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Morphological and Cultural Characteristics of Fungi Causing Rice Sclerotial Diseases

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水稻菌核病을 일으키는 眞菌의 形態的 및 培養的 特性

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ABSTRACT: Morphological and cultural characteristics of fungi causing rice sclerotial diseases were examined. Hyphal widths of *R. solani* and *R. oryzae* were same and ranged 6.0-12.0 μm with average 9.0μm, the widest among those of the sclerotial fungi examined. Hyphal width of *R. oryzae-sativae* ranged 6.0-9.0μm with average 7.4μm. Hyphal width of *R. cerealis* was the narrowest among those of *Rhizoctonia* species examined, and the same was hyphal width of *S. oryzae* among those of *Sclerotium* species. Nuclear staining by HCL-Giemsa method showed that *R. solani* and *R. oryzae* had many nuclei within one hyphal cell, *S. oryzae* one nucleus, and the other sclerotial fungi mostly two nuclei. The nuclear number of *R. solani* was the largest, which ranged 2-17 with average 6.3. Average size of sclerotia of the sclerotial fungi except *S. hydrophilum* and *S. oryzae* produced in lesions ranged 1.0-2.0mm. Average size of sclerotia of *S. hydrophilum* and *S. oryzae* was 0.5mm and 0.24mm, respectively. Sclerotia of *R. solani* and *R. oryzae* produced in culture were more variable in size and larger than those produced in lesions. However, the sclerotial sizes of the other sclerotial fungi produced in culture were almost the same as those produced in lesions. Sclerotial colors of sclerotial fungi produced in lesions were similar to those produced in culture, but sclerotial shapes of some sclerotial fungi exhibited somewhat difference between the sclerotia produced in lesions and in culture. Optimum temperature for mycelial growth of *R. cerealis* was 23℃, and that of the other sclerotial fungi ranged from 27 to 33℃. Maximum temperature for mycelial growth of some sclerotial fungi was as high as 41℃, while that of *R. cerealis* was as low as 31℃. Minimum temperature for mycelial growth of *R. cerealis* was 2℃, and that of the other sclerotial fungi ranged from 6 to 10℃.

KEYWORDS: Sclerotial fungi, rice, morphology, cultural characteristics.

Various sclerotial fungi are associated with occurrence of rice sclerotial diseases in the paddy field (Hashioka and Makino, 1969; Kim and Yu, 1990; Nakata and Kawamura, 1939; Nonaka *et al.*, 1980; Oniki, 1979; Ou, 1985). It is very difficult to identify the sclerotial fungi because that they rarely produce the perfect or conidial states on the host or in culture. Special culture methods have been improved for the production of the perfect states of some sclerotial fungi (Oniki *et al.*, 1986). However, cultural conditions for the production of the perfect states are very limited. Therefore, descriptions on the systematic morphological and cultural characteristics of the mycelial states of the sclerotial fungi could be very helpful to identify them. Some morphological or cultural characteristics of several sclerotial fungi from rice plants have been reported (Hashioka and Makino, 1969; Inagaki and Adachi, 1987; Oniki, 1979; Ou, 1985) but not thoroughly. In this study, morphological and cultural characteristics of the mycelial states of the sclerotial fungi from rice plants were

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examined and compared.

Materials and Methods

Isolates: Isolates of *Rhizoctonia cerealis* van der Hoeven, *R. fumigata* (Nakata ex Hara) Gunnell and Webster, *R. oryzae* Ryker *et* Gooch, *R. oryzae-sativae* (Sawada) Mordue, *R. solani* Kühn, *Sclerotium hydrophilum* Sacc., *S. oryzae* Catt., and *Sclerotium* sp. obtained from diseased rice plants were used for the investigation of their morphological and cultural characteristics. Their original source was described in the previous report (Kim and Yu, 1990).

Investigation of hyphal width and nuclear number: The isolates of sclerotial fungi were cultured on sterile slide glasses coated with potato dextrose agar (PDA) for the investigation of hyphal widths and nuclear numbers within hyphal cells. The slide-glass cultures were incubated two to four days at 25℃. Hyphal width of each sclerotial fungus was measured under a light microscope with a micrometer. Fifty cells of main hyphal strands per isolate were measured from hyphal tip cells to lower hyphal cells.

Nuclear number in hyphal cells of each sclerotial fungus was examined by the HCL-Giemsa staining method used by Herr (1979). One hundred hyphal cells per isolate except hyphal tip cells were examined by light microscopy.

Investigation of sclerotial morphology: Two isolates of each sclerotial fungus were used for sclerotial formation. Each isolate was cultured in PDRC media (100 m*l* of potato dextrose broth plus 30g of rice chaff) in a 500 m*l*-flask at 25-28℃ for 50 days. Fifty sclerotia produced in PDRC media per isolate were measured by a micrometer caliper or a light microscope with a micrometer, and their color and shape were examined.

Each inoculum cultured in PDRC media was inoculated to rice plants at the booting stage of cultivars Shingkwang and Dongjin cultivated in 1/5000a wagner pots with sterile soil. Size of sclerotia produced in lesions was measured 60 days after inoculation, and the color and shape of the sclerotia were examined.

Investigation of temperature range for mycelial growth: Temperature range for mycelial growth of the isolates of sclerotial fungi was investigated in PDA culture. Optimum temperature for mycelial growth of the isolates was examined from 21 to 33℃ at 2℃ intervals, minimum temperature for that from 2 to 10℃ at 2℃ intervals, and maximum temperature for that from 33 to 42℃ at 1℃ intervals. The positive criterion for the mycelial growth at the minimum and maximum temperatures was above the growth rate of 1mm per 24hr.

Results

Hyphae of most sclerotial fungi were variable in their width (Table 1). Hyphal widths of *R. solani* and *R. oryzae* were same and ranged 6.0-12.0 μm, the widest among those of the sclerotial fungi examined. Hyphal width of *R. oryzae-sativae* ranged 6.0-9.0μm with average 7.4μm. Hyphal width of *R. cerealis* was the narrowest among those of *Rhizoctonia* species examined, and the same was hyphal width of *S. oryzae* among those of *Sclerotium* species. Hyphal widths of *R. fumigata* and *S. hydrophilum* were almost same.

Nuclear staining by HCL-Giemsa method sho-

Table 1. Hyphal width of sclerotial fungi isolated from rice plants

Sclerotial fungi	No. of isolates examined	Hyphal width(μm)	
		Range	Average
Rhizoctonia cerealis	3	4.0-7.0[a]	5.2
R. fumigata	3	4.0-8.0	6.0
R. oryzae	4	6.0-12.0	9.0
R. oryzae-sativae	4	6.0-9.0	7.4
R. solani	8	6.0-12.0	9.0
Sclerotium hydrophilum	4	5.0-8.0	6.2
S. oryzae	3	2.4-4.0	3.2
Sclerotium sp.	4	4.0-7.0	5.6

[a]Fifty cells of main hyphal strands per isolate were measured from hyphal tip cells to lower hyphal cells by light microscopy.

Table 2. Karyological characteristics of sclerotial fungi isolated from rice plants

Sclerotial fungi	No. of isolates examined	No. of nuclei per hyphal cell	
		Range	Average
Rhizoctonia cerealis	3	1-3[a]	2.0
R. fumigata	3	1-3	2.0
R. oryzae	4	1-9	4.2
R. oryzae-sativae	4	1-3	2.0
R. solani	8	2-17	6.3
Sclerotium hydrophilum	4	1-3	2.0
S. oryzae	3	1	1.0
Sclerotium sp.	4	1-3	2.0

[a]One hundred hyphal cells per isolate were examined by HCL-Giemsa method. Hyphal tip cells were excluded from the examination.

wed that *R. solani* and *R. oryzae* had many nuclei within one hyphal cell, *S. oryzae* one nucleus, and the other sclerotial fungi mostly two nuclei (Table 2). The nuclear number of *R. solani* was the largest, which ranged 2-17 with average 6.3. Distribution of the nuclei in the hypal cells of the sclerotial fungi was shown in Fig. 1 through Fig. 8.

Sclerotia produced in lesions and in culture were very variable in their size (Table 3). Average size of sclerotia of the sclerotial fungi except *S. hydrophilum* and *S. oryzae* produced in lesions ranged 1.0-2.0mm. Average size of sclerotia of *S. hydrophilum* and *S. oryzae* was 0.5mm and 0.24mm, respectively. Sclerotia of *R. solani* and *R. oryzae* produced in culture were more variable in size and larger than those produced in lesions. However, the sclerotial sizes of the other sclerotial fungi produced in culture were almost the same as those produced in lesions.

Sclerotial colors of sclerotial fungi produced in lesions were similar to those produced in culture, but sclerotial shapes of some sclerotial fungi exhibited somewhat difference between the sclerotia produced in lesions and in culture (Table 4). *R. cerealis*, *R. oryzae*, and *R. oryzae-sativae* produced cylindrical sclerotia besides irregular or globular sclerotia in lesions but not those in culture. *R. fumigata* produced globular or irregular sclerotia in lesions but only irregular ones in culture. Sclerotial shapes of the other sclerotial fungi exhibited no difference between the sclerotia produced in lesions and in culture.

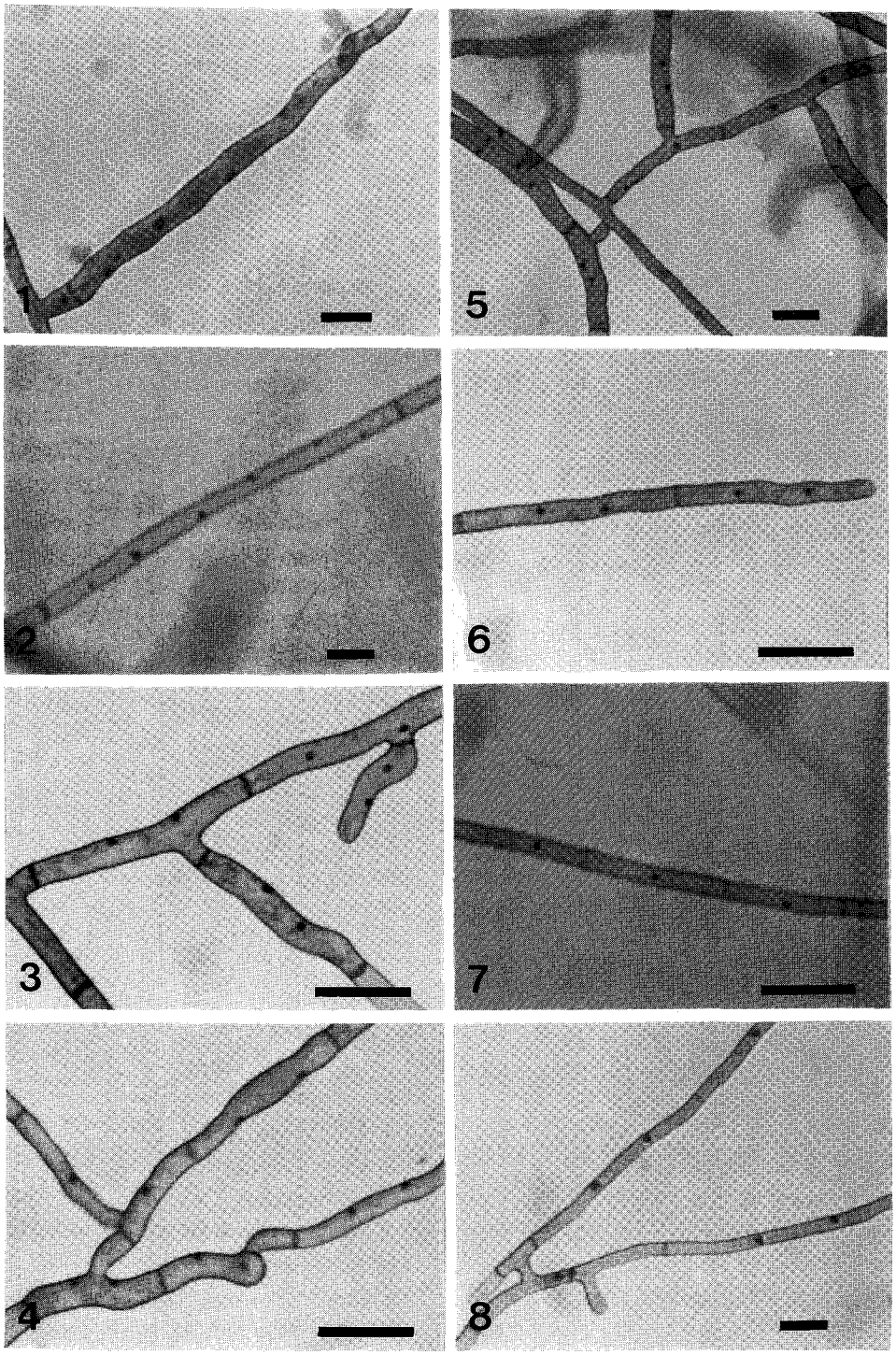
Optimum temperature for mycelial growth of *R. cerealis* was 23℃, and that of the other sclerotial fungi ranged from 27 to 33℃ (Table 5). Maxi-

Table 3. Sclerotial size of sclerotial fungi isolated from rice plants

Sclerotial fungi	Size(mm) of sclerotia produced in			
	Lesion		Culture	
	Range	Average	Range	Average
Rhizoctonia cerealis	0.3 -2.2[a]	1.0	0.4 -2.5[b]	1.1
R. fumigata	0.3 -2.0	1.2	0.3 -2.0	1.2
R. oryzae	0.5 -4.5	1.6	0.5 -8.0	2.1
R. oryzae-sativae	0.3 -2.2	1.2	0.3 -2.5	0.9
R. solani	0.5 -5.0	2.0	0.5-10.0	3.0
Sclerotium hydrophilum	0.3 -1.0	0.5	0.3 -2.0	0.5
S. oryzae	0.15-0.32	0.24	0.15-0.4	0.2
Sclerotium sp.	0.5 -3.0	1.0	0.4 -3.5	0.9

[a]Two isolates and two rice cultivars Shinkwang and Dongjin were used. Each isolate was inoculated to rice plants at the booting stage. The size of sclerotia was measured 60 days after inoculation.

[b]Two isolates per species were cultured in PDRC media at 23-28℃ for 50 days. Fifty sclerotia per isolate were measured.



Figs 1-8. Nuclear distribution in the hyphal cells of sclerotial fungi. The scale bars represent 20μm. 1, *Rhizoctonia solani*; 2, *R. oryzae*; 3, *R. oryzae-sativae*; 4, *R. cerealis*; 5, *R. fumigata*; 6, *Sclerotium hydrophilum*; 7, *S. oryzae*; 8, *Sclerotium* sp.

Table 4. Description on color and shape of sclerotia of sclerotial fungi isolated from rice plants

Sclerotial fungi	Color of sclerotia produced in		Shape of sclerotia produced in	
	Lesion	Culture	Lesion	Culture
Rhizoctonia cerealis	Dark brown, black	Brown, dark brown	Irregular, cylindrical	Irregular
R. fumigata	Grey, brownish grey	Brownish grey, pale brown	Globular, irregular,	Irregular,
R. oryzae	Yellow, pale red	Pale red, red, salmon	Irregular, cylindrical	Irregular,
R. oryzae-sativae	Pale brown, brown, dark brown	Pale brown, brown	Globular, irregular, cylindrical	Globular, irregular
R. solani	Brown, dark brown	Brown, dark brown	Globular, irregular	Globular, irregular
Sclerotium hydrophilum	Brown, dark brown, black	Brown, dark brwon, black	Globular, irregular	Globular, irregular
S. oryzae	Black	Black	Globular	Globular
Sclerotium sp.	Pale brown, brown, dark brown	Pale brown, brown, dark brown	Globular, irregular	Globular, irregular

Table 5. Temperature range for mycelial growth of sclerotial fungi isolated from rice plants

Sclerotial fungi	No. of isolates examined	Temperature(℃) for mycelial growth		
		Minimum	Optimum	Maximum
Rhizoctonia cerealis	3	2[a]	23	31
R. fumigata	3	6	29-31	35
R. oryzae	4	10	31-33	41
R. oryzae-sativae	4	8	31-33	41
R. solani	8	10	27-29	35
Sclerotium hydrophilum	4	8	31-33	41
S. oryzae	3	6	27-29	34
Sclerotium sp.	4	10	29-31	40

mum temperature for mycelial growth of some sclerotial fungi was as high as 41℃, while that of *R. cerealis* was as low as 31℃. Minimum temperature for mycelial growth of *R. cerealis* was 2℃, and that of the other sclerotial fungi ranged from 6 to 10℃.

Discussion

Hyphal widths of most sclerotial fungi are different among the species except those of *R. solani* and *R. oryzae* which are same. The hyphal width of *R. solani* examined by the author is almost consistent with other reports (Nonaka *et al.*, 1979; Ou, 1985). The hyphal width of *R. oryzae* is consistent with that reported by Inagaki and Adachi (1987) but wider than those by other workers (Nonaka *et al.*, 1979; Ou, 1985). It has been reported that hyphal widths of *R. fumigata*, *R. oryzae-*

sativae, and *S. hydrophilum* are almost same (Inagaki and Adachi, 1987; Nonaka *et al.*, 1979). This study also reveals that hyphal widths of *R. fumigata* and *S. hydrophilum* are almost same. However, they are narrower than that of *R. oryzae-sativae*. The hyphal width of *R. oryzae-sativae* examined by the author is wider than those reported by other workers (Inagaki and Adachi, 1987; Nonaka *et al.*, 1979; Ou, 1985). It is probable that hyphal widths of *R. oryzae* and *R. oryzae-sativae* vary among the isolates.

Nuclear numbers within the hyphal cells of sclerotial fungi examined by the author are similar to those reported by other workers (Flentje *et al.*, 1963; Fukano, 1932; Hashioka and Makino, 1969; Inagaki and Makino, 1974; Nonaka *et al.*, 1980). The nuclear numbers within the hyphal cells should be a character for the classification of sclerotial fungi, although those of some *Rhizoctonia* and *Sclerotium* species are same. It is certainly revealed that *R. solani* and *R. oryzae* are multinucleate, *R. cerealis*, *R. fumigata*, *R. oryzae-sativae*, *S. hydrophilum*, and *Sclerotium* sp. binucleate, and *S. oryzae* mononucleate. It is not thoroughly possible to distinguish the sclerotial fungi into *Rhizoctonia* or *Sclerotium* species with only the nuclear number.

Sclerotia of sclerotial fungi are different or occasionally same in their size, color, and shape according to the species. Morphological characteristics of the sclerotia are similar to those described by other workers (Nonaka and Kaku, 1973; Oniki, 1979; Ou, 1985). Kim and Kim (1988) reported that lots of sclerotia were distributed in paddy fields, and various species of sclerotial fungi were identified from the sclerotia collected. It is very difficult to identify directly the species of sclerotia collected from the field. The descriptions on the morphological characteristics of sclerotia should be very helpful to identify the sclerotia of some sclerotial fungi but not sufficient to identify clearly all the sclerotia.

The temperature range for mycelial growth of sclerotial fungi from rice plants is broad and generally agrees with other reports (Hashioka and Makino, 1969; Inagaki and Adachi, 1987; Oniki, 1979; Ou, 1985). The maximum temperature of some sclerotial fungi is as high as 41℃. In addition, the optimum temperature for mycelial growth of most sclerotial fungi except *R. cerealis* ranges from 27 to 33℃. Accordingly it is likely that the growing season of rice is suitable for the outbreak of most sclerotial diseases in terms of temperature. The optimum temperature for mycelial growth of *R. cerealis* is as low as 23℃, suggesting that the fungus mostly attacks rice plants at low temperatures. The minimum temperature for mycelial growth of the sclerotial fungi ranges from 2 to 10℃. Most sclerotial fungi from rice plants also are tolerant to sub-freezing temperatures (Makino and Inagaki, 1977). It is considered that sclerotial fungi adapt themselves to broad range of temperatures for their subsistence.

The morphological characteristics of some sclerotial fungi from rice plants are same or similar in their hyphal widths, nuclear numbers or sclerotial shapes, but those of the others not. The temperature ranges for mycelial growth of the sclerotial fungi show the same tendency. Accordingly it is very difficult to identify clearly the sclerotial fungi with a few data on the morphological and cultural characteristics. The data of this study should be very helpful to identify clearly the mycelial states of the sclerotial fungi.

摘 要

水稻菌核病을 일으키는 眞菌의 형태적 및 배양적 특성을 조사하였다. *R. solani*와 *R. oryzae*의 菌絲幅은 같았으며, 6.0-12.0μm, 平均 9.0μm로서 조사한 菌核眞菌들의 菌絲幅중에서 가장 넓었다. *R. oryzae-sativae*의 菌絲幅은 6.0-9.0μm, 平均 7.4μm였다. *R. cerealis*의 菌絲幅은 조사한 *Rhizoctonia*種들의 菌絲幅중에서 가장 좁았으며, *S. oryzae*의 菌絲幅은 조사한 *Sclerotium*種들의 菌絲幅중에서 가장 좁았다. HCL-Giemsa法으로 核染色을 하여 조사한 결과, *R. solani*와 *R. oryzae*는 한 菌絲細胞內에 많은 核을 가지고 있었으며, *S. oryzae*는 1개의 核을, 그리고 다른 菌核眞菌들은 대부분 2개의 核을 가지고 있었다. *R. solani*의 核數는 2-17개, 平均 6.3개로서 가장 많았다. *S. hydrophilum*과 *S. oryzae*를 제외한 菌核眞菌들의 병반에서 형성된 菌核의 平均크기는

1.0-2.0mm였다. *S. hydrophilum*과 *S. oryzae*의 平均 菌核크기는 각각 0.5mm와 0.24mm였다. *R. solani*와 *R. oryzae*의 배양에 의해 형성된 菌核들은 병반에서 형성된 菌核들 보다 크고, 크기도 더 일정하지 않았다. 그러나 다른 菌核眞菌들의 배양에 의해 형성된 菌核의 크기는 병반에서 형성된 菌核의 크기와 거의 같았다. 菌核眞菌들의 병반에서 형성된 菌核의 색깔은 배양에 의해 형성된 菌核의 색깔과 비슷하였으나, 일부 菌核眞菌들의 병반에서 형성된 菌核의 모양은 배양에 의해 형성된 菌核의 모양과 다소 차이가 있었다. *R. cerealis*의 菌絲生長 適溫은 23℃였으며, 다른 菌核眞菌들의 菌絲生長 適溫은 27-33℃였다. 일부 菌核眞菌들의 菌絲生長 最高溫度는 41℃로서 높은 반면, *R. cerealis*의 菌絲生長 最高溫度는 31℃로서 낮았다. *R. cerealis*의 菌絲生長 最低溫度는 2℃였으며, 다른 菌核眞菌들의 菌絲生長 最低溫度는 6-10℃였다.

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