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Original Research Paper

Evaluation of *Solanum* species and eggplant cultivated varieties for bacterial wilt resistance

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* is one of the major diseases in *Solanum* species including cultivated Eggplant (*Solanum melongena* L.). Bacterial wilt (BW) disease management in eggplant is difficult due to high survival rate of pathogen in soil and chemical application is not eco-friendly. The best way to avoid bacterial wilt in eggplant is using disease-resistant varieties. However, only a limited number of bacterial wilt resistant varieties are available and, there is a necessity to identify and/or develop new resistant varieties. In the current study, wild *Solanum* species, and eggplant cultivated varieties were evaluated against *Ralstonia solanacearum*, and disease incidence was recorded. The cultivated varieties IIHR-108, Pusa Purple Long and Rampur Local were identified as susceptible, whereas, IIHR-7 and CARI-1 were identified as resistant to bacterial wilt. These resistant wild and cultivated varieties can be used as a root-stock in bacterial wilt disease resistant breeding programmes.

Keywords: Wild *Solanum* species, Eggplant, *Ralstonia solanacearum*, Disease scoring, Grafting

INTRODUCTION

Eggplant (*Solanum melongena* L.) (2n=24) is one of the most popular Solanaceous vegetable crop cultivated globally. Asia is the major producer (93%) of eggplant (FAO, 2016). It is originated from India and South China (Daunay and Hazra, 2012). Eggplant is generally cultivated in open fields with hot and humid conditions. Eggplant is susceptible to numerous diseases viz., bacterial wilt; fusarium wilt; verticillium wilt, early blight, leaf spot, *potato virus-Y* (PVY), *tobacco ring spot virus*, *tomato spotted wilt virus* (TSWV), phytoplasma, and root-knot nematode. Duo to these diseases, quality and quantity of eggplant production is adversely affected.

Eggplant bacterial wilt (*Ralstonia solanacearum*) is a major concern in India. The pathogen is race specific and has five different races. *Ralstonia solanacearum* strains are divided into five different biovars based on the biochemical analysis. In India, eggplant bacterial wilt is mainly caused by Race 1 and Biovar 3 (Gopalakrishnan *et al.*, 2005). The pathogen (*Ralstonia solanacearum)* can survive in soil upto ten years without any host plant and have the ability to colonize in non-host plants including a vast range of symptomless weeds (Gopalakrishnan et.al., 2014). The pathogen enters into the plants through wounds or secondary root initiation points leading to pathogen colonization in the vascular parenchyma and cell wall breakage (Ramesh, 2008). The initial wilt symptoms are leaf drooping, followed by full-plant wilting and vascular discoloration. When cut ends of wilted plant placed in water, milky white ooze out can be observed. There are different methods of artificial inoculation/ screening of bacterial wilt like root cut inoculation, leaf or stem pricking and natural field infestation (Ramesh, 2008). These pathogenic spores remain viable and active in the soil for several years making the disease control almost impossible through any means of chemical treatments especially in the regions

where repeated eggplant cultivation is taken up. Many of the commercial varieties/hybrids are highly susceptible to bacterial wilt. Chemical management often leads to the presence of chemical residues in the fruits, thus, raises the concern of food safety. The most economical way of bacterial wilt control is to develop bacterial wilt resistant varieties/hybrids. Recently, grafting strategy using resistant root-stock has been proposed for soil-borne pathogens like bacterial wilt, and it will help in bacterial wilt resistant varieties/hybrids development with the different genetic backgrounds. Hence, identification of a bacterial wilt root-stock will be of higher use and any preferable variety/hybrid can be grafted on it. Here, we evaluated wild solanum species and cultivated varieties for bacterial wilt resistance through artificial inoculation, and these can be used as root-stock in grafting.

MATERIALS AND METHODS

Plant Material and Phenotypic Characters

In this study, five eggplant cultivated varieties viz., IIHR-7 (IC395334), IIHR-662 (CARI-1, IC0585684), IIHR-108 (Arka Kusumakar), IIHR-663 (Rampur Local), and seven wild species viz., *Solanum gilo* (RS-3), *Solanum indicum* (RS-4), *Solanum viarum* (RS-6), *Solanum aethiopicum* (RS-7), *Solanum mammosum* (RS-8), and *Solanum torvum* (RS-9 and RS-9a) were used for bacterial wilt screening (**Fig.1**). These germplasm accessions were maintained at Indian Institute of Horticultural Research, Bengaluru.

Fig. 1. Eggplant cultivated varieties [IIHR-7 (IC395334), IIHR-662 (CARI-1, IC0585684), IIHR-108 (Arka Kusumakar), IIHR-663 (Rampur Local)] and seven wild species [*Solanum gilo, S. indicum, S. viarum, S. aethiopicum, S. mammosum,* and *S. torvum* used for bacterial wilt screening.

Table 1. Phenotypic traits of elite germplasm line

S;. No.	Name of the line	Phenotypic Characters
1	IIHR-108 (Arka Kusumakar)	Accession IIHR-108, is a pure line selection from IIHR-193 (A local collection from Karnataka). Phenotypic characters viz., Plant height: medium/tall; growth habit: spreading; stem and foliage color: green; flower color: white; fruit: small (medium-long) green; cooking qualities: good; Yield: 40t/ha; crop duration: 140-150 days and bacterial wilt incidence: high (susceptible).Quantitative traits: plant height (85-100 cm); fruit length (13-14 cm); fruit diameter (3.0-3.5 cm); average fruit weight (30-40 g).
2	IIHR-7 (IC395334)	It is an advanced breeding line derived from cross between Arka Keshav, and IIHR-322. Phenotypic characters viz., plant height: tall; growth habit: spreading; stem and foliage color: green; flower color: purple; fruit: long-green; cooking qualities: good and bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (100-120 cm); fruit length (18-20 cm); fruit diameter (3.2-3.5 cm); average fruit weight (40-45 g).
3	IIHR-662 (CARI-1, IC0585684)	This germplasm line was collected from Andaman & Nicobar (Port Blair) islands. Phenotypic characters viz., Plant height: medium/tall; growth habit: spreading; fruit: round-green; bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (115-130 cm); fruit length (10-12 cm); fruit diameter (14-16 cm); average fruit weight (300-350 g).
4	IIHR- 663 (Rampur Local)	This germplasm line was collected from Rampur village of UP, India. Phenotypic characters viz., Plant height: tall; growth habit: spreading; fruit: long-violet purple; fruit bearing: mixed (fruits are borne in clusters and solitary); cooking qualities: good and bacterial wilt incidence: high (highly susceptible).Quantitative traits: plant height (115-130 cm); fruit length (20-24 cm); fruit diameter (4.0-4.5 cm); average fruit weight (70-75 g).
5	Pusa Purple Long	This variety was developed through pure-lone selection by IARI, New Delhi. Phenotypic characters viz., Plant height: Medium; growth habit: spreading; fruit: long-purple (glossy); cooking qualities: good and bacterial wilt incidence: high (highly susceptible). Fruits are born in clusters.Quantitative traits: plant height (30-40 cm); fruit length (15-18 cm); yield about 40-45tonnes/ha.
6	*Solanum gilo*	Plant height: tall; growth habit: erect; fruit: small- round (look like tomato and have both ridge/ridge-less traits) cooking qualities: bad and bacterial wilt incidence: low (highly resistant). Quantitative traits: plant height (100-115cm); fruit length (3.5-4.0 cm); fruit diameter (3.0-3.5 cm); average fruit weight (25-30 g).
7	*Solanum indicum*	Plant height: medium/tall, growth habit: spreading (thorns on stems and mid ribs); fruit: small and purple (pea size, and red to orange when ripe) and bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (80-90 cm); fruit length (1.0-1.5 cm); fruit diameter (0.9-1.0 cm); average fruit weight (2-3 g).
8	*Solanum viarum*	Plant height: tall, growth habit: spreading (dense thorns are present on the stem mid ribs); fruit: small-round (white stripes during immature stage and turn yellow after ripening) and bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (100-120 cm); fruit length (2.5-3.0 cm); fruit diameter (2.0-3.0 cm); average fruit weight (5-8 g).
9	*Solanum aethiopicum*	Plant height: medium/tall, growth habit: spreading (have purple tinge); fruit: small-red, oblate round (look like tomato) and bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (85-95 cm); fruit length (5.5-6.5 cm); fruit diameter (4.5-5.0 cm); average fruit weight (40-50 g).
10	*Solanum mammosum*	Plant height: tall, growth habit: spreading (dense thorns are present on the stem mid ribs); fruit: cow's udder shape (turn yellow on ripening) and bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (125-135 cm); fruit length (5.5-6.0 cm); fruit diameter (3.5-4.0 cm); average fruit weight (15-20 g).
11	*Solanum torvum*	Plant height: tall, growth habit: spreading (dark green foliage); flower color: white; fruit: dark green at immature stage and turn yellow on ripening) and bacterial wilt incidence: low (highly resistant).Quantitative traits: plant height (130-150 cm); fruit length (1.5-2.5 cm); fruit diameter (1.0-1.5 cm); average fruit weight (10-15 g).

Basic information of *Solanum* species and eggplant varieties were described in **Table 1**.

Artificial Screening of Bacterial Wilt

R. solanacearum inoculum was prepared according to Urquhart *et al*. (1998) from bacterial wilted eggplants. The bacterium colonies were collected from wilted plant using ooze out test, and streaked on Triphenyl Tetrozolium Chloride (TZC or TTC) plate (**Fig. 2**). The plates were incubated at 28°C - 30°C for 48 hours, and the TZC plates were checked for development of avirulent and virulent colonies. The separated virulent colonies were selected and suspended in sterile distilled water. The concentration of inoculum was recorded using a spectrophotometer and stored at 4°C for further use. Soil drenching method was used for inoculating the 25 days seedlings with *R. solanacearum* suspension, with concentration

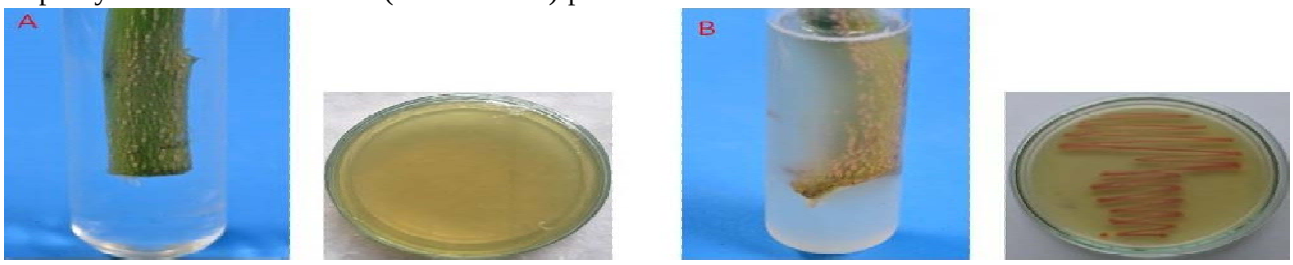

Figure 2. Ooze out test and *R. Solanacearum virulence* identification in TZC plate.

of 1.0 x10^8 CFU / ml (OD 600 nm = 0.3) (Rashmi *et al.*, 2012).

A) Resistant B) Susceptible

Observation and Bacterial Wilt Disease Screening

According to Hussain *et al.* (2005), bacterial wilt symptoms and total number of wilted plants per germplasm/variety were recorded on a 0-5 scale, with minor modifications. Based on the percentage of wilted plants, accessions were categorized as highly resistant to highly susceptible.

Disease scoring scale

S.No.	Percentage of disease Incidence (%)	Scale (0-5)
1	No wilt symptom (0%)	Highly Resistant (HR) (0)
2	1 - 10% wilted plants	Resistant (R) (1)
3	11 -20% wilted plants	Moderately Resistant (MR) (2)
4	21-30% wilted plant	Moderately Susceptible (MS) (3)
5	31- 40% wilted plants	Susceptible (S) (4)
6	> 40% wilted plants	Highly Susceptible (HS) (5)

RESULTS AND DISCUSSION

Ralstonia solanacearum race 1 Biovar 3 is more devastating and causes a severe problem in eggplant cultivation mainly in hot & humid areas like India (Markose, 1996). Controlling the bacterial wilt disease is practically not possible with the help of chemicals, and hence growing the resistant variety/hybrid is the best approach. In the breeding or grafting, identification of best-durable resistant root-stock is the first step. In various studies, high level of *Ralstonia solanacearum* resistance is reported in IIHR-7, CARI-1 and wild *Solanum* species *Solanum torvum*, LS 174, *Solanum sisymbriifolium*, *Solanum viarum*, *Solanum mammosum*, *Solanum nigrum*, *Solanum maroniense* and *Solanum stramonifolium* (Mochizuki and Yamakawa, 1979, Gousset *et al.*, 2005; Reddy *et al.*, 2015; Bainsla *et al.*, 2016). Eggplant cultivated varieties and wild *Solanum* species were artificially inoculated with *R. solanacearum* inoculum (Concentration ~1.0 x 10^8 cfu/ml). After 25-35 days post-inoculation period, wilt incidence was observed on IIHR-108, Rampur Local and Pusa Purple Long. Up to 7-8 weeks, no wilting symptoms were observed on IIHR-7, CARI-1 and all wild *Solanum* species.

In the present study, susceptible check variety IIHR-108 has shown ~100% bacterial wilt incidence on artificial inoculation. In the test accessions, bacterial wilt incidence ranged from 0 to 99.33 %. In general, the resistance level of eggplant cultivated varieties to bacterial wilt in is limited (Bhavana and Singh, 2016). Resistant source from cultivated eggplant accessions will be highly useful in breeding due to their advantage in crossing, and no negative effects of fruits will hinder the breeding. Among the cultivable eggplant accessions, IIHR-7 (IC-395334) and IHR-662

Table 2. Mean performance of genotypes for bacterial wilt incidence and yield attributing traits

Sl. No.	Name of the genotype	No. of primary branches	Plant height	Fruit length (cm)	Fruit diameter (cm)	No. of fruits / plant	Average fruit wt. (g)	Fruit yield (t/ha)	% Bacterial wilt incidences
1	IIHR-108	6.63	94.67	14.32	3.09	37.33	42.67	6.22	99.33 (85.70*)
2	IIHR-7	5.74	106.00	19.67	3.50	39.67	42.67	37.47	0.00 (0.00*)
3	IIHR-662 (CARI-1)	6.64	95.17	13.84	10.48	18.00	291.67	29.15	0.00 (0.00*)
4	IIHR-663(	6.37	91.16	17.27	3.61	31.22	36.72	27.56	21.54 (27.64*)
5	Pusa Purple Long	4.03	94.78	19.49	2.94	6.79	32.84	4.52	92.32 (75.46*)
6	*Solanum gilo*	3.57	93.45	5.13	3.86	27.67	25.67	4.56	26.45 (30.93*)
7	*Solanum indicum*	3.71	84.81	1.61	0.38	174.33	0.29	0.79	0.00 (0.00*)
8	*Solanum viarum*	7.13	123.33	3.42	2.46	176.00	19.84	1.80	0.00 (0.00*)
9	*Solanum aethiopicum*	3.20	76.00	5.57	3.31	26.00	33.17	8.22	4.38 (12.03*)
10	*Solanum mammosum*	5.32	111.99	5.65	4.05	35.00	30.83	4.39	4.50 (12.19*)
11	*Solanum torvum*	3.70	143.33	2.12	1.52	223.33	0.19	0.82	0.00 (0.00*)
G. MEAN		5.10	101.34	9.83	3.56	72.30	50.60	11.41	66.53
SEM		0.35	3.79	0.67	0.27	4.93	2.69	0.80	1.24
SD		0.49	5.37	0.94	0.38	6.97	3.80	1.14	1.76
CD		1.49	16.27	2.85	1.16	21.15	11.54	3.45	5.18
CV (%)		9.64	5.29	9.57	10.72	9.64	7.52	9.97	2.65

*Values in parenthesis are angular transformed values

(CARI-1) were found to have high resistance with no incidence of bacterial wilt.

Among the wild species, bacterial wilt incidence ranged from 0 (*Solanum torvum*, *Solanum viarum*, and *Solanum indicum*) to 26.45 % (*Solanum gilo*). Wild species are hardy in nature, and will have the special advantage of strong root system for combating other abiotic stresses. These wild species cannot be used in breeding programme easily because of negative fruit traits. However, these can be used as a root-stock for eggplant and tomato cultivation in bacterial wilt prone areas. These wild species can be effectively used for grafting the commercial varieties and hybrids of eggplant and tomato. Gopalakrishnan *et al.* (2005) identified high phenolic content, and some special root cortical cells in bacterial wilt resistant cultivar root system, and are thought to be responsible for controlling of *R.solanacearum* spreading and multiplication. Already, the success of using *Solanum torvum* in grafting eggplant for bacterial wilt and yield advantage has been proved. The same can be used for tomato and other *Solanum* cultivation. *Solanum torvum* have graft advantages and compatibilities with cultivated eggplant, hence, it can be effectively used in grafting programs of bacterial wilt resistant eggplant varieties/hybrids (Ashok *et al.*, 2017). Ashok *et al.* (2017) reported *Solanum torvum* resistance with 5.7% susceptibility, whereas, in our study it was found highly resistant with no incidence. This variation in level of resistance may be due to the variability in the *Solanum torvum* accessions or difference in *Ralstonia solanacearum* races.

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