Selection of Eggplant Cultivars and Combination with Graft Cultivation for Effective Biological Control of Vascular Wilt Diseases Using a Phenotypic Conversion Mutant of *Ralstonia solanacearum*

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**Introduction**

Eggplant (*Solanum melongena* L.) is a major global vegetable crop worldwide, with a production of approximately 55.2 million tons (1,847,787 ha) and is an economically important vegetable crop in tropical and subtropical countries (FAOSTAT, 2019). Eggplants are generally cultivated in open fields under hot and humid conditions and are susceptible to soil-borne vascular wilt diseases such as bacterial wilt, *Verticillium* wilt, and *Fusarium* wilt (Daunay, 2008).

*Verticillium* wilt, caused by the soil-borne fungal pathogen *Verticillium dahliae*, occurs in a wide range of hosts, including Solanaceae, Brassicaceae, and Cucurbitaceae crops (Pegg and Brady, 2002). This fungal pathogen infects the roots and invades the vascular tissues. The fungus forms conidia that are carried by the sap stream and migrate to the upper tissues of plants.
After sporulation, the fungus enters the saprophytic stage during tissue necrosis or plant senescence (Frardin and Thomma, 2006). In eggplants with *Verticillium* wilt disease, the vascular tissue in the stem turns brown, and the symptoms of yellowing, wilting, and defoliation progress from the lower to the upper leaves (Hashimoto, 1989).

Bacterial wilt is caused by the soil-borne bacterium *Ralstonia solanacearum*. It occurs in tomato, eggplant, potato, and other crops and is widely distributed in tropical, subtropical, and warm temperate regions (Hayward, 1991). This bacterial pathogen infects the root and invades the vascular system, where it rapidly multiplies and produces extracellular polysaccharides (EPS) that lead to clogged vessels, wilting symptoms, and plant death (Kao et al., 1992; Saile et al., 1997).

To control vascular wilt diseases in eggplants, plants have been cultivated using soil fumigants and grafted onto resistant rootstocks (McKee and Mountain, 1967; Yamakawa, 1982; Duniew, 2002; Bletlos et al., 2003). However, completely controlling these diseases is difficult, because *R. solanacearum* can survive in moist soil, water microcosms, weeds, and plant residues for many years (Hayward, 1991); additionally, *V. dahiae* produces microsclerotia that can survive in soil and plant residues for several years (Pegg and Brady, 2002; Frardin and Thomma, 2006). Further, soil fertilizers harm human health and the environment (Duniew, 2002). In the cultivation of eggplants by grafting onto resistant rootstocks, some wild species of *Solanum* plants such as *S. integrifolium* and *S. torvum* have been used as rootstock cultivars in Japan. However, no cultivars are completely resistant to *Verticillium* wilt and bacterial wilt (Yamakawa, 1982; Monma et al., 1997; Yoshida, 2007; Miki et al., 2011). A breakdown of resistance to bacterial wilt in rootstock cultivars has occurred because of new virulent strains of *R. solanacearum* and temperature increases in the culture conditions (Krausz and Thurston, 1975; Date et al., 1994). Additionally, the virulent strain of *R. solanacearum* has infected rootstocks and has been transferred to the scion, causing wilt symptoms in grafted seedlings (Nakaho et al., 1996). Therefore, new, environmentally friendly controls for vascular wilt diseases must be developed.

Biological control using beneficial microorganisms has attracted attention as an eco-friendly method for control, and endophytic bacterial genera (e.g., *Pseudomonas*, *Bacillus*, and *Paenibacillus*) are known to be effective biological control agents (BCAs) against vascular wilt diseases (Eljounaidi et al., 2016; O’Brien, 2017). In previous studies, we developed a method for the biological control of bacterial wilt in eggplants, tomatoes, and potatoes (Ogawa et al., 2011; Kuroki et al., 2016; Nakahara et al., 2016a, b) and *Verticillium* wilt in eggplants (Maehara et al., 2017; Nakahara et al., 2021b) using phenotypic conversion (PC) mutants of *R. solanacearum*. The wild-type strain of *R. solanacearum* spontaneously undergoes PC from fluidal colonies to non-fluidal colony morphology in broth culture, soil, plants, and water extracts of plants (Kelman and Hruschka, 1973; Mori et al., 2011, 2012; Nakahara et al., 2021a). PC mutants have several characteristics related to virulence factors, such as reduced EPS production, reduced endoglucanase and pectin methyl-esterase activities, and enhanced polygalacturonase and siderophore activities and motility; PC mutants can colonize the host tissue, but are either weakly virulent or avirulent (Kelmen and Hruschka, 1973; Brumley and Denny, 1990; Denny and Baek, 1991; Genin and Denny, 2012; Nakahara et al., 2021a). Previous studies showed that biological controls of bacterial wilt and *Verticillium* wilt differ depending on the selection of PC mutants (Nakahara et al., 2016a, b, 2021b). Moreover, varietal differences in the biological control of bacterial wilt are induced by PC mutants in eggplants, and the selection of eggplant cultivars is considered important for controlling bacterial wilt by PC mutants (Ogawa et al., 2011; Nakahara et al., 2016b). However, the existence of varietal differences in the biological control of *Verticillium* wilt in eggplant remains unclear; therefore, clarifying cultivars that demonstrate good biological control by PC mutants is necessary. In plants infected with the pathogen, defense-related enzymes are induced and are higher in cultivars with higher disease resistance (Vanitha and Umesh, 2008; Vanitha et al., 2009). Therefore, good control against the effects of bacterial wilt and *Verticillium* wilt diseases may be achieved by combining grafting cultivation and BCA inoculation, such as PC mutants; however, the control effects have not been verified.

In this study, we investigated varietal differences in the biological control of *Verticillium* wilt among 13 commercial cultivars and six rootstock cultivars and clarified the eggplant cultivars with good biological control by PC mutants to select those that stably prevent *Verticillium* wilt using PC mutants. Furthermore, to promote the use of grafting and BCA inoculation as a new disease control technique, we investigated the biological control of grafted seedlings inoculated with PC mutants against bacterial wilt and *Verticillium* wilt and determined the effectiveness of controlling vascular wilt diseases with the combined use of graft cultivation and PC mutant inoculation.

**Materials and Methods**

**Fungal strain**

The fungal pathogen *Verticillium dahliae* strain No. 5, which was isolated from eggplants, was provided by the Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization (NARO), Japan. The fungal suspension was prepared at a concentration of 10^6 conidia·mL^{-1} according to the method described by Nakahara et al. (2021b).
Bacterial strains

*Ralstonia solanacearum* strains 8103 (MAFF 730126) and 8107 (MAFF 107632), which are classified as race 1, biovar 4, phylotype I, and sequevar 15, were used in this study. The wild-type strain 8103 was used as a virulent strain of bacterial wilt. 8103PC was previously selected as an effective PC mutant for biological control of bacterial wilt in eggplants and tomatoes (Nakahara et al., 2016a, b) and was used as an effective PC mutant to control bacterial wilt in this study. 8107PC was previously selected from 27 PC mutants as an effective PC mutant for biological control of *Verticillium* wilt in eggplants (Nakahara et al., 2021b) and was used as a control for *Verticillium* wilt in this study. PC mutants were selected based on the shift from fluidal white to non-fluidal red colony morphology in a BG broth (BG medium without 1.5% agar and 50 mg·L\(^{-1}\) tetrazolium chloride [TZC]) after 14 days in a BG broth (BG medium without 1.5% agar and 50 mg·L\(^{-1}\) TZC) static culture. Bacterial suspensions were prepared according to the method described by Nakahara et al. (2016b). Colony-forming units (CFU) per milliliter of inoculum were prepared by dilution plating on a selective medium (BG medium supplemented with 50 mg·L\(^{-1}\) cycloheximide, 50 mg·L\(^{-1}\) polymyxin B, 25 mg·L\(^{-1}\) TZC, 10 mg·L\(^{-1}\) chloramphenicol, and 5 mg·L\(^{-1}\) crystal violet [Hara and Ono, 1983]).

Plant materials

Thirteen commercial eggplant (*Solanum melongena*) cultivars were used in this study, including ‘Senryo nigo’, ‘Saitama ao daimaru’, ‘Chikuyo’, ‘Shoya onaga’, ‘Mizu nasu’, ‘Wase daimaru’, ‘Shitamachi bijin’ (Takii & Co., Ltd., Kyoto, Japan), ‘Kurume naga’, ‘Sadowara’, ‘Aichi honnaga’ (Asahi Noen Seed Co., Ltd., Aichi, Japan), ‘Black Beauty’ (Fuku Tane Co., Ltd., Fuku, Japan), ‘Hitoshibo’ (Hokuetsu Noji Co., Ltd., Niigata, Japan), and ‘Sendai naga’ (Watanabe Seed Co., Ltd., Miyagi, Japan). Six rootstock cultivars (*S. melongena* ‘Meet’ and ‘Daitaro’, *S. integrifolium* ‘Akanasu’, *S. integrifolium* × *S. melongena* ‘Taibyo-VF’, *S. torvum* ‘Tonashimu’, and ‘Torvum vigor’ [Takii]) were used in this study (Table 1). Seeds were surface-disinfected with 70% ethanol for 10 s and 1% sodium hypochlorite for 10 min and washed twice with SDW. The seeds were germinated and grown in vermiculite in a growth chamber maintained at 28°C for 12 h in light and 22°C for 12 h in the dark. Seedlings were treated once or twice with liquid fertilizer (1/2 strength OAT B solution; OAT Agrio Co., Ltd., Tokyo, Japan).

| Classification | Cultivar          | Low-temperature tolerance | Plant vigor | Bacterial wilt | Verticillium wilt | Fusarium wilt |
|----------------|-------------------|---------------------------|-------------|----------------|--------------------|---------------|
| *S. melongena* | ‘Meet’            | Strong                     | Medium      | Weak           | Strong             | Strong        |
| *S. melongena* | ‘Daitaro’         | Medium                    | Strong      | Strong         | Weak               | Strong        |
| *S. integrifolium* | ‘Akanasu’ | Strong                     | Strong      | Weak           | Weak               | Strong        |
| *S. integrifolium* × *S. melongena* | ‘Taibyo-VF’ | Very strong                | Strong      | Weak           | Strong             | Strong        |
| *S. torvum*    | ‘Torvum vigor’    | Medium                    | Very strong | Strong         | Strong             | Strong        |
| *S. torvum*    | ‘Tonashimu’       | Medium                    | Very strong | Strong         | Strong             | Strong        |

* This table is based on the information about rootstock cultivars from Takii <https://www.takii.co.jp/sk/hinshu/daigi/ana.html>.

Varietal difference in the suppression of *Verticillium* wilt among eggplants by PC mutants

We used 8107PC, which is an effective PC mutant for biological control of *Verticillium* wilt in eggplants, from a selection of 27 PC mutants. Second-leaf stage seedlings of 19 eggplant cultivars were inoculated with a bacterial suspension of 8107PC (10\(^6\) CFU·mL\(^{-1}\)). The seedlings were cut at approximately one third of their root length and then soaked in 20 mL of SDW or bacterial suspensions for 30 min as pre-inoculation. Inoculated seedlings were transplanted into 128-cell trays (3 × 3 × 4 cm\(^3\)) filled with commercial soil contaminated with *V. dahliae* (ca. 10\(^{6}\) conidia·g\(^{-1}\) fresh weight [FW]) as a challenge inoculation. Inoculated plants were grown in a growth chamber (25°C for 12 h of light and 12 h of darkness). Plants were scored daily for disease symptoms on each leaf using an assessment key with four classes (0, no symptoms; 1, yellowing; 2, wilting; 3, defoliation). The disease severity of *Verticillium* wilt was calculated at 28 days after inoculation using the following formula: disease severity (%) = \(\Sigma\) (disease symptoms of each leaf)/(3 × total number of leaves) × 100. The biological control efficiency was calculated using the following formula: biological control efficiency (%) = (\(A - B\)/\(A\)) × 100, where A and B are the percentages of disease severity in the control and PC treatments, respectively. Experiments were performed with 12 plants in each treatment and were conducted in duplicate or triplicate in successive trials.

Suppression of *Verticillium* wilt in grafted eggplants inoculated with a PC mutant

We used *S. melongena* ‘Senryo nigo’ as the scion and...
Suppression of bacterial wilt in grafted eggplants inoculated with a PC mutant

We used *S. melongena* ‘Senryo nigo’ as the scion and *S. torvum* ‘Tonashimu’ and *S. melongena* ‘Daitaro’ as rootstocks in this experiment. The grafted seedlings were prepared as described previously. 8103PC was used as an effective PC mutant to control bacterial wilt in eggplants (Nakahara et al., 2016b). The seedlings grafted onto *S. torvum* ‘Tonashimu’ and *S. melongena* ‘Daitaro’ and the non-grafted seedlings of ‘Senryo nigo’ were removed from the trays; approximately one third of the root length was cut, and the roots were soaked in 20 mL of SDW or a bacterial suspension (10⁶ CFU·mL⁻¹) of 8103PC for 30 min. Inoculated plants were transplanted into 10.5-cm pots filled with commercial soil contaminated with *V. dahlieae* (ca. 10⁷ conidia·g⁻¹ FW) and grown in a growth chamber (25°C for 12 h of light and 12 h of darkness). The disease severity of *Verticillium* wilt was calculated 28 days after inoculation. Experiments were performed with six plants in each treatment and repeated twice in successive trials.

**Results and Discussion**

**Varietal differences in the suppression of Verticillium wilt among commercial eggplant cultivars by PC mutants**

In a previous study, 8107PC was selected as the most effective PC mutant for the biological control of *Verticillium* wilt in eggplants from 27 PC mutants (Nakahara et al., 2021b). In this study, we tested the varietal differences among 19 eggplants (13 commercial cultivars and six rootstock cultivars) in the biological control of *Verticillium* wilt using 8107PC. The potential resistance and biological control differed between the eggplant cultivars (Tables 2 and 3).

In the commercial cultivars of *S. melongena*, the biological control efficiencies of *Verticillium* wilt varied from 3.2 to 67.1 (Table 2). Disease severity was significantly suppressed by 8107PC inoculation in 10 cultivars, except ‘Chikuyo’, ‘Sendai naga’, and ‘Shitamachi bijin’. In ‘Mizu nasu’, the disease severity was the highest in the control (88.2%) and the lowest in the PC mutant inoculation (29.0%), resulting in the highest biological control efficiency of all the cultivars. In ‘Shitamachi bijin’, the disease severity was low (33.1%) in the PC mutant inoculation, but lower in the control (52.4%) than in any other cultivar, resulting in a lack of significant suppression. Varietal differences exist in the biological control of bacterial wilt induced by PC mutants in commercial eggplant cultivars (Ogawa et al., 2011; Nakahara et al., 2016b). In ‘Kurume naga’,

**Table 2.** Varietal differences in suppression of *Verticillium* wilt among 13 commercial eggplant cultivars (*Solanum melongena*) by 8107PC inoculation.

| Cultivar                | Disease severity (%) | Biological control efficiency (%) |
|-------------------------|----------------------|----------------------------------|
|                         | Control             | Treatment                        |                                |
| ‘Mizu nasu’             | 88.2 ± 2.5          | 29.0 ± 6.1*                      | 67.1                           |
| ‘Hitohio’               | 86.0 ± 3.2          | 36.2 ± 6.1*                      | 57.9                           |
| ‘Aichi honnaga’         | 82.8 ± 3.0          | 36.6 ± 7.9*                      | 55.8                           |
| ‘Black Beauty’          | 76.1 ± 3.9          | 35.7 ± 5.5*                      | 53.1                           |
| ‘Wase daimaru’          | 71.7 ± 2.3          | 36.9 ± 4.8*                      | 48.5                           |
| ‘Kurume naga’           | 57.5 ± 3.4          | 29.7 ± 5.2*                      | 48.4                           |
| ‘Senryo nigo’           | 63.9 ± 2.9          | 33.2 ± 4.3*                      | 48.0                           |
| ‘Sadowara’              | 65.6 ± 4.4          | 36.7 ± 7.1*                      | 44.0                           |
| ‘Shoya onaga’           | 60.6 ± 2.9          | 37.1 ± 4.8*                      | 38.7                           |
| ‘Shitamachi bijin’      | 52.4 ± 3.5          | 33.1 ± 9.0                       | 36.8                           |
| ‘Saitama ao daimaru’    | 87.1 ± 2.6          | 66.2 ± 5.1*                      | 24.0                           |
| ‘Sendai naga’           | 74.0 ± 3.8          | 59.7 ± 8.0                       | 19.4                           |
| ‘Chikuyo’               | 72.6 ± 3.3          | 70.2 ± 4.8                       | 3.2                            |

* Data show mean ± standard error of 12 plants in each treatment done in duplicate or triplicate in successive trials (n = 24–36) at 28 days after inoculation. Asterisks indicate significant differences (P < 0.05) between the control and 8107PC treatments in each cultivar according to the Student’s *t*-test; the percentage values were obtained through arcsine transformation.

* Data show the average of two or three replicates.
controlled was inoculated in the rootstock cultivars, resistance to Verticillium wilt was relatively high (Table 2). Conversely, in ‘Saitama sugiyar’ have strong resistance (Table 1; Yamakawa, 1982; Bletsos et al., 2003). Varietal differences in resistance to Verticillium wilt were obtained through arcsine transformation.

Data show the average of two replicates.

### Table 3. Varietal difference in suppression of Verticillium wilt among six rootstock cultivars in eggplants by 8107PC inoculation.

| Classification | Cultivar          | Disease severity (%) | Biological control efficiency (%) |
|----------------|-------------------|----------------------|-----------------------------------|
|                |                   | Control             | Treatment                         |
| S. torvum      | ‘Tonashimu’       | 18.7±3.6            | 3.5±2.0*                          | 81.5                           |
| S. integrifolium| ‘Akanasu’         | 29.2±4.9            | 11.6±4.1*                         | 60.4                           |
| S. melongena    | ‘Meet’            | 32.3±3.7            | 13.8±2.5*                         | 57.4                           |
| S. torvum      | ‘Torvum vigor’    | 32.3±3.9            | 15.6±3.2*                         | 51.7                           |
| S. melongena    | ‘Daitaro’         | 70.7±3.6            | 45.5±7.0*                         | 35.7                           |
| S. integrifolium× S. melongena | ‘Taibyo-VF’  | 39.5±4.8            | 27.6±4.7                          | 30.1                           |

* Data show mean ± standard error of 12 plants in each treatment that was repeated twice in successive trials (n = 24) at 28 days after inoculation. Asterisks indicate significant differences (*P* < 0.05) between the control and 8107PC treatments in each cultivar according to the Student’s *t*-test; the percentage values were obtained through arcsine transformation.

### Figure 1. Relationship between the disease severities of Verticillium wilt in the control and PC treatments among 19 eggplant cultivars. The disease severities show the average of 12 plants in each cultivar in duplicate or triplicate in successive trials. Correlation (*r*) with *P* < 0.01 is indicated as a double asterisk by the Pearson’s correlation coefficient; the percentage values were obtained through arcsine transformation.

### Relationship between the disease severity of Verticillium wilt among eggplant cultivars in the control and PC treatments

In the control of bacterial wilt by PC mutants, eggplant cultivars with higher potential resistance tend to have better control effects by PC mutants (Ogawa et al., 2011; Nakahara et al., 2016b). In this study, a significant positive correlation (*r* = 0.752, *P* < 0.01) was observed between the disease severity of Verticillium wilt in the control and PC treatments among 19 eggplant cultivars (Fig. 1), suggesting that suppression by PC treatment tended to be higher in cultivars with higher resistance to Verticillium wilt. Disease defence-related enzymes such as phenylalanine ammonia-lyase, polyphenol oxidase, peroxidase, and lipoxygenase are at higher levels in cultivars with higher resistance to disease (Vanitha and Umesha, 2008; Vanitha et al., 2009). Furthermore, the expression of these defense-related enzymes is also induced by pre-inoculation with...
Pseudomonas fluorescens, which is known as a BCA against several diseases and is stronger against bacterial wilt in resistant cultivars than in susceptible cultivars (Vanitha and Umesha, 2011). In our previous study, expression of pathogenesis-related (PR) protein genes (e.g., basic β-1,3-glucanase (PR-2b), basic chitinase (PR-3b), thaumatin-like protein (PR-5), and proteinase inhibitor II (PR-6)) were induced in tomatoes inoculated with PC mutants (Nakahara et al., 2016a). The PR-2b and PR-3b proteins are involved in the degradation of chitin and glucan, which are cell wall constituents of fungi; the PR-5 protein has antifungal activity (van Loon et al., 1994; Tjamos et al., 2005; Bu et al., 2014). In this study, a similar mechanism may have been involved in the suppression of Verticillium wilt by PC mutants, and higher resistance is likely induced by inoculation with the PC mutant into cultivars with high potential resistance. Future studies need to clarify varietal differences in the expression of disease defense-related genes by PC mutant treatments.

Suppression of Verticillium wilt and bacterial wilt in grafted eggplants inoculated with PC mutants

Solanum torvum is strongly resistant to bacterial wilt and Verticillium wilt (Monma et al., 1997; Yoshida, 2007; Miki et al., 2011). In seedlings grafted with S. melongena ‘Senryo nigo’ onto S. torvum ‘Tonashimu’ and those inoculated with 8107PC, Verticillium wilt was significantly suppressed compared to that in the non-grafted seedlings and those inoculated with 8017PC (Table 4). The biological control by the PC mutant increased in grafted seedlings, suggesting that inoculation of grafted seedlings with PC mutants enhanced the resistance of plants against Verticillium wilt.

Ralstonia solanacearum has been classified into five bacterial groups (I–V) based on differences in virulence to four Solanum species (S. melongena, S. mammosum, S. integrifolium, and S. torvum) in Japan. Additionally, bacterial group IV isolates have the strongest pathogenicity and affect all four Solanum species (Ozaki and Kimura, 1992). The resistance to bacterial wilt in S. torvum is destroyed by bacterial group IV isolates and high temperatures (Date et al., 1994). In a previous study, 8103PC was selected as an effective PC mutant for biological control of bacterial wilt in eggplant cultivars (Nakahara et al., 2016b). In this study, we tested biological control of bacterial wilt caused by the virulent wild-type strain 8103, which is classified in bacterial group IV of R. solanacearum, in the grafted seedlings of S. melongena ‘Senryo nigo’ grafted onto ‘Tonashimu’ or ‘Daitaro’ of rootstock cultivars with 8103PC inoculation. The resistance to bacterial wilt in ‘Tonashimu’ was destroyed by the virulent wild-type strain 8103. In the seedlings grafted onto ‘Tonashimu’, bacterial wilt was not controlled without PC mutant inoculation; conversely, the disease was completely suppressed by the PC mutant inoculation (Table 5). Seedlings grafted with ‘Daitaro’ effectively controlled bacterial wilt without PC mutant inoculation (Table 5). However, ‘Daitaro’ was not suitable to control Verticillium wilt because it had the highest disease severity among rootstock cultivars in both the control and PC mutant treatments (Table 3). Therefore, the combination of grafting with ‘Tonashimu’ and inoculation of PC mutants most effectively controlled bacterial wilt and Verticillium wilt diseases in eggplants.

Two mechanisms have been considered for the induction of resistance in grafted seedlings. The first is that rootstocks induce high resistance and suppresses the transfer of pathogens into the scion (Guan et al., 2012). The second is the transfer of signaling substances and defense-related substances from rootstocks to scions. Signaling substances (e.g. salicylic acid and jasmonic acid) from the rootstock are transmitted to the scion, and PR proteins are expressed in the scion (Li et al., 2002; Verberne et al., 2003; Heil and Ton, 2008). The PR proteins expressed in the rootstock are transferred to the scion (Bortolotti et al., 2005). In this study, grafted seedlings inoculated with PC mutants may have enhanced rootstock functions.

Conclusions

In this study, varietal differences in the biological control of Verticillium wilt among 13 commercial cultivars and six rootstock cultivars by inoculation with a PC mutant were confirmed. Verticillium wilt was sup-

| Table 4. Biological control of Verticillium wilt in grafted eggplants by 8107PC inoculation. |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| Treatment*                             | Disease severity (%)y                  | Biological control efficiency (%)z      |
| Control                                 | Treatment                               |                                        |
| Non-grafted                             | 79.9±4.7a                               | 57.8±7.3b                              |
| Grafted onto ‘Tonashimu’                | 20.1±7.7c                               | 8.3±4.0c                               |

* Non-grafted seedlings of S. melongena ‘Senryo nigo’, a commercial cultivar, and the grafted seedlings of ‘Senryo nigo’ grafted onto S. torvum ‘Tonashimu’, a rootstock cultivar, were used. In the PC treatment, the seedlings were soaked in water or bacterial suspensions (10^7 CFU·mL^−1) of 8107PC for 30 min, and then transplanted into pots filled with commercial soil contaminated with P. dahliae (ca. 10^6 conidia·g^−1 FW).

* Data show the mean ± standard error of six plants in each treatment that was repeated twice in successive trials (n = 12) at 28 days after inoculation. Different letters indicate significant differences (P<0.05) according to one-way ANOVA following the Tukey-Kramer test; the percentage values were obtained through arcsine transformation.

* Data show the average of two replicates.
pressed by inoculation with the PC mutant in some eggplant cultivars, and the cultivars with a higher potential resistance to *Verticillium* wilt tended to be more suppressed by the PC mutant. In rootstock cultivars, *S. torvum* ‘Tonashimu’ and *S. melongena* ‘Daitaro’ effectively controlled *Verticillium* wilt and bacterial wilt, respectively. Selecting effective cultivars is important to achieve stable biological control of *Verticillium* wilt and bacterial wilt by PC mutants. Furthermore, we proved that bacterial wilt and *Verticillium* wilt can be effectively controlled by inoculation with PC mutants in seedlings grafted with ‘Tonashimu’. The combination of grafting and inoculation with PC mutants is expected to be an effective technique for the combined control of vascular wilt diseases in eggplants.

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**Table 5.** Biological control of bacterial wilt in grafted eggplants by 8103PC inoculation.

| Treatment               | Disease severity (%) | Biological control efficiency (%) |
|-------------------------|----------------------|----------------------------------|
|                         | Control              | Treatment                        |                                  |
| Non-grafted             | 66.7±14.2 a          | 13.9±9.6 b                       | 79.2                             |
| Grafted onto ‘Tonashimu’| 70.8±12.7 a          | 0.0±0.0 a                        | 100.0                            |
| Grafted onto ‘Daitaro’  | 8.3±8.3 bc           | 0.0±0.0 c                        | 100.0                            |

* Data show mean ± standard error of six plants in each treatment that was repeated twice in successive trials (n = 12) at 28 days after inoculation. Different letters indicate significant differences (P<0.05) according to one-way ANOVA following the Tukey-Kramer test; the percentage values were obtained through arcsine transformation.

* Data show the average of two replicates.
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