Evaluation of 29 *Lisianthus* Cultivars (*Eustoma grandiflorum*) and One Inbred Line of *E. exaltatum* for Resistance to Two Isolates of *Fusarium solani* by Using Hydroponic Equipment

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Fusarium root rot of lisianthus (*Eustoma grandiflorum*) caused by *Fusarium solani* is one of the most important and damaging lisianthus diseases. It occurs commonly in Japan and worldwide and causes serious crop losses. However, little effort has been made to breed lisianthus for resistance to this disease. We initiated a breeding program for resistance to *F. solani* in 2014. Twenty-nine lisianthus cultivars (*E. grandiflorum*) and one inbred line of *Eustoma exaltatum* were evaluated for resistance to two isolates (MAFF712388 and MAFF712411) of *F. solani*, as a first step toward the breeding of resistant cultivars. Seedlings were inoculated following injury by needle, then grown using hydroponic equipment—an efficient and reliable method for evaluating resistance. We found large differences in resistance among the 29 cultivars and the one inbred line tested. ‘Papillon Pink Flash’ was highly resistant to both isolates and showed no disease symptoms in a total of four tests. Furthermore, *E. exaltatum* Ohkawa No. 1 was highly resistant to isolate MAFF712388, showing no disease symptoms, and resistant to isolate MAFF712411. On the other hand, ‘Mink’, ‘Nagisa A’, ‘Nagisa B’, and ‘Vulcan Marine’ were stably susceptible with 70% to 100% of plants of these four cultivars wilting in all tests. MAFF712411 had greater pathogenicity than MAFF712388, but it is not clear whether the two isolates belong to different races.

Key Words: fusarium root rot, hydroponic culture, Russell prairie gentian, Texas bluebell.

Introduction

Lisianthus, *Eustoma grandiflorum* (Raf.) Shinn., commonly known as the Russell prairie gentian or Texas bluebell, is one of the most important cut flowers in Japan and worldwide. It has gained economic importance and has been in increasing demand over the last three decades (McGovern, 2016). Cut lisianthus flowers are produced year-round in Japan and are ranked fourth in production value (12.7 billion yen, 2017) and third in cultivation area (426 ha, 2018) among cut flowers in Japan (Statistics of Agriculture, Forestry and Fisheries in Japan; [http://www.affrc.go.jp/j/tokei/kouhyou/](http://www.affrc.go.jp/j/tokei/kouhyou/sakumotu/sakkyou_kaki/index.html)

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The genus *Eustoma* is a member of the family Gentianaceae and includes three species, *E. grandiflorum*, *E. exaltatum* (L.) Salisb. ex G. Don. and *E. barkleyi* Standl. ex Shinn. (Barba-Gonzalez et al., 2015; Ohkawa, 2003a; Shinn, 1957). *Eustoma grandiflorum*, from which most commercial cultivars have been derived, is native to the midwestern and southern prairies of the USA, inhabiting moist prairie habitats from Nebraska to Colorado and Texas, as well as in northern Mexico (Halevy and Kofranek, 1984; Ohkawa, 2003a). *Eustoma exaltatum* is distributed in the southernmost USA, Mexico, British Honduras (now called Belize) and the West Indies (Ohkawa, 2003a; Shinn, 1957). *Eustoma barkleyi* is known only from a limited area in Coahuila, Mexico (Ohkawa, 2003a; Wood and Weaver, 1982).

Lisianthus was introduced into Japan from the USA in the 1930s for ornamental use (Fukuta, 2016; Ohkawa, 2003b). Since then, Japanese seed companies and individual private breeders have produced many

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cultivars for cut flowers. In 1963, the first purebred lisianthus cultivar, ‘Shihai’ was bred by Fukukaen Nursery & Bulb Co., Ltd., Nagoya, Japan (Fukuta, 2016; Ohkawa, 2003b). In 1981, the first F_1 cultivars of lisianthus were bred by Sakata Seed Corporation, Yokohama, Japan. Today, eight seed companies in Japan intensively breed many commercial cultivars with vast diversity every year. The popularity of lisianthus is based on its gorgeous flowers—either elegant and rose-like doubles or neat and clear-shaped singles—its excellent postharvest life, and its wide range of flower colors, including white, pale yellow, pale green, pink, red, and purple.

Recently, improving disease resistance has become one of the major breeding objectives of many ornamental plant improvement programs. Use of cultivars with improved disease resistance can help growers improve the stability of flower production and reduce production costs. The NARO Institute of Vegetable and Floriculture Science (NIVFS, Japan) has improved the resistance of carnations (*Dianthus caryophyllus* L.) to bacterial wilt (*Burkholderia caryophylli*), one of the most damaging diseases of these plants (Onozaki, 2018). To breed carnations with improved resistance to bacterial wilt, a sequence-tagged site marker closely linked to a major resistance gene derived from a highly resistant wild species, *Dianthus capitatus* ssp. *andrzejowskianus*, was developed (Onozaki et al., 2004). Moreover, resistant standard-type cultivar ‘Karen Rouge’ and resistant spray-type cultivars, ‘Momo Karen’ and ‘Hime Karen’, have been developed by using both disease screening and marker-assisted selection techniques (Takebe et al., 2020; Yagi et al., 2010; Yamaguchi, 2019).

Fusarium root rot of lisianthus caused by *Fusarium solani* is one of the most economically important and damaging lisianthus diseases. It occurs commonly in Japan and worldwide and causes serious crop losses. Root infection of lisianthus by *F. solani* has been described in Argentina (Wolcan et al., 2001), Japan (Chikuo et al., 2003; Tomioka et al., 2002, 2011), South Africa (Truter and Wehner, 2004), Korea (Choi et al., 2017), and China (Xiao et al., 2018). Continuous monocropping of lisianthus often leads to serious crop losses, with plants displaying severe symptoms despite soil disinfection with steam or chemicals (Zhou et al., 2019). Therefore, there is a need to develop effective approaches to control this disease.

The initial symptoms are wilting of the lower leaves, which turn yellow to brownish. Yellowing and leaf wilt progress from the lower to the upper leaves, and eventually the entire plant wilts and dies. Roots and stem bases of the diseased plants rot, and soon after, the rot extends into the root system (McGovern, 2016; Tomioka et al., 2002, 2011).

Most commercial breeding efforts by seed companies have focused on flower morphology (e.g. double or sin-

gle, corolla shape, fringed or non-fringed), flower color, and earliness, and efforts to breed for resistance to fusarium root rot in lisianthus have been rare. Breeding for *Fusarium* resistance would greatly decrease production losses and improve landscape performance (Harbaugh, 2006). Therefore, in 2014 we initiated a breeding program for resistance to *F. solani*. In this paper, we report on the resistance of 29 lisianthus cultivars and one inbred line of *E. exaltatum* to two isolates of *F. solani*, as a first step toward the breeding of resistant cultivars.

Materials and Methods

Plant materials

Twenty-nine cultivars of *E. grandiflorum* and one inbred line of *E. exaltatum*, Ohkawa No. 1, were used in this study (Table 1). Cultivars were selected mainly by asking specialists at Japanese seed companies and prefectural agricultural improvement extension officers which cultivars under current cultivation are resistant or susceptible to fusarium root rot.

Seeds were sown in 200-cell plastic germination trays (Plant Plug; Sakata Seed Corporation, Yokohama, Japan). The seeded trays were kept in a dark cool-room at 10°C for five weeks or two weeks to prevent the formation of rosettes and promote subsequent stem elongation (Tanigawa et al., 2002). Trays were then transferred to a growth chamber (KCLP-1400IIC; NIPPON MEDICAL & CHEMICAL INSTRUMENTS Co., Ltd., Osaka, Japan), and plants were grown under a constant day/night preset temperature of 28°C/18°C in a 12-h photoperiod supplemented with fluorescent lamps with a photosynthetic photon flux density of 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Testing for *F. solani* resistance by using hydroponic equipment

After about two months of growth in the growth chamber, four to 20 seedlings at the three-pair leaf stage of each cultivar or the inbred line were transplanted to hydroponic equipment (Home Hyponica Sarah; Kyowa Co., Ltd., Takatsuki, Japan) in a greenhouse at NIVFS, Tsukuba, Japan. All plants were grown in commercial liquid fertilizer solution for hydroponic culture (High Tempo Ar 1 $\text{mL}\cdot\text{L}^{-1}$ and High Tempo Cu 2 $\text{mL}\cdot\text{L}^{-1}$; Sumitomo Chemical Co., Ltd., Tokyo, Japan) with an electrical conductivity (EC) of approximately 1.5 $\text{dS}\cdot\text{m}^{-1}$. During the inoculation test, half-strength liquid fertilizer solution was added as appropriate to replace depleted nutrients and to avoid increases in the nutrient solution concentration. The water temperature was maintained at 25 to 26°C with an auto-heater (Pre-set Auto-heater, 100 W; EVERES, Tokyo, Japan) in spring, autumn, and winter. With this auto-heater, the temperature around the roots in each test in spring, autumn, and winter could be kept constant. In summer (July to August), the water temperature was not con-

Table 1. Disease incidence in 29 lisianthus cultivars and one inbred line tested for resistance to *Fusarium solani* isolates MAFF712388 and MAFF712411.

Cultivar or line	Isolate MAFF712388						Isolate MAFF712411					
	1×10 ⁷ (bud-cells/mL)			5×10 ⁷ with re-inoculation (bud-cells/mL)			5×10 ⁷ (bud-cells/mL)			1×10 ⁷ (bud-cells/mL)		
	2015 autumn % ^y	DJ ^x	2016 spring % ^y	DJ ^x	2016 winter % ^y	DJ ^x	2018 spring % ^y	DJ ^x	2018 autumn % ^y	DJ ^x	2019 spring % ^y	DJ ^x
Annie Light Pink	KA ^z											
Blue Fizz	KA	90 (9/10) 80.0			10 (1/10) 5.0		10 (1/10) 10.0		0 (0/5) 0.0		60 (6/10)	40.0
Bolero Blue Flash	MI								95 (19/20) 93.8			
Bolero White	MI	50 (5/10) 45.0			90 (9/10) 70.0				60 (12/20) 51.3			
Bon Voyage Type Pink	SA	40 (4/10) 32.5			30 (3/10) 25.0				95 (19/20) 91.3		100 (10/10)	100.0
Cecil Passion ME	KA		20 (2/10) 5.0									
Clare Pink	KA	0 (0/10) 0.0			10 (1/10) 10.0		10 (1/10) 10.0		50 (3/6)		90 (9/10) 82.5	
Clariss Pink	SA		50 (5/10) 42.5								50 (3/6)	37.5
Cocktail Blue	KA											
<i>E. exaltatum</i> Ohkawa No.1	—	0 (0/10) 0.0			0 (0/9) 0.0		20 (2/10) 15.0		70 (7/10) 60.0		80 (8/10) 65.0	
Endless Love	KA	10 (1/10) 10.0			80 (8/10) 45.0		0 (0/10) 0.0		20 (2/10) 15.0		0 (0/10)	0.0
First Love	KA	50 (5/10) 42.5			100 (10/10) 85.0							
Kiki	MI								75 (15/20) 71.3			
Marble Pink	MI								45 (9/20) 38.8			
Mink	TA											
Nagisa A	MI	80 (8/10) 70.0			87.5 (7/8) 71.9		80 (8/10) 67.5		100 (10/10) 100.0		100 (10/10)	100.0
Nagisa B	MI	70 (7/10) 65.0			83.3 (5/6) 70.8		70 (7/10) 65.0		90 (9/10) 85.0		70 (7/10) 67.5	
New Lination Pink Flash	MI	100 (10/10) 100.0			77.8 (7/9) 66.7				90 (18/20) 88.8		83.3 (5/6) 83.3	
Papillon Pink Flash	SA	0 (0/10) 0.0			0 (0/10) 0.0		30 (3/10) 30.0		100 (4/4) 87.5		100 (10/10) 100.0	
Piccorosa Green (Ver. 2)	SA	20 (2/10) 10.0			10 (1/10) 5.0						0 (0/10) 0.0	
Pink Fizz	KA	80 (8/10) 62.5			0 (0/10) 0.0							
Reina White	SA	50 (5/10) 25.0			20 (2/10) 12.5							
Rosina (Type 3) Green	SA	80 (8/10) 70.0			30 (3/10) 25.0				100 (10/10) 95.0			
Rosina Lavender	SA	50 (5/10) 45.0			20 (2/10) 12.5							
Shihai	FU	50 (5/10) 45.0			50 (4/8) 31.3		90 (9/10) 85.0		100 (10/10) 100.0		70 (7/10) 62.5	
Suzukaze	KA	0 (0/10) 0.0			10 (1/10) 10.0				80 (16/20) 72.5			
Umihonoka	SU											
Voyage (Type 2) Blue	SA	20 (2/10) 10.0			50 (5/10) 12.5							
Voyage (Type 2) White	SA	0 (0/10) 0.0			20 (2/10) 10.0							
Yulecan Marine	TA		70 (7/10) 55.0				90 (9/10) 77.5		80 (8/10) 65.0		70 (7/10) 65.0	
Control group (no inoculation)												
Annie Light Pink	KA				0 (0/10) 0.0							
Bolero White	MI								0 (0/5) 0.0		0 (0/5) 0.0	
Clare Pink	KA										0 (0/5) 0.0	
Cocktail Blue	KA											
Mink	TA	0 (0/10) 0.0			10 (1/10) 10.0						0 (0/10) 0.0	
Nagisa B	MI				0 (0/10) 0.0							
New Lination Pink Flash	SA						0 (0/10) 0.0					
Reina White	SA											
Rosina (Type 3) Green	SA	0 (0/10) 0.0			0 (0/20) 0.0						0 (0/10) 0.0	
Shihai	FU											

^z Breeding companies: FU, Fukukaen Nursery & Bulb Co., Ltd.; KA, Kaneko Seeds Co., Ltd.; ML, Miyoshi Co., Ltd.; SA, Sakata Seed Corporation; SU, Sumika Agrotech Co., Ltd.; TA, Takii & Co., Ltd.

^y Percentage of wilted plants. Numbers in parentheses indicate (number of wilted plants/number of tested plants).

$$DI = \Sigma (\text{number of plants} \times \text{disease severity rating (0 to 4)}) / (\text{total number of plants} \times 4) \times 100.$$

Control group was irrigated with 1 mL of distilled water instead of bud cell solution.

trolled because the hydroponic equipment did not have a water cooler (the temperature range was 25 to 36°C).

To prevent wilting caused by other root rot diseases, such as that caused by *Pythium spinosum* or *Pythium irregulare*, metalaxyl granules (Ridomil 2; containing metalaxyl 2%, Syngenta Japan, K. K., Tokyo, Japan; no longer available) were added to the nutrient solution at a rate of 1 g·L⁻¹. In the 2018 and 2019 tests, Metalaxyl M (Subdue Maxx Fungicide; containing metalaxyl M 22%, Syngenta Japan) was added to the nutrient solution at a rate of 0.1 mL·L⁻¹, because metalaxyl granules were no longer available.

Three weeks after the seedlings were transplanted into the hydroponic equipment, they were inoculated with bud cell suspensions of *F. solani*. Pathogenic fungi (isolate MAFF712388, isolated from naturally infected lisianthus plants in Yamagata Prefecture, Japan; or isolate MAFF712411, isolated from naturally infected lisianthus plants in Iwaki City, Fukushima Prefecture, Japan) were cultured on 200 mL of potato dextrose (PD) liquid medium in 300-mL Erlenmeyer flasks at 25°C for seven to 10 days on a reciprocal shaker (160 strokes per min) (Fig. 1A). The resulting culture was passed through absorbent cotton gauze (Type I; Hakujiji Co., Ltd., Tokyo, Japan), and the filtrate was centrifuged at 5000 × g at 4°C for 15 min (Fig. 1B). Bud cell pellets were resuspended in distilled water to

produce a concentration of 1 × 10⁷ or 5 × 10⁷ bud cells·mL⁻¹ (Fig. 1C). The pathogenic fungi were cultured under the same conditions in each test as described above; however, the number of bud cell pellets obtained differed between tests. Therefore, the concentration was adjusted to 1 × 10⁷ or 5 × 10⁷ bud cells·mL⁻¹ depending on the total amount of inoculated solution required for inoculation.

After penetration of the stems at two points within 2 mm of the ground with an injection needle (25 G × 1" (0.50 × 25 mm); Terumo Corporation, Tokyo, Japan) (Fig. 1F), 1 mL of bud cell suspension was irrigated onto each stalk with a pipette (Fig. 1G). In each test there was a control group (Table 1) that was irrigated with 1 mL of distilled water instead of bud cell solution.

Inoculation tests were performed four times with isolate MAFF712388 and twice with isolate MAFF712411. Dates of planting and inoculation, inoculation concentration, and average temperature during each test are shown in Table 2. In the winter (January) 2016 test, the hydroponic equipment was covered with a thermal insulation vinyl film (Tokan Ace NH Nukumori; TOKANKOSAN Co., Ltd., Tokyo, Japan) and heated to > 10°C by panel heaters (SPZ-250 and SP-250; SHOWA SEIKI Co., Ltd., Kikukawa, Japan) to maintain the appropriate temperature for disease onset (Fig. 1H). In the 2016 winter and 2018 spring tests, as

Table 2. Details of inoculation with *Fusarium solani* isolates MAFF712388 and MAFF712411.

(A) Isolate MAFF712388

	2015 autumn	2016 spring	2016 winter	2018 spring
Date of planting	October 1	April 15	January 8	April 17
Date of inoculation	October 22	May 20	January 28	May 8
Experiment end date	December 31	June 24	April 11	August 7
Inoculation concentration (bud-cells/mL)	1 × 10 ⁷	1 × 10 ⁷	5 × 10 ⁷	5 × 10 ⁷
Re-inoculation	—	—	March 7	June 19
Inoculation concentration (bud-cells/mL)	—	—	5 × 10 ⁷	5 × 10 ⁷
Duration of investigation	70	35	74 (35) ^Y	91 (49) ^Y
Initial air temperature ^Z (°C)	20.2	23.8	15.3	21.1
Average air temperature (°C)	17.7	23.6	18.3 (19.6) ^X	25.9 (28.5) ^X
Average water temperature (°C)	25.5	27.2	26.6	29.2

(B) Isolate MAFF712411

	2018 autumn	2019 spring
Date of planting	August 28	April 15
Date of inoculation	September 18	May 7
Experiment end date	November 19	July 23
Inoculation concentration (bud-cells/mL)	5 × 10 ⁷	1 × 10 ⁷
Duration of investigation	62	77
Initial air temperature ^Z (°C)	23.9	21.6
Average air temperature (°C)	21.7	23.5
Average water temperature (°C)	26.7	26.7

^Z Initial air temperature: average air temperature in the week after inoculation.

^Y Numbers in parentheses indicate the numbers of days after re-inoculation.

^X Values in parentheses indicate average air temperatures after re-inoculation.

no disease symptoms were observed until 39 days and 42 days, respectively, after inoculation, plants were re-inoculated on March 7 and June 19, respectively, under the conditions described in Table 2.

Measures of disease severity and resistance

Disease severity in seedlings was scored weekly after inoculation, with wilting levels rated as 0 = no symptoms, 1 = wilt of lower leaves or poor growth, 2 = wilt of lower and upper leaves, 3 = wilt of all leaves and the whole plant, and 4 = dead, according to the criteria of Satou and Fukuta (2016, 2019). In addition, the disease index (DI) was calculated by using the following formula: $DI = \Sigma (\text{number of plants} \times \text{disease severity rating}) / (\text{total number of plants} \times 4) \times 100$. The percentage of wilted plants (%) was calculated by using the following formula: $\text{Percentage of wilted plants} = \text{number of plants with disease severity rating} \geq 1 / \text{total number of plants} \times 100$. Because the rate of disease progression showed a large seasonal variation, the evaluation was performed until the disease incidence of the most susceptible cultivars reached 90% to 100%; the final evaluation results (percentage of wilted plants and DI) are summarized in Table 1. The level of resistance of each cultivar or inbred line was expressed as follows: highly resistant, no symptoms (0% wilted); resistant, 1% to 20% wilted; moderately susceptible, 21% to 69% wilted; susceptible, 70% to 100% wilted (Table 1).

Results

The 29 lisianthus cultivars and one inbred line showed large differences in disease incidence (percentage of wilted plants and DI) when inoculated with the *F. solani* isolates MAFF712388 and MAFF712411 (Table 1). The percentage of wilted plants ranged between 0% and 100%, and DI ranged between 0 and 100. No disease occurred in the control group, with the exception of one wilted plant out of 10 tested plants in ‘Mink’ in the spring 2016 test. Wilting of this one plant began on day 28 (seven days before the end of the test), but the cause was unknown.

‘Papillon Pink Flash’ was highly resistant to both isolates and showed no disease symptoms in the four tests. Furthermore, *E. exaltatum* Ohkawa No. 1 was highly resistant to isolate MAFF712388, showing no disease symptoms in the four tests. It was classified as resistant to MAFF712411 in the autumn 2018 test and as highly resistant in the spring 2019 test, with 20% and 0% wilted plants and DIs of 15 and 0.

In contrast, ‘Mink’, ‘Nagisa A’, ‘Nagisa B’, and ‘Vulcan Marine’ were stably susceptible to both isolates, with 70% to 100% wilted plants in all tests (Table 1). Although ‘Bolero White’ and ‘Shihai’ were susceptible in two tests of MAFF712411, their percentages of wilted plants ranged between 50% and 90% upon exposure to MAFF712388.

Although ‘Annie Light Pink’ was resistant in the

spring 2018 test to MAFF712388 and highly resistant in the autumn 2018 test to MAFF712411, its percentage of wilted plants reached 60% in the spring 2019 test with MAFF712411.

Comparing disease incidences (percentages of wilted plants and DIs) revealed that MAFF712411 had greater pathogenicity than MAFF712388 (Table 1). For example, although ‘Cecil Passion ME’, ‘Clare Pink’, ‘Cocktail Blue’, ‘Suzukaze’, and ‘Voyage (Type 2) White’ were highly resistant or resistant to MAFF712388, at least 50% of plants of these five cultivars wilted upon exposure to MAFF712411.

Figure 2 gives examples of the disease severity in plants in the autumn 2015 test using isolate MAFF712388, at 70 days after inoculation. The 10 plants in the back row were of the highly resistant inbred line, *E. exaltatum* Ohkawa No. 1, which showed no disease symptoms. On the other hand, all 10 plants of the susceptible cultivar ‘Nagisa B’ (Fig. 2, front) wilted and died.

In tests with either isolate, plants of susceptible cultivars showed severe stem rot and wilt, and brownish-white masses of conidia in sporodochia were often observed on the basal surface of rotted stems at the end of each test (Fig. 3).

Discussion

In a preliminary test conducted in spring (March to June) 2015, we used the cut-root soaking method, which is effective for determining resistance of carnation to bacterial wilt (Onozaki et al., 1999). Seedlings with roots damaged by a razor were soaked in a bud cell suspension (5×10^6 bud cells·mL⁻¹) for 30 min and then transplanted into the hydroponic equipment. However, as this method did not cause any disease until 45 days after inoculation with *F. solani* isolate MAFF712388 (data not shown), all subsequent tests were performed by using the needle injury method, as described in Materials and Methods.

‘Papillon Pink Flash’ (Fig. 4A), with pink-flashed, small double flowers, bred by Miyoshi & Co., Ltd., Hokuto, Japan, was highly resistant to isolates MAFF712388 and MAFF712411 (Table 1). This cultivar showed no disease symptoms in any of the four tests. In contrast, the lisianthus breeder Miyoshi & Co., Ltd. informed us that ‘Papillon Pink Flash’ seemed to be susceptible to *Fusarium* disease, because most plants of this cultivar in a bed found to be contaminated with *F. oxysporum* f. sp. *eustomae* wilted and died (personal communication: T. Akimoto, July 2014, July 2016). Our results and their observation indicate that different genes may be involved in the resistance of ‘Papillon Pink Flash’ to *F. solani* and *F. oxysporum* f. sp. *eustomae*.

Eustoma exaltatum Ohkawa No. 1 (Fig. 4B) had no disease symptoms when infected with isolate MAFF712388, and was resistant or highly resistant to

isolate MAFF712411 (20% or 0% wilted plants; DI of 15 or 0). In August 1986, Dr. K. Ohkawa investigated the growth situation and ecological characteristics of wild *Eustoma* species in Colorado and Texas, USA, in areas where species of *Eustoma* are distributed, and collected seeds of *E. exaltatum* for introduction into Japan (Harbaugh, 2006; Ohkawa, 1988, 2003a). *Eustoma exaltatum* Ohkawa No. 1 is an inbred line derived from these seeds. We chose this line to identify DNA markers of resistance to *F. solani* because it is a homozygous

inbred line.

Furthermore, we were able to identify four cultivars —‘Mink’, ‘Nagisa A’, ‘Nagisa B’, and ‘Vulcan Marine’—that were stably susceptible to both isolates. These four cultivars should be useful as control cultivars to confirm disease incidence in inoculation testing of *F. solani*.

Use of hydroponic equipment for inoculation of seedlings to evaluate resistance has many advantages. Satou and Fukuta (2016, 2019) examined the control of root rot caused by *Pythium* spp. in hydroponic culture and established a chemical method to control *Pythium* root rot. The use of hydroponic culture to screen for resistant cultivars allows tests to be conducted under uniform root temperature conditions for most of the year. Our detailed observations over five years of trials indicate that an air temperature of about 25°C is suitable for disease evaluation and allows for rapid disease onset. During the summer, the daytime air and water temperatures exceeded 30°C, and the disease and its progres-

Fig. 1. Procedure for inoculation with *Fusarium solani* by using hydroponic equipment. A: Shaking culture in potato dextrose liquid medium (25°C for 7–10 days). B: The filtrate was centrifuged at $5000 \times g$ at 4°C for 15 min. C: Bud cell pellets were resuspended in distilled water to produce a concentration of 1×10^7 or 5×10^7 bud cells mL^{-1} . D: Bud cells of *F. solani*. E: Hydroponic equipment and test plants. F: The stem of each plant at two points within 2 mm of the ground was penetrated with an injection needle. G: After penetration, 1 mL of bud cell suspension was irrigated onto the stalk with a pipette. H: In the winter 2016 test, the equipment was covered by a vinyl film and heated to above 10°C.

Fig. 2. Disease severity 70 days after inoculation with *Fusarium solani* (MAFF712388) in autumn 2015. Back: Highly resistant line, *Eustoma exaltatum* Ohkawa No. 1 (no disease symptoms), Front: Highly susceptible cultivar, ‘Nagisa B’ (all plants wilted and died). Experimental period: 22 October to 31 December 2015.

Fig. 3. Basal stem rot and brownish-white masses of conidia in sporodochia on rotted stem of the susceptible cultivar ‘Vulcan Marine’ 77 days after inoculation with *Fusarium solani* (MAFF712411) in the spring 2019 test.

Fig. 4. (A) Lisianthus cultivar ‘Papillon Pink Flash’ and (B) *Eustoma exaltatum* Ohkawa No. 1.

sion were extremely inhibited in our preliminary test conducted in summer (July to September) 2015 (data not shown). Summer was not suitable for evaluating resistance because it was difficult to maintain the appropriate temperature. In winter, the water temperature could be maintained at around 25°C—the optimal temperature for disease—but the air temperature dropped to 10°C at night, slowing disease progression.

In the winter 2016 and spring 2018 tests, as no disease was observed until 39 and 42 days, respectively, after inoculation, re-inoculation was required. In the winter 2016 test, the average air temperature in the week after inoculation was 15.3°C (Table 2), too low for disease occurrence. In contrast, in the spring 2018 test, it was 21.1°C, close to that in the autumn 2015 test (20.2°C), when disease progression occurred. This apparent contradiction may have occurred because of low temperatures in the two days immediately after inoculation in the spring 2018 test, although we were unable to confirm this association.

Thus, in addition to water temperature, the air temperature above the ground was also important for disease onset. In winter tests, it is necessary to maintain a suitable temperature by using a vinyl cover and a heater. Therefore, evaluating disease resistance in lisianthus is possible at most times of the year except summer, although the average air temperature varies with the season, and it was necessary to adjust the survey period depending on disease progression.

We identified several cultivars with resistance to isolate MAFF712388: ‘Papillon Pink Flash’ that was highly resistant and seven cultivars, namely ‘Annie Light Pink’, ‘Cecil Passion ME’, ‘Clare Pink’, ‘Cocktail Blue’, ‘Piccorosa Green (Ver. 2)’, ‘Suzukaze’, and ‘Voyage (Type 2) White’, were highly resistant or resistant. Of these, the highly resistant cultivar ‘Papillon Pink Flash’ and two highly resistant or resistant cultivars, ‘Voyage (Type 2) White’ and ‘Piccorosa Green (Ver. 2)’ are normal *grandiflorum*-type double-flowered cultivars. ‘Annie Light Pink’, ‘Cecil Passion ME’, ‘Clare Pink’, ‘Cocktail Blue’, and ‘Suzukaze’ were developed by Kaneko Seeds Co., Ltd., Maebashi, Japan and are small- and single-flowered cultivars with funnel-shaped corollas; judging from their plant forms they are likely derived from the wild species *E. exaltatum*. *Eustoma exaltatum* has been used to develop lisianthus with smaller flowers by interspecific crossing (Harbaugh, 2006), although lisianthus cultivars for cut flowers are derived mostly from *E. grandiflorum*. Moreover, we found that *E. exaltatum* Ohkawa No. 1 was highly resistant to isolate MAFF712388, with no disease symptoms. These results suggest that these cultivars may possess resistance genes derived from *E. exaltatum*.

In conclusion, we found that ‘Papillon Pink Flash’ and *E. exaltatum* Ohkawa No. 1 were highly resistant to *F. solani*. To our knowledge, this is the first report on

resistance to *F. solani* in lisianthus. Although almost all resistant lisianthus cultivars, including ‘Papillon Pink Flash’, are F₁ hybrids, ‘Shihai’ is a purebred cultivar and *E. exaltatum* Ohkawa No. 1 is an inbred line. Barba-Gonzalez et al. (2015) reported that interspecific crosses between *E. grandiflorum* and *E. exaltatum* can be performed with ease in both directions (female or male progenitors). Our group plans to analyze resistance heritability and develop DNA markers linked to resistance genes by using ‘Shihai’ and *E. exaltatum* Ohkawa No. 1.

We tested two *F. solani* isolates. However, it is not yet clear whether they belong to different races. Further studies are needed to analyze the resistance levels of the 30 tested accessions statistically, because they have not been tested at the same time using the same concentration of bud cells. We also plan to continue investigating resistance by using more *F. solani* isolates collected from major lisianthus production areas in Japan, as well as resistance to another *Fusarium* species, *F. oxysporum* f. sp. *eustomae*.

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