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Research Article

Rootstocks for the Management of Bacterial Wilt in Eggplant (*Solanum melongena* L.) and Tomato (*Solanum lycopersicum* L.) in the Coastal Regions of India

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Cultivation of solanaceous vegetables such as eggplant and tomato is severely affected by bacterial wilt in the coastal regions of India. The causal agent *Ralstonia pseudosolanacearum* is soilborne bacterium, highly diverse, and able to survive in soil for many years without a host. Five bacterial wilt resistant eggplant (*Solanum melongena*) rootstock lines were evaluated by challenge inoculation and were found to show different levels of wilt incidence. Grafts of eggplant made on two rootstocks (S0004 and Surya) recorded reduced incidence of bacterial wilt (10 to 40%) during greenhouse evaluation while in nongrafted seedlings, the wilt incidence was 80 to 100%. Field evaluation of eggplant grafts made on *Solanum torvum* (Turkey berry), S0004, and Surya reduced the incidence of bacterial wilt compared to nongrafted seedlings. The lowest wilt incidence (0 and 15–40%) was observed in the field evaluations where *S. torvum* was used as rootstock, while the nongrafted control recorded 93–100% wilt. Tomato seedlings grafted on *S. torvum*, Surya, and S0004 recorded very low bacterial wilt incidence (0 to 15%) under greenhouse evaluation while the nongrafted seedlings recorded 80 to 100% wilt. Reduced bacterial wilt incidence (23 to 40%) was observed in grafts of polyhouse-grown tomato hybrid (GS-600) made on *S. torvum* while the nongrafted seedlings were severely affected (80 to 100%) in evaluation trials conducted for two years. From this study, it is evident that grafting of susceptible eggplant and tomato on resistant rootstocks, viz., *S. torvum* and Surya, could be a promising strategy in bacterial wilt management.

1. Introduction

Bacterial wilt disease is caused by *Ralstonia pseudosolanacearum* (Smith) Yabuuchi and is a major constraint in the production of solanaceous vegetables. The disease is widespread in different geographical locations of the world [1] and is a limiting factor in the production of vegetables in India [2–5]. The pathogen is soilborne and survives for many years [6]. Many commercial varieties and locally preferred cultivars of eggplant and tomato grown in India are highly susceptible to the disease [7, 8]. Management of this disease poses a huge challenge due to the presence of diverse *R. pseudosolanacearum* strains and the ability of the bacterium to survive longer even in adverse conditions. The pathogen is easily spread and can be introduced into noninfested areas either by water, soil, and infected plant material or by mechanical means [9]. Bacterial wilt could lead to 30–100% yield loss in eggplant [10] and more than 90% yield losses in tomato [11].

Several management strategies of bacterial wilt have been reported. Host resistance is the cheapest and easiest means of control. However, development of bacterial wilt resistant cultivars is difficult due to a variety of reasons like complex and polygenic inheritance of resistance, association between resistance and poor fruit quality, highly variable pathogen strains, and complex interaction between bacterial wilt resistance and environmental factors [12]. Other management strategies such as soil amendments [13], soil solarization [14], bio-fumigants [15–18] plant growth promoting rhizobacteria [19, 20], use of SAR inducers [18, 21, 22], and biological control [23, 24] had been developed with varying levels of success.

Grafting has been practiced for several decades to control *Fusarium* wilt in watermelon and its use has been realized later in other cucurbits and solanaceous crops [25]. Ioannou [26] reported the improved resistance to root-knot nematodes in eggplant and tomato grafts made on resistant rootstock. *Solanum torvum* (Turkey berry) has been reported as bacterial wilt resistant rootstock in controlling the disease in eggplant [27]. In tomato, grafting technique was used to manage bacterial wilt [18, 28–31]. This study reports screening of other promising resistant rootstocks along with *S. torvum* and their use in the management of bacterial wilt in eggplant and tomato in the coastal regions of India.

2. Materials and Methods

2.1. Screening of Eggplant Rootstock Lines to Bacterial Wilt in Greenhouse

2.1.1. Eggplant Rootstocks and Scion Material. Five bacterial wilt eggplant (*Solanum melongena*)-resistant rootstock lines (AVRDC-World Vegetable Centre Accession Nos. S00019 (EG 195), S00022 (EG 203), S00004 (EG 219), S00003, and TS02257 (EG 190)) received from the Indian Institute of Vegetable Research (IIVR), Varanasi (Supplementary Figure 1), were screened for bacterial wilt resistance. Seeds of five lines along with bacterial wilt susceptible local cultivar, Agassaim, were sown in a nursery tray. Twenty five days old seedlings of eggplant (cv. Agassaim and other rootstocks) were transplanted into pots containing pot mixture (2 : 1 : 1 red soil, farm yard manure, and sand) in greenhouse maintained at 30°C. In a second experiment, two additional lines (cv. Taleigao and variety, Surya) as additional susceptible and resistant check respectively were included. The plants were inoculated one week after transplanting.

2.1.2. R. pseudosolanacearum Strain and Inoculation to Rootstocks. R. pseudosolanacearum Rs-09-161, Phylotype I, and biovar 3 isolate [3] were used in the inoculation studies. *R. pseudosolanacearum* was grown in 50 ml casamino acid-peptone-glucose (CPG) broth [32] for 18–24 h, centrifuged at 8000 rpm for 10 min, and the pellet was resuspended in the same volume (50 ml) of phosphate buffered saline (PBS) and this was used as inoculum. The rootstock lines and other susceptible and resistant lines were inoculated with *R. pseudosolanacearum* by pouring (soil drenching) ten ml of the population adjusted inoculum (8 Log CFU mL^{-1}) around the base of each plant [2]. The inoculated plants were watered regularly to maintain sufficient soil moisture for plant growth and were monitored every day for the occurrence of wilt up to 21 days. Presence of milky bacterial ooze in the wilted plant was considered as confirmation that the plant died due to infection with *R. pseudosolanacearum*. *R. pseudosolanacearum* was reisolated from the wilted plant by streaking the ooze from xylem tissues on CPG medium and the colonies were compared with the original culture.

2.2. Field Screening of Eggplant Rootstock Lines to Bacterial Wilt.

The seedlings of bacterial wilt resistant eggplant rootstock lines (S00019 (EG 195), S00022 (EG 203), S00004 (EG 219), S00003, and TS02257), a bacterial wilt resistant variety (Surya), and two bacterial wilt susceptible cultivars (Agassaim and Taleigao) were planted in a bacterial wilt sick plot at ICAR-CCARI, Old Goa. Three replications and 10 plants per replication were maintained. Crop cultivation practices, viz., fertilizer application, weeding, and watering, were followed as per ICAR-CCARI's recommendation. Incidence of bacterial wilt was recorded at weekly intervals for the entire cropping season.

2.3. Evaluation of Eggplant Grafts for Bacterial Wilt Incidence in Greenhouse

2.3.1. Grafting of Cultivated Eggplant on S. torvum/Surya/Other Rootstocks. S. torvum, bacterial wilt resistant eggplant rootstock line (S0004), and Surya were selected as rootstocks for making grafts and evaluation in greenhouse and field. Surya is a proven bacterial wilt resistant eggplant variety in India. Agassaim and Taleigao lines were highly preferred regionally but susceptible to bacterial wilt. So, Surya was selected as rootstock; Agassaim and Taleigao were selected as scions. Apart from Surya, S0004 was selected as other resistant rootstock as it was reportedly resistant to nematode as well. Seeds of these were sown in nursery beds and standard nursery management practices were followed. About 5–6 cm tall seedlings were transplanted into nursery bags (70 × 140 mm size) containing pot mixture (2 : 1 : 1 red soil, farm yard manure, and sand). About 45–50 days old seedlings of *S. torvum* and 30–35 days old seedlings of Surya and S0004 (stem: 3 mm dia) were selected for making grafts. Locally preferred and bacterial wilt susceptible cultivars, Agassaim and Taleigao, were used as scion material. Seeds Agassaim and Taleigao were sown in nursery beds/trays with the abovementioned pot mixture. About 30–35 days old seedlings (stem: 3 mm dia) were selected as scion. Top wedge grafting method was used to prepare the grafts. The graft union was tied with thin polythene sheet (50–100 microns) to keep the union intact till the tissues were healed. The grafted plants were maintained in high humidity chambers (3 × 2 × 2 M) with less direct sunlight for 8–10 days and the new leaf emergence was noticed after 5–7 days. Hardening of the grafts was done in open field conditions for 3–4 days and then used in the pathogen inoculation studies and field planting [27].

2.3.2. Evaluation of Eggplant Grafts for Bacterial Wilt. Two experiments were carried out with local cultivars, Agassaim and Taleigao, as scion material. In experiment 1, Surya was used as rootstock and 13–14 grafts and seedlings of each cultivar were inoculated with the *R. pseudosolanacearum* Rs-09-161 (8 Log CFU mL^{-1} and 9 Log CFU mL^{-1}) by soil drenching method as described earlier. All the grafts were reinoculated with 10 mL of inoculum (8 Log CFU mL^{-1}) on 14^{th} day after first inoculation. In experiment 2, Surya and S0004 were used as rootstocks; five seedlings and 10 grafts of each cultivar were inoculated with the pathogen (8 Log CFU mL^{-1} and 9 Log CFU mL^{-1}) by soil drenching method. Wilt incidence in the grafted and nongrafted seedlings were recorded up to 21 days after inoculation and was analysed.

2.4. Field Evaluation of Eggplant Grafts for Bacterial Wilt Incidence. Field evaluations of the eggplant grafts were conducted during 2016 to 2017 in the research farm at ICAR-CCARI, Old Goa, Goa, and in the farmer's field at Sangolda Village, Bardez, Goa, India. Grafts of eggplant cultivars, viz., Agassaim and Taleigao, were made as described earlier using *S. torvum*, Surya, and S0004 as rootstock. *S. torvum* has been included in field evaluations based on the research findings [27]. Totally three field experiments were conducted.

In experiment 1, S0004 was used as rootstock; Agassaim and Taleigao were used as scion and the experiment was conducted at research farm of ICAR-CCARI during March 2016. The experiment was conducted with a minimum of six replications and each replication consists of 8–14 plants. In experiment 2, *S. torvum*, Surya, and S0004 were used as rootstocks; Agassaim and Taleigao were used as scion material; the experiment was conducted at research farm of ICAR-CCARI during December 2016. The experiment had three replications and each replication consists of 16 plants. In experiment 3, *S. torvum*, Surya, and S0004 were used as rootstocks; Agassaim and Taleigao were used as scion material; the experiment was conducted at farmer's field in Sangolda, North Goa, during December 2016. The experiment had three replications and each replication consists of 13–16 plants.

Grafted plants and seedlings were planted in the fields where severe incidence of bacterial wilt was regularly recorded. Planting of grafts was done in such a way that the graft union remains at least 10 cm about the ground level to avoid any contact with the soil. Crop cultivation practices, viz., fertilizer application, weeding, and watering were followed as per ICAR-CCARI's recommendation. Incidence of bacterial wilt was recorded at weekly intervals and mean wilt incidence on 90 days after planting was analysed.

2.5. Evaluation of Tomato Grafts for Bacterial Wilt Incidence in Greenhouse. Seeds of *S. torvum*, Surya, and S0004 were sown in nursery beds and standard nursery management practices were followed. Tomato hybrid/lines, Red Queen (Balaji seeds), Mose, and Myca (Syngenta) were used as scion material. Grafting of tomato was done similar to eggplant grafting as described earlier and the hardened grafts were used in the pathogen inoculation studies and also for planting in polyhouse. *R. pseudosolanacearum* isolate Rs-09-161 was used as inoculum (8 Log CFU mL^{-1}) by soil drenching method.

Three experiments were conducted with three tomato hybrid/lines, Red Queen, Mose, and Myca as scion material. In experiment 1, *S. torvum* was used as rootstock and 10 seedlings and grafts of Red Queen were inoculated. In experiment 2, *S. torvum* was used as rootstock; 10 seedlings and 8 grafts of Red Queen, Mose, and Myca were inoculated. In experiment 3, Surya and S0004 were used as rootstocks; 10 seedlings and 8 grafts of Red Queen, Mose, and Myca were inoculated.

2.6. Evaluation of Tomato Grafts in Polyhouse. Tomato hybrid (GS-600-golden seeds) was used as scion material and *S. torvum* was used as rootstock. Grafting of tomato was done as described earlier [27, 33] and the hardened grafts were used for planting in the polyhouse in two experiments in a factorial design.

During 2017–18, the grafts were planted along with nongrafted seedlings with two drip irrigation levels (I1: 120 mL/plant, 6 minutes twice daily; I2: 100 mL/plant, 5 minutes twice daily) and two fertigation levels (F1: recommended dose of NPK and micronutrients thrice a week; F2: 50% of the recommended dose of NPK and micronutrients thrice a week). Planting materials included grafts (P1: graft), transplanting of 20–25 days old nongrafted seedling on the bed (P2: transplanted), and directly sowing of the seed on the beds (P3: direct seeded). The dates of sowing and planting were adjusted in such a way that all plants were at a uniform stage when planting of seedlings and grafts were done. The experiment was conducted with four replications and the incidence of bacterial wilt was recorded regularly for the entire crop period.

During 2018–19, the grafts (P1) and nongrafted seedlings (P2) were planted with two fertigation levels (F1: recommended dose of NPK and micronutrients thrice a week; F2: 50% of the recommended dose of NPK and micronutrients thrice a week) on two planting media (soil and coco peat in a grow bag). The experiment was conducted with four replications. Crop cultivation practices were followed as per ICAR-CCARI's recommendation. Incidence of bacterial wilt was recorded regularly for the entire crop period.

2.6.1. Data Analysis. All the data were statistically analysed using ANOVA (WASP 2.0 statistical programme (https://ccari.res.in/wasp2.0/index.php) and data analysis option in Microsoft Office Excel) and the treatment means were compared using least significant difference.

3. Results

3.1. Screening of Eggplant Rootstock Lines to Bacterial Wilt in Greenhouse. Five eggplant rootstock lines were screened against bacterial wilt to identify a resistant rootstock to use in grafting. In greenhouse study, wilting symptoms were observed on the fifth day after inoculation. In experiment 1, the wilt incidence in the rootstock lines ranged from 0 to 40 per cent on 9 days after inoculation (DAI) and from 20 to 50 per cent on 21 DAI. However, the susceptible cultivar Agassaim recorded 90 per cent wilt on 9 DAI. In experiment

2, wilt incidence in the resistant rootstock lines ranged from 0 to 30 per cent on 9 DAI and 30 to 90 per cent on 21 DAI. However, the susceptible cultivars, *viz.*, Agassaim and Taleigao, recorded 100 per cent wilt on 9 DAI. Wilt incidence in rootstock lines was significantly lesser compared to the susceptible cultivars on 9 DAI (Table 1). Hence, these lines can be considered as rootstock material for making grafts of bacterial wilt susceptible lines of eggplant. Among the rootstocks screened, S0003 and S00022 and S0004 recorded significantly reduced wilt incidence.

3.2. Field Screening of Eggplant Rootstocks to Bacterial Wilt. All the resistant rootstock lines, Surya, and the susceptible cultivars, viz., Agassaim and Taleigao, were planted in the sick plot and no wilting was observed in all the resistant rootstock lines as well as in Surya. However, in Agassaim and Taleigao, 100 per cent wilt was recorded (data not shown). As none of the rootstock lines wilted in the field evaluation, only two rootstocks, *viz.*, Surya, pedigree of S00022 (EG 203), and S0004 (EG219), were selected for further studies in greenhouse.

3.3. Evaluation of Eggplant Grafts for Bacterial Wilt Incidence in the Greenhouse. In experiment 1 (Surya + Agassaim/Taleigao), no wilting was observed in any of the grafts up to 9 days, whereas in nongrafted seedlings, 100% plants wilted. All the grafts were reinoculated with 10 mL of (8 Log CFU mL^{-1}) on 14th day after first inoculation. Wilting started after 10 days of second inoculation and maximum of 50 per cent wilt was observed in Agassaim grafts inoculated with 9 Log CFU mL^{-1} until 45 days. In all other cases, 28 per cent wilt was recorded (data not shown). In experiment 2 (Surya/S0004 + Agassaim/Taleigao), eggplant (Taleigao and Agassaim) grafted on Surya did not wilt. However, the plants grafted on S0004 showed wilting up to 50 per cent. Higher inoculum load (9 Log CFU mL^{-1}) caused higher percentage wilt (50% in Agassaim grafts and 40% in Taleigao grafts) compared to relatively lower inoculum (8 Log CFU mL^{-1}) which recorded 30% wilt in Agassaim grafts and 20% wilt in Taleigao grafts. However, the nongrafted seedlings of Agassaim and Taleigao recorded 80% to 100% wilt. Wilt incidence in grafts was significantly less ($p < 0.05$) compared to nongrafted seedlings in both the inoculum levels. Further, comparison of mean wilt incidence indicated that no wilt was recorded in grafts made on Surya and significantly reduced wilt ($p < 0.05$) was recorded on grafts made on S0004 (Figure 1). Based on these results, Surya and S0004 were selected for making grafts of susceptible varieties for evaluation in field. Further, grafts on *S. torvum* had been included in field evaluations based on previous research findings [27].

3.4. Field Evaluation of Eggplant Grafts for Bacterial Wilt Incidence. In experiment 1, eggplant grafts made on S0004 recorded less wilt compared to the nongrafted seedlings. Taleigao grafts recorded 9.0 per cent wilt and Agassaim grafts recorded 4.0 per cent wilt, whereas the seedlings of Taleigao and Agassaim recorded 80.0 and 95 per cent wilt, respectively. Wilt incidence in grafts was significantly less ($p < 0.05$) compared to nongrafted seedlings in each variety (Figure 2).

In experiment 2, Agassaim grafts made on Surya, S0004, and *S. torvum* recorded 38 to 50 per cent wilt while the Agassaim nongrafted seedlings recorded 94 per cent wilt. Taleigao grafts made on Surya, S0004, and *S. torvum* recorded 15 to 64 per cent wilt, whereas Taleigao nongrafted seedlings recorded 96 per cent wilt (Figure 3). Incidence of wilt in nongrafted seedlings was significantly higher ($p < 0.05$) compared to grafts. More than 50% of nongrafted seedlings wilted within two months of planting. However, the grafts showed wilting during later stage of the crop.

In experiment 3, Agassaim grafts made on Surya and S0004 recorded 64 to 71 per cent wilt. Taleigao grafts made on Surya and S0004 recorded 47 to 69 per cent wilt. However, no wilt incidence was observed in both Agassaim and Taleigao grafts made on *S. torvum*, whereas the seedlings of Agassaim and Taleigao recorded 100 per cent wilt (Figure 4). Incidence of wilt in nongrafted seedlings was significantly higher ($p < 0.05$) compared to grafts. More than 40% seedlings wilted within two months of planting. However, the grafts showed wilting during later stage of the crop.

3.5. Evaluation of Tomato Grafts to Bacterial Wilt Incidence in Greenhouse. In experiment 1, nongrafted seedlings of Red Queen started wilt three days after inoculation and 100 per cent plants wilted four days after inoculation. However, wilting started on 4th day and the maximum of 20 per cent plants wilted in case of grafts made on *S. torvum*. In experiment 2, wilt started five days after inoculation and 100 per cent plants wilted after eight days of inoculation in case of nongrafted seedlings of Red Queen. In case of Myca and Mose, wilt started five days after inoculation and 80 per cent nongrafted seedlings wilted after seven (Mose) and ten (Myca) days of inoculation. However, in grafts, wilting started on 10th day in Red Queen and Myca and the maximum of 12.5 per cent plants wilted. No wilting was observed in Mose grafts. When nongrafted seedlings and grafts made on *S. torvum* are compared, 80–100% plants wilted in nongrafted seedlings of Myca, Mose, and Red Queen. Grafts recorded only 12.5 to 20 per cent wilt and no wilt was observed in Mose grafts. Mean wilt incidence in grafts (11.25%) was significantly less ($p < 0.05$) compared to nongrafted seedlings (90%) (Figure 5). In experiment 3, grafts of Red Queen, Mose, and Myca made on Surya and S0004 did not wilt till 21 days. However, 80 to 100 per cent plants wilted in the nongrafted seedlings. Mean wilt incidence in grafts (0%) was significantly less ($p < 0.05$) compared to nongrafted seedlings (86.67%) (Figure 5).

3.6. Evaluation of Tomato Grafts in Polyhouse. During 2017–18, higher number of plants wilted in the direct seeded planting method (84.38%) and transplanted seedlings (63.84%) while the incidence of wilt in graft was only 35.7%. Significant reduction ($p < 0.05$) of wilt was observed in

Table 1: Incidence of wilt in the rootstock lines, local cultivars/varieties inoculated with *R. pseudosolanacearum*.

		% of plants wilted			
		First evaluation		Second evaluation	
S. No.	Rootstock lines/varieties	9 DAI	21 DAI	9 DAI	21 DAI
1	S00019	20 ± 20^{b}	50 ± 30^{ab}	20 ± 0^{b}	90 ± 10^{a}
2	S00022	10 ± 10^{b}	30 ± 10^{b}	20 ± 20^{b}	30 ± 10^{cd}
3	S00004	40 ± 20^{b}	50 ± 30^{ab}	0 ± 0^{b}	50 ± 10^{bc}
4	S00003	0 ± 0^{b}	20 ± 0^{b}	20 ± 0^{b}	30 ± 10^{cd}
5	TS002257	20 ± 0^{b}	30 ± 10^{b}	30 ± 30^{b}	70 ± 10^{ab}
6	Surya	ND	ND	0 ± 0^{b}	0 ± 0^{e}
7	Taleigao	ND	ND	100 ± 0^{a}	100 ± 0^{a}
8	Agassaim	90 ± 10^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
	SE (d)	18.25	25.82	18.03	11.18
	LSD (0.05)	44.67	63.17	41.57	25.78

Mean of 2 replications; 5 plants per replication; inoculum: Log 8 CFU/mL; ND: not done; in a column, values followed by the same letter are not significantly different at 5% level determined by LSD ($p < 0.05$).

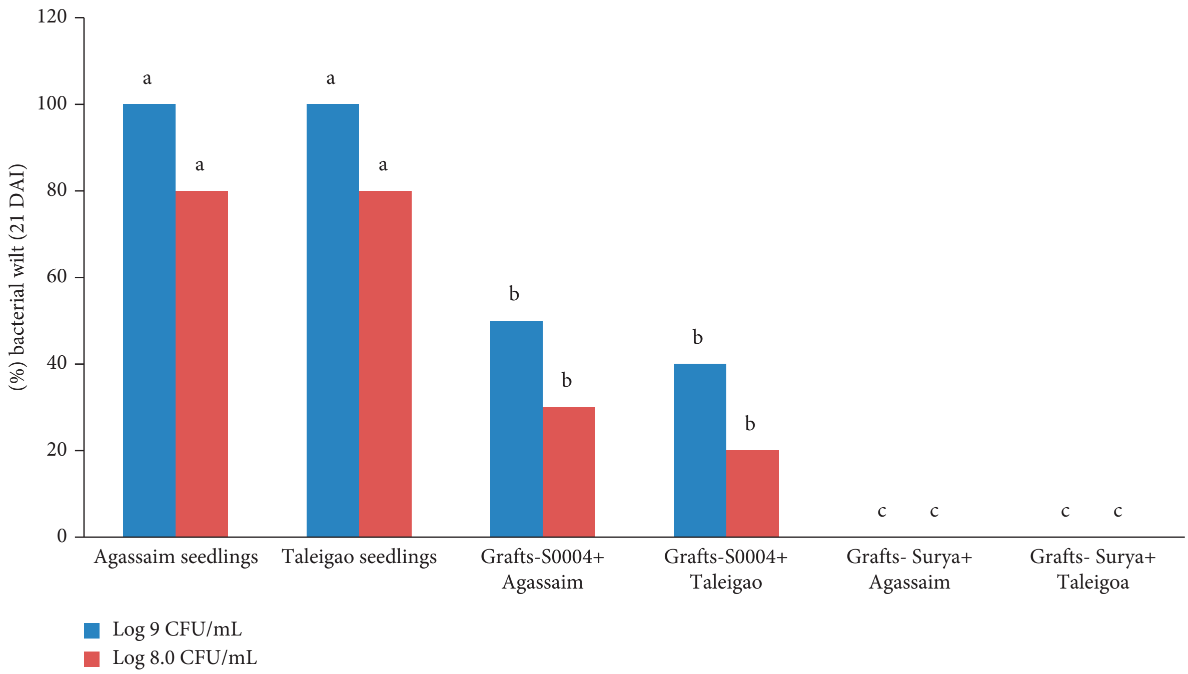

Figure 1: Incidence of bacterial wilt in eggplant grafts on S0004 and Surya in the greenhouse evaluation. % of plants wilted 21 days after the inoculation of *R. pseudosolanacearum* (8 Log CFU mL^{-1} and 9 Log CFU mL^{-1}). Treatments are significantly different at 5% (LSD = 26.54). Wilt % in the column indicated by the same letter are not significantly different at 5% level.

grafted plants compared to nongrafted seedlings (Table 2). No significant difference in the wilt incidence with the different levels of irrigation as well as fertilizer application treatments was observed (Table 3).

During 2018–19, tomato grafts were least affected by bacterial wilt, whereas the nongrafted seedlings were severely affected. Wilt incidence in the grafts ranged between 3 and 14% (10 DAP), 19 and 33% (40 DAP), 23 and 39% (70 DAP), and 26 and 44% (100 DAP). However, wilt incidence in the nongrafted seedlings was 0 (10 DAP), 22–73% (40 DAP), and 63–97% (70 and 100 DAP). Incidence of wilt at 40 DAP and 70 DAP is presented and discussed. Less number of plants wilted in the case of grafts (25.0% and 32.59%) as compared to nongrafted seedlings (43.61% and 82.3%) at 40 DAP and 70 DAP, respectively. There was a significant reduction ($p < 0.05$) of wilt in grafted plants as compared to nongrafted seedlings at 40 DAP and 70 DAP. However, no significant difference in the wilt incidence with the different levels fertilizer application treatments and planting medium was observed (Tables 4 and 5).

4. Discussion

Various methods including soil disinfection, soil amendment, biological and chemical controls, and resistant cultivars or rootstocks have been reported for bacterial wilt management [22, 34, 35]. Use of chemicals for bacterial wilt management is not practical as the pathogen resides inside

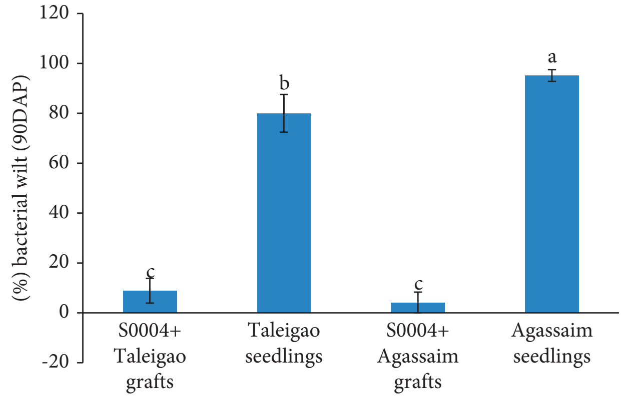

Figure 2: Incidence of bacterial wilt in eggplant grafts on S0004 and nongrafted seedlings in the field evaluation at research farm of ICAR-CCARI (March 2016). S0004+ Taleigao grafts: mean of 6 replications; mean No. of plants per replication: 7.5; Taleigao seedlings: mean of 8 replications; mean No. of plants per replication: 9.75; S0004+ Agassaim grafts: mean of 6 replications; mean No. of plants per replication: 8.0; Agassaim seedlings: mean of 10 replications; mean No. of plants per replication: 13.8; bar in the column represents the standard error. Treatments are significantly different at 5% (LSD = 12.69). Wilt % in the column indicated by the same letter are not significantly different at 5% level.

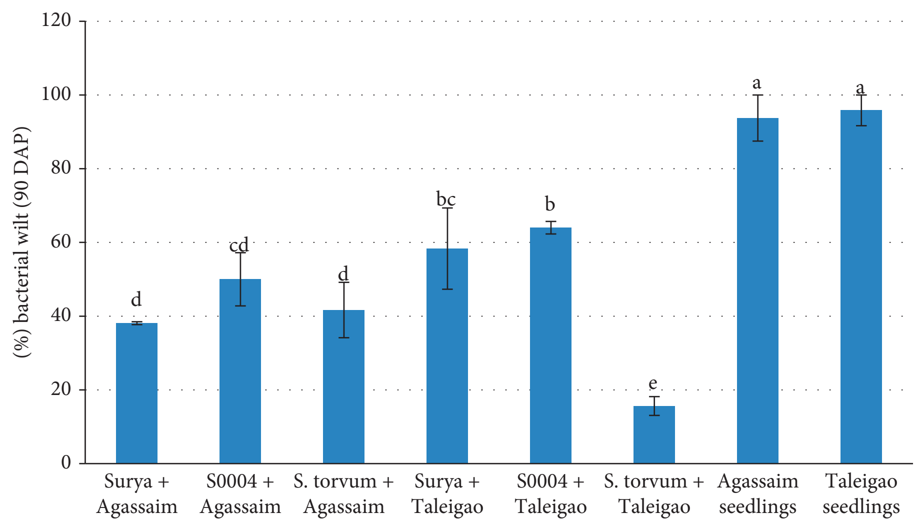

Figure 3: Incidence of bacterial wilt in eggplant grafts on Surya, S0004, and *S. torvum*; nongrafted seedlings in the field evaluation at research farm of ICAR-CCARI (Dec 2016). Mean of three replications; each replication consists of 16 plants. Treatments are significantly different at 5% (LSD = 14.16) and at 1% (LSD = 19.59) level. Bar in the column represents the standard error. Wilt % in the column indicated by the same letter are not significantly different at 5% level.

the xylem [36]. To date, there are no chemicals or antibiotics available to efficiently control bacterial wilt in the field. Biological control using different strains of bacteria suppressed bacterial wilt with limited success in the field studies [20, 23, 24]. Resistant varieties are the best bet for the management of bacterial wilt but has been hampered by polygenic inheritance and sometimes the association of resistance with undesirable traits of wild species [37, 38]. Grafting onto resistant rootstocks could also provide an alternative solution to manage soilborne pathogens, including bacterial wilt, in Solanaceous crops [27, 39, 40]. Grafting has traditionally been practiced to control *Fusarium* wilt in watermelon and later its use has been realized to control diseases in other cucurbits and Solanaceous crops [25]. In Japan, grafting of eggplant on *S. torvum* is widely used accounting for 50% of the total acreage [41]. *Verticillium* and *Fusarium* wilt disease control in crops of eggplant and melon had been well proven [42, 43].

S00022 (EG 203), S0004 (EG219), and TS02257 (EG190) were reported as potential rootstocks for root-knot nematode and bacterial wilt management studies conducted at AVRDC [44]. S00019 (EG195) has been used as a rootstock for the *Fusarium* wilt management in tomato at AVRDC [33]. In this study, S0003, S00022, and S0004 were comparatively resistant; S00019 and TS2257 were susceptible to bacterial wilt in challenge inoculation studies.

Objective of this study was to study additional eggplant resistant lines that can be used as potential rootstocks for grafting. From these screening studies, S0003 and S00022 appear to be a good source for use as rootstock in grafting.

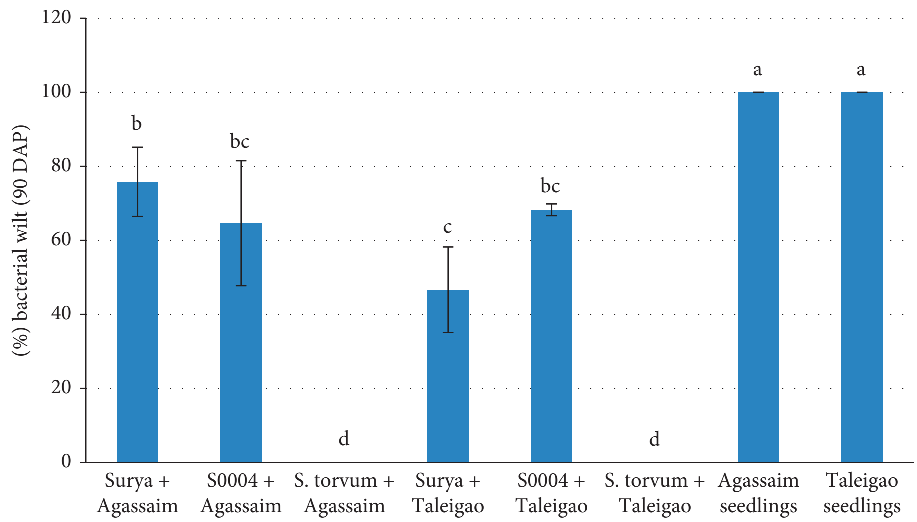

FIGURE 4: Incidence of bacterial wilt in eggplant grafts on Surya, S0004, and *S. torvum*; nongrafted seedlings in the field evaluation at farmers field in Sangolda (Dec 2016). Mean of three replications; each replication consists of 13–16 plants. Treatments are significantly different at 5% (LSD = 14.61) and at 1% (LSD = 20.27) level. Bar in the column represents the standard error. Wilt % in the column indicated by the same letter are not significantly different at 5% level.

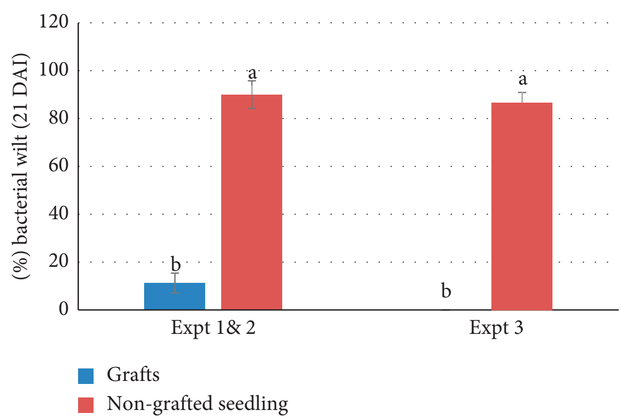

FIGURE 5: Incidence of bacterial wilt in tomato grafts on *S. torvum* (Expt. 1 and 2); on Surya and S0004 (Experiment 3) and nongrafted seedlings in the greenhouse evaluation. Two-sample *T*-test analysis indicated that wilt incidence in grafts is significantly less ($p < 0.05$) compared to wilt incidence in the nongrafted seedlings in all the three experiments. Mean wilt incidence in grafts is significantly less compared to wilt incidence in nongrafted seedlings at 5% level (LSD = 8.696 for experiments 1 and 2, 3.835 for experiment 3). Bar in the column represents the standard error. Wilt % in the column indicated by the same letter are not significantly different at 5% level.

TABLE 2: Incidence of bacterial wilt in tomato (GS-600) grafts and nongrafted seedlings planted with two drip irrigation levels and two fertigation levels in polyhouse (2017–18; 6 MAP).

Planting method ∗	I1: irrigation-1		I2: irrigation-2	
	F1: fertigation-1	F2: fertigation-2	F1: fertigation-1	F2: fertigation-2
P1: grafts	21.43	33.93	51.79	35.71
P2: transplanted	62.50	51.79	57.14	83.93
P3: direct seeded	87.50	83.93	71.43	94.64

Mean of four replications; MAP: months after planting. Mean comparison of the treatments using ANOVA.

As none of the rootstocks wilted in the field screening, only two rootstocks, viz., Surya and S0004, were selected for further studies. Surya is a bacterial wilt resistant variety from KAU, Kerala, India, and is a pedigree of S00022 (EG 203) and S0004 (EG219) along with *S. torvum* were used for field evaluation of grafts of two susceptible cultivars (Agassaim and Taleigao). Results of field screening of all the rootstocks in a bacterial wilt sick plot showed that these lines were resistant at field inoculum concentration.

Namisy et al. [8] reported that EG 203 and *S. torvum* were resistant or moderately resistant to bacterial wilt

Table 3: Mean comparison of bacterial wilt incidence (%) in tomato (GS-600) grafts and nongrafted seedlings (2017-18).

Irrigation	I1	56.85
	I2	65.77
Fertigation	F1	58.63
	F2	63.99
Planting material	P1	35.71 *
	P2	63.84 *
	P3	84.38 *

* Significantly different at 5% level ($p < 0.05$); LSD = 20.495; wilt incidence in irrigation and fertigation levels are not significantly different.

Table 4: Incidence of bacterial wilt in tomato (GS-600) grafts and nongrafted seedlings planted with two fertigation levels on two planting media in polyhouse (2018–19).

Planting medium	Fertigation	Planting material *	% wilt			
			10 DAP	40 DAP	70 DAP	100 DAP
Soil-S1	F1: fertigation-1	P1: graft	8.93	25.00	39.29	42.86
		P2: seedling	0.00	48.35	63.24	63.24
	F2: fertigation-2	P1: graft	7.14	19.64	23.21	26.79
		P2: seedling	0.00	73.53	81.88	86.56
Coir pith-S2	F1: fertigation-1	P1: graft	14.29	33.93	35.71	44.64
		P2: seedling	0.00	30.30	86.36	95.45
	F2: fertigation-2	P1: graft	3.57	21.43	32.14	37.50
		P2: seedling	0.00	22.25	97.73	97.73

Mean of four replications; DAP: days after planting. Mean comparison of the treatments using ANOVA.

pathogen in the screening studies at AVRDC. Ramesh et al. [27] reported that grafts made on *S. torvum* were completely resistant to bacterial wilt when the pathogen was inoculated even at higher concentrations. However, in this study, higher wilt incidence (15 and 41%) was observed in the grafts made on *S. torvum* in one of the field trials (Figure 3). Grafts made on Surya and S0004 recorded wilt ranging from 38 to 64% (Figure 3) and 46 to 75% (Figure 4) indicating that these lines may not be very much useful as rootstocks for bacterial wilt management where the incidence is very severe although partial yield could be obtained due to the delayed incidence. Resistance to bacterial wilt could vary depending on the pathogenic strains present in that field or geographical location. Namisy et al. [8] documented this varying degree of resistance of different cultivated eggplant and wild relatives when screened against two *R. pseudosolanacearum* strains. Hence, it is very imperative to understand the pathogenic potential of the *R. pseudosolanacearum* strain present in the location and the response of the resistant variety/rootstock before management recommendations are made.

Susceptible tomato lines grafted on *S. torvum*, Surya, and S0004 significantly reduced the incidence of bacterial wilt under greenhouse evaluation studies (Figure 5). Bacterial wilt susceptible tomato grafted on resistant rootstocks resulted in 85–95 per cent survival in Sri Lanka [45]. In tomato, grafting susceptible scions onto bacterial wilt resistant hybrid rootstocks reduced wilt incidence in the field trails in heavily infested *R. pseudosolanacearum* soils [46]. Hong and Yang [47] reported that the grafting of tomato on eggplant rootstocks effectively controlled bacterial wilt. Manickam et al. [30] reported that bacterial wilt resistant eggplant rootstocks showed good graft compatibility with tomato and the grafted tomato plants showed low wilting percentage (0.0–20.0%). However, based on the results of eggplant in different field evaluations, we choose to use only *S. torvum* as rootstock for tomato in polyhouse evaluation studies. Relatively higher incidence of wilt (35–37%) in polyhouse grown tomato grafts is reported in this study. However, the incidence of bacterial wilt in the grafted plants is significantly less compared to nongrafted plants during both the seasons (Tables 2 and 4).

Although disease control mechanism by grafting is not well understood, it is presumed that physical avoidance of pathogen from soil [25] could be the main reason. Studies on the population *of R. pseudosolanacearum* in graft evaluation indicated that the pathogen was present in the rhizosphere soil, but absent in the stem of the grafts (scion part) [27]. However, examination of the diseased tomato plants in this study revealed that the pathogen was present only in the scion part and not in the rootstock part in some samples and was present in both parts in some samples. This could be due to the practice of pruning the lower leaves regularly using nonsterilized knife which might have contaminated with the pathogen from a susceptible and infected seedling. Further emergence of lateral roots from the scion part of tomato and entering into the soil also could have been a cause for direct entry of pathogen to scion.

In conclusion, results of this study reveal that *S. torvum* as rootstock significantly reduced the incidence of bacterial wilt in eggplant and tomato grafts in the field evaluations. Hence, this could be exploited for bacterial wilt management in eggplant and tomato wherever there is no resistant varieties are available. Other rootstocks showed varying levels of resistance in the study location that could also be used in the regions where the disease incidence is moderate. Hence, it is very important to understand the level of resistance of

Table 5: Mean comparison of bacterial wilt incidence (%) in tomato (GS-600) grafts and nongrafted seedlings (2018-19).

		40 DAP	70 DAP
Planting medium	S1	41.63	51.903
	S2	26.978	62.986
Fertigation	F1	34.394	56.149
	F2	34.214	58.74
Planting material	P1	25.0 *	32.589 *
	P2	43.608 *	82.3 *

* Significantly different at 5% level ($p < 0.05$); LSD = 16.963 at 40 DAP and 17.971 at 70 DAP; wilt incidence in planting medium and fertigation levels are not significantly different.

rootstock to *R. pseudosolanacearum* as the resistance level could vary depending on the strain present in the location. Elaborate studies on the host-pathogen interaction using different strains present in the geographical location are required before recommending any resistant line or rootstock for bacterial wilt management.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Authors' Contributions

RR conceived the study and analysed and interpreted the data and has written the manuscript. MJG has made major contributions to the concept and design of the study and contributed in manuscript writing. MS, TA, and GA executed the experiments.

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Supplementary Materials

Supplementary Figure 1. Fruit types of bacterial wilt resistant rootstocks received from AVRDC. (*Supplementary Materials*)

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