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Screening Method to Identify Watermelon Cultivars Resistant to *Acidovorax citrulli*, the Cause of Bacterial Fruit Blotch

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***Acidovorax citrulli* is a causative pathogen for bacterial fruit blotch (BFB) in *Cucurbitaceae*, including watermelon. The most effective method to control this plant disease is to cultivate resistant cultivars. Herein, this study aimed to establish an efficient screening method to determine the resistance of watermelon cultivars against *A. citrulli*. To this end, we explored the virulence of seven *A. citrulli* isolates belonging to clonal complex group I or II based on *gltA* gene analysis. Furthermore, we evaluated the BFB occurrence in the seedlings of two arbitrarily selected watermelon cultivars according to the growth stage of the watermelon seedlings, inoculum concentration, and incubation temperature after inoculation in a humidity chamber. Taken together, we established the following method to determine the resistance of watermelon cultivars against *A. citrulli*: watermelon seedlings at the fully expanded two-leaf stage can be spray-inoculated with an *A. citrulli* bacterial suspension at a concentration of 1.0×10^6 cfu/ml; after incubation for 48 h at 28°C in a humidity chamber, the plants were cultivated in a growth chamber at 25°C with 80% relative humidity under a 12-h light/dark cycle; and BFB occurrence on the plants can be investigated at 7 dpi by visual estimation of the diseased leaf area (%). Based on these experimental methods, we investigated the resistance degree of 43 commercial watermelon cultivars against *A. citrulli* KACC 17005. Therefore, our results can provide important information for the development of resistant cultivars against *A. citrulli*.

Keywords : bacterial fruit blotch, bioassay, clonal complex, disease resistance, environmental condition

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Bacterial fruit blotch (BFB) is caused by the Gram-negative bacterium *Acidovorax citrulli* in the *Cucurbitaceae* family, such as watermelon, melon, cucumber, and pumpkin, which results in severe economic loss in seed, seedling, and fruit production (Burdman and Walcott, 2012; Schaad et al., 2008; Walcott et al., 2004). BFB disease was first identified in Georgia in 1965 (Webb and Goth, 1965). Subsequently, outbreaks occurred in the watermelon fields of Florida, Indiana, Delaware, and Texas during the 1990s (Black et al., 1994; Evans and Mulrooney, 1991; Latin and Rane, 1990; Somodi et al., 1991). In South Korea, BFB was first found in the watermelon fields of Gochang, Jeollabuk-do in 1991 (Song et al., 1991). It has also been reported that severe BFB occurred in the watermelon fields of Goryeong, Gyeonsangbuk-do in 2005 and the melon fields of Gwangju and Naju, Jeollanam-do in 2006 (Seo et al., 2006). BFB, a seed-borne disease, is mainly transmitted from seed to seedlings but can also be spread by grafting, diseased residue, and rainfall (Dutta et al., 2012b; Rane and Latin, 1992; Walcott, 2017). BFB development in watermelon plants is highly influenced by temperature and relative humidity. Because environmental conditions in nurseries exhibit high temperatures, high relative humidities, and high plant densities, seedlings cultivated in nurseries can be infected more easily than seedlings grown in fields (Latin

and Hopkins, 1995).

The BFB causative agent *A. citrulli* infects the aboveground part of watermelon plants through the stomata and wounds. Symptoms on the leaves begin as dark brown water-soaked lesions on the back of the cotyledon and then extend along the hypocotyl to reach the true leaves (Latin and Hopkins, 1995). The disease progresses to the stems and branches through the veins to cause necrosis and blight (Latin and Hopkins, 1995). On fruits, *A. citrulli* infects through the stomata of 2- or 3-week-old immature fruits after blooming (Frankle et al., 1993). After infection, although symptoms do not appear at the immature stage of the fruits, disease symptoms appear just before the fruit matures. Irregular water-soaked lesions initially appear on the upper surfaces of mature fruits, and as the disease progresses, the mature fruits may split or exhibit rotting symptoms (Somodi et al., 1991). In the case of seed infection, it occurs when *A. citrulli*, invading the shoots and leaves, moves to the fruits through the xylem. Pathogens that enter through the flower can penetrate deeper into the seeds compared to those introduced via fruit infection, leading to extended bacterial survival within the seeds (Dutta et al., 2012a; Lessl et al., 2007; Walcott et al., 2003). In particular, pistil infection causes seed infection in symptomless fruits, whereas ovary pericarp infection causes seed infection along with disease symptoms. Therefore, seed decontamination is an important strategy for controlling BFB (Kubota et al., 2012).

Management of BFB on watermelon relies on cultural and chemical practices. However, the use of chemicals such as copper compounds is known to interfere with the growth of cucurbits, and the drug efficacy has been reduced due to the resistance of *A. citrulli* against copper compounds (Hopkins, 1991; Walcott, 2008). Currently, cultivation of disease-resistant cultivars has been considered the most effective control method for BFB of watermelon. Hopkins and Thompson (2002) reported that PI 482279 and PI 494817 from Zimbabwe and Zambia, respectively, were highly resistant resources against *A. citrulli*. Wechter et al. (2011) screened 332 resources of *Cucumis* spp. and consequently selected four *C. melo* and one *C. ficifolius* resources that exhibit high resistance against *A. citrulli*. Despite these efforts to develop resistant cultivars, there are no commercially known watermelon cultivars that represent significant resistance against *A. citrulli*.

To develop resistant cultivars through crossbreeding, it is important to select high-resistant genetic resources against *A. citrulli* based on an effective screening method to determine resistance. Nevertheless, there is limited information on efficient testing methods to determine the resistance of

watermelon against *A. citrulli*. Herein, we aimed (1) to explore the virulence of seven *A. citrulli* isolates belonging to clonal complex I (CC group I) and II (CC group II) on two arbitrarily selected watermelon cultivars, (2) to investigate the disease severity of watermelon seedlings according to the growth stage, inoculum concentration, and cultivation temperature after inoculation with *A. citrulli* KACC 17005, and (3) to examine the resistance degree of 43 watermelon cultivars against *A. citrulli* KACC 17005. Our screening method established in this study can provide important information for the development of resistant cultivars against *A. citrulli*.

Materials and Methods

***A. citrulli* isolates and their clonal complex.** Six isolates of *A. citrulli*, KACC 17000, KACC 17005, KACC 17909, KACC 18649, KACC 18783, and KACC 18785, were provided by the Korean Agricultural Culture Collection (KACC) of the Rural Development Administration (Jeonju, Korea), and the *A. citrulli* NWBSC074 isolate was obtained from Nongwoo Bio Co. Ltd. (Suwon, Korea). Each isolate was cultured on nutrient agar (Becton, Dickinson and Co., Sparks, MD, USA) medium at 30°C for 24 h and stored at –80°C with 20% glycerol.

To confirm the clonal complexes of seven *A. citrulli* isolates, the gDNA of each isolate was extracted and used to amplify the *gltA* gene with the primer set gltA1 (5'-GAAGTCCACGTTCGGGTAGA-3') and gltA2 (5'-TACATGTACCCGCAGAACCA-3'). For the polymerase chain reaction (PCR), each reaction contained 0.25 µl of Ex Taq polymerase (Takara Korea Biomedical Inc., Seoul, Korea), 5 µl of 10× Ex Taq Buffer, 4 µl of dNTP mixture, 500 nM each of forward and reverse primers, 50 ng of gDNA and nuclease-free water added to a final volume of 50 µl. The PCR cycling conditions were as follows: 95°C for 2 min followed by 35 cycles of PCR consisting of denaturation at 95°C for 1 min, annealing at 56°C for 30 s, and extension at 72°C for 2 min. After confirmation of the resulting PCR products on 1.5% agarose gel, the PCR products were sequenced by Macrogen, Inc., Seoul, Korea.

Plants and growth condition. The 43 commercial watermelon cultivars used in this study were as follows: 'Santakkul', 'Speedkkul', 'Hwansangkkul', 'Bravokkul', and 'Wonderfulkkul' from Nongwoo Bio Co. Ltd.; 'Nakdongkkul', 'Wellbeing', 'Jijonkkul', 'Sambokggul', 'Jinhansambokggul', 'Hanyeoreumkkul', 'Goodtime', 'Onsesang', 'Acekkul', 'Goodchoice', and 'Dangdanghan' from FarmHannong (Seoul, Korea); 'Nunettinekkul',

'Heukho', and 'Dalgonakkul' from Syngenta Seed (Seoul, Korea); 'Seotaeja', 'Seolkang102', 'Supergold', 'Shinseolkang102', 'Blackrubi', 'PlushoneyQ', 'Numberonekkul', 'Yeoreumen', 'Newkkokkoma' and 'Heukgwajeok' from Asia Seed (Seoul, Korea); 'Bestkkul', 'Chilbokggul', 'Supergranfree', 'Hwangkuemkkul', 'Soknorankkul', 'Noranbok', 'Noranbusibok' and 'Joeunbok' from Jeil Seed (Jeungpyeong, Korea); 'Backmagold' and 'Choikangkkul' from Koregon Seed (Anseong, Korea); 'Jangchunkkul' and 'Kamcheonkkul' from Jangchun Seed (Chilgok, Korea); 'Chodangkkul' from Samsung Seed (Pyeongtaek, Korea); and 'PMR Perfect' from Partner Seed (Gimje, Korea).

To measure the development of BFB in the watermelon seedlings, horticultural soil No. 2 (Punong, Gyeongju, Korea) was placed into a 4 × 8 plug pot (80 ml/pot; Bumnong, Jeongeup, Korea), and then, the seeds of the watermelon cultivars were sown in the soil of each pot and cultivated in a greenhouse at 25 ± 5°C for 13 days. A fully expanded two-leaf stage of the watermelon seedling was used for the experiment. For the BFB occurrence experiment based on the watermelon growth stage, the seeds were cultivated in a greenhouse at 25 ± 5°C for 9 (fully expanded one-leaf stage), 13 (fully expanded two-leaf stage), 19 (fully expanded three-leaf stage), 24 (fully expanded four-leaf stage), and 27 (expanded five-leaf stage) days (Supplementary Fig. 1).

Inoculum preparation and pathogen inoculation. *A. citrulli* isolates grown in a tryptic soy agar (Becton, Dickinson and Co.) medium at 30°C for 24 h were inoculated into a 5-ml tryptic soy broth (TSB; Becton, Dickinson and Co.) for the seed cultures. The resulting culture was added into a new TSB medium at a concentration of 1% and then incubated at 30°C with shaking (200 rpm). After incubation for 2 days, the bacterial culture was centrifuged at 8,000 rpm for 10 min, and the pellet of bacterial cells was resuspended in sterile water. The bacterial suspension was adjusted to an optical density of 0.1 at 600 nm (OD_{600}), corresponding to $1.0 × 10^8$ cfu/ml and then diluted (10^{-2}). A 2 ml of bacterial suspension (about $1.0 × 10^6$ cfu/ml) was spray-inoculated on both sides of the leaves of the watermelon seedlings. The inoculated seedlings were incubated in a humidity chamber at 28°C for 48 h, and then, the plants were transferred and grown in a growth chamber (25°C, 80% relative humidity) with a 12-h light/dark cycle.

Analysis of BFB occurrence. To assess the occurrence of BFB in the watermelon seedlings, the disease severity based on the lesion area of the leaves was daily investigated from 4 days post-inoculation (dpi) or 5 dpi depend-

ing on the experiment. The area under the disease progress curve (AUDPC) was also calculated according to the following equation (Jeger and Viljanen-Rollinson, 2001; Madden et al., 2007): AUDPC = $\Sigma ni = 1$ [$t(i+1) – ti$] × [$DS(i+1) + DSi$]/2, where *n* is the number of assessments; *ti* is the number of days elapsed from the inoculation day to the assessment date *i*, and *DSi* is the disease severity (%) on the assessment date *i*. All the experiments were performed in 10 replicates with two runs. All data were subjected to a one-way analysis of variance (ANOVA), and the significance of treatments was determined by Duncan's multiple range test ($P = 0.05$).

Results and Discussion

Clonal complex and virulence of the *A. citrulli* isolates. It has been reported that *A. citrulli* isolates can be divided into two groups: group I is moderately aggressive on all cucurbit host plants except watermelon, whereas group II causes more severe symptoms on watermelon than on other host plants (Walcott et al., 2004). These groups correspond to the clonal complex (CC) groups I and II, based on multilocus sequence typing analysis (Feng et al., 2009). Furthermore, the DNA sequence in the housekeeping gene *gltA* easily distinguishes the CC groups: isolates in CC group I have the nucleotides C, G, and A at positions 439, 442, and 451 from the start codon, respectively, whereas isolates in CC group II have G, A, and C at these same positions (Song et al., 2015; Yan et al., 2013). When we confirmed the *gltA* gene sequence of the seven *A. citrulli* isolates, two isolates KACC 18649 and KACC 18785 were classified into CC group I, and the other five isolates KACC 17000, KACC 17005, KACC 17909, KACC 18783, and NWBSC074 were classified into CC group II (Table 1). Among the seven *A. citrulli* isolates used in this study, our result that the two isolates KACC 17005 and NWBSC074 classified as CC group II was the same as the previous report by Song et al. (2020).

To investigate the virulence of the seven *A. citrulli* isolates on watermelon seedlings, we inoculated the isolates on the watermelon seedlings using the spray method, considering that the symptoms caused by *A. citrulli* are water-soaking in leaves followed by necrosis. When two arbitrarily selected watermelon cultivars 'Seotaeja' and 'Busibok' were inoculated with the seven *A. citrulli* isolates, KACC 18783 exhibited the highest average lesion area on the two cultivars at 7 dpi, followed by NWBSC074, KACC 17000, KACC 17005, KACC 18785, KACC 17909, and KACC 18649; KACC 18783, NWBSC074, KACC 17000, KACC 17005, and KACC 17909, belonging to CC group II exhib-

Table 1. *Acidovorax citrulli* isolates used in this study

Isolate	Isolated host	Isolation site	DNA polymorphism (439, 442, and 451)[a]	Clonal complex[b]	Source
KACC 17000	Watermelon	Buyeo, Korea	G, A, C	II	KACC
KACC 17005	Watermelon	Suwon, Korea	G, A, C	II	KACC
KACC 17909	Watermelon	Andong, Korea	G, A, C	II	KACC
KACC 18649	Watermelon	Buyeo, Korea	C, G, A	I	KACC
KACC 18783	Watermelon	Jinju, Korea	G, A, C	II	KACC
KACC 18785	Watermelon	Milyang, Korea	C, G, A	I	KACC
NWBSC074	Watermelon	Kimje, Korea	G, A, C	II	Nongwoo Bio Co.

[a]DNA polymorphism at the indicated base pair position from the start codon of the *gltA* gene.
[b]Based on DNA polymorphism in the housekeeping gene *gltA* (Song et al., 2020; Yan et al., 2013).

ited disease severity values of 100%, 98%, 74%, 60%, and 42%, respectively, whereas the isolates KACC 18649 and KACC 18785 belonging to CC group I exhibited disease severity values of 39% and 49%, respectively (Table 2). Furthermore, when we calculated the AUDPC based on the results of BFB development in watermelon seedlings, the average AUDPC values by KACC 18649 and KACC 18785 were the lowest among the tested isolates (Table 2). These results suggest that the isolates belonging to CC group II have stronger virulence in watermelon seedlings than the isolates belonging to CC group II.

It has been reported that the two clonal complex groups have different virulence against cucurbit plants: CC group I has moderate pathogenicity against cucurbits except for watermelon, and CC group II causes more severe symptoms in watermelon compared to other host plants (Burd-

Table 2. Virulence of the seven *Acidovorax citrulli* isolates on watermelon seedlings

Isolate	Cultivar	Disease severity (%)[a]				AUDPC[b]
		4 dpi	5 dpi	6 dpi	7 dpi	
KACC 18649	Seotaeja	16.2 ± 3.9	22.1 ± 3.1	31.2 ± 6.6	39.2 ± 7.0	49.8 c
	Busibok	16.4 ± 3.8	24.4 ± 3.8	30.6 ± 5.8	38.9 ± 6.2	52.1 c
KACC 17909	Seotaeja	20.0 ± 2.3	27.5 ± 4.6	33.3 ± 5.3	41.1 ± 5.9	58.1 c
	Busibok	19.8 ± 2.6	27.1 ± 4.5	33.9 ± 6.0	43.1 ± 4.4	58.5 c
KACC 18785	Seotaeja	23.1 ± 4.4	25.3 ± 4.0	44.4 ± 4.9	49.2 ± 4.8	61.4 c
	Busibok	21.8 ± 3.0	25.0 ± 4.4	42.8 ± 8.8	49.6 ± 6.8	60.7 c
KACC 17005	Seotaeja	24.7 ± 5.1	32.2 ± 4.5	46.7 ± 6.8	60.3 ± 6.3	74.7 b
	Busibok	23.9 ± 4.6	32.6 ± 3.9	50.9 ± 6.1	60.2 ± 5.6	74.6 b
KACC 17000	Seotaeja	27.6 ± 5.9	36.2 ± 5.3	59.0 ± 7.8	74.9 ± 4.3	87.4 b
	Busibok	25.7 ± 6.5	36.5 ± 6.9	57.3 ± 7.8	72.1 ± 6.6	85.4 b
NWBSC074	Seotaeja	33.0 ± 3.3	45.8 ± 4.8	71.7 ± 3.7	98.0 ± 2.1	111.4 a
	Busibok	32.4 ± 4.1	47.2 ± 6.2	70.0 ± 6.1	97.7 ± 2.4	112.2 a
KACC 18783	Seotaeja	37.8 ± 6.9	52.3 ± 2.6	78.3 ± 4.8	100 ± 0.0	121.2 a
	Busibok	32.5 ± 5.0	50.3 ± 6.7	77.5 ± 8.3	99.7 ± 0.9	116.4 a

[a]Thirteen-day-old watermelon seedlings were spray-inoculated with a bacterial suspension (1×10^6 cfu/ml). The infected plants were incubated in a humidity chamber at 28°C for 48 h and then transferred to a growth chamber at 25°C (80% relative humidity with a 12-h light/dark cycle). Diseased leaf area (%) was investigated daily from 4 days post-inoculation (dpi), and disease severity was calculated based on the diseased leaf area. Each value represents the mean ± standard deviation of two runs with 10 replicates.
[b]Area under the disease progress curve (AUDPC) = $\Sigma ni = 1 \ [t(i+1) – ti] \times [DS(i+1) + DSi]/2$; n = number of assessments, ti = number of days elapsed from the inoculation day to the assessment date i, DSi = disease severity (%) on assessment date i. Different small letters in a column indicate a significant difference at $P < 0.05$ according to Duncan's multiple range test.

man et al., 2005; Feng et al., 2009; Walcott et al., 2004; Yan et al., 2013). Similarly, our results showed that the isolates belonging to CC group II isolates had a greater disease severity compared to the CC group I isolates. From these results, for the establishment of a screening method to determine resistance against *A. citrulli* on watermelon, we selected the KACC 17005 isolate belonging to the CC group II with medium virulence among the tested isolates.

Effect of the plant growth stage on BFB occurrence. To investigate BFB occurrence according to the growth stage of watermelon seedlings, the KACC 17005 isolate was spray-inoculated onto five different growth stages of the watermelon cultivars 'Seotaeja' and 'Busibok.' When we measured the average disease severity of the two cultivars, the disease severity values at 7 dpi were 100%, 86%, 57%, 15%, and 6% from 9-day-old (fully expanded one-leaf), 13-day-old (fully expanded two-leaf), 19-day-old (fully expanded three-leaf), 24-day-old (four-leaf and five-leaf), and 27-day-old (expanded five-leaf stage) seedlings, respectively (Table 3). Furthermore, based on the disease severity observed at 5 to 8 dpi, the average AUDPCs of the two cultivars were 283, 236, 161, 44, and 18 for the 9-, 13-, 19-, 24-, and 27-day-old seedlings, respectively (Table 3). Namely, the watermelon seedlings at the fully expanded one-leaf stage were the most sensitive to *A. citrulli* KACC 17005, whereas the seedlings at the expanded five-leaf stage exhibited the lowest sensitivity to this bacterium.

Therefore, our results show that BFB occurrence decreased as the watermelon seedlings grew.

Bahar et al. (2009) reported that young seedlings of melon were highly sensitive to *A. citrulli*, and the mature plants were relatively resistant to this bacterium. They also suggest that 10- to 14-day-old seedlings of melon spray-inoculated with *A. citrulli* can be used to investigate the resistance of melon cultivars. Furthermore, Hopkins et al. (1993) investigated the resistance degree of 21 watermelon cultivars against *A. citrulli* on 14-day-old seedlings and consequently found 5 cultivars exhibiting resistance. Based on the results of this study, we suggest that a virulence assay can be performed with the fully expanded two-leaf stage of watermelon seedlings (approximately 13-day-old seedlings) for an efficient bioassay to investigate the resistance degree.

Effect of the inoculum concentration on BFB occurrence. To investigate BFB occurrence according to the inoculum concentration of *A. citrulli*, two watermelon cultivars 'Seotaeja' and 'Busibok' were spray-inoculated with five different concentrations of *A. citrulli* KACC 17005 bacterial cells. At 7 dpi, the average disease severities of the two cultivars inoculated at concentrations of 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 cfu/ml were 2.3%, 20%, 75%, 100%, and 100%, respectively (Table 4). Furthermore, based on the disease severity observed at 5 to 8 dpi, the average AUDPCs of the two cultivars were 6.4,

Table 3. Occurrence of bacterial fruit blotch on the watermelon cultivars according to the plant growth stage

Plant growth stage	Cultivar	Disease severity (%)[a]				AUDPC[b]
		5 dpi	6 dpi	7 dpi	8 dpi	
9-day-old	Seotaeja	79.0 ± 16.2	93.8 ± 8.1	100.0 ± 0.0	100.0 ± 0.0	283.3 a
	Busibok	77.5 ± 22.9	93.5 ± 7.5	100.0 ± 0.0	100.0 ± 0.0	282.3 a
13-day-old	Seotaeja	63.3 ± 14.5	73.0 ± 9.4	85.8 ± 6.3	94.0 ± 4.6	237.4 b
	Busibok	51.0 ± 11.5	75.0 ± 8.3	86.8 ± 8.8	96.0 ± 4.4	235.3 b
19-day-old	Seotaeja	27.8 ± 8.2	52.5 ± 14.5	59.5 ± 10.0	78.0 ± 9.4	164.9 c
	Busibok	23.8 ± 5.8	50.5 ± 6.9	53.5 ± 6.7	81.0 ± 8.1	156.4 c
24-day-old	Seotaeja	6.0 ± 3.1	9.8 ± 3.8	14.5 ± 4.6	33.5 ± 7.6	44.0 d
	Busibok	6.0 ± 4.2	9.5 ± 3.2	15.5 ± 4.3	30.0 ± 11.0	43.0 d
27-day-old	Seotaeja	0.5 ± 1.5	4.0 ± 3.8	5.8 ± 3.7	12.5 ± 3.4	16.3 e
	Busibok	2.5 ± 2.6	4.3 ± 4.8	6.3 ± 3.6	14.0 ± 4.9	18.8 e

[a]Watermelon seedlings were spray-inoculated with an *Acidovorax citrulli* KACC 17005 bacterial suspension (1×10^6 cfu/ml). The infected plants were incubated in a humidity chamber at 28°C for 48 h and then transferred to a growth chamber at 25°C (80% relative humidity with a 12-h light/dark cycle). Diseased leaf area (%) was investigated daily from 5 days post-inoculation (dpi), and disease severity was calculated based on the diseased leaf area. Each value represents the mean ± standard deviation of two runs with 10 replicates.
[b]Area under the disease progress curve (AUDPC) = $\Sigma ni = 1 \, [t(i+1) - ti] \times [DS(i+1) + DSi]/2$; n = number of assessments, ti = number of days elapsed from the inoculation day to the assessment date i, DSi = disease severity (%) on assessment date i. Different small letters in a column indicate a significant difference at $P < 0.05$ according to Duncan's multiple range test.

Table 4. Occurrence of bacterial fruit blotch on the watermelon cultivars according to the inoculum concentration

Inoculum concentration (cfu/ml)	Cultivar	Disease severity (%)[a]				AUDPC[b]
		5 dpi	6 dpi	7 dpi	8 dpi	
1.0×10^4	Seotaeja	2.0 ± 2.5	2.3 ± 2.6	2.8 ± 3.4	2.8 ± 3.4	7.4 d
	Busibok	1.8 ± 2.4	1.8 ± 2.4	1.8 ± 2.4	2.0 ± 2.5	5.4 d
1.0×10^5	Seotaeja	10.3 ± 3.8	13.3 ± 3.7	17.3 ± 7.3	19.3 ± 8.0	45.3 c
	Busibok	12.0 ± 5.9	19.0 ± 2.5	23.3 ± 7.7	26.0 ± 6.4	61.3 c
1.0×10^6	Seotaeja	49.8 ± 10.8	65.0 ± 12.0	71.5 ± 15.6	94.5 ± 4.6	208.6 b
	Busibok	46.0 ± 11.9	69.3 ± 10.8	77.5 ± 15.9	96.0 ± 5.0	217.8 b
1.0×10^7	Seotaeja	67.5 ± 7.3	92.0 ± 9.5	100.0 ± 0.0	100.0 ± 0.0	275.8 a
	Busibok	73.0 ± 9.8	95.0 ± 7.6	100.0 ± 0.0	100.0 ± 0.0	281.5 a
1.0×10^8	Seotaeja	80.8 ± 6.6	96.8 ± 8.0	100.0 ± 0.0	100.0 ± 0.0	287.1 a
	Busibok	82.3 ± 6.5	97.0 ± 5.7	100.0 ± 0.0	100.0 ± 0.0	288.1 a

[a]Watermelon seedlings at the fully expanded two-leaf stage were spray-inoculated with various inoculum concentrations of the *Acidovorax citrulli* KACC 17005 bacterial suspension. The infected plants were incubated in a humidity chamber at 28°C for 48 h and then transferred to a growth chamber at 25°C (80% relative humidity with a 12-h light/dark cycle). Diseased leaf area (%) was investigated daily from 5 days post-inoculation (dpi), and disease severity was calculated based on the diseased leaf area. Each value represents the mean ± standard deviation of two runs with 10 replicates.
[b]Area under the disease progress curve (AUDPC) = $\Sigma ni = 1 [t(i+1) - ti] \times [DS(i+1) + DSi]/2$; n = number of assessments, ti = number of days elapsed from the inoculation day to the assessment date i, DSi = disease severity (%) on assessment date i. Different small letters in a column indicate a significant difference at $P < 0.05$ according to Duncan's multiple range test.

53, 213, 279, and 288 for the seedlings inoculated with the concentrations of 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7, and 1.0×10^8 cfu/ml, respectively (Table 4). Therefore, our results show that the BFB occurrence increased as the concentration of the inoculated *A. citrulli* bacterial suspension increased.

Resistance of the host plants can be divided into qualitative resistance and quantitative resistance (Corwin and Kliebenstein, 2017; Pilet-Nayel et al., 2017). If the plant has a qualitative resistance, disease occurrence in resistant cultivars depends on the race of the pathogen, but there is no significant difference in disease occurrence depending on the inoculation concentration (Baik et al., 2011; Lee et al., 2015; Park et al., 2012). In contrast, it has been reported that the disease occurrence of plants with quantitative resistance increased as the inoculation concentration of the pathogenic isolate increased (Jo et al., 2014, 2016; Lee et al., 2017, 2020, 2022). Therefore, the inoculum concentration of pathogens is important for the bioassay to determine the resistance of cultivars showing quantitative resistance properties. In this study, when the watermelon seedlings were inoculated at concentrations of 1.0×10^7 or more, the disease severity values of the seedlings were 100% at 7 dpi (Table 4). However, the seedlings inoculated at concentrations of 1.0×10^4 and 1.0×10^5 cfu/ml represented disease severities of 2.4% and 23%, respectively, at 8 dpi. Given

that the disease severity of the watermelon seedlings inoculated with a concentration of 1.0×10^6 cfu/ml was 72-78% and 95-96% at 7 dpi and 8 dpi, respectively (Table 4), our results suggest that an inoculum concentration of 1.0×10^6 cfu/ml is effective for the bioassay of resistant watermelon to BFB.

Effect of environmental conditions on BFB occurrence of watermelon. To determine the incubation temperature after inoculation in the bioassay, the plants inoculated with the KACC 17005 isolate were incubated in a humidity chamber at 20°C, 25°C, 28°C, and 30°C for 48 h. The BFB at 20 and 25°C poorly occurred with disease severity values of 1.8% and 10% at 7 dpi, respectively (Table 5). However, when the inoculated seedlings were cultivated in a humidity chamber at 28 and 30°C, BFB severely occurred with disease severity values of 78% and 94% at 7 dpi, respectively. Based on the disease severity observed at 5 to 8 dpi, the average AUDPCs of the two cultivars were 4.8, 25, 208, and 250 at 20°C, 25°C, 28°C, and 30°C, respectively (Table 5).

Additionally, we compared BFB occurrence according to the incubation periods in a humidity chamber after the inoculation of *A. citrulli*. At 7 dpi, the average disease severities of the two cultivars incubated at 25°C for 24 and 48 h were 3% and 13%, respectively (Table 6). When the inocu-

Table 5. Occurrence of bacterial fruit blotch on the watermelon cultivars according to the temperature after inoculation

Temperature (°C)	Cultivar	Disease severity (%)[a]				AUDPC[b]
		5 dpi	6 dpi	7 dpi	8 dpi	
20	Seotaeja	1.3 ± 2.2	1.5 ± 2.4	2.0 ± 2.5	2.0 ± 2.5	5.1 d
	Busibok	1.3 ± 2.1	1.5 ± 2.4	1.5 ± 2.4	1.8 ± 2.4	4.4 d
25	Seotaeja	4.5 ± 5.1	5.3 ± 4.1	10.8 ± 3.7	14.8 ± 3.8	25.6 c
	Busibok	5.3 ± 4.1	6.0 ± 4.2	8.5 ± 5.2	12.5 ± 3.0	23.4 c
28	Seotaeja	48.0 ± 11.5	63.0 ± 12.3	79.3 ± 11.6	88.5 ± 10.8	210.5 b
	Busibok	44.3 ± 18.0	62.0 ± 17.9	75.9 ± 21.5	92.0 ± 8.3	205.9 b
30	Seotaeja	69.3 ± 12.6	75.0 ± 11.9	91.8 ± 6.7	95.0 ± 5.1	248.9 a
	Busibok	66.5 ± 15.0	74.0 ± 9.9	95.5 ± 5.1	97.8 ± 4.4	251.5 a

[a]Watermelon seedlings at the fully expanded two-leaf stage were spray-inoculated with an *Acidovorax citrulli* KACC 17005 bacterial suspension (1×10^6 cfu/ml). The infected plants were incubated in a humidity chamber at 20, 25, 28, and 30°C for 48 h, and then, each plant was transferred to a growth chamber at 25°C (80% relative humidity with a 12-h light/dark cycle). Diseased leaf area (%) was investigated daily from 5 days post-inoculation (dpi), and disease severity was calculated based on the diseased leaf area. Each value represents the mean ± standard deviation of two runs with 10 replicates.
[b]Area under the disease progress curve (AUDPC) = $\Sigma ni = 1$ [$t(i+1) - ti$] × [$DS(i+1) + DSi$]/2; n = number of assessments, ti = number of days elapsed from the inoculation day to the assessment date i, DSi = disease severity (%) on assessment date i. Different small letters in a column indicate a significant difference at $P < 0.05$ according to Duncan's multiple range test.

Table 6. Occurrence of bacterial fruit blotch on the watermelon cultivars according to the incubation period in a humidity chamber

Incubation period	Cultivar	Disease severity (%)[a]				AUDPC[b]
		4 dpi	5 dpi	6 dpi	7 dpi	
24 h at 25°C	Seotaeja	0.0 ± 0.0	1.0 ± 2.1	1.3 ± 2.2	2.5 ± 3.4	3.5 d
	Busibok	0.0 ± 1.5	2.5 ± 2.6	2.8 ± 2.6	3.5 ± 3.3	7.0 d
48 h at 25°C	Seotaeja	1.5 ± 2.4	8.0 ± 3.4	9.5 ± 2.2	12.0 ± 3.0	24.3 c
	Busibok	2.0 ± 2.5	7.0 ± 5.7	8.0 ± 5.2	13.0 ± 3.4	22.5 c
24 h at 28°C	Seotaeja	6.0 ± 3.1	10.5 ± 3.2	22.5 ± 10.9	33.0 ± 4.7	52.5 b
	Busibok	8.0 ± 4.1	10.5 ± 2.8	19.3 ± 7.7	27.3 ± 9.1	47.4 b
48 h at 28°C	Seotaeja	49.0 ± 17.3	62.0 ± 17.0	84.5 ± 9.4	96.8 ± 5.4	219.4 a
	Busibok	45.5 ± 18.9	60.0 ± 12.1	71.3 ± 7.4	83.3 ± 7.8	195.6 a

[a]Watermelon seedlings at the fully expanded two-leaf stage were spray-inoculated with an *Acidovorax citrulli* KACC 17005 bacterial suspension (1×10^6 cfu/ml). The infected plants were incubated for 24 or 48 h in a humidity chamber at 25 and 28°C, and then, each plant was transferred to a growth chamber at 25°C (80% relative humidity with a 12-h light/dark cycle). Diseased leaf area (%) was investigated daily from 5 days post-inoculation (dpi), and disease severity was calculated based on the diseased leaf area. Each value represents the mean ± standard deviation of two runs with 10 replicates.
[b]Area under the disease progress curve (AUDPC) = $\Sigma ni = 1$ [$t(i+1) - ti$] × [$DS(i+1) + DSi$]/2; n = number of assessments, ti = number of days elapsed from the inoculation day to the assessment date i, DSi = disease severity (%) on assessment date i. Different small letters in a column indicate a significant difference at $P < 0.05$ according to Duncan's multiple range test.

lated plants were incubated at 28°C, the average disease severities at 7 dpi were 30% for the 24-h incubation and 90% for the 48-h incubation (Table 6). Based on the disease severity observed at 5 to 8 dpi, the average AUDPCs of the two cultivars incubated at 25°C for 24 and 48 h were 5.3 and 23, respectively, whereas the average AUDPCs of the two cultivars incubated at 28°C for 24 and 48 h were 50 and 208, respectively (Table 6). Therefore, our results show that at both 25 and 28°C, when the inoculated plants were incubated for 48 h in a humidity chamber, more BFB occurred in the watermelon seedlings compared to the 24-h incubation.

Temperature is one of the important environmental factors in the disease occurrence and development of plant

Table 7. Resistance degree of the 43 commercial watermelon cultivars against *Acidovorax citrulli* KACC 17005

Cultivar	Diseased leaf area (%)[a]	Cultivar	Diseased leaf area (%)
Supergold	62.5 ± 29.9	Nunettinekkul	94.7 ± 9.4
Wellbeing	64.1 ± 41.3	Onsesang	95.0 ± 10.6
Blackrubi	84.5 ± 19.9	Heukho	95.0 ± 8.9
Chilbokggul	85.3 ± 21.5	Soknorankkul	95.3 ± 10.7
Chodangkkul	85.5 ± 19.3	Heukgwajeok	95.3 ± 7.4
Joeunbok	86.1 ± 17.5	Kamcheonkkul	95.3 ± 8.7
PMR Perfect	87.8 ± 18.3	Hanyeoreumkkul	95.6 ± 8.1
Santakkul	88.9 ± 12.4	Acekkul	95.6 ± 9.1
Yeoreumen	89.4 ± 12.5	Busibok	96.3 ± 8.9
Noranbok	89.4 ± 16.6	Supergranfree	96.3 ± 9.0
Sambokggul	89.4 ± 19.5	Backmagold	96.4 ± 5.9
Numberonekkul	90.3 ± 19.4	Newkkokkoma	96.7 ± 4.9
PlushoneyQ	90.8 ± 13.1	Dalgonakkul	97.1 ± 5.9
Speedkkul	91.6 ± 11.7	Noranbusibok	97.1 ± 8.5
Jinhansambokggul	91.7 ± 12.9	Bravokkul	97.2 ± 8.3
Goodchoice	91.7 ± 12.9	Goodtime	97.3 ± 4.6
Dangdanghan	92.1 ± 11.3	Seotaeja	97.5 ± 7.9
Jijonkkul	92.9 ± 9.2	Seolkang102	97.6 ± 6.6
Hwansangkkul	93.0 ± 11.3	Nakdongkkul	98.0 ± 6.2
Jangchunkkul	93.5 ± 12.3	Hwangkuemkkul	98.2 ± 7.3
Wonderfulkkul	94.4 ± 11.0	Choikangkkul	99.2 ± 2.6
Bestkkul	94.4 ± 9.2		

[a]Thirteen-day-old watermelon seedlings were spray-inoculated with an *A. citrulli* KACC 17005 bacterial suspension (1×10^6 cfu/ml). The infected plants were incubated in a humidity chamber at 28°C for 48 h and then transferred to a growth chamber at 25°C (80% relative humidity with a 12-h light/dark cycle). Diseased leaf area (%) was investigated at 7 days post-inoculation. Each value represents the mean ± standard deviation of two runs with 10 replicates.

disease resistance (Fox et al., 1971; Lee et al., 2013, 2018; Staub and Williams, 1972). For example, it has been reported that soft rot occurrences on radish plants and potatoes increased as the cultivation temperature after inoculation increased (Fox et al., 1971; Lee et al., 2018). In addition, Lee et al. (2013) reported that cabbage cultivars exhibited different resistance against *Xanthomonas campestris* pv. *campestris* at 22°C, but most cultivars were sensitive to this pathogen at 30°C. Similarly, Staub and Williams (1972) reported that black rot disease caused by *X. campestris* pv. *campestris* occurred in both resistant and susceptible cabbage cultivars at 28°C although the resistant cultivars were significantly resistant to the pathogen at 20-24°C. In this study, we evaluated BFB occurrence in watermelon seedlings according to the incubation temperature and periods after pathogen inoculation. Although the disease severity was greatest at 30°C, 28°C is thought to be an optimal incubation temperature for BFB development on watermelon

seedlings in a humidity chamber with a 48-h incubation, considering that there are no commercial resistant cultivars to *A. citrulli*.

Resistance degree of the 43 commercial watermelon cultivars. In this study, we established an effective bioassay method to determine the resistance of watermelon plants against *A. citrulli*. Briefly, watermelon seedlings were grown until the fully expanded two-leaf stage in a greenhouse (25 ± 5°C), and then, the seedlings were spray-inoculated with an *A. citrulli* bacterial suspension at a concentration of 1.0×10^6 cfu/ml. After incubation for 48 h at 28°C in a humidity chamber, the plants were moved to a growth chamber (25°C, relative humidity 80%) and cultivated under a 12-h light/dark cycle. BFB occurrence on the plants was investigated at 7 dpi based on the diseased leaf area (%).

Based on the established bioassay method mentioned

above, we evaluated BFB occurrence of the 43 commercial watermelon cultivars with the *A. citrulli* KACC 17005 isolate. Among the tested cultivars, there was no cultivar that had strong resistance to the isolate (Table 7). However, the cultivars 'Supergold' and 'Wellbeing' exhibited a diseased leaf area of 63% and 64%, respectively, and the other cultivars exhibited diseased leaf areas ranging from 85% to 99% (Table 7). It was found that most commercially available watermelon cultivars are susceptible to *A. citrulli*. Considering that the cultivars 'Supergold' and 'Wellbeing' showed weak resistance, they can be used for a bioassay to develop resistant watermelon against *A. citrulli* causing BFB.

Herein, we established the screening methods by which the disease severity of watermelon seedlings was systemically investigated according to the growth stage, inoculum concentration, and cultivation temperature after inoculation with *A. citrulli*, making it more comprehensive than the current method (Song et al., 2020). However, despite these efforts, the screening method established in this study is still labor-intensive and needs to be improved.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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