韓 國 菌 學 會 誌 第 6 卷 第 2 號
Kor. J. Mycol. Vol. 6, No. 2, 19~24 1978

Studies on the Identification of *Pythium* spp. and Sclerotial Fungi isolated from Rice plants in Korea (Ⅱ)

Young Hee Lee, Eung Kwon Lee and Byung Soo Kim

Institute of Agricultural Science, O.R.D.
Suweon, Korea

水稻에 關與하는 *Pythium* spp와 菌核病菌類의 分類同定에 關한 硏究 (Ⅱ)

李 永 熙·李 應 權·金 柄 洙

農村振興廳 農業技術硏究所

Abstract : Four species of *Pythium* and two species of *Sclerotium* previously not recorded in Korea during 1976 and *Leptosphaeria salvinii* which previosly reported but reidentified. *Pythium aristosporm* Vantery, *Pythium* sp. and *Pythium* myriotylum Drechsler were isolated from diseased rice seedlings and from green withered rice plants and *Pythium irreglare* Buisman was isolated from paddy soil. Three species of *Pythium* except *P. irregulare* grew well at 40°C on Potato dextrose agar and were confirmed as highly pathogenic but *P. irregulare* showed low pathogenicity on Yushin variety of rice.

Sclerotium hydrophilum and Sclerotium oryzae-sativae appeared to be weakly pathogenic, but *Leptosphaeria salvinii* was confirmed as a highly pathogenic. Ordinally the two species of *Sclerotium* grew and produced many sclerotia on dead sheath and stems of rice. There are still some problems to clarify and reconsider in regard to the pathogenicity of the sclerotial fungi because their populations were so very high in paddy fields, but their role might be wound parasite.

Introduction

Since Tongil rice and its related varieties have been distributed throughout the country, new phenomena have occurred on rice plants for the first time in Korea in 1976 one was basal stem rot associated with *Pythium* spp. about one month after transplanting of rice seedlings and the other was a green withering phenomenon which occurred after the heading stage of rice and continued development until the ripening stage.

Both *Pythium* spp. and sclerotial fungi were isolated from diseased specimens obtained in the field. Among the sclerotial fungi, *Leptosphaeria salvinii* was reidentified (1, 2) but two others were new and unrecorded in Korea. Park (1976) in a recent review showed that *Pythium oryzae* caused seed and seedling rot of rice. Cho (1965) in his studies on black rot of rice seedlings showed *Pythium*

spp. were associated with damping off of rice, but this report did not give the name of the species.

Wei (1957) reviewed the descriptions of 12 species of *Pythium* associated with seedling damping off of rice throughout the world which was described in rice disease by Ou (1972)

This key studies of Sclerotial Fungi Nakada and Kawamura devised a key to mophological was used for the identification of the sclerotial fungi which were isolated from diseased rice plants and paddy fields in Korea. In this paper we report four species of Pythium and three sclerotial fungi as being new to Korea.

We would like to express our appreciation to the Commonwealth Mycological Institute, Kew, England for confirming the identification of the *Pythium* spp. and to thank Dr. O.H. Calvert for reading the manuscript.

Material and Methods

a) Isolation of Pathogens

The diseased rice specimens showing symptoms of basal stem rot and green withering which were brought to the laboratory for identification of the pathogens. Pieces of the basal stem and root were cut off from the upper part of the plant and plated on water agar and incubated at 28°C over night. The *Pythium* and *Sclerotium* organisms grew rapidly on the surface of the water agar. The fungi isolated after 24 hrs of incubation and transferred to Smitthenner's (1967) medium to get the sexual state.

To identify the species of *Pythium* isolated from the diseased rice seedlings. The colonies growing on water agar were transferred to Smitthener's media, which was slightly modified by the addition of 0.05 sterol and cultured for 15 days where the sexual state orgarnism was ready for identification. To stimulate sporangial production, small pieces of mycelium including some media, were cultured in sterilized pond water for 2 additional days. Colonies growing on the medium were then mounted in water and lacto phenol solution with cotton blue. Fifty spore measurments were made of each fungus at a magnification of 900X using an eye piece micrometer, and Photomicrographs taken with an Olympus PM 7 camera unit for future reference.

b) Pathogenicity tests

The pathogenicity tests were carried out as follows: rice paddy soil was sterilized by autoclaving at 1.1kg/squ. cm for 1 hr and 650g(about 6cm deep) of sterilized soil were placed in each soft glass cylinders (9cm diam.×18cm height). Until water was poured to 2 cm depth of water above the surface of the soil in the cylinders and this water level was maintained as closely as possible throughout the test period.

Seeds of the Yushin variety were soaked for 24 hrs in a 1% solution of sodium hypochlorite and washed with tap water for an additional 24 hrs before sowing. Twenty seed were sown evenly over the surface of the soil in each cylinders. Inoculum for each *Pythium* isolate was prepared by blending mycelium at the rate of 100ml of distilled water per each 10 day old petri-culture dish using a Waring blender. Fifteen ml of mycelial suspension was poured into each seeded cylinders. The cylinders were placed in a greenhouse of phytotron with tamperature controlled at 17~18°C. The percentage of withered seedlings were checked after month.

Results

The distribution of the isolated species were determined from surveys that were carried out in the area around Suweon, Kimpo, Daejeon and Seoul Korea (Table 1).

Three *Pythium* isolates were obtained from diseased rice plants and from paddy soil and the sclerotial fungi from diseased rice plants.

The *Pythium* species were identified using the key to *Pythium* Pringsheim and the genus *Pythium* by G.M. Waterhouse (1967, 1968) as shown in the Table 2.

Isolate description

Pythium aristosporum had somewhat of lustrous mycelium with slight aerial development that soon

Table 1. Distribution of *Pythium* spp. and sclerotial fungi isolated during this study

Isolate	Date isolated	Source	Locality
Pythium aristosporum	July 1976	Rice plant	Kimpo
Pythium sp	September 1976	Rice plant	Seoul
Pythium myriotylum	December 1976	Rice plant	Suweon
Pythium irregulae	December 1976	Paddy soil	Suweon
Sclerotium oryzaesativae	September 1976	Rice plant	Kimpo
Sclerotium hydrophilum	September 1976	Rice plant	Kimpo
Leptosphaeria salvinii	September 1976	Rice plant	Taejeon

Table 2. Morphological, Pathological and physiological differences encountered in the *Pythium* species

Isolate characters measured	*Pythium aristosporum* Vanterpool	*Pythium sp*	*Pythium myriotylum* Drechsler	*Pythium irregulare* Buisman
Hyphal diam. (μm)	3.7	3.9	4.0	4.6
Type of sporangium	lobulate	lobulate	lobulate	conidia
Vesicle production	ǂa	ǂǂ	ǂǂ	—
Oogonial diam. (μm)	20.4	—	32.9	23.2
Oospore diam. (μm)	16.1	—	26.3	17.8
Antheridial diam. (μm)	5.5	—	6.4	6.3
No. of antheridia per oogonium	2.2	—	3.7	1.5
Pathogenicity b/(%)	95	75	60	35
Growth on PDA(cm)				
20°C	4.3	4.6	3.8	4.5
28°C	8.0	6.2	9.0	6.7
40°C	5.0	6.0	8.0	1.3

a/ — : No. vesicle produced
ǂ : A few vesicles produced
ǂǂ : Many vesicles produced
b/ Percent of Yushin rice seedlings infected
c/ Diameter of colony measured after 35 hr incubation on PDA medium

became granular in appearance and creamy in color. The hyphae averaged 3~7μm in diameter and formed numerous appressoria. Sporangial lobulations developed very well on Smitthenner's medium. At first the individual elements were digitate but in older complexies they more often became swollen lumps; their germination was by numerous tubes. Sporangial vesicles were produced after the additional pond water culture. Oogonia were plerotic smooth, subspherical, terminal or intercalary; most often they were produced abundantly on short side-branches (average, 20.4μm): they formed in fine days on PDA and Somitthenner's media and each had a few of diclinous antheridia. This species was highly pathogenic to Yushin rice seedlings and grew best at 28°C (Table 2).

Pythium sp was very similar to *P. aristosporum* except no oogonia were produced when cultured on the same media and pond water culture. This species was somewhat less aggressive on Yushin rice but grew well at 20, 28 and 40°C (Tabe 2)

The isolate *P. myriotylum* developed well and the aerial mycelium was abundant on the PDA and Smitthenner's media.

Inflated filamentous sporangia were produced on media and they germinated by extending long tubs before forming vesicles and many zoospores. Oogonia were produced terminaly or intercalarily, smooth subspherical average 32.9 in diameter, and were aplerotic; diclinous antheridia usually produced up to 10 (average 3 to 6) to an oogonium; the antheridia were terminally expanded, clavate, often crook-necked or arched in shape. Pathogenicity was lower than the first two isolates. The growth on PDA at 20°C was very slow but the fastest encounted at 28 and 40°C. T he Oospores produced by *P. myriotylum* were colorless or yellowish, subspperical averaging only 26.3μm in diameter.

According to Lee et al (1975) report or in their studies of *Pythium* spp in Korea. The isolate of *P. myriotylum* had been isolated from kidney bean and it's pathogenicity was reported to be very high on kidney bean. T he isolate from a rice seedlings in their study was identical to *P. myriotylum* in the key by G.M. waterhouse. (1968)

P. irregulae had hyphae that was relatively regular which averaged 4.6μm in diameter; asexual reproduction was without vesicle formation but conidia like body were formed averaging 10~15μm rarely in diameter. Oogonia mostly intercalary, spherical to subspherical averaging in diameter, smooth aplerotic sometimes globous, sometimes irregular in shape; monoclinous antheridia clavate and slightly curved.

The sclerotia from the three sclerotial fungi isolated from rice plants were sectioned by hand, viewed microspically in detail (T able 3).

Sclerotium hydrophilum was first described by Saccado in Japan in 1930 and was reported a *Sclerotium sphaeroides* Nakata from Japan. In addition to Japan it has been reported from Eastern China, U.S.A. and Bulgaria, but it was found to be identical with *S. hydrophilum* after acomparative study by Nakata and Kawamura (1939). This fungus caused indistint yellowish lesions on the outer leaf sheath. The sclerotia are globous or spherical and the sclerotial dimensions were 210~440μm. In cross section the sclerotium is composed of two layers, a dark brown outer layer and the hyaline or light yellow inner layers. The pathogenicity of this *Sclerotium* sp. was low (Table 3).

Sclerotium oryzae-sativae was first described by Sawada in Taiwan but has since also been reported in Japan, Vietnam, East, Northwest and South east China reviewed by Ou (1972). The sclerotial dimension were very variable and because of their aggregation became very large (300~1600μm). The sclerotia were composed of swollen mycelium with no distint outer or inner layers visible in cross section. This species was able only to cause a few brownish lesions on Yushin rice stems (Table 3).

Nakatea sigmoidium is the imperfect state of *Leptosphaeria salvinii* which causes stem rot of rice usually after the heading stage in rice. The perfect stage was never observed during the test period. The small black sclerotia and it were identical to those of *L. salvinii* in the sclerotium fungi key described by Nakada & Kawamura (1939). The

Table 3. Morphological and pathological differences encountered sclerotial fungi

Isolatce	Sclerotia				Conidial production	Pathogenicity a/
	Daimeter (μm)	type	Color	Shape		
Sclerotium hydrophilum Saccado	Min-Max.(Average) 210—440(360)	Cortex and medulla cell	black	spherical	none	low
Sclerotium oryzae-sativae Sawada	300—1600(620)	No cortex medulla cell only	brow	irregular	none	low
Leptosphaeria salvinii Cattaneo	190—310(250)	Cortex and regular *modulla cell*	black	spherical	nakatae state	high

a/ Yushin rice seedlings

pathogenicity of this to Yushin rice stems was very high causing black streaks with sclerotial formation in the stems of killed plants.

Discussion

The fow species of *Pythium* had not previously been recorded as occurring in Korea. According to Park's (1976) review on damping off of rice, only P. oryzae was described in his text. Among the species of *Pythium* three of them were isolated from rice seedlings or mature rice plants except *P. irregulae* which was isolated from paddy soil. The pathogenicity of the later was much weaker than the others. Cho (1965) studied black rot of rice seedlings in which he described *Pythium* spp associated with damping off of rice seedlings, but he did not describe the species of *Pythium*.

Pythium aristosporum was found to occurr very commonly in paddy fields. The ratio of this species was high during isolations made from the diseased specimens about one month following transplanting and after the heading stage. In the case of *Pythium* sp. although it produced no cogonia, the aggressiveness same as *P. aristosporum* and it was very similar in other morphological characteristics.

Pythium myriotylum was isolated from rice seedlings under conditions of the high temperature in a greenhouse environment. The oogonia were very distinct in shape and size in comparison with the other species of *Pythium*. It grew well on PDA at high temperature (over 40°C) while *P. irregulae* didn't grow well at all under the same temperature. According to Lee et al (1975) *P. myriotylum* was isolated during the summer time from kidney bean and also grew very well at high temperature on PDA. Saladim et al (1975) reported that *P. aristosporum* and *P. vanterpoolii* were essentially non-infectious at high temperatures. Therefore when the pathogenicity with the *Pythium* spp was tested, We considered such variables as inoculation temperatures and the degree of soil moisture.

Of the sclerotial fungi, *L. salvinii* been reported in 1928, but the other two have not been reported as occurring in Korea. Since the introduction of Indica type varieties of rice throughout Korea these kind of sclerotial diseases have attracted our attention. The varieties are cultivated under much hoavier mitrogen application than the other Japonica type varieties.

Although stem rot is caused by two different organisms asexual states *Nakatae sigmoideum* and *N. sigmoideum* var irregular, in the present study only sclerotia formed by *N. sigmoideum* were investigated. Here the sclerotia were smooth and shiny and grew only on the surface of the PDA medium and the conidia had only shortbe aked. To differentiate between the three sclerotial fungi color, cutural characteristics, and cell structure of the sclerotia were compared and checked with the key of Nakadd et al (1939).

Results of the pathogenicity test using the Yushin varety of rice *L.* salvinii showed the highest degree of pathogenicity, but the others showed a much lower degree of pathogenicity as in the sence of Ou (1972). In this test the two *Sclerotium* spp seemed to be wound parasite. The distribution of these two Sclerotium spp was very wide and in much higher numbers in the paddy fields. Many more ecological and pathological studies should be tried under high nitrogen level application practices for rice. More than neo variety and one isolate should be used in determining pathogen virulence and host reaction in each association.

要 約

1976年 9月 日新品種系인 水稻의 維新品種에 主로 發生된 萎凋버와 箱子育苗代 및 畓土壤 等을 對象으로 病害調査를 實施한 結果 *Pythium* spp와 몇가지 菌核病菌類를 分離同定하였다.

*Pythium*屬 가운데는 病原性이 있고 高溫에서 잘자라는 *Pythium aristosporum* Vanterpool과 *Pythium* sp. *Pythium myriotylum* Drechsler 그리고 病原性이 弱하고 低溫性으로 畓土壤에서 分離된 *Pythium irregulare* Buisman을 同定하였으며 菌核病菌으로는 우리나라의 病害目錄에는 未記錄種인 *Sclerotium hydrophilum*(球狀菌核病)과 *Sclerotium oryzae-sativae*(褐色菌核病)를 同定하였다. 그리고 이미 밝혀진 小粒菌核病中 小球菌核病(*Leptosphaeria salvinii*)을 再同定하

였으며 이들 菌核病들의 病原性을 보면 小粒菌核病이 强한 便이며 그의 菌核病들은 오래된 下部葉鞘와 傷病寄生性의 弱한 病原性을 보여주었다. 그러나 이들 菌核病의 分布는 畓土壤中에 相當한 密度를 차지하고 있으므로 發生生態에 關한 硏究가 이루어져야 할 것으로 믿는다.

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