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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Ralstonia solanacearum causes bacterial wilt and undergoes spontaneous phenotypic conversion (PC) from its wild-type to an avirulent form. In previous studies, we reported that pre-inoculation of plants with PC mutants suppressed bacterial wilt and *Verticillium* wilt in eggplants. In addition, we previously revealed bacterial differences in the control effects in eggplant among PC mutants; 8107PC and 8103PC were selected as effective strains for controlling *Verticillium* wilt and bacterial wilt diseases, respectively. In this study, we investigated the biological control efficiencies of 8107PC inoculation among 19 eggplant cultivars, including 13 commercial cultivars and six rootstock cultivars, to select eggplant cultivars that stably prevent *Verticillium* wilt using PC mutants. Varietal differences in biological control were confirmed. Additionally, cultivars with a higher potential resistance to *Verticillium* wilt tended to be more suppressed by the PC mutant, suggesting that cultivar selection is important to achieve effective biocontrol. Furthermore, we found that the biocontrol with 8107PC against *Verticillium* wilt in grafted eggplant seedlings of *Solanum melongena* 'Senryo nigo' onto *S. torvum* 'Tonashimu' was the most effective rootstock cultivar against *Verticillium* wilt. The biocontrol was more effective than the non-grafted seedlings inoculated with the PC mutant and the grafted seedlings not inoculated with the PC mutant. We also investigated biocontrol by the 8103PC inoculation against bacterial wilt using grafted seedlings ('Senryo nigo' as the scion) with 'Tonashimu' or *S. melongena* 'Daitaro' as rootstocks. Although 'Daitaro' had a low control effect against *Verticillium* wilt, the grafted seedlings showed a high control effect against bacterial wilt, regardless of the PC mutant inoculation. Conversely, seedlings grafted with 'Tonashimu' were vulnerable to bacterial wilt; however, inoculation with the PC mutant completely suppressed the disease. Therefore, this study demonstrated that bacterial wilt and *Verticillium* wilt could be effectively controlled by inoculation with PC mutants in seedlings grafted with 'Tonashimu'. The combination of grafting and PC mutant inoculation is expected to be effective for the combined control of *Verticillium* wilt and bacterial wilt diseases in eggplants.

Key Words: avirulent bacteria, bacterial wilt, rootstock, soil-borne diseases, *Verticillium* wilt.

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.

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After sporulation, the fungus enters the saprophytic stage during tissue necrosis or plant senescence (Fradin 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 (McKeen and Mountain, 1967; Yamakawa, 1982; Duniway, 2002; Bletsos 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. dahliae* produces microsclerotia that can survive in soil and plant residues for several years (Pegg and Brady, 2002; Fradin and Thomma, 2006). Further, soil fumigants harm human health and the environment (Duniway, 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 (Kelman and Hruschka, 1973; Brumbley 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 Umesha, 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 BGT medium (1% bacto peptone, 0.5% glucose, 0.1% yeast extract, 0.1% casamino acid, 1.5% agar, and 50 mg·L⁻¹ tetrazolium chloride [TZC]) after 14 days in a BG broth (BGT 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., Fukui, Japan), ‘Hitoshio’ (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* ‘Taiby-

VF’, *S. torvum* ‘Tonashimu’, and ‘Torvum vigar’ [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 at 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).

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 (10⁸ 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 cm³) filled with commercial soil contaminated with *V. dahliae* (ca. 10⁶ 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 (%) = Σ (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

Table 1. Low-temperature tolerance, plant vigor, and disease tolerance against soil-borne wilt diseases in eggplants of rootstock cultivars used in this study.^z

Classification	Cultivar	Low-temperature tolerance	Plant vigor	Disease tolerance		
				Bacterial wilt	<i>Verticillium</i> wilt	<i>Fusarium</i> wilt
<i>S. melongena</i>	‘Meet’	Strong	Medium	Weak	Strong	Strong
<i>S. melongena</i>	‘Daitaro’	Medium	Strong	Strong	Weak	Strong
<i>S. integrifolium</i>	‘Akanasu’	Strong	Strong	Weak	Weak	Strong
<i>S. integrifolium</i> × <i>S. melongena</i>	‘Taiby-VF’	Very strong	Strong	Weak	Strong	Strong
<i>S. torvum</i>	‘Torvum vigar’	Medium	Very strong	Strong	Strong	Strong
<i>S. torvum</i>	‘Tonashimu’	Medium	Very strong	Strong	Strong	Strong

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

S. torvum ‘Tonashimu’ as the rootstock in this experiment. Second-leaf stage seedlings were grafted and grown for 10 days to securely connect the scion and rootstock. The seedlings grafted onto *S. torvum* ‘Tonashimu’ and 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 (10^8 CFU·mL⁻¹) of 8107PC for 30 min. Inoculated plants were transplanted into 10.5-cm pots filled with commercial soil contaminated with *V. dahliae* (ca. 10^6 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.

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. Then, approximately one third of the root length was cut, and the roots were soaked in 20 mL of SDW or a bacterial suspension (10^8 CFU·mL⁻¹) of 8103PC for 30 min. Inoculated plants were transplanted into 10.5-cm pots filled with commercial soil contaminated with the virulent wild-type strain 8103 of *R. solanacearum* (ca. 10^7 CFU·g⁻¹ FW) and grown in a growth chamber (30°C for 12 h of light and 28°C for 12 h of darkness). The disease severity of bacterial wilt was calculated at 28 days after inoculation using the following formula: disease severity (%) = (number of wilted leaves)/(total number of leaves) × 100. Experiments were performed with six plants in each treatment and repeated twice in successive trials.

Statistical analysis

To ensure the homogeneity of the variances and symmetry of the distribution of each variable, data recorded as percentages were arcsine-transformed before performing a Student’s *t*-test, one-way analysis of variance (ANOVA) following the Tukey-Kramer test, and Pearson’s correlation coefficient, which was used to analyze the relationship between the disease severity of *Verticillium* wilt in the control and the PC treatments among 19 eggplant cultivars. Statistical analysis was performed using SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

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 (%) ^z		Biological control efficiency (%) ^y
	Control	Treatment	
‘Mizu nasu’	88.2±2.5	29.0±6.1*	67.1
‘Hitoshio’	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

^z 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.

^y Data show the average of two or three replicates.

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

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

^z 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.

^y Data show the average of two replicates.

‘Senryo nigo’, ‘Sadowara’, and ‘Shoya onaga’—cultivars with strong control effects against bacterial wilt by PC mutants (Ogawa et al., 2011; Nakahara et al., 2016b)—the biological control of *Verticillium* wilt was also relatively high (Table 2). Conversely, in ‘Saitama ao daimaru’, ‘Sendai naga’, and ‘Chikuyo’—cultivars with low biological control of bacterial wilt (Ogawa et al., 2011; Nakahara et al., 2016b)—the biological control of *Verticillium* wilt was also low. In ‘Mizu nasu’, ‘Hitoshio’, and ‘Black Beauty’—cultivars with low biological control of bacterial wilt (Ogawa et al., 2011; Nakahara et al., 2016b)—the biological control against *Verticillium* wilt was relatively high in this study. Therefore, selecting cultivars with strong effects is important to obtain stable biological control of *Verticillium* wilt and bacterial wilt by PC mutants.

Varietal difference in suppression of *Verticillium* wilt among rootstock cultivars of eggplant by PC mutants

Grafting cultivation has become widespread as a control for soil-borne vascular wilt diseases; however, rootstock cultivars have partial resistance to *Verticillium* wilt, causing disease in grafted plants (Yamakawa, 1982; Bletsos et al., 2003). Varietal differences in resistance to *Verticillium* wilt exist among rootstock cultivars; the resistance in *S. melongena* ‘Daitaro’ and *S. integrifolium* ‘Akanasu’ is weak, whereas *S. melongena* ‘Meet’, *S. integrifolium* × *S. melongena* ‘Taibyo-VF’, *S. torvum* ‘Tonashimu’, and ‘Torvum vigar’ have strong resistance (Table 1; Yamakawa, 1982; Monma et al., 1997; Yoshida, 2007; Miki et al., 2011). In this study, no rootstock cultivars completely controlled *Verticillium* wilt; the potential resistance in the rootstock cultivars was consistent with Table 1, except for *S. integrifolium* ‘Akanasu’, which showed relatively strong resistance (Table 3). When 8107PC was inoculated in the rootstock cultivars, resistance to *Verticillium* wilt tended to increase. In *S. torvum* ‘Tonashimu’, the potential resistance was the highest in this study; the resistance was further enhanced by inoculation with 8107PC and biological control efficiency was the highest at 81.5%.

Fig. 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 8107PC (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 pre-

vious 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 ^z	Disease severity (%) ^y		Biological control efficiency (%) ^x
	Control	Treatment	
Non-grafted	79.9 ± 4.7 a	57.8 ± 7.3 b	27.7
Grafted onto ‘Tonashimu’	20.1 ± 7.7 c	8.3 ± 4.0 c	58.7

^z 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 (10^8 CFU·mL⁻¹) of 8107PC for 30 min, and then transplanted into pots filled with commercial soil contaminated with *V. dahliae* (ca. 10^6 conidia·g⁻¹ FW).

^y 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.

^x Data show the average of two replicates.

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

Treatment ^z	Disease severity (%) ^y		Biological control efficiency (%) ^x
	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 c	100.0
Grafted onto ‘Daitaro’	8.3± 8.3 bc	0.0±0.0 c	100.0

^z Non-grafted seedlings of *S. melongena* ‘Senryo nigo’, a commercial-cultivar, and the grafted seedlings of ‘Senryo nigo’ grafted onto *S. torvum* ‘Tonashimu’ or *S. melongena* ‘Daitaro’, rootstock cultivars, were used. In the PC treatment, the seedlings were soaked in water or bacterial suspensions (10^8 CFU·mL⁻¹) of 8103PC for 30 min, and then transplanted into pots filled with commercial soil contaminated with the pathogenic strain 8103 of *R. solanacearum* (ca. 10^7 CFU·g⁻¹ FW).

^y 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.

^x 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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