Thesis for the Degree of Master of Agriculture

Diagnosis of pathogenic fungus causing dimple rot on Gold3 kiwifruits in Jeju Island

DEPARTMENT OF AGRICULTURE

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제주 Gold3 키위에 발생하는 부패병 원인균 동정

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Diagnosis of pathogenic fungus causing dimple rot on Gold3 kiwifruits in Jeju Island

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ABSTRACT

Since the past few years dimple rot has been one of the most serious diseases affecting *Actinidia chinensis* var. *chinensis* Gold3 kiwifruit in Korea, which causes low quality of production and consequently losses of income. In the current study, the causal pathogen of dimple rot on Gold3 kiwifruit was identified as *Botryosphaeria dothidea*, a widespread pathogen which is able to penetrate the fruit directly without wounding. Correspondingly, pathogenicity of the fungus was evaluated on other kiwifruit cultivars such as Hayward and Hort16A. Among the other two cultivars, Gold3 was the most susceptible one to infection. Since dimple-rot symptoms have been previously associated with other pathogens from the *Pseudocercospora* genus, in this study *B. dothidea* was ratified as the causal agent of this disease in Jeju Island. To illustrate the high pathogenicity of the fungus on Gold3, further studies such as the observation of the infection structures of the fungus may be needed.

I. INTRODUCTION

Actinidia spp. is an ample genus of berries with more than 70 individual species that grow in nature, mostly on the temperate forests of mountainous areas in south-western China (Warrington et al. 1990). In the early 1900s, botanists became interested in these plants and introduced the fruits to Europe, United States and New Zealand. Two species were recognized as potential marketable crops because of their sweetness, tastiness and nutritious properties; *Actinidia chinensis* (*Actinidia chinensis* -A. Chev.- var. *chinensis* -C.F. Liang et A.R. Ferguson-) characterized by its yellow flesh and the green-fleshed *A. deliciosa* (*Actinidia deliciosa* -A. Chev.- var. *deliciosa* -C.F. Liang et A.R. Ferguson-), although only the later variety survived, mainly in New Zealand, and became a profitable success in 1930 promoted as Hayward. Over the years, the Chinese gooseberry production grew and exports began to prosper outside New Zealand, giving to this berry the common name of kiwifruit (UNECE Standard for Kiwifruit FFV-46).

The commercial success of the Hayward kiwifruit brought an increasing production interest, and plantations began to prosper in China and Italy to be later introduced to other latitudes, responding to the rocketing customers demand. In countries like Chile or Greece, now some of the biggest kiwifruit exporters, this berry was introduced at the beginning of the decade of 1970 (Ferguson, 2010). In the later decades, its production has been increasing in Asian countries like Japan, Korea and Taiwan. Although four *Actinidia* species grow naturally in South Korea, *A. arguta*, *A. kolomita*, *A. pollygama* and *A.rufa*, which grow only in Jeju Island (Shim and Ha, 1999), in the middle 1970s, kiwifruit plants of the Hayward cultivar from New Zealand, were imported to the country and began to be cultivated in the southern areas, Jeju Island included. Later, in 2004, the yellow-fleshed variety, *Actinidia*

chinensis var. *chinensis* cultivar “Hort16A” was introduced mostly to Jeju Island and the production quantities promptly augmented multiplying the amount of orchards (Shim and Ha, 1999). Unfortunately, a severe bacterial canker disease caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) vastly attacked orchards inside and outside New Zealand. In Korea, Psa was first detected on Hayward in 1988 and on Hort16A in 2006, resulting in a severe epidemic on kiwifruit orchards of the south provinces, including Jeju Island (Kim et al., 2017a).

In the following years, the search for sweeter varieties with less allergenic reactions and the response to previous dire diseases such as the canker by Psa, brought to Korea the introduction of a new yellow fleshed variety, the Gold3 kiwifruit (*Actinidia chinensis* var. *chinensis* cultivar 'Zesy002') grown especially in the southern part of the Korean peninsula and the island of Jeju (Zespri Annual Review 2015/16). The growing industry brought several challenges, among them, in-orchard and storage diseases that undermine the profits of the farmers.

During the last decades in Korea (RDA, 1993), postharvest ripe dimple rot has been a growing problem in commercial orchards of yellow Gold3 kiwifruit. This disease, defined as dimple rot (syn. side rot) begins as a dimple, a small circular scabbed depression, generally developed on the side or near the stem end of the fruit and darkened from tan to dark brown. Dimples seem to appear during late fruit development and, if not recognized during the harvest season, they can develop into fruit rots during cold storage, shortening shelf-life and threatening whole kiwifruit stocks (Pennycook, 1985). These symptoms were similar to those described on 'Hayward' (Pennycook, 1985) and on 'Hort16A' (Manning et al., 2003) kiwifruit. Particularly, dimple-rot symptoms were reported to link the disease with two main Ascomicota families; *Pseudocercospora* (Yano et al., 2015) and *Botryosphaeria* (Koh et al., 2005), proved as causal pathogens in other cultivars, but in Gold3 kiwifruit, the causal

agent is not yet known. Accordingly, an effective protection strategy has not been devised yet due to lack of knowledge of the causal pathogen.

In the present study, the diagnosis of dimple rot present on Gold3 kiwifruits from Jeju orchards was carried out. Furthermore, bio-test of the isolated fungus *B. dothidea* was performed on different kiwifruit cultivars. Similarly, to find the causal fungus on the dimple rot, pathogenicity of both fungi *B. dothidea* and *Pseudocercospora* sp. was tested by inoculation on Gold3 kiwifruits.

II. MATERIALS AND METHODS

1. Morphological diagnosis of the pathogenic isolates

During 2018 and 2019 harvest seasons, a total of 150 kiwifruits showing dimples were collected from thirteen Gold3 kiwifruit orchards in Jeju Island (33.499621° N, 126.531188° E), South Korea. The fruits were surface sterilized by dipping in 1 % (v/v) sodium hypochlorite for 60 s, followed by 70 % (v/v) ethanol for 60 s, rinsed twice in sterile distilled water for 60 s and air-dried for 1 h. The kiwifruit skin was peeled back and four segments (4 mm^2) were detached from the margins of symptomatic underlying tissue and transferred to potato dextrose agar (PDA) plates containing 100 μg/ml of streptomycin sulphate (Sigma Aldrich, Inc., St. Louis, Missouri, United States). After incubation at 28°C for 6 days, representative fungal colonies from each plate were transferred to PDA and oatmeal agar (OMA) medium and were incubated at 28°C for 7 days. A total of 126 isolates were obtained to be cultural and morphologically analyzed. Because of their common features, 50 representative isolates were selected and characterized.

2. Molecular identification using molecular marker

Genomic DNA was extracted from approximately 200 mg of fungal hyphae scraped directly from the PDA-grown cultures. The cetyltrimethylammonium bromide – CTAB – method (Aamir et al., 2015) was followed with little modifications. The hyphae were lyophilized in liquid nitrogen and grinded with mortar and pestle. The powder obtained was placed in a 2μl vial and 700μl of lysis buffer (100 mM Tris HCl [pH8.0], 50mM EDTA, 3%

SDS) were added. This mixture was homogenized with a WiseMix Multifunction Vortex Mixer (DAIHAN Scientific, Wonju, South Korea) for 20 minutes and centrifuged at 13,000 rpm for 10 min. This supernatant was transferred to a fresh 2µl microcentrifuge tube, then 2µl of RNase A (10mg/ml) were added and the whole solution was incubated at 37°C for 15 min. Later, according to the volume in each vial, equal volume of cleaning buffer (phenol: chloroform: Isoamyl alcohol (25:24:1)) was added, homogenized and centrifuged at 13,000 rpm for 10 min. This step was repeated once. The upper aqueous layer was taken in a fresh 1.5 µl micro centrifuge tube and equal volume of 100% ethanol was added. The DNA in this solution was precipitated at -20°C for 30 min, followed by centrifugation at 12,000 rpm for 10 min. The pellet of DNA was washed with 70% ethanol and centrifuged again at 12,000 rpm for 5 min. Finally the DNA pellets were air dried overnight and dissolved in 1X TE buffer.

For each sample, 50 µl of PCR amplification cocktail were prepared, containing 2 µl of DNA template (5~10 ng/ml), 10 ρmol of each forward and reverse primer, 25 mM of MgCl, 2.0 mM of each of the four dNTPs and 5 units of Taq DNA polymerase. The Internal Transcribed Spacer (ITS) rDNA region was amplified for all selected isolates using universal primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and 4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The reaction for ITS, was performed on a thermal cycler (Biometra TOne 96G; Analytik Jena AG, Jena, Germany) and the PCR cycling parameters were an initial preheat at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing for 30 s, at 56°C and extension at 72°C for 1 min, with a final extension stage at 72°C held for 10 min.

PCR products were detected in 1% agarose electrophoresis gel and stained with 0.01 % ethidium bromide to UV light visualization using an UVP transilluminator (UVsolo touch: Analytik Jena AG, Jena, Germany). Nucleotide sequences of PCR products were

determined using an ABI 3730xl DNA Analyzer (Macrogen Inc., Seoul, South Korea) and the resulting sequences were compared with sequences in the GenBank database using BLAST program of NCBI for primary identification (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All of the isolates were identified as *B. dothidea*.

3. Pathogenicity test of the isolates and other reported pathogens.

A. B. dothidea inoculum

One representative isolate was transferred to OMA medium and incubated in aerobic conditions under 5000 lux, at 28°C for 7 days. Later, the plates were flooded with 10 ml of sterile distilled water and conidia were harvested with a sterile plastic loop. The conidial suspension was filtered through two layers of Miracloth (Calbiochem, San Diego, CA, United States), the concentration was adjusted to 10^5 conidia / ml and Tween-20 (Yakuri Pure Chemicals Co. LTD, Kyoto, Japan) was added to a concentration of 0.01% (v/v).

B. Koch's postulates confirmation

Pathogenicity test on wounded versus unwounded Gold3 kiwifruit

A total of 54 healthy mature Gold3 kiwifruits (Lowe, 2011, NZKGI, 2019) were surface-disinfected with 1 % (v/v) sodium hypochlorite for 60 s, followed by 70 % (v/v) ethanol for 60 s, rinsed twice in sterile distilled water for 60 s and air-dried for 1 h. From the total, 27 fruits were wounded with a sterilized wood stick (Ø 0.05 mm), while the other 27 were left unwounded. For each fungal type, a mycelial plug (Ø 5 mm) or a 60 µl drop of the conidial suspension was placed in the central side of each fruit, directly above the wound previously done, when wounded. A 0.01 % (v/v) Tween-20 solution and sterile PDA plugs were used as negative controls. All the fruits were placed in baskets and incubated at 28°C

and >90% humidity for 7 days. After the incubation period, grade of disease was assessed, measuring the size of symptoms on the fruit with a ruler.

Then, for pathogen re-isolation, symptomatic skin on the kiwifruits was peeled back and two pieces (4 mm^2) from lesion margins of the underlying decayed flesh were transferred to PDA plates containing 100 μg/ml streptomycin sulphate (Sigma Aldrich, Inc.). The re-isolated fungus was identified by the morphological and molecular analysis previously described. For this experiment, three identical repetitions in different times were performed.

Pathogenicity test on Hayward, Hort16A and Gold3 kiwifruit cultivars

A total of 36 healthy mature kiwifruits (Lowe, 2011, NZKGI, 2019), 12 from each of the varieties Hayward (*A. deliciosa* var. *deliciosa*), Hort16A (*A. chinensis* var. *chinensis* cultivar 'Hort16A') and Gold3 (*A. chinensis* var. *chinensis* cultivar 'Zesy002'), all supplied by Zespri Korea Group Ltd., were surface-disinfected and inoculated with 60 μl of the conidial suspension as described before. A 0.01 % (v/v) Tween-20 solution was used as negative control. All the fruits were placed in baskets and incubated at 28°C and >90% humidity for 9 days. After the incubation period, dimple-rot disease severity was assessed in four levels according to the development of symptoms: Level 0 = No dimples and the fruit is completely healthy. Additionally, fruits show signs of fungal growth over the skin, but no evident pathogen penetration nor dimples. 1 = One or two small dimples were localized in or nearby the inoculation site. 2 = One main dimple or several dimples beginning to develop to darkened or soft areas around, but easily differentiable from the fruit tissue around, which still looks firm and healthy. 3 = One big well developed dimple or a cluster of small dimples localized inside and around the inoculation site with soft areas nearby. 4 = Cluster of well-developed dimples with collapsed tissue and generalized nodes of infection outside the

inoculation site. Disease assessment and pathogen re-isolation was performed in the same way as previously described. The experiment had three repetitions in different times, replicating the same conditions described.

Pathogenicity test of other pathogens associated to dimple rot

Since several *Pseudocercospora* species have been previously reported as commonly associated with this kind of side-rot disorder in other kiwifruit varieties, a *Pseudocercospora* sp. isolate was facilitated by the department of Plant Medicine of Sunchon National University, to compare its pathogenicity with *B. dothidea*. A total of 18 near-ripe healthy Gold3 kiwifruits were surface-disinfected as described before. *Pseudocercospora* sp. and *B. dothidea* mycelial plugs (Ø 5 mm) were respectively placed in the central side of the fruits. Sterile PDA plugs were used as negative controls. All the fruits were placed in baskets and incubated at 28°C and >90% humidity for 7 days. Disease assessment and pathogen re-isolation was performed in the same way as previously described. The experiment had three repetitions in different times, replicating the same conditions described.

C. Data analysis

For all experiments the data were analyzed with Duncan's multiple range test (DMRT) using statistical analysis system (SAS) program (SAS Institute, version 9.0). Statistical significance was considered at $p < 0.05$.

III. RESULTS

1. Symptom of dimple rot

Typical symptoms of dimple rot were found among the kiwifruit recollected. They began with a superficial dimple, a small scabbed depression on the side or near the stem end of the fruit that appeared often during the harvest or initial storage (Fig. 1A and 1B). The symptoms developed internally, first into a milky area under the dimple and then into a side rot, characterized by a brownish darker area, enclosed by a dark circle (Fig. 1C). Internally, the diseased tissues often collapsed, with a soft and watery texture of a darker tone than the surrounding firm healthy area (Fig. 1D).

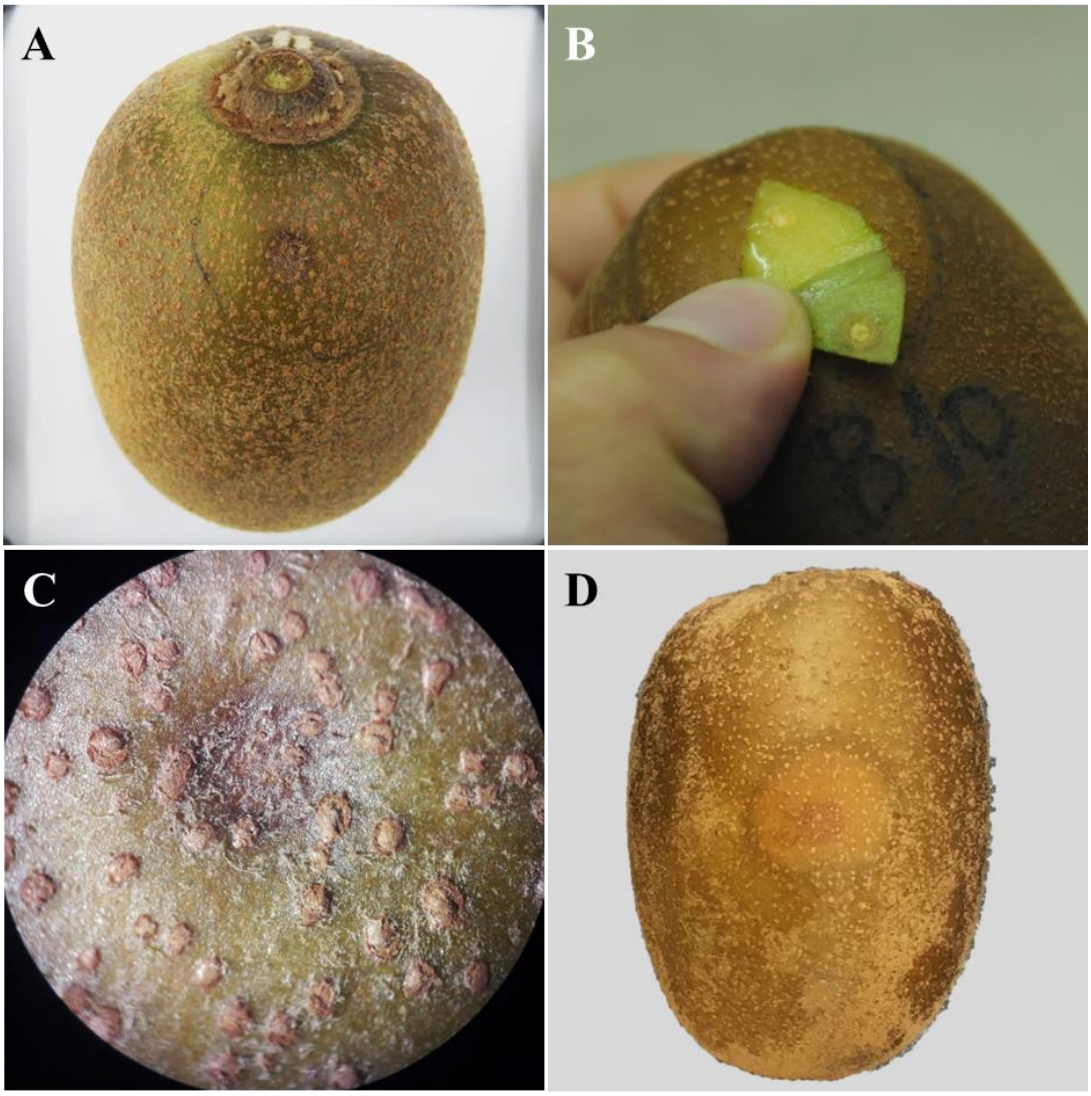

Figure 1. Dimple rot characteristic symptoms documented on Gold3 kiwifruit form thirteen orchards in Jeju Island, South Korea during 2018-2019 harvest seasons. (A) External and (B) internal symptoms of the first stages of infection on the fruit, found during early storage, (C) magnification of dimple, and (D) dimple rot developed.

2. Morphological and molecular identification of isolates

The resulting 126 fungal isolates were obtained from the growing edge of symptomatic areas. After observing their morphological and cultural characteristics, nearly 95% (n=120) showed similar characteristics. On PDA plates, cultures presented a fast growing rate of abundant aerial mycelium mats that appear light to dark gray (Fig. 2A). On OMA plates, cultures produced olivaceous mycelium (Fig. 2B) and formed well defined pycnidia after approximately 10 days (Fig. 2C), which grew individually or in small groups and exuded masses of hyaline unicellular α-conidia of oval to fusoid shape, aseptate, measuring 7.1 ±0.83 μm wide per 34.2 ±2.8 μm long. This morphology matched with the Ascomycota genus, *Botryosphaeria*.

From the fungal isolates, a sample of 50 representative cultures was selected, its total DNA was extracted by the CTAB method and then amplified using a universal ITS primer set (ITS1 and ITS4). Visualization in 1% agarose gel resulted in bands of 600 ±70 bp (Fig. 3A). After sequencing, the BLASTn identification showed that all the isolates held between 96-100% homology with *B. dothidea* strains (Fig 3B).

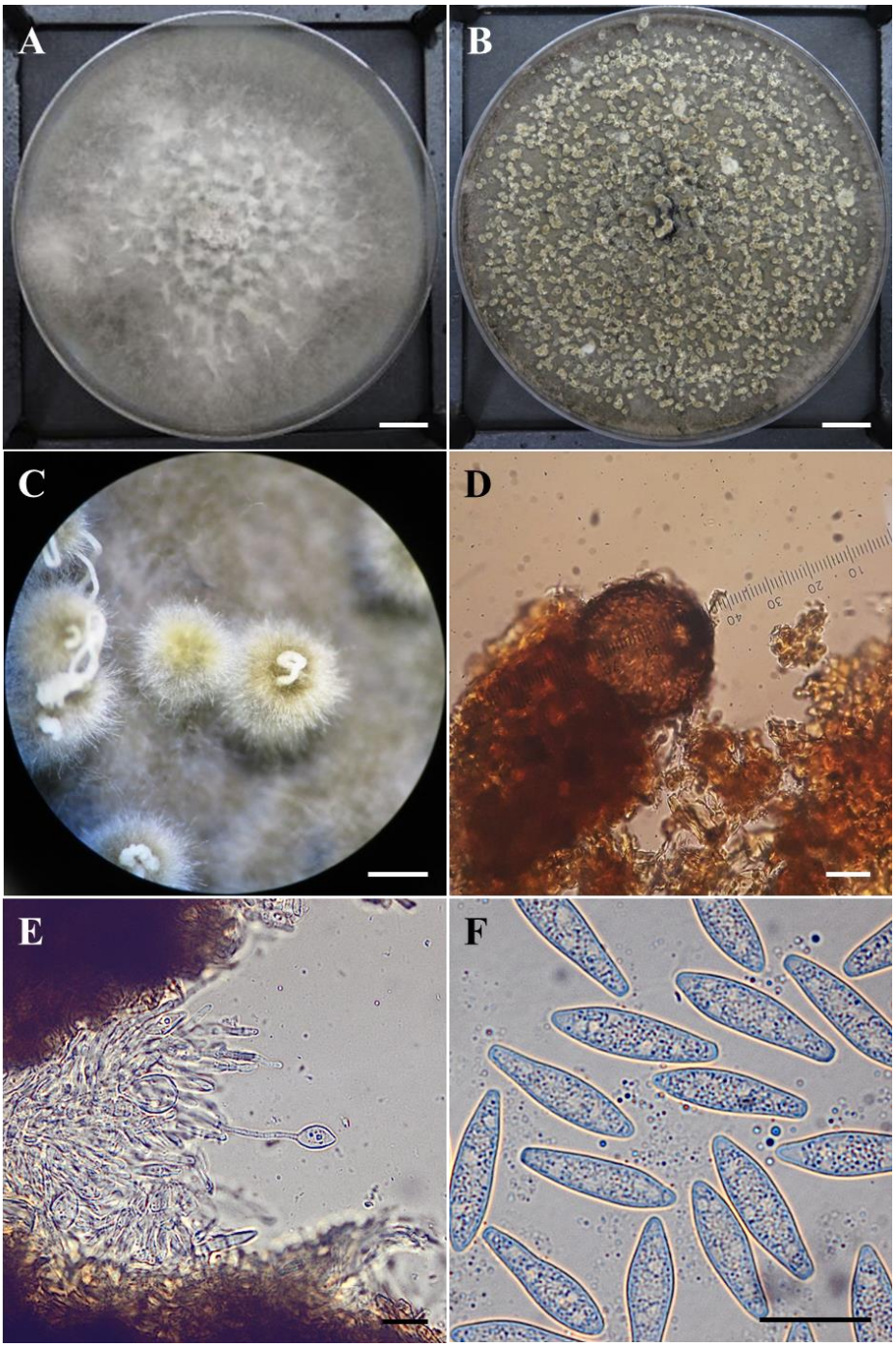

Figure 2. Morphological characteristics of the pathogen causing dimple rot identified as *B. dothidea*, extracted from naturally infected kiwifruit. (A) Colony growing fluffy dark to light gray aerial mycelium on PDA. (B, C) Colony sporulating on OMA. (D, E) Conidiomata, conidiogenous cells and paraphyses. (F) Hyaline conidia, unicellular and fusoid. Scale bars: A, B = 1cm; B, C = 1000 μm; E, F = 20 μm.

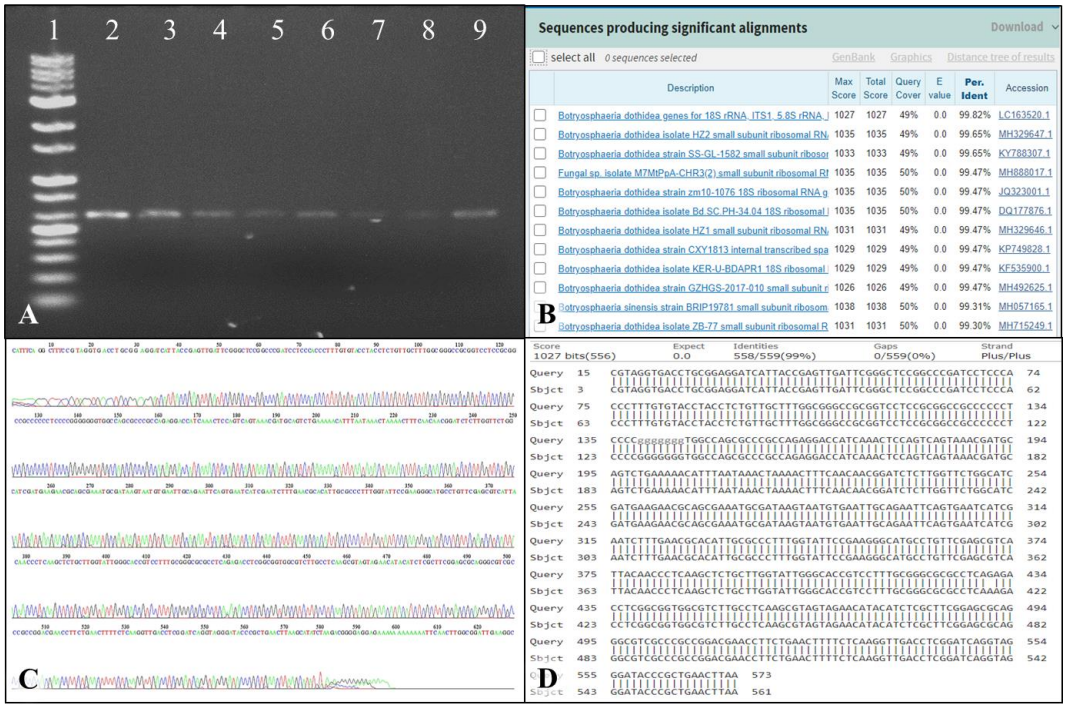

Figure 3. Molecular identification of the pathogen causing dimple rot identified as *B. dothidea*, extracted from naturally infected Gold3 kiwifruit. (A) Representative PCR-amplification product of the isolates. Lane: 1; ladder, lane 2; ITS1/4 amplification product from a sample re-isolated from artificially inoculated kiwifruit, lanes 3, 4, 5, 6, 7, 8, and 9, ITS1/4 amplification product from samples isolated from naturally infected kiwifruit on orchard. (B) Representative MEGABLAST analysis of ITS1 DNA sequence of the isolates, showing 99% of identity with *B. dothidea*. (C) Representative chromatogram of DNA sequences of the isolates. (D) Representative sequence alignment between the query sequence of the isolates and a *B. dothidea* strain with accession number LC163520.1

3. Pathogenicity test

A. Re-inoculation on Gold3 kiwifruit

Typical dimple rot symptoms were observed on all inoculated fruits not only wounded but also unwounded ones (Fig. 4A and 4B) and the inoculated fungus was re-isolated in all the cases; hyphal or conidia-inoculation in wounded or unwounded fruit, this reconfirmed by molecular identification (Fig 3A).

B. Koch's postulates confirmation.

Pathogenicity test on wounded versus unwounded Gold3 kiwifruit.

For wounded fruit, symptoms of side rot had no difference between inoculation methods; showing generalized watery, soft and disorganized tissue, which externally appears as a darker area. Unwounded fruit presented dimples of equal behavior as the observed in naturally infected fruit, with internal symptoms showing disorganization in a lesser extent and significantly slower development of the disease (Fig. 4). The morphology of re-isolated fungi form either wounded or unwounded fruits revealed identical characteristics with those first isolated from naturally infected fruit. Likewise, total DNA from these fungi resulted in bands of 600 ±70 bp which were reconfirmed as *B. dothidea* after sequencing and BLASTn analysis.

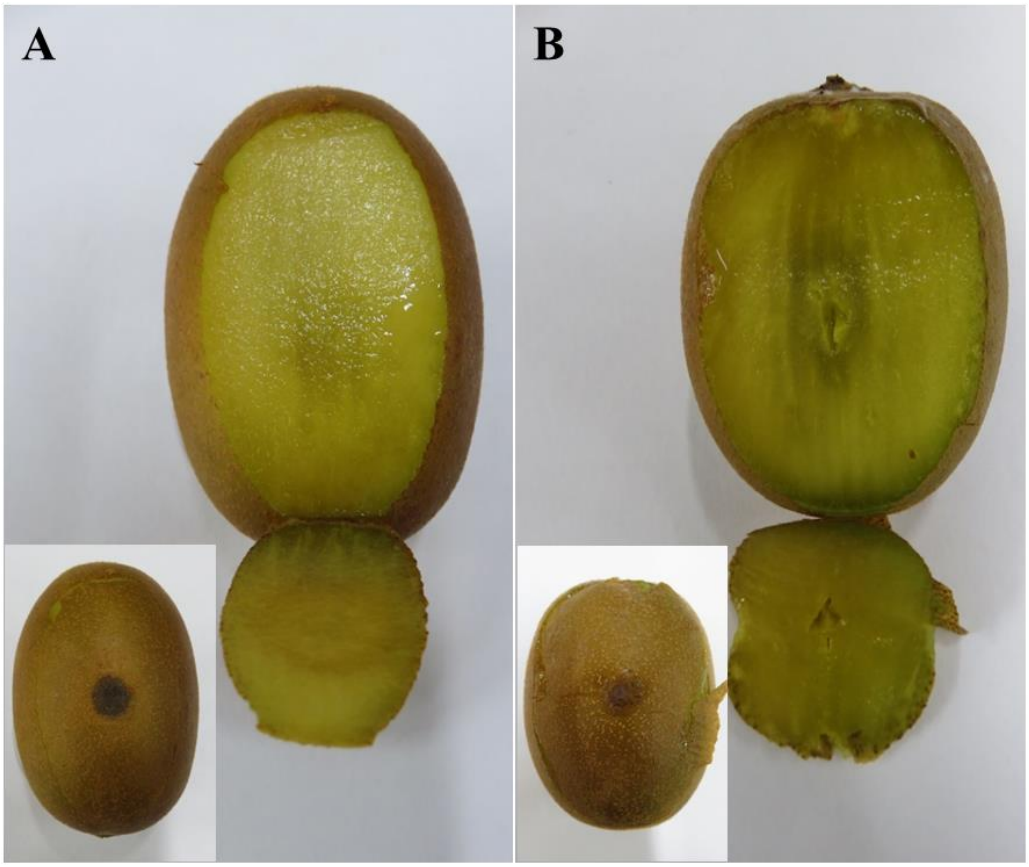

Figure 4. Internal symptoms of unwounded (A) and wounded (B) Gold3 kiwifruit artificially inoculated with *B. dothidea* conidial suspension. The presented photos were taken at 13 days after inoculation. The small boxes show respective external symptoms.

Pathogenicity test on Hayward, Hort16A and Gold3 kiwifruit cultivars

Fruits without wounding from the three cultivars were infected by *B. dothidea* presented typical dimple-rot symptoms (Fig. 5). The development of disease showed higher severity in Gold3 cultivar than in the two other cultivars (Fig. 6). Moreover, Gold3 kiwifruit showed to be prone to formation of dimple clusters. The inoculated fungi were re-isolated and re-identified from fruits of the three cultivars in the same way as previously described. Control fruit showed no disease symptoms in any of the experiments, neither any fungus was recovered from them.

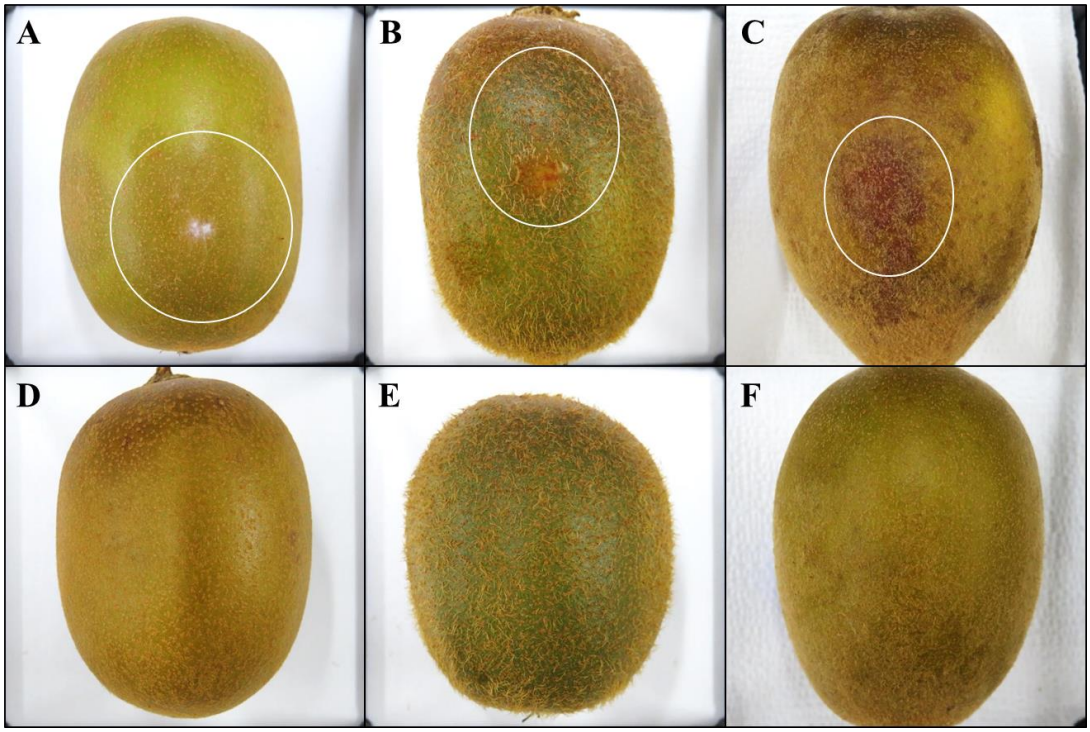

Figure 5. External symptoms of dimple rot on artificially inoculated kiwifruits with conidial suspension of *B. dothidea*. (A) Unwounded Gold3, (B) Hayward and (C) Hort16A kiwifruit, compared with respective untreated Gold3 (D), Hayward (E) and Hort16A (F) controls. The presented photos were taken at 9 days after inoculation. Circled areas show the external symptoms.

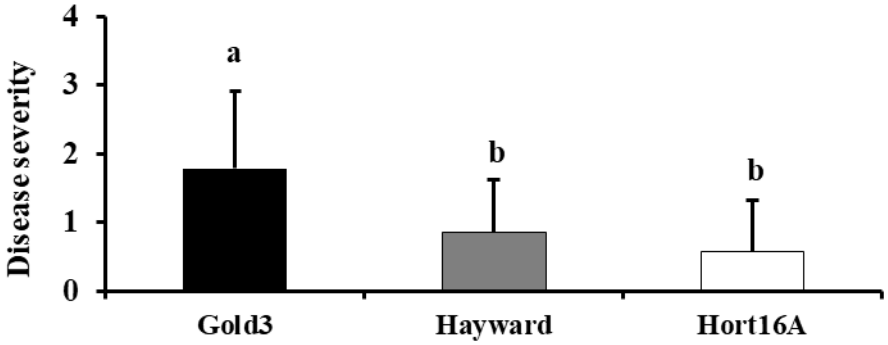

Figure 6. Disease severity on Gold3, Hayward and Hort16A kiwifruit cultivars at 9 days after inoculation with *B. dothidea*. The index of disease severity is presented in materials and methods. Different letters on the columns indicate a significant difference ($P < 0.05$) according to DMRT.

Pathogenicity test of other pathogens associated to dimple rot

After seven days of incubation, unwounded fruits inoculated with *B. dothidea* agar plugs showed dimple-rot symptoms (Fig. 7A, Fig. 8) with typical lesion diameter, while fruits inoculated with *Pseudocercospora* sp. remained healthy (Fig. 7B) without any sign of infection by the inoculated fungus (Fig. 8).

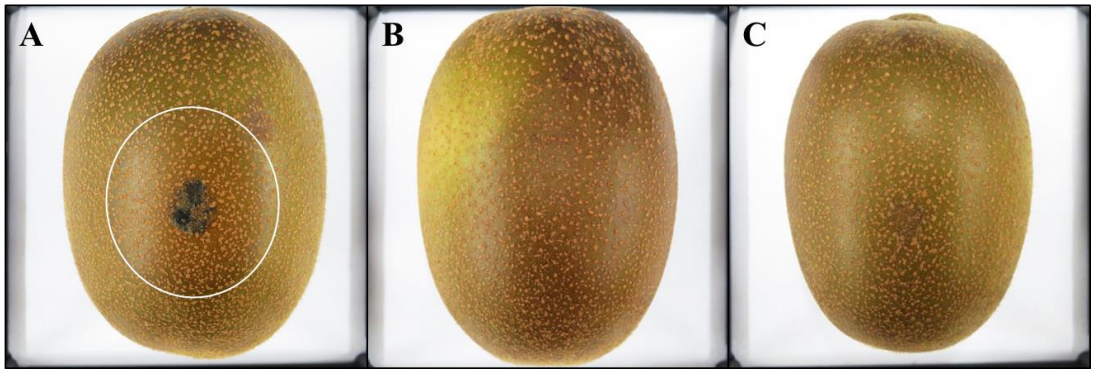

Figure 7. External symptoms of dimple rot on artificially inoculated Gold3 kiwifruits with agar plugs of (A) *B. dothidea*, (B) *Pseudocercospora* sp. and (C) sterile agar plugs as control. The presented photos were taken at 7 days after inoculation. Circled areas show the external symptoms.

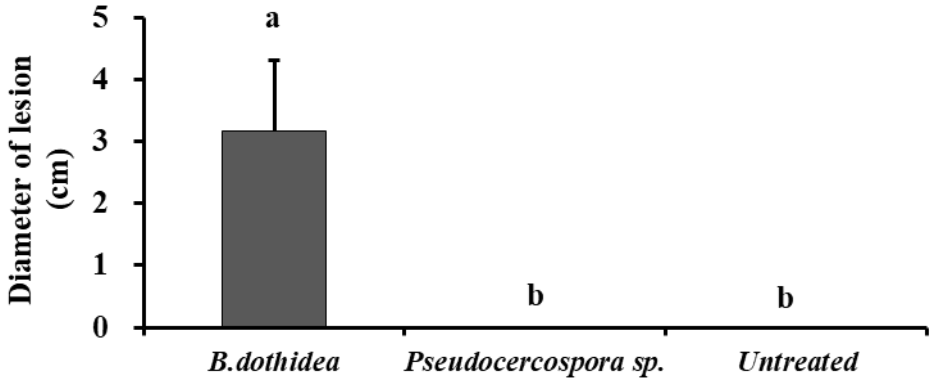

Figure 8. Diameter of external symptoms of dimple rot on artificially inoculated Gold3 kiwifruits, with agar plugs of *B. dothidea*, *Pseudocercospora* sp. and sterile agar plugs (Untreated). Different letters on the columns indicate a significant difference ($P < 0.05$) according to DMRT.

B. dothidea fungi were recovered from all its respective inoculated fruits, but *Pseudocercospora* sp. was not found in any of them. This indicates that *B. dothidea* may be comfortable with Gold3 kiwifruit, but *Pseudocercospora* sp. may not. Furthermore, control fruit showed no disease symptoms in any of the experiments, neither any fungus was recovered from them (Fig. 7C).

IV. DISCUSSION

Postharvest dimple rot constitute a serious threat to the kiwifruit production in Jeju Island, Korea. The pathogenic fungi associated with this disease on Gold3 kiwifruit needed to be identified, in order to decrease economical loss on kiwifruit farms.

Dimple-like lesions were recognized (Whiteman, et al., 2018) in orchard or storage during 2018-2019 harvest seasons, on 150 Gold3 kiwifruits from 13 greenhouses around Jeju Island. Disease symptoms consisted of small scabbed depressions on the side or near the stem end of the kiwifruits that develops internally into a side rot. Identical signs have been reported in several other kiwifruit cultivars in Korea, China and New Zealand among other countries (Koh, et al., 2003, Manning, et al., 2010, Kwon, et al., 2011, Zhou, et al., 2015, Kim, et al., 2017b), but not in Gold3 kiwifruit. In the present work, these symptoms were excised and cultured, resulting in 126 isolates. Among them, 95% showed similar morphological characteristics (Fig. 2). Their abundant gray to olive aerial mycelia and the hyaline unicellular α-conidia of oval shape matched with the *Ascomycota* genus, *Botryosphaeria*. DNA sequence identification of a sample of 50 isolates gave a 100% result of *B. dothidea* strains (Fig. 3).

B. dothidea is a globally widespread pathogen, which has been associated to dieback, canker and severe fruit rots in an extensive host range (Tang, et al., 2012, Pitt, et al., 2010, Beckman and Reilly, 2005, Milholand, 1972). Through pathogenicity testing, in this study was found that *B. dothidea* is able to penetrate the fruit directly without wounding. But the presence of a wound exacerbates the disease, accelerating its development and intensifying its severity, as previously reported (Zhou et al., 2015). After re-isolation from

artificially inoculated kiwifruits, same symptoms in the fruit, as well as the same cultural behavior, morphology and also DNA sequence identity were found (Fig. 3), in this way confirming Koch's Postulates and therefore ratifying *B. dothidea* as causal agent of postharvest dimple rot on Gold3 kiwifruit in Jeju Island.

In Korea, yellow fleshed varieties have shown to be less resistant to post-harvest rots than green fleshed ones (Kwon et al., 2011). Previous experiences showed that the ploidy level might play a fundamental role in breeding resistance against the bacterial canker caused by *P. syringae* pv. *actinidiae* or Psa (Tahir, 2019), a pathogenic bacterium that can lead to severe crop loss. Therefore, the evaluation of susceptibility to *B. dothidea* among Hort16A (diploid), Gold3 (tetraploid) and Hayward (hexaploid) varieties may provide insights about a possible relationship between predisposition to dimple rot and ploidy level. In the current study, Gold3 kiwifruit presented more acute symptoms compared to Hayward and Hort16A kiwifruit varieties (Fig. 6), suggesting that dimple-rot susceptibility might not follow the ploidy hypothesis applied for Psa. Additional research could help to find candidate alleles for susceptibility and resistance to dimple-rot disease.

Alternatively, phenological and structural development could influence susceptibility to dimple rot. Kim et al. (2017) observed that flesh firmness declined faster, while sugar contents are generally higher on yellow varieties, making them more susceptible to infection compared to the green-fleshed cultivars and linking incidence of disease in kiwifruit with firmness and Brix degree. Dimple rot incidence also increases during Gold3 kiwifruit maturity stage (Green fractile 112.9º hue and 16.3% of soluble solids content on average at harvest. Lowe, 2011, NZKGI, 2019), which compared with mature Hort16A kiwifruit (Green fractile 110° hue and 15.6% of soluble solids on average at harvest. Lowe, 2011, NZKGI, 2019) and mature Hayward kiwifruit (Brix level of 6.2% and no more than

one out of 30 fruits with 3 or more not-black seeds. NZKGI, 2019), shows higher soluble solids content, suggesting a higher susceptibility to infection during maturity and storage ripening. Phenological studies on Gold3 kiwifruit would help to draw conclusions in this matter.

In addition, compared with Hayward, the yellow varieties Gold3 and Hort16A kiwifruit have shorter and thinner hairs present in lower density, but Gold3 differs from Hort16A in that its skin is covered with numerous and conspicuous lenticels. In the present research, it was observed that Gold3 kiwifruit dimples were more probably beginning to develop with a lenticel as epicenter, while dimples in Hort16A and Hayward were harder to form (Fig. 6). Besides, Gold3 showed to be prone to formation of dimple clusters, i.e. group of dimples that develop into a big rotten area. This suggests that lenticels may play a decisive role in the infection development process, since *B. dothidea* is able to easily enter on hosts through lenticels and surface cracks under favorable conditions (Guan et al., 2015). Additionally, this pathogen has proven to achieve direct penetration through germ tube and appressoria formation in apple fruits (Kim, 1999), but further research needs to be done to prove this behavior on kiwifruits.

In the other hand, solitary dimples on the side of kiwifruits have been previously reported as characteristic symptoms caused by *Mycosphaerellaceae* pathogens, *Pseudocercospora actinidiae* and *P. hangzhouensis* (Yano et al., 2015). This possibility was explored, comparing the pathogenicity capacity of *Pseudocercospora* sp. with *B. dothidea*, under the same conditions. While *B. dothidea* was able to penetrate the unwounded fruit skin, this strain of *Pseudocercospora* sp. did not originated any symptoms and was not re-isolated from any of the inoculated kiwifruits, indicating that *Pseudocercospora* sp. might not be the major pathogen for this form of dimple rot in Gold3.

These results open new possibilities for the integral management of the dimple rot disease, in which protecting the fruit during crucial developmental stages and designing of different storage conditions may better complement the control strategies for Gold3 kiwifruit disease. Further experiments regarding *B. dothidea* life cycle, overwintering of secondary inoculum and the possibility of symbiotic relations with fruit feeding insects may be needed, to respond with a successful strategy for Gold3 kiwifruit protection in Jeju Island.

Ⅴ. 적 요

최근 몇 년간 *Actinidia chinensis* var. *chinensis* 골드3 키위열매의 과일썩음병은 한국에서 가장 심각한 질병 중 하나로 키위의 품질 저하 원인이 되고 결과적으로 소득 손실을 초래한다. 이 과일썩음병의 원인 병원균으로 *Botryospaeria dothidea*로 밝혀졌으며 이 곰팡이는 상처 없이 과일에 직접 침투하는 것으로 확인되었다. 또한, 헤이워드와 호트16A와 같은 다른 키위의 다른 품종을 포함하여 이 곰팡이의 병원성을 알아본 결과 골드3가 다른 두 품종에 비해 가장 감수성인 것으로 밝혀졌다. 한편, *Pseudocercospora* 속에 속하는 병원균이 과일썩음병을 유발한다고 보고됨에 따라 본 연구에서 제주도에서 발생하는 이 병의 원인이 *B. dothidea*라는 것을 밝혔다. 골드3 키위 열매에 이 병원균의 병원성을 정확하게 밝히기 위해서는 이 곰팡이의 감염구조를 비교하기 위한 추가 연구가 필요하다.

VI. References

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