Occurrence of Leaf Blight on Cosmos Caused by *Alternaria cosmosa* in Korea

Jian Xin Deng¹, Ji Hye Lee², Narayan Chandra Paul³, Hye Sun Cho⁴, Hyang Burm Lee^{3*} and Seung Hun Yu^{2*}

¹Department of Plant Protection, College of Agriculture, Yangtze University, Jingzhou 434025, China

²Department of Applied Biology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea

³Division of Applied Bioscience & Biotechnology, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 500-757, Korea

⁴Ginseng Resources Institute, Korea Ginseng Corp., Daejeon 305-805, Korea

(Received on September 24, 2014; Revised on November 24, 2014; Accepted on November 29, 2014)

In 2011, a leaf blight disease was observed on cosmos (*Cosmos bipinnatus*) leaves in Nonsan, Korea. The causal pathogen was isolated and identified based on morphological and molecular approaches. Morphological characteristics of the pathogen matched well with the *Alternaria cosmosa* and also easily distinguishable from *Alternaria zinniae* reported from cosmos seeds by producing branched beak. Phylogenetically, the pathogen could not be distinguished from *A. passiflorae* based on the sequence analysis of a combined data set of *Alt a1* and *gpd* genes. However, *A. passiflorae* was distinguished from the present species by having conidiophores with 4 to 5 conidiogenous loci. The results indicate that the present *Alternaria* species is *A. cosmosa*. Pathogenicity tests revealed that the isolate was pathogenic to the leaves of *Cosmos bipinnatus*. This is the first report of *Alternaria* blight disease caused by *A. cosmosa* on cosmos in Korea.

Keywords : asteraceae, morphology, pathogenicity, phylogenetic analysis

Cosmos bipinnatus, known as the garden cosmos or Mexican aster, is a half-hardy annual herbaceous plant native to Mexico. The species belonging to the family Asteraceae offers flowers of solitary red, white, pink or purple color blooms (Dubey and Singh, 2011). The cosmos has been widely cultivated as a valuable ornamental plant since its first introduction in Korea in 1938 (Park, 2009). Now, it is commonly to be found in plant gardens and

along the sides of road or river in Korea. Fruits and aerial parts of the plant are useful as traditional herbal medicine to reduce various diseases such as jaundice, intermittent fever, and splenomegaly (Jang et al., 2008). Jang et al. (2008) reported that the cosmos flowers showed a significant antioxidant activity and protective effect against oxidative DNA damage

Anthrachnose, powdery mildew and white smut diseases of *Cosmos bipinnatus* have been reported in Korea caused by *Colletotrichum acutatum*, *Podosphaer fusca* and *Entyloma cosmic*, respectively (Park et al., 2012). Cho (2004) isolated a large-spored *Alternaria* containing narrow-taper long beak from *C. bipinnatus* in Gongju and Naju, Korea, in 2000. The *Alternaria* species was considered as a new species based on the morphological and molecular characteristics. However, the author did not assign scientific name of the pathogen. Soon afterwards, a new species of *Alternaria*, *A. cosmosa* Y.L. Li & W.S. Wu was reported from cosmos seeds in Taiwan on the basis of conidial morphology, which showed a specific pathogenicity on *Cosmos bipinnatus* and *C. sulphureus* (yellow cosmos) (Wu and Li, 2005). Simmons (2007) summarized his results of a lifetime study on *Alternaria* taxonomy based on morphology and described 275 species totally. But he did not include the species in his publication because he unsuccessfully obtained the ex-type material of *A. cosmosa* for the comparison with the other morphologically similar taxa. Meanwhile, *Alternaria danida* on *Ageratum houstonianum* is suggested as a similar taxon for *A. cosmosa* (Simmons, 2007).

In 2011, a large-spored *Alternaria* was isolated again from blight leaves of *Cosmos bipinnatus* in Nonsan, Korea. The morphological characteristics were identical to previously reported species of *Alternaria* (Cho, 2004). The objective of the present study was to report a new

*Corresponding author.

Phone) +82-42-821-5762, FAX) +82-42-823-8679

E-mail) shunyu@cnu.ac.kr, hblee@jnu.ac.kr

Fig. 1. *Alternaria* leaf blight on *Cosmos bipinnatus* and colony of the causal fungus. (A) discolored and necrotic symptoms on leaves of *C. bipinnatus*. (B) obverse (left) and reverse (right) of colony grown on potato dextrose agar at 25°C for 7 days.

Alternaria disease of *Cosmos bipinnatus* in Korea and to identify the causal pathogen based on morphological and molecular approaches.

Symptoms and isolation of the fungus. Blight symptoms on lower leaves of *Cosmos bipinnatus* were observed during the period of August–September 2011 in Nonsan, Chungnam, Korea (Fig. 1A). Infected leaves showed small and brown necrotic spots along with leaf discoloring. The necrotic lesions were enlarged gradually, finally resulting in the leaf blight and defoliation. The diseased leaves were cut and placed in a moist Petri dish for conidial sporulation. Pure cultures (including CNU 111459) of the *Alternaria* species were obtained by single spore isolation method. Another isolate (CNU 3437) of *Alternaria* sp. collected in 2000 (Cho, 2004) and preserved in the Culture Collection of Chungnam National University (CNU) in Korea was also used in this study. The new isolates were deposited in the CNU Culture Collection, and the Korean Agricultural Culture Collection (KACC) in Suwon, Korea.

Morphological characteristics. The isolate CNU 111459 was grown on potato dextrose agar (PDA; Difco, Montreal, Canada) incubated at 25°C in the dark. Colony diameters were measured after 7 days of incubation and other characteristics such as texture, color and pigmentation were also recorded. The isolate was also cultured on potato carrot agar (PCA: 20 g white potato, 20 g carrot, and 20 g agar in 1 l) under fluorescent lights (8/16 light/dark) at 22°C to induce sporulation (Simmons, 2007). Randomly

Fig. 2. Conidial morphology of the present isolate CNU 111459 from *Cosmos bipinnatus*. (A) sporulation patterns, (B) conidiophores, (C) conidia. Scale bars = 50 µm.

selected conidia (50) from 7-day-old cultures were used to obtain conidial measurements and photograph was taken using an OLYMPUS BX50 light microscope (OLYMPUS, Tokyo, Japan) with an Artcam 300MI digital camera (ARTRAY, Tokyo, Japan).

The colonies on PDA were cotton or woolly, effuse, white or pale to dark grey; reverse yellow to orange, secreting a yellow pigment into the medium, 65–74 mm in diameter (Fig. 1B). Conidiophores on PCA were simple, erect or curved, septate (1–7), pale brown, smooth-walled, usually only one pigmented terminal conidiogenous site, measuring 25–120 (–140) × 4.5–8.5 µm (Fig. 2A, 2B). Conidia were solitary, straight or slightly curved, mostly long obclavate, olivaceous brown to dark yellowish brown, smooth-walled, rostrate, tapering towards a long beak; conidium body 60–130 (–140) × 5–15 (–22) µm, with 2–13 (–16) transverse septa, 1–4 longitudinal septa; beaks narrowly filamentous, filiform, sub hyaline to pale brown, often branched once, twice or more, 50–200 (–220) µm long, 2–3 µm wide, with 0–8 septa (Fig. 2C).

The cultural, conidial and sporulation characteristics of the present *Alternaria* species was identical with the species described by Cho (2004) in Korea and *A. cosmosa* reported by Wu and Li in Taiwan (2005) even though there were some variation in the size of conidia (Table 1). Some morphological characteristics of *Alternaria* (e.g., color, size, shape of conidia, and sporulation patterns) that are used for identification sometimes overlap among species and vary depending on the cultural conditions (e.g., temperature and substrate) (Simmons and Roberts, 1993).

The first report of *A. cosmosa* was from cosmos seeds (Wu and Li, 2005). Richardson (1979) also reported another *Alternaria* species, *A. zinniae*, from cosmos seeds. But *A. zinniae* is easily differentiated from the present *Alternaria* species by producing unbranched beak. Morphologically, the present *Alternaria* species was distinct to *A. carthami* and similar with *A. agripestis* and

Table 1. *Alternaria* species used for phylogenetic analyses

Species	Isolate	GenBank accession No.	
		Alt a 1	gpd
<i>A. agripestis</i>	CBS 577.94	JQ646440	JQ646356
<i>A. carthami</i>	CBS 635.80	JQ646421	JQ64633
<i>A. cichorii</i>	CBS 102.33	JQ646424	JQ646340
<i>A. cirsinioxia</i>	CBS 113261	JQ646435	JQ646351
<i>A. cosmosa</i>	CNU 3437	KM655806	KM655808
<i>A. cosmosa</i>	CNU 111459	KM655807	KM655809
<i>A. cretica</i>	CBS 109164	JQ646426	JQ646342
<i>A. cyphomandrae</i>	CBS 109155	JQ646444	JQ646360
<i>A. danida</i>	EGS 07-029	JQ646420	JQ646336
<i>A. dichondrae</i>	CBS 199.74	JQ646441	JQ646357
<i>A. grandis</i>	CBS 109158	JQ646425	JQ646341
<i>A. hawaiiensis</i>	CBS 630.93	JQ646436	JQ646352
<i>A. linicola</i>	CBS 103.46	JQ646447	JQ646363
<i>A. multirostrata</i>	CBS 712.68	JQ646446	JQ646362
<i>A. passiflorae</i>	CBS 113.38	JQ646437	JQ646353
<i>A. porri</i>	ATCC 58175	AY563296	AY278806
<i>A. protenta</i>	CBS 116696	JQ646419	JQ646335
<i>A. scorzonerae</i>	CBS 478.83	JQ646418	JQ646334
<i>A. solani</i>	EGS 44-098	GQ180096	GQ180080
<i>A. solani-nigri</i>	CBS 113403	JQ646417	JQ646333
<i>A. subcylindrica</i>	CBS 109161	JQ646429	JQ646345
<i>A. tomatophila</i>	EGS 42-156	GQ180101	GQ180085

A. passiflorae (Table 2). However, *A. agripestis* differed from the present species by having ornamented conidial body, and *A. passiflorae* distinguished from the present species by having conidiophores with 4 to 5 conidiogenous loci (Simmons, 2007). Simmons (2007) mentioned that *A. cosmosa* and *A. danida* are morphologically similar species. However, conidial beak tip of *A. danida* is possible to convert into a secondary conidiophore yielding a chain of two conidia, the morphological phenomenon was not observed among the present *A. cosmosa* isolates, and not

Table 2. Morphological comparison among the present *Alternaria* species and the other related species

Species	Conidial body		Beak (µm)	Culture media	Temperature	References
	Size (µm)	Transverse septa				
<i>A. agripestis</i>	80–110 × 11–18	8–11	190–260	PCA	22°C	Simmons (2007)
<i>A. carthami</i>	75–90 × 17–22	8–10	150–200	V8A	22°C	Simmons (2007)
<i>A. danida</i>	95–120 × 23–26	6–11	175–250 (>250)	V8A	22°C	Simmons (2007)
CNU 111459	60–130 (–141) × 5–15 (–22)	2–13 (–16)	50–200 (–220)	PCA	22°C	In the study
CNU 3437	50–100 × 12–18	6–13	75–163	V8A	20°C	Cho (2004)
<i>A. cosmosa</i>	50–110 × 15–25	7–12	112.5–287.5	V8A	25°C	Wu and Li (2005)
	50–100 × 15–22.5	6–11	137.5–287.5	PCA	25°C	Wu and Li (2005)
<i>A. passiflorae</i>	70–115 × 16–22	7–10 (>10)	100–220	V8A	22°C	Simmons (2007)

Fig. 3. Maximum likelihood tree of the present *Alternaria* species from *Cosmos bipinnatus* based on a combined dataset of Alt a1 and *gpd* gene sequences. Bootstrap values are shown above branches. The bar indicates nucleotide substitutions per site.

described by Wu and Li (2005).

Phylogenetic analysis. Genomic DNA of the present isolates (CNU 3437 and CNU 111459) was extracted using a modified protocol of Park et al. (2005). Fragments containing *Alternaria* allergen a 1 (Alt a1) gene and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene were amplified with primers Alt-a1-for & Alt-a1-rev (Hong et al., 2005), and *gpd1* & *gpd2* (Berbee et al., 1999), respectively. PCR products were purified using a Wizard PCR prep kit (Promega, Madison, WI, USA) and sequenced using commercial sequencing service provider (Macrogen, Seoul, Korea). Resultant sequences were deposited in GenBank (Table 1), from which the molecular sequences for the other species were retrieved and included in the study. A pair-wise alignment of all sequences was completed using the ClustalX program (Thompson et al., 1997). Phylogenetic relationships of a combined dataset were estimated by a maximum likelihood analysis using the RAXML program (Stamatakis et al., 2008).

PCR amplification resulted DNA fragments in size of 452 and 564 bp from Alt a1 and *gpd* genes, respectively. The present *Alternaria* isolates CNU 111459, CNU 3437 and *A. passiflorae* could not be differentiated by the sequences of these two genes (Fig. 3). Phylogenetic analysis of the dataset revealed that the present *Alternaria*

species was closely related to *A. carthami* and *A. agripes*, genetically distant to *A. danida* which was accordance to the results of Lawrence et al. (2013).

Although the present *Alternaria* species could be delimited from *A. passiflorae* based on the phylogenetic analysis in the study, the morphology of conidial body and condiophore was an obvious delimitation characteristic. Hence, the present *Alternaria* isolates CNU 111459 and CNU 3437 were all confirmed as *Alternaria cosmosa* Y.L. Li & W.S. Wu (2005). This is the first report of *Alternaria* blight caused by *A. cosmosa* on *Cosmos bipinnatus* in Korea.

Pathogenicity. The pathogenicity of the species was conducted by spraying conidial suspension (1×10^5 conidia/ml) to the detached surface-sterilized leaves. Necrotic spots were induced after two to three days and enlarged the size with age (data not shown). Conidia of *A. cosmosa* were re-isolated from the diseased leaves. *A. cosmosa* were pathogenic to *Cosmos bipinnatus* and *C. sulphureus* (yellow cosmos), which is readily infected the lower leaves of yellow cosmos and caused brownish spots (Wu et al., 2007). During our observation in Korea, the causal disease was also commonly found on the lower leaves of *C. bipinnatus*.

Acknowledgment

This study was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea.

References

- Berbee, M. L., Pirseyedi, M. and Hubbard, S. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91:964–977.
- Cho, H. S. 2004. Taxonomic studies on Korean species of *Alternaria* based on morphology and molecular phylogenetic analysis. Ph.D. Thesis. Chungnam National University, Deajeon, Korea.
- Dubey, S. and Singh, V. K. 2011. Population dynamics of *Aphis spiraeicola* Patch (Homoptera: Aphididae) on medicinal plant *Cosmos bipinnatus* in eastern Uttar Pradesh, India. *Adv. Life Sci.* 1:54–58.
- Hong, S. G., Cramer, R. A., Lawrence, C. B. and Pryor, B. M. 2005. Alt a 1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. *Fungal Genet Biol.* 42:119–129.
- Jang, I. C., Park, J. H., Park, E., Park, H. R. and Lee, S. C. 2008.

- Antioxidative and antigenotoxic activity of extracts from cosmos (*Cosmos bipinnatus*) flowers. *Plant Foods Hum Nutr.* 63:205–210.
- Lawrence, D. P., Gannibal, P. B., Peever, T. L. and Pryor, B. M. 2013. The sections of *Alternaria*: formalizing species-groups concepts. *Mycologia* 105:530–546.
- Park, S. H. 2009. New Illustrations and Photographs of Naturalized Plants of Korea. Ilchokok Publisher, Seoul. Korea. 575 pp. (in Korean)
- Park, J. H., Cho, S. E., Park, M. J., Hong, S. H. and Shin, H. D. 2012. Occurrence of white smut caused by *Entyloma cosmi* on *Cosmos bipinnatus* in Korea. *Res. Plant Dis.* 18:250–254 (in Korean).
- Park, M. S., Seo, G. S., Bae, K. S. and Yu, S. H. 2005. Characterization of *Trichoderma* spp. associated with green mold of Oyster mushroom by PCR-RFLP and sequence analysis of ITS regions of rDNA. *Kor. J. Plant Pathol.* 21:229–236.
- Richardson, M. J. 1979. An annotated list of seed-borne disease. CAB International, Surrey, UK. 320 pp.
- Simmons, E. G. 2007. *Alternaria*: An Identification Manual. CBS Fungal Biodiversity Centre, Utrecht, Netherlands.
- Simmons, E. G. and Roberts, R. G. 1993. *Alternaria* themes and variations (73). CBS Fungal *Mycotaxon* 48:109–140.
- Stamatakis, A., Hoover, P. and Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Syst. Biol.* 57:758–771.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25:4876–4882.
- Wu, W. S. and Li, Y. L. 2005. A new species of *Alternaria* on cosmos seeds. *Mycotaxon* 91:15–20.
- Wu, W. S., Li, Y. L. and Wu, H. C. 2007. Potential of *Bacillus amyloliquefaciens* for control of *Alternaria cosmosa* and *A. patula* of *Cosmos sulfurous* (Yellow Cosmos) and *Tagetes patula* (French Marigold). *Phytopathology* 155:670–675.