et al.* 2014, Bashyal *et al.* 2014). The typical symptoms of bakanae are slender, chlorotic and abnormally elongated plants. However, crown rot is also seen, resulting in stunted rice plants (Bashyal 2018). The seed treatment with chemical fungicides can effectively control the disease, however resistant varieties are the most economical and eco-friendly approach to manage the disease. India has rich genetic diversity of aromatic rice genotypes grown in

localized pockets in almost all states of the country (Malik *et al.* 1994, Roy *et al.* 2016). However, level of resistance against bakanae disease in these genotypes is not known. Most of the methods used for evaluation of resistant varieties against bakanae disease were based on disease incidence (DI) or number of healthy plants (Fiyaz *et al.* 2014, Bashyal *et al.* 2016a). These scoring methods may not be suitable for quantitative resistance, where it is governed by polygenes. Identification of different components for the bakanae disease resistance will be helpful in quantifying the resistance in different genotypes. Therefore, the objective of the present study was the screening of short grain aromatic rice genotypes for bakanae resistance by artificial inoculation with a virulent isolate of *F. fujikuroi* and identification of resistance components against the disease.

MATERIALS AND METHODS

Plant material and growing conditions: The virulent *Fusarium fujikuroi* isolate “F250” (GenBank Accession no. KM50526; MBPO00000000) was used for the study (Bashyal *et al.* 2016a, 2020). Seeds of short grained aromatic rice genotypes were procured from Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi. Disease was evaluated under artificial inoculated glasshouse conditions during the rainy (*kharif*) seasons of 2019–20

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Inoculum preparation: *Fusarium fujikuroi* isolate “F250” was multiplied in sterilized sorghum grains by incubating at 25°C for 15 days. Spore suspension was prepared with sterilized distilled water using 15 days old culture of *F. fujikuroi* and filtered through two layers of sterile muslin cloth and brought to a final concentration of approximately 1×10^6 spore’s/ml and used for seed inoculation.

Inoculation and disease assessment: For the disease evaluation, methodology of Bashyal and Aggarwal (2013) was followed. Briefly, one 100 rice seeds were soaked in 20 ml of inoculum suspension for 18 h at room temperature of $25 \pm 2^\circ\text{C}$. Control seeds were soaked in sterile water. Inoculated and control seeds were sown in pots (25 seeds/pot or 4 pots/genotypes) containing autoclaved mixture of soil and sand in the ratio of 3:1 (Fig. 1). The greenhouse temperature was maintained at 24–26°C during the day and 16–18°C during the night. During the year 2020, experiment was conducted in pro-trays (Fig. 1). Bakanae disease incidence observations were taken at the weekly interval starting with 7 days to 30 days. Final per cent disease incidence was calculated as no. of plants infected/no. of plants transplanted \times 100. Pusa Basmati 1121 was used as a susceptible check for the bakanae disease. Genotypes were classified as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) based on incidence value of 0, ≤ 1 , 1–6, 6–25, 25–50 and 50–100% respectively (Supplementary Table 1; International Network for Genetic Evaluation of Rice 1996, Sunder *et al.* 1998).

Assessment of different plant growth parameters: Symptoms of elongation, stunting or rotting and disease severity were assessed. Disease severity and other parameters were used to calculate area under the disease progress curve (Madden *et al.* 2007). For the AUDPC, disease incidence is considered to be equal to the disease severity and severity data of different time interval was used for AUDPC by using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{n_i-1} \frac{y_i + y_{(i+1)}}{2} (t_{(i+1)} - t_i)$$

where, n_i = the number of assessment times; y_i = disease severity and t_i = time (in days).

Further, growth parameters like root length (cm), shoot length (cm), root threads (no.) and days required for the symptom appearance were assessed at 30 days of seed inoculation.

Statistical analysis: All the experiments were conducted in randomized block design (RBD). All the individual evaluation treatments conducted with 5 replications and modules were evaluated with 3 replications. Data were analyzed with statistical analysis software (OPSTAT, CCS Haryana Agricultural University, Haryana, Sheoran *et al.* (1998). All data were first subjected to analysis of variance (ANOVA).

Box plots of all the rice genotypes were prepared using pooled disease severity data (Table 1). Three replications were used for each genotype and box plots were prepared using Origin (Pro) vs. 2023 software (Origin Lab Corporation, Northampton, MA, USA). Disease severity scale of 0 to 100 percent was plotted in vertical axis and genotypes in horizontal axis. All genotypes were divided into 6 graphs, each containing 15 genotypes.

RESULTS AND DISCUSSION

Resistant sources for bakanae disease of rice: Disease response of all the rice genotypes was recorded based on pooled disease severity. Genotypes were classified into different resistance categories based on pooled disease severity (Table 1). Out of 90 rice genotypes evaluated against bakanae disease, Kankjeer A, Lectimanchi-A, Sumati, Pankhali-203, GR-102, NWGR-3042, Geetanjali, R 1432-261-105-2-1-2, Khaskani, C-4-63-G, Calrose 76, JJ 92, Koliha, Hari Shankar, Kusuma, IR 74717-3-3-1-3, IR 74725-115-3-3-3, IR 74728-134-1-1-3, Hansraj, Anterved and GAR-1 were identified as moderately resistant with the disease rating of 3 in both the years of evaluation. Whereas, genotypes Dindli, RAU 3036, Amrit Bhog, Dubraj, HUR-ASG-MJ 72505, RD 1205, Jaipulla, IR 74721-47-3-2-2, Kankjeer A and KonBogi Joha were observed highly susceptible with the rating of 9 for the disease (Supplementary Table 2). A susceptible check PB1121 shown a highly susceptible reaction with average disease severity of 97.22% (Table 1). Sunder *et al.* (1998) evaluated the scented and non-scented rice genotypes against the bakanae disease using standard evaluation system of the International Rice Research Institute and identified

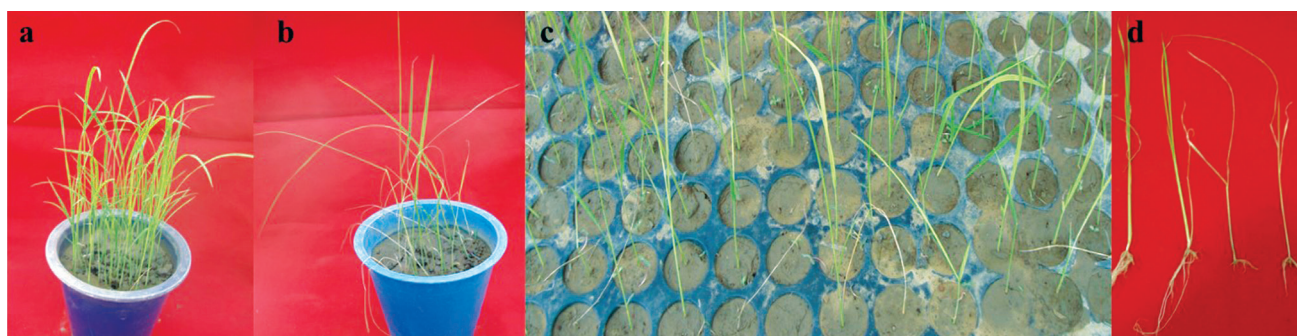


Fig. 1 Bakanae disease evaluation in short grained aromatic rice in glasshouse conditions.

A, Uninoculated control; b and c, evaluation in pots and pro-trays; d, diseased and healthy seedlings of cv. Hankesh

2 scented rice genotypes were resistant against the disease, whereas, 8 were moderately resistant. Interestingly, genotype Hansraj identified as moderately resistant (MR) in the year 1998 has given similar disease response in our study also (Table 1). A high throughput screening protocol was used for identifying novel sources of resistance to bakanae disease based on per cent infected seedlings. Genotypes such as Athad apunnu, C101A51, Chandana, IR 58025B, Panchami, PAU 201, Pusa 1342, and Varun Dhan were highly resistant and BPT 5204, Himju, Peeli badam, Suphala were found resistant (Fiyaz *et al.* 2014). Apart from this, several studies reported QTLs for bakanae disease resistance and attempted to utilize QTLs in breeding programme for developing resistant varieties (Fiyaz *et al.* 2016, Lee *et al.* 2022).

Area Under Disease Progress Curve (AUDPC): AUDPC was calculated for all the rice genotypes in the

year 2019 (Table 1, Supplementary Fig. 1). AUDPC of 0 to 20 was observed in rice genotypes Chanan, JJ 92, Koliha, IR 74725-115-3-3-3, IR 74728-134-1-1-3, Anterved, Pankhali-203, Geetanjali, Hari Shankar, Sumati, Kusuma, Govindbhog and C-4-63-G. Similarly, genotypes, viz. Lectimanchi-A, R 1432-261-105-2-1-2, IR 74717-3-3-1-3, GAR-1, Maharaji, Hansraj, Khaskani, Kankjeer A, Calrose 76, NWGR-3042, GR-102, Kalimooch, Della, IGSR-3-1-5, Kamod and Samundchini were identified as moderately resistant with AUDPC range of 21 to 50. The rice genotypes with AUDPC value of 401 to 700 were identified as susceptible.

A uniform disease severity scale of 0 to 100 was used in box plot analysis for all the rice genotypes. Genotypes were distributed based on response of the genotype to the bakanae disease. Red line in the graph indicates 10% disease

Table 1 Disease severity (%) of rice genotypes evaluated during the years 2019–20

Genotype	Disease severity 2019 (%)	Disease severity 2020 (%)	Pooled disease severity (%)	Disease response	AUDPC
Banspatri	21.87	13.10	12.48	MS	87.50
Dindli	83.30	58.30	70.80	HS	495.83
RAU 3055	25.83	28.33	27.08	S	189.58
RAU 3036	97.14	37.14	67.14	HS	470.00
Sonachoor	22.22	20.22	21.22	MS	155.56
AmritBhog	83.87	74.19	79.03	HS	553.23
Kankjeer A	6.09	2.00	4.05	MR	24.42
Lectimanchi-A	4.00	3.03	3.52	MR	21.21
Kheersai	20.00	25.00	22.50	MS	560.00
IGSR-3-1-5	12.50	9.00	10.75	MS	43.75
Hankesh	46.51	35.00	40.76	S	162.79
IGSR-2-1-6	44.18	11.62	27.90	S	195.35
Sonth	50.00	33.33	41.67	S	291.67
BanthaPhool B	69.56	21.733	45.65	S	319.57
Dubraj (Raipur)	9.09	9.09	9.09	MS	63.64
Kamod	12.5	8.50	10.50	MS	43.75
CB 06550	15.00	5.00	10.00	MS	70.00
Govindbhog	3.57	2.27	2.92	MR	15.91
Kapoosar	11.36	9.09	10.23	MS	71.59
Kalikamod	34.14	2.43	18.29	MS	128.05
Chhatri	45.45	45.45	45.45	S	318.18
Jiradhan	32.43	18.91	25.67	S	179.73
Chinoor	50.00	45.00	47.50	S	350.00
Elayachi	16.60	8.33	12.47	MS	116.67
ShyamJira	16.65	10.00	13.33	MS	140.00
Tulsi Prasad	9.09	6.00	7.55	MS	56.76
Kubrimohar	59.35	25.92	42.64	S	219.77
Lalsumbhog	19.51	14.63	17.07	MS	51.22

Contd.

Table 1 (Continued)

Genotype	Disease severity 2019 (%)	Disease severity 2020 (%)	Pooled disease severity (%)	Disease response	AUDPC
Maharaji	13.33	6.66	10.00	MS	23.33
Dubraj	95.23	85.71	90.47	HS	633.33
Samundchini	12.90	10.00	11.45	MS	45.16
Sumati	6.08	3.44	4.76	MR	12.07
ChittiMutyalu (Small grain)	11.76	5.88	8.82	MS	61.76
Krishna Kamod	17.50	10.00	13.75	MS	96.25
Pankhali-203	5.50	2.70	4.10	MR	9.72
GR-102	5.26	5.00	5.13	MR	36.84
GR-104	10.86	8.69	9.78	MS	68.48
NWGR-3042	5.00	2.50	3.75	MR	33.33
NWGR-3045	33.50	16.60	25.05	MS	175.00
AmrutBhog	32.00	25.00	28.50	S	182.00
Geetanjali	3.22	3.00	3.11	MR	11.29
JGL 11609	11.53	9.00	10.27	MS	364.29
NDR 8022	22.22	18.18	20.20	MS	63.64
R-1498-747-358-2-1	10.71	7.14	8.93	MS	62.50
RRB 2005-1	14.89	8.40	11.65	MS	134.04
HUR-ASG-MJ 72505	90.00	50.00	70.00	HS	175.00
R 1432-261-105-2-1-2	4.160	2.08	3.12	MR	21.88
NDR 8399-2	56.52	33.04	44.78	S	243.48
PTB 13	65.51	31.03	48.27	S	337.93
Acharamati	17.60	10.52	14.06	MS	73.68
RD 1205	75.00	66.66	70.83	HS	495.83
Mayur Kranti	15.00	10.00	12.50	MS	87.50
Tulsi Manjari	16.66	11.11	13.89	MS	97.22
Khaskani	4.54	2.27	3.41	MR	23.86

Contd.

Table 1 (Concluded)

Genotype	Disease severity 2019 (%)	Disease severity 2020 (%)	Pooled disease severity (%)	Disease response	AUDPC
Mohan Bhog	31.81	24.13	27.97	S	84.48
Kalamaniya	25.0	13.50	19.25	MS	87.50
BR-34	38.46	34.61	36.54	S	255.77
Keda Gauri	14.70	11.76	13.23	MS	92.65
Gayasu	15.38	3.84	9.61	MS	67.31
Tulasiful	16.66	4.16	10.41	MS	116.67
Gopalbhog	22.72	20.83	21.78	MS	79.55
Dudhkhosa	33.00	26.00	29.50	S	70.00
C-4-63-G	2.50	2.00	2.25	MR	17.50
Kalimooch	9.09	5.55	7.32	MS	38.89
Begami T 1	42.42	36.36	39.39	S	275.76
Calrose 76	4.16	4.00	4.08	MR	29.17
Della	12.5	11.11	11.81	MS	38.89
Chanan	7.140	6.66	6.09	MS	0.00
JJ 92	6.00	4.00	5.00	MR	0.00
Koliha	3.00	2.00	2.50	MR	0.00
Hari Shankar	3.33	2.00	2.67	MR	11.67
Bastul	50.00	41.66	45.83	S	175.00
Kusuma	2.12	2.12	2.12	MR	14.89
PKV Marakand	9.00	5.55	7.28	MS	233.33
Chatianaki	33.33	22.11	27.72	S	155.56
Jaipulla	62.50	50.00	56.25	HS	393.75
IR 74717-3-3-1-3	3.22	3.12	3.17	MR	21.88
IR 74719-23-3-2-2	14.70	8.82	11.76	MS	82.35
IR 74720-13-1-2-2	10.41	8.33	9.37	MS	65.63
IR 74721-47-3-2-2	60.00	52.00	56.00	HS	392.00
IR 74724-82-2-2-3	15.00	12.50	13.75	MS	52.50
IR 74725-115-3-3-3	2.70	2.50	2.60	MR	0.00
IR 74728-134-1-1-3	6.06	4.00	5.03	MR	0.00
Kankjeer A	100.00	84.61	92.31	HS	646.15
RAU 3041	7.14	6.97	7.06	MS	375.00
Tulasiphulla	28.09	25.04	26.57	S	375.00
KonBogi Joha	71.00	70.00	70.50	HS	497.37
Hansraj	3.33	2.0	2.67	MR	23.33
Anterved	3.50	2.00	2.75	MR	0.00
GAR-1	6.25	3.00	4.63	MR	21.88
PB1121	94.44	100	97.22	HS	766.66
(Susceptible check)					

severity cut off value for classification of the resistant and susceptible genotypes (Fig. 2)

Evaluation of rice genotypes for different parameters:

Twenty rice genotypes were selected from different disease response category for the evaluation of different parameters like root length, shoot length, no. of fibrous threads of roots and days required for the symptom appearance (Table 2). Significant differences were observed in rice genotypes for different parameters. Root length was recorded maximum in genotype Sumati (8.50 cm) and minimum in RAU3036 (1.90 cm). Shoot length was observed maximum for the genotype RD1205 (35.16 cm) and minimum in RAU3036 (7.50 cm). Similarly, number of days required for the bakanae symptom appearance was maximum in the genotype IR74725-115-3-3-3 (25 days). The disease symptoms were observed within 6 days in Dindli and RAU3036 (Table 2).

Correlation between different parameters evaluated in susceptible and resistant rice genotypes: In susceptible genotypes, a positive and highly significant correlation was observed between disease severity and AUDPC ($r = 1.00$, Supplementary Table 3). Similarly, significant positive correlation was observed between disease severity/AUDPC and days required for the symptom appearance ($r = 0.95$). Whereas, in resistant genotypes no correlation was observed between most of the parameters except disease severity and AUDPC ($r = 0.99$).

The disease severity method takes only the per cent infected plants as the sole criteria for response of genotypes to the disease. It ignores the disease progression in the individual genotype/plants. Whereas, the AUDPC includes the disease severity and its progression over the time in the host and useful for identification/evaluation of resistant genotypes. Also, authors observed no increase in disease after 40 days' post inoculation indicating that AUDPC is needed to know the behaviour of the genotypes to the disease (Zainudin *et al.* 2008a). To evaluate the *Fusarium* wilt of different crops apart from per cent disease incidence/severity other resistance parameters were also considered by different authors (Burlakoti *et al.* 2012, Bani *et al.* 2012). Similarly, disease index based on 0 to 4 scale was used to measure the severity of the bakanae disease (Matic *et al.* 2021). Recently, both disease incidence (%) and AUDPC criteria have been considered in evaluation of bakanae disease (Bashyal *et al.* 2022). This evaluation method detected the existence of quantitative resistance to the pathogen within rice accessions evaluated. In general, more susceptible genotype induced disease symptoms earlier compared with less susceptible genotype. To determine the most adaptable and easiest method of disease scoring for future screening, correlation between the different parameters evaluated was also examined and significant correlation was observed between disease severity and AUDPC.

A total of 90 short grained aromatic rice genotypes were evaluated against the bakanae disease, all these were grouped into different disease response categories such as moderately resistant, moderately susceptible, susceptible and highly susceptible genotypes by using disease severity

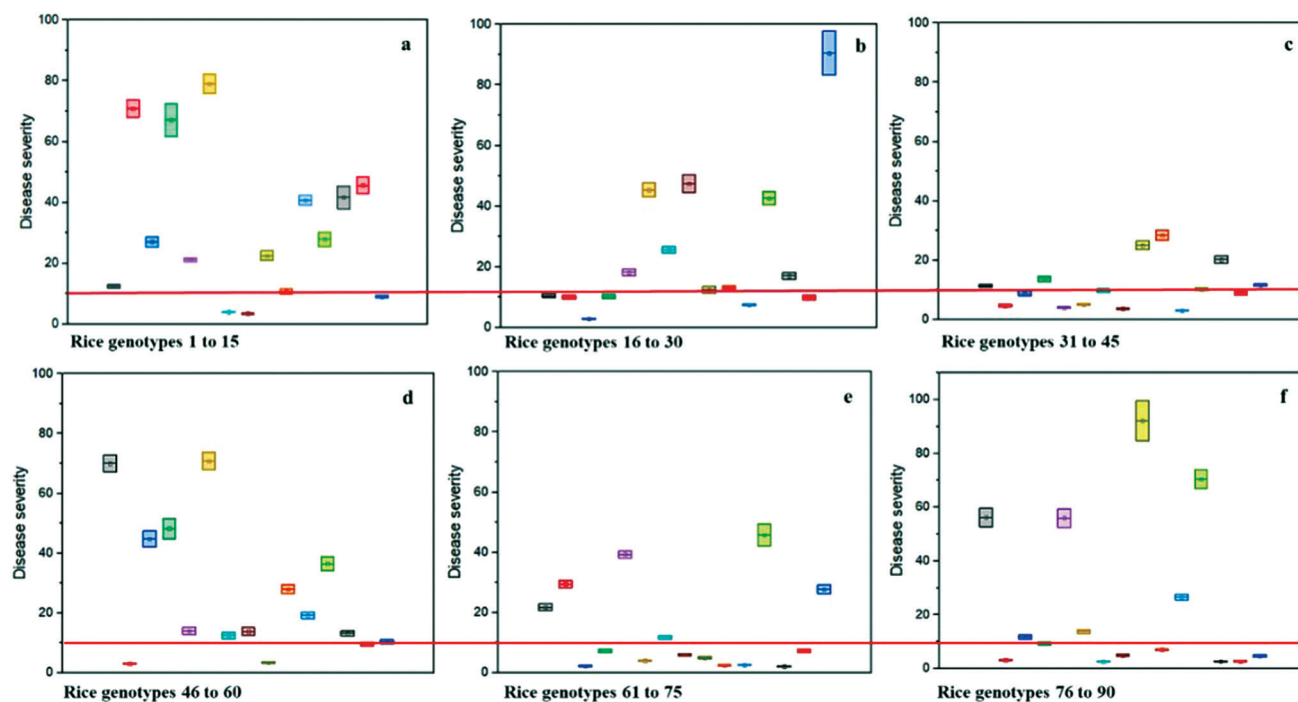


Fig. 2 Distribution of rice genotypes with box plot analysis according to the response of the genotypes to bakanae disease. a to f: box plot based on pooled disease severity of rice genotypes with sequential order 1 to 90 (15 rice genotypes were presented in each graph). Red line in the graph indicates 10% disease severity cut off for classification of resistant and susceptible genotypes.

Table 2 Different parameters evaluated for the rice genotypes during the year 2019

Genotype	Root length (cm)	Shoot length (cm)	No. of fibers	Days required for the symptom appearance
RD1205	3.03	35.16	12.66	7
Amrit Bhog	2.17	16.60	12.00	8
Dindli	3.33	18.46	10.66	6
RAU3036	1.90	7.50	8.00	6
Dubraj	3.30	22.50	10.00	9
Hankesh	3.20	30.67	9.67	12
Begami T1	4.00	34.50	10.00	8
Chinoor	3.50	34.00	8.66	13
Chhatri	2.80	24.93	12.66	8
BR-34	2.50	23.26	10.00	8
Kalamaniya	4.66	32.00	14.00	15
Gopalbhog	6.33	33.83	11.33	21
Banspatri	4.33	28.47	15.33	15
ChittiMutyalu (Small grain)	5.77	29.26	18.67	21
R-1498-747-358-2-1	6.23	27.87	16.33	15
Hari Shankar	7.86	34.33	17.33	21
Kusuma	6.66	33.00	19.67	21
IR 74725-115-3-3-3	6.33	31.33	18.67	25
Sumati	8.50	30.10	18.67	21
Pankhali-203	7.00	29.83	24.00	21
CD (P=0.05)	3.02	6.77	7.27	-

method. No highly resistant or resistant rice genotypes were observed through this method. However, AUDPC evaluation method revealed a no disease or zero AUDPC in the moderately resistant genotypes such as Chanan, JJ 92, Koliha, IR 74725-115-3-3-3, IR 74728-134-1-1-3 and Anterved. Interesting observations/conclusions can be made from the present study, i.e., the parameters of disease severity and AUDPC were positively correlated in susceptible ($r = 1$) and resistant genotypes ($r = 0.99$) but no correlation was observed in resistant genotypes for most of the parameters. This study indicates that both per cent disease incidence/severity and AUDPC could be used in bakanae disease evaluation. The disease severity criteria alone will lead to wrong conclusions. However, detailed study needs to be conducted considering the different isolates and pathotypes of the pathogen. The resistant genotypes can be utilized in rice breeding programme to develop resistant variety against bakanae disease.

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