Incidence of *Alternaria* Species Associated with Watermelon Leaf Blight in Korea

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Alternaria leaf blight is one of the most common diseases in watermelon worldwide. In Korea, however, the *Alternaria* species causing the watermelon leaf blight have not been investigated thoroughly. A total of 16 *Alternaria* isolates was recovered from diseased watermelon leaves with leaf blight symptoms, which were collected from 14 fields in Korea. Analysis of internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and RNA polymerase II second largest subunit (*RPB2*) were not competent to differentiate the *Alternaria* isolates. On the contrary, analysis of amplicon size of the histone H3 (*HIS3*) gene successfully differentiated the isolates into three *Alternaria* subgroups, and further sequence analysis of them identified three *Alternaria* spp. *Alternaria tenuissima*, *A. gaisen*, and *A. alternata*. Representative *Alternaria* isolates from three species induced dark brown leaf spot lesions on detached watermelon leaves, indicating that *A. tenuissima*, *A. gaisen*, and *A. alternata* are all causal agents of Alternaria leaf blight. Our results indicate that the *Alternaria* species associated watermelon leaf blight in Korea is more complex than reported previously. This is the first report regarding the population structure of *Alternaria* species causing watermelon leaf blight in Korea.

Keywords: *Alternaria*, disease, *HIS3*, leaf blight, watermelon

Watermelon (*Citrullus lanatus* Thunb.), a member of the Cucurbitaceae, is an economically important fruit crop and its annual production in 2018 was estimated at 103 million tons worldwide (Food and Agriculture Organization of the United Nations, 2018). It is widely grown in the most parts of Africa, the Caribbean, the southern United States, and Southeast Asia (Ma et al., 2021).

Dramatic problems in watermelon production have been caused by various pathogens, such as bacterial fruit blotch by *Acidovorax citrulli*, Fusarium wilt by *Fusarium oxysporum*, powdery mildew by *Podosphaera xanthii*, gummy stem blight by *Didymella bryoniae*, anthracnose by *Colletotrichum orbiculare*, and leaf spot disease by *Nigrospora sphaerica* (Burdman and Walcott, 2012; Ismail and Abd Razak, 2021; Noh et al., 2014). On the top of that, watermelon leaf blight caused by the genus *Alternaria* is an epidemic disease worldwide, resulting in reduced fruit size, quality, and yield (Chopra et al., 1974; Kim et al., 1994; Zhao et al., 2016b). *Alternaria cucumerina* was initially considered to be the causal agent of leaf blight in watermelons and other Cucurbitaceae crops (Jackson and Weber, 1959). Recently, however, various other *Alternaria* spp. including *A. cucumerina*, *A. alternata*, *A. tenuissima*, *A. gaisen*, and *A. infectoria* have also been identified from watermelons in China, Serbia, and the United States (Blagojević et al., 2020; Ma et al., 2021; Zhao et al., 2016b; Zhou and Everts, 2008). In Korea, after *A. cucumerina* was first reported as a causal agent of watermelon leaf blight (Kim et al., 1994), additional studies concerning leaf blight have been rarely reported. Therefore, there is no further information about the presence of additional *Alternaria* spp.
on watermelon in Korea. Although the survey conducted in Korea during 2008 to 2012 to examine different watermelon diseases reported *Alternaria* spp. to be one of 18 fungal pathogens, its species has not been identified (Noh et al., 2014).

Identification of the *Alternaria* species in watermelons is essential for setting up disease-management programs. The taxonomy of species within *Alternaria* have been previously based on morphological and physiological characteristics and differences in their host plants (Simmons, 2007). However, even in a single host, their morphological variation caused by environmental conditions, prolonged sub-culturing, and cultivation medium complicated species recognition. Molecular methods, therefore, have been more recently used to complement morphology-based approaches (Kang et al., 2001; Nishikawa and Nakashima, 2019). In the current study, we investigated what kinds of *Alternaria* species are associated with watermelon leaf blight in Korea and how to distinguish their species by comparing nucleotide sequences of key loci, internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), RNA polymerase II second largest subunit (*RPB2*), and histone H3 (*HIS3*), commonly used for *Alternaria* species separation. Sequence analysis of the *HIS3* gene successfully differentiated causal agents of Alternaria leaf blight into three different species, *A. tenuissima*, *A. alternata*, and *A. gaisen*. In addition to *A. cucumerina*, a previously reported causal agent, at least four different *Alternaria* spp. are now associated with watermelon leaf blight in Korea, which should be considered to set up effective management strategies.

### Materials and Methods

**Sample collection and isolation of *Alternaria* species.** In 2019 and 2020, a total of 14 fields from nine cities among the major watermelon production areas in Korea were selected for *Alternaria* spp. survey. Watermelon leaves and stems showing *Alternaria*-induced leaf blight lesions were harvested. Small pieces of tissue (10 × 10 mm) were removed from the margins between the healthy and symptomatic tissues, and their surfaces were disinfected with 70% ethanol for 1 min and rinsed with sterilized dH₂O three times. The pieces were then placed on potato dextrose agar (PDA) (MBcell, Seoul, Korea) plates and incubated at 28°C in light for seven days. Multiple fungal colonies were obtained from each symptomatic tissue, and only those with *Alternaria* morphologies were selected as isolates. The isolates were sub-cultured onto new PDA plates for single spore purification.

**Alternaria genomic DNA extraction.** Genomic DNA was extracted following a previously described protocol (Chen and Ronald, 1999). For the extraction, *Alternaria* mycelia were scrapped from the surface of the seven-day cultures grown on PDA and placed in a 2 ml microcentrifuge tube containing 700 µl cetyltrimethyl-ammonium bromide (CTAB) extraction buffer (3% (w/v) CTAB, 20 mM EDTA pH 8.0, 1.42 M NaCl, 100 mM Tris-HCl pH 8.0, 2% (w/v) polyvinylpyrrolidone, and 0.2% β-mercaptoethanol). After 10 min incubation at 65°C, the addition of 570 µl of chloroform:isoamyl alcohol (24:1) to the tube, and 10 min in a centrifuge at 13,000 rpm, genomic DNA was precipitated with 0.7 volume isopropanol. After centrifuging for 10 min at 13,000 rpm, the genomic DNA pellet was washed with 75% ethanol and then dried at room temperature. The genomic DNA was dissolved in 20 µl sterilized dH₂O and stored at −20°C.

**Polymerase chain reaction amplification.** The ITS region of ribosomal DNA, *GAPDH* gene, *RPB2* gene, and *HIS3* gene were amplified using ITS1 (5’-TCCTCCGTAAGGTGAAACCTGCGG-3’)/ITS4 (5’-TCTTATTGATATGCG-3’) (White et al., 1990), *gpd1* (5’-CAACGGCTTCCGTCGATTG-3’)/gpd2 (5’-GGAAGCAGTTGTTGTGCG-3’)/frRPB2-7cR (5’-CCCATRGCTTGTYYRCCCAT-3’) (Berbee et al., 1999), *RPB2-5F2* (5’-GGGGWGYACAGAGAAGGC-3’)/fRPB2-7cR (5’-CCCATRGCTTGTYYRCCCAT-3’) (Liu et al., 1999), and *H3-1a* (5’-ACTAAGCAGACC-GCCCCGAGG-3’)/H3-1b (5’-GCCGGCGAGCTGGATTGTCCTT-3’) (Glass and Donaldson, 1995) primers, respectively. Polymerase chain reaction (PCR) was performed in a 20 µl reaction mixture containing 0.1 µl FIREPol DNA Polymerase (0.5 units) (Solisbiodyne, Tartu, Estonia), 1 µl each primer (10 µM), 0.2 µl dNTP mix (200 µM), 2 µl 10× buffer, 1 µl template (approximately 100 ng), 4 µl 5X Q-solution, and 10.7 µl sterilized dH₂O. Amplifications were performed with an initial denaturation step at 94°C for 3 min followed by 35 cycles of 15 s at 94°C, 15 s at 53°C and 60 s at 72°C for ITS region, 35 cycles of 15 s at 94°C, 15 s at 57°C and 60 s at 72°C for *GAPDH*, 35 cycles of 15 s at 94°C, 15 s at 56°C and 2 min at 72°C for *RPB2*, 35 cycles of 15 s at 94°C, 15 s at 67°C and 60 s at 72°C for *HIS3*, and final extension at 72°C for 7 min. Amplified fragments were loaded on an agarose gel (0.8% w/v or 1.5% w/v) stained with RedSafe Nucleic Acid Staining Solution (20,000×) (Intronbio, Seongnam, Korea) and visualized under UV light.

**Morphological characterization.** The morphology of *Alternaria* isolates was examined as previously described.
Different *Alternaria* Species Causing Watermelon Leaf Blight

isolates were pure-cultured on PDA plates at 28°C in the Alternaria modification. Briefly, to prepare the inoculum, according to the previously described detached-leaf inoculation method (Pryor and Michailides, 2002) with minor modification, leaves from symptomatic leaves were detached and used as inoculum source. The detached leaves were placed on papers soaked with distilled water in plastic boxes (25 × 19 × 5 cm [length × width × height]) and three mycelial plugs per leaf were placed on the detached leaves. The plastic boxes were individually covered with transparent plastics to maintain 100% humidity and incubated at 25°C with a 12 h photoperiod per day for seven days. The disease index was calculated after each disease area was divided by the whole leaf area using ImageJ. *Alternaria* isolates were re-isolated consistently from all the inoculated leaf to fulfill Koch’s postulates.

**Results**

**Sampled locations and *Alternaria* isolates.** Watermelon leaves and stems showing *Alternaria*-induced leaf blight lesions were collected from 14 fields in nine cities of Korea for *Alternaria* spp. survey (Table 1, Fig. 1A). Multiple fungal colonies were obtained from each symptomatic tissue (Fig. 1B), and only those with *Alternaria* morphologies were selected as isolates. A total of 16 *Alternaria* isolates were obtained and cultured to homogeneity. All isolates developed loosely cottony and light brown to grey colonies on PDA after incubation at 28°C for seven days in the dark. (Supplementary Fig. 1).

**Classification of *Alternaria* species based on ITS region.** It has been reported that the amplicon lengths of the ITS regions varied among the *Alternaria* genus (Blagojević et al., 2020; Woudenberg et al., 2013). The ITS regions of *Alternaria* isolates were amplified with primers, ITS1 and ITS2.

**Pathogenicity tests.** *Alternaria* inoculation was performed according to the previously described detached-leaf inoculation method (Pryor and Michailides, 2002) with minor modification. Briefly, to prepare the inoculum, *Alternaria* isolates were pure-cultured on PDA plates at 28°C in the dark for seven days and each mycelial plug was cut from the actively growing margin of the fungal colonies. Inoculations were conducted on detached leaves of watermelon cultivar, ‘Charleston gray’ (Wu et al., 2019). Fully expanded leaves were collected from six- to seven-week-old watermelon plants grown in a growth chamber. For the inoculation of each *Alternaria* isolate, six leaves from ‘Charleston gray’ were placed on papers soaked with distilled water in plastic boxes (25 × 19 × 5 cm [length × width × height]) and three mycelial plugs per leaf were placed on the detached leaves. The plastic boxes were individually covered with transparent plastics to maintain 100% humidity and incubated at 25°C with a 12 h photoperiod per day for seven days. The disease index was calculated after each disease area was divided by the whole leaf area using ImageJ. *Alternaria* isolates were re-isolated consistently from all the inoculated leaf to fulfill Koch’s postulates.

**Table 1.** Origins of *Alternaria* isolates and GenBank accession numbers of ITS region

| Origin (city) | Isolate | ITS region Accession no. | Genus |
|--------------|---------|--------------------------|-------|
| Chungju      | CJ191-1 | MW474904                 | *Alternaria* sp. |
| Chungju      | CJ191-3 | MW474905                 | *Alternaria* sp. |
| Cheongyang   | CY202-11| MW474906                 | *Alternaria* sp. |
| Cheongyang   | CY202-16| MW474907                 | *Alternaria* sp. |
| Cheongyang   | CY202-18| MW474908                 | *Alternaria* sp. |
| Cheongyang   | CY202-25| MW474909                 | *Alternaria* sp. |
| Cheongyang   | CY202-29| MW474910                 | *Alternaria* sp. |
| Gochang      | GC191-1 | MW474911                 | *Alternaria* sp. |
| Gochang      | GC191-14| MW474912                | *Alternaria* sp. |
| Gochang      | GC192-7 | MW474913                | *Alternaria* sp. |
| Gochang      | GC192-13| MW474914                | *Alternaria* sp. |
| Gwangju      | GJ192-1 | MW474915                | *Alternaria* sp. |
| Gwangju      | GJ192-2 | MW474916                | *Alternaria* sp. |
| Incheon      | IC191-1 | MW474917                | *Alternaria* sp. |
| Incheon      | IC191-2 | MW474918                | *Alternaria* sp. |
| Yongin       | Y1201-2 | MW474919                | *Alternaria* sp. |

ITS, internal transcribed spacer.
and ITS4. However, there was no length variation and sequence diversity in the ITS region throughout all isolates, except for one single-nucleotide substitution (T to C) in isolate CY202-29 (Supplementary Fig. 2A and B). Phylogenetic analyses based on the ITS region was able to divide seven references retrieved from GenBank into two different clades, *A. cucumerina* and *A. infectoria*. However, our isolates and many other references were not successful in dividing into clades and all were grouped into one clade containing *A. alternata*, *A. tenuissima*, and *A. gaisen*.

**Table 2.** Subgroups of *Alternaria* isolates and GenBank accession numbers of HIS3, GAPDH, and RPB2

| Isolate  | HIS3 size (bp) | Subgroup | Accession no. | Origin (city) |
|----------|----------------|----------|---------------|---------------|
| CY202-11 | 546            | I        | HIS3          | -             | Cheongyang   |
| CY202-16 | 546            | I        | GAPDH         | -             | Cheongyang   |
| CY202-25 | 546            | I        | RPB2          | -             | Cheongyang   |
| GC191-1  | 546            | I        | HIS3          | -             | Gochang      |
| GC191-14 | 546            | I        | GAPDH         | -             | Gochang      |
| GC192-7  | 546            | I        | RPB2          | -             | Gochang      |
| GJ192-1  | 546            | I        | HIS3          | -             | Gwangju      |
| GJ192-2  | 546            | I        | GAPDH         | -             | Gwangju      |
| IC191-1  | 546            | I        | RPB2          | -             | Incheon      |
| YI201-2  | 546            | I        | HIS3          | -             | Yongin       |
| GC192-13 | 484            | II       | HIS3          | -             | Gochang      |
| CJ191-1  | 440            | III      | GAPDH         | -             | Chungju      |
| CJ191-3  | 440            | III      | RPB2          | -             | Chungju      |
| CY202-18 | 440            | III      | HIS3          | -             | Cheongyang   |
| CY202-29 | 440            | III      | GAPDH         | -             | Cheongyang   |
| IC191-2  | 440            | III      | RPB2          | -             | Incheon      |

HIS3, histone H3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RPB2, RNA polymerase II second largest subunit.
Different Alternaria Species Causing Watermelon Leaf Blight

These results indicate that the ITS region cannot be used for assaying genetic diversity in the Alternaria spp. causing leaf blight on watermelons in Korea.

Classification of Alternaria species based on HIS3 gene.

Partial coding sequence of HIS3 was successfully used to divide Alternaria into subgroups based on size analysis of PCR amplicons (Zhao et al., 2016a, 2016b; Zheng et al., 2015). After PCR analysis using a primer set, H3-1a and H3-1b, all 16 Alternaria isolates were successfully divided into three subgroups (Sub I, II, and III) based on their sizes (Table 2, Fig. 2). The largest amplicon with 546 bp was produced by Sub I, consisting of CY202-11, CY202-16, CY202-25, GC191-1, GC191-14, GC192-7, GJ192-1, GJ192-2, IC191-1, and YI201-2. The second largest one with 486 bp was amplified by Sub II GC192-13. Sub III producing the smallest amplicon with 440 bp was the dominant isolates, consisting of CJ191-1, CJ191-3, CY202-18, CY202-29, and IC191-2. Two isolates from dominant Sub I (CY202-11 and GC191-1) and one isolate from each Sub II (GC192-13) and Sub III (CJ191-1) were randomly selected for further analysis.

Morphological characterization of Alternaria species.

Four representative isolates, CY202-11, GC191-1, GC192-13, and CJ191-1, from three subgroups were subjected to morphological analysis on PDA and V8 juice agar plates. On the V8 juice agar plate, all isolates produced loosely cottony and dark grey colonies after incubation at 28°C in dark condition for seven days. On PDA plates, three isolates, CY202-11, GC191-1, and GC192-13, in Sub I and Sub II developed cotton light brown colonies, while CJ191-1 from Sub III produced a cotton dark grey colony (Table 3, Fig. 3A). In all isolates, conidiophores were short and arising singly (Fig. 3B). The conidia of them were mainly short ovoid to obclavate with a rounded apical cell, with no significant difference among the four representative isolates. A similar number of transverse septa and longitudinal septa of their conidia were also observed from 1 to 6 and from 0 to 2, respectively. All isolates had a similar size of conidiophores but slightly differed in sporulation on PDA and V8 juice agar (Table 4).

Molecular genetic differentiation of subgroup I, II, and III of Alternaria spp.

To examine whether the three subgroups can be identified into the Alternaria species, their HIS3 sequences were compared (Fig. 4, Supplementary...
Fig. 4. As a reference for sequence comparison, the HIS3 gene (accession no. XM_018526023) of an A. alternata isolate was used. Its full-length cDNA is 411 bp in length and the cDNA fragment amplified with primers H3-1a and H3-1b is 378 bp. In the analysis of Sub I isolates, CY202-11 and GC191-1, the HIS3 gDNA amplicon was 546 bp in length and contained three introns 54 bp (Int A), 62 bp (Int B), and 52 bp (Int C) (Fig. 3). The HIS3 gDNA amplicon from GC192-13 isolate in Sub II was 484 bp with two introns 54 bp (Int A) and 52 bp (Int C). CJ191-1 isolate in Sub III was 440 bp in length with only one intron 62 bp (Int B).

Phylogenetic analyses based on HIS3 was performed together with available reference sequences retrieved from GenBank (eight from A. alternata, eight from A. tenuissima, one from A. gaisen, four from A. infectoria, three from A. cucumerina, two from Alternaria sp., and one outgroup Fusarium proliferatum) (Fig. 5). Sub I isolates, CY202-11 and GC191-1, were located in one clade with the known A. tenuissima from GenBank. Sub II GC192-13 isolate was together with A. gaisen and Sub III CJ191-1 isolate was located in the A. alternata clade. Occurrence rates of each Alternaria sp. were relatively high with quite wide range depending on where the isolates were harvested (Supplementary Table 1). Including A. infectoria references and A. cucumerina references, the entire species-group within the Alternaria spp. was successfully split into five clades, suggesting that HIS3 can be used for classifying Alternaria

| Subgroup | Isolate         | Sporulation (per ml) | Length (μm) | Width (μm) |
|----------|-----------------|----------------------|-------------|------------|
|          |                 | PDA                  | V8 juice agar |            |
| I        | CY202-11        | +++                  | 12.5-32.4 (23.3 ± 6.7) | 7.2-14.4 (10.8 ± 2.4) |
| I        | GC191-1         | ++                   | 15.7-48.4 (27.4 ± 9.7) | 7.9-16.2 (10.0 ± 3.5) |
| II       | GC192-13        | +++                  | 16.2-43.4 (27.7 ± 7.9) | 6.9-18.1 (11.0 ± 3.1) |
| III      | CJ191-1         | +                    | 14.6-35.1 (24.0 ± 5.9) | 4.8-15.4 (10.1 ± 2.9) |

*Sporulation: ‘+++’, (>10^5); ‘++’, (<10^5, >10^3); ‘+’, (<10^3).*
Different *Alternaria* Species Causing Watermelon Leaf Blight

**Fig. 4.** Schematic of histone H3 gene in Subgroup I, II, and III. Solid lines indicate introns. Intron (Int) A, B, and C are 54 bp, 62 bp, and 52 bp in size, respectively. Gray and white rectangles indicate cloned and noncloned histone H3 exons, respectively. Primers, H3-1a and H3-1b, used for the cloning of the exons were indicated as arrows. Coding region sequence of histone H3 inferred from *Alternaria alternata* (GenBank accession no. XM_018526023) was used as a reference for this comparison.

**Fig. 5.** Maximum likelihood tree constructed based on histone H3 of four *Alternaria* isolates and 27 reference sequences retrieved from GenBank. Subgroup I (CY202-11 and GC191-1), II (GC192-13), and III (CJ191-1) were grouped into *A. tenuissima*, *A. gaisen*, and *A. alternata*, respectively. Red dots indicate *Alternaria* spp. isolated in this study. Bootstrap values are shown below branches. The bar indicates nucleotide substitutions per site.
spp. causing leaf blight on watermelon.

**Classification of *Alternaria* species based on *GAPDH* and *RPB2*.** The amplicon lengths of *GAPDH* and *RPB2* varied among some of *Alternaria* spp. associated with leaf spot and leaf blight disease in Solanaceae and Brassica (Bessadat et al., 2020; Blagojević et al., 2020; Lawrence et al., 2016). Although the sequences of *GAPDH* and *RPB2* were not identical among four isolates and references retrieved from GenBank, none of the variations was specific for the *Alternaria* spp. (Supplementary Fig. 5). Therefore, four isolates and the reference *Alternaria* spp. were not in separate clades based on the phylogenetic tree of *GAPDH* and *RPB2*.

**Pathogenicity tests.** The pathogenicity of the four representatives isolates from three *Alternaria* species, *A. tenuissima*, *A. gaisen*, and *A. alternata*, was evaluated on a watermelon cultivar, ‘Charleston gray’ using the detached-leaf inoculation method (Pryor and Michailides, 2002). About seven days after inoculation, dark brown lesions around agar disks of the *Alternaria* spp. appeared on the detached leaves (Fig. 6), similar to those observed on naturally infected leaf samples. No symptoms were observed in the control detached leaves treated with agar disk only. The average disease incidence and average disease index caused by *A. tenuissima*, *A. gaisen*, and *A. alternata* ranged from 42.9% to 100% and 39.4% to 68.7%, respectively (Table 5). The *Alternaria* spp. were re-isolated consistently from all the leaf lesions to fulfill Koch’s postulates, while no *Alternaria* isolates were re-isolated from the control leaves.

**Discussion**

In this study, we describe the occurrence and identification of the *Alternaria* spp. obtained from watermelon plants displaying leaf blight lesions. The identified species were *A. tenuissima*, *A. gaisen*, and *A. alternata*, and all of them displayed pathogenicity on a watermelon cultivar, ‘Charleston gray’. Although *A. cucumerina* has been reported as the prevalent species causing leaf blight on Cucurbitaceae including watermelon and muskmelon in Korea and China (Jackson and Weber, 1959; Kim et al., 1994; Liu et al., 2010; Ma et al., 2021; Zhao et al., 2016a, 2016b), this was not obtained in our study. Similarly, no *A. cucumerina* isolates were recovered from watermelon and muskmelon in the Beijing municipality of China (Zhao et al., 2016a, 2016b). Instead, *A. tenuissima* and *A. alternata* were reported as the predominant species of *Alternaria* leaf blight of watermelon in China (Ma et al., 2021; Zhao et al., 2016a, 2016b). These previous reports are consistent with our observation that 10 and five among the 16 *Alternaria* isolates were identified as *A. tenuissima* and *A. alternata*, respectively. These results suggest that the composition

![Fig. 6. Pathogenicity of four representative isolates of three *Alternaria* species on detached watermelon leaves. Three agar disks growing each *Alternaria* species were placed on the detached of ‘Charleston gray’. Images were obtained seven days after inoculation. (A) Control with agar disks only. (B) *A. tenuissima* (CY202-11). (C) *A. tenuissima* (GC191-1). (D) *A. gaisen* (GC192-13). (E) *A. alternata* (CJ191-1). Scale bars = 2 cm.](image)

**Table 5.** Disease incidence and disease index of the four *Alternaria* isolates on detached watermelon leaves

| Isolate  | Subgroup | Species       | Total leaves | Infected leaves | Disease incidence (%) | Disease index (%)   |
|----------|----------|---------------|--------------|-----------------|-----------------------|---------------------|
| CY202-11 | I        | *A. tenuissima* | 7            | 7               | 100                   | 19.3-98.0 (56.6 ± 29.6) |
| GC191-1  | I        | *A. tenuissima* | 7            | 5               | 71.4                  | 7.4-100.0 (60.3 ± 40.4) |
| GC192-13 | II       | *A. gaisen*    | 7            | 6               | 85.7                  | 8.0-99.3 (39.4 ± 33.8) |
| CJ191-1  | III      | *A. alternata* | 7            | 3               | 42.9                  | 19.4-99.0 (68.7 ± 35.2) |
of the *Alternaria* species may have changed and become more complex, probably due to recent breeding programs for disease resistant cultivars.

Recently, *A. gaisen* was reported as new *Alternaria* sp. causing watermelon leaf blight in China (Ma et al., 2021). In our study, although there was only one isolate, *A. gaisen* was also obtained from Gochang city. These results suggest a possibility that *A. gaisen* is becoming one of the Agents of watermelon leaf blight worldwide. *A. gaisen* had been reported as a causal agent of black spot disease in Japanese pears (*Pyrus pyrifolia*) previously discovered only in Japan, Korea, and China (Simmons and Roberts, 1993). However, now, it has been discovered in various hosts—wintersweet (*Chimonanthus praecox*), gerbera daisy (*Gerbera jamesonii*), and watermelon—of many countries including France, Australia, China, Pakistan, and the US (Akhtar et al., 2014; Baudry et al., 1993; Ma et al., 2021; Perveen et al., 2018; Tian et al., 2020). Additional study will be necessary to determine whether *A. gaisen* can be another predominant species of *Alternaria* leaf blight of watermelon in Korea.

Information on the species of plant pathogens is necessary for disease management because different species often display different degrees of resistance to fungicides (Iacomi-Vasilescu et al., 2004). Morphological trait analysis has been the common method to identify and differentiate *Alternaria* species (Simmons and Roberts, 1993). However, previous studies indicate that morphological characters were often uninformative and insufficient to accurately distinguish *Alternaria* species (Pryor and Michailides, 2002; Zheng et al., 2015). To complement morphological trait analysis, phylogenetic analysis after combining DNA sequences has been suggested (Kang et al., 2001). Previously, the *Alternaria* species had been successfully differentiated using the ITS region, *GAPDH*, and *RPB2* (Bessadat et al., 2020; Blagojević et al., 2020; Lawrence et al., 2016; Woudenberg et al., 2013). However, our results suggested that each of them and the combination of their sequences were not competent to differentiate the *Alternaria* spp. causing watermelon leaf blight in Korea. On the other hand, PCR amplification and sequence analysis of the *HIS3* gene successfully differentiated *Alternaria* isolates. The PCR products amplified from *A. tenuissima*, *A. gaisen*, and *A. alternata* differed in size, depending on the presence and type of introns. Therefore, we recommend the analysis of the sequences of *HIS3* rather than the ITS region, *GAPDH*, and *RPB2* to identify *Alternaria* species causing leaf blight on watermelons in Korea.

### Conflicts of Interest

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

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### Electronic Supplementary Material

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

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