Plant Pathol. J. 18(2) : 77-80 (2002)

The Plant Pathology Journal

A Simple Method for Sporangial Formation of the Rice Downy Mildew Pathogen, *Sclerophthora macrospora*

Hyeong-Jin Jee*, Seong-Sook Han and Jin-Hyeuk Kweon[1]

Plant Pathology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea

[1]Kyeongnam Agricultural Research and Extension Service, Jinju 660-370, Korea

(Received on March 2, 2002)

A simple method for sporangial formation of the rice downy mildew pathogen, *Sclerophthora macrospora*, on infected leaf tissues was developed to facilitate diagnosis of the disease. Freshly infected young leaves showing whitish to yellowish small spots were selected and cut into small pieces about 2-3 cm in length. About 10-20 pieces were surface sterilized in a 100 ml Duran bottle with 40 ml of 70% ethanol by vigorous shaking for 30 seconds. After washing three times with distilled water, the leaf cuts were submerged in 10 ml of Millipore-filtered paddy water and incubated at 20°C in the dark. After 8-10 h of incubation, the bottle was vigorously agitated on a voltex mixer. Aliquot amount of the suspension, 0.1-1.0 ml, was spread on a slide glass and examined under a light microscope at 50 or 100x magnification. It was found that light and 1% NaClO strongly inhibit sporangial formation of *S. macrospora*. Meanwhile, the use of freshly infected young leaves and washing with 70% ethanol stimulated sporangial formation of the fungus on rice leaves.

Keywords : rice, *Sclerophthora macrospora*, sporangial formation.

Sclerophthora macrospora is an Oomycetes causing downy mildew on a wide variety of cereals, turf grasses, and weedy grasses over an extensive geographic range (Erwin and Ribeiro, 1996; Ou, 1985; Frederiksen and Renfro, 1977). The pathogen, however, is reported to cause downy mildew only on rice in Korea. Jung et al. (1974) reported that the disease was first observed in Gyeonggi province in 1966 and spread nationwide in the nurseries in 1974. Since then, the disease has occurred sporadically with substantial yield loss. Yet, it has been given little attention considering its minor importance and infrequent occurrence in the country.

From late June to early August of 1998 and 1999, rice downy mildew occurred in several areas of Chungnam, Jeonnam, Gyungbuk, and Gyungnam provinces. The disease caused significant damage in some heavily infected fields, partly because of erroneous or belated diagnosis. Isolation and observation of the causal pathogen are essential for disease diagnosis, but are difficult to get done because the causal pathogen cannot be cultured *in vitro* as an obligatory parasite (Erwin and Ribeiro, 1996; Ou, 1985; Wester and Gunnel, 1992).

Diseased rice samples showing yellowing, stunting, malformation, excessive tillering, and whitish to yellowish spots on leaves were brought to the laboratory for diagnosis. The plants were suspected to be infected by downy mildew based on symptoms and references (Ou, 1985; Wester and Gunnel, 1992). However, reproduction structures of the causal pathogen, especially sporangia, were not observed from the samples and little information was available to determine the pathogen on diseased leaf tissues. To facilitate diagnosis of the disease upon observation of the fungal structures, factors affecting sporangial formation of *S. macrospora* were investigated and a simple method for sporangial formation on infected plant leaves is presented in this paper.

Materials and Methods

Sampling. Rice plants showing typical symptoms of downy mildew such as dwarfing, yellowing, malformation, excessive tillering, and whitish to yellowish small spots on leaves were considered as infected by the downy mildew pathogen, *Sclerophthora macrospora*. Infected plants were collected from several fields in Naju, Boryung, and Nonsan from July to August of 1999. The field samples were transplanted in Wagner pots and kept in a greenhouse for further study.

Effect of light and incubation time on sporangial formation. Rice leaves showing typical downy mildew symptoms were collected from the field samples. The leaves were cut into small pieces about 2-3 cm in length by using scissors. Ten to twenty (10-20) pieces of the leaf cuts were weighed, placed in a 100 ml Duran bottle, and washed with 40 ml of 70% ethanol for 30 seconds by vigorous shaking. After discarding the ethanol, the leaf cuts were washed three times with sufficient amount of

*Corresponding author.
Phone) +82-31-290-0436, FAX) +82-31-290-0453
E-mail) hjjee@rda.go.kr

distilled water (DW). Then, the leaf cuts were submerged in 10 ml DW in a 100 ml Duran bottle and incubated at 20°C in the dark or under light (500 lux). After incubation for certain hours, the bottle was agitated vigorously on a voltex mixer for 30 seconds to detach the sporangia that have formed on the surface of the leaf cuts. Aliquot amount of the suspension, 0.1-1.0 ml, was spread on a slide glass and observed under a microscope at 50 or 100x magnification. Total number of sporangia per gram of rice leaf was calculated by converting the counted numbers in the aliquot.

Effect of leaf age on sporangial formation. Freshly infected young leaves and relatively old maturing leaves showing whitish to yellowish spots were collected separately. The leaves were cut into small pieces as described above and 10-20 pieces of the leaf cuts were weighed before washing and placed in a 100 ml Duran bottle. The leaf cuts were washed with 40 ml of washing materials: 70% ethanol, 1% NaClO, or DW as described above.

Effect of leaf washing materials on sporangial formation. Infected rice leaves were surface sterilized with each of 70% ethanol, 1% NaClO, and DW. Young leaves showing typical symptoms were selected and cut into small pieces as described above. Ten to twenty (10-20) leaf cuts were placed in a 100 ml Duran bottle containing 40 ml of the washing agent. The leaf cuts were washed, incubated, and the suspension was observed as described above.

Effect of incubation water on sporangial formation. Paddy water, rainwater, soil extract, and DW were used to test their effect on sporangial formation of *S. macrospora* on rice leaf. Soil extract was prepared as follows: 500 g of fertile soil was mixed in an amount of DW that yielded 1.0 liter of extract; the soil mixture was agitated for 1.0 h and allowed to stand overnight before filtering through a filter paper; first cloudy filtrate was re-filtered by returning it to the same filter. Relatively young rice leaves showing typical symptoms of downy mildew were selected and cut into small pieces and 10-20 leaf cuts were placed in a 100 ml Duran bottle containing 40 ml of 70% ethanol. The washed leaf cuts were submerged in 10 ml incubation water in the bottle and incubated for 10 h. All experiments were carried out at 20°C in dark unless otherwise indicated. The number of sporangia in the suspension was counted at least three times in all experiments.

Results

Effect of light and incubation time on sporangial formation. The rice downy mildew pathogen, *Sclerophthora macrospora,* readily produced sporangia in the dark, but not under light at 500 lux (Table 1). In the dark, the fungus started to form sporangia 4.0 h after incubation at 20°C and the total number of sporangia increased with incubation time up to 24 h. Empty sporangia that have released zoospores appeared at 8.0 h and over 50% have become husks at 14 h after incubation (Table 1). Total number of sporangia ranged from 516 to 4738 per gram of rice leaf after incubation for 8-24 h under the condition.

Effect of leaf age and washing materials on sporangial formation. The number of sporangia produced on young leaves was much higher than those of old leaves. Especially when the leaves were surface sterilized with 70% ethanol, the young leaves produced the highest number of sporangia in the dark as 601 sporangia/g, but the old leaves did not produce any (Table 2). However, both young and old leaves did not or rarely produced sporangia when incubated under light. These results are similar to those in Table 1, although the sporangial numbers varied. Among washing materials used for surface sterilization of the rice leaves, 70% ethanol was the best in both experiments (Tables 2 and 3). A total of 1560 sporangia/g was produced when the samples were washed with 70% ethanol, while no sporangia was found when the samples were washed with 1% NaClO (Table 3).

Effect of incubation water on sporangial formation. Paddy water, soil extract, and DW were better in inducing sporangial formation of the pathogen on rice leaves than tap water and rain water (Table 4). The fungus produced higher number of sporangia in Millipore-filtered water than in autoclaved water, except in the case of paddy water. The number of sporangia ranged from 2070 to 3204 per gram of rice leaf cuts when incubated in Millipore filtered water (Table 4).

Table 1. Effect of light and incubation time on sporangial formation of *Sclerophthora macrospora* on infected rice leaf

Incubation time (h)	No. of sporangia/g of leaf*			
	Light (500 lux)		Dark	
	Total	Husk	Total	Husk
0	0	0	0	0
4	0	0	64	0
6	0	0	42	0
8	0	0	516	152
10	66	0	734	236
12	5	0	1,252	446
14	0	0	777	588
19	0	0	2,776	1,882
24	0	0	4,738	3,326

*Samples were incubated at 20°C.

Table 2. Effect of leaf age on sporangial formation of *Sclerophthora macrospora* on infected rice leaf

Leaf washing material	No. of sporangia/g of leaf*			
	Light (500 lux)		Dark	
	Young leaf	Old leaf	Young leaf	Old leaf
70% ethanol	8	0	601	0
1% NaClO	9	0	35	0
Distilled water	0	0	75	11
No washing	0	0	66	21

*Samples were incubated at 20°C in the dark for 10 h.

Table 3. Effect of leaf washing materials on sporangial formation of *Sclerophthora macrospora* on infected rice leaf

Washing material	No. of sporangia/g of leaf*	
	Light (500 lux)	Dark
70% ethanol	34	1,560
1% NaClO	0	0
Distilled water	0	660
No washing	0	160

*Samples were incubated at 20°C in the dark for 10 h.

Table 4. Effect of incubation water source on sporangial formation of *Sclerophthora macrospora* on infected rice leaf

Water source	No. of sporangia/g of leaf*	
	Millipore filtered	Autoclaved
Paddy water	2,817	3,843
Rain water	2,070	1,746
Soil extract	2,925	2,349
Distilled water	3,204	2,519
Tap water	2,241	1,098

*Samples were incubated at 20°C in the dark for 10 h.

Discussion

The rice downy mildew pathogen, *Sclerophthora macrospora,* readily formed sporangia on infected rice leaves when the tissues were incubated under conditions favorable to sporangial formation. Several factors such as light, leaf age, washing material, and incubation water considerably affected sporangial formation of the pathogen. Among these factors, light seemed to be a critical inhibitory factor for sporangial formation of the fungus (Tables 1, 2, and 3). In general, light stimulates formation of asexual and sexual reproduction structures in most plant pathogenic fungi. In particular, ultraviolet (UV) or near ultraviolet (NUV) wavelengths of less than 340 nm usually induce sporulation of many plant pathogenic fungi (Dhingra and Sinclair, 1995). Sporangial production of *Phytophtora* that is closely related fungus to *S. macrospora* in morphology and phylogeny is also induced by light (Erwin and Ribeiro, 1996; Jee et al., 2000). Thus, it is unusual that reproduction of the rice downy mildew pathogen was strongly inhibited by light in this study.

Surface sterilization of infected plant tissues is an essential step for isolation of a causal pathogen. Ethanol 70% and NaClO 1% are the most commonly used chemical agents for disinfection. However, NaClO 1% seemed toxic to the rice downy mildew pathogen according to results obtained in this study (Tables 2 and 3). Meanwhile, plant tissues washed with 70% ethanol readily formed sporangia. Sampling freshly infected young leaves also facilitated sporangial formation of the fungus. However, sources of incubation water did not play an important role in sporangial production of the pathogen, although the number of sporangia produced in various incubation water varied. In general, Millipore filtered water induced more sporangia than autoclaved water.

Sclerophthora macrospora is known to infect more than 43 genera in the Gramineae including many cereal crops such as rice, oats, barley, wheat, rye, millet, and maize (Erwin and Ribeiro, 1996; Jackson and Dernoeden, 1980; Ou, 1985), although it has been reported only on rice in Korea. The rice downy mildew is considered of minor importance because it does not cause serious damage to the plant (Ou, 1985). However, the disease is favored by cool temperature from 15 to 25°C, high humidity, and deficient sunlight, and has caused over 50% yield loss without control measure (Kang and Lee, 1987).

Symptoms of the rice downy mildew such as dwarfing, yellowing, malformation, excessive tillering, and whitish to yellowish spots on leaves are to some extent similar to viral diseases (Shirako and Ehara, 1985). This similarity often leads to erroneous diagnosis by growers and inspectors. Accordingly, accurate diagnosis of the disease largely relies on observation of the fungal structures on infected tissues since the fungus is not culturable. Consequently, the method developed in this study for sporangial formation on infected rice leaves will facilitate the disease diagnosis and the control strategy development. In addition, this technique can also be used in the preparation of inoculum of the pathogen.

References

Dhingra, O. D. and Sinclair, J. B. 1995. *Basic plant pathology methods.* 2nd ed. *CRC Press*, Inc. 434 p.

Erwin, D. C. and Ribeiro, O. K. 1996. *Phytophthora Diseases Worldwide. APS press*, St. Paul. Minn. 562 p.

Frederiksen, R. A. and Renfro, B. L. 1977. Global status of maize downy mildew. *Ann. Rev. Phytopathol.* 15:249-275.

Jackson, N. and Dernoeden, P. H. 1980. *Sclerophthora macrospora:* the incitant of yellow turf disease of turf grasses. *Plant Dis.* 64:915-916.

Jee, H. J., Cho, W. D. and Kim, C. H. 2000. *Phytophthora Diseases in Korea. National Institute of Agricultural Science and Technology, Rural Development Administration*, Suwon 441-707, Korea. 226 p.

Jung, B. J., Lee, S. H. and Lee, Y. S. 1974. Downy mildew of rice plant caused by *Sclerophthora macrospora. Korean J. of Plant Protection* 13(4):232-234.

Kang, S. W. and Lee, K. H. 1987. Efficacy of metalaxyl for control of rice downy mildew caused by *Sclerophthora macrospora* Saccardo. *Korean J. Plant Pathol.* 3:17-19.

Ou, S. H. 1985. *Rice diseases*. 2nd ed. *Commonwealth Mycological Institute*, Kew, England. 380 p.

Shirako, Y. and Ehara, Y. 1985. Composition of viruses isolated from *Sclrophthora macrospora*-infected rice plants. *Ann. Phytopath. Soc. Japan*. 51(4):459-464.

Wester R. K. and Gunnel, P. S. 1992. *Compendium of rice diseases*. St. Paul, Minn. *APS press*.