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Identification and classification of pathogenic *Fusarium* isolates from cultivated Korean cucurbit plants

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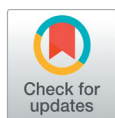
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Abstract

Fusarium wilt disease caused by *Fusarium* species is a major problem affecting cultivated cucurbit plants worldwide. *Fusarium* species are well-known soil-borne pathogenic fungi that cause *Fusarium* wilt disease in several cucurbit plants. In this study, we aimed to identify and classify pathogenic *Fusarium* species from cultivated Korean cucurbit plants, specifically watermelon and cucumber. Thirty-six *Fusarium* isolates from different regions of Korea were obtained from the National Institute of Horticulture and Herbal Science Germplasm collection. Each isolate was morphologically and molecularly identified using an *internal transcribed spacer of ribosomal DNA*, *elongation factor-1 α* , and the *beta-tubulin* gene marker sequence. *Fusarium* species that infect the cucurbit plant family could be divided into three groups: *Fusarium oxysporum* (*F. oxysporum*), *Fusarium solani* (*F. solani*), and *Fusarium equiseti* (*F. equiseti*). Among the 36 isolates examined, six were non-pathogenic (*F. equiseti*: 15-127, *F. oxysporum*: 14-129, 17-557, 17-559, 18-369, *F. solani*: 12-155), whereas 30 isolates were pathogenic. Five of the *F. solani* isolates (11-117, 14-130, 17-554, 17-555, 17-556) were found to be highly pathogenic to both watermelon and cucumber plants, posing a great threat to cucurbit production in Korea. The identification of several isolates of *F. equiseti* and *F. oxysporum*, which are both highly pathogenic to bottle gourd, may indicate waning resistance to *Fusarium* species infection.

Key words: cucurbit plants, *Fusarium* species, identification, pathogenicity



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Introduction

Cucurbitaceae plants are among the most important vegetable crops cultivated worldwide and are the second-largest economically important horticultural family after *Solanaceae*. Agricultural products from cucurbit plants are known for their high nutritional value and are a crucial source of vitamins (Paris, 1989; Salehi et al., 2019). In Korea, the production of cucumber and watermelon fruits is approximately 254,276 and 672,914 tons per year, respectively (Seo and Kim, 2017). Cucurbit plants

are susceptible to various diseases caused by viruses, bacteria, nematodes, and fungi (Song et al., 2018; Kwak et al., 2021). Soil-borne pathogens such as *Fusarium* species and root-knot nematodes are extremely difficult to control in cucurbit plants (Seo and Kim, 2017). *Fusarium* species causing Fusarium wilt is one of the most harmful pathogens to the cucurbit family. *Fusarium oxysporum*, *F. solani*, *F. graminearum*, *F. moniliforme*, *F. proliferatum*, *F. sambucinum*, and *F. semitectum* have been reported to cause diseases in cucurbit plants (Kim and Kim, 2004). In Korea, cucurbit plants such as cucumber and watermelon are mostly cultivated in greenhouses. Unfortunately, greenhouse facilities also protect pathogens from harsh winter conditions. Therefore, cultivation fields and greenhouses will continue to be infected due to the capability of *Fusarium* species to persist for long periods (Seo and Kim, 2017). Hence, accurately identifying pathogenic *Fusarium* species against cucurbit plants to effectively implement control measures is crucial. To this end, we aimed to identify and classify pathogenic *Fusarium* species in cultivated Korean cucurbit plants in this study.

Materials and Methods

Fungal isolates

Typical symptoms of Fusarium wilt disease in cucurbit plants include yellowing, withering, and wilting leaves. Upon excision of the infected plant stem, browning of vascular tissues was observed due to colonization by the fungal pathogen. Symptomatic tissue samples in this study were collected from cultivated cucurbit plants that exhibited the aforementioned symptoms. Thirty-six fungal isolates belonging to the *Fusarium* family were obtained from the National Institute of Horticulture and Herbal Science (NIHHS) germplasm fungal collection. Each isolate was re-cultured on potato dextrose agar (Difco Laboratories, Detroit, MI, USA) and carnation leaf agar (Difco Laboratories, Detroit, MI, USA) at 25°C for 10 days in the dark. The potato dextrose agar (PDA) culture was used as source material for molecular and pathogenicity analyses, whereas carnation leaf agar (CLA) was used for morphological examination.

Morphological and molecular identification

The morphology of the fungal isolates was examined from the CLA culture using a light microscope (Carl-Zeiss, Oberkochen, Germany). The morphological characteristics examined included the shape and size of the conidia, septa formation, conidiophores, and colony appearance on the medium. For molecular identification, the isolate was identified using an *internal transcribed spacer of ribosomal DNA (ITS-rDNA)*, *elongation factor-1 α (EF-1 α)*, and *beta-tubulin (β -tub)* gene marker sequences. Amplification of gene markers, *ITS-rDNA*, *EF-1 α* , and *β -tub* was performed using the primer pairs ITS1/ITS4 (White et al., 1990), EF1-728F/EF1-986R (Glass and Donaldson, 1995), and Bt2a/Bt2b (Carbone and Kohn, 1999) respectively. Polymerase chain reaction (PCR) amplification was performed using the GoTaq DNA polymerase kit (Promega, Madison, WI, USA) following the suggested standard protocol. A phylogenetic tree was constructed using MEGA-X bioinformatics software (Kumar et al., 2018).

Pathogenicity test

Pathogenicity tests were performed on two weeks old of watermelon (*var.* Speedggul), cucumbers (*var.* baekdadaki), and bottle gourds (*var.* dongjanggoon). Microconidia were harvested by flooding the isolated culture PDA with sterile water and scraped with a sterile triangle scraper before filtering with double layers of sterile cheesecloth. The root systems of the plant host seedlings were inoculated with a conidial suspension (1×10^6 conidia $\cdot \mu\text{L}^{-1}$) for 30 min before returning to the soil pot. Inoculated seedlings were then transferred to a greenhouse (temperature: 30°C, daylight: 14 h) for observation. Control plants were inoculated with sterile water. Fungal pathogens from the symptomatic seedling root system were re-isolated and their morphological characteristics and molecular identities were re-identified to fill Koch's postulate.

Results and Discussion

The characteristics of each of the 36 selected *Fusarium* species were examined and grouped based on morphologies described by Hyun et al. (2019) and Booth (1971). Following the morphological assessment, molecular identification was performed to confirm the identity of the isolates and their groups. A BLASTN and phylogenetic analysis results showed that the selected fungal isolates were clustered into three groups: *F. oxysporum*, *F. solani*, and *F. equiseti* (Fig. 1). Among the 36 isolates examined, 23 were identified as *F. oxysporum*, nine as *F. solani*, and four as *F. equiseti*. However, as a species complex, *Fusarium* species were indistinguishable as pathogenic and non-pathogenic species based on morphological and molecular identification using housekeeping gene markers alone. Therefore, pathogenicity tests were conducted to assess the pathogenicity of these isolates (Fig. 2). Among the 36 fungal isolates examined, six of them are non-pathogenic to any of the cucurbit plants host used, whereas the remaining 30 isolates exhibited a pathogenicity trait range from low to highly virulence (Table 1). Approximately 72% of the isolates selected are pathogenic to watermelon, 38% pathogenic to bottle gourd and only 27% pathogenic to cucumber. This result indicated that generally, the watermelon plant is more susceptible to *Fusarium* pathogen infection compared to bottle gourd and cucumber plants. Therefore the implementation of disease management should be given more attention to the watermelon cultivation area.

Five of the *F. solani* isolates (11-117, 14-130, 17-554, 17-555 and 17-556) that were isolated from symptomatic tissues of cucumber) were found to be highly pathogenic to both cucumber and watermelon plants. This result may indicate that besides *F. oxysporum*, *F. solani* species may also pose a greater threat to the cucurbit agricultural production in Korea. Another interesting finding in this study was the identification of *F. oxysporum* and *F. equiseti* as causal agents to wilt disease in bottle gourd plants. Results show that four *F. oxysporum* (14-065, 14-066, 14-077, 19-050) and one *F. equiseti* (14-079) isolates are highly pathogenic to bottle gourd plant. Interestingly, this is the first time *F. equiseti* identified pathogenic to bottle gourd in Korea. Bottle gourds are widely used as rootstocks for grafted watermelons in Korea because of their resistance to *Fusarium* infection (Lee et al., 2010). However, this result suggests that the bottle gourd resistance to *Fusarium* infection is slowly waning. Therefore, the identification of these fungal species as pathogenic to bottle-gourd plants is crucial for both farmers and researchers. The geographical distribution of pathogenic *F. oxysporum* and *F. equiseti* species to grafted watermelons will allow implementing effective management strategies to manage *Fusarium* wilt disease in a specific region. This also provides crucial information to develop a quick and efficient identification protocol to identify *Fusarium* species that cause wilt disease in grafted watermelon. Bottle-gourd resistance against *Fusarium* invasion is waning. This information is also crucial for assessing new cucurbit species or cultivars that are highly resistant to *Fusarium* species and serve as rootstocks for grafted watermelon in the future.

Table 1. Pathogenicity test conducted using the National institute of horticulture and herbal science (NIHHS) fungal isolates on ten roots seedlings for each cucurbit plant host.

No	Host	Fusarium species	Regions	Isolate No. (NIHHS)	Cucurbit plants		
					Bottle gourd (Dongjanggoon)	Watermelon (Speedggul)	Cucumber (Backdadaki)
1	Cucumber	<i>F. equiseti</i>	Chungnam	16-126	10	1	0
2	Cucumber	<i>F. oxysporum</i>	Chungbuk	16-271	0	5	5
3	Cucumber	<i>F. oxysporum</i>	Gangwon	14-233	0	4	7
4	Cucumber	<i>F. oxysporum</i>	Jeonnam	17-557	0	0	0
5	Cucumber	<i>F. solani</i>	Chungnam	14-130	0	10	10
6	Cucumber	<i>F. solani</i>	Gyeongbuk	11-117	0	3	4
7	Cucumber	<i>F. solani</i>	Jeonnam	17-554	0	10	10
8	Cucumber	<i>F. solani</i>	Jeonnam	17-555	0	10	10
9	Cucumber	<i>F. solani</i>	Jeonnam	17-556	0	10	10
10	Watermelon	<i>F. equiseti</i>	Gyeongbuk	15-127	0	0	0
11	Watermelon	<i>F. equiseti</i>	Jeonbuk	14-079	8	1	0
12	Watermelon	<i>F. equiseti</i>	Jeonbuk	14-080	0	1	0
13	Watermelon	<i>F. oxysporum</i>	Chungnam	12-029	0	0	1
14	Watermelon	<i>F. oxysporum</i>	Chungnam	14-065	10	0	0
15	Watermelon	<i>F. oxysporum</i>	Chungnam	14-066	10	6	0
16	Watermelon	<i>F. oxysporum</i>	Chungnam	14-077	10	5	0
17	Watermelon	<i>F. oxysporum</i>	Chungnam	14-078	10	7	0
18	Watermelon	<i>F. oxysporum</i>	Chungnam	14-129	0	0	0
19	Watermelon	<i>F. oxysporum</i>	Chungnam	19-144	9	6	0
20	Watermelon	<i>F. oxysporum</i>	Gangwon	18-369	0	0	0
21	Watermelon	<i>F. oxysporum</i>	Gyeonggi	06-062	10	0	0
22	Watermelon	<i>F. oxysporum</i>	Gyeonggi	19-050	10	4	0
23	Watermelon	<i>F. oxysporum</i>	Gyeonggi	19-051	10	5	0
24	Watermelon	<i>F. oxysporum</i>	Gyeonggi	20-236	0	8	0
25	Watermelon	<i>F. oxysporum</i>	Jeonbuk	14-087	10	0	0
26	Watermelon	<i>F. oxysporum</i>	Jeonbuk	14-090	10	7	0
27	Watermelon	<i>F. oxysporum</i>	Jeonbuk	15-075	0	10	0
28	Watermelon	<i>F. oxysporum</i>	Jeonbuk	20-237	1	10	0
29	Watermelon	<i>F. oxysporum</i>	Jeonbuk	20-238	1	10	0
30	Watermelon	<i>F. oxysporum</i>	Jeonnam	17-559	0	0	0
31	Watermelon	<i>F. oxysporum</i>	Jeonnam	19-022	0	10	0
32	Watermelon	<i>F. oxysporum</i>	Jeonnam	19-465	0	10	0
33	Watermelon	<i>F. solani</i>	Gyeonggi	12-143	0	1	0
34	Watermelon	<i>F. solani</i>	Gyeonggi	12-155	0	0	0
35	Watermelon	<i>F. solani</i>	Jeonnam	18-009	0	10	10
36	Watermelon	<i>F. solani</i>	Unknown	15-041	0	10	10

Fungal virulence level: 10 - 30% infection, 1 - 3; 40 - 70% infection, 4 - 7; 80 - 100% infection, 8 - 10.

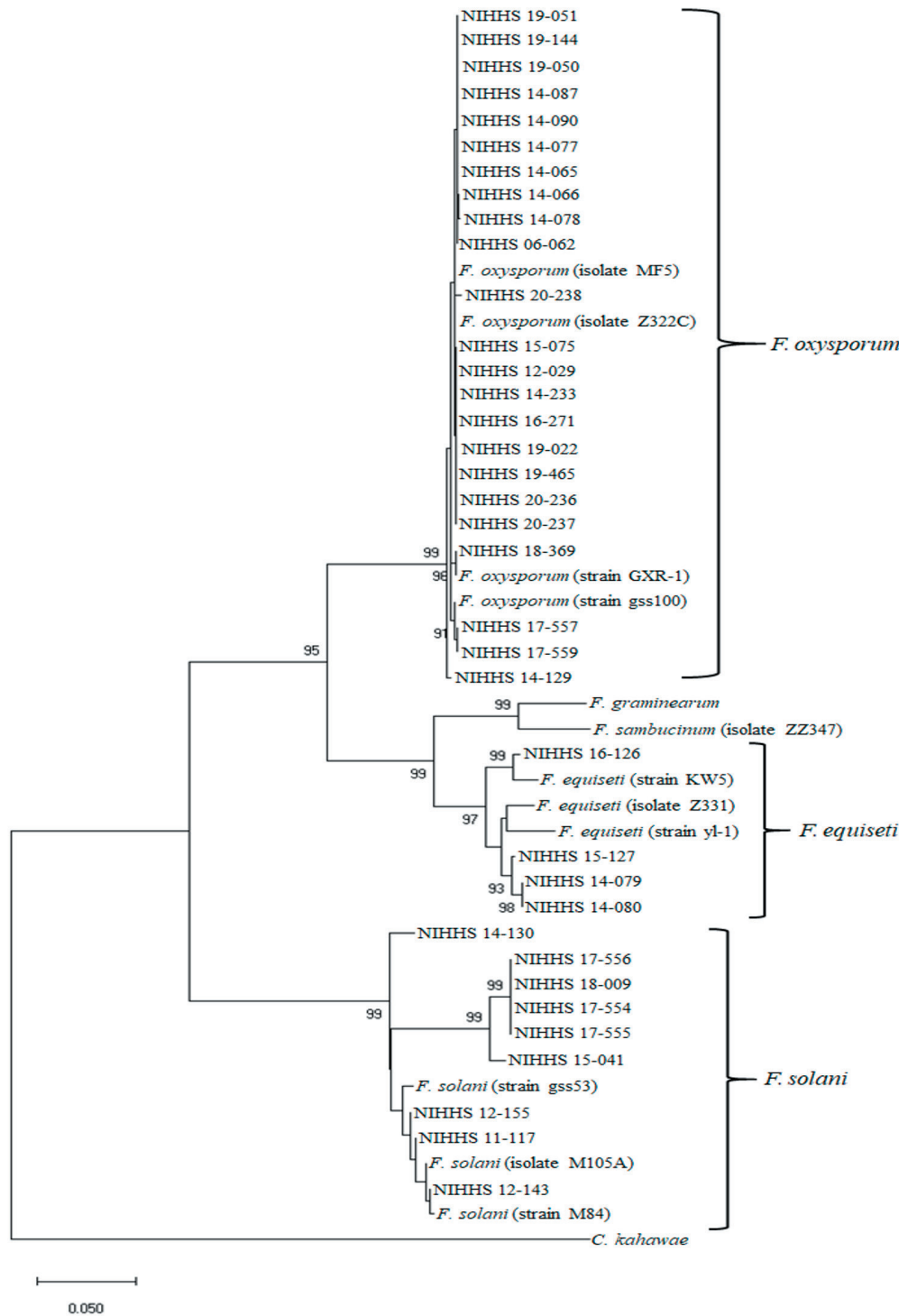


Fig. 1. Phylogenetic tree constructed based on concatenated sequences of *ITS-rDNA*, *EF-1α*, and *β-tub* of 36 fungal isolates from National institute of horticulture and herbal science (NIHHS). Reference sequences of *Fusarium oxysporum*, *F. solani*, *F. graminearum*, *F. sambucinum*, and *F. equiseti* were obtained from Guan et al. (2020), Li et al. (2015), Lu et al. (2014), Stefańczyk et al. (2016), and Zhang et al. (2017), respectively. The tree rooted to *Colletotrichum kahawae* has been reported by Sharma and Shenoy (2014).

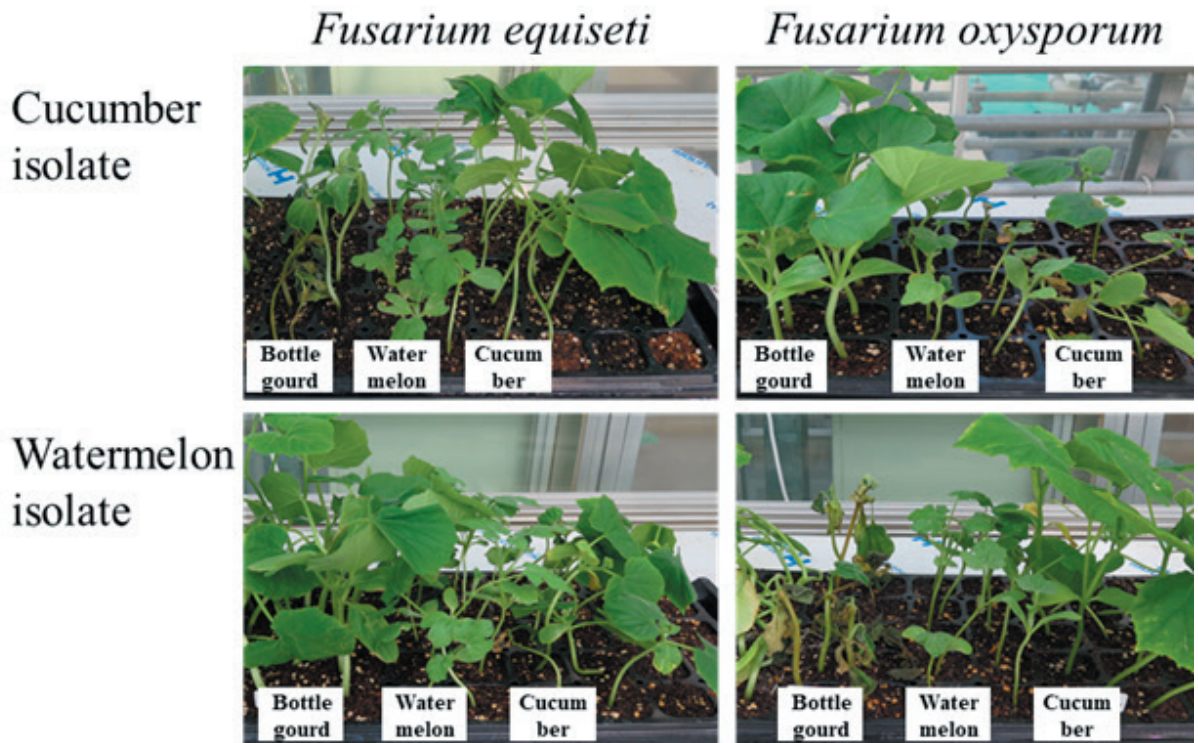


Fig. 2. Pathogenicity test of two isolates of *Fusarium* species on three different cucurbit plants at 25°C in warehouse.

Conclusion

In this study, *F. oxysporum*, *F. solani*, and *F. equiseti* were problematic to cultivated cucurbit plants in Korea. Among this group, *F. solani* was shown to be highly pathogenic to both watermelon and cucumber plants, whereas *F. equiseti* was identified as a novel pathogen to bottle gourd. A high number of *F. oxysporum* isolates together with *F. equiseti*, which have been identified as pathogenic to bottle gourds, may indicate that bottle gourd resistance to *Fusarium* species is waning and a new rootstock for grafted watermelon is needed.

Conflict of Interest

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

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References

- Booth C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute Kew, Surrey, England 237:130-155.
- Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91:553-556.
- Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied And Environmental Microbiology* 61:1323-1330.
- Guan YM, Deng JC, Ma YY, Li Y, Zhang YY. 2020. Seed-associated fungal diversity and the molecular identification of *Fusarium* with potential threat to ginseng (*Panax ginseng*) in China. *Plant Disease* 104:330-339.
- Hyun IH, Chang SY, Kim MK. 2019. Illustrated manual on identification of seed-borne fungi. Animal and Plant Quarantine Agency, Gimcheon, Korea. [in Korean]
- Kim JW, Kim HJ. 2004. *Fusarium* fruit rot of postharvest oriental melon (*Cucumis melo* L. var. *makuwa* Mak.) caused by *Fusarium* spp. *Research in Plant Disease* 10:260-267.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549.
- Kwak HR, Byun HS, Choi HS, Han JW, Kim CS, Wintermantel WM, Kim JE, Kim M. 2021. First report of cucurbit chlorotic yellows virus infecting cucumber in South Korea. *Plant Disease* 105:1862.
- Lee JM, Kubota C, Tsao S, Bie Z, Echevarria PH, Morra L, Oda M. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Scientia Horticulturae* 127:93-105.
- Li ZY, Wang N, Dong ZP, Dong L, Bai H, Quan JZ, Liu L. 2015. First report of *Fusarium graminearum* causing ear rot of foxtail millet in China. *Plant Disease* 99:1856.
- Lu NH, Huang QZ, He H, Li KW, Zhang YB. 2014. First report of black stem of *Avicennia marina* caused by *Fusarium equiseti* in China. *Plant Disease* 98:843.
- Paris HS. 1989. Historical records, origins, and development of the edible cultivar groups of *Cucurbita pepo* (*Cucurbitaceae*). *Economic Botany* 43:423-443.
- Salehi B, Sharifi-Rad J, Capanoglu E, Adrar N, Catalkaya G, Shaheen S, Jaffer M, Giri L, Suyal R, Jugran AK, et al. 2019. Cucurbita plants: From farm to industry. *Applied Sciences* 9:3387.
- Seo Y, Kim YH. 2017. Potential reasons for prevalence of *Fusarium* Wilt in oriental melon in Korea. *The Plant Pathology Journal* 33:249-263.
- Sharma G, Shenoy BD. 2014. *Colletotrichum fructicola* and *C. siamense* are involved in chili anthracnose in India. *Archives of Phytopathology and Plant Protection* 47:1179-1194.
- Song JY, Oo MM, Park SY, Seo MW, Lee SC, Jeon NB, Nam MH, Lee YS, Kim HG, Oh SK. 2018. Analysis of intraspecific genetic diversity in *Acidovorax citrulli* causing bacterial fruit blotch on cucurbits in Korea. *Korean Journal of Agricultural Science* 45:575-582.
- Stefańczyk E, Sobkowiak S, Brylińska M, Śliwka J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. *European Journal of Plant Pathology* 145:871-884.

- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. In PCR Protocols: A Guide to Methods and Applications Innis MA, Gelfand DH, Sninsky JJ, White TJ. Academic Press, Inc., New York, USA.
- Zhang YQ, Lin BY, Zou MY, Liang JX, Hu HQ. 2017. First report of Fusarium Wilt of *Dendrobium officinale* caused by *Fusarium oxysporum* in China. Plant Disease 101:1039.