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Cultural Characteristics of a Seedborne Fungus, *Bipolaris spicifera* Detected from Imported Grass Seeds into Korea

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The study on the cultural characteristics of *Bipolaris spicifera* was conducted to provide with information for the identification, and inoculation studies, etc. *B. spicifera* grew well at 30~35°C and wide range of pH 5.0~9.0. However, the fungal growth was retarded at pH 4.0 and 10.0, respectively. Conidia were germinated with 70% at 30°C but maintained 50% germination even at 40°C, indicating that this pathogen could infect plants at relatively high temperature. The pathogen could not produce conidia under 24 hr fluorescent light condition for 7 days. In contrast, it produced many more conidia at 24 hr dark condition.

KEYWORDS: ***Bipolaris spicifera*, Conidia, Grass seed, Seedborne fungi**

Bipolaris spicifera (Bainier) Subramanian is not currently present in Korea (Anonymous, List of Plant Diseases in Korea, 1994). This fungus is often detected in the gramineae family during entry inspection and its morphological characteristics has already been reported (Koo, 2003). Identification and often pathogenicity test of this pathogen is necessary for quarantine inspection. To meet this purpose, we need to know cultural characteristics and best conditions for conidia production. However, specific cultural characteristics and best condtions for conidia production are not really reported in detail. Domsch *et al.* (1980) described the cultural characteristics on *B. spicifera* but the report did not have any detailed data.

B. spicifera is distributed worldwide and occurs mainly in tropical and subtropical regions (Sivanesan and Holliday, 1981; Alcorn, 1988). This fungus was first named as *Brachucladium spoiciferum* Bainier in 1908, then renamed as *Curvularia spicifera* (Bainier) Boedijin in 1933, then *Helminthosporiun spiciferum* (Bainier) Nicot in 1953 followed by *Dreschslera spicifera* (Bainier) Arx in 1970 and renamed again as *Bipolaris spicifera* (Bainier) by Subramanian 1971 (Alcorn, 1988; Sivanesan and Holliday, 1981).

B. spicifera has been recorded in more than 77 plant species including 51 grass genera and could be isolated from soil and air (Domsch *et al.*, 1980).

The objective of this study was to determine the cultural characteristics and best conditions for condidia production of *B. spicifera* to provide with information for the identification and inoculation study.

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Materials and Methods

Isolate. Single conidia of *B. spicifera* were isolated from the seeds of a bermuda grass from overseas collected from the Seoul Branch Station of National Plant Quarantine Service of Korea located at Kimpo airport and maintained on potato dextrose agar (PDA) for the studies of physiological characteristics.

Effects of temperature and pH on mycelial growth. Mycelial plugs (5 mm in diameter) were transferred to PDA and incubated at 5 to 40°C for 7 days under dark condition to determine colony diameters. The PDAs adjusted with different pHs of 4.0~9.0 for the study on effect of pH on mycelial growth were inoculated with mycelial plugs (5 mm in diameter) and incubated at 30°C for 5 days to determine mycelial growth.

Effect of temperature on conidia germination. The mycelial plug of the fungi was inoculated on V-8 agar and incubated at 30°C for 5 days. Conidia suspensions were made with sterilized distilled water 5 days after incubation and adjusted to 1×10^5 conidia/*ml* with sterilized distilled water to determine the rate of germination. Conidia suspension was dropped on a hole slide glass and incubated at 5~40°C under humid condition for 12 hr to determine the rate of germination on a haemocytometer.

Effect of different media and light conditions on conidia production. Mycelial plugs (1 cm in diameter) were transferred to PDA, oat meal agar (OA), corn meal agar (CMA), water agar (WA) and V-8 juice agar (V-8)

and incubated at 30°C for 7 days under near ultraviolet (near u.v.) and dark (12 hr/12 hr). Three mycelial plugs (1 × 1 cm) per plate were punched and suspended in 1 *ml* of sterilized distilled water to determine conidia concentration per cm^2 of agar plate on a haemocytometer. There were three plates for each treatment. To study effect of different light sources on conidia production, the PDA plates inoculated with mycelial plugs (1 × 1 cm) were incubated at 30°C for 7 days under 24 hr fluorescence light, 24 hr continuous dark, 24 hr continuous fluorescent light + 12 hr dark, 12 hr near u.v. + 12 hr dark. Three mycelial plugs (1 × 1 cm diameter) per plate were punched and suspended in 1 *ml* of sterilized distilled water to determine conidia concentration on a haemocytometer. There were three plates for each treatment.

Results and Discussion

Mycelial growth was best at 30~35°C, reaching 8~9 cm in diameter (Fig. 1) in PDA at 7 days after incubation. The growth was linearly increased from 5°C to 35°C. However, growth was sharply retarded above 35°C. Optimum temperature for mycelial growth of the fungi was little higher compared to that of most other fungi which is 15~30°C (Deacon, 1996; Moore-Landecker, 1982). This may explain why *B. spicifera* occurs much more often in subtropical and tropical region (Domsch *et al.*, 1980). In contrast, the growth was retarded at 5~15°C and 40°C.

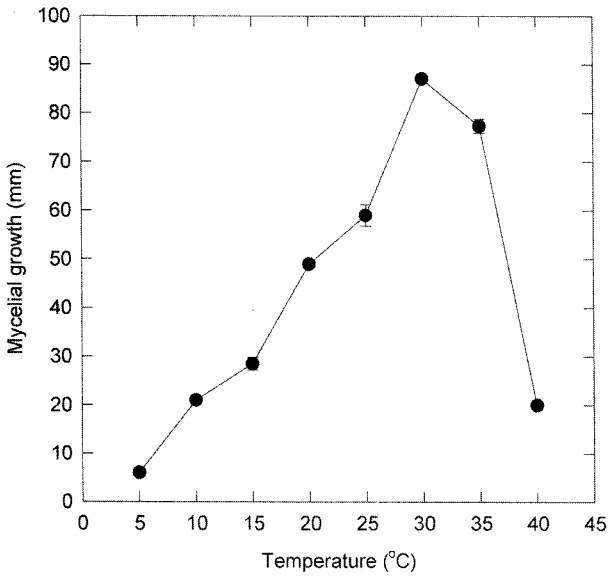

Fig. 1. Effect of temperature on mycelial growth of *B. spicifera*. Mycelial plugs (5 mm diameter) were transferred to PDA. The growth was determined at 7 days after incubation at 30°C. Data points and error bars indicate the means of radial growth on plates and standard errors of the means. There were four replications for each temperature.

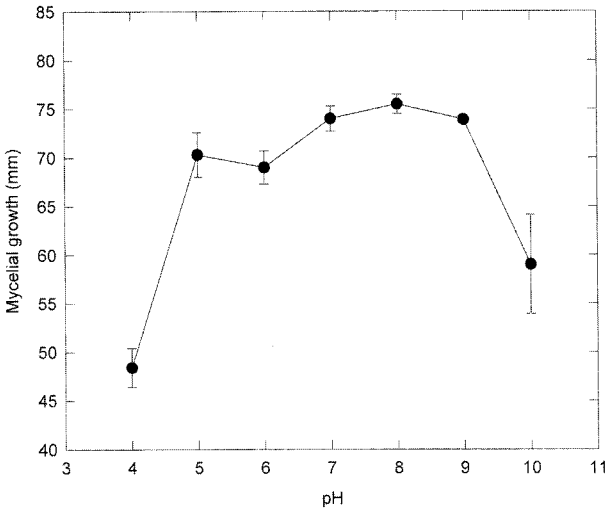

Fig. 2. Effect of pH on mycelial growth on potato dextrose agar. Mycelial plugs (5 mm diameter) were transferred to the solidified PDA adjusted with respective pHs. The plates were incubated at 30°C for 5 days and the growth was determined to measure the diameter of colonies. The experiment repeated twice. Error bars indicate SEM. Four replications were conducted for each treatment.

The results were similar to what Domsch *et al.* (1980) described.

B. spicifera grew well at the wide range of pH 5.0~9.0, reaching 65~75 mm in diameter in the plates. However, the mycelial growth was very retarded at pH 4.0 and 10.0, reaching 47 mm and 60 mm, respectively (Tukey, $P = 0.05$) compared to pH 5.0~9.0 (Fig. 2). *B. spicifera* grew well at the wide range of pH 5.0~9.0, reaching 65~75 mm in diameter in the plates. However, the mycelial growth was very retarded at pH 4.0 and 10.0, reaching 47 mm and 60 mm, respectively (Tukey, $P = 0.05$) compared to pH 5.0~9.0 (Fig. 2). Our results were different from what Domsch *et al.* (1980) described that optimum pH of *B. spicifera* was 6.0. This might be due to a difference between strains. Moore-Landecker (1982) reported that least, optimum and maximum pH for fungal growth could be changed easily and this might be due to hydrogen ion concentration complicated with other types of growth processes.

The rate of germination of conidia was increased up to 70% in response to temperature with optimum at 30°C (Fig. 3). However, the rate of germination reduced when temperature increased at above 30°C.

Conidia were produced best at V-8 followed by OA and PDA, resulting in 119.3, 75.7, 19.3 × 10^4 conidia per square centimeter, respectively (Fig. 4). Conidia production was very low at WA and CMA as below 1.0 × 104 conidia per square centimeter. There was a significant difference in conidia production depending on media in which V-8 and

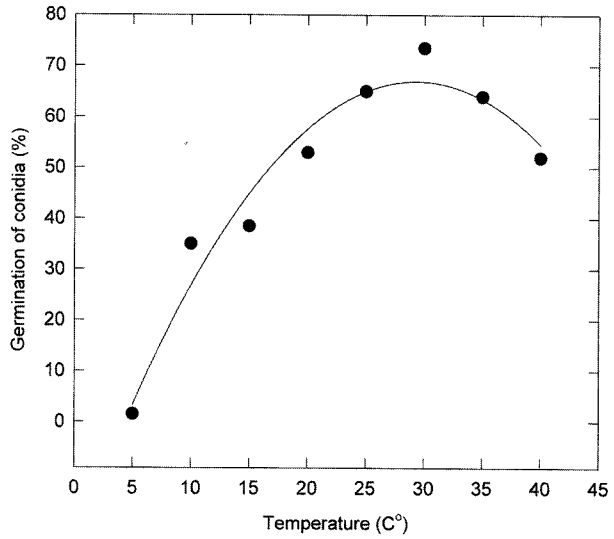

Fig. 3. Effect of temperature on conidia germination. Thirty micro-liter of conidial suspension (10^5 conidia per *ml*) on hole slide glass was incubated under humid condition at 5, 10, 15, 20, 25, 30, 35 and 40°C, respectively. The percentage of germination was measured 12 hr after incubation and hyperbolically correlated to the increase of temperature ($R^2 = 0.96$). Equation for polynomial regression was $Y = -16.5 + 4.4X - 0.0089X^2 - 0.0014X^3$.

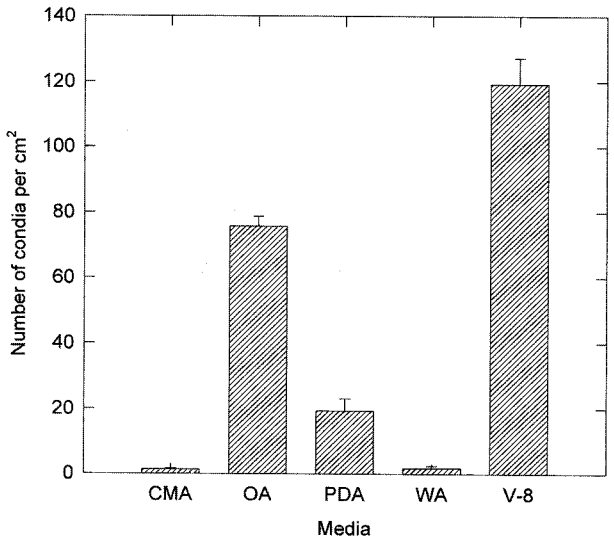

Fig. 4. Effect of different media on conidia production of *B. spicifera*. Mycelial plugs (5 mm diameter) were transferred to PDA, CMA, OA, WA and V-8 agar and incubated under near u.v. and dark (12 hr/12 hr) at 30°C for 7 days. Three places per plate were punched (1 cm diameter) following preparations of conidia suspensions of 1 *ml* per punched area to determine conidia production per cm^2. There were 3 replications per treatment. Error bars indicate SEM.

OA was best (Tukey, $P = 0.5$). This information could be used for reference to inoculation studies and culture condition, etc. Fifty percentage of conidia could germinate even at 40°C, suggesting that the fungus could infect hosts at even high temperature above 35°C (Fig. 3).

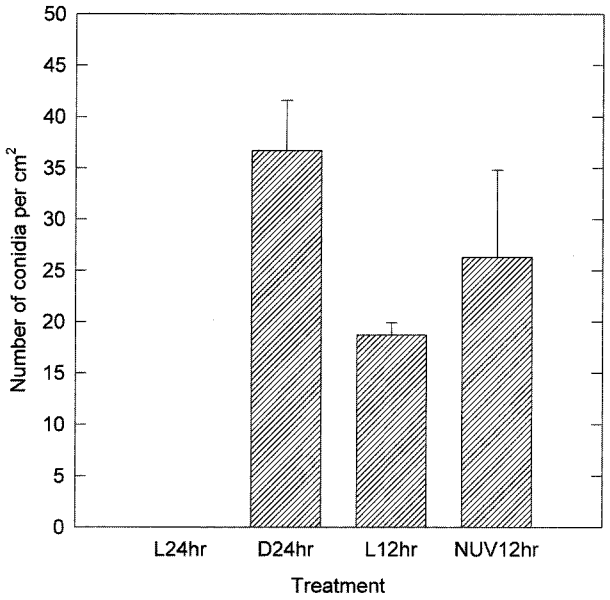

Fig. 5. Effect of different light on conidia production of *B spicifera*. Mycelial plugs (5 mm diameter) were transferred to respective media. The plates were incubated under different light condition at 30 °C for 7 days to determine conidia production. L24 hr, D24 hr, L12 hr, and NUV 12 hr indicate fluorescent light 24 hrs, dark 24 hrs, fluorescent light/dark (12 hr/12 hr) and near ultraviolet (light and dark, 12 hr/12 hr), respectively. Error bars indicate SEM.

Conidia were well produced in the treatments of dark (24 hr), dark and fluorescent light (12 hr/12 hr), near u.v. and dark (12 hr/24 hr) (Fig. 5). In contrast, no conidium was produced in the treatment of continuous fluorescent light (24 hr). There was significant difference in conidia production between the light regimes. Dark condition for 24 hr induced more conidia production that alternated dark and light condition. It was unexpected that no conidium was produced at continuous fluorescent light condition for 24 hrs. In general, it is known that light affect reproduction and differentiation of organ (Duncan and Elsyn, 1966). Alternate light induces asexual conidia production of many other fungi in WA and also often round stripes with conidia on agar in *Neurospora crass* and *Trichoderma* spp. (Duncan and Elsyn, 1966). Also, round stripes are formed by ascomata in *Podospora anserina* and some other ascomycetes. This phenomenon is induced by the effect of near u.v. (330~380 nm) or blue light (450 nm) on receptors containing fravin. Duncan and Elsyn (1966) reported that there were significant differences in light reaction of organism in nature on conidia production. Conidia production is induced in *Alternaria* spp. by uv (280~290 nm) and near u.v. in *Botrytis cinerea*. However, blue light inhibits spore production of *B. cinerea*

(Duncan and Elsyn, 1966).

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