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Development of high throughput screening protocol and identification of novel sources of resistance against bakanae disease in rice (*Oryza sativa* L.)

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Abstract

Bakanae or foot rot disease caused by *Fusarium fujikuroi* (teleomorph: *Gibberella fujikuroi,* Sawada,Wollenweber) is emerging as a serious disease of rice. A simple, reliable and high-throughput method for screening the disease would enable rapid screening of germplasm aimed at identifying resistance sources, mapping QTLs/genes and developing resistant rice cultivars. In the present study, a high-throughput, reliable bioassay to screen rice germplasm for resistance to bakanae disease was developed and compared with the conventional screening technique. This technique involves soaking of rice seeds in fungal spore suspension ($1.0x10^6$ spores ml^{-1}) for 24 hours at room temperature. Seedling growth at 30°/25° (±3)°C day/night temperature and 60/80(±10)% day/night relative humidity in glasshouse gave the best results. The new protocol described here produces consistent and reproducible bakanae disease symptoms and enables screening of hundreds of rice germplasm within 15 days without any loss of precision in screening of rice genotypes against bakanae disease. The resistant and susceptible genotypes can be used for developing mapping population and identification of QTLs/genes conferring resistance to bakanae disease.

Key words: Rice, bakanae, *Fusarium fujikuroi,* high throughput, resistance, screening

Introduction

Bakanae or foot rot disease of rice (*Oryza sativa* L.) caused by the fungal pathogen *Fusarium fujikuroi* (teleomorph: *Gibberella fujikuroi,* Sawada, Wollenweber) occurs in many parts of the world, especially in South-East Asia including Japan, Korea, Thailand, Philippines and India. The disease is known by several names such as “elongation disease”, “white stalk”, “bakanae” disease and “man rice”, etc. while in India it is commonly called as “foot rot”. The disease causes severe quantitative and qualitative losses to the produce with severe losses under the field conditions [1]. The typical symptom of this disease is yellowing and abnormal elongation of infected rice seedlings, which led to the name “*Bakanae*”, a Japanese word meaning “foolish seedling” [2]. The elongation is attributed to gibberellins (a plant growth hormone) and stunting to fusaric acid, both of which are produced by the fungus. The pathogen is both a seed-borne and soil-borne and has a wide host range [3]. The sexual stage of the fungus causing bakanae disease was first reported in 1919 and named *Lisea fujikuroi*, which was later amended to *Gibberella fujikuroi* by Ito and Kimura [4] who also identified the asexual stage as *Fusarium moniliforme* [5]. However, this taxon comprises a number of distinct species, collectively termed as *Gibberella fujikuroi* species complex. The sexual stage of *Gibberella* can be distinguished by mating populations or biological species. *Fusarium fujikuroi* (teleomorph: *Gibberella fujikuroi;* synonym, *G. fujikuroi* mating population C) is a biologically and phylogenetically distinct species within *Gibberella fujikuroi* species complex and causes bakanae disease of rice [6-10].

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Fusarium fujikuroi induces seedling elongation, foot rot, seedling rot, grain sterility, and grain discoloration in rice plants [11]. In older plants, the roots, crowns, stems, leaf sheaths, and panicles may also get infected. Rice seedlings after transplanting can get infected, resulting in weak tillering and poor grain filling capacity [11-12]. Disease at a later stage usually causes a yield loss of ~ 10-20%, and the loss could even be higher than 70% under severe outbreak of the disease [4, 11, 13]. During the past decade, monoculture of single variety and the use of new methods for seedling raising, especially the increasing application of dry seed-bed raising for hybrid rice, have increased the incidence of bakanae disease [14-15] and has led to outbreaks of the disease in many countries like Japan, Korea and California. The disease has become a serious threat across major rice growing regions of India and the Philippines [16-17].

Basmati is the specialty rice of India, which is highly priced in the international market for its unique cooking quality characteristics and aroma. Indian Agricultural Research Institute, New Delhi has developed the world's longest cooked kernel Basmati rice variety Pusa Basmati 1121 (PB 1121) which alone occupies > 65% (1.35 mha) of the total area (2.0 mha) under Basmati cultivation with an annual foreign exchange earning of ~ $ 4.0 billion [18]. Pusa Basmati 1121 is highly susceptible to various diseases and pests among which bakanae or foot rot disease is of major concern. Recently, outbreaks of bakanae disease have also been observed in other varieties such as CSR-30 and Pusa Basmati 6 [19]. Though, the disease can be managed through seed treatment with chemical fungicides, the identification of sources of resistance for developing varieties with inbuilt resistance to bakanae disease represents the most cost effective and eco-friendly approach to manage the disease.

Till date, there has been limited progress in identifying resistant sources against the bakanae disease due to lack of reliable, reproducible and rapid assay to screen large number of germplasm against the disease. The available screening methods are time taking, laborious and require larger area. The field inoculation techniques are inherently poor in terms of reproducibility as a result of uneven inoculum distribution, interaction with other pathogens, and variations in weather and other environmental factors which may affect disease severity. Therefore, it is of utmost importance to develop a high throughput screening technique to screen large number of germplasm and to identify resistance sources against bakanae disease. Considering these facts, the present study was carried out with the objective of developing and standardizing a high-throughput seedling screening assay for precise, reliable and reproducible screening against the disease.

Material and methods

Collection of fungal isolates

Surveys were conducted across the Basmati growing areas of north western India during 2011 to 2014 in order to collect bakanae disease infected plants. The plants showing bakanae symptoms such as, stem elongation, white heads, and root rot were put in paper packets and brought to the laboratory in the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. The infected stems, roots and heads of the symptomatic plants were cut into 1 cm^2 pieces, sterilized with 2% solution of sodium hypochlorite, rinsed twice with distilled water and then placed on potato dextrose agar medium (PDA). The plates were incubated at 28 ±1°C for a week (Fig. 1). The growing colonies of fungi were transferred to new plates for purification and were identified according to Nelson *et al*. [20].

Preparation of inoculum

All collected isolates were subjected to virulence assay to check their ability to induce symptoms of bakanae on rice seedlings. Based on pathogenicity test, a highly virulent and aggressive isolate (F250) isolated from Basmati growing areas of north western India was selected for this study. The plates of PDA inoculated with pure culture of fungus were incubated for 12-15 days at room temperature before the mycelium in the plate was scraped. After 15 days, plates were flooded with sterile water and scraped with a sterile spatula. The resulting suspensions were filtered through two layers of sterile muslin cloth and spore concentration was adjusted to $5x10^5$, $1x10^6$, $5x10^6$ and $1x10^{10}$ conidia/ml for testing the pathogenicity.

Reactions of rice cultivars to treatment

The reaction of highly susceptible genotype, PB 1121 and the resistant rice genotype, Pusa 1342 was assessed against the highly virulent isolate, F250 (NCBI Gene bank accession number - KM50526). Inoculation was done using two methods namely, seed inoculation method and seedling dip method. For seed inoculation, the disinfected seeds of ninety-eight rice

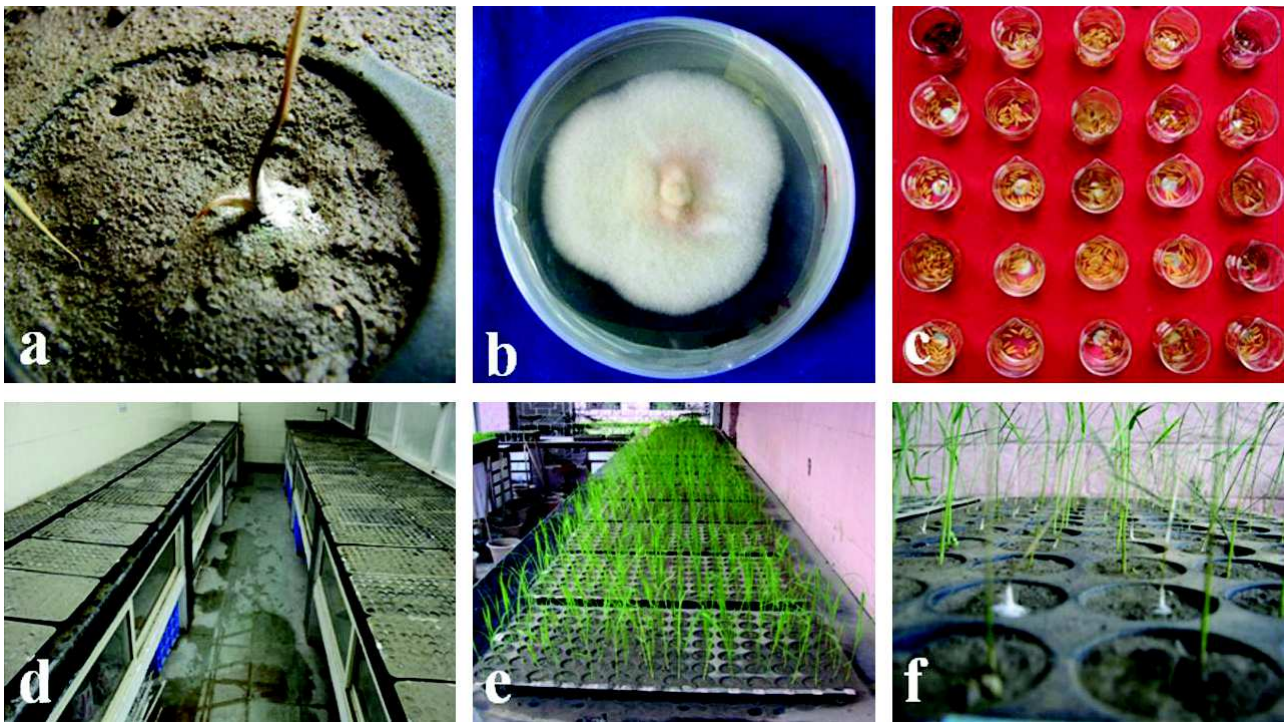

Fig. 1. High throughput protocol for screening rice accessions against bakanae disease. (a) Mycelial growth of *Fusarium fujikuroi* at the base of infected seedling; (b) *Fusarium fujikuroi* grown on PDA to harvest microconidia for inoculum; (c) Soaking of seeds in microconidial suspension; (d) Inoculated seeds sown in soil in protrays; (e) Plants growing in greenhouse until reactions are recorded; (f) Plants showing disease symptom after 15 days of inoculation

entries including the highly susceptible and resistant genotypes were soaked in 10 ml of inoculum suspension for 24 hours at room temperature. Control seeds were soaked in sterile water. Inoculated and control seeds were sown in protrays (four protrays per treatment/ ninety-eight seeds per protray) containing autoclaved mixture of soil and sand in the ratio of 3:1. For conventional screening through seedling inoculation method, the surface sterilized seeds of each genotype were incubated in a petri plate (90 mm diameter) lined with moist filter paper for germination. The petriplates were incubated for seven days at 25°C and watered daily to maintain moisture. Seven days old seedlings of each genotype were inoculated by dipping their roots in freshly prepared inoculum suspension for two hours. Inoculated seedlings were transplanted in pots containing autoclaved mixture of soil and sand in the ratio of 3:1. The greenhouse was maintained at 30°/ 25° (±3)°C day/night temperature, 60/80(±10)% day/ night relative humidity, and natural sunlight levels with variable photoperiod depending on the time of year. Protrays and pots with seedlings were watered everyday to keep them in saturated condition. Post inoculation, the seedlings were carefully observed for the symptoms of bakanae disease. Data on seedling height and number of seedling mortality was recorded everyday upto 15 days [21]. Percent elongation was determined as seedling height under inoculation over control on 15th day after inoculation.

Screening of rice germplasm against bakanae disease

The rice germplasm consisting of 92 diverse genotypes from across India which includes improved varieties and landraces were screened in the Division of Genetics, Indian Agricultural Research Institute, New Delhi for resistance against bakanae disease using highly virulent isolate, F250.

Statistical analysis

The experiment was laid out in complete randomised block design with four replications for each genotype and each treatment consisted of 98 entries. Germination data were recorded one week after sowing. The basal nodes of dead seedlings were examined under microscope for the fungus. The data of discoloured,

elongated, stunted and dead seedlings were recorded daily starting from 7th day to 15th day after inoculation. The data for disease incidence was recorded as percent infected seedlings (including elongated and dead seedlings) to assess the level of resistance or susceptibility of each test genotype following disease rating scale:

Disease incidence (Per cent infected seedlings)	Disease reaction
0-10	Highly resistant
11-20	Resistant
21-40	Moderately resistant
41-60	Moderately susceptible
61-80	Susceptible
Above 80	Highly susceptible

Disease incidence = (Number of infected seedlings/ Total number of plants)*100

Data collected were analyzed through appropriate procedures of statistics using SAS software Version 9.2 (SAS, 2009, SAS Institute Inc., Cary, NC, USA).

Results

The most characteristic symptom of bakanae disease is abnormal elongation of seedlings with chlorotic stems and leaves. Across the four inoculum concentration treatments, disease severity slightly increased with an increase in inoculum concentration up to 1×10^6 spores ml^{-1}. There was no significant difference between the three highest spore concentrations. At this concentration, there was higher abnormal seedling elongation and mortality, which are indication of higher susceptibility of rice variety to bakanae disease. Therefore, the spore concentration of 1×10^6 ml^{-1} was considered best for screening rice against bakanae disease.

It was observed that the infection percentage (seedling elongation) in seedlings was higher in seed inoculation method as compared to seedling inoculation (Fig. 2). The genotype Pusa 1342, did not show increase in seedling height due to abnormal elongation under both seed and seedling inoculation. While in genotype PB 1121, inoculated seeds resulted in abnormal seedling elongation compared to un-inoculated control. The mean seedling height at 15th day in seed inoculated PB 1121 was 23.53±1.86 cm compared to 18.5±2.60 cm in control. Student's *t*-test for mean seedling height in seed treatment was significantly different when compared to control (t_{18} = –4.97; P<0.001; n=20). The mean seedling height in PB 1121 under seedling inoculation method was

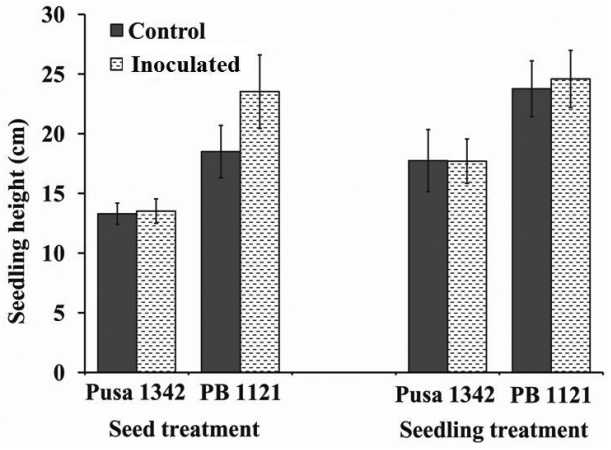

Fig. 2. Seedling height (15 days after inoculation) in the resistant genotype Pusa 1342 and susceptible genotype, PB 1121 following seed and seedling inoculation for screening against bakanae disease

24.59±2.40 cm compared to 23.77±2.34 cm in control, revealing there by that seedling height was not affected under conventional seedling treatment method (t_{18} = –0.77; P>0.45; n=20).

Data on per cent elongation after 15 days of inoculation in both the varieties is presented in Fig 3; per cent elongation was more pronounced in seed inoculation than in seedling inoculation method. No significant difference observed in the elongation percentage in resistant parent Pusa 1342 under seed inoculation (1.65%) as compared to seedling inoculation (0.28%) while highly significant difference was observed in per cent elongation of PB 1121 under seed inoculation (27.19%) as compared to seedling inoculation (3.45%). The seed inoculation method produced clear cut and distinct symptoms of the disease than seedling inoculation method.

The primary purpose of the present study was to develop a bioassay that could consistently distinguish minor differences among rice germplasm for reaction against bakanae disease. The results showed that, the isolate F250 was able to clearly distinguish the response of the resistant genotype (Pusa 1342) and susceptible genotype (PB 1121) against bakanae disease. Seedling mortality was observed in seed inoculated treatment for both PB 1121 and Pusa 1342, whereas no seedling mortality was recorded in the controls. The susceptible genotype, PB 1121 showed seedling mortality from 9th day after inoculation and mortality increased up to

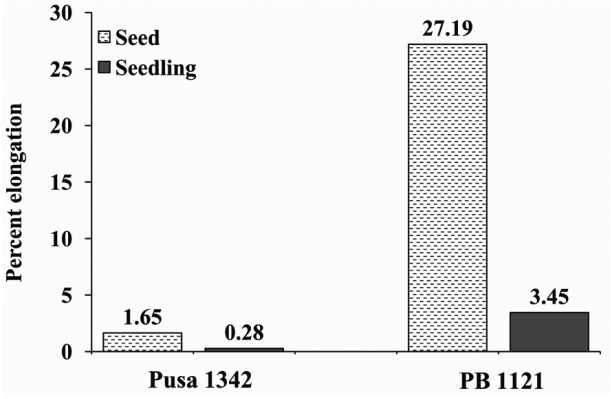

Fig. 3. Per cent increase in seedling height over control under seed and seedling inoculation method after 15 days of inoculation in Pusa 1342 and PB 1121

99% by the end of 15th day of inoculation. In the resistant genotype Pusa 1342, seedling mortality was observed from 10 days after inoculation and it was consistent upto 15 days. The final count of dead seedlings was 8/98 in case of resistant genotype Pusa 1342, whereas in the susceptible genotype PB 1121, it was 97/98 seedlings (Table 1). The disease reactions of Pusa 1342 and PB 1121 are shown in Fig. 4. The seedlings of PB 1121 showed significant differences in height following seed inoculation compared to control from the 11th day onwards after inoculation (Table 1). By contrast, no significant difference in seedling height was observed in the resistant genotype, Pusa 1342 in seed inoculated and control samples (Fig. 5). Based on these results, seed inoculation method was selected as the standard protocol for screening rice for bakanae disease.

Out of ninety-two genotypes screened following the high throughput screening protocol developed in present study, eight were highly resistant, four were resistant, thirty-three were found to be moderately resistant, fourteen moderately susceptible, thirteen susceptible and twenty genotypes as highly susceptible to the disease (Table 2). The mean per cent elongation of the genotypes ranged from 0.2 % in Mirzag to 68.5 % in Jaya. Genotypes such as Athad apunnu, C101A51, Chandana, IR 58025B, Panchami, PAU 201, Pusa 1342 and Varun Dhan were found to be highly resistant, while BPT 5204, Himju, Peeli badam and Suphala were resistant. Rasi and TKM 6 were found to be highly susceptible exhibiting cent per cent mortality with elongation and mortality symptoms as early as on 8th day after inoculation. Genotypes, Heibao, Indira sona, Lal Basmati, PRR 78, Pungreila, Pusa Basmati 1121, Punjab Basmati 3, Pusa 1568, Pusa 33, Saathi, Sahpasand, SN 46, Sonasal, Suraniat, Swarna, Tetep, Type-3, Tilak Chandan, were highly susceptible to bakanae disease.

Discussion

In the present study, we developed a rapid, reliable and high-throughput seed inoculation assay for screening against bakanae disease in rice. The developed assay produced disease symptoms

Table 1. Mean seedling height and seedling mortality in Pusa 1342 and PB 1121 after seed inoculation

Days after inoculation	Pusa 1342			PB 1121		
	Seedling height (cm)		Seedling mortality in treated	Seedling height (cm)		Seedling mortality in treated
	Control	Treated		Control	Treated	
7	4.6±0.17	4.1±0.10	0	5.5±0.22	5.7±0.33	0
8	5.5±0.15	5.4±0.17	0	7.5±0.37	7.4±0.41	0
9	8.2±0.26	7.0±0.19	0	9.1±0.36	9.4±0.31	6
10	9.8±0.20	8.2±0.21	1	11.1±0.49	11.1±0.51	23 (29)
11	11.1±0.26	9.9±0.32	2 (3)	15.1±0.53	14.9±1.06	21 (50)
12	11.6±0.27	10.3±0.35	1 (4)	17.0±0.49	19.5±1.24	22 (72)
13	12.0±0.26	10.9±0.45	2 (6)	18.7±0.47	21.4±1.91	15 (87)
14	12.4±0.23	12.0±0.48	1 (7)	20.1±0.48	24.3±2.30	5 (92)
15	12.9±0.21	12.3±0.46	1 (8)	20.7±0.42	24.0±0.00	5 (97)

*Numbers in parenthesis indicates cumulative number of dead seedlings on a given day after inoculation

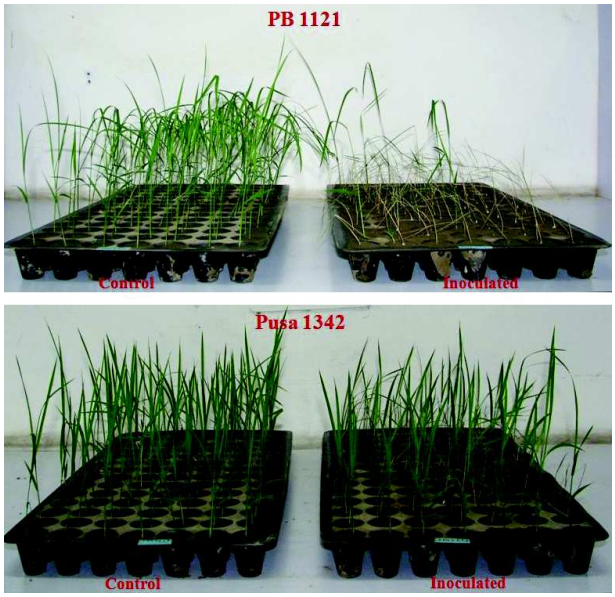

Fig. 4. Control and seed inoculated seedlings in the resistant genotype, Pusa 1342 and susceptible genotype, PB 1121 in protrays

typically associated with bakanae disease such as discolouration, elongation, stunting and in severe cases mortality of the seedlings. Disease symptoms produced by seed inoculation method and seedling dip method were compared to test their efficiency in screening for bakanae disease.

Abnormal increase in seedling height is one of the main symptoms of bakanae disease. Significant differences were observed in seedling height due to abnormal elongation of seedlings in treated samples as compared to the control. In both seed and seedling inoculation, the genotype Pusa 1342, did not show abnormal elongation of seedlings, while in PB 1121, inoculated seeds resulted in abnormal elongated seedlings, when compared to control. Seedling treatment did not show any significant effect on seedling height between the treatment and control. This abnormal elongation of treated seedlings may be attributed to high levels of gibberellin, a plant hormone produced by the fungus [5, 22-23].

In general, per cent elongation was more in seed inoculation compared to seedling inoculation. A significant difference between seed and seedling inoculation methods could be clearly established based on the disease symptoms in the susceptible genotype PB 1121 and the resistant genotype Pusa 1342. The seedling mortality at 15th day was low in resistant genotype Pusa 1342 compared to nearly total kill recorded in PB 1121. Agarwal *et al*. [24] also reported that diseased plants at advanced stages of infection showed collar infection followed by death within 14 to 42 days due to bakanae disease. In the present study, we were able to clearly demonstrate that the seed inoculation could be reliably utilized in screening for bakanae resistance as compared to seedling inoculation. In contrast to this, Haque et al. [25] reported that maximum disease development, both qualitative and quantitative, was obtained by dipping the sprouted seeds in spore suspension before sowing for 12 hours. Results of the present investigation are in accordance with the findings of Hossain et al. [26]; Rajagopalan and Bhuvaneswari [27] where, they reported that sowing ungerminated seeds in infected soil resulted in rapid progress of the disease and a high percentage of mortality.

Several bakanae screening methods such as, *In vitro* seedling screening assay [28], sprouted seedling assay [25], dipping dry seeds in gibberellic acid [26, 29], infested soil pot or field method [27, 30] reported the use of higher inoculum concentration (5.0×10^{10} ml^{-1}) to ensure heavy disease pressure and requires more than 35 days to two months for screening rice germplasm against bakanae disease. The high-throughput bioassay used in present study has several advantages over these conventionally used protocols: Firstly, uniform and healthy seeds are used, minimizing the influence of physiological conditions of seedling and interference of other pathogens in assessment of the bakanae resistance of the genotypes. Secondly, it requires less space and labour for testing and does not require any *in vitro* conditions for screening. Thirdly, the symptoms produced are easily diagnosable hence it is more precise. Fourthly, the new assay is rapid for screening large number of rice genotypes against bakanae disease; it can be completed within 15 days after sowing. It would be possible to screen as many as 968 genotypes (14 genotypes per protray measuring 0.53 cm x 0.27 cm with seven plants per genotype) in an area as less as 10 sq.m within 15 days time. Finally, the disease pressure can be ensured even with low inoculum concentration (1.0×10^{6} ml^{-1}).

Large scale germplasm screening is critical for identifying genotypes resistant to bakanae disease, gene mapping studies as well as in mining novel alleles for bakanae resistance. The highly resistant genotypes such as Athad apunnu, C101A51, Chandana, IR 58025B, Panchami, PAU 201, Pusa 1342 and Varun Dhan can be utilized as resistance donors in breeding programs, whereas the highly susceptible genotypes

Table 2. Mean per cent elongation, disease incidence and disease reaction of 92 rice genotypes screened against *Fusarium fujikuroi* isolate F250 using seed inoculation method

S.No.	Genotype	Mean % elongation	Disease incidence (% seedling mortality)	Disease reaction
1	Pusa Basmati 1121	27.2	100.0	HS
2	Pusa 1342	1.7	8.0	HR
3	Apo	21.9	23.8	MR
4	ASD 18	34.2	28.6	MR
5	Athad apunnu	5.5	0.0	HR
6	Basmati 370	12.6	76.2	S
7	BPT 5204	4.8	14.3	R
8	Brown gore	8.1	71.4	S
9	Bungpat subdmei	9.7	28.6	MR
10	C101A51	3.1	0.0	HR
11	Chakkad	6.9	61.9	S
12	Chandana	10.9	0.0	HR
13	Chuhata	31.0	52.4	MS
14	CO 39	12.5	23.8	MR
15	CSR 23	31.8	47.6	MS
16	CSR 27	21.1	61.9	S
17	CSR 30	16.6	23.8	MR
18	Domsiah	19.4	33.3	MR
19	FL 478	32.0	38.1	MR
20	Haryana Basmati 1	54.2	33.3	MR
21	Hassan serai	7.5	57.1	MS
22	Heibao	32.3	95.2	HS
23	Himju	7.5	7.1	HR
24	Indira sona	55.3	81.0	HS
25	IR 24	19.5	38.1	MR
26	IR 36	7.9	71.4	S
27	IR 50	31.6	33.3	MR
28	IR 58025 B	7.3	0.0	HR
29	IR 64	9.2	57.1	MS
30	IR 72	32.3	33.3	MR
31	Jaya	68.5	42.9	MS
32	Jeeni	24.0	61.9	S
33	Jhulhat	26.7	33.3	MR
34	Kala Fultas	38.6	38.1	MR
35	Karad	7.8	42.9	MS
36	Katheri	58.0	61.9	S
37	Kaw kaver	5.8	38.1	MR
38	Khao khao	29.6	42.9	MS
39	Lal Basmati	27.4	81.0	HS
40	Lalat	20.5	28.6	MR
41	Lypya	24.2	57.1	MS
42	Malviya dhan	28.9	38.1	MR
43	Mazari dhan	33.9	28.6	MR
44	Mirzag	0.2	38.1	MR
45	MTU 1001	48.0	47.6	MS
46	MTU 1010	37.0	28.6	MR
47	Nagina 22	15.1	33.3	MR
48	Panchami	12.9	0.0	HR
49	Pathara	17.6	21.4	MR
50	PAU 201	21.8	9.5	HR
51	Peeli badam	2.4	14.3	R
52	Pokkali	2.1	28.6	MR
53	PRR 78	20.0	81.0	HS
54	Pungreila	8.7	81.0	HS
55	Punjab Basmati 3	16.0	95.2	HS
56	Punjab mehak	12.0	38.1	MR
57	Pusa 1568	10.2	95.2	HS
58	Pusa 33	9.3	100.0	HS
59	Pusa 44	6.8	52.4	MS
60	Pusa 6B	1.8	33.3	MR
61	Pusa Basmati 1	11.1	33.3	MR
62	Pusa Basmati 6	32.3	76.2	S
63	Improved Pusa Basmati 1	17.4	33.3	MR
64	Pusa Basmati 1509	16.5	57.1	MS
65	Pusa 1602	7.4	23.8	MR
66	Pusa Basmati 1609	22.8	61.9	S
67	Pusa 1790	3.1	76.2	S
68	Pusa Sugandh 2	5.3	71.4	S
69	Pusa Sugandh 5	15.4	57.1	MS
70	Ramjawayan	5.0	28.6	MR
71	Rangoli	7.6	28.6	MR
72	Rasi	14.6	100.0	HS
73	Saathi	10.1	100.0	HS
74	Safed luchai	4.8	38.1	MR
75	Sahpasand	5.6	81.0	HS
76	Sarjao	40.8	42.9	MS
77	SN 46	20.7	81.0	HS
78	Sona Mahsuri	8.3	28.6	MR
79	Sonasal	18.4	100.0	HS
80	Sanwal Basmati	25.8	66.7	S
81	Suhagmani	12.5	28.6	MR
82	Sulandas	9.0	76.2	S
83	Suphala	35.3	14.3	R
84	Suraniat	38.5	100.0	HS
85	Swarna	26.3	95.2	HS
86	Taipei 309	19.3	52.4	MS
87	Tetep	24.6	81.0	HS
88	Tilak Chandan	NA	100.0	HS
89	TKM 6	16.3	100.0	HS
90	Type 3	10.8	81.0	HS
91	Vandana	29.6	28.6	MR
92	Varun Dhan	4.7	0.0	HR

*HR=highly resistant, R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible, HR=highly susceptible and NA=not available.

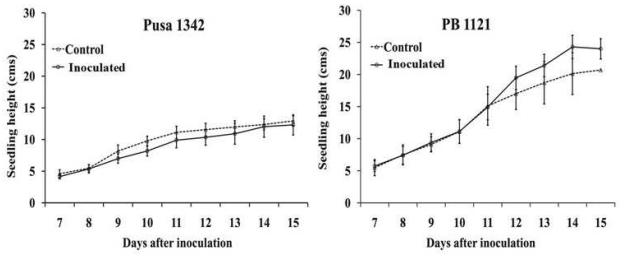

Fig. 5. Influence of seed inoculation with *Fusarium fujikuroi* isolate F250 on seedling height in Pusa 1342 and PB 1121

such as Rasi, TKM 6, and PB1121 can be crossed with resistant genotypes for development of mapping populations for mapping QTLs/genes conferring resistance to bakanae disease. The new bioassay reported in this study is simpler, quick, space-effective and accurate than the existing methods available for screening bakanae disease. Using our method, the screening of several hundreds of rice germplasm can be completed within 15 days. The seed soaking method produced more severe disease symptom even with lesser concentration of inoculum than those obtained using seedling dip method. With the sequencing of *Fusarium fujikuroi* genome [31], the new rapid bioassay developed here may find wider application in both pathogen and host studies, including the identification of bakanae resistant sources, mapping QTLs/genes governing resistance and to develop resistant varieties with inbuilt resistance and minimize the damage caused by the pathogen and enhance rice production especially in Basmati rice.

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