

**SCREENING OF BETELVINE CULTIVARS AGAINST *SCLEROTIUM*
ROLFSII CAUSING FOOT AND ROOT ROT DISEASE**

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Abstract

Thirteen betelvine cultivars (PB 001 to PB 013) collected from different locations of Bangladesh showed remarkable variations in disease reaction to foot and root rot caused by *Sclerotium rolfsii*. The lowest incubation period 8 days was required for the cultivars PB 005 (BARI line), PB 006 (Misti pan), PB 009 (BARI Pan-1) and PB 010 (Bangla pan) and the highest incubation period 22 days was required for the cultivars PB 001 (Laldingi pan) and PB 013 (Gayasur pan) for appearance of 1st disease symptoms. The disease incidence ranged 8.33 to 100% at 30 days after inoculation. The lowest disease incidence was recorded in PB 001 (Laldingi pan), while the highest disease incidence was recorded from PB 002, PB 003 (Chalitaguti pan), PB 004 (Sanchi pan), PB 005, PB 006 (Misti pan), PB 007, PB 008, PB 009 (BARI Pan-1), PB 010 (Bangla pan) and PB 012 (Bhabna pan) followed by PB 011 (Jhal pan) and PB 013 (Gayasur pan). Based on incidence of foot and root rot on those cultivars, PB 001 (Laldingi) was graded as resistant, PB 011 (Jhal pan) and PB 013 (Gayasur pan) as moderately susceptible and rest of the cultivars as susceptible.

Keywords: Betelvine, cultivars, foot and root rot, resistance

Introduction

Betelvine (*Piper betle* L.) is an important cash crop mainly for its leaves, which are used for chewing purpose (Maity and Shivashankara, 1998). Total cultivated area under the crop in Bangladesh in 2016-17 was about 23,803.20 hectares and the total annual production was about 2,14,252 metric tons. The average yield per hectare is 9.0 metric tons (BBS, 2018). Betelvine is one of the least land-intensive and highly labour-intensive crops (Sen and Roy, 1984). This crop has great market value both inside and outside Bangladesh and its export started to Europe in 1974-75 and to Saudi Arabia in 1991. Bangladesh annually exports betel leaves worth around TK.123 crore to Saudi Arabia. The country exported 4,984 tons betel leaves in the year 2017-18 mainly to Middle East countries including Saudi Arabia (Jasim Uddin, 2018). Betelvine is a dioecious plant, propagated vegetatively for commercial cultivation. There are about 100 varieties of betelvine across the world, out of which 30 varieties are from West Bengal and Bangladesh (Guha and Jain, 1997). The origin of betelvine is thought to be Malayasia (Das *et al.*, 2016). Betel leaves contain some vitamins, enzymes,

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thiamine, riboflavin, tannin, iodine, iron, calcium, minerals, protein, essential oil and medicine for liver, brain and heart diseases (Chopra *et al.*, 1956).

Despite of the tremendous potential of the crop, cultivation of betelvine is highly risky and returns are uncertain because of its vulnerability to several pests and diseases, aggravated by the moist and humid conditions in the plantation. Among them foot and root rot caused by *Sclerotium rolfsii* is the major constraints for cultivation of the betelvine crop (Goswami *et al.*, 2002). The fungus *S. rolfsii* is a facultative parasite and can maintain continuity of its generation under adverse situation by the formation of resting structure called sclerotia. In severely infected field, loss ranges from 10 to 25% and sometimes, it reaches up to 80% (Mehan and McDonald, 1990).

Detection of *S. rolfsii* resistance betelvine cultivars is of paramount importance for betelvine improvement and cost-effective cultivation. Till date, there is limited information on the resistance source of betelvine against *S. rolfsii* causing foot and root rot disease. The present study was conducted to evaluate the response of betelvine entries against *S. rolfsii* for the identification of resistance sources in available genotypes in Bangladesh.

Materials and Methods

A total of 13 betelvine cultivars were collected from different betelvine growing areas of Bangladesh as described in Table 1. The cultivars were screened for their resistance against *S. rolfsii*, causal pathogen of foot and root rot of betelvine. The experiment was conducted *in-vivo* in earthen pot in a betelvine yard, commercially known as ‘baroj’. The baroj was constructed in the experimental field of Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh. The experiment was conducted during March, 2016 to March, 2017. The experiment was laid out in a randomized complete block design (RCBD) with 4 replications. Data were recorded on various resistance components and analyzed statistically by using computer package program (Statistix 10).

Table 1. List of betelvine cultivars used in screening experiment against *Sclerotium rolfsii* causing foot and root rot disease

Entry	Name of Cultivar	Location of collection (Upazilla and Zilla)
PB 001	Laldingi pan	Pakundia, Kishoreganj.
PB 002	-	Spices Research Centre, BARI, Bogura.
PB 003	Chalitaguti pan	Gouronadi, Barisal.
PB 004	Sanchi pan	Kaliganj, Jhenaidah
PB 005	-	Spices Research Centre, BARI, Bogura.
PB 006	Misti pan	Mohanpur, Rajshahi
PB 007	-	Spices Research Centre, BARI, Bogura.
PB 008	-	Spices Research Centre, BARI, Bogura.
PB 009	BARI Pan-1	RARS, Barisal.
PB 010	Bangla pan	Mirpur, Kushtia.
PB 011	Jhal pan	Sitakunda, Chattogram.
PB 012	Bhabna pan	Kaligonj, Jhenaidah
PB 013	Gayasur pan	Pakundia, Kishoreganj.

Growing betelvine plants

Apparently healthy betelvines of different cultivars were used to prepare cuttings. Forty centimeter long cutting having five nodes each was prepared and grown in 14 inches diameter earthen pots containing potting medium at one plant per pot. The pots were placed inside the betelvine orchard (baroj) and allowed to grow providing necessary care and management practices. One to two internodes below the bud point was dipped into the soil, kept touching with surface soil. Potting medium was prepared by mixing soil, sand and well decomposed cowdung in the proportion of 2:1:1 by volume and were sterilized with formaldehyde. Formalin solution (4%) @ 200 ml/cft soil was mixed with the soil heap and the soil was covered with a polythene sheet for 48 hours for sterilization. After 7 days, surface sterilized earthen pots were filled with the sterilized soil (Dashgupta, 1988). Betelvine plants were fasten with bamboo sticks used in the baroj.

Inoculum preparation and inoculation

A virulent isolate of *S. Rolfsii* was multiplied on barley grains (Gupta and Kolte, 1982). Barley grains were soaked in 2% sucrose solution overnight, drained off excess solution and boiled in fresh water for 30 minutes and drained off again. The grains were transferred into 250 ml conical flasks @ 80 g per flask and autoclaved at 121.6°C under 1.1 kg/cm² pressures for 20 minutes. The conical flasks were allowed to cool at room temperature and the grains inside were inoculated with 5 mm discs of 3 to 4 days old culture of *Sclerotium rolfsii* grown on PDA. Seven discs were added into each flask and the flasks were incubated for three weeks at 25±2°C.

After twelve months of plantation the betelvine cultivars were inoculated with freshly multiplied isolate of *S. rolfsii*. The cultivars were prepared for inoculation by removing top soil within 5 cm of the stem to a depth of 2 cm. A table spoon (5 g) of inoculum was placed in direct contact of entire circumference of the exposed stem. Finally, the inoculum was lightly covered with top soil for infection and disease development. The symptomatology was studied to screen the betelvine entries for resistance to foot and root rot disease.

Data collection and grading of cultivars

Data on days to appearance of visible symptom and incidence of foot and root rot on different cultivars including susceptible check were recorded at 10, 15, 20, 25 and 30 days after inoculation. The tested cultivars were graded as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) based on disease incidence determined on a 1 - 4 scale, where disease incidence 0-10% (scale 1) = resistant (R), 11-30% (scale 2) = Moderately resistant (MR), 31-60% (scale 3) = Moderately susceptible (MS) and more than 61% (scale 4) = Susceptible (S).

The incidence of the disease was computed based on the following formula:

$$\% \text{ Disease incidence} = \frac{\text{Number of infected plant in the area covered}}{\text{Number of inspected plant}} \times 100$$

Results and Discussion

Days to appearance of disease symptom

The time interval required for appearance of first disease symptoms after inoculation differed considerably among the betelvine cultivars. The lowest incubation period of 8 days was required for the cultivars PB 005, PB 006 (Misti pan), PB 009 (BARI Pan-1) and PB 010 (Bangla pan). The highest incubation period of 22 days was required for the cultivars PB 001 (Laldingi pan) and PB 013 (Gayasur pan) (Table 2 and Plate 1). The incubation period for other cultivars ranged 9-20 days.

Disease incidence

Among the 13 betelvine cultivars, no disease symptom was observed in cultivars PB 001, PB 002, PB 003, PB 007, PB 008, PB 011 and PB 012 after 10 days of pathogen inoculation whereas PB 009 showed 66.66% disease incidence at the same days after inoculation (Table 2).

At 15 days after inoculation of pathogen, 100% disease incidence was observed in cultivar PB 009 (BARI Pan-1), no disease incidence (0%) was found in PB 001, PB 011 and PB 013.

At 20 days after inoculation, the highest disease incidence was observed in PB 005, PB 006, PB 009 and PB 010, followed by PB 007 and the lowest disease incidence was recorded in PB 001 and PB 013 followed by PB 011.

At 25 days after inoculation, the betelvine cultivars showed more or less similar disease incidence as observed at 20 days after inoculation. The minimum disease incidence was recorded in PB 001 (Laldingi) (8.33%) which was followed by PB 008, PB 011 and PB 013. The maximum disease incidence (100%) was recorded from PB 005, PB 006, PB 007, PB 009 and PB 010.

The final disease incidence recorded at 30 days after inoculation showed remarkable variations among different cultivars. The lowest disease incidence (8.33%) was recorded in cultivar PB 001 (Laldingi pan). The highest disease incidence (100%) was observed in PB 005, PB 006, PB 007, PB 009 and PB 010 which was followed by PB 004, PB 012, PB 002, PB 003, PB 008, PB 011 (58.33%) and PB 013 (49.99%) (Table 2).

Grading of cultivars

The betelvine cultivars were graded into different categories of response to foot and root rot disease based on final disease incidence. Among the cultivars, only

one cultivar PB 001 (Laldingi pan) showed resistant reaction (R), while cultivars PB 011 (Jhal pan) and PB 013 (Goyasur pan) displayed moderately susceptible response and rests of the cultivars were found susceptible (Table 2 and Plate 1)). The results revealed that the sources of resistance response to foot and root rot disease are limited in the available cultivars of betelvine in Bangladesh.

Table 2. Response of different betelvine cultivars of foot and root rot disease caused by *Sclerotium rolfsii*

Betelvine cultivars	Days to appearance of symptom	Percent disease incidence at different days after inoculation (DAI)					Disease reaction ^o
		10	15	20	25	30	
PB 001 (Laldingi pan)	22	0.00c ^a (0.083) ^b	0.00f (0.083)	0.00d (0.083)	8.33d (8.56)	8.33d (8.56)	R
PB 002	12	0.00c (0.083)	33.33e (34.017)	33.33c (34.017)	66.66b (52.79)	83.33ab (69.81)	S
PB 003 (Chalitaguti pan)	11	0.00c (0.083)	66.66cd (52.799)	66.66b (52.799)	66.66b (52.80)	83.33ab (69.81)	S
PB 004 (Sanchi pan)	9	41.66b (38.71)	58.33de (48.104)	66.66b (52.799)	66.66b (52.80)	91.66a (78.32)	S
PB 005	8	41.66b (38.71)	75.00bc (65.115)	100a (86.822)	100a (86.82)	100a (86.82)	S
PB 006 (Misti pan)	8	58.33a (48.10)	91.67ab (78.316)	100a (86.822)	100a (86.82)	100a (86.82)	S
PB 007	11	0.00c (0.083)	66.66cd (52.799)	91.67a (78.316)	100a (86.82)	100a (86.82)	S
PB 008	11	0.00c (0.083)	33.33e (34.017)	33.33c (34.017)	33.33c (34.02)	66.66bc (52.80)	S
PB 009 (BARI Pan-1) (Control)	8	66.66a (52.79)	100a (86.822)	100a (86.822)	100a (86.82)	100a (86.82)	S
PB 010 (Bangla pan)	8	33.33b (34.02)	83.33b (69.811)	100a (86.822)	100a (86.82)	100a (86.82)	S
PB 011 (Jhal pan)	20	0.00c (0.083)	0.00f (0.083)	8.33d (8.56)	33.33c (34.02)	58.33c (48.10)	MS
PB 012 (Bhabna pan)	11	0.00c (0.083)	49.99de (43.408)	66.66b (52.799)	75.00b (61.31)	91.66a (78.32)	S
PB 013 (Gayasur pan)	22	0.00c (0.083)	0.00f (0.083)	0.00d (0.083)	25.00c (25.91)	49.99c (43.41)	MS

^aValues within the same column with a common letter(s) do not differ significantly (P=0.01).

^bData within parenthesis are square root or arc-sine transformed values

^oDisease reaction : R= Resistant, MS= Moderately susceptible, S= Susceptible



A

B



C Susceptible plant Resistant plant

D

Plate 1. *Sclerotium rolfsii* inoculated plant (A), mycelia formation on inoculated earthen pot (B), susceptible and resistant plant against *Sclerotium rolfsii* (C) and lesion on foot and root of betelvine plant (D).

Conclusion

Most of the betelvine cultivars screened in the present study were found susceptible to foot and root rot disease, only Laldingi pan (PB 001) was found resistant and Jhal pan and Gayasur pan showed moderately susceptible reaction. The information generated from the present study will help development of betelvine against foot and root rot disease.

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