Evaluation of eggplant rootstocks for grafting eggplant to improve fruit yield and control bacterial wilt disease

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Abstract Bacterial wilt caused by *Ralstonia* solanacearum is a major devastating soil-borne disease impeding eggplant cultivation worldwide. The present investigation was conducted to recognize and assess bacterial wilt resistant rootstocks among *Solanum melongena* (Haritha, Surya, SM 1, SM 2, SM 3, SM116, and SM 398), *Solanum torvum* (St TNAU 1 and St KAU 1), and *Solanum sisymbrifolium* (SS 1) for vigor, yield and qualitative traits of 'Green Long Hybrid' scion through grafting. The artificial inoculation method as well as the sick plot method of bacteria wilt screening were adopted. The root dip method of artificial inoculation was found to be the most reliable method as compared to media drenching and stem inoculation methods. All the rootstocks except *Solanum*

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Department of Floriculture and Landscaping, College of Horticulture, Kerala Agricultural University, Trichur, Kerala, India sisymbrifolium possessed a high degree of bacterial wilt resistance (in artificial as well as sick plot conditions). Maximum plant spread, stem girth, number of primary branches, yield per plant (6.69 kg), number of fruits per plant (94.80), fruit length (22.22 cm), root length (63.65 cm), and root spread (87.05 cm) were exhibited by Green Long Hybrid scion when grafted onto Solanum melongena cv. Haritha rootstock. Taller plants with high fruit girth (10.97 cm) and average fruit weight (78.00 g) of scion were recorded on SM 116 rootstock. The highest dry matter content (11.12%) and total phenolic content (113.30 mg/ 100 g) of fruits were observed in scion when SM 398 and SM 3 were used as rootstocks, respectively. Overall, 'Haritha' cultivar was found to be the best rootstock for grafting in the current study. Thus, grafting technology can be effectively used for the control of bacterial wilt as well as for acquiring higher yield in eggplant.

Keywords Bacterial wilt · Eggplant · Rootstock evaluation · Vegetable grafting

Introduction

Eggplant (*Solanum melongena* L.) is a popular, widely cultivated warm-season vegetable crop grown extensively in tropical, subtropical as well as temperate regions of the world (Fegan & Prior, 2005; Genin & Denny, 2012). Eggplant cultivation in India especially the coastal regions, is severely affected by the incidence of bacterial



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wilt disease caused by Ralstonia solanacearum (Ramesh et al., 2016). The preponderance of bacterial wilt is due to the prevalence of high temperature, high humidity, highly virulence strains and acidic soil conditions (Li et al., 2017; Santhosha et al., 2015). It accounts for 11.67-96.67% of crop loss of eggplant worldwide (Bainsla et al., 2016). R. solanacearum is primarily a soil-borne bacterium that enters through wounds or secondary root initiation points (Champoiseau et al., 2009; Pradhanang et al., 2005). Then, the pathogen multiplies inside the plant and blocks the vascular bundles, the chief conducting tissue of water and nutrients, thereby causing sudden wilting of plants (Elphinstone, 2005; Saile et al., 1997). Failure to mitigate bacterial wilt disease is mainly due to the highly diverse, persistent, and pervasive nature of the R. solanacearum and its species complex (Aloyce et al., 2017; Ravelomanantsoa et al., 2018; Sarkar & Chaudhuri, 2016).

Integrated crop protection strategies involving crop rotation, chemical, and biological methods of control cultural practices have not yielded any accomplishment to control bacterial wilt disease (Namisy et al., 2019; Van Elsas et al., 2001; Yuliar et al., 2015). Hence, the cultivation of resistant cultivars/hybrids is the best economically viable option. However, lack of resistant genotypes with desirable agronomical traits and wide climatic adaptability has prompted expanding consideration towards eggplant grafting (Bletsos, 2005; Davis, Perkins-Veazie, Hassell, et al., 2008a; King et al., 2008; Miguel et al., 2004).

Grafting in vegetables has emerged as a promising and surgical alternative tool to the relatively long and slow conventional breeding methods planned for expanding resilience to biotic and abiotic stresses as well as high productivity (Gisbert et al., 2011; Narayanankutty et al., 2015). For grafting purpose, Solanum torvum, one of the wild relatives of eggplant, is being utilized as rootstock to combat a wide range of soil pathogens especially bacterial wilt disease caused by Ralstonia solanacearum (Bletsos et al., 2003; Daunay, 2008; King et al., 2010). Be that as it may, the utilization of S. torvum as a rootstock has been constrained due to absence of quick and homogeneous seed germination (Ginoux & Laterrot, 1991). Also, hypocotyl length in S. torvum is short and might be challenge for grafting (Miceli et al., 2014). Germination problem in Solanum torvum can be improved by chemical treatments with gibberellic acid and potassium nitrate treatments, and light irradiation (Ranil et al., 2015), nevertheless, there is a need to distinguish new alternate rootstocks impervious to bacterial wilt in eggplant. Keeping these things in mind, a study was conducted to evaluate best bacterial wilt resistant rootstocks and investigate field performance of grafted eggplants for vigor, yield, and fruit quality exhibited by bacterial wilt resistant rootstocks on the scion.

Materials and methods

Experimental site

The experiment was conducted at ARS (Agricultural Research Station), Mannuthy and Centre for Hi-Tech Horticulture and Precision farming, Kerala, India during the year 2018–2019. The experimental site is situated at $76^{\circ}26'$ E longitude and $10^{\circ}54$ 'N latitude at an altitude of 22.5 m above MSL. The experimental area enjoys a tropical humid climate which provides high congenial environment for bacteria wilt infestation. The average temperature varied from 26.7 ° C in winter, 32.2 ° C in summer and 27.4 ° C in rainy season. Relative humidity varies between 53% and 89%. The soil in which the experiment was conducted was highly acidic with pH-5.2 and contained a medium quantity of organic carbon. The available nitrogen (N), phosphorus (P), and potassium (K) content were 283, 3.12, and 164 kg/ha soil, respectively.

Plant materials

Ten rootstocks, *Solanum sisymbrifolium cv.* SS 1, *Solanum torvum* cv. St TNAU 1, St KAU 1 and *Solanum melongena* cv. Haritha, Surya, SM 1, SM 2, SM 3, SM 116, and SM 398, were used for the study. The details of the genotypes and their sources are presented in Table 1. A tomato cultivar 'Pusa Ruby' was used as a susceptible check for bacterial wilt screening. After bacterial wilt resistance screening, nine bacterial wilt resistant rootstocks were identified and selected for evaluation through grafting. Green Long Hybrid (M/s Sungro Seeds, West Bengal, India) was used as scion in the grafting due to high consumer preference in South India (Sidhu & Dhatt, 2006).

Evaluation of rootstock seedlings for assessment of bacterial wilt resistance

Evaluation in sick plot condition

Rootstock seedlings were spot planted with susceptible check tomato variety Pusa Ruby in bacterial wilt sick plot during September, 2018– December 2018. The treatments were laid out in Randomized Block Design (RBD) in three replications at a spacing of 75 cm \times 60 cm. The plant population of 20 plants per accession and 200 plants of susceptible check were maintained per replication. The mean population of *Ralstonia solanacearum* infested sick field was found to be 1.9×10^6 CFU/mL (Gopalakrishnan et al., 2014).

The number of wilted plants due to *Ralstonia solanacearum* was recorded after confirming through the ooze test followed by isolation on TTZ (2, 3, 5, Triphenyl Tetrazolium Chloride) medium (Zheng et al., 2017) (Fig. 1). The severity of the disease incidence was calculated based on the accumulated observation up to 90 days after transplanting for statistical analysis (Umesh et al., 2018).

Bacterial wilt incidence (%)

 $=\frac{\text{Number of dead plants due to bacterial wilt disease}}{\text{Total number of plants established}} \times 100$

Selected genotypes were scored into five categories viz., Immune (I) - 0% wilt symptoms; HR (Highly Resistant) - 1-10% wilt; MR (Moderately Resistant) - 11-50% wilt; MS (Moderately susceptible) - 51-75% wilt and HS (Highly susceptible) - 76-100% wilt (Sitaramiah et al., 1981).

Artificial inoculation

Bacterial isolate maintenance and inoculation preparation Bacterial wilt infected plants was collected from the bacterial wilt sick plot, Kerala Agricultural University, Kerala. One cm length stem portion at the collar region of the plant was rinsed with distilled water and surface sterilized with the help of 1% Sodium Hypochlorite solution (NaOCl) accompanied by repeated rinsing with distilled water. Then by using mortar and pestle, the sample was macerated and streaked on to the nutrient agar (HiMedia) petri plates containing glucose (0.4 g/ 100 ml) and 1% TTC (2, 3, 5 Triphenyl Tetrazolium Chloride) (70 μ l/ 100 ml) solution (Buyela et al., 2017). The incubation of Petri plates was done at room temperature (26 ± 2 °C) and waited for the visual confirmation of the proliferation of pathogen. The bacterial colonies appeared as creamy white growth with pointed pink color at the center of the colony within 48–72 DPI.

For the artificial inoculum purpose, pure culture of *Ralstonia solanacearum* was prepeared. From the freshly cultured *Ralstonia solanacearum* colonies, two loops were taken and suspended into 150 ml of nutrient agar media accompanied by shaking in an incubator maintained at 28 °C and 150 rpm for 24 h. Then, bacterial cultures were centrifuged at 4000 rpm at 4 °C for 15 min and the supernatants were discarded (Singh et al., 2018). The bacterial pellets were re-suspended in the sterile distilled water to prepare bacterial suspension and pathogen population was estimated by the help of serial dilution followed by isolation on TTZ (2, 3, 5, Triphenyl Tetrazolium Chloride) medium.

Ralstonia solanacearum isolate belonged to Phylotype I (Gaitonde & Ramesh, 2014), race I and biovar III (Chandrashekara et al., 2012).

Raising of seedlings

In this experiment seedlings of the 10 rootstocks seedlings along with the susceptible check (Pusa Ruby) were raised in pro-trays filled with a sterilized soilless medium comprising of coco peat + perlite + vermiculite (3:1:1) during July, 2018 and 30 days old seedlings were transplanted in the pots filled with sterilized soilless medium. The pots were kept in a chamber maintaining temperature of 28–30 ⁰ C and relative humidity of 90–95%.

Inoculation of bacterial suspension

Seedlings of all the genotypes were artificially inoculated with *R. solanacearum* suspension containing bacterial population at 3.8×10^4 colony forming units per ml (CFU ml⁻¹) (Gopalakrishnan et al., 2005). Three methods of inoculation viz., media drenching, stem inoculation, and root dipping were carried out (Fig. 2). The experiment was conducted in Completely Randomized Design (CRD) with 33 treatment combinations (11 genotypes × 3 methods of inoculation) in 3 replications. The seedling population of 30 plants were maintained per genotype in each replication.

Genotypes	Specification	Sources	Bacterial wilt incidence (%)	No. of days taken to express wilt symptoms (Days)	Disease Reaction [¶]
Surya	OP Variety, Kerala Agricultural University(K AU), Kerala, India)	Agricultural Research Station (ARS), Mannuthy, Kerala, India	0.0 ^g	0.0 ^g	Ι
Haritha	Released variety (KAU, Thrissur, Kerala, India)	ARS, Mannuthy, Kerala, India	0.0 ^g	0.0 ^g	Ι
SM1	Local variety	ARS, Mannuthy, Kerala, India	23.3 ^d	30.3 ^{ab}	MR
SM2	Local variety	ARS, Mannuthy, Kerala, India	40.0 ^c	28.7 ^{abcd}	MR
SM3	Local variety	ARS, Mannuthy, Kerala, India	0.0 ^g	0.0 ^g	Ι
SM116	Local collection	ARS, Mannuthy, Kerala, India	0.0^{g}	0.0 ^g	Ι
SM398	Local variety	College of Horticulture, Vellanikkara, Kerala, India	40.0 ^c	32.4 ^a	MR
SS 1	Solanum sisymbrifolium	ARS, Mannuthy, Kerala, India	96.7 ^a	23.4 ^{ef}	HS
St KAU 1	Solanum torvum	TNAU, Tamil Nadu, India	16.7 ^{ef}	26.8 ^{bcde}	MR
St TNAU 1	Solanum torvum	NBPGR, Regional Station, Thrissur, Kerala, India	20.0 ^{de}	29.0 ^{abc}	MR
Pusa Ruby (Susceptible check)	ICAR- Indian Agricultural Research Institute (IARI), New Delhi	ICAR- Indian Agricultural Research Institute (IARI), New Delhi	93.3 ^{ab}	21.2	HS
Critical difference $(p \le 0.05)$			14.80**	4.61**	
Standard error of mean (\pm)			5.02	1.56	
Standard error of difference (±)			7.10	2.21	
Coefficient of variation			28.97	15.52	

Table 1 Reactions of different genotypes of eggplant to bacterial wilt in sick plot condition

Values followed by different letters indicate significant ($p \le 0.05$) differences; NS: Non-significant.; *- statistically significant differences at p value below 0.05, **- statistically significant differences at p value below 0.01 (Duncan's multiple range test); ¶ I- Immune; MR-Moderately Resistant; HS-Highly Susceptibl

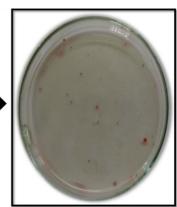
Media drenching method 1 cm of the root tip of seedlings were trimmed with sterile scissors before planting in pots and 10 ml inoculum was poured into the pots

Fig. 1 visual detection and isolation of bacterial wilt pathogen (*R. solanacearum*)

near the base of plants after planting (Xian-Gui et al., 2006). One day after planting, the second dose of inoculation was poured at the rate of 15 ml per pot.



Presence of pathogen by ooze test



Isolated bacteria on TTZ media

Stem inoculation method Small prick was given on the main stem close to the leaf axil by using a syringe. A small piece of cotton was dipped in the bacterial suspension and kept in the leaf axil and the cotton was kept moist by periodically spraying bacterial suspension (Gizi et al., 2011).

Root dipping method Seedlings were uprooted and the root system was thoroughly washed before inoculation. Root tips of one cm were trimmed with sterile scissors to make a wound and then immediately dipped in 50 ml of the bacterial suspension for 2 min and planted in pots (Liu et al., 2015).

The inoculated plants were kept in the mist chamber (28–30 °C & 90–95% RH) and watered with 20 ml of distilled water whenever the media was dry. No nutrients were provided to the plants and plants were monitored daily for 42 days from the date of inoculation. The wilted plants of each genotype were collected and bacterial wilt was confirmed through ooze test and isolation of *R. solanacearum* on TTZ medium. The severity of disease incidence in selected genotypes was scored according to the standard score chart (Sitaramiah et al., 1981) as done in the previous experiment. Observations were recorded on the number of days to bacterial wilt incidence (incubation period) and percentage of bacterial wilt incidence as of the previous experiment.

Field evaluation of grafted plants

Raising of grafted eggplants plants Rootstocks which showed resistance to bacterial wilt in the field evaluation studies as well as in artificial inoculation experiments (Surya, Haritha, SM 1, SM 2, SM 3, SM 116, SM 398, St KAU 1 and St TNAU 1) were selected for grafting. Grafting was done during January, 2019 by using the wedge or cleft method of grafting (Lee, 1994). The top portion of 40 days old seedlings of rootstocks was cut and removed. The top portion of the scion was cut and removed by retaining 4-5 leaves and the base was made into the shape of a wedge by giving a slant cut from both sides. A vertical slit was made in rootstocks and the wedge-shaped scion was inserted into a matching vertical slit made on the rootstock and the joint was secured by using a grafting clip. Immediately after grafting, the grafted plants were transferred to a mist chamber maintained at a temperature of 20-25 °C, 90-95% relative humidity for one week for the healing of graft union. After that grafted plants were transferred to the acclimatization chamber for hardening. Hardened grafted plants were transplanted in the main field and evaluated in the field following the recommended package of practices under precision farming for yield and quality parameters.

Agronomic practices for grafted eggplants The experimental area was cleaned, the land was thoroughly ploughed and farmyard manure at 25 t/ha was incorporated into the soil. Beds of size 45 m \times 0.9 m \times 15 cm were taken at a spacing of one meter between the adjacent beds. The beds were mulched with a 30 μ silver black polythene film. Fertilizers were applied as per the dose recommended for eggplant. Basal dose of FYM 25 t/ha along with NPK 50:30:30 kg/ha and top dressing with 50 kg/ha after 30 days of transplanting was done. The grafted plants were laid out in Randomized Block Design (RBD) in four replications. The plant population of 20 plants per treatment were maintained per replication. The crop was irrigated through drip irrigation and fertigation started one week after planting of seedlings. A total number of 45 fertigations were given at three days interval during the entire duration of the crop. Staking of plants was provided to the plants one month after planting. Removal of root suckers (suckers which were produced below the graft union) was done at regular intervals to avoid the diversion of nutrients to the water shoots. Plant protection measures were undertaken to control aphids, whiteflies, jassids, thrips, red spider mite, and shoot and fruit borer. Harvesting was done at weekly intervals as and when fruits attained marketable stage. Harvested fruits were used for recording fruit characters.

Evaluation of grafted plants for bacterial wilt resistance For assessment of bacterial wilt resistance, grafted plants along with the non-grafted plants, were planted in the well-prepared sick plot at a spacing of 50 cm \times 50 cm. The experiment was laid out in Randomized Block Design (RBD) with four replications of 20 plants each. Proper nutrient and water management as well as weeding operations were done as per recommendations.

Observations on plant vigor, yield, fruit and root traits Plant height (cm), plant spread (cm), stem girth (cm) at 2 cm above the graft union, and the number of primary branches per plant were recorded at 30 and 60 DAT (Days After Transplanting) for five random plants per treatment per replication and the average was

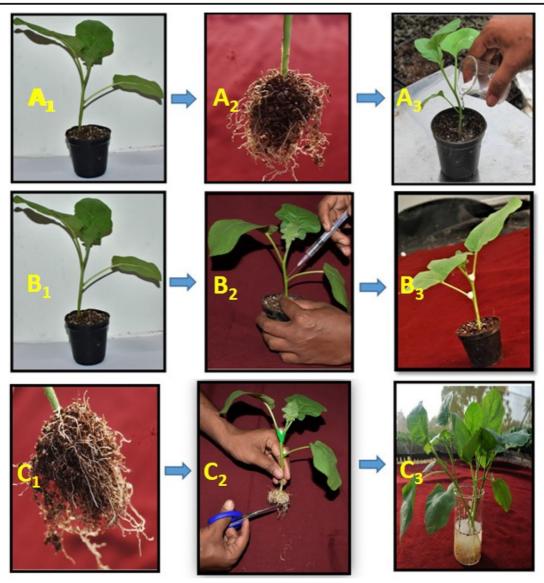


Fig. 2 Different artificial screening methods adopted for bacterial wilt resistance $[A_{1-1}: Media Drenching method; B_{1-3}: Stem inoculation method; C_{1-3}: Root dip method]$

calculated. Plant spread (cm) was measured in east-west and north- south direction in randomly selected plants at harvesting stage with the help of scale and average was taken in centimeter after summing up of the observations. The number of days taken for first flower anthesis, number of fruits, fruit length, and fruit girth were recorded at regular intervals. Fruit length was taken from blossom end to stalk end in each harvest from one randomly selected fruit from an individual plant and fruit girth was measured by measuring the circumference of the fruit at the posterior end in each harvest from one randomly selected fruit from each plant. Average fruit weight was calculated by dividing total yield by total number of fruits harvested in each replication. Total yield was calculated as kg per plant (taking into account only the plants alive at the end of the experiment). For root traits study, five plants per treatment from each replication were uprooted carefully without damaging the roots (Fig. 3). Then after repeated cleaning with water and sun-drying, the measurement for length and spread of roots was carried out.

Estimation of phenol content of fruits Estimation of phenols was done according to the method suggested

by Sadashivam and Manickam (1992). 1 g of sample was macerated by pestle and mortar with 10 times the volume of 80% ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 min. The supernatant was collected and re-extracted with five times the volume of 80% ethanol followed by centrifuge and collection of the supernatants. The supernatant was then evaporated to dryness in the water bath and the residue was dissolved in 5 ml of distilled water. Different aliquots of standards (0.1–0.5 ml and blank) and sample (0.2 ml) were pipetted out into the test tubes and the volume in each test tube was adjusted to 3 ml by adding distilled water. 0.5 ml of folin-ciocalteau reagent was added and after 3 min, 2 ml of 20% sodium carbonate was added to each test tubes. After mixing thoroughly, each test tube was kept in the dark for one hour at room temperature and the absorbance at 650 nm was measured by using a spectrophotometer. The standard curve was prepared by the use of different concentrations of catechol. The mean was calculated and expressed in mg/100 g. Dry matter (%) was determined in samples by drying at 105 °C temperature until a constant weight was achieved.

Dry Matter content (%)

= fresh weight-dry weight/fresh weight x 100

The total soluble solids (%) was determined by using refractometer (0-32 range) (Bidaramali et al., 2020).

Statistical analysis Data collected were subjected to analysis of variance. The mean separation was carried out utilizing the Duncan multiple range test (DMRT). The data analysis for bacterial wilt screening of rootstocks was conducted using OP-STAT software (Sheoran et al., 1998) (available at http://14.139.232.166/opstat/default. asp). The graft evaluation study was analyzed by using WASP 2.0 software.

Results

Evaluation of rootstocks for bacterial wilt resistance in the sick plot

A significant difference existed among rootstocks for reaction to bacterial wilt disease (Table 1). Among the rootstocks, Surya, Haritha, SM 3, and SM 116 showed an immune reaction to bacterial wilt exhibiting no symptom of the disease, while *Solanum sisymbrifolium* recorded the highest bacterial wilt incidence (96.6%). Remaining genotypes viz., SM 1 (23.3% wilt), St KAU 1 (16.6% wilt), and St TNAU 1 (20.0% wilt) were categorized as moderately resistant to bacterial wilt disease. Susceptible check 'Pusa Ruby' showed expected disease reaction to bacterial wilt in which 93.3% plants succumbed to death. Among the rootstocks, SM 398 took maximum days to show symptoms of bacterial wilt (32.3 days), while the lowest incubation period of the pathogen was observed in *Solanum sisymbrifolium* (23.40 days), followed by Pusa Ruby (21.20 days).

Evaluation of rootstocks for bacterial wilt resistance through artificial inoculation

A significant difference was observed concerning the bacterial wilt incidence and number of days taken to express wilt symptoms (incubation period) of the pathogen in the rootstocks for different methods of inoculation (Table 2). Out of the three artificial inoculation methods, the root dipping method was found to be the best for artificial screening for bacterial wilt resistance followed by the stem inoculation method. The root dipping method recorded the highest percentage of disease incidence in both susceptible genotypes Solanum sisymbrifolium (86.67%) and the susceptible check Pusa Ruby (100%). Similarly, the least days were taken by the susceptible genotypes viz., Solanum sisymbrifolium (11.7) and Pusa Ruby (7.2) for the appearance of the wilt symptoms when root dip screening protocol was followed. The artificial screening revealed the existence of immune reaction (0% wilt) of Surya, Haritha, SM 1, SM 2, SM 3, SM 116 and SM 398, St KAU 1, and St TNAU 1 genotypes towards bacterial wilt disease.

Evaluation of grafted eggplants for vigor

A significant difference was observed among the rootstocks for the vigor of the scion, in terms of plant height, plant spread, and number of primary branches. Green Long hybrid grafted onto SM 116 rootstock resulted in maximum plant height at 30 DAT (36.65 cm) and 60 DAT (109.4 cm). The lowest plant height was recorded in the non-grafted Green Long Hybrid plants at 30 DAT (30.55 cm) and 60 DAT (95.6 cm) (Table 3). Haritha (107.10 cm), St KAU 1 (106.50 cm) and St TNAU 1 (108.20 cm) rootstocks performed statistically on par



Fig. 3 Root traits of various rootstocks used in grafting [1-Surya, 2-Haritha, 3- SM 1, 4-SM 2, 5-SM 3, 6-SM 116, 7-SM 398, 8- St KAU 1, 9-St TNAU 1, 10-Non-grafted Green Long Hybrid]

with SM 116 for plant height at 60 days after transplanting.

Similarly, plant spread differed significantly among the grafted and control plants as well as within the rootstock-scion combinations (Table 3). The grafted

Fig. 4 Impact of rootstocks on number of fruits and fruit yield of Green Long Hybrid scion [1-Grafted onto Haritha; 2-Grafted onto Surya; 3-Grafted onto SM 398; 4- Non-grafted Green Long Hybrid]



plants produced significantly higher plant spread than

non-grafted control plants. Haritha when used as root-

stock produced a maximum plant spread of 46.62 cm at

30 DAT and 110.13 cm at 60 DAT, while the minimum

plant spread was recorded in non-grafted Green Long

Table 2 Reactions of different genotypes of eggplant against artificial inoculation of Ralstonia solanacearum

Genotypes	Bacterial wilt incidence (%)				No. of days taken to express wilt symptoms (days)			
	Media drenching	Stem inoculation	Root dip	Mean A (Genotypes)	Media drenching	Stem inoculation	Root dip	Mean A (Genotypes)
Surya	0 ^c	0 ^c	0 °	0 ^c	0 ^c	0 °	0 °	0 °
Haritha	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c
SM1	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c
SM2	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c
SM3	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c
SM116	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 °
SM398	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 °
SS1	26.5 ^a	60.00 ^a	86.67 ^a	73.33 ^a	26.5 ^a	12.25 ^a	11.70 ^a	16.82 ^a
St KAU1	0 ^c	0 °	0 ^c	0 ^c	0 ^c	0 °	0 ^c	0 °
St TNAU1	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 °
Pusa Ruby (susceptible check)	11.25 ^b	73.33 °	100.00 ^c	86.67 ^c	11.25 ^b	10.86 ^b	7.20 ^b	9.77 ^b
Mean B (Methods)	3.432	12.12	16.97		3.432	2.101	1.718	
Factors		C.D. (0.01)			C.D. (0.01)			
Factor (A)		0.157**			0.482**			
Factor (B)		0.301**		0.922**				
Factor (A×B)		0.522**			1.598**			

Values followed by different letters indicate significant ($p \le 0.05$) differences; NS: Non-significant.; *- statistically significant differences at p value below 0.05, **- statistically significant differences at p value below 0.01 (Duncan's multiple range test)

Genotypes	Plant height (cm)		Plant spread (cm)		Stem girth (cm)		Number of primary branches		Days to first flowering
	30 DAT [¥]	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT	
Surya	35.10 ^{ab}	100.00 ^e	45.82 ^a	106.00 ^b	3.10 ^{bc}	7.30 ^{bcd}	2.65 ^a	7.30 ^{cd}	41.80 ^{bcd}
Haritha	35.50 ^{ab}	107.10 ^{abc}	46.62 ^a	110.13 ^a	3.25 ^a	7.53 ^a	3.00 ^a	8.20 ^a	41.45 ^{cd}
SM 1	33.85 ^{abc}	101.65 de	43.27 ^{abc}	107.90 ^{ab}	3.18 ^{ab}	7.28 ^{cd}	1.95 ^b	7.15 ^{cd}	42.10 ^{bcd}
SM 2	34.00 ^{abc}	104.15 ^{cd}	43.57 ^{ab}	106.55 ^b	3.10 ^{bc}	7.35 ^{bc}	2.05 ^b	7.85	42.85 ^{bc}
SM 3	31.90 ^{cd}	102.15 de	41.42 ^{bcd}	107.60 ^{ab}	2.95 de	7.35 ^{bc}	2.10 ^b	7.00 ^d	43.60 ^b
SM 116	36.35 ^a	109.40 ^a	43.32 ^{abc}	107.63 ^{ab}	3.18 ^{ab}	7.45 ^{ab}	2.95 ^a	8.05 ^a	42.80 ^{bcd}
SM 398	35.10 ^{ab}	106.20 ^{bc}	46.05 ^a	106.43 ^b	3.03 ^{cd}	7.15 ^{de}	2.60 ^a	7.45 ^{bc}	41.00 ^d
St KAU1	33.85 ^{abc}	106.50 ^{abc}	39.87 ^{bcd}	105.60 ^b	2.90 ^e	7.00 ^e	2.00 ^b	7.40 ^{cd}	47.65 ^a
St TNAU1	33.10 ^{bcd}	108.20 ^{ab}	39.62 ^{cd}	104.68 ^{bc}	2.90 ^e	7.18 ^d	2.05b	7.05 ^{cd}	48.65 ^a
Non-grafted Green Long Hybrid	30.55 ^d	95.60 ^f	38.17 ^d	101.73 ^c	$2.63^{\rm f}$	$6.00^{\text{ f}}$	1.00 ^c	5.25 °	41.85 ^{bcd}
Critical difference (p≤0.05)	3.00*	3.13**	4.16**	3.22**	0.10**	0.16**	0.44**	0.41	1.81*
Standard error of mean (\pm)	1.03	1.08	1.43	1.11	0.03	0.06	0.15	0.14	0.62
Standard error of difference (\pm)	1.47	1.54	2.03	1.57	0.05	0.08	0.21	0.20	0.88
Coefficient of variation	6.11	2.09	6.82	2.09	2.22	1.55	13.44	3.92	2.87

 Table 3 Effect of grafting on vigour and flowering traits of 'Green Long Hybrid' scion

Values followed by different letters indicate significant ($p \le 0.05$) differences; NS: Non-significant.; *- statistically significant differences at p value below 0.05, **- statistically significant differences at p value below 0.01 (Duncan's multiple range test); * Days after transplanting

Hybrid plants at 30 DAT (38.17 cm) as well as 60 DAT (101.73 cm). Scion grafted onto rootstocks viz., SM 1 (107.9 cm) and SM 116 (107.63 cm) were statistically on par with Haritha rootstock for plant spread.

Irrespective of the rootstocks used, all the grafted plants produced significantly higher the number of primary branches than non-grafted control plants at 30 and 60 days after transplanting (Table 3). Green Long hybrid grafted onto Haritha produced the maximum number of primary branches at 30 DAT (3.52) as well as 60 DAT (7.53), while the minimum number of branches were found in case of the non-grafted Green Long Hybrid plants at 30 DAT (2.63) and 60 DAT (6.00). SM 116 (7.45) was statistically on par for the number of primary branches with Haritha at 60 days after transplanting.

Stem girth varied significantly among the grafted and control plants (Table 3). All the grafts plants produced significantly higher stem girth than the non-grafted control plants. Maximum stem girth of scion at 30 DAT (3.25 cm) and 60 DAT (7.52 cm) were observed when Haritha was used as rootstock. The lowest stem girth was recorded in non-grafted Green Long Hybrid plants at 30 DAT (2.63 cm) and 60 DAT (6.00 cm). At 60 DAT rootstocks SM 116 performed statistically on par for stem growth with Haritha at 60 DAT.

Evaluation of grafted eggplants for earliness, yield, and yield attributing traits

Grafting and rootstocks had a significant impact on the early flowering occurrence of the scion (Table 3). The earliest opening of the first flower was shown when Green Long was grafted onto SM 398 rootstock (41.00 days), while St TNAU 1 caused much delayed first flower opening in the scion (48.65 days). Upon used as rootstock for imparting earliness in flowering, SM 398, Surya (41.80 days), Haritha (41.45 days), SM 116 (42.80 days) performed statistically on par with each other as well as the non-grafted scion of Green Long.

Statistical analysis revealed the existence of a significant difference among rootstocks for fruit yield and yield attributing traits (Table 4). The maximum number of fruits per plant was produced when Green Long was grafted on to Haritha rootstock (94.80) followed by SM 398 rootstock (94.35), while the minimum number of fruits were produced by non-grafted Green Long plants (63.55). Similarly, yield per plant significantly varied among the grafted and control plants irrespective of the rootstocks used. The highest yield was obtained from Green Long when Haritha (6.70 kg) was used as rootstock, followed by SM 398 rootstock (6.17 kg). The lowest yield was exhibited by the non-grafted plants (4.08 kg).

Rootstocks had a statistically significant influence on both fruit length and fruit girth of the scion under study. All the grafted plants produced bigger fruits as compared to non-grafted control plants (Table 4). Haritha rootstock exhibited the maximum fruit length of 22.22 cm in the scion which was statistically on par with SM 116 (22.16 cm) and SM 398 (22.15 cm) rootstocks, respectively. Similarly, maximum fruit girth was recorded when SM 116 was used as rootstock (10.97 cm), which was statistically on par with Haritha (10.94 cm), SM 398 (10.91 cm), and SM 3 (10.86 cm) rootstocks. The lowest fruit length (20.31 cm) and fruit girth (10.43 cm) were recorded in non-grafted control plants. The highest average fruit weight of scion was exhibited by SM 116 rootstock (78.00 g) which was on par with Haritha rootstock (76.00 g) (Table 4). However, the lowest average fruit weight was found in nongrafted control (67.85 g) plants.

Evaluation of grafted eggplants for fruit quality traits

Statistical analysis ensured a significant impact of graft combinations on the total phenolic content of fruits of Green Long hybrid. The highest phenol content of fruits was observed when SM 3 (113.30 mg) was used as rootstock followed by SM 116 rootstock (103.2 mg), while the lowest phenol content in fruits was observed in non-grafted eggplants (61.90 mg)(Table 4). The maximum dry weight was exhibited by SM 398 rootstock (11.43%) followed by St TNAU 1 rootstock (11.12%). The lowest dry matter was found when Surya was used as rootstock (8.85%), which was statistically on par with the non-grafted control (8.98) (Table 4). There was no significant difference observed concerning total soluble solids (TSS) of fruits among all the grafted and control plants (Table 4). Maximum TSS was recorded in the fruits of SM 116 (4.42%) rootstock, while minimum TSS was recorded in the fruits of control plants (4.27%).

Evaluation of grafted eggplants for bacterial wilt resistance in sick plot

There existed a significant variation among grafted and non-grafted eggplants with respect to disease reaction

Table 4 Impact of grafting combinations on yield,	abinations on		traits, fruits bioche	mical properties	and bacterial	yield attributing traits, fruits biochemical properties and bacterial wilt resistance of 'Green Long Hybrid' scion	sn Long Hybrid' scion	_	
Genotypes	No. of fruits per plant	No. of fruits Yield per plant (Kg) Fruit length (cm) Fruit girth (cm) Average fruit per plant weight (Fruit length (cm)	Fruit girth (cm)	Average fruit weight (g)	Total phenolic content (mg/100 g)	Dry matter (%) TSS (%)		Bacterial wit incidence $(\%)^{*}$
Surya	79.80 °	5.48 °	21.73 ^b	10.76 ^{bc}	69.49 ^{bc}	63.20 °	8.85 ^d 4.5	4.35 0.00 ^c	
Haritha	94.80 ^a	6.70 ^a	22.22 ^a	10.95 ^a	76.00 ^a	66.35 °	9.98 ^b 4.38	38 0.00 °	
SM 1	76.75 ^d	5.17 ^e	20.93 °	10.63 ^{cd}	69.90 ^{bc}	90.20 ^{cd}	9.80 ^b 4.35	35 0.00 °	
SM 2	78.00 ^{cd}	5.30 ^{de}	21.22 ^c	10.59^{cde}	70.89 ^b	97.20^{bc}	9.98 ^b 4.30	30 0.00 °	
SM 3	77.85 ^{cd}	5.42 ^{cd}	21.73 ^b	10.87^{ab}	71.39 ^b	113.30 ^a	8.85 ^d 4.35	35 0.00 °	
SM 116	91.20 ^b	6.29 ^b	22.16 ^a	10.97 ^a	78.00 ^a	103.20 ^b	9.48 ^{bc} 4.43	43 0.00 °	
SM 398	94.35 ^a	6.17 ^b	22.15 ^a	10.92^{ab}	69.28 ^{bc}	66.15 ^e	11.43 ^a 4.33	33 0.00 °	
St KAU1	70.05 °	4.84 ^f	20.96 °	10.52 ^{de}	71.65 ^b	89.48 ^d	9.15 ^{cd} 4.35	35 6.25 ^b	
St TNAU1	70.00 °	4.82 ^f	21.17 °	10.63 ^{cd}	69.94 ^{bc}	68.63 °	11.13 ^a 4.30	30 5.00 ^{be}	
Non-grafted Green Long Hvbrid	63.55 ^f	4.08 ^g	20.32 ^d	10.44 °	67.85 °	61.91 ^e	8.98 ^{cd} 4.28	28 28.75 ^a	
Critical difference $(p \le 0.05)$	2.91^{**}	0.16^{**}	0.46**	0.18^{**}	2.85**	7.04**	0.54** 0.2	$0.23^{\rm NS}$ 9.54^{**}	
Standard error of mean (\pm)	1.00	0.05	0.16	0.06	0.98	2.43	0.19 0.0	0.08 3.24	
Standard error of difference (\pm)	1.42	0.08	0.22	0.09	1.39	3.43	0.27 0.11	11 4.65	
Coefficient of variation	2.51	1.97	1.46	1.17	2.75	5.92	3.84 3.6	3.60 164.39	
Values followed by different letters indicate significant ($p \le 0.05$) differences; NS: Non-significant; *- statistically significant differences at p value below 0.05, **- statistically significant differences at p value below 0.01 (Duncan's multiple range test) [*] Grafted plants were screened for bacterial wilt resistance under sick plot condition	ters indicate si ₁ 1 (Duncan's n	gnificant ($p \le 0.05$) diff nultiple range test) [¥] Gr	ferences; NS: Non- rafted plants were s	significant.; *- st screened for bact	atistically sigrerial wilt resis	iffcant differences at p tance under sick plot c	value below 0.05, **- ondition	- statistically sig	nificant

against bacterial wilt in sick plot condition (Table 4). Haritha, Surya, SM 398, SM 1, SM 2, SM 3, SM 116 when used as rootstock, no wilt symptoms appeared in any of the grafts. The high susceptible reaction towards bacterial wilt was observed in non-grafted Green Long hybrid in which 28.75% plants showed disease symptoms, followed by Green Long Hybrid grafted on St KAU 1 (6.25%) and St TNAU 1 (5.00%).

Evaluation of grafted eggplants for root traits

There were significant differences among the root traits of the rootstocks in this study (Table 5). The maximum root length was exhibited by Haritha (63.65 cm) rootstock followed by *St TNAU 1* (62.20 cm) and SM 116 (61.05 cm). The highest root spread was also produced on Haritha rootstock (87.05 cm). The minimum root length (46.05 cm) and root spread (63.45 cm) were recorded in non-grafted control plants.

Discussion

Bacterial wilt disease is one of the most destructive and devastating diseases of eggplant, which has been a

 Table 5 Root traits of the cultivated and wild genotypes (root-stocks and scion) used in the current experiment

Genotypes	Root length (cm)	Root spread (cm)
Surya	55.65 ^d	75.00 ^c
Haritha	63.65 ^a	87.05 ^a
SM 1	51.20 ^e	70.80 ^d
SM 2	52.05 ^e	71.95 ^d
SM 3	59.85 °	81.60 ^b
SM 116	61.05 ^{bc}	82.20 ^b
SM 398	60.20 ^c	81.95 ^b
St KAU 1	59.60 °	81.80 ^b
St TNAU 1	62.20 ^{ab}	82.30 ^b
Green Long Hybrid	46.05 ^f	63.45 ^e
Critical difference (p≤0.05)	2.00**	2.23**
Standard error of mean (±)	0.69	0.77
Standard error of difference (±)	0.97	1.08
Coefficient of variation	2.41	1.97

Values followed by different letters indicate significant ($p \le 0.05$) differences; NS: Non-significant.; *- statistically significant differences at p value below 0.05, **- statistically significant differences at p value below 0.01 (Duncan's multiple range test)

global challenge for the researchers (Mansfield et al., 2012). Hence, recognition of high yielding genotypes with enhanced climatic adaptability and resistance to bacterial wilt is the need of the hour. These can either be used directly in crop improvement programs or as rootstocks for grafting onto high yielding commercial hybrids (Satyaprakash et al., 2020). Thus, for this purpose, various screening methods can be adopted for sorting out bacterial wilt resistant genotypes. To do this, mostly the sick plot method of screening is adopted (Gopalakrishnan et al., 2014), however, it is not 100% reliable due to the uneven distribution of pathogens in the sick plot (Artal et al., 2012). This leads to the erratic appearance of symptoms among the screening population, which creates hindrances on early disease reaction scoring of the undergoing experiment. For this purpose, spot planting of susceptible check variety is done along with the genotype (s) under study all over the field to ensure uniform distribution of the pathogen. During the sick plot screening susceptible spot planted Pusa Ruby showed 93.3% bacterial wilt symptoms referring to the uniform distribution of the pathogen in the sick plot. In the current experiment, a significant difference was observed among the rootstocks for bacterial wilt resistance (Jhangta, 2015; Malshe et al., 2016). Genotypes viz., Surya, Haritha, SM 3, and SM 113 were categorized as immune to bacterial wilt disease, whereas Solanum sisymbrifolium showed a highly susceptible reaction, contradicting studies by previous researchers (Mochizuki & Yamakawa, 1979; Rahman et al., 2002). This might cause due to the reason that mechanism of resistance in these wild Solanum species is tolerance rather than immunity (Date et al., 1994). Hence, degree of tolerance might be fluctuated with enhanced temperature and humidity prevailed during experiment period, as these two factors promotes faster multiplication and virulence of Ralstonia solanacearum bacterium as reported in other Solanaceous crops (Hernández-Romano et al., 2012).

Artificial methods of screening were standardized for screening programs to reduce errors from the experiment by distributing experimental material more uniformly. In artificial screening methods, bacterial suspension of *Ralstonia solanacearum* is disseminated into the plant vascular system through various methods, root dipping, stem inoculation, media drenching, and leaf clipping (Artal et al., 2012), etc. In the current study, artificial inoculation of bacterial suspension through the root dip method recorded the highest bacterial wilt incidence (86.67%) and took the lowest number of days for appearance of symptoms (9.35 days) in susceptible check as compared to media drenching and stem inoculation methods. Hence, the root dipping method can be considered to be the most efficient artificial inoculation method for screening eggplant genotypes for bacterial wilt resistance. A significant difference was observed between artificial inoculation and sick plot method of screening of germplasm for bacterial wilt resistance. This difference in reaction of the host plant to the pathogen may be due to the genetic potential of host to resist the pathogen, age of the plant, concentration of the inoculum, and environment in which the plants were grown (Gousset et al., 2005; Sakata et al., 1996). Such varying reactions towards bacterial wilt incidence under artificial inoculation have been reported by Dutta and Rahman (2012), Kim et al. (2016), and Sadarunnisa et al. (2018).

Grafting is an efficient way to manage soil borne diseases and improve growth vigor and well as fruit yield and quality (Sabatino et al., 2016). The current study revealed the existence of a significant impact of rootstocks on the vigor of the scion. Plant vigor was manifested in terms of plant height, the number of primary branches, plant canopy spread and stem girth in the scion. Maximum plant height at 60 DAT was observed when SM 116 was used as rootstock, followed by St TNAU 1, which performed far better than the nongrafted plants. This shows that rootstocks had a significant influence on the vigor of the plants (Khah et al., 2006; Passam et al., 2005). Similarly, maximum plant spread, number of primary branches, and stem thickness was exhibited upon the use of Haritha as rootstock, which may be due to the prevalence of the vigorous root system of Haritha rootstock causing efficient absorption of water, minerals, and nutrients (Alan et al., 2007; Davis & Perkins-Veazie, 2005; Gisbert et al., 2011; Khatun, 2011).

Irrespective of the rootstocks used, all the grafted plants showed a significant difference concerning earliness in flowering when compared to control plants. Days taken for first flower opening varied from 41.80 days to 48.65 days among the rootstocks when compared to non-grafted control (41.85 days). Early flowering in the grafted plants is easy due to the movement of endogenous flowering substances of rootstock to scion across the graft union. These results conform to the findings of Ibrahim et al. (2014). Increased earliness in melon plants, when grafted onto *Cucurbita* rootstocks, has been reported by Cohen et al. (2002) and Fita et al. (2007).

Grafting vegetables on vigorous rootstocks improves the content of phytohormone (cytokinins) in the scion which are transported through the xylem from rootstock to scion and this in turn improves the number of fruits per plant (Djidonou et al., 2013; Fernandez et al., 2013; Gisbert et al., 2010; Khah et al., 2006). Similar findings were obtained in our current experiment as all the grafted plants produced a higher number of fruits per plant when compared to non-grafted control plants. Haritha rootstock resulted in the highest number of fruits in Green Long Hybrid scion followed by SM 398, which were statistically on par with each other.

Grafting significantly increased the yield and all the grafts produced significantly higher yield per plant when compared with non-grafted plants (Fig 4). The maximum yield per plant was recorded in Haritha rootstock, while the minimum yield per plant was recorded in non-grafted control plants. Correlation studies were conducted between various qualitative and quantitative characters observed in the study (Table 6). Correlation study revealed the existence of a positive correlation of yield per plant with plant spread, number of primary branches, stem girth, number of fruits per plant, fruit length, fruit girth, average fruit weight, root length, and root spread. Khatun (2011) reported a significant positive correlation between yield and the mentioned traits in eggplant genotypes. The highest yield in grafted plants may be due to better and strong root system which helped the grafts with efficient absorption of water, minerals, nutrients, increased vigor, and increased photosynthesis (Attia et al., 2003; Davis, Perkins-Veazie, Sakata, et al., 2008b; Khah, 2011; Kumar et al., 2018; Marsic & Osvald, 2004; Moncada et al., 2013; Sabatino et al., 2018; Voutsela et al., 2012). It was also observed that Haritha as rootstock performed even better than Solanum torvum in terms of yield. Superiority in fruit yield by Haritha as compared to S. torvum may be due to superior compatibility and nutrient flow from rootstock to scion as both rootstock and scion are of the same species (Lee & Oda, 2003). Hence, Haritha can be exploited as a reliable substitute for the traditionally used Solanum torvum rootstock in commercial scale. Besides the promise of cost efficient, regular and uniform rootstock production for grafting purpose, this substitute rootstock has also immense potential to provide high yield as well as quality produce. Agri-based enterprises can also be benefited as the grafted plants

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can be produced in a short period of time and according to the demand of farmers' community.

Grafting significantly influenced fruit length and girth. Maximum fruit length and fruit girth were recorded by Haritha and SM 116 rootstocks, which were statistically on par with each other. An increase in fruit length and girth might be influenced by the changes in the concentration of plant growth regulators induced by the rootstock (Jang et al., 2012; Moncada et al., 2013). Similarly, all the grafts recorded significantly higher average fruit weight when compared to control. The highest average fruit weight was exhibited by SM 116 rootstock and the minimum in non-grafted control plants. Enhanced uptake of minerals, nutrients, and water was influenced by rootstock-scion interaction which eventually led to increased fruit length and girth in plants grafted onto SM 116 and Haritha rootstocks. The increased fruit length and girth, in turn, resulted in increased fruit weight (Djidonou et al., 2013; Fernandez et al., 2013; Kumar et al., 2018).

Among phytochemicals, polyphenols content in fruits and vegetables have several beneficial traits related to human health, due to their scavenging potential of free radicals and inactivation property of other pro-oxidants. Solanum torvum is one of the mostly adopted rootstock by farmer community for its vigor as well as wilt disease resistance; however, information on increase in quality of grafted eggplant are yet to be unraveled. Cürük et al. (2005) concluded that the impact of grafted eggplants on fruit quality are scion/rootstock specific. In some studies grafting was reported not to influence the fruit quality (Bletsos et al., 2003) and even caused negative effect on scion fruit quality (Nisini et al., 2002). Nevertheless, in the current study, rootstocks significantly determined total phenol content in fruits of the scion. SM 3 rootstock caused higher total phenolic content in fruits of scion as compared to nongrafted plants. Higher total phenolic content in grafted plants may be due to additional stress in rootstock/scion combination. Phenol content of fruits of scions grafted onto SM 3 was significantly higher even as compared to the other rootstocks including Solanum torvum, which may be due to divergence between allied eggplants species (Sabatino et al., 2016; Stommel & Whitaker, 2003). A significant difference was observed with respect to dry matter content of fruits in grafted plants when compared to control. The highest dry matter was found in the fruits of SM 398 rootstock. Overall, except for Surva and SM 3, remaining rootstocks performed superior than non-grafted plants for dry matter content of fruits (Miceli et al., 2014; Wei et al., 2009). However, the Total Soluble solids (TSS) content of fruits was not influenced by grafting, which is similar to the observations found by Miguel et al. (2004), Davis, Perkins-Veazie, Hassell, et al. (2008a), and Sabatino et al. (2013).

Rootstocks maintained their immune reaction as per preliminary screening in bacterial wilt sick plot. Rootstock had a significant impact on bacterial wilt resistance of capacity of the scion. Evaluation of grafted plants under sick plot conditions showed significant success of minimizing soil borne bacterial wilt infestation in grafted Green Long Hybrid as compared to the non-grafted controls (Bhavana & Singh, 2016; Gisbert et al., 2011).

Conclusion

From the above studies, it could be concluded that the root dipping method of artificial inoculation can be used as an efficient and reliable alternate to sick plot screening against bacterial wilt disease. In our study, all rootstocks except one i.e. Solanum sisymbrifolium exhibited a high degree of resistance towards bacterial wilt disease. Solanum melongena cv. 'Haritha' was found to be the most promising rootstock for grafting in eggplant as it recorded significantly higher yield per plant, number of fruits per plant, plant spread, stem girth, number of primary branches, fruit length, root length, and root spread followed by SM 116 rootstock which recorded the highest plant height, fruit girth, and average fruit weight. Grafting had significant impact on the total phenol content and dry matter content of the fruits, although total soluble solids (TSS) content was not affected. Hence, Grafting technology could be successfully utilized in eggplant not only for bacterial wilt resistance but also for obtaining enhanced productivity with highly nutritious fruits.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

The study presented in the manuscript does not involve human or animal subjects.

All authors have reviewed the final version of the manuscript and agree to its submission to your journal.

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