



Research Article

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Screening of Six Parents and 15 Cross Combinations against Bacterial Wilt (*Ralstonia solanacearum*)



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Abstract

The study on the screening for resistance to bacterial wilt (*Ralstonia solanacearum*) of six parents and 15 cross combinations/hybrids were evaluated under artificial epiphytotic conditions at the Plant Pathology lab and glass house of Plant Pathology Section of Horticulture Research Centre (HRC) of Bangladesh Agricultural Research Institute (BAIR), Gazipur, Bangladesh during the period from September 2016-17 to develop high yielding F₁ eggplant varieties resistant to bacterial wilt. Amongst the six parents and 15 hybrids, six hybrids viz., F₁ 4x17, F₁ 4x5, F₁ 18x17, F₁ 3x17, F₁ 3x5, F₁ 17x5 showed resistant (R) reaction. The cross F₁ 3x17, F₁ 17x5 exhibited the lowest percent of wilt (10%) followed by F₁ 4x17 (16.67%), F₁ 3x5 (13.33%). In case of parents, three parents showed resistant (R) reaction viz. BL083 (20.00% wilting), BL081 (16.67% wilting) and BLS2 (16.67% wilting). Incubation period ranges from 4 days to 12 days. The lowest incubation period (4 days) was observed from the parent BL034 followed by Islampuri (5 days) and Uttara (6 days), hybrids F₁ 4x14 (6 days) and F₁ 18x14 (6 days).

Keywords: *Ralstonia solanacearum*; Hybrids; Epiphytotic; Plant Pathology; Horticulture

Abbreviations: HRC: Horticulture Research Centre; BAIR: Bangladesh Agricultural Research Institute; TZC: Triphenyl Tetrazolium Chloride; CRD: Completely Randomized Design; HR: Highly Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; HS: Highly Susceptible

Introduction

Bacterial wilt caused by *Ralstonia solanacearum* [1], synonym *Pseudomonas solanacearum* Smith (1914), is widespread in the tropical, subtropical and temperate region, where it represents a major constraint to the production of numerous crops [2,3]. Recent reviews showed the very extensive host range of *R. solanacearum* which includes several hundred species representing about 53 families of plants [4]. Host species include ornamentals, weeds and important crops such as tomato (*Lycopersicon esculentum* L.) potato (*Solanum tuberosum* L.) eggplant (*Solanum melongena* L.), chilli pepper (*Capsicum* spp.), tobacco (*Nicotiana tabacum* L.), ginger (*Zingiber officinale* L.), peanut (*Arachis hypogea* L.) etc.

The incidence of soil borne diseases particularly bacterial wilt caused by *Ralstonia solanacearum* E.F. Sm. is the most limiting factor to produce eggplant in Bangladesh. Not only in Bangladesh, bacterial wilt is a disease of world wide importance in eggplant production [5]. An early crop of eggplant is very profitable for the farmers in Bangladesh, but the early crop is particularly vulnerable

to this disease. Once the eggplants are affected by bacterial wilt, they die within three to four days. The organism when injected in the susceptible hosts through the root systems cause vascular wilt and ultimate death of the plant [6]. The characteristics symptoms of bacterial wilt in most hosts are wilting, stunting and yellowing of the foliage. With susceptible infected eggplants, the most striking symptom is the rapid and complete wilting of the plants following field infection. Distinct epinasty of the petioles prior to wilting may be apparent if disease development is slow. Internal symptoms include discoloration of vascular tissue and decay of portions of the pith at the advanced stages of infection. It is the most destructive bacterial plant pathogen especially in the warm regions [6]. In recent years, bacterial wilt has become a great problem for eggplant cultivation in Bangladesh. The main effort to control this disease has been directed towards the development of resistance cultivars. But available information suggests that very few of the cultivars were reported as resistant to this disease. Various attempts to control bacterial wilt of different crops have

been reported [7] but success was limited. Breeding for resistance, therefore, remains the best control strategy, even though varietal resistance differs in different localities due to extreme variability and adaptation of the pathogen [8].

The grafting of cultivated variety on wild brinjal root-stock can successfully control the disease [9], but grafting technology is not well adopted to the farmers due to some technological aspects and it is some time troublesome. So, development of disease resistant cultivar is the easiest way to solve the problem, but development of disease resistant variety is not easy. Many workers are still working in the world to develop disease resistant variety. The development of resistant cultivars for the control of bacterial wilt has been successful in the case of tobacco and peanut [3]. Many cultivars of tomato are resistant for certain environments, but their resistance was not stable under conditions of high temperature and humidity in the lowland tropics. Similarly, some chilli accessions which were found to be resistant in India were susceptible in Japan [10]. Thus, the development of resistant variety in specific locations of the crops to certain pathogen remained a continuous process. Available information suggests that no appreciable attempt has yet been made to study the local as well as the available exotic plant material against bacterial wilt resistance in Bangladesh. Besides, no hybrid variety has been screened against bacterial wilt disease. The present study has been undertaken to evaluate the performance of the hybrid variety against bacterial wilt disease.

Materials and Methods

The present study was carried out to find out the resistance against bacterial wilt of the 15 cross combinations/hybrids and six parents and screened them to find out suitable cross combination of inbred lines for developing hybrid variety with high yield and wilting resistant. The materials required and methodology are described below under the following sub-heading.

Experimental site

The present experiment was conducted at the Plant Pathology lab and glass house of Plant Pathology Section of Horticulture Research Centre (HRC) of Bangladesh Agricultural Research Institute (BAIR), Gazipur, Bangladesh during the period from September 2016-17.

Materials: Six parents and 15 cross combinations/hybrids total 21 eggplant genotypes were used in this experiment. The parents were selected not only based on high yield and other yield contributing characters and quality but also on the basis bacterial wilt resistant. Therefore, the selected parents exhibited some degrees of resistant and the main objective of the experiment was to find out how the intensity of resistance turns to the hybrids form the parents.

Seeding production: Seeds of the parents and cross combinations were sown in steel tray containing sterilized soils mixture (soil: sand: compost) in the ratio of 3: 2: 1 on 1st September 2016. Before sowing, the seeds were treated with GA₃

solution (100ppm) for 24 hours at room temperature for quick and uniform germination. Seedlings were germinated on trays with sterilized soils mixture for a period of ten days. Ten days after seed germination young seedlings were transferred to the individual plastic pot (9cm diameter) containing sterilized soil mixed with 50% organic matter (v/v).

Bacterial cultures, preparation of inoculum and stock cultures: Pure culture of *R. solanacearum* strain used in this study was isolated from wilted eggplant showing typical symptoms of bacterial wilt from the research field of Plant Pathology Section, HRC, BARI. The pathogen on isolation on triphenyl tetrazolium chloride (TZC) agar medium [6] yielded grayish white, fluidal colonies with light pink centers. After purification, the isolate was characterized as race1 according to pathogenicity and host range [2], and biochemical test [4]. The identification of the isolates was also confirmed by Cock's Postulate. All isolates were kept as stock culture in lyophilized form and in 5ml sterilized tap water kept at 15 °C. Before preparation of inoculum, the bacteria was streaked on TZC from the stock culture. The inoculum was prepared by culturing the virulent and fluidal colony of *R. solanacearum* on casamino acid-peptone-glucose (CPG) agar medium.

Experimental design

The plants of 15 cross combinations and six parents were arranged in the glass house following Completely Randomized Design (CRD) with three replications and ten plants were inoculated in each replication. Line to line and plant to plant distance were maintained 30cm and 25cm respectively. The plants were inoculated when they were approximately 35 days old (15 days after transplanting in plastic pot).

Inoculation: Suspension of *R. solanacearum* was prepared from the stock culture which was grown on CPG for 48 hours at 30°C. A sterile toothpick was used to inoculate the bacterial suspension containing approximately 2.0×10^8 cfu/ml (colony forming unit per milliliter) was inserted at the axil of 3rd fully expanded leaf of the plants. Plants were observed regularly for the initiation of symptoms of wilt, incubation period, 25% and 50% plant wilt (days after inoculation). After recording the 50% wilted plant data regularly, data recorded at seven days interval and final data recorded at 42 days after inoculation. The initiation of the first wilt-symptoms after inoculation and the number of wilted plants for each accession was recorded and graded on a 0-5 rating scale of Winstead & Kelman [5] with some modifications. The modified rating scale is;

- 0 = Plants did not show any symptoms of wilt; highly resistant (HR)
- 1 = 1 - 20% plants wilted; resistant (R)
- 2 = 21 - 40% plants wilted; moderately resistant (MR)
- 3 = 41 - 60% plants wilted; moderately susceptible (MS)
- 4 = 61 - 80% plants wilted; susceptible (S)

5 = more than 80% plants wilted; highly susceptible (HS)

The reaction of the hybrids and parents were categorized as highly resistant, resistant, moderately resistant, moderately susceptible to highly susceptible, depending on the percentage of wilt of the plants. The incubation period, time taken to produce 25% and 50% wilt at days after inoculation (DAI) were also recorded. Final data on wilting (recorded at 42 DAI) were converted into percent wilt and the reaction of the genotypes was graded on a 0-5 rating scale according to Winstead & Kelman [5].

Results and Discussion

The reaction of the eggplant parents and hybrids against bacterial wilt shown in Table 1. It was revealed from the result reaction against bacterial wilt exhibited different degrees of variation. Among the 15 cross combinations, six hybrids showed resistant (R), seven hybrids showed moderately resistant (MR) and two hybrids showed moderately susceptible (MS) reaction against wilting. But in case of the parents three showed resistant (R) and three parents showed moderately susceptible (MS) reaction against wilting.

Table 1: Reaction of 15 hybrids combinations and six parents to *Ralstonia solanacearum*.

Hybrids and Parents	% of Wilt	Reaction*
F ₁ 4x18	40	MR
F ₁ 4x3	23.33	MR
F ₁ 4x17	16.67	R
F ₁ 4x5	20	R
F ₁ 4x14	43.33	MS
F ₁ 18x3	26.67	MR
F ₁ 18x17	20	R
F ₁ 18x5	23.33	MR
F ₁ 18x14	46.67	MS
F ₁ 3x17	10	R
F ₁ 3x5	13.33	R
F ₁ 3x14	36.67	MR
F ₁ 17x5	10	R
F ₁ 17x14	26.67	MR
F ₁ 5x14	36.67	MR
Uttara (4)	43.33	MS
Islampuri (18)	46.67	MS
BL083 (3)	20	R
BL081 (17)	16.67	R
BLS ₂ (5)	16.67	R
BL034 (14)	56.67	MS

N.B. 4 = Uttara, 18 = Islampuri, 3 = BL083, 17 = BL081, 5 = BLS₂, 14 = BL034

*0 = Plants did not show any symptoms of wilt, highly resistant (HR); 1 = 1 - 20% plants wilted, resistant (R); 2 = 21 - 40% plants wilted, moderately resistant (MR); 3 = 41 - 60% plants wilted, moderately susceptible (MS); 4 = 61 - 80% plants wilted, susceptible (S); 5 = more than 80% plants wilted; highly susceptible (HS).

Among the 15 hybrids six hybrids viz., F₁ 4x17, F₁ 4x5, F₁ 18x17, F₁ 3x17, F₁ 3x5, F₁ 17x5 showed resistant (R) reaction. The cross F₁ 3x17, F₁ 17x5 exhibited the lowest percent of wilt (10%) followed by F₁ 4x17 (16.67%), F₁ 3x5 (13.33%). The 20.00% wilting was observed from hybrids F₁ 4x5, F₁ 18x17. Seven hybrids showed moderately resistant (MR) reaction against bacterial wilt disease and the percent wilting plants ranged from 23.33 to 40.00%. The hybrids exhibited 23.33% wilting were F₁ 4x3, F₁ 18x5; 26.67% wilting observed from the hybrids F₁ 18x3, F₁ 17x14; 36.67% from F₁ 3x14, F₁ 5x14 and 40.00% from F₁ 4x18. Moderately susceptible (MS) reaction showed only by two hybrids they were F₁ 4x14 (43.33% wilting) and F₁ 18x14 (46.67% wilting).

In case of parents, three parents showed resistant (R) reaction viz. BL083 (20.00% wilting), BL081 (16.67% wilting) and BLS₂ (16.67% wilting). Three parents exhibited moderately susceptible (MS) reaction they were Uttara (43.33% wilting), Islampuri (46.67% wilting) and BL034 (56.67% wilting). Rahman [9] reported from an experiment against bacterial wilt (*R. solanacearum*) that line BL081 showed resistant reaction while the genotypes BL099 showed moderately resistant reaction. Six genotype BL117, ISD011, ISD001, BLS18, BLS₂ and Tarapuri (hybrid variety) were found moderately susceptible to bacterial wilt.

From the above discussion it was revealed that the hybrids along with the parents BL083, BL081 and BLS₂ showed resistant to moderately resistant reaction against bacterial wilt and increased the resistant reaction against the disease intensity. All the hybrids performed better than the lower parent and mid parent and sometime better parent also. When the parent crossed with BL034 performance was not satisfactory. Among the 15 cross combinations the hybrids F₁ 3x17, F₁ 17x5, F₁ 4x17, F₁ 3x5, F₁ 4x5, F₁ 18x17 performed better and showed resistant reaction (R; wilting 0-20% plant) than other hybrids against bacterial wilt disease. Rashid et al. screened twenty-three local and exotic cultivars of eggplant and three wild relatives of the crop against bacterial wilt in the glass house. The wild relatives *Solanum mammosum* was found resistant while the cultivar Dingaras Multiple Purple and Khakhatia long were found moderately resistant, the cultivars Nayankazal, Kahkhatia round, Tiwan naga, Nagasaki naga and Kurume naga were found moderately susceptible, while the rest of the cultivars as highly susceptible to bacterial wilt.

Mochizuki & Yamakawa [11] studied the resistant of selected eggplant cultivars and wild *Solanum* species to bacterial wilt. They reported that among eggplant cultivars tested "Dingras Multiple Purple" (from India) and "Aubergine" (from USA) showed higher resistant to bacterial wilt "Taiwan naga" one of the most resistant cultivars in Japan. Eggplant varieties No. 1 (from Malaysia) and "Sinampiro", "Makiling" and "Mayon" (from the Philippines) showed resistance like that of "Tiwan naga".

Incubation period ranges from 4 days to 12 days (Table 2). The lowest incubation period (4 days) was observed from the parent BL034 followed by Islampuri (5 days) and Uttara (6 days), hybrids

F₁ 4x14 (6 days) and F₁ 18x14 (6 days). The highest days (12) for incubation was recorded for hybrids F₁ 4x17 and F₁ 17x5 followed by F₁ 3x17 (11 days), parent BL081 (11 days). From the discussion it was revealed that moderately susceptible genotypes required fewer days (4 to 6 days) and moderately resistant to resistant genotypes required more days (7 to 12 days) for incubation.

Table 2: Incubation period and % wilt form DAI of 15 hybrids combinations and six parents to *Ralstonia solanacearum*.

Hybrids and Parents	Incubation Period (days)	25% Wilt DAI*	50% Wilt DAI*
F ₁ 4x18	7	10	-
F ₁ 4x3	9	-	-
F ₁ 4x17	12	-	-
F ₁ 4x5	10	-	-
F ₁ 4x14	6	9	-
F ₁ 18x3	8	-	-
F ₁ 18x17	9	-	-
F ₁ 18x5	9	-	-
F ₁ 18x14	6	8	-
F ₁ 3x17	11	-	-
F ₁ 3x5	10	-	-
F ₁ 3x14	7	12	-
F ₁ 17x5	12	-	-
F ₁ 17x14	9	-	-
F ₁ 5x14	7	11	-
Uttara (4)	6	9	-
Islampuri (18)	5	7	-
BL083 (3)	10	-	-
BL081 (17)	11	-	-
BLS ₂ (5)	10	-	-
BL034 (14)	4	6	9

N.B. 4 = Uttara, 18 = Islampuri, 3 = BL083, 17 = BL081, 5 = BLS₂, 14 = BL034

*DAI = Days after inoculation.

Only parent BL034 exhibited 56.67% wilting and days after incubation was nine days. The rest of the other resistant, moderately resistant and moderately susceptible genotypes did not show 50% wilt even at 42 days after inoculation. Among the 15 cross combinations 13 hybrids did not show 25% wilting and among the six parents three parent did not 25% wilting at final data collection (42 DAI). Six hybrids exhibited 25% wilting within 8 to 12 days and three parents showed 25% wilting within 6 to 11 days.

Conclusion

Pure culture of *R. solanacearum* strain used in this study was isolated from wilted eggplant showing typical symptoms of bacterial wilt from the research field. Considering the reaction to *Ralstonia solanacearum* the six parents and 15 hybrids, six hybrids viz., F₁ 4x17, F₁ 4x5, F₁ 18x17, F₁ 3x17, F₁ 3x5, F₁ 17x5 showed resistant (R) reaction. The cross F₁ 3x17, F₁ 17x5 exhibited the lowest percent of wilt (10%) followed by F₁ 4x17 (16.67%), F₁ 3x5 (13.33%). In case of parents, three parents showed resistant (R) reaction viz. BL083 (20.00% wilting), BL081 (16.67% wilting) and BLS₂ (16.67% wilting). Incubation period ranges from 4 days to 12 days (Table 2). The lowest incubation period (4 days) was observed from the parent BL034 followed by Islampuri (5 days) and Uttara (6 days), hybrids F₁ 4x14 (6 days) and F₁ 18x14 (6 days).

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