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Anthracnose of Safflower Caused by *Colletotrichum acutatum*

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Anthracnose occurred severely on safflower plants grown in Euiseong and Jecheon areas of Korea in 1997 and 1998. The disease incidence was up to 100% in some fields, and symptoms developed on seedlings, leaves, stems, roots and hulls of the plants. *Colletotrichum* sp. was consistently present on the diseased plant parts, and all the isolates from the lesions were identified as *Colletotrichum acutatum* based on the morphological and cultural characteristics. Similar symptoms were produced on the host plants by artificial inoculation with isolates of the fungus. The fungus was reisolated from lesions on the plants inoculated. This is the first report that *C. acutatum* causes anthracnose of safflower.

Keywords : anthracnose, *Colletotrichum acutatum*, safflower.

Safflower (*Carthamus tinctorius* L.) is cultivated worldwide as an ornamental plant or oil crop. In Korea, the plant has been cultivated as a medicinal crop, and cultivation area of the plant is increasing because of high price of the seeds. Recently severe outbreaks of anthracnose on the plants were encountered very frequently during survey of plant diseases in two locations of Korea. The disease occurred at the seedling stage of the plant and continuously developed on leaves, stems, hulls and roots until the harvesting season. Since it rained quite often in Korea during the spring and summer in 1998, many farmers had to give up growing the safflower because most of the plants in some fields were destroyed due to the severe outbreaks of the disease during the growing season.

Colletotrichum spp. cause anthracnose on a variety of plants (Arx, 1957b; Arx, 1970; Farr et al., 1989; Sutton, 1980; Sutton, 1992). *Gloeosporium carthami* (Fukui) Hori & Hemmi was named as an anthracnose pathogen of safflower in Japan (Hemmi, 1919). Since then, occurrence of safflower anthracnose caused by this fungus has been reported in other countries (Farr *et al.*, 1989; Mankin, 1969; Tai, 1979). However, the genus *Gloeosporium* was invalidated, and many species belonging to the genus were transferred to *Colletotrichum* or other genera (Arx, 1957a; Arx, 1957b; Arx, 1970; Sutton, 1980; Sutton, 1992). Arx (1957b) recorded *G. carthami* as a synonym of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

Inoculation tests showed that some isolates of *C. gloeosporioides* f. sp. *malvae* and *C. orbiculare* (Berk. & Mont.) Arx can attack safflower plants (Mortensen and Makowski, 1997; Walker et al., 1991). There has been no report on identification and pathogenicity of *Colletotrichum* species except *G. carthami* causing anthracnose of safflower in fields. This study was carried out to identify the fungus which causes anthracnose of safflower in the fields.

Materials and Methods

Field survey. Safflower fields in Euiseong and Jecheon areas of Korea were surveyed during the growing seasons in 1997 and 1998. Incidence of anthracnose on 100 safflower plants in each field was investigated in three replicates, and symptoms on the plants were observed.

Isolation of the pathogen. Diseased safflower plants were collected from the locations investigated. Three to 5 mm-lesion pieces cut from the diseased plants were plated on 2% water agar (WA) after surface-sterilizing with 1% sodium hypochlorite solution for 1 min. The lesion pieces were observed under a stereomicroscope after two days of incubation at 24°C. Using an inoculating needle, conidial mass on each lesion piece was suspended in 200 μl of sterile distilled water in an 1.5 ml-microtube to make a conidial suspension. A loopful of the conidial suspension was streaked on WA surface with a platinum wire loop to distribute the conidia. After 24 hr incubation at 24°C, agar fragments bearing a single germinated conidium were transferred to fresh WA and incubated at 24°C for 5 days. Monoconidial isolates obtained from the WA plates were cultured on potato dextrose agar (PDA) and used for the identification and pathogenicity tests.

Morphological characteristics. Conidia and setae produced on host plants and in PDA cultures were examined by light microscope. Fifty conidia and 25 setae chosen randomly from each lesion or culture were observed and measured under the light microscope. Appressoria of the isolates were examined using a

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modified slide culture method of Smith (1990). A drop of conidial suspension (3~5×10⁶/ml) from each isolate was placed onto 2% WA in a 9 cm-diameter petri dish. A sterile coverslip was placed over the drop and incubated at 24°C for 10 days in the dark. Fifty randomly chosen appressoria per isolate were observed and measured by light microscope.

Cultural characteristics. Five isolates were used for the examination of cultural characteristics. Six millimeter-diameter mycelial disks from PDA cultures of the isolates were transferred to

Table 1. Incidence of anthracnose in safflower fields in two locations of Korea from April to July in 1997 and 1998

Location	No. of fields surveyed	No. of fields infected	% infected plants	
			Range	Average
Euiseong	18	15	1~100	28.6[a]
Jecheon	22	20	20~100	45.2

[a]One hundred plants in each field were investigated in three replicates.

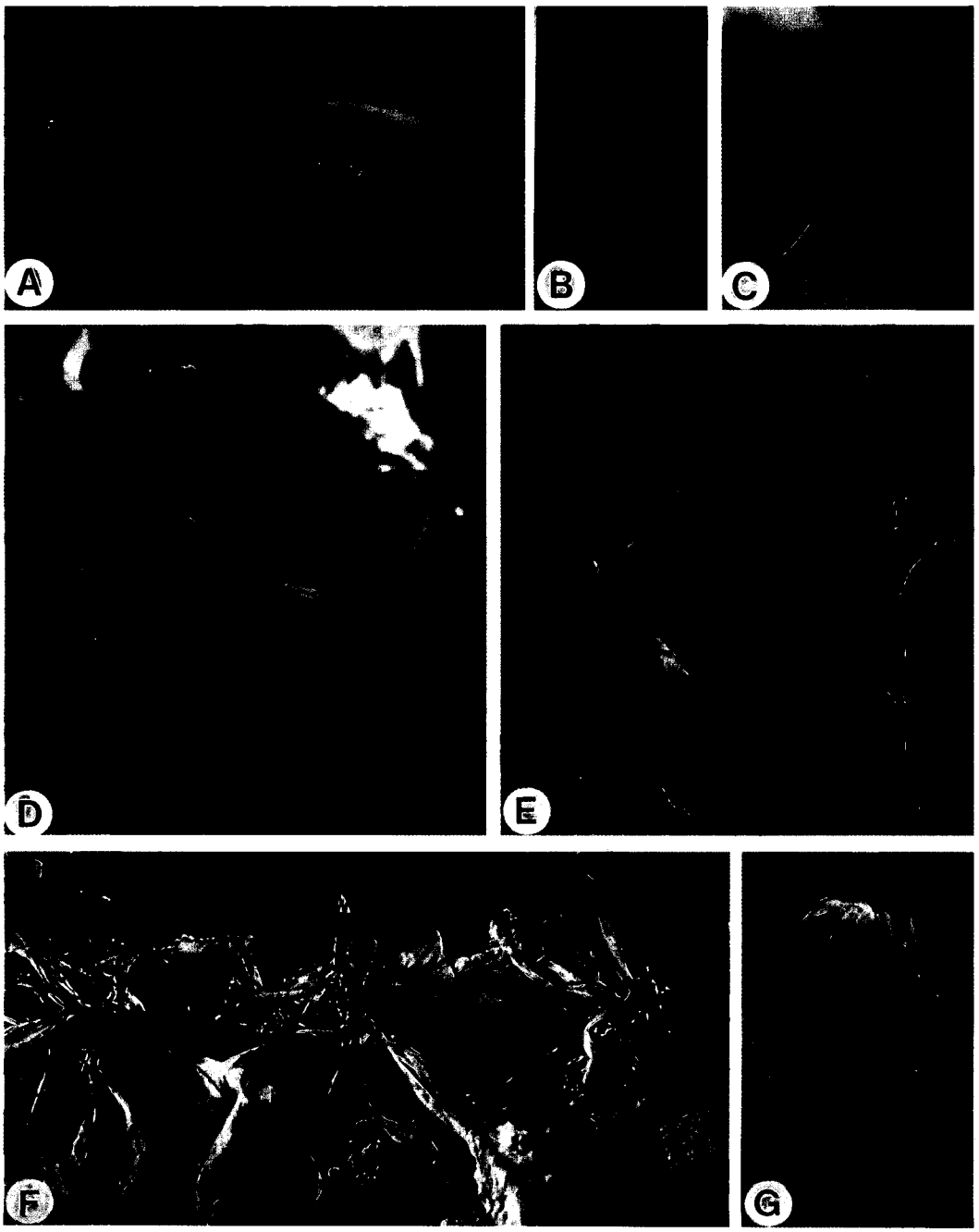

Fig. 1. Symptoms of anthracnose on safflower plants in the field. A and B, lesions on seedlings; C, a circular spot on a leaf; D, lesions on leaves and stems; E, wilt due to root rot of a diseased plant; F, severely infected plants at the early stage of growth; G, an infected hull showing rot and blight at the late stage of growth.

PDA in 9 cm-diameter petri dishes. Cultural appearance of the isolates was observed after 20 days of incubation at 26°C in the dark. Optimum tremperature for mycelial growth of the isolates was examined in three replicate PDA cultures at 2°C intervals from 20 to 30°C, minimum temperature at 1°C intervals from 6 to 10°C, and maximum temperature at 1°C intervals from 32 to 37°C. The positive criterion for the mycelial growth at the minimum and maximum temperatures was above the linear growth rate of 1 mm for 10 days.

Pathogenicity test. Healthy safflower seeds were sown in circular plastic pots (21 cm in diameter and 29 cm in height) containing sterile soil, and the pots were placed in a greenhouse at 18~32°C. Safflower plants were used for inoculation tests at the 23- and 46-day-old stages.

Five isolates were used for the inoculation tests. Conidial suspensions were prepared from 20-day-old PDA cultures, then diluted with sterile distilled water to make a concentration of $3\sim5\times10^6$ conidia/ml. Twenty milliliter conidial suspension of each isolate was sprayed onto 23-day-old plants, and 40 ml onto 46-day-old plants, respectively. Control plants were treated with sterile distilled water. The plants were placed in dew chambers with 100% relative humidity at 24~26°C for 2 days, then moved into the greenhouse. This experiment was performed in three replicates. Disease severity was rated based on the degree of symptoms induced 8 days after inoculation. Reisolation of the pathogen from the lesions on the plants was conducted as described previously.

Results

Disease incidence and symptoms. Anthracnose on safflower plants occurred severely in Euiseong and Jecheon areas of Korea during the disease survey from April to July in 1997 and 1998 (Table 1). The disease incidence ranged from 1 to 100% with average of 28.6% in 15 of 18 fields in Euiseong and from 20 to 100% with average of 45.2% in 20 of 22 fields in Jecheon during the growing seasons of safflower.

Symptoms developed on seedlings, leaves, stems, hulls and roots of safflower. Symptoms on seedlings appeared as a damping-off (Fig. 1A and B). As seedlings emerged, water-soaked tan lesions developed on hypocotyls and cotyledons. The infected seedlings rotted and died in a few days after appearance of the symptoms. Symptoms on leaves appeared as circular to irregular spots with gray to dark brown discoloration, and severely infected leaves blighted (Fig. 1C and D). Symptoms on stems appeared as elliptical to irregular sunken cankers with dark brown discoloration (Fig. 1D). The infected stems bent toward the lesions. Pale yellow conidial masses developed on the lesions at the late stages. Infected roots rotted with dark brown to black discoloration, and aerial parts of the plant wilted (Fig. 1E). Severely infected plants blighted and died at the early stage of growth (Fig. 1F). Infected hulls rotted and blighted with dark brown discoloration resulting in poor or no seed production (Fig. 1G).

Isolation and identification of the pathogen. *Colletotrichum* sp. was consistently isolated from anthracnose lesions on safflower plants. A total of 156 isolates were obtained from seedlings, leaves, stems, roots and hulls of the plants (Table 2). The fungus was isolated from the lesions of roots collected in Euiseong, but not from those in Jecheon.

All the isolates were identified as *Colletotrichum acutatum* Simmonds ex Simmonds based on the morphological and cultural characteristics. The morphological characteristics of *C. acutatum* examined by the authors were similar to those reported by previous workers (Table 3). Colonies on PDA were gray to dark gray, and pale yel-

Table 2. Isolation of *Colletotrichum* sp. from anthracnose lesions on safflower plants

Plant part	No. of isolates from locations		Total
	Euiseong	Jecheon	
Seedling	7	8	15
Leaf	8	12	20
Stem	40	28	68
Root	41	0	41
Hull	5	7	12

Table 3. Morphological characteristics of *Colletotrichum acutatum* isolated from safflower and other hosts examined by previous workers

Source	Shape and size (μm) of conidia		Septation and size (μm) of setae		Shape and size (μm) of appressoria
	Host	Culture	Host	Culture	
The present isolate	Fusiform 8~18×3~5 (12.7×4)[a]	Fusiform 8~16×3~5 (12×4)	0, 1- or 2-septate 20~86×3~4	0, 1- or 2-septate 24~78×3~4	Clavate to obovate or slightly lobed 6~12×5~8
Simmonds (1965)	–[b]	Pointed ends 8.3~14.4×2.5~4 (11.1×3.1)	–	–	Rarely lobed
Dyko and Mordue (1979)	–	Fusiform 8~16×2.5~4	Septate 46.5~85×3~4	–	Clavate to obovate 6.5~11×4.5~7.4
Walker et al. (1991)	Fusiform 11~16×2.5~4	Fusiform 10~18×2.5~4.5	–	–	Clavate unlobed to slightly lobed 7~16×5~8

[a] The data in parentheses indicate averages of measurements.
[b] –: no datum

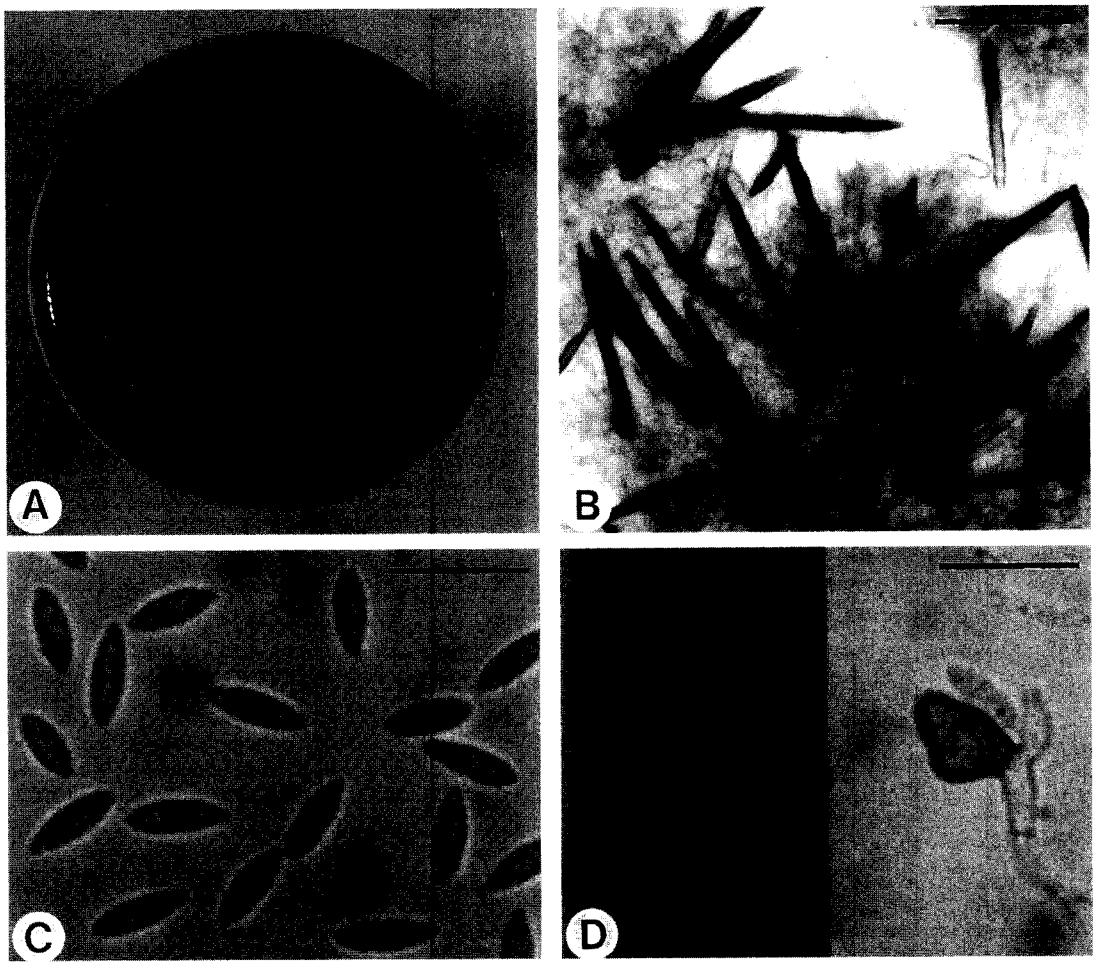

Fig. 2. Cultural and morphological features of *Colletotrichum acutatum* isolated from safflower. A, colonies after 20 days of incubation on PDA at 26°C in the dark; B, setae produced in PDA culture (scale bar=30 μm); C, conidia (scale bar=15 μm); D, appressoria (scale bar=15 μm).

low conidial masses were scattered on the surface of cultures (Fig. 2A). The cultures showed pale yellow and dark patches with partially black streaks and dots on the bottom. Setae were occasionally produced on host plants and PDA cultures, which were dark brown to black, needle-shaped, aseptate, one or two septate (Fig. 2B), and measured 20~86×3~4 μm on the host plants. Conidia were hyaline, aseptate, fusiform (Fig. 2C) and measured 8~18×3~5 μm on the host plants. The conidial ends were pointed or attenuated. The sizes of setae and conidia produced on the host plants were similar to those in PDA culture. Appressoria were gray to grayish brown, clavate to obovate or slightly lobed (Fig. 2D) and measured 6~12×5~8 μm. Temperature range for mycelial growth of the fungus was 8~35°C, and optimum temperature was 24~26°C. The linear length of mycelial growth at the optimum temperature was 3.4~3.6 mm per day.

Pathogenicity. All of the five isolates tested were virulent on safflower plants (Table 4). Anthracnose symptoms induced on the plants by artificial inoculation with the isolates were similar to those observed in the field. The fungus was reisolated from lesions on the plants inoculated. The result of inoculation tests showed that 23-day-old plants were more susceptible to the fungus than 46-day-old plants.

Table 4. Pathogenicity of *Colletotrichum acutatum* isolates on safflower plants by artificial inoculation

Isolate No.	Source	Disease severity[a]				
		23-day-old plants		46-day-old plants		
		Leaf	Stem	Leaf	Stem	Hull
C98-01	Stem	+++[a]	+++	++	++	++
C98-30	〃	+++	+++	++	++	++
C98-81	〃	+++	+++	+	+	++
C98-82	〃	+++	+++	+	++	++
C98-87	Root	+++	+++	++	++	++
Control		−	−	−	−	−

[a]Disease severity was rated eight days after inoculation. +++: severely rotted and blighted, ++: abundant lesions, +: a few lesions, −: no symptom.

There was no significant difference in pathogenicity among the isolates.

Discussion

Colletotrichum acutatum causes anthracnose on a large number of plants (Simmonds, 1965; Sutton, 1980; Sutton, 1992). Simmonds (1965) named it first to the fungus isolated from papaw, strawberry and tomato. Since then, several new hosts of the fungus have been reported (Chellemi and Knox, 1993; Fitzell, 1979; Gibson and Munga, 1969; Griffin, 1979; Reed et al., 1996; Sato et al., 1997; Smith, 1993; Walker et al., 1991). Walker et al. (1991) recognized the fungal hosts in 34 genera of 22 families of plants. The present study first reveals that *C. acutatum* causes anthracnose of safflower. It was reported that some isolates of *C. orbiculare* from *Xanthium* spp. caused symptoms on a safflower cultivar by inoculation tests (Walker et al., 1991), and an isolate of *C. gloeosporioides* f. sp. *malvae* from *Malva pusilla* Sm. on some safflower cultivars (Mortensen and Makowski, 1997). It is probable that the two *Colletotrichum* species cause anthracnose on safflower plants in fields. On the other hand, *Gloeosporium carthami* which was revised as a synonym of *C. gloeosporioides* (Arx, 1957b) has been recorded as a pathogen of safflower anthracnose (Farr et al., 1989; Hemmi, 1919; Mankin, 1969; Tai, 1979). Hemmi (1919) described that conidia of *G. carthami* were elongated ellipsoidal or elongated fusiform and 8~23× 3.2~6.0 μm in size. The conidial size is larger than that of *C. acutatum* examined by the present authors and other workers (Arx, 1970; Dyko and Mordue, 1979; Simmonds, 1965; Walker et al., 1991) but similar to that of *C. gloeosporioides* described by other workers (Arx, 1970; Arx, 1981; Sutton, 1980), although the conidial shape is somewhat similar to that of *C. acutatum*. Therefore, it is considered that *G. carthami* is a synonym of *C. gloeosporioides* as previously reported (Arx, 1957b).

A typical morphological feature for a specific recognition of *C. acutatum* is the fusiform conidium. The conidial ends are pointed or attenuated (Arx, 1970; Fitzell, 1979; Kulshrestha et al., 1976; Simmonds, 1965). In our study, the sharpness of the pointed ends was variable with the isolates. Conidia of some isolates showed very pointed ends, but others slightly pointed or attenuated ends. However, overall shapes of the conidia were consistently fusiform for all the isolates.

It has been reported that many isolates of *C. acutatum* produce pigment in culture (Baxter et al., 1983; Gunnell and Gubler, 1992; Shi et al., 1996; Walker et al., 1991). A chromogenic type produces pink or red pigment, but a non-chromogenic one does not. All the isolates from safflower plants produced neither pink nor red pigment in culture. Colonies of the isolates examined by the authors were consistent with those of non-chromogenic isolates reported by the previous workers (Baxter et al., 1983; Gunnell and Gubler, 1992; Shi et al., 1996; Walker et al., 1991). The slow growing character of mycelia and the temperature response to the mycelial growth of the isolates from safflower plants were similar to those of the isolates from other hosts (Simmonds, 1965; Smith and Black, 1990).

Anthracnose symptoms on safflower plants appeared as damping-off, leaf spot, stem canker, root rot and hull blight. The causal fungus, *C. acutatum* attacks all parts of the safflower plants. Major inoculum source of the disease is conidia. The conidia disseminate in wind and water droplets. It is known that *C. acutatum* is seed-borne (Kulshrestha et al., 1976). The fungus was detected from seeds of some plants (Britton, 1995; Kulshrestha et al., 1976; Reed et al., 1996). Anthracnose symptoms developed on safflower seedlings at the early stage, suggesting that the primary inoculum source may be originated from the seeds. Accordingly it needs to examine the infection and transmission of the fungus in safflower seeds.

Two formae speciales of *C. acutatum* were reported (Baxter et al., 1983; Dingley and Gilmour, 1972), but have not been accepted because the names were not validly published (Sutton, 1992). We did not examine host specificity of *C. acutatum* isolates from safflower plants. There was only one report on *C. acutatum* causing anthracnose on apple fruits in Korea (Lee, 1994). It is possible that anthracnose caused by the fungus occurs on other plants in Korea. Further study on the host range of the safflower isolates is required.

Incidence of anthracnose differed with the safflower fields surveyed. During the survey, we found that there was very rare or no occurrence of the disease in some fields near the fields with very severe disease outbreaks. Isolates of *C. acutatum* were classified into two groups based on disease severity on strawberry cultivars (Denoyes and Baudy, 1995), and some isolates varied greatly in virulence to various strawberry cultivars (Smith and Black, 1990). Thus it is suggested that there are some races specificity to the strawberry cultivars. Reactions of inoculation tests with *C. orbiculare* and *C. gloeosporioides* f. sp. *malvae* differed with the cultivars of safflower (Mortensen and Makowski, 1997; Walker et al., 1991). There has been no information on definite cultivars of safflower in Korea. It needs to study race differentiation of the isolates from safflower plants.

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