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Identification and Pathogenicity of *Pythium* spp. Associated with Seedling Damping Off of Rice

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벼 모 마름病을 일으키는 *Pythium*屬 菌과 病原性

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Abstract: Five species of the genus *Pythium* were identified from 91 isolates which were collected from rice seedlings in machine transplanting nursery boxes and paddy fields in Korea. They included *P. graminicola* complex, *P. monospermum* complex, *P. rostratum* complex, and *P. ultimum* complex. The most frequently found species of the genus *Pythium* was *P. graminicola* complex followed by *P. monospermum* complex. Rice seed germinability was greatly decreased by being infested with *P. graminicola* complex at nursery box. *P. graminicola* complex was the most virulent in terms of prohibiting germinations and stunting plants in nursery boxes.

Key words: *Pythium*, Rice seedlings, *Pythium graminicola*, *P. monospermum*, *P. rostratum*, *P. ultimum*.

The rapid increase of machine transplantation of rice seedlings in the paddy fields has brought about the out-breaks of various diseases in box nurseries. Box nursery diseases are rapidly spreaded because nursery systems in machine transplanting are extremely favorable conditions with high seed density, high temperature and high humidity for disease occurrences (Fujii 1981, Ohata 1981). The organisms of soil borne diseases occurring in box nurseries are *Fusarium* spp., *Pythium* spp., *Rhizoctonia* spp., *Trichoderma* spp. and *Mucor* spp. (Ibaraki 1976, Ohata 1981, Sung *et al.* 1982). Although some of them were known as non-pathogenic, all the organisms cause seedling diseases in nursery boxes. Especially, most of *Pythium* spp. cause damping off and seedling blight symptom. These fungi infect through wounded parts of seeds, roots and coleptiles. The *Pythium* spp. are susceptible to environmental condition and has wide host range (Watanabe 1981). Iwayama (1933) first described in 1929 a snow rot of cereals caused by a *Pythium* species in Japan. Middleton (Middleton 1943) reviewed and discussed *Pythium* taxonomy on basis of size, shape and number of various vegetative and sexual structures. Hendrix (Hendrix & Papa 1974) established the system of identification based on morphological characteristics.

The objective of this investigation is to identify *Pythium* spp. associated with seedling blight in nursery boxes and to determine their pathogenicity in the laboratory and in the nursery box conditions.

Materials and Methods

Collection and Identification of the *Pythium* spp.

Diseased seedlings in nursery boxes and plants in the fields were collected from various locations in

mid-June 1981 and brought the samples kept in open plastic bags to the laboratory. The collected samples were washed briefly in tap water and then immersed in running tap water for 24 hrs. Diseased tissues were cut into piece 0.5cm long and pressed between paper towels to remove free water. Four pieces of roots and crowns were placed in separate petri dishes with 2% water agar. After 2~3 days held at 20°C, hyphal tips grown from diseased tissues on water agar were isolated. The fungi for identification were cultured on PDA at 20°C and then maintained as stock cultures. Media used in taxonomic studies included water agar and PDA. Identification of *Pythium* spp. was based on the characteristics of sporangia formed on water agar.

Pathogenicity Trials

In the laboratory conditions, seeds of Milyang 23 were sterilized by putting in 1% sodium hypochloride for 5 min and rinsed three times in distilled water. Four rice seeds were placed on water agar with small pieces of *Pythium* species inoculum and kept in incubator with light at 20°C for 2 weeks.

In the nursery box condition, seeds of Akibare and Milyang 23 were disinfected with 1000× Busan 30 for 24 hurs., kept in water at 20°C incubator for 5 days, and incubated at 28°C incubator for 2 days. Inocula of *Pythium* spp. were prepared on a cornmeal sand mixture of which was combined with 15g cornmeal, 485g sand and 120ml distilled water, and autoclaved 45 min at 121°C. The cornmeal sand mixture infested with mycelial colony of each species in flask was incubated at 20°C and shaken occasionally to make uniform colonization in the medium. The 3 week-old inocula were mixed with the autoclaved paddy soil in ratio of one to thirty. One hundred and twenty grams of sprouted seeds for each box of two cultivars were sowed in infested soil with suspected pathogenic organisms, and held in greenhouse without direct sunlight for 2 days. An autoclaved cornmeal sand mixture was used as the control. Three grams of seeds were weighed and sowed in each box with 50cm^2. Number of rice seedlings emerged in 50cm^2 were counted. Plant heights were measured from crown to leaf tip.

Results

Symptoms in Nursery Box

Pythium spp. caused the symptoms of damping off and seedling blight diseases. The symptoms were generally developed from the hypocotyl or upper root of rice seedlings just below the soil surface level. The fibrous root system and rootlet were infected with the disease pathogen. Extension of brownish lesions developed to the first leaf. The symptoms produced above ground included stunting, sudden wilting or death. The crown tissues of infected seedlings were seriously rotted and the root system became separated easily from the culm.

Identification of *Pythium* species

Ninty-one isolates were collected from rice seedlings in nursery boxes and paddy fields at 16 different locations. Among *Pythium* spp. identified, thrity four isolates belonged to *P. graminicola*

Table I. Characteristics of propagules on water agar medium of *Pythium* species isolated from rice seedlings.

Pythium species	Characteristics of propagule	No. of isolates*
P. graminicola complex	Lobate or inflated sporangia	34 (37)
P. monospermum complex	Filamentous sporangia	18 (20)
P. ultimum complex	Conidia	8 (9)
P. rostratum complex	Spherical	7 (8)
P. pyrilobum complex	Lobate, filamentous, sphrical	9 (10)
Unidentified	—	15 (16)
Total	—	91(100)

* Number in the parenthesis is percentage of isolates isolated.

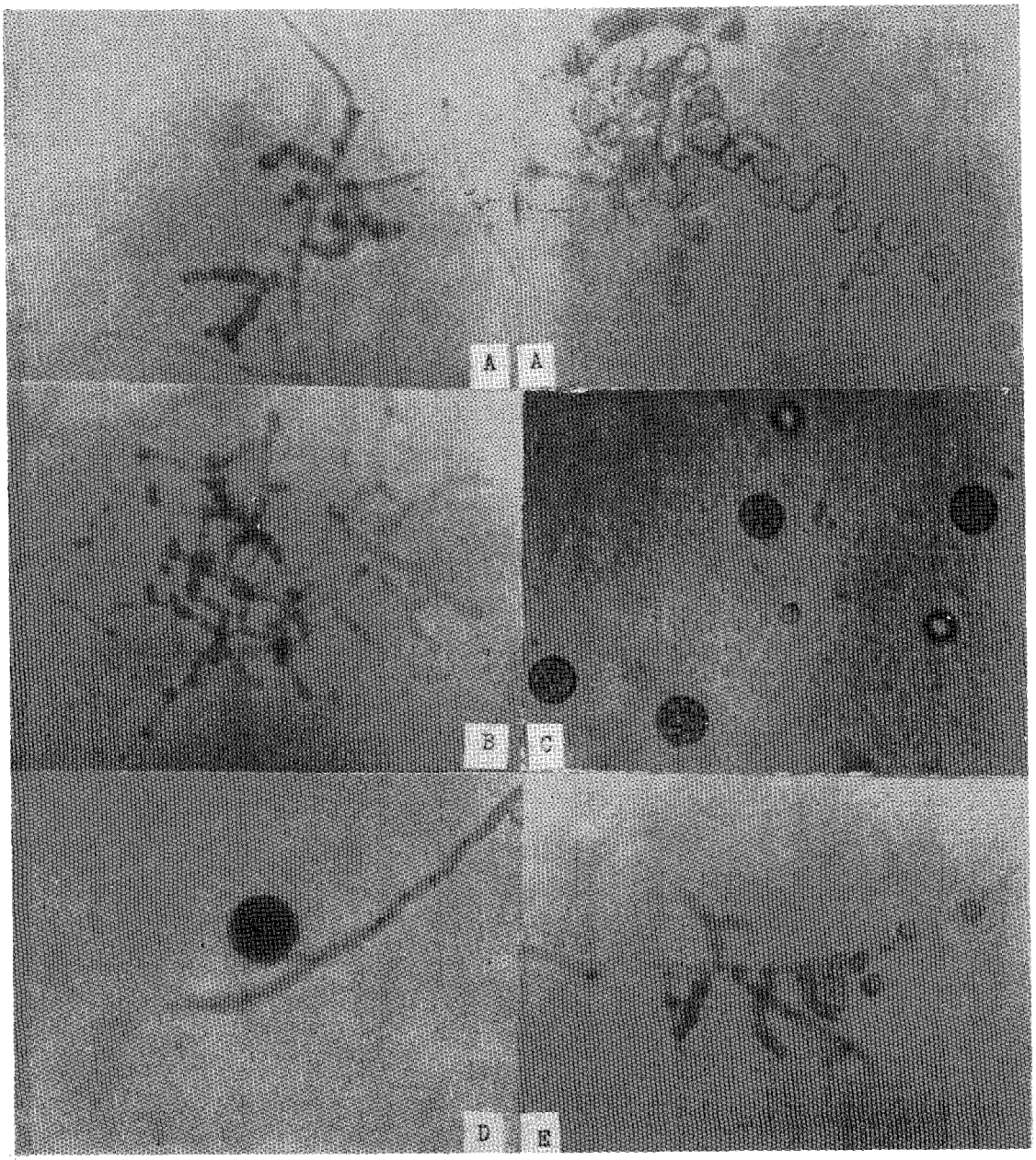

Fig. 1. Sporangia of *Pythium* spp. obtained from water agar.
A) lobate B) filamentous C) conidia D) spherical
E) filamentous lobate and spherical on the same hyphae.

complex, 18 isolates to *P. monospermum* complex, 9 isolates to *P. pyrilobum* complex, 8 isolates to *P. ultimum* complex, 7 isolates to *P. rostratum* complex, and 15 isolates to unknown (Table I). *P. graminicola* complex were characterized by forming lobate or inflated sporangia (Fig. 1A), spherical smooth oogonia, and monoclinous or diclinous antheridia. *P. monospermum* complex formed filamentous sporangia (Fig. 1B), and spherical smooth oogonia bearing one or two monoclinous and diclinous antheridia when grown on water agar. *P. ultimum* complex were characterized by forming conidia (Fig. 1C), monoclinous antheridia, aplerotic oospores, and formed smooth oogonia at the end of mycelium. *P. rostratum* complex constituted spherical sporangia (Fig. 1D), plerotic oospores, oogonia with smooth and either mycelial terminal or intercalary and monoclinous or diclinous antheridia. *P. pyrilobum* complex produced filamentous, lobate, and spherical sporangia on the same hyphae (Fig. 1E). They formed plerotic oospore, smooth oogonia on the hyphae terminally or intercalarily, and oogonium with one to six antheria. Fifteen isolates of *Pythium* sp. were unable to identify because of continuous vegetative growth on water agar.

Pathogenicity

In the laboratory test, seeds of Milyang 23 were placed on the water agar with small mycelium pieces of *Pythium* spp., *P. graminicola* brought about the lowest germination rate among *Pythium* spp. tested (Table II). Ungerminated seeds were covered with white mycelium of *P. graminicola* complex.

Table II. Number of seeds germinated of rice cultivar Milyang 23 placed on water agar with infestation of each *Pythium* species.

Pythium species	No. seeds tested	No. seed germinated (%)
P. graminicola complex	170	44 (21)
P. pyrilobum complex	45	32 (71)
P. monospermum complex	120	106 (88)
P. rostratum complex	70	52 (88)
P. ultimum complex	35	34 (97)
Unidentified	45	45(100)

In the nursery box test, two rice cultivars, Akibare and Milyang 23, were seeded in the nursery boxes infested with inoculum of *Pythium* spp., *P. graminicola* complex caused the lowest germination rate and also showed the most stunting effect of plant height among *Pythium* spp. tested on Akibare and Milyang 23 (Tables III). Rice seed germination and plant growth were most greatly affected by infestation of *P. graminicola* complex as compared to the other species.

Table III. Percent germination of seeds and plants growth of two rice cultivars Akibare and Milyang 23 in nursery boxes infested with each *Pythium* species.

Pythium species	% germination of seeds		Av. plants height (cm)	
	Akibare	Milyang 23	Akibare	Milyang 23
P. graminicola complex	55 (73)*A**	49 (62) A	4.7 A	4.0 A
P. monospermum complex	75 (99) B	57 (73) AB	8.7 B	7.7 CD
P. ultimum complex	78(103) B	61 (78) B	8.0 B	6.7 BC
P. rostratum complex	81(106) B	65 (83) BC	9.7 C	8.5 D
Unidentified	78(103) B	64 (81) BC	7.9 B	6.2 B
Control	100(132) C	100(127) D	10.9 D	9.0 D

* Figures in parenthesis are number of germinated seeds.

** Means in a column followed by the same letter do not differ significantly by Duncan's multiple range test ($p>0.05$).

Discussion

The results of this study clearly indicated that all the *Pythium* spp. were investigated rice seedlings. The *Pythium* spp. were isolated from rice seedlings collected from 16 different locations grown in nursery boxes and paddy fields. On the basis of identification system proposed by Hendrix (1974), ninety-one isloates were identified as *P. graminicola* complex, *P. ultimum* complex, *P. rostratum* complex, *P. monospermum* complex and *P. pyrilocum* complex. *P. graminicola* complex, with which were 35 of 91 isolates identified, was widely distributed in the paddy soils as the resting propagule. Sporangial forms of *Pythim* were isolated from rice (Iwayama 1933). Watanabe (1981) Fujii. (1981) reported that *Pythim* spp. were largely distributed in Japan and Taiwan. Three species of the genus *Pythium* were isolated from rice seedlings in Korea (Lee *et al.* 1978) but they formed lobate sporangia only. Thus, isolates forming lobate were classified into *P. graminicola* complex as suggested by Hendrix (1974). This result together with the previous works (Iwayama 1933, Littrell & Marcanter 1971) indicated that the isolates of *P. graminicola* complex appeared to be commonly present in any of paddy soils in Korea.

Five species of *Pythium* were confirmed as pathogenic fungi under laboratory and nursery box conditions. Expecially, *P. graminicola* complex reduced greatly germination rate of rice seeds and stunted plant height of two rice cultivars Akibare and Milyang 23 under nursery box conditions. The other species tested were recognized to have considerable pathogenicity. Isolates of *Pythium* spp. such as *P. monospermum* and *P. oryzae* from rice seedlings caused seedling blight and damping off diseases in seedling stages in Japan (Ohata *et al.* 1981). Ohata (1981) and Yunoki (1973) stated the symptoms of infected rice seedlings, epidemiology, and control of rice seedling diseased caused by *Pythium* spp. Low temperature and excessive soil humidity during hardening periods incited the disease caused by *Pythium* spp. (Ohata, *et al.* 1981). The results indicated that *Pythium* spp. caused seedling blight in the unrsery boxes and root rot in paddy fields under favorable conditions with low temperature, high density of seeds and high humidity. Expecially, *P. gramicola* complex which were morphologically similar isolates, attacked rice under nursery boxes for machine transplanting.

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