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Potential of host resistance as an important tool in the management of bacterial wilt in tomatoes

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Abstract

Bacterial wilt is a major bacterial disease that impacts tomato (*Solanum lycopersicum*) production in Tanzania. The disease is caused by members of soil borne *Ralstonia solanacearum* with complex pathogen variability and a wide host range complicating its management. We established the use of tomato lines MT56 and WG120 and eggplant line EG190 from the pre-screening experiment to evaluate resistance against *Ralstonia pseudosolanacearum* strains collected from key tomato-growing regions of Tanzania**.** Among the three lines resistant to bacterial wilt infection, MT56 and EG190 were selected as rootstocks for grafting with the susceptible tomato variety Moneymaker. Grafted seedlings were challenged with a mixture of strains that were previously used in the evaluation of rootstocks. Grafted seedlings had significantly reduced bacterial wilt incidence compared to self-grafted Moneymaker. Bacterial wilt disease progress as measured by area under disease progress curves (AUDPCs) varied significantly among rootstock/scion combinations (*P*=0.0190). The area under the disease progress curve values was consistently low for self-grafted rootstock and rootstock/scion grafted seedlings. This study demonstrates the potential of using host resistance as an efficient and environmentally friendly management option for bacterial wilt disease.

Key words: *Bacterial wilt; Host; Ralstonia pseudosolanacearum; Ralstonia solana*cearum; *Resistance; Rootstock*

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Introduction

Tomato is among the most valuable horticultural crops in Tanzania contributing 51% of the total vegetable crop production in the country (De Putter *et al.,* 2011; URT 2012; Luzi-Kihupi *et al.,* 2015, Mutayoba and Ngaruko, 2018). However, tomato production of 2.2-16.5 t/ha in Tanzania is far lower than the 27.5 t/ha global average (FAO, 2005; Maerere *et al.,* 2006). Important factors such as deteriorating soil fertility, the use of vulnerable and low-yielding varieties, unreliable rainfall, diseases, pests, and poor farming practices contribute to reduce tomato production (Minja *et al.,* 2011). Bacterial wilt is among the most damaging soilborne diseases of tomatoes that lead to total wilting of plants especially at the flowering and fruiting stage; hence causing huge yield losses (Elphinstone, 2005, Ayana *et al.*, 2016).

As with many soilborne diseases, bacterial wilt management is complex and difficult to accomplish using popularly known disease management strategies. This is attributed in part to the ability of the pathogen to survive long term in the soil with favorable humidity and temperature conditions (French *et al.,* 1998; Arwiyanto *et al.,* 2015, Choudhary *et al.,* 2018). Bacterial wilt management approaches range from phytosanitary measures, cultural practices, biological control, and chemical treatments to host resistance (Saddler, 2005; Elphinstone, 2005; Champoseau and Momol, 2009, Shutt *et al.*, 2018). Host resistance is the preferred means of bacterial wilt management of tomatoes; however, most popular tomato varieties are susceptible to bacterial wilt (Opena *et al.*, 1990; Wang *et al.,* 1998; Huang *et al.*, 2015; Du *et al.,* 2019). Host resistance has been explored in solanaceous crops that include pepper (*Capsicum* spp.), eggplant, and tomato (Wang *et al.,* 1998; Oda, 1995; Lin *et al.,* 2008; Du *et al.,* 2016; Salgon *et al.,* 2017). Many solanaceous rootstocks have been identified and explored for their performance in reducing bacterial wilt on susceptible horticultural preferable varieties. Researchers in China deployed wild tomato species lines (CH-2-21, 25 and 26) as rootstocks for grafting fresh market tomatoes with 80-100% success in controlling bacterial wilt disease (Lu *et al.,* 1992). Tomato breeding lines Hawaii 7996, Hawaii 7997, and Hawaii 7998 are known for reduced bacterial wilt incidence and severity in worldwide locations (Grimault *et al.*, 1995, Hanson *et al.,* 1996, Scott *et al.,* 2005, Jacobs *et al.,* 2013). Rahmawat and Arwiyanto (2020) reported about 40% bacterial wilt incidence when Hawaii 7996 was grafted to susceptible tomato scions in Indonesia. Rivard and Louws (2008) reported a 100% survival rate in heirloom tomato plants grafted onto Hawaii 7996 and CRA-66 rootstocks and exposed to *R. solanacearum*. Similar results were observed in Brazil using Hawaii 7996 as rootstock for commercial tomato varieties (Cardoso *et al.,* 2012) and Louisiana, USA against phylotype I and II isolates (Ivey *et al.,* 2020). In the same context, Scott *et al*. (1995) crossed a susceptible tomato variety with Hawaii 7997 to obtain the variety Neptune with bacterial wilt resistance. However, a limited spectrum of resistance was observed and in 2009 they released lines Fla8109 and Fla8109b that had similar pedigrees as Hawaii 7997 (Scott *et al.*, 2009). In a similar context Phiri *et al.,* 2024 evaluated 40 tomato accessions provided by the United States Department of Agriculture (USDA) for their potential to resist bacterial wilt disease. Among these accessions only five namely: PI 645370, PI 647306, PI 600993, PI 355110, and PI 270210 showed some pedigree of resistance to bacterial wilt with a ranging incidence of 42.8% to 59.9%. Other commercial tomato rootstocks Cheong Gang (Seminis), Shield (Rijk Zwaan), and RST-04-106-T (DP Seeds) were reported to fully control bacterial wilt disease when grafted to susceptible tomato scions (Suchoff *et al.,* 2019).

Eggplant is preferred to tomato as a rootstock because of eggplant's durable resistance to *Ralstonia* spp. and ability to survive in flooded soils (Lee *et al.,* 2013). In eggplant, bacterial wilt resistance genes segregate as single genes (*ERs1* and *RE-bw*) (Salgon *et al.,* 2017). The eggplant rootstock EG203 (AVRDC- the World Vegetable Center) was reported to survive at a rate of >95% in bacterial wilt-infested soils. Fresh market wilt-susceptible tomato varieties TStarE and Victoria grafted onto five eggplant rootstock accessions VI041979A, VI041809A, VI041984, VI041945, and VI041943 from AVRDC exhibited 0-20% bacterial wilt incidence (Manickam *et al.,* 2021). Other eggplant lines or varieties including SM164, SM6, Surya, and AF9125 exhibited promising resistance to phylotype I and II *Ralstonia* spp. Strains (Ivey *et al.,* 2020).

This study was focused on identifying bacterial wilt-resistant rootstocks for use in grafting susceptible farmer-preferred tomato varieties in Tanzania. The study hypothesized that the impact of bacterial wilt on susceptible tomato varieties will be reduced significantly when they are grafted to rootstocks with host resistance to *R. pseudosolanacearum* strains collected from Tanzania's key tomato producing regions. We aimed to identify eggplant and tomato breeding lines resistant to *R. pseudosolanacearum* for use as rootstocks for grafting with the most horticulturally preferred tomato varieties identified during the tomato farmers' survey.

Materials and methods

Preliminary rootstock screening

Eleven tomato breeding lines from the OSU Tomato Breeding Program and tomato MT56 and eggplant EG190 were screened for resistance to bacterial wilt in two separate experiments conducted in Ohio in 2017- 2018 (Table 1). A split plot randomized block design was used for these experiments in which rootstock lines were the main plots and *R. pseudosolanacearum* strains were subplots. Each of the four blocks contained three plants (one plant per pot). Plants of each test line were inoculated separately with each of the test strains, or mock-inoculated with sterile distilled water as a negative control. The bacterial wilt-susceptible tomato variety Moneymaker was used as a control in this experiment. The experiments were replicated twice and blocked by time.

Seedlings, inoculum preparation, and inoculation

Tomato and eggplant seeds (Table 1) were sown in fertilizer-amended (NPK 20:20:20) autoclaved muck soil collected from the OSU CFAES Muck Crops Experiment Station in Willard, OH. Seedlings were grown in 15cm-diameter plastic pots in a greenhouse set at 28°C, with 16 hrs of light and an average relative humidity of 80% for 4 weeks. Seedlings were inoculated by drenching the soil in each pot with 50ml of 10^8 CFU/ml *R. pseudosolanacearum* (Table 2) cell suspension prepared from 48 hr cultures grown on Casamino acid-Peptone-Glucose (CPG) medium. The concentration of inoculum was adjusted to 10^8 CFU/ml (OD_{600}= 0.1). Control plants were drenched with 50 ml of sterile water. Control and inoculated plants were arranged in a split plot RCBD described above with three replications and maintained in a BSL2 greenhouse room set at 28°C ± 2°C for eight weeks. Plants were watered once daily and fertilized with NPK 20:20:20 once every two weeks.

Disease assessment

Plants were assessed weekly for incidence and severity of wilting symptoms. Disease incidence was assessed by counting the total number of plants (N) and the number of plants with bacterial wilt symptoms (n) for each line and isolate inoculated. The incidence of bacterial wilt was calculated using the equation 1.

$$Incidence\ of\ bacterial\ wilt = \frac{n}{N} * 100 \quad (1)$$

Disease severity values were obtained by rating diseased plants for each line x strain combination using a 1-5 scale (Horita and Tsuchiya, 2001) in which 1=asymptomatic, 2=two leaves withered, 3=three leaves wilted, 4=at least four leaves wilted, and 5=dead plant. Each severity score was converted to a leaf damage scale (0-100 %), where 0 represented asymptomatic leaves and 1, 2, 3, 4, and 5 represented 20, 40, 60, 80, and 100% wilted leaves, respectively. Mean disease severity was expressed as the mean of all leaf damage percentages for each line x isolate combination. The area under the disease progress curve (AUDPC) was calculated according to the Excel formula (Madden *et al.*, 2007).

$$\mathrm{AUDPC} = \sum_{i=1}^{n}\left(\left(\frac{y_i + y_{i+1}}{2}\right) + (t_i + 1 - t_i)\right) \quad (2)$$

Where y_i = measures of disease level at ith observation and t_i time of disease measure at ith observation.

To assess latent infection, plants remaining asymptomatic 8 weeks after inoculation were sampled by cutting a 2 cm stem section from the base of the plant and placing it in a tube containing 2.5 ml of sterile distilled water to allow for bacterial streaming for one hour. Suspensions

(100µl) were pipetted into wells of a 96-well microtiter plate and an enzyme-linked immunosorbent assay (*R. solanacearum* ELISA kit; Agdia Inc. Elkhart, IN, USA) was conducted according to manufacturer instructions. The percentage of plants with latent infection was calculated by dividing the number of plants that tested positive *for R. pseudosolanacearum* by the total number of plants sampled for latent infection x 100.

Selected rootstock evaluation in Tanzania

From the preliminary rootstock evaluation, bacterial wilt-resistant rootstocks WG120, EG190, and MT56 were selected for further evaluation at the Department of Horticulture and Crop Science, Sokoine University of Agriculture, Morogoro, Tanzania from November 2019 to June 2020. The protocol for the evaluation of these rootstocks was modified by Lebeau *et al*. (2011). The exception was in the change of the experimental design to split plot to accommodate time and resource limitations and the inoculation method to soil drenching. A split plot in a completely randomized block design (RCBD) with four replications (blocks) was used to assess the resistance of tomato MT56 and WG120 and eggplant EG190 against six selected virulent *R. pseudosolanacearum* strains collected from the five key tomato producing regions of Tanzania during a 2019 farm survey (Table 2). In the experiment, *R. pseudosolanacearum* strains were the main plots and lines were subplots randomized within the main plots, with five seedlings per line in separate pots per block. Tomato variety Tanya F1 (Seminis, Holland) was used as a susceptible control. Tomato seedlings and the inoculum were prepared as described in the preliminary rootstock evaluation except for the use of pre-autoclaved forest soil, polythene bags (0.5kg) instead of pots, and NPK fertilizer (YaraMila Winner 15-9-20). Inoculated plants were maintained in a screenhouse with a daily average temperature of 30°C and nighttime of 22°C and plants were watered once daily and fertilized once every two weeks. Disease scoring and latent infection assessments were as described in the preliminary evaluation experiment. The experiment was conducted twice.

Rootstock-scion graft compatibility evaluation in Tanzania

The grafting experiment was conducted in a Sokoine University of Agriculture screenhouse with a daily average temperature of 30°C and nighttime of 22°C in February and June 2020. Tomato line MT56 and eggplant line EG190 were selected as rootstocks and the tomato variety Moneymaker was the scion. Fifty seeds of each of the rootstocks and scion were sown into 0.5kg polythene bags filled with pre-sterilized (autoclaved) moist field soil and kept for 3 to 4 weeks on the screenhouse bench. Fifteen seedlings that had the same stem diameter from each of the lines were selected for each grafting treatment. The treatments were self-grafted plants of MT56, EG190, and Moneymaker, and Moneymaker grafted to MT56 and EG190. Seedlings were grafted using the tube grafting method by joining the scion to the rootstock cut above the cotyledon with silicone clips to support the graft union (Black *et al.*, 2003). Grafting was conducted in a screenhouse room precleaned by mopping with soapy water and wiped with a solution of sodium hypochlorite (2% sodium hypochlorite). All the grafting tools and the grafter's hands were cleaned and disinfected using 70% alcohol before and during grafting to minimize contamination. Immediately after grafting seedlings were placed in a healing chamber on the benchtop of a screenhouse room. The chamber was misted twice daily with a hand sprayer to maintain high relative humidity. Plants were removed from the healing chamber two weeks after grafting and arranged on benches of a screenhouse with a daily average temperature of 30°C and a night temperature of 26°C for four weeks. Plants were watered once daily and fertilized with Yara NPK fertilizer once every two weeks. A randomized complete block design (RCBD) was used to lay 75 seedlings, five from each of the self-grafted rootstocks and scions, and rootstock-scion combinations in three blocks. The number of live and dead plants was recorded twice weekly for two weeks. The experiment was conducted twice.

Response of grafted plants to bacterial wilt inoculation

Fully recovered 4-week-old grafted seedlings from the grafting experiment were tested for resistance to bacterial wilt. The experimental design was an RCBD with three blocks each containing five seedlings from each treatment of grafted plants inoculated with 10^8 CFU/ml *R.*

pseudosolanacearum cells as described above for rootstock screening. The inoculum was a combination of the six *R. pseudosolanacearum* strains (Table 6) used to screen rootstocks. Inoculated plants were maintained in a screenhouse as described above for eight weeks. Disease incidence was assessed for eight weeks, and then stem tissue was sampled for latent infection using the Agdia *Ralstonia* ELISA kit as described above. The experiment was conducted twice.

Statistical analysis

Statistical analyses were carried out using SAS statistical software (SAS 94.4.4 2017) with Proc GLM (SAS Institute). All data were checked with Levene's test for treatments and experiments as fixed effects. When no significant difference was observed between treatment and replications and their interaction the two experiments were combined for analysis. Analysis of variance (ANOVA) was used to compare a variety of responses (rootstocks and scion) and isolates used for evaluation using F and t-tests where applicable. Means were separated using the Fisher's Tukey's Least Significant Difference (LSD) test in SAS using Proc GLM.

Table 1

Tomato and eggplant lines/varieties screened for resistance to Ralstonia pseudosolanacearum in these studies

Line/Variety	**Source**	**Experiment**
SGH06 220(WG12-128A)	D. Francis, OSU	Preliminary
WG2 121	D. Francis, OSU	Preliminary
SGH06 215(WG12-108A)	D. Francis, OSU	Preliminary
SGH06-211(WG12-139A130)	D. Francis, OSU	Preliminary
SGH06-216(FG12-608A)	D. Francis, OSU	Preliminary
WG12-110	D. Francis, OSU	Preliminary
WG12-130	D. Francis, OSU	Preliminary
WG12-120	D. Francis, OSU	Preliminary and Tanzania rootstock evaluation
FGH06- 304	D. Francis, OSU	Preliminary
FGH06- 301	D. Francis, OSU	Preliminary
FGH06-302	D. Francis, OSU	Preliminary
MT56	Makerere University, Uganda	Preliminary and Tanzania rootstock evaluation
EG190	AVRDC, Taiwan	Preliminary and Tanzania

		rootstock evaluation
Moneymaker	Growseed, Bristol, UK	Preliminary
Tanya	Seminis, Holland	Tanzania rootstock evaluation

Table 2

Ralstonia pseudosolanacearum strains used in these studies

Strain	**Origin**	**Host**	**Phylotype**	**Year of collection**	**Experiment**	**Reference**
TZ 9	Rungwe, Mbeya	Tomato	III	2017	Preliminary	This study
TZ 130	Rungwe, Mbeya	Tomato	III	2017	Preliminary	This study
TZ 48	Mbeya rural, Mbeya	Eggplant	III	2017	Preliminary	This study
SM 716	Comilla, Bangladesh	Pepper	I	2012	Preliminary	Subedi, 2015
SM 747	Chitwan, Nepal	Eggplant	I	2012	Preliminary	Subedi, 2015
TZ 55	Kilolo, Iringa	Tomato	III	2017	Preliminary	This study
TZ 57	Iringa rural, Iringa	Tomato	III	2017	Preliminary	This study
TZ 58	Mvomero, Morogoro	Soil	III	2017	Preliminary	This study
TZ 70	Arumeru, Arusha	Potato	III	2017	Preliminary	This study

TZ 71	Arumeru, Arusha	Tomato	III	2017	Preliminary	This study
TZ 80	Lushoto, Tanga	Potato	III	2017	Preliminary	This study
SM 727	Tangail, Bangladesh	Eggplant grafted on *S. sisymbriifolium*	I	2012	Preliminary	Subedi, 2015
SM 732	Tangail, Bangladesh	Eggplant grafted on *S. sisymbriifolium*	I	2012	Preliminary	Subedi, 2015
SM 738	Bogra, Bangladesh	Eggplant	I	2012	Preliminary	Subedi, 2015
TZ 22	Mvomero, Morogoro	Tomato	I	2017	Preliminary	This study
TZ 71	Misufini, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 72	Misufini, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 73	Misufini, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study
TZ 25	Mlali, Morogoro	Tomato	I	2019	TZ rootstock; grafted plants	This study

TZ 24	Mlali, Morogoro	Sweet pepper	I	2019	TZ rootstock; grafted plants	This study
TZ 95	Mlali, Morogoro	Sweet pepper	I	2019	TZ rootstock; grafted plants	This study

Results

Preliminary rootstock screening

There were significant differences between tomato lines in bacterial wilt incidence, severity and latent infection (Table 3). In this experiment, none of the eleven rootstocks showed complete resistance to the five Tanzanian and Asian strains tested. Bacterial wilt incidence was significantly ($P<0.0001$) lower in nine of the rootstocks evaluated l, higher in one line (80%) and not different in two lines (78% and 64%) than in the bacterial wilt-susceptible variety Moneymaker (66%). Disease severity was significantly ($P<0.0001$) lower in eight rootstock lines than in Moneymaker. Latent infection ranged from 2.5% to 90% of surviving plants at the end of the experiment. Of the eleven lines screened, WG12-120 exhibited amongst the lowest wilt incidence (36%) and latent infection (2.5%), and the lowest disease severity (31%).

Table 3

Bacterial wilt incidence, severity, and latent infection in Ohio tomato rootstock breeding lines and scion variety 'Moneymaker' during preliminary screening with five selected Tanzanian and South Asian Ralstonia pseudosolanacearum strains

Variety / line	Bacterial wilt ^{x,y}		
	Incidence (%)	**Severity (%)**	**Latent infection (%)**
Moneymaker	66 bc	66.8 a	25.0 c
SGH06-211	40 ed	44.4 g	17.5 dc
SGH06-215	80 a	50.4 a	20.1 dc
SGH06-216	40 ed	58.4 bc	7.5 dc
SGH06-220	78 ab	64.4 a	17.5 dcd
WG12- 110	40 ed	62.4 ab	46.0 b
WG12- 120	36 e	31.0 h	2.5 d
WG12- 130	50 d	52.4 dfe	55.8 b
WG12- 121	64 c	57.1 dc	5.8 b
SGH06-301	40 ed	56.0 dce	90.0 a
SGH06-302	36 e	42.0 g	60.0 b
SGH06-304	48 ed	52.0 fe	5.0 d

P value	<0.0001	<0.0001	<0.0001
LSD	13.4	5.1	18.8

[x] Values are means across individual inoculations of three Tanzanian and two South Asian strains of *R. pseudosolanacearum*.
[y] Means of two combined experiments conducted under similar greenhouse conditions; means with the same letters in a column are not significantly different at 5% alpha value

In the second experiment, there were significant differences between tomato/eggplant lines in bacterial wilt incidence, severity, and latent infection (Table 4) and a significant line*strain interaction for all three variables ($P<0.0001$). Neither eggplant EG190 nor tomato MT56 was completely resistant to all 15 *R. pseudosolanacearum* strains used to screen them (Table 4). Averaged across all 15 strains, disease incidence, severity and latent infection were significantly ($P<0.0001$) lower in EG190 and MT56 than in the susceptible variety Moneymaker. Disease incidence was lowest in EG190 (5.9%) followed by MT56 (22.9%) and high in Moneymaker (56.4%). Bacterial wilt disease severity and latent infection were statistically similar in EG190 and MT56, but low in Moneymaker.

Table 4

Bacterial wilt incidence, severity, and latent infection in tomato MT56 and eggplant EG190 rootstock lines and the susceptible variety 'Moneymaker' inoculated individually with 15 selected Tanzanian and South Asian Ralstonia pseudosolanacearum strains

Variety / line	Incidence (%)	Bacterial wilt [x, y] Severity (%)	Latent infection (%)
Moneymaker	56.4 a	40.1 a	51.3 a
MT56	22.9 b	16.2 b	23.3 b
EG190	5.9 c	11.1 b	16.6 b
P value	<0.0001	<0.0001	<0.0001

[x] Averaged across all ten Tanzanian and five South Asian *R. pseudosolanacearum* strains used as inoculum.
[y] Means of two combined experiments; means with same letters in a column are not significantly different at 5% alpha value.

Selected rootstock evaluation in Tanzania

None of the rootstocks were completely resistant to the six *R. pseudosolanacearum* strains tested (Table 3). There were significant ($P<0.0001$) differences between rootstocks and between strains in bacterial wilt incidence, but no significant line*strain interaction ($P=0.107$) (Table 5). There was, however, a significant ($P< 0.0001$) rep*line interaction. Bacterial wilt incidence eight weeks after inoculation was significantly lower in all three rootstock lines averaged across *R. pseudosolanacearum* strains than in the wilt-susceptible variety Tanya (64.4%). Disease incidence was lowest (8.9%) in EG190, followed by WG120 (28.9%), and MT56 (43.9%). Similar results were observed for disease progress ($P<0.0001$), except that AUDPC values for wilt incidence over eight weeks post-inoculation did not differ significantly for tomato lines MT56 and WG120. Bacterial wilt AUDPC was lowest in line EG190 (44.1) followed by WG120 (150.7) and MT56 (166.93) and highest in the susceptible control Tanya (368.3). The majority of surviving plants exhibited latent infection, ranging from 62.7% to 80.3% of the plants tested positive for

Ralstonia ELISA. There were no significant differences in latent infection between rootstocks and the scion variety Tanya (*P*=0.1161).

Table 5

Bacterial wilt incidence, area under the disease progress curve (AUDPC), and latent infection in tomato MT56 and WG120 and eggplant EG190 rootstock lines and tomato variety 'Tanya' eight weeks post-inoculation with six Ralstonia pseudosolanacearum strains collected from key tomato producing regions of Tanzania

	Bacterial wilt [x, y]		
Variety/line	**Incidence (%)**	**AUPDC**	**Latent infection (%)**
Tanya	64.4 a	368.3 a	73.6
MT56	43.9 b	166.7 b	72.6
WG120	28.9 c	175.9 b	62.7
EG190	8.9 d	41.1 c	80.3
P-value	<0.0001	<0.0001	0.1161
LSD	7.3	72.3	-

[x] Means of two combined experiments across individual inoculations of six Tanzanian strains of *R. pseudosolanacearum*.
[y] Means with the same letter in a column are not significantly different at $P \leq 0.05$.

Grafted plant survival and response to R. pseudosolanacearum inoculation

Survival of grafted plants before inoculation by *R. pseudosolanacearum* strains and subsequent bacterial wilt incidence, AUDPC, and latent infection after inoculation are summarized in Table 6. There were significant differences between seedlings of Moneymaker scion grafted onto either rootstock and self-grafted Moneymaker seedlings in bacterial wilt incidence (*P*= 0.0024) and AUDPC (*P*=0.019) after challenge with six combined Tanzanian *R. pseodosolanacearum* strains. Disease incidence percentage eight weeks post-inoculation did not differ significantly for plants that were grafted onto tomato MT56 and eggplant EG190 rootstocks and were significantly higher in self-grafted Moneymaker (70%). Similar results were observed for disease progress where AUDPC values over eight weeks post-inoculation did not differ significantly for tomato lines seedlings grafted to MT56 and EG190 and were significantly higher in self-grafted Moneymaker. Latent infection by *R. pseudosolanacearum* was detected using ELISA at the termination of the experiment eight weeks after inoculation and no significant differences (*P*=0.4533) were observed between graft types.

Table 6

Grafted tomato seedling survival and bacterial wilt incidence, area under the disease progress curve (AUDPC), and latent infection in response to challenge with a combination of six Tanzanian Ralstonia pseudosolanacearum strains. The seedlings were self-grafted or grafted using tomato MT56 and eggplant EG190 rootstocks and Moneymaker (MM) scion and tested under screenhouse conditions

	Bacterial wilt [x, y]			
Graft type (rootstock/scion)	**Survival (%) [z]**	**Incidence (%)**	**AUDPC**	**Latent infection (%)**
Moneymaker/Moneymaker	75	70 a	446.9 a	30
MT56/MT56	60	16 b	121.9 b	83.5
EG190/EG190	65	0 b	0.0 b	37.5
MT56/Moneymaker	65	10 b	81.3 b	66.5
EG190/Moneymaker	70	0 b	0.0 b	44
P value	0.5535	0.0024	0.019	0.4533
LSD	-	19.7	216.6	-

[x] Means across grafts from EG190, MT56 rootstocks and Moneymaker scion inoculated with combined six Tanzanian strains of *R. pseudosolanacearum*.
[y] Means of two combined experiments conducted under similar greenhouse conditions; means in a column with same letters are not significantly different at 5% alpha value.
[z] Percentage grafted seedlings that survived healing chamber and weaning process for four weeks post grafting.

Discussion

Selected tomato and eggplant lines were evaluated for resistance to *R. pseudosolanacearum* strains collected from Tanzania's main tomato producing regions for use as rootstocks for grafting farmer-preferred susceptible tomato varieties. From the preliminary screening of one eggplant and twelve tomato lines with Tanzanian and South Asian *R. pseudosolanacearum* strains, we selected three rootstocks for further evaluation. The three rootstocks exhibited a moderate to high degree of resistance to the selected strains, of which 93% were phylotype I and 7% were phylotype III. Eggplant line EG190 was highly resistant while tomato lines MT56 and WG 120 were moderately resistant to the *R. pseudosolanacearum* strains tested. The pedigree of line WG120 includes Hawaii 7997 (Dr. D. Francis personal communication), a tomato breeding line resistant to bacterial wilt in multiple locations with resistance presented by a single dominant gene (Grimault and Prior, 1995; Wang *et al.*, 1998). Line MT56 exhibited moderate to high resistance to bacterial wilt when tested in various agroecological zones of Uganda (Asiimwe *et al.*, 2013). The response of rootstocks to bacterial wilt can be variable depending on the source of resistance genes and edaphic soil and environmental factors (Scott *et al.*, 2005, Lebeau *et al.*, 2011). The complexity and variability of *R. pseudosolanacearum* may lead to non-durable resistance and limitation of global use of identified resistant lines or varieties (Wang *et al.*, 1998). Therefore, screening with local populations of *R. pseudosolanacearum* is important to identify reliable rootstocks for use in Tanzania or areas with similar soil and *Ralstonia* population characteristics. In this study, we also observed variability in the aggressiveness of *R. pseudosolanacearum* strains against the lines tested. This either describes the reaction of resistance genes against the strains or the virulence of the strains. Rootstocks such as Hawaii 7996 were observed to restrict the movement of the pathogen beyond the lower stem (Grimault and Prior, 1995). Variability in resistance of rootstocks was also reported by MacAvoy *et al*. (2012) and Scott *et al*. (2005, 2009). Strain virulence is highly influenced by population density as well as the expression of virulence genes that directly determine the aggressiveness of the pathogen (Grimault and Prior, 1995; Shutt *et al.,* 2018; Manickam *et al.,* 2021). Variability in pathogen virulence was also reported by Shutt *et al.,* 2018. Thus, Lebeau *et al.,* (2011) described resistance based on surviving plants because it is almost impossible to achieve 100% survival when screening for bacterial wilt resistance, especially in disease hotspots.

Tomato plants grafted onto resistant tomato and eggplant rootstocks have shown high potential to resist bacterial wilt (Rivard and Louws, 2008; Waiganjo *et al.,* 2011; Kanyua, 2018; Ivey *et al.,* 2020). Our findings indicated 100% survival of seedlings grafted with resistant rootstock EG190 after challenge by strains of *R. pseudosolanacearum* collected from the major tomato growing regions of Tanzania. Rivard and Louws (2008) had similar observations with CR66 and Hawaii 7996 grafted to heirloom tomatoes. Rivard *et al*. (2012) also demonstrated the potential of Dai Honmei rootstock to resist bacterial wilt disease. However, they also reported that grafted plant survival was inconsistent across different locations. We also observed differences in aggressiveness of Tanzanian strains to the rootstocks EG190, MT56 and WG120 but in our study strain*line interactions were lacking among the rootstocks. Strain virulence is highly influenced by population density as well as the expression of virulence genes that directly determine the aggressiveness of the pathogen (Grimault and Prior, 1995; Shutt *et al.,* 2018; Manickam *et al.,* 2021). We observed differences in bacterial wilt incidence with a significant strain*line interaction in the prescreening experiments, where South Asian strains were more virulent than Tanzanian strains. Although the Tanzanian strains were also phylotype I little is known about their ability to attack multiple solanaceous crops as our results indicated they were virulent on tomato but not eggplant. Asian phylotype I members are known for their high degree of virulence and ability to attack several solanaceous hosts, including eggplant, sweet pepper, tomato, hot pepper, and black nightshade (Chesneau *et al.,* 2018). All South Asian strains tested in this study were virulent to eggplant and tomato lines with variation in the percentage of surviving plants (incidence) but no variation in latent infection or disease severity. Significant interactions are not only subject to change with pathogen variability but also the environmental conditions of the test (Lebeau *et al.,* 2011). Controlled environmental conditions may have contributed to the behavior of the strains. Wang *et al. (*2013) explained the effect of environmental conditions on the strains and accessions during their experiments on tomatoes. However, more experiments are needed to establish how environmental conditions contribute to the interaction observed during our study.

Similar to other reports (Grimault and Prior, 1995; Lebeau *et al.,* 2011), in this study bacterial wilt-resistant rootstocks and susceptible scions grafted onto these rootstocks supported latent infection by *R. pseudosolanacearum*. The rootstocks EG190, MT56, and WG120 can be regarded as potential candidates for grafting for bacterial wilt resistance in Tanzania. Lebeau *et al*. (2011) described good rootstocks as ones that can adapt to the pathogen (tolerance) or restrict the movement of *R. pseudosolanacearum* to the lower part of the seedlings. Resistant rootstocks enhance plant survival in pathogen-infested soils by inhibiting plant colonization or restricting pathogen movement in the xylem (Lebeau *et al.,* 2011). Research with bacterial wilt-resistant eggplant and/or tomato rootstocks demonstrated improved plant vigor, increased fruit yield, size, and weight of the grafted plants (McAvoy *et al.,* 2012; Rivard and Louws, 2008; Manickam *et al.,* 2021) through robust root systems as well as their strong disease resistance (Salgon *et al.,* 2017; Manickam *et al.,* 2021). The robust root system improves nutrient and water uptake and enhances the signalling and translocation of defense hormones and proteins (Kumar *et al.,* 2017, Manickam *et al.,* 2021). With these findings, the main challenge remains the availability of seeds from the three candidates identified in this study. Seeds for these experiments were obtained directly from breeders via special requests. Thus, breeding programs should focus on increasing the number of seeds for community use because currently, only breeders volunteer to provide seeds for research purposes. In addition to seed availability, this work has been done in a controlled greenhouse environment and large-scale and field testing is required. Future research should concentrate on field experiments to assess the performance of grafted plants with a broad representation of strains and natural environmental factors for better rootstock recommendations based on ecological characteristics or individual regions.

Conclusion and recommendations

The study findings give the potential of using tested rootstocks for grafting bacterial wilt susceptible agronomic valued tomato varieties. Resistance rootstocks can efficiently restrict bacterial wilt pathogens from causing wilts and reduce their devastating effect. Regarding their highest potential, we recommend the use of EG 190 and MT56 as a tool in integrated pest management packages that are aimed at managing bacterial wilt disease in Tanzania. The lines should be used as rootstocks in grafting agronomically preferred tomato varieties that are highly susceptible to bacterial wilt to reduce the devastating disease effect and yield losses. It is recommended that responsible institutes and the Ministry of Agriculture consider arranging and initiating special programs on seed production and increase for these lines as well as training farmers on tomato grafting.

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