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First Report of Chrysanthemum (*Chrysanthemum morifolium*) Crown Rot Caused by *Fusarium solani* in Korea

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In August 2010, a severe crown rot was observed on chrysanthemum (*Chrysanthemum morifolium* Ramat., variety Sinro) in several greenhouses located at Damyang and Muan, Jeonnam province, Korea. Three isolates (EML-CHS1, -CHS2, and -CHS3) of *Fusarium* were isolated from the affected plants and identified based on morphological characteristics and rDNA internal transcribed spacer (ITS) sequence analysis. Sequence analysis by BLAST indicated that EML-CHS1, -CHS2 and CHS3 were closest to a *Fusarium* species, *F. solani* with > 99% sequence similarity. Pathogenicity tests were performed on chrysanthemum with spore suspensions containing 3.4 × 10^6 spores/ml using the dipping method. Ten days after inoculation, similar symptoms to those observed in the greenhouses were seen on the inoculated plants. The causal fungus was re-isolated from the artificially inoculated basal stems, fulfilling Koch's postulates. To our knowledge, this is the first report of crown rot by *Fusarium solani* on chrysanthemum (*Chrysanthemum morifolium*) in Korea.

Keywords **:** Asteraceae, rDNA sequence, outbreak, pathogenicity

Chrysanthemums are a genus (*Chrysanthemum morifolium* Ramat.) of about 30 species of perennial flowering plants in the family Asteraceae, which is native to Asia and northeastern Europe. In Korea, the cultivation area covers 700-800 ha and the annual monetary yield reaches approximately $400 million. Generally, chrysanthemums are considered a beautiful cut flower in regards to its figure and fragrance. Also, some varieties of chrysanthemums have been used to make tea as well as a natural insect repellent. Chrysanthemum tea is a flower-based tisane made from chrysanthemum flowers of the species *C. morifolium* or *C. indicum*, which are highly popular in East Asia including China and Korea. The extracts of chrysanthemum plants (stem and flower) have been shown to have a wide variety of potential medicinal properties, including anti-viral, anti-bacterial and anti-mycotic activities (Hu et al., 1994; Collins et al., 1997; Sassi et al., 2008; Marongiu et al., 2009).

In August 2010, a severe outbreak of wilt due to crown rot was observed on chrysanthemums (*C. morifolium*, variety Sinro) in several greenhouses located at Damyang and Muan, South Korea. To date, 7 viral, 10 fungal and 2 bacterial diseases on chrysanthemums have been reported in Korea. The literature concerning the major diseases on chrysanthemums in Korea have included phytophthora rot by *Phytophthora cactorum*, powdery mildew by *Golovinomyces cichoracearum*, rust by *Puccinia tanaceti* and verticillium wilt by *Verticillium dahliae* (KSPP, 2009). To date, 78 records of diseases caused by 23 *Fusarium* species including *F. solani*, *F. oxysporum*, *F. poae*, *F. roseum* and *Fusarium* sp. have been reported to cause stem or root rot and wilt on chrysanthemums (Farr and Rossman, 2011). Several records of stem rot or wilt on *Chrysanthemum* spp. by *F. solani* have been reported in the USA, Hongkong, Thailand, India and Papua New Guinea (Farr and Rossman, 2011).

The objectives of this study were to investigate the morphological characteristics of *Fusarium* species isolated from the crown rot lesion on chrysanthemums, identify the causal fungus based on rDNA internal transcribed spacer (ITS) sequence analysis, and evaluate its pathogenicity to chrysanthemum plant.

Occurrence of crown rot on chrysanthemum. In August 2010, a severe wilt due to crown rot was observed on chrysanthemums at greenhouses located at Damyang and Muan, Jeonnam province, Korea (Fig. 1). The disease outbreak ranged from 20% to 35% in affected greenhouses, causing an enormous economic loss for farmers. The early symptoms included slight wilt and brown to blackish-brown discoloration surrounding basal stems. The late symptoms included crown rot, root rot, wilting, leaf blight and dying plants. The infected surfaces often became covered with white tufts of spores and mycelia (Fig. 1C, D). When basal stems were split, they showed tan to brown discoloration inside (Fig. 1E).

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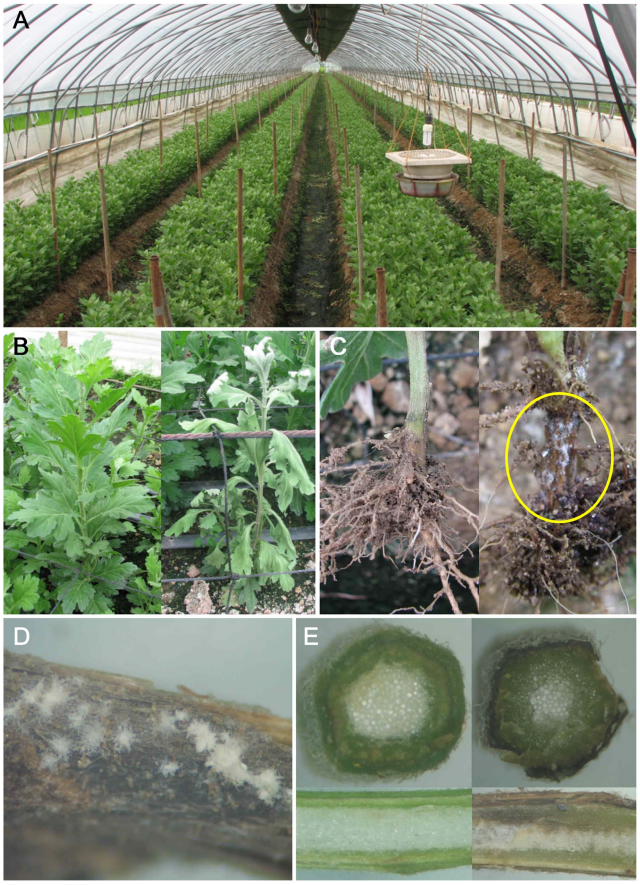

Fig. 1. Occurrence of crown rot on *Chrysanthemum morifolium* (variety Sinro) and morphology of the causal pathogen, *Fusarium* sp. EML-CHS1. (A) healthy chrysanthemum growing in the greenhouse, (B) typical wilt symptom due to crown rot (right) and healthy (left) chrysanthemum, (C) rot symptom on basal stem and root with white fungal mass (right, in yellow circle) and healthy stem (left), (D) infected basal stem covered with white mycelia and spores, (E) cutted stem healthy (left) and infected (right).

Morphological characteristics of crown rot fungus. Three isolates were asceptically isolated from the crown rot lesions of wilted chrysanthemums using a thin capillary tube and subcultured on PDA. The diseased basal stems were collected from the greenhouses and the fungal masses including spores and mycelia were directly taken from the lesions for slide preparation using a capillary tube. Microscopic examination of three representative samples (EML-CHS1, -CHS2 and -CHS3) was conducted to identify the pathogen based on references (Booth, 1971; Nelson et al., 1983). The specimens examined were deposited at EMLH (Environmental Microbiology Lab Herbarium, Chonnam National University, Gwangju, Korea). The microconidia was oval to kidney shaped and the conidia on the natural stem lesions were 13.8–24.3 (avg. 17.6) μm in length × 3.3–6.5 (avg. 5.2) μm in width. Macroconidia on natural lesions were slightly curved, falcate-shaped with 3–5 septa,

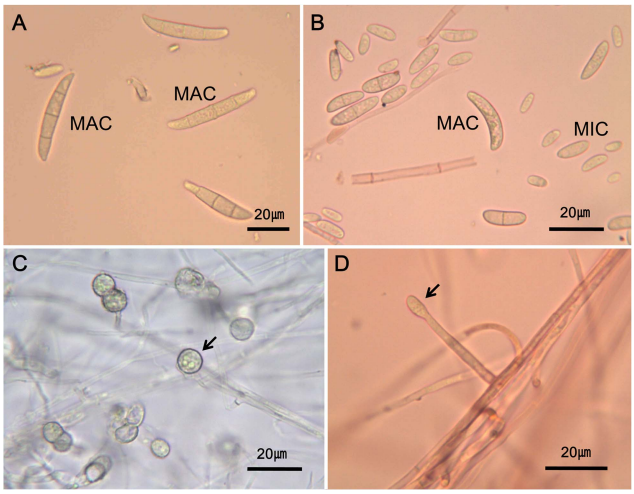

Fig. 2. *Fusarium solani* EML-CHS1 from crown rot lesion on *C. morifolium*. (A & B) Macro-conidia (MAC) and microconidia (MIC) of *F. solani* formed on crown rot lesion of the basal stem, (C) chlamydospores (black arrow) and (D) phialide (black arrow) formed on PDA at 25°C for 7 days (× 400).

and the conidia were 30.4–43.3 (avg. 36.3) μm in length × 3.9–6.2 (avg. 5.4) μm in width. Conidiophores were 43.9–82.9 (avg. 59.3) μm in length. The chlamydospores were globose, hyaline, smooth to rough and the diameter were 7.7–10.6 (avg. 9.0) μm (Fig. 2).

Molecular phylogenetic analysis of the crown rot fungus. To confirm the tentative identification based on the morphological characteristics, molecular analysis of internal transcribed spacer (ITS) rDNA sequences from the three representative isolates was performed. The isolates were cultured on PDA overlaid with cellophane. From the extracted genomic DNA, the complete ITS region including the 5.8S rDNA region was amplified with ITS5 (5'-GGAAGTAAAAGTCGTAACAA-3') and ITS4 (5'-TCC-TCCGCTTATTGATATGC-3') primers as described by Lee et al. (2006). Phylogenetic analyses based on the ITS sequences of *Fusarium* isolates were conducted, using BioEdit ver. 5.0.9.1, Clustal X ver. 1.83 (Thompson et al., 1997). Their phylogenies were assessed using 60 taxa (Table 1) by employing programs available in the MEGA 4 (Tamura et al., 2007). EML-CHS1 (accession number, HQ439150), EML-CHS2 (accession number, HQ439151) and EML-CHS3 (accession number, HQ439152) were completely matched to *F. solani*, which belongs to the *F. solani* species complex (Summerbell and Schroers, 2002), by BLASTN search (Fig. 3). The Neighbour-Joining (NJ) phylogenetic tree based on the ITS sequences of 60 taxa, including the three isolates obtained from diseased *C. morifolium* in Korea was constructed using. The percent sequence identity (the number of matches/the complete

Table 1. *Fusarium* taxa used for molecular phylogenetic analysis in this study

Taxon name	Collection no. (isolate no.)	Source (host)	Origin	GenBank accession no.
Fusarium sp.	EML-CHS1*	*Chrysanthemum morifolium*	Korea	HQ439150
Fusarium sp.	EML-CHS2*	*Chrysanthemum morifolium*	Korea	HQ439151
Fusarium sp.	EML-CHS3*	*Chrysanthemum morifolium*	Korea	HQ439152
F. solani	d16	NI**	Netherlands	GQ922560
F. solani	Tu1-1	Roots of *Cymbidium* sp.	China	HM214456
F. solani	MAFF731042	*Daucus carota* root	Japan	AB513851
F. solani	MOD-5	NI	China	EU625405
F. solani	FMR7988	Human keratitis	Brazil	AM412642
F. solani	FMR4391	Human Blood	USA	AM412635
F. solani	SUF209	*Solanum tuberosum*	NI	AF150466
F. solani	FMR7992	Human keratitis	Brazil	AM412625
F. solani	FMR8688	Nematode	Spain	AM412602
F. solani	FMR8340	Human fungemia	Qatar	AM412594
F. solani	FMR7140	Aquarium sand	Spain	AM412636
F. solani	NRRL22825	*Glycine max*	NI	AF178419
F. solani	NRRL31156	NI	NI	AY220236
F. solani	FMR7141	Aquarium sand	Spain	AM412637
F. solani	FMR7985	Human keratitis	Brazil	AM412641
F. solani	FMR7991	Human keratitis	Brazil	AM412624
F. solani	NRRL22277	*Xanthoxylum* sp.	NI	AF178401
F. solani	NRRL22142	*Cucurbita* sp.	NI	AF178411
F. venenatum	QP	NI	USA	AF006359
F. venenatum	NRRL22198	NI	Germany	AF006350
F. sambucinum	NRRL22187	NI	Germany	U85540
F. sambucinum	NRRL22203	NI	Germany	AF006346
F. sporotrichioides	NRRL25479	NI	Netherlands	AF006348
F. sporotrichioides	FSU85541	NI	USA	U85541
F. culmorum	OTU180	NI	Canada	GU934521
F. culmorum	MAFF241212	NI	NI	AB586990
F. culmorum	CBS122445	*Orthotomicus erosus*	Spain	DQ655726
F. graminearum	LH184	*Camellia sinensis*	China	HQ832817
F. graminearum	ATCC MYA-4620	NI	USA	GU327636
F. graminearum	NRRL25797	NI	Netherlands	AF006344
F. poae	FPU85538	NI	NI	U85538
F. poae	NRRL25799	NI	Netherlands	AF006345
F. poae	FRC T-0796	NI	Japan	AB586983
F. chlamydosporum	CanS-26	Healthy canola stem	China	JF817304
F. chlamydosporum	037	Diseased cocoa tree	Ghina	FJ545407
F. chlamydosporum	dx-6	Unknown mushroom	China	FJ441006
F. chlamydosporum	Ppf29	*Paris polyphylla* Smith var. *yunnanensis*	China	GU586833
F. oxysporum f. sp. *benincasae*	NI	NI	China	FJ943656
F. oxysporum f. sp. *melonis*	NI	NI	NI	AY188919
F. oxysporum f. sp. *melonis*	ISPaVe1070	Melon	Italy	FR852561
F. oxysporum f. sp. *phase*	DM091019-1	Common bean root	China	HM756257
F. annulatum	10p	Bean root rot	Mexico	FJ627998
F. oxysporum f. sp. *vasin*	PA3	*Vitis vinifera*	USA	AY462580
F. oxysporum f. sp. *cepae*	CSC6035ITS	Onion	USA	HQ658961
F. proliferatum	CanR-8	Healthy canola root	China	JF817300

Table 1. Continued

Taxon name	Collection no. (isolate no.)	Source (host)	Origin	GenBank accession no.
F. proliferatum	ATCC42112 (=NRRL13569)	*Zea mays*	USA	GQ167231
F. proliferatum	F25	*Cicer arietinum*	Mexico	EU091039
F. annulatum	CBS258.54	NI	NI	AY213654
F. annulatum	NRRL13614	NI	NI	U61670
F. avenaceum	xsd08063	Poplar	China	FJ478097
F. avenaceum	MAFF239206	NI	NI	AB587016
F. avenaceum	F64	Healthy bulbs of *Lilium longiflorum*	USA	HQ379700
F. dimerum	CBS110320	Human toe nails	Chile	EU926273
F. dimerum	CBS116527	Human, scalp lesion of leukemi patient	USA	EU926284

*Korean strains isolated from *C. morifolium*. **NI: no information. ATCC: American Type Culture Collection, USA. CBS: Centraalbureau voor Schimmelcultures, The Netherlands. EML: Environmental Microbiology Laboratory Culture Collection, Chonnam National University, Korea. FMR: Facultat de Medicina de Reus Culture Collection, Spain. FRC: Fusarium Research Center, Penn State Univ., USA. MAFF: Ministry of Agriculture, Forestry and Fisheries Culture Collection, Japan. NRRL: Agricultural Research Service (ARS) Culture Collection, USDA, USA. SUF = Culture Collection of *Fusarium* in Sinshu University, Japan.

alignment length) values were obtained *via* a NCBI BLASTN search of each isolate. As shown in Fig. 3, identities of EML-CHS1, EML-CHS2 and EML-CH3 isolates were 536/537 (> 99%), 535/536 (> 99%) and 537/539 (> 99%) with AM412635, AM412635 and AM412642 retrieved from NCBI, respectively.

Pathogenicity test. To evaluate the pathogenicity of the three *Fusarium* isolates on *C. morifolium* (variety Sinro), the isolates were cultivated on PDA and then the spores were harvested. The pathogenicity test was performed using the dipping method. Healthy roots and stems of chrysanthemums were soaked in a conidial suspension adjusted to approximately 3.4×10^6 conidia per ml (distilled water containing 0.005% Tween 80) for 15 min. The plants were then potted in sterile soil, kept in a humid chamber for 72 hours and finally moved to a greenhouse. The experiment was carried out in duplicate and repeated two times. Ten days after inoculation, similar symptoms to those observed in the greenhouses were seen on the inoculated plants (Fig. 4). The causal fungus was re-isolated from the artificially inoculated basal stems, fulfilling Koch's postulates. No crown rot or wilt symptoms were observed on control plants whose roots and stems were dipped in sterile water. The disease severity was evaluated using a severity score index 3 to 15 days after inoculation. Out of three isolates tested, EML-CHS1 showed the strongest pathogenicity to chrysanthemum (Fig. 4). Inoculated plants resembled those observed on naturally infected plants within 10 days.

It has been shown that chrysanthemums are subject to two vascular wilt diseases caused by *F. oxysporum* f.sp. *chrysanthemi* and *Verticillium dahliae*, which persist in the soil for many years. The first signs of Fusarium wilt are yellowing of foliage, stunting, and wilting often along one side of the plant. Plants sometimes appear water stressed and foliage brown and die. In addition, the vascular system of the stems appears a reddish brown color (Agriculture & Landscape Program, 2011).

On the other hand, symptoms of Verticillium wilt often appear only after blossom buds have formed and young vigorous plants may be symptomless. Foliage becomes yellow and wilted, sometimes only along leaf margins and on one side of the plant. Leaves begin to die from the base of the plant upward and often remain attached. Stems may exhibit dark streaks in the vascular system (Agriculture & Landscape Program, 2011).

The genus *Fusarium* including *F. solani*, has been shown to cause various diseases on a wide range of hosts including Asteraceae (Summerell et al., 2003). Thus far, there have been no reports of *Fusarium*-induced diseases including crown rot or basal stem rot by *F. oxysporum* or *F. oxysporum* f. sp. *chrysanthemi* on chrysanthemums in Korea. Only Verticillium wilt by *V. albo-atrum* and *V. dahliae* was reported to occur on chrysanthemums in Korea. To the best of our knowledge, this is the first report of crown rot caused by *F. solani* on chrysanthemums in Korea.

Strider (1985a, b) reported that the susceptibility of the chrysanthemum varied with the cultivars. The vast majority of chrysanthemums are highly resistant to both *F. oxysporum* f. sp. *chrysanthemi* and f. sp. *tracheiphilum*; however, Excel, Foxy, Luv, and Fortune are highly susceptible and Applause, Circus, Remarkable, and Tempter are susceptible to *F. oxysporum* f. sp. *chrysanthemi*. In addition, Foxy was highly susceptible and Luv was susceptible to *F. oxysporum* f. sp. *tracheiphilum*. Fusarium wilt of chrysanthemums is

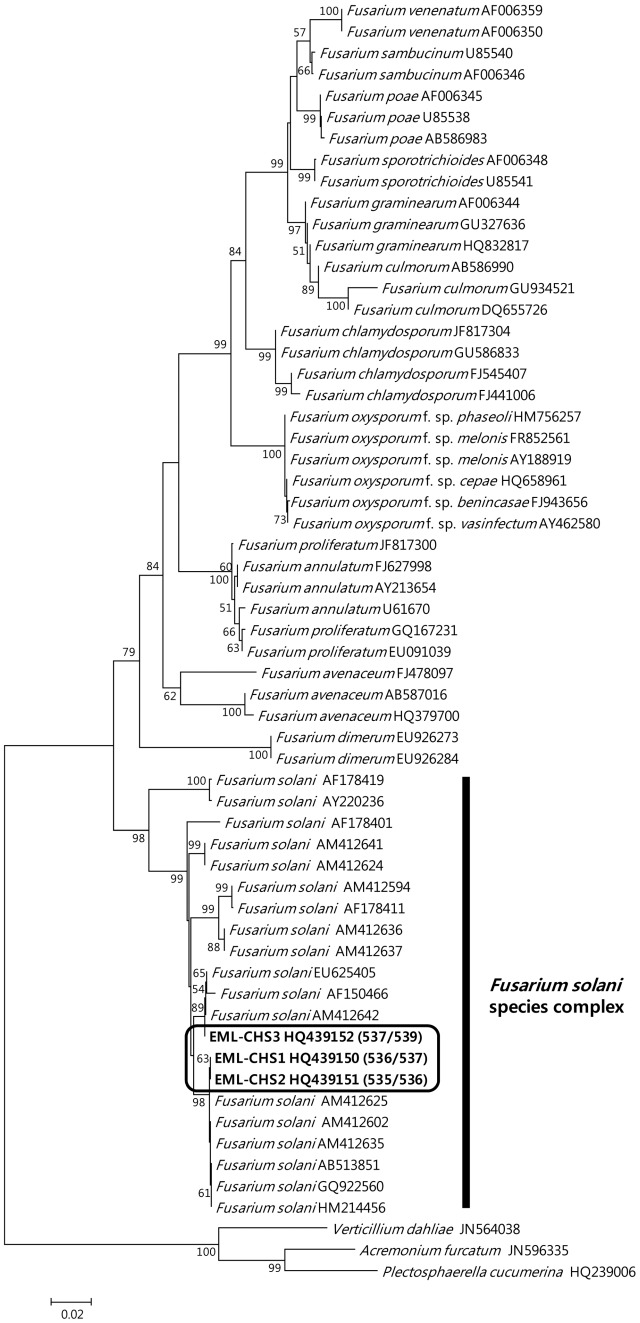

Fig. 3. Phylogentic analysis of the ITS sequences of 60 taxa, including three causal isolates (EML-CHS1, EML-CHS2 and EML-CHS3) obtained from diseased *C. morifolium* in Korea. The three isolates were observed in this NJ tree relative to other strains of different *Fusarium* sp. using *Verticillium dahliae*, *Acremonium furcatum* and *Plectosphaerella cucumerina* as outgroups. Bootstrap values were shown above branches supported by more than 50% from 1,000 replications.

commonly caused by *F. oxysporum* f. sp. *chrysanthemi* and *F. oxysporum* f. sp. *tracheiphilum*. Fusarium crown rot of chrysanthemums is often difficult to be diagnosed because its symptoms are similar to those caused by nutrient deficiencies, improper watering, or diseases such as Pythium root rot or Fusarium wilt (Fisher, 1983; Ren et al., 2008). Fusarium spreads in contaminated soil and infected cuttings and can thrive in warm temperatures, high relative humidity, overwatering, and poor drainage. To manage this disease, pathogen free cuttings or plants are recommended and highly susceptible cultivars should be avoided.

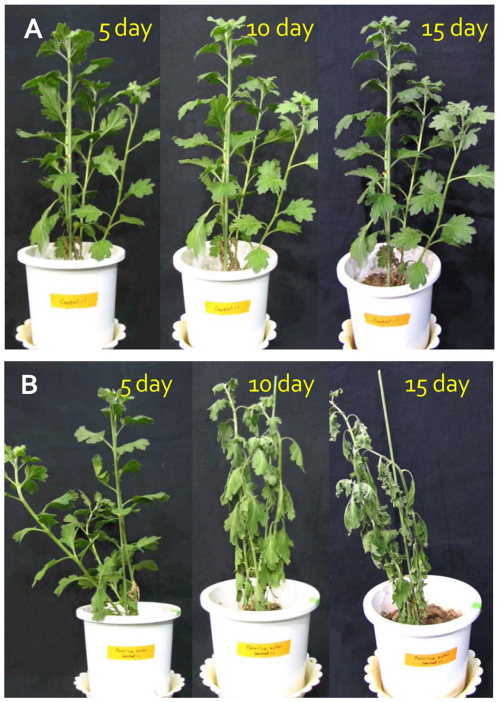

Fig. 4. Pathogenicity of EML-CHS1 on *C. morifolium* (variety Sinro). (A) control, (B) treated.

More studies on the ecological characteristics, host range of *F. solani*, relationship between the outbreak of wilt and crown rot and climate change, and appropriate methods to control the diseases on chrysanthemums are needed in future.

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