Horticulture, Environment, and Biotechnology
https://doi.org/10.1007/s13580-019-00190-0

RESEARCH REPORT

Molecular characterization of *Acidovorax citrulli* strain NIHHS15-280 causing bacterial fruit blotch disease in Korea and screening of resistance sources in melon

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Received: 9 May 2019 / Revised: 24 September 2019 / Accepted: 9 October 2019

Abstract
Bacterial fruit blotch (BFB), caused by *Acidovorax citrulli*, is an economically devastating disease in melon. Molecular characterization of causal strains and identification of sources of resistance are necessary to control this important disease. Many strains of BFB were identified by partial sequencing and documented in GenBank. Recently, there was an outbreak of BFB strain NIHHS15-280 in melon fields (Goksung, Jeonnam, Korea) that severely hampered melon production in Korea. For this reason, we characterized NIHHS15-280 by partially sequencing the 16S rRNA gene using PCR-based markers. The partial sequence of the 16S rRNA gene of NIHHS15-280 was much more similar to *A. citrulli* strains associated with melon and watermelon hosts than other cucurbit hosts. A PCR-based assay identified the strain (NIHHS15-280) as belonging to *A. citrulli* group I, which predominantly infects non-watermelon cucurbits (virulent to melon). Thirty-five melon genotypes were screened against NIHHS15-280 in a detached-leaf inoculation assay. The lack of visible disease symptoms 11 days after inoculation in lines PI353814 and PI140471 indicated these to be highly resistant lines, whereas moderate resistance was observed in lines PI420145 and SCNU1154 and cultivars ME6 and ME5. Also, the most resistant lines were corroborated by a fruit inoculation assay. The characterized *A. citrulli* strain NIHHS15-280 (group I) and identified resistant lines will serve as a valuable resource for further genetic studies and improvement of BFB resistance in melon.

Keywords 16S rRNA gene · *Acidovorax citrulli* · Bacterial fruit blotch · Melon · Multivariate analysis · Partial sequencing

1 Introduction

Melon (*Cucumis melo* L., Cucurbitaceae) is a delicious and musky-flavored fruit rich in vitamins, minerals, and health-promoting antioxidants, and is an economically important horticultural crop in terms of worldwide cultivation and consumption. However, the production of this fruit is greatly hampered by bacterial fruit blotch (BFB) disease, caused by the bio-trophic, gram-negative, seed-borne bacterium *Acidovorax citrulli* (formerly *Acidovorax avenae* subsp. *citrulli*) (Schaad et al. 2008). BFB was first reported in watermelon seedlings in Georgia, United States, in 1965 (Sowell Jr and Schaad 1979), and further studied following devastating outbreaks in other parts of the United States in the late 1980s (Latin and Rane 1990; Somodi et al. 1991; Schaad et al. 2003). The disease became widespread in Brazil during the late 1990s (Robbs et al. 1991; Assis et al. 1999; Halfeld-Vieira and Nechet 2007) and in Israel from 2000 to 2003 (Burdman et al. 2005). Initially, most of the economic loss due to BFB was reported in watermelon (Wall and Santos 1988; Wall et al. 1990; Somodi et al. 1991; Hopkins 1995; Schaad et al. 2003); however, in recent years, melons have also been severely affected by the disease (Isakeit et al. 1997, 1998; O'brien and Martin 1999; Burdman et al. 2005).

The ability of BFB to spread via inadvertent distribution of contaminated commercial seeds and affect many cucurbit species has recently made it an even more serious

Communicated by Sung-Chur Sim.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13580-019-00190-0) contains supplementary material, which is available to authorized users.

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Published online: 27 November 2019

threat (Bahar et al. 2009; Burdman et al. 2005; Burdman and Walcott 2012). The disease has now been reported in 22 countries in melon and other cucurbits—such as citron melon, prickly paddy melon, cucumber, pumpkin, and squash, as well as several types of gourds (Isakeit et al. 1997; Langston Jr et al. 1999; Martin and Horlock 2002; Martin et al. 1999; Bahar and Burdman 2010; Burdman and Walcott 2012). In South Korea, BFB in melon was first reported in Gochang (Noh et al. 2014) in 1990. An outbreak occurred recently because of the use of contaminated seeds and the expansion of the BFB's host range to other cucurbits, causing substantial losses in marketable yield (Noh et al. 2014). Under favorable environmental conditions, especially during rainy seasons and highly fluctuating temperature regimes, the disease can cause 80 to 100% loss in melon production (Conceição et al. 2014; de Melo et al. 2015; Sales Júnior and Menezes 2001). A survey of watermelon diseases in Korea reported BFB in 3.9% of farmers' fields, revealing it to be an important disease, along with gummy stem blight, fruit and vine rot, and powdery mildew (Noh et al. 2014).

The recent global outbreak of the disease in diverse cucurbits can be attributed to changes in the population structure of the causal agent, which is evident from reports of intra-specific or intra-sub-specific variations among strains and from the global distribution of the pathogen (Burdman et al. 2005). *A. citrulli* strain diversity was first reported in 1991 (Somodi et al. 1991), and the species was later categorized into two distinct groups (I and II) on the basis of DNA fingerprinting and bio-chemical properties. Group II strains were predominantly collected from watermelon, while group I strains were collected from non-watermelon cucurbits (Walcott et al. 2004), with each group showing aggressive virulence towards its corresponding host(s) (Burdman et al. 2005; Oliveira et al. 2007). Such group-specific diversity in virulence necessitates the identification of the prevalent *A. citrulli* strains prior to undertaking any crop improvement program. A total of 62 (33 and 29) Korean *A. citrulli* isolates collected from various cucurbits were analyzed by multi-locus phylogeny using five conserved loci (16S rRNA, *adk*, *gltA*, *glyA*, *pilT*) and two distinct groups (KCC1 and KCC2) were identified in the population based on group-specific genetic variation (Song et al. 2015, 2018). PCR-based assays were developed to target specific genes, such as 16S rDNA, internally transcribed spacer (ITS) regions, YD-repeat proteins, and *hrp* genes, which often lack substantial polymorphisms between closely related bacterial species and thus may not be suitable for the detection of host crop-wise virulent *A. citrulli* strains (Slovareva et al. 2019; Eckshtain-Levi et al. 2016; Han et al. 2016). In this regard, only a few studies have been conducted on Korean *A. citrulli* strains, only six to seven of which were found to be melon specific (Adhikari et al. 2017; Rahimi-Midani et al. 2018b; Choi et al. 2016).

Despite the use of chemical seed disinfectants and chemical control measures in infested fields, widespread outbreaks of BFB frequently occur, especially under wet field conditions (Burdman and Walcott 2012). These require the use of resistant cultivars to safeguard melon production against the disease. Screening of 1344 U.S. *Citrullus* spp. and *Praecitrullus fistulosus* accessions identified five showing varying degrees of resistance to *A. citrulli* (Hopkins and Thompson 2002). Bahar et al. (2009) reported only three melon accessions to be relatively resistant to *A. citrulli.* No cultivars with complete, monogenic resistance have been reported in melon so far (Rahimi-Midani et al. 2018a). Until now, no Korean cultivars have been screened for resistance towards BFB.

In this study, we characterized a prevalent Korean *A. citrulli* strain, NIHHS15-280, via partial sequencing of 16S rRNA, determined the group of the strain via available PCR-based molecular markers, and identified sources of resistance against this strain in promising Korean melon cultivars and several global melon genotypes.

2 Materials and methods

2.1 Bacterial strains: collection and inoculum preparation

Sixteen bacterial strains belonging to three different genera (*Acidovorax*, *Xanthomonas*, and *Pseudomonas*) were collected from various sources for this study (Table 1). The *A. citrulli* strain NIHHS15-280 was obtained from the National Institute of Horticultural and Herbal Science (NIHHS), South Korea. This strain was originally collected from an oriental melon plant from Goksung, Jeonnam, South Korea. All bacteria were cultured on King's B (KB) media supplemented with 100 µg ml^{-1} ampicillin (Schaad et al. 2001) for 36 to 48 h at 28 °C until formation of bacterial colonies. A bacterial suspension was prepared by adding 5 ml of sterile, double-distilled water to the culture plate and gently scraping the bacterial colonies using a sterile L-shaped rubber spreader. For inoculation, the bacterial suspension was diluted to a final concentration of ~ 1×10^6 colony forming units (cfu) ml^{-1} (Bahar et al. 2009).

2.2 Bacterial DNA extraction and partial sequencing of 16S rRNA of strain NIHHS15-280

Genomic DNA of all 16 bacterial strains was extracted using a Qiagen DNeasy kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. A 1349-bp PCR-amplified segment of the 16S rRNA gene was directly sequenced using the sequencing service Macrogen Inc., Seoul, South Korea. The resulting sequence was subjected to NCBI BLAST

Table 1 Plant pathogenic bacterial strains belonging to Acidovorax and other bacterial genera along with their hosts and presence/absence of amplification by model group II A. citrulli strain specific primers

Sl.	Bacterial strain	Bacterial species	Host plant	Source	PCR amplification							
					Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5	Fr. 6	Fr. 7	Fr. 8
1	NIHHS15-280	*Acidovorax. citrulli*	Melon	South Korea	−	−	−	−	−	−	−	−
2	KACC18782	*A. citrulli*	Melon	South Korea	−	−	−	−	−	−	−	−
3	NIHHS16-088	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
4	KACC17000)	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
5	KACC17001	*A. citrulli*	Watermelon	South Korea	+	+	+	+	−	+	+	+
6	KACC18785	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
7	KACC17005	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
8	KACC17909	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
9	KACC17910	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
10	KACC17911	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
11	KACC17912	*A. citrulli*	Watermelon	South Korea	+	+	+	+	+	+	+	+
12	KACC18648	*A. avenae*	Rice	South Korea	−	−	−	−	−	−	−	−
13	KACC17130	*A. avenae* subsp. *avenae*	Hog millet	South Korea	−	−	−	−	−	−	−	−
14	HRIW3811	*Xanthomonas campestris* pv. *campestris*	*Brassica oleracea*	USA	−	−	−	−	−	−	−	−
15	WHRI8305	*X. campestris* pv. *raphani*	*B. rapa* var. *perviridis*	United Kingdom	−	−	−	−	−	−	−	−
16	ICMP13051	*Pseudomonas syringae* pv. *maculicola*	*B. oleracea* var. *capitata*	New Zealand	−	−	−	−	−	−	−	−

NIHHS National Institute of Horticultural and Herbal Science (NIHHS), South Korea; *KACC* Korean Agricultural Culture Collection, South Korea; *ICMP* International Collection of Microorganisms from Plants (ICMP), Landcare Center, Auckland, New Zealand; *HRIW* culture collection of the School of Life Sciences, Wellesbourne Campus, University of Warwick, UK. Fr.1 to Fr.8 indicate eight pairs of primers originally designed by Eckshtain-Levi et al. (2016) to detect model group II *A. citrulli* strain AAC00-1. Primer sequences are given in Table S2. The "+" and "−" indicate amplification and nonamplification of target genomic fragments, respectively (also presented as agarose gel picture in Fig. S3)

(https://blast.ncbi.nlm.nih.gov/Blast.cgi). The matching sequences were aligned using ClustalW version 1.83 (Thompson et al. 1994) to identify sequence polymorphism, and a phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates using BioEdit version 7.2.5 (Hall 1999).

2.3 PCR-based assay

The bacterial group of the test strain NIHHS15-280 was determined using 'Group II' and 'Group I & II'-type *Acidovorax* strain-specific primers (Table S1) via a PCR-based assay (Zivanovic and Walcott 2016). NIHHS15-280 was further tested in a PCR-based assay using eight pairs of markers (Table S2) originally developed by Eckshtain-Levi et al. (2016) to detect the model group II strain AAC00-1. The PCR reactions were performed as described by the respective authors.

2.4 Plant materials and growing conditions

Thirty-five diverse melon genotypes were tested. Of these, 14 lines were collected from the U.S. National Plant Germplasm System (https://npgsweb.ars-grin.gov/gringlobal/search.aspx); one line was provided by the Horticulture Laboratory of Sunchon National University, South Korea; and 20 cultivars were collected from Asia Seed Co., Ltd. South Korea (Table 2). Seedlings of all 35 genotypes were grown in 32-cell trays containing artificial soil mix in a controlled plant growth chamber at 25 ± 2 °C with a 16-h day length and 440 µmol m^{-2} s^{-1} light intensity at the bench level.

2.5 Detached-leaf inoculation assay

Fully grown melon leaves with long petioles were cut from greenhouse-grown 5-week-old seedlings of the 35 melon genotypes. Each detached leaf was then laid on top of a closed 14-cm Petri dish filled with water, with the petiole inserted through a hole in the lid to allow for the uptake of water from inside the petri dish (Hassan et al. 2018). The bacterial inoculum was spray-inoculated onto the detached leaves until it began to run off. The Petri dishes with inoculated leaves were placed in a sealed plastic box to maintain high relative humidity. The water in the Petri dishes was replenished as needed. Disease response of the 35 genotypes was assessed based on the days until the first symptom appeared, disease severity score, number of lesions/leaf, and percentage of diseased leaf area at 7,

Table 2 Melon genotypes and their disease severity scores for BFB caused by A. citrulli strain NIHHS15-280 as determined by detached-leaf inoculation assay

Genotype	Taxonomy	Origin	Disease score	Statistical significance
PI353814	*Cucumis melo* subsp. *melo*	Israel	1.0	d
PI140471	*C. melo*	USA	1.0	d
PI614525	*C. melo* subsp. *agrestis*	India	6.3	a
PI614596	*C. melo* subsp. *melo*	India	6.3	a
PI420145	*C. melo*	USA	1.3	cd
PI482398	*C. melo* subsp. *melo*	USA	3.3	bcd
CornellZPPM	*C. melo*	USA	3.0	bcd
PI482399	*C. melo* subsp. *melo*	USA	4.3	ab
PI536473	*C. melo* subsp. *agrestis*	Maldives	3.3	bcd
PI504558	*C. ficifolius*	India	3.6	abcd
PI343701	*C. pustulatus*	Nigeria	4.0	abc
PI614401	*C. melo* subsp. *melo*	India	3.3	bcd
PI614601	*C. melo* subsp. *melo*	India	3.0	bcd
PI147065	*C. anguria* var. *longaculeatus*	Brazil	4.3	ab
SCNU1154	*C. melo*	South Korea	2.0	bcd
ME1, ME4, ME5, ME6, ME11, ME16	*C. melo*	South Korea	2.3	bcd
ME2, ME3, ME7, ME9, ME10, ME12, ME13, ME14, ME15, ME18, ME21, ME25, ME28	*C. melo*	South Korea	3.3	bcd
ME 8	*C. melo*	South Korea	3.6	abcd

PI plant introduction. All PI accessions, including CornellZPPM and SCNU 1154, are lines and all ME accessions are cultivars. All PI accessions including CornellZPPM were obtained from the U.S. Department of Agriculture and the remaining genotypes from Sunchon National University, South Korea. Values followed by the same letter are not significantly different ($P < 0.05$), as per Tukey's pairwise comparisons

9, and 11 days after inoculation (DAI). Disease severity was scored on a scale of 1–6, indicating a diseased leaf area of ≤ 10%, 11–25%, 26–50%, 51–75%, 76–90%, and > 90%, respectively (Bahar et al. 2009) (Fig. S1). Plants with scores of 1 and 2 were considered highly resistant and resistant, respectively, while plants with scores of 3–5 and 6 were considered susceptible and highly susceptible, respectively. The experimental design was completely randomized with three replicates (plants) per genotype.

2.6 Fruit inoculation assay

The harvested mature fruits of four highly or moderately resistant and four highly susceptible genotypes selected based on the detached-leaf assay (see Results) were inoculated by injecting 1 ml of bacterial suspension at 1×10^8 cfu/ml through the rind with a 5-ml syringe, to a depth of no more than 1 cm, in two different sites per fruit. Inoculated fruits were placed in plastic boxes (one fruit/box) and incubated at 25–28 °C. Disease severity was evaluated at 7 DAI according to the following scale: 0, no symptoms; 1, small water-soaked lesions on the rind (surface lesions); 2, large lesions penetrating into the rind; 3, lesions penetrating into the flesh of the fruit; 4, extensive penetration and collapse of fruit tissues; and 5, complete fruit rot (Burdman et al. 2005).

2.7 Statistical analysis

Analysis of variance (ANOVA) was performed to test the statistical significance of the genotypic variability for disease response against NIHHS15-280 in both the detached-leaf assay and the fruit inoculation assay. Tukey's pairwise comparison (post hoc) was performed to separate genotypic means. Overall genotypic variability was analyzed by principal component analysis (PCA) using all parameters of the detached-leaf inoculation assay. All analyses were performed using Minitab 18 statistical software (Minitab Inc., State College, PA, USA).

2.8 Data availability

A partial sequence of the 16S rRNA gene of *A. citrulli* strain NIHHS15-280 was deposited into the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) under GenBank accession number MH542625.1.

3 Results

3.1 Molecular identification of bacterial strain NIHHS15-280

To confirm the identity of NIHHS15-280 at the molecular level, a 1349-bp portion of the 16S rRNA gene was sequenced (GenBank accession number MH542625.1). This partial sequence was 100% identical to the corresponding partial sequences of strains XD0611-7, WM0922-3, xjgb-wy6, pslb-25, ZZ-1, SY-3, FC376, JZT8, and FC374, and 99% identical to strains AACHN-1, A1, RKFB 794, JXZS4, and FC455, all belonging to *A. citrulli* (Fig. 1 and Table S3). The test strain NIHHS15-280 differs from this latter group of strains by only two SNPs, at base pairs 194 and 699 of the sequence of GenBank accession MH542625.1 (Fig. S2). The fact that the test strain had high percentages of sequence identity to a large number of *A. citrulli* strains confirmed the molecular identity of the test strain NIHHS15-280 as *A. citrulli* (Fig. 1).

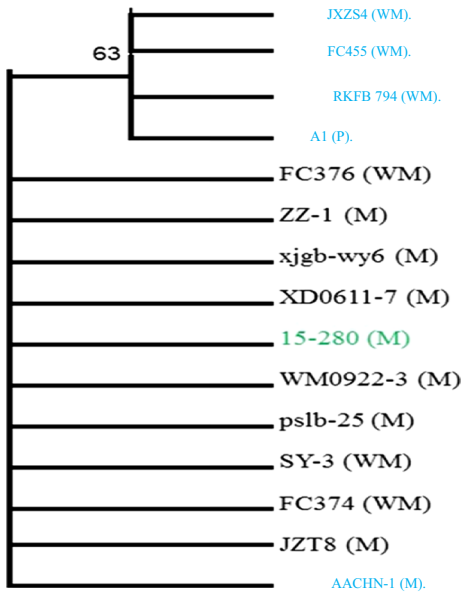

Fig. 1 Phylogenetic tree confirming the test strain NIHHS15-280 as *A. citrulli.* The tree was constructed based on the sequence similarity of the 16S rRNA gene (partial sequence) of strain NIHHS15-280 to those of other highly similar *A. citrulli* strains, as identified by NCBI BLAST searching. The partial sequence of the 16S rRNA gene of the test strain NIHHS15-280 (green text) is 99% and 100% identical to those of strains, indicated by blue and black text, respectively (see the sequence alignment in Table S3). ClustalW version 1.83 was used to identify sequence polymorphisms, and a phylogenetic tree was constructed using the neighbor-joining method by BioEdit version 7.2.5. The number at the branch indicates the percent bootstrap value based on 1000 replications. M, WM, and P in parentheses indicate that the host of the indicated strain is melon, watermelon, and pumpkin, respectively

3.2 Determination of the group of the test strain NIHHS15-280 via PCR assay

To classify the test strain NIHHS15-280 into one of the two *A. citrulli* bacterial groups, the genomic DNA of 16 bacterial strains was amplified in PCR assays using the markers for 'Group I & II' and 'Group II' originally designed by Zivanovic and Walcott (2016) (see Table S1). The fact that DNA was amplified from NIHHS15-280 (along with a known group I *A. citrulli* strain, KACC18782) by the 'Group I & II' primer pair, but not by the 'Group II' primer pair, clearly suggests that NIHHS15-280 was of the group I type of *A. citrulli* (Fig. 2). As expected, the control strain DNA from *Acidovorax* species other than *A. citrulli* (*A. avenae*, *A. avenae* subsp. *avenae*) and from non-*Acidovorax* species was not amplified by either of the primer pairs.

To further confirm the group of the test strain, all 16 bacterial strains were tested using eight PCR-based markers (see Table S2) that were previously reported to detect the model group II strain AAC00-1 (Eckshtain-Levi et al. 2016). In this case, DNA from the test *A. citrulli* strain NIHHS15-280 and known group I strain KACC18782 was also not amplified by any of the eight primer pairs (Fig. S3). DNA from all of the other strains, which were previously reported to be collected from watermelon (i.e., related to group II), was amplified by these markers. The control strains belonging to other *Acidovorax* species and non-*Acidovorax* species showed no amplification. Together, these data suggest that strain NIHHS15-280 is associated with non-watermelon cucurbits (i.e., group I). This molecular characterization is consistent with the preliminary record of NIHHS, which shows that NIHHS15-280 was collected from melon plants in Goksung, Jeonnam, South Korea. Subsequent pathogenicity tests on diverse melon lines were performed using this strain.

3.3 Disease response of melon genotypes in detached-leaf inoculation assay

Disease responses of 35 melon genotypes to *A. citrulli* strain NIHHS15-280 were assessed in the detached-leaf assay in terms of days to the appearance of the first symptom, number of lesions per leaf, diseased leaf area, and disease severity score. Significant variation ($P < 0.01$) was observed among the 35 genotypes for these traits, as determined by one-way ANOVA (Table S4). Among the lines showing symptoms during the 11-DAI assay period, symptoms first appeared in ME18, ME14, and PI614596 (4 DAI) and last appeared in CornellZPPM (9 DAI; Fig. 3a). No clear symptoms were observed in PI353814 or PI140471 within the 11-DAI assay period. In the remaining genotypes, the first symptoms were observed between 5 and 7 DAI. In terms of the number of lesions per leaf at 11 DAI (maximum duration of the assay), no lesions were visible in nonsymptomatic lines PI353814 and PI140471. Only one lesion was observed by 11 DAI in PI420145, CornellZPPM, and ME1, and two lesions were observed in PI614601, ME2, ME25, and ME28 (Fig. 3b). The highest numbers of lesions were observed in PI614525 (13 lesions), PI504558 (11 lesions), and ME8 (9 lesions). Only 7% of the leaf area of PI420145 was affected by the disease and hence, along with non-symptomatic lines PI353814 and PI140471, affected by 1.3% and 1.0, respectively and showed the lowest disease severity score, while 16% of

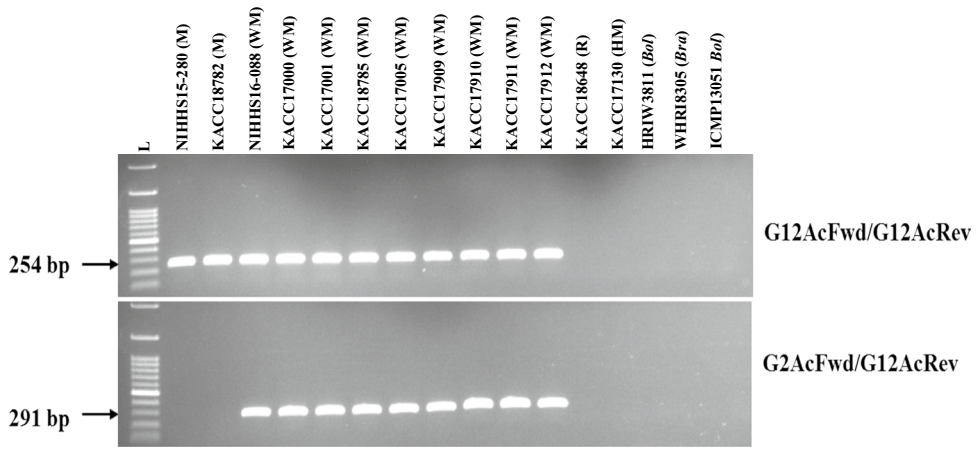

Fig. 2 Amplification of bacterial strains by primers for 'Group I & II' and 'Group II' type *A. citrulli* strains. Known 'Group I'-type *A. citrulli* strain KACC18782 and known 'Group II'-type strains NIHHS16-088, KACC17000, KACC17001, KACC18785, KACC17005, KACC17909, KACC17910, KACC17911, and KACC17912 were used along with the test strain NIHHS15-280. Lane L contains a size marker consisting of 12 chromatography-purified DNA fragments ranging from 3000 to 100 bp. M, WM, R, HM, *Bol* and *Bra* in parentheses indicates strains from melon, watermelon, rice, hog millet, *Brassica oleracea* and *B. rapa*, respectively

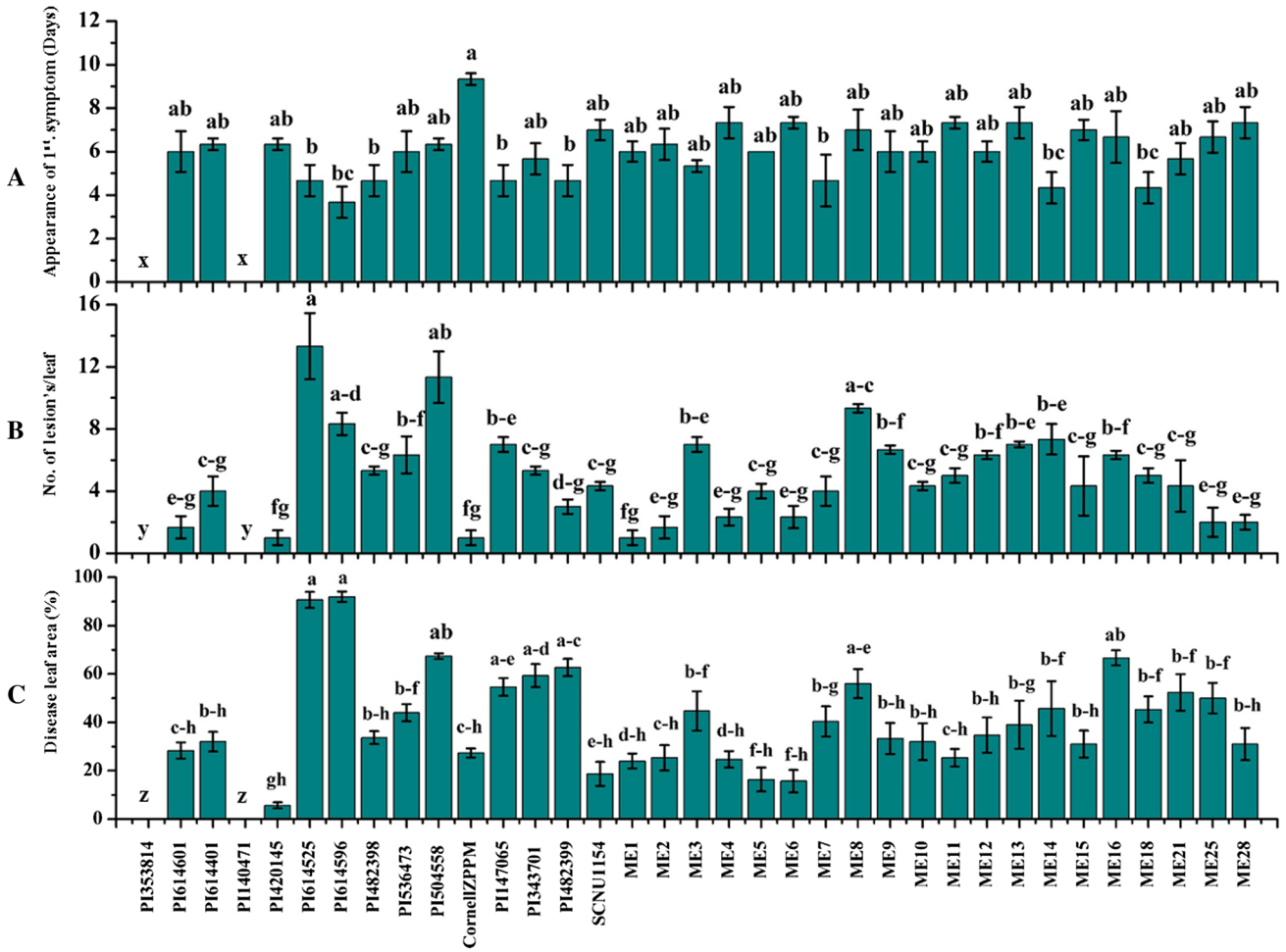

Fig. 3 Disease responses of 35 melon genotypes in terms of days to appearance of first symptom in leaf (**a**), number of lesions/leaf at 11 DAI (**b**), and percent diseased leaf area at 11 DAI (**c**) after inoculation with *A. citrulli* strain NIHHS15-280 in a detached-leaf assay. Days to the appearance of the first symptom, the number of lesion's/leaf, and disease leaf area (%) in PI353814 and PI140471 during the 11-day assay period is indicated by x, y and z in figure a, b and c, respectively. Data are presented as mean ± SD, $n = 3$. For each trait, statistical values marked with the same letter are not significantly different ($P < 0.05$), as determined by Tukey's pairwise comparison

the leaf area of ME5 and ME6 (score 2.3) and 19% of the leaf area of SCNU1154 (score 2.0) was affected (Fig. 3c and Table 2). More than 50% of the leaf area of PI504558, PI147065, PI343701, PI482399, ME8, ME16, ME21, and ME25 was affected, while the highest diseased leaf area (> 90%) was observed in lines PI614525 and PI614596 (score 6.3).

The correlation among disease response parameters from the detached-leaf inoculation assay was determined by Pearson's correlation analysis. Days to the appearance of the first symptom did not show consistently significant correlation with diseased leaf area at any time points, as it was only significantly correlated with diseased leaf area at 9 DAI, but not with diseased leaf area at 7 or 11 DAI (Table S5). Days to the appearance of the first symptom was also not significantly correlated with the number of lesions per leaf at 11 DAI. However, the number of lesions at 11 DAI showed significant positive correlation with the diseased leaf area at 11 DAI.

3.4 Overall variability among melon genotypes as assessed by PCA

We performed PCA to illustrate the overall variability among the 35 melon genotypes for all the studied disease response parameters from the detached-leaf inoculation assay. The first three extracted principal components (PCs) explained 83.5% of the total variation in the datasets (Fig. 4). Principal component 1 (PC1) accounted for 61.5% of the total variability, which can largely be attributed to higher positive coefficients of diseased leaf area at 9 and 11 DAI and number of lesions per leaf at 11 DAI (Table S6). It can thus be inferred that PC1 separated the genotypes mainly based on their disease response towards *A. citrulli* strain

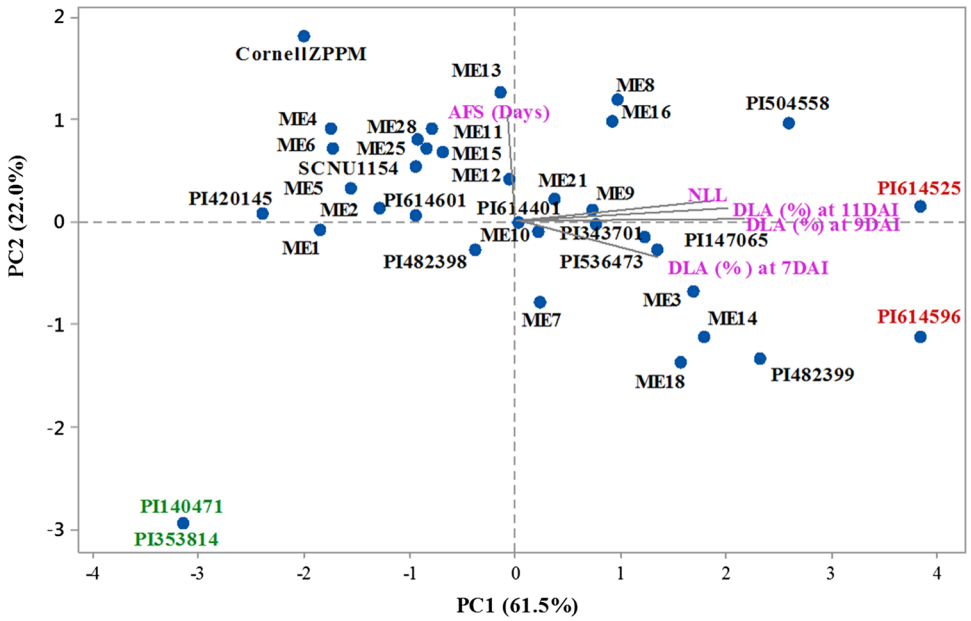

Fig. 4 Biplot showing the association among 35 melon genotypes (highly resistant and susceptible are in green and red text, respectively, and all remaining are in black text) and disease response parameters (pink text) of detached leaves upon inoculation with *A. citrulli* strain NIHHS15-280, as determined by PCA. Principal component 1 (PC1) clearly distinguished the genotypes based on their resistance against the strain. *AFS* appearance of first symptom (Days); *NLL* number of lesions/leaf; *DLA* disease leaf area (%)

NIHHS15-280, which is also evident from the positioning of the two highly resistant lines (PI353814 and PI140471) and two highly susceptible lines (PI614525 and PI614596) in opposite quadrants of the PCA biplot (Fig. 4). PC2 accounted for 22.0% of the overall variation, which can be attributed to higher positive coefficient of days to the appearance of the first symptom.

3.5 Disease progression in selected resistant and susceptible genotypes

Differences in disease progression between resistant and susceptible genotypes were assessed by comparing the increase in percentage of diseased leaf area over time between four highly or moderately resistant genotypes (lines PI353814, PI140471, PI420145, and SCNU1154 and cultivar ME6) and four highly susceptible genotypes (lines PI614525, PI504558, PI614596, and cultivar ME16). The percentage of diseased leaf area increased more rapidly in the susceptible genotypes than in the resistant genotypes (Fig. 5). Less than 5% leaf area in the resistant lines was affected by BFB at 7 DAI, which increased to a maximum of only 15% at 11 DAI (Figs. 5, 6a). In contrast, the diseased leaf area of the susceptible lines increased from 5 to 25% at 7 DAI, to 67 to 68% in ME16 and PI504558 and 91 to 92% in PI614525 and PI614596 at 11 DAI (Figs. 5, 6a).

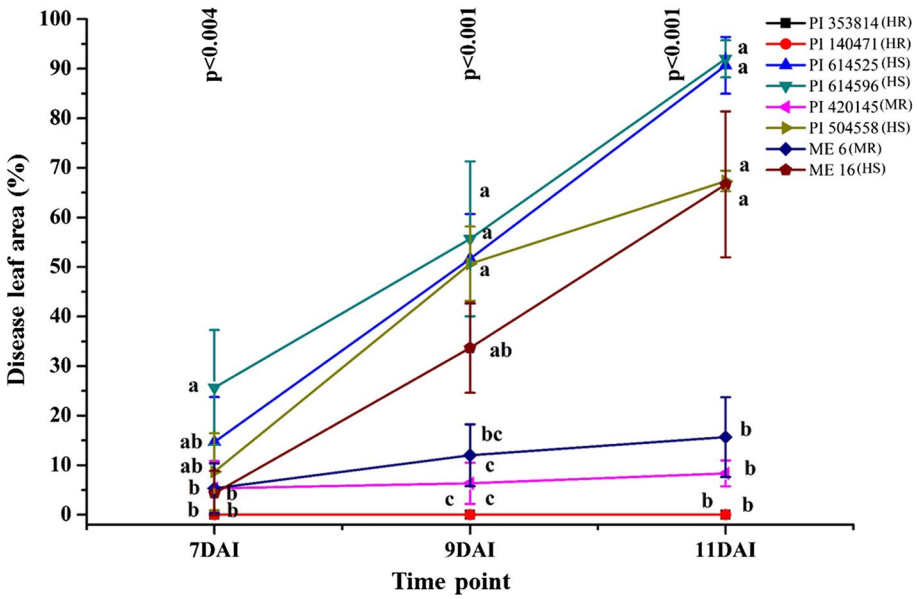

Fig. 5 Progression of disease in the four highly or moderately resistant (PI353814, PI140471, PI420145, and ME6) and four highly susceptible (PI614525, PI504558, PI614596, and ME16) genotypes against *A. citrulli* strain NIHHS15-280, measured in terms of percent diseased leaf area at 7, 9, and 11 DAI. The Y and X axis indicates diseased leaf area (%) and time point, respectively. Data are presented as mean ± SD, $n = 3$. Different letters across genotypes at each time point indicate statistically different diseased leaf areas ($P < 0.05$), as per Tukey's pairwise comparisons. HR, HS, and MR denotes highly, moderately resistant and highly susceptible, respectively

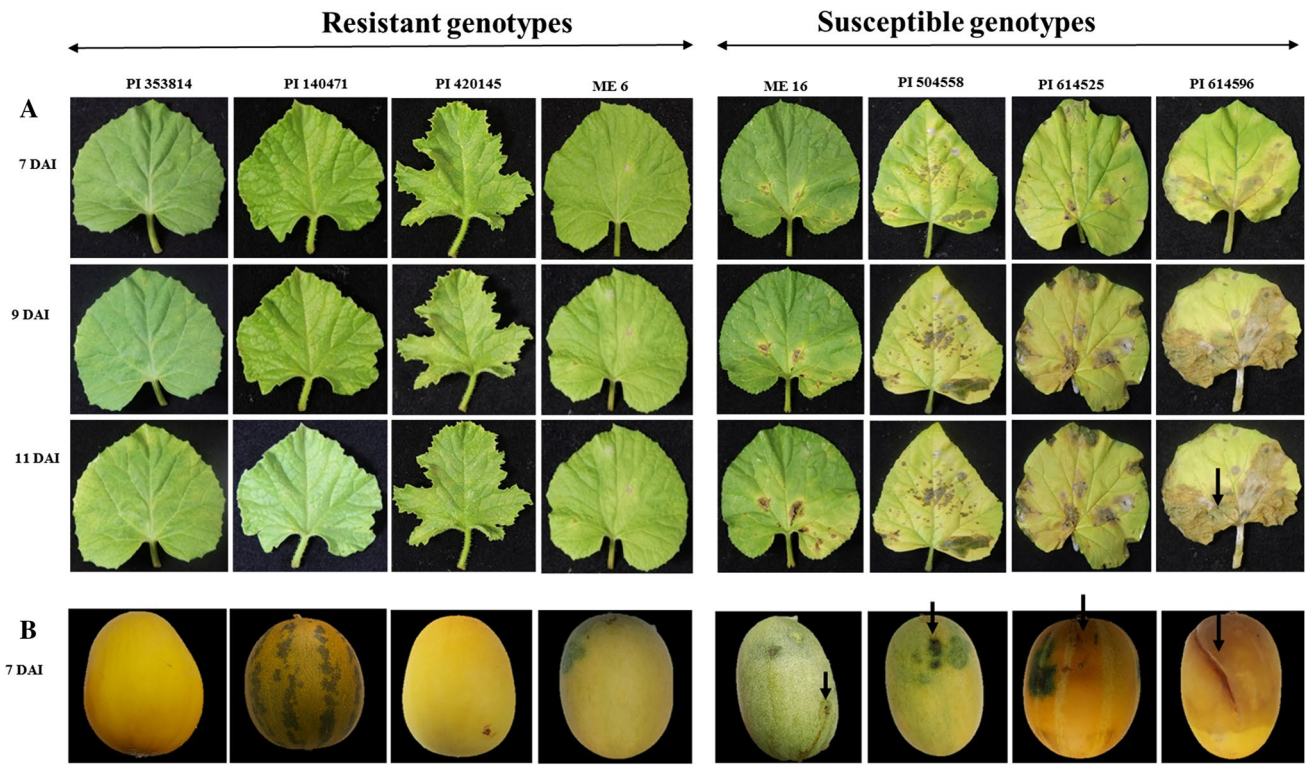

Fig. 6 BFB disease symptoms in the leaves (**a**) and fruits (**b**) of selected resistant and susceptible melon genotypes caused by *A. citrulli* strain NIHHS15-280 at 7, 9, and 11 DAI. Black arrows indicate typical BFB symptoms in leaf and fruit

3.6 Disease response of melon genotypes in fruit inoculation assay

The disease response of the selected resistant and susceptible genotypes was also investigated in mature fruits inoculated with NIHHS15-280. The inoculated fruits showed typical BFB symptoms that ranged from small lesions barely penetrating the rind to complete rot of the fruit, and disease severity varied significantly across genotypes ($P < 0.05$). Among the four resistant cultivars, no symptoms were visible in lines PI140471 or PI353814 (disease severity score in fruit of 0.0) while 10.33% and 20.33% of the diseased area was visible in the fruits of ME6 (severity score of 1.3) and PI420145 (severity score of 2.3), respectively (Table 3 and Fig. 6b). In contrast, the diseased fruit surface area of the susceptible genotypes ranged from 37.67% (in ME16, severity score of 3.3) to 94.33% (almost complete fruit rot in PI614596, severity score of 5.3) after inoculation with NIHHS15-280. The diseased fruit surface area at 7 DAI in the fruit inoculation assay showed significant linear correlation with diseased leaf area at 11 DAI in the detached-leaf inoculation assay (Fig. S4).

Table 3 Disease response of selected resistant and susceptible melon genotypes against A. citrulli strain NIHHS15-280 in fruit inoculation assay

Genotype	Infected fruit (%)	Disease severity score	Statistical significance
PI614596	94.33	5.3	a
PI614525	70.67	3.6	ab
PI504558	44.67	3.0	abc
ME 16	37.67	3.3	abc
PI420145	20.33	2.3	bc
ME 6	10.33	1.3	c
PI140471	0.00	0.0	c
PI353814	0.00	0.0	c

Four highly or moderately resistant (PI353814, PI140471, PI420145, and ME6) and four highly susceptible (PI614525, PI504558, PI614596, and ME16) genotypes (selected based on the results of detached-leaf inoculation assay) were tested in a fruit inoculation assay. Scores of 0 to 5 indicate "no symptoms" to "complete fruit rot" (see Materials and methods section for details). Data presented are means of three replicates. Statistical values followed by the same letter are not significantly different ($P < 0.001$), as per Tukey's pairwise comparisons

4 Discussion

BFB, caused by *A. citrulli*, has become a devastating disease in melon that completely destroys its marketability (Hopkins and Thompson 2002; Walcott et al. 2004; Silva et al. 2016; Eckshtain-Levi et al. 2016). In this study, we established the molecular identity of a South Korean *A. citrulli* strain, NIHHS15-280, via partial sequencing of the 16S rRNA gene, and determined its bacterial group by using previously reported PCR-based molecular markers.

Partial sequencing of the 16S rRNA gene, which is found in all prokaryotic organisms and is highly conserved among members of a particular species, offers a precise and reproducible method to identify microbes at a molecular level (Clarridge 2004; Wilson et al. 1991; Relman et al. 1992; Srinivasa et al. 2012; Patel 2001). The high similarity (99–100% identity) of the partial sequence of the strain NIHHS15-280 with other known *A. citrulli* strains confirmed its identity (Fig. 1 and Table S3).

A. citrulli has been categorized into two genetically and physiologically distinct groups, I and II, using an array of techniques, such as gas chromatography-fatty acid methyl ester (GC-FAME) and substrate-utilization profiling, restriction enzyme (*SpeI*) digestion and pulse field gel electrophoresis (PFGE) DNA fingerprinting, multilocus sequence typing of housekeeping genes (HKG), and repetitive element palindromic polymerase chain reaction (rep-PCR) (Burdman and Walcott 2012; Eckshtain-Levi et al. 2016; Feng et al. 2009; Walcott et al. 2004; Walcott and Gitaitis 2000). Group II strains have been isolated predominantly from—and are highly virulent to—watermelon, but are only mildly aggressive to non-watermelon hosts, while group I strains have been isolated mainly from non-watermelon hosts (Walcott and Gitaitis 2000; Walcott et al. 2004; Burdman et al. 2005; Feng et al. 2009). We tested NIHHS15-280 using PCR-based 'Group I & II' and 'Group II' markers that were originally designed from the *Aave_2166* gene (Zivanovic and Walcott 2016). The fact that NIHHS15-280 DNA was only amplified by 'Group I & II' primers indicated that this strain belongs to group I (Fig. 2). To further validate this, we investigated the amplification of the 16 bacterial strains by eight sets of primers developed from eight unique genomic fragments from model group II strain AAC00-1 that are absent in the genome of model group 1 strain M6 (Eckshtain-Levi et al. 2016). As expected, DNA from test strain NIHHS15-280 and group I strain KACC18782 was not amplified by the eight primer sets for model group II strain AAC00-1 (Fig. S3). These data suggest that the test strain (NIHHS15-280) is a group I-type strain.

Identifying sources of resistance to BFB in cucurbits and harnessing them to develop resistant commercial cultivars is necessary because chemical and agronomic control measures can be ineffective, especially in wet growing seasons (Adhikari et al. 2017; Burdman and Walcott 2012; Hopkins 1995). The few germplasm-screening studies conducted so far have indicated only limited resistance to BFB in members of cucurbits, including melon (Bahar et al. 2009; Burdman et al. 2005; Hopkins and Thompson 2002; O'Brien and Martin 1999; Patrick Wechter et al. 2011). Among the 12 resistant genotypes identified by greenhouse-based screening of more than 1300 *Citrullus* spp. and *Praecitrullus fistulosus* accessions, only five were found to show a moderate to high level of resistance against *A. avenae* subsp. *citrulli* in a field evaluation (Hopkins and Thompson 2002). In melon, Wechter et al. (2011) identified four lines (PI353814, PI381171, PI536573, and PI614401) out of 332 accessions that showed significantly higher resistance than susceptible cultivars when tested using seed vacuum-infusion assays and standard spray inoculation tests. Bahar et al. (2009) identified only one resistant cultivar (cv. ADIR339) by screening 35 melon genotypes using seed transmission, seedling, and mature plant inoculation experiments. In this study, we identified two highly resistant lines (PI353814 and PI140471) and four moderately resistant lines (PI420145, ME6, ME5, and SCNU1154) against the BFB-causing *A. citrulli* strain NIHHS15-280. Wechter et al. (2011) identified lines PI343701 and PI614401 as highly resistant and resistant, respectively, whereas we observed substantial susceptible responses (60% and 32% diseased leaf area, respectively) in these genotypes. This disparity might be due to the differences in assay procedures, strain virulence, and environmental conditions.

Identification of these genotypes with resistance to the Korean *A. citrulli* strain NIHHS15-280 will surely be helpful in developing elite cultivars with increased resistance to BFB. However, screening of melon germplasm against other prevalent strains under field conditions may help to identify genotypes that confer broad-spectrum resistance. Segregating populations are being developed by crossing genotypes with contrasting levels of BFB resistance as a means to decipher the inheritance pattern of the trait and to map loci responsible for resistance to BFB. This can pave the way for fine-mapping the genes that confer resistance against this devastating disease along with development of functional-gene-based molecular markers to aid in breeding programs aimed at improving BFB resistance in melon, for an example core sets of EST-SSR markers have been developed for seed purity test from segregating population, which could be used in QTL mapping for candidate gene identification (Nam et al. 2019).

Acknowledgements This study was supported by the Center for Horticultural Seed Development (Golden Seed Grant Number:

213007-05-3-CG100) of the Ministry of Agriculture, Food and Rural Affairs in the Republic of Korea (MAFRA). We thank the Korean Agriculture Culture Collection (KACC), National Institute of Horticultural and Herbal Science (NIHHS), South Korea; the International Collection of Microorganisms from Plants (ICMP), New Zealand; and the School of Life Sciences, University of Warwick, UK, for providing bacterial strains.

Author contributions MRI prepared and cultured the samples, isolated DNA, performed wet lab experiments, analyzed the data, interpreted the results, and wrote the first draft of the manuscript. UKN and HJJ assisted in sequence analysis and PCR-based assays. MA assisted in wet lab experiments and in preparing the tables and figures. MRH extensively revised and finalized the manuscript. ISN, JIP, HTK, and MRH conceived the project and designed the study. All authors read and approved the final draft of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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