Effects of Two Rootstocks on Bacterial Wilt Resistance and Rhizosphere Microbial Quantity in Cherry Tomato (Postprint)

Authors: Zhao Wenzong, Zheng Xuyang, Zhang Yingqing, Zhong Chuan, Yang Yanjuan, Wenjin Yu

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Abstract

Bacterial wilt is one of the most serious soil-borne diseases affecting tomato production in South China. To investigate the effects of grafting with two different rootstocks on resistance to tomato bacterial wilt and rhizosphere microbial populations under artificial inoculation with Ralstonia solanacearum. Grafting was performed using rootstocks ‘Fanzhen No. 1’ and ‘Qiezhen No. 21’ with cherry tomato ‘Fenbeibei’ , and the dilution plate method was employed to isolate rhizosphere microorganisms from each grafting combination. The results demonstrated that grafting with disease-resistant rootstocks significantly enhanced tomato disease resistance, reduced disease incidence and disease index, and delayed disease onset. Ralstonia solanacearum exhibited dynamic changes during plant infection, with its population gradually decreasing from the root system to the above-ground stems. The population of R. solanacearum in the rhizosphere substrate and root system of scion-rootstock grafted plants and rootstock self-grafted plants was reduced, and the pathogen population in above-ground stems was significantly lower than that in scion self-grafted plants. Following inoculation with R. solanacearum, the populations of bacteria, fungi, and actinomycetes in the rhizosphere substrate of each grafting combination exhibited a trend of initial increase followed by decrease with disease progression. Grafting with disease-resistant rootstocks generally increased total rhizosphere microbial populations, as well as bacterial and actinomycete numbers, decreased fungal populations, improved the rhizosphere microenvironment of grafted plants, and played an important role in reducing the incidence of tomato bacterial wilt.

Effects of Two Rootstocks on Bacterial Wilt Resistance and Rhizospheric Microorganisms in Cherry Tomato

ZHAO Wenzong, ZHENG Xuyang, ZHANG Yingqing, ZHONG Chuan, YANG Yanjuan, YU Wenjin*
College of Agriculture, Guangxi University, Nanning 530004, China

Abstract

Bacterial wilt is one of the most devastating soil-borne diseases threatening tomato production in South China. This study investigated the effects of grafting with two different rootstocks on tomato bacterial wilt resistance and rhizospheric microbial populations under artificial inoculation conditions. Cherry tomato "Fenbeibei" was grafted onto rootstocks "Fanzhen No.1" and "Qiezhen No.21," and rhizospheric microorganisms were isolated using the dilution plate method. The results demonstrated that grafting with disease-resistant rootstocks significantly enhanced tomato resistance, reduced disease incidence and disease index, and delayed disease onset. *Ralstonia solanacearum* exhibited dynamic changes during plant infection, with pathogen populations gradually decreasing from roots to aboveground stems. Grafted plants (both scion/rootstock combinations and rootstock self-grafted plants) showed reduced *R. solanacearum* populations in rhizospheric substrate and roots, with pathogen levels in aboveground stems significantly lower than those in scion self-grafted control plants. Following inoculation, bacterial, fungal, and actinomycete populations in the rhizospheric substrate of all grafting combinations initially increased then decreased as disease progressed. Overall, grafting with disease-resistant rootstocks increased total rhizospheric microbial populations, enhanced bacterial and actinomycete abundance, reduced fungal populations, and improved the rhizospheric microenvironment, playing a crucial role in reducing tomato bacterial wilt incidence.

Keywords: tomato, grafting, bacterial wilt, disease resistance, rhizospheric microorganisms

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Introduction

Bacterial wilt, caused by *Ralstonia solanacearum* (hereinafter referred to as the pathogen), is a soil-borne vascular disease prevalent in tropical and subtropical regions, causing particularly severe damage to Solanaceae crops. The pathogen can spread through soil and water, survive long-term in soil without host plants, and is difficult to eradicate, often resulting in significant agricultural economic losses (Guo et al., 2004; Liu et al., 2005; Wicker et al., 2007). Research on tomato bacterial wilt control has explored chemical pesticides, antagonistic bacteria, and antibiotics (Yan et al., 2004; Li et al., 2006). However, chemical control is costly and its efficacy is affected by soil and climatic conditions, with no long-term effective chemical measures available. Biological control shows some potential but suffers from poor stability and unsatisfactory field application results (Lin et al., 2005; Xiao et al., 2007). Breeding resistant tomato varieties represents another effective approach, but few commercial varieties combine good market traits with disease resistance (Wang et al., 2004). Currently, using disease-resistant rootstocks for grafting is the most effective control measure, significantly improving tomato resistance and reducing field incidence (Bie, 2012; Huang et al., 2013; Wang et al., 2018). Commercial rootstocks resistant to bacterial wilt generally fall into two categories: *Solanum lycopersicum* (tomato) and *Solanum melongena* (eggplant) rootstocks (Zhang et al., 2010; Mo et al., 2013).

While numerous studies have reported improved bacterial wilt resistance through grafting, most have focused on disease resistance evaluation, rootstock germplasm screening, and cultivation effect comparisons, with limited research on the underlying mechanisms. Studies indicate that unique sieve plate structures in rootstock roots can inhibit pathogen growth and proliferation, while grafting induces isozyme restructuring and functional reorganization in plants, enabling expression of resistance genes (Zhou et al., 1998; Wang, 2017). Plant resistance to soil-borne diseases is closely related to rhizospheric microbial diversity and community structure (Feng et al., 2010; Eisenhauer et al., 2012). Resistant tomato varieties have been reported to maintain significantly higher total rhizospheric microbial populations than susceptible varieties after pathogen inoculation (Liu et al., 2006). Yin et al. (2008) found that grafting increased eggplant rhizospheric bacterial and actinomycete populations while decreasing fungal populations, significantly reducing verticillium wilt incidence and disease index. Yang et al. (2013) observed that tomato plants infected with bacterial wilt showed dramatically reduced rhizospheric bacterial and actinomycete populations but significantly increased fungal populations. However, the relationship between grafted tomato resistance to bacterial wilt and rhizospheric microbial population changes remains poorly documented. This study compared the distribution characteristics of the pathogen in grafted tomato plants and rhizospheric substrate, and the dynamic changes in rhizospheric microorganisms at different disease stages under artificial inoculation conditions, aiming to elucidate the relationship between grafting-induced resistance and rhizospheric microbial populations, and further reveal the mechanism of disease resistance from the perspective of the plant rhizospheric microenvironment.

1.1 Experimental Materials

Rootstock and scion varieties: The rootstocks were "Fanzhen No.1" (No.1, hereafter referred to as Fanzhen), a tomato-specific rootstock with high resistance to bacterial wilt developed by the College of Agriculture, Guangxi University, and "Qiezhen No.21" (No.21, hereafter referred to as Qiezhen), a universal rootstock for both tomato and eggplant. The scion was the highly susceptible cherry tomato variety "Fenbeibei" (Fb).

Pathogen: A highly virulent strain isolated, purified, and preserved from infected tomato plants was used for inoculation, confirmed through plate culture and hypersensitivity reaction tests on tobacco leaves.

Rhizospheric microbial culture media: Nutrient agar (NA) medium, Martin's medium, and modified Gause No.1 medium were used for isolating bacteria, fungi, and actinomycetes, respectively. Triphenyltetrazolium chloride (TTC) medium was used as the differential medium for *R. solanacearum*.

1.2.1 Experimental Design

Five grafting combinations were established: scion/rootstock grafts (Fb/No.1, Fb/No.21), rootstock self-grafts (No.1/No.1, No.21/No.21), and scion self-grafted control (Fb/Fb, CK). Rootstocks were grown in 21-cell trays (62 mm × 30 mm), while scions were grown in 72-cell trays using a substrate mixture of peat and coconut fiber (1:1 ratio). Grafting was performed using the C-type sleeve grafting method when rootstocks developed three true leaves.

After graft establishment, some plants were transferred to a phytotron. When scions developed 4–5 true leaves, they were inoculated with *R. solanacearum* using the root-wounding irrigation method at a concentration of 5 × 10 CFU · mL ^{1}. Each grafting combination consisted of 40 plants arranged randomly with three replications. Additional plants were transplanted to a natural bacterial wilt nursery (40 plants per combination, three replications) for field observation and disease statistics.

1.2.2 Disease Investigation

Disease symptoms were investigated every 5 days for 30 days post-inoculation to determine the disease cycle and calculate incidence and disease index. Based on the methods of Lai (1998) and Xie et al. (2010), disease severity was rated on a 0–4 scale: 0 = no symptoms; 1 = one wilted leaf; 2 = 2–3 wilted leaves; 3 = all leaves wilted except the top 2–3; 4 = whole plant wilted and dead.

Disease incidence (%) = (Number of diseased plants / Total inoculated plants) × 100
Disease index = Σ(Disease grade × Number of plants in grade) / (Highest disease grade × Total inoculated plants) × 100

1.2.3 Rhizospheric Substrate Sampling

Under phytotron inoculation conditions, rhizospheric substrate was collected at four stages: pre-inoculation (S1), early disease stage (S2), disease peak (S3), and late disease stage (S4) using the root-shaking method. Ten plants were randomly selected per combination and sampled three times consecutively. After removing the top 1 cm of substrate, entire root systems were excavated and gently shaken to remove excess substrate. Clean brushes were used to collect substrate within 4 mm of the root surface as rhizospheric substrate (Shen et al., 2011). Samples were passed through a 10-mesh sieve, placed in 50 mL sterile centrifuge tubes, and stored at -20°C.

1.2.4 Plant Tissue Sampling

Root systems were washed to remove attached impurities, then rinsed with deionized and sterile water, and dried with sterile gauze. Root tissue was collected, along with 1 cm stem sections above and below the graft union (designated as scion stem and rootstock stem, respectively). Each tissue type (10 g) was sealed and stored at -20°C.

1.2.5 Microbial Isolation and Counting

Rhizospheric substrate suspension preparation: Ten grams of rhizospheric substrate were placed in a flask containing 90 mL sterile water, sealed, and shaken horizontally at 30°C and 200 rpm for 30 minutes to create a 10 ^{1} suspension. Serial dilutions were prepared by transferring 1 mL of suspension to 9 mL sterile water tubes, repeated to achieve 10 ^{2}, 10 ^{3}, etc.

Plant sample dilution preparation: Ten-gram samples were surface-sterilized (70% ethanol for 30 s, 1% NaClO for 15 min, rinsed three times with sterile water), chopped, ground, mixed with 90 mL sterile water, and serially diluted as described above.

The dilution plate method was used to isolate rhizospheric microorganisms and internal *R. solanacearum*. Dilutions of 10 ^{3}, 10 , and 10 were spread-plated (0.1 mL per plate) onto appropriate media, with three plates per combination. Plates were sealed, inverted, and incubated at 28°C for 2 days (bacteria), 3 days (fungi), or 6 days (actinomycetes). Colony counts were recorded and converted to average colony-forming units per gram of substrate. Total microbial population was calculated as the sum of all microbial groups (Lin, 2010; Yao, 2018).

Microbial colonies (CFU · g ^{1}) = (Average colony count × Dilution factor × 20 × Fresh substrate weight) / Dry substrate weight

R. solanacearum colonies (CFU$\cdot$g ^{1}) = (Average colony count × Dilution factor) / [Volume plated × Fresh substrate (or tissue) weight]

1.2.6 Data Processing

Data were processed using Microsoft Excel 2010, and statistical analysis including ANOVA and multiple comparisons was performed using SPSS 20.0.

2.1 Disease Resistance Performance of Different Grafting Combinations

As shown in , 30 days after phytotron inoculation, both scion/rootstock grafted plants (Fb/No.1, Fb/No.21) and rootstock self-grafted plants (No.1/No.1, No.21/No.21) exhibited significantly lower disease incidence and disease index compared to the scion self-grafted control (Fb/Fb). Both rootstocks significantly improved tomato resistance, with Fanzhen-grafted plants (Fb/No.1) showing high resistance (HR) and Qiezhen-grafted plants (Fb/No.21) showing resistance (R). Under natural disease nursery conditions, Qiezhen-grafted plants showed high resistance (HR), while other combinations maintained consistent resistance levels with phytotron results, though mortality and disease index were lower than in phytotron conditions. Scion/rootstock grafted plants showed slightly higher incidence and disease index than rootstock self-grafted plants, indicating that scion-rootstock interaction influenced disease resistance. Additionally, Fanzhen-grafted plants demonstrated slightly lower incidence, mortality, and disease index than Qiezhen-grafted plants, indicating marginally stronger resistance.

2.2 Dynamic Changes in Disease Development After Inoculation

Under phytotron conditions, disease incidence and index changes are illustrated in [Figure 1: see original paper]. Symptoms began appearing 3–5 days post-inoculation. With increasing days after inoculation, the control (CK) showed significant increases in both incidence and disease index, while other grafting combinations exhibited minimal increases. At day 10, CK incidence reached 38.71%, while scion/rootstock and rootstock self-grafted plants showed 4.84% (Fb/No.1), 3.17% (Fb/No.21), 6.45% (No.1/No.1), and 6.35% (No.21/No.21), respectively. By day 25, CK incidence peaked at 96.77%, while other combinations reached 11.29% (Fb/No.1), 12.70% (Fb/No.21), 9.68% (No.1/No.1), and 9.52% (No.21/No.21), with disease progression slowing and remaining stable through day 30. Based on these results, the post-inoculation disease cycle was defined as: early stage (days 5-10), peak stage (days 10-25), and late stage (after day 25).

2.3 Dynamic Changes in *Ralstonia solanacearum* Populations in Rhizosphere and Plant Tissues

Under phytotron inoculation, *R. solanacearum* populations in rhizospheric substrate of all grafting combinations showed a dynamic pattern of initial increase, subsequent decrease, and final stabilization (FIGURE:2). Pre-inoculation (S1), all tissues contained zero pathogens. During the early disease stage (S2), pathogen populations peaked in rhizospheric substrate, then decreased significantly during the peak disease stage (S3). At S2, CK showed the highest rhizospheric substrate pathogen population at 84.85 × 10 CFU · g^{1}, while scion/rootstock grafted plants (Fb/No.1) showed the lowest at 42.42 × 10 CFU · g^{1}. Analysis of different plant tissues revealed that CK pathogen populations in roots, rootstock stems, and scion stems peaked at S3, while grafted plants peaked at S2. At S2, Qiezhen-grafted plants (No.21/No.21 and Fb/No.21) showed root pathogen populations of 48.48 × 10 CFU · g^{1}, significantly higher than Fanzhen-grafted plants (No.1/No.1: 24.24 × 10 CFU · g^{1}; Fb/No.1: 30.30 × 10 CFU · g^{1}) but not significantly different from CK (54.55 × 10 CFU · g^{1}). At S3, CK root pathogen populations reached 75.76 × 10 CFU · g^{1}, significantly higher than all grafted combinations. From peak to late disease stage (S4), aboveground stem pathogen populations showed no significant differences among grafted combinations except CK, with Fanzhen-grafted plants slightly higher than Qiezhen-grafted plants. Pathogen populations decreased progressively from roots to aboveground stems in all combinations. At S4, scion stems of Fb/No.1 and Fb/No.21 contained 3.03 × 10 CFU · g^{1}, significantly lower than CK (21.21 × 10 CFU · g^{1}), while No.1/No.1 and No.21/No.21 approached zero. These results demonstrate that disease-resistant rootstock grafting effectively prevents pathogen invasion into roots and aboveground stems, inhibits pathogen proliferation, and reduces disease incidence or slows disease progression.

2.4 Total Rhizospheric Microbial Populations of Different Grafting Combinations

As shown in [Figure 3: see original paper]A, total rhizospheric microbial populations increased significantly after inoculation, showing an overall trend of initial increase, decrease, and subsequent increase from S1 to S4. In [Figure 3: see original paper]B, at S2, No.1/No.1 showed the highest total microbial population at 206.68 × 10 CFU · g^{1}, followed by No.21/No.21 at 195.83 × 10 CFU · g^{1}, with scion/rootstock grafted plants at 162.45 × 10 CFU · g^{1} (Fb/No.1) and 186.81 × 10 CFU · g^{1} (Fb/No.21), all significantly higher than CK (134.75 × 10 CFU ·

g ^{1}). At S3, total microbial populations in four rootstock-grafted combinations decreased but remained higher than CK, with no significant difference between Fb/No.1 and Fb/No.21. At S4, total microbial populations increased again in all combinations, with all rootstock-grafted treatments significantly higher than CK except No.21/No.21. These results indicate that disease-resistant rootstock grafting increased total rhizospheric microbial populations under pathogen infection.

2.5 Rhizospheric Bacterial Populations of Different Grafting Combinations

As shown in [Figure 4: see original paper]A, rhizospheric bacterial populations followed similar trends to total microbial populations, peaking at S1 for No.1/No.1 and No.21/No.21, and at S4 for other combinations. Bacterial populations in scion/rootstock and rootstock self-grafted plants remained higher than CK throughout S2–S4, indicating that disease-resistant rootstock grafting promoted bacterial proliferation. In [Figure 4: see original paper]B, at S2, No.1/No.1 showed the highest bacterial population at 155.63 × 10 CFU · g ^{1}, significantly higher than other combinations, while Fb/No.1 showed the lowest at 113.44 × 10 CFU · g ^{1}, not significantly different from CK (104.12 × 10 CFU · g ^{1}). At S3, bacterial populations decreased to varying degrees across all combinations, with No.1/No.1 showing the largest decline but remaining significantly higher than CK, while other combinations showed no significant differences. At S4, bacterial populations increased again, with Fb/No.1 and Fb/No.21 reaching peak values of 149.47 × 10 CFU · g ^{1} and 139.84 × 10 CFU · g ^{1}, respectively, significantly higher than other combinations.

2.6 Rhizospheric Fungal Populations of Different Grafting Combinations

As shown in [Figure 5: see original paper]A, pre-inoculation fungal populations showed no significant differences among combinations. Post-inoculation, fungal populations generally increased then decreased, remaining substantially lower than bacterial populations. In [Figure 5: see original paper]B, at S2, No.1/No.1 fungal populations increased significantly from S1, reaching 2.55 × 10 CFU · g ^{1}, higher than other combinations. At S3, CK fungal populations increased significantly while grafted combinations decreased markedly, with Fb/No.1 and Fb/No.21 dropping to 0.37 × 10 CFU · g ^{1} and 0.21 × 10 CFU · g ^{1}, respectively. At S4, scion/rootstock grafted plants showed extremely significant fungal population reductions, decreasing by 86.77% (Fb/No.1) and 85.53% (Fb/No.21) compared to S1, while rootstock self-grafted plants decreased by 56.47% (No.1/No.1) and 27.17% (No.21/No.21), and CK increased by 21.70%. These results demonstrate that disease-resistant rootstock grafting inhibited fungal proliferation and reduced fungal populations under pathogen infection.

2.7 Rhizospheric Actinomycete Populations of Different Grafting Combinations

Post-inoculation actinomycete populations showed different trends from bacteria and fungi, increasing significantly at S2, then decreasing and stabilizing. Fb/No.1 and Fb/No.21 showed significant increases at S4 compared to S1 ([Figure 6: see original paper]A). As shown in [Figure 6: see original paper]B, scion/rootstock and rootstock self-grafted plants showed extremely significant actinomycete population increases at S2 compared to CK, reaching 47.13 × 10 CFU · g^{1} (Fb/No.1), 55.90 × 10 CFU · g^{1} (Fb/No.21), 48.50 × 10 CFU · g^{1} (No.1/No.1), and 62.47 × 10 CFU · g^{1} (No.21/No.21), with Qiezhen-grafted plants generally showing higher relative increases than Fanzhen-grafted plants. At S3, actinomycete populations decreased substantially across all combinations, with Qiezhen-grafted plants showing the most pronounced declines of 59.55% (No.21/No.21) and 56.23% (Fb/No.21). Except for Fb/No.1, which was slightly higher than CK, all grafted combinations remained significantly higher than CK. At S4, changes were minimal, with Fb/No.1 actinomycete populations significantly higher than Fb/No.21, while other combinations showed no significant differences.

2.8 Correlations Between Disease Resistance and Rhizospheric Microbial Populations

As shown in , correlations existed between disease index, incidence, and rhizospheric microbial group populations across grafting combinations. Disease index and incidence showed positive correlations with rhizospheric *R. solanacearum*, bacterial, and actinomycete populations, but extremely significant negative correlations with fungal populations. *R. solanacearum*, bacteria, and actinomycetes showed extremely significant positive correlations with total microbial populations. *R. solanacearum* showed extremely significant positive correlations with both bacteria and actinomycetes, indicating that pathogen stress promoted proliferation of rhizospheric bacteria and actinomycetes, thereby increasing total microbial populations.

Discussion and Conclusion

Grafting technology for controlling tomato bacterial wilt has been widely reported and applied. Disease-resistant rootstock grafting effectively reduces field incidence and disease index, enhances disease resistance, and improves tomato quality and yield. In this study, the susceptible tomato variety self-grafted control (CK) showed bacterial wilt incidence exceeding 93%, while disease-resistant rootstock grafting dramatically reduced incidence and disease index to high resistance (HR) or resistance (R) levels.

Grafting can alter rhizospheric microbial community diversity and inhibit pathogen growth and reproduction. Our results demonstrate that under artificial inoculation, at the late disease stage (S4), pathogen populations in rootstock stems and scion stems of both scion/rootstock and rootstock self-grafted plants were significantly lower than in CK, indicating that disease-resistant rootstock grafting inhibits pathogen proliferation and effectively prevents invasion into root and aboveground stem tissues. Notably, pathogen populations in grafted plants peaked at the early disease stage (S2), whereas CK peaked later at the disease peak stage (S3). At the early stage, Qiezhen-grafted plants (No.21/No.21, Fb/No.21) showed significantly higher pathogen populations in rhizospheric substrate and roots than Fanzhen-grafted plants (No.1/No.1, Fb/No.1), suggesting weaker initial suppression of pathogen proliferation and root invasion in Qiezhen-grafted plants. However, during the disease peak stage, Qiezhen-grafted plants showed significantly reduced pathogen populations in rhizospheric substrate and roots, with no significant differences from Fanzhen-grafted plants. These findings indicate that pathogen infection and proliferation rates differ among grafting combinations, possibly related to rootstock root tissue structure or antimicrobial substances secreted by grafted plants. Additionally, pathogen populations decreased progressively from roots to aboveground stems, showing dynamic changes.

Liu et al. (2012) reported that grafting increased actinomycete and bacterial populations in pepper rhizospheric soil. Liu et al. (2014) found that disease-resistant rootstock grafting increased beneficial microorganisms such as ammonifying bacteria and free-living nitrogen-fixing bacteria while decreasing fungal populations in tomato rhizospheric soil. The shift from bacterial-dominated to fungal-dominated soil often indicates deteriorating microecological conditions, and increased rhizospheric fungal populations may be an important factor contributing to higher soil-borne disease incidence (Yin et al., 2008). In this study, pre-inoculation (S1) rhizospheric substrate conditions were consistent across combinations with no significant differences in total microbial populations. Post-inoculation, rootstock-grafted plants showed significantly increased total microbial populations that remained high throughout disease progression. During the early disease stage, dramatic changes occurred in bacterial, fungal, and actinomycete populations, with rootstock-grafted plants showing significantly increased bacterial and actinomycete populations compared to CK. From disease peak to late stage, scion/rootstock grafted plants showed significantly reduced fungal populations compared to other combinations, likely due to scion-rootstock interactions. In contrast, CK fungal populations increased by 49.06% and 21.70% during this period, while disease index increased by 44.36% and 59.68%. Correlation analysis revealed significant positive correlations between disease index/incidence and bacterial/actinomycete populations, but extremely significant negative correlations with fungal populations. These results demonstrate that disease-resistant rootstock grafting significantly increased bacterial and actinomycete populations while reducing fungal populations, improving rhizospheric soil conditions.

Numerous studies have shown that plants can create and maintain a unique rhizospheric microenvironment through root exudates, which interact synergistically with rhizospheric microorganisms to resist biotic and abiotic stresses, closely relating to plant disease resistance (Baetz & Martinoia, 2014; Haichar et al., 2014). Whether the disease-resistant rootstocks and grafted tomatoes in this study inhibit pathogen growth directly or indirectly through root exudates, and what specific active substances are involved, requires further investigation.

In conclusion, disease-resistant rootstock grafting significantly improved tomato resistance to bacterial wilt, effectively inhibited rhizospheric pathogen proliferation and plant invasion, thereby reducing disease incidence or slowing disease progression. Under artificial inoculation, rootstock-grafted plants showed dynamic changes in rhizospheric bacterial, fungal, and actinomycete populations, with bacterial and actinomycete populations increasing significantly while fungal populations decreased markedly. Disease-resistant rootstock grafting plays an important role in improving the rhizospheric microenvironment and reducing field bacterial wilt incidence in tomato.

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