Pathogenicity of *Pythium* Species Isolated from Ungerminated Corn Seeds Planted at a Low Temperature

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Abstract

Five *Pythium* species (*P. paroecandrum*, *P. spinosum*, *P. sylvaticum*, *P. ultimum*, and an unidentified *Pythium* sp.) were isolated from the embryos and the endosperms of ungerminated corn seeds grown in corn monoculture soils at 10 C. In inoculation tests, all five *Pythium* species were pathogenic to corn. In the Hokkaido district of Japan, *Pythium* spp. are considered to be responsible for the occurrence of seed rot of corn.

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Key words : *Pythium paroecandrum*, *Pythium spinosum*, *Pythium sylvaticum*, *Pythium ultimum*, *Pythium* sp., corn.

Introduction

In the Hokkaido district of Japan, germination of corn (*Zea mays* L.) seeds is affected adversely by low temperatures^{4,5,7)}. Although a number of soilborne fungi are known to cause seed rots and seedling blight of corn, *Pythium* spp. were found to attack germinating corn seeds and cause seed rot under low temperature conditions^{2,7)}.

The purpose of this study was to identify the *Pythium* spp. isolated from ungerminated corn seeds and to determine their pathogenicity to corn.

Materials and Methods

Isolations. The three corn cultivars (Wasehomare, Daiheigen, Heigenwase) were planted (10 seeds/pot) in five potted soils each collected from corn monoculture plots at the Hokkaido Central Agricultural Experiment Station (Naganuma soil) and the Hokkaido Prefectural Tokachi Agricultural Experiment Station (Memuro soil). The percent germination of the seeds was determined by incubating the pots for 7 days at 10 C and subsequently 7 days at 25 C. The control seeds were incubated 7 days at 25 C. Following surface sterilization of the seeds with 3% NaOCl, embryos and endosperms were dissected from the ungerminated seeds and incubated on corn meal agar (CMA) in plates at 10 C. Hyphal tips were selected at random from the fungal growth and transferred to CMA to obtain pure cultures.

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Pathogenicity. To determine the pathogenicity of the *Pythium* isolates, corn seeds were inoculated in two ways and the percent seedling emergence was determined: (1) The suspension of mycelial fragments was prepared by cutting-up a hyphal mat that developed on CMA after incubation for 7 days at 25 C. Twenty seeds (Heigenwase) were at first soaked in a suspension of mycelial fragments of each *Pythium* species for 3 days at 10 C. Then the seeds (10 seeds/dish) were incubated 3 days at 15 C and transferred to 20 C. (2) Ten seeds (Heigenwase) were planted in soil artificially infested with *Pythium* species in each of three clay pots (11 cm diameter). Each potted soil was infested with one hyphal mat that was homogenized after growing in corn meal culture (25 ml medium/200 ml flask) for 3-wk at 25 C. The pots were incubated 7 days at 10 C, subsequently 7 days at 15 C and transferred to 25 C.

The control for the first inoculation procedure consisted of soaking seeds in sterilized water instead of mycelial suspension and for the second incubating seeds in uninfested soil for 7 days at 25 C.

Results

Influence of a low temperature

The percent germination of corn seeds decreased significantly in both Naganuma and Memuro soils, when incubated at 10 C (Table 1). In contrast, seeds incubated only at 25 C had a high percent germination (94-100%).

Table 1. Effect of low temperature on germination of three varieties of corn (*Zea mays* L.) in the potted corn monoculture soils

Soil	Incubation time (days)		Varieties		
	10C	25C	Heigenwase	Wasehomare	Daiheigen
Naganuma	7	7	34 ^{a)}	72	—
	0	7	94	94	—
Memuro	7	7	14	38	24
	0	7	94	100	98

a) Average % germination of 50 seeds (10 seeds/pot) in 5 replications. Data for each soil at each varieties were significantly different ($P=0.05$) based on Student's t-test.

Identification of Pythium spp.

Pythium spp. were isolated from both embryos and endosperms of ungerminated corn seeds (Table 2) and identified according to descriptions by previous workers^{1,3)}. Sometimes *Pythium* spp. were isolated from buds of corn seeds that had germinated slightly in soil. In the Naganuma soil *P. sylvaticum* Campbell and Hendrix and *P. ultimum* Trow were isolated from ungerminated seeds and in the Memuro soil *P. sylvaticum*, *P. ultimum*, *P. paroecandrum* Drechsler, *P. spinosum* Sawada and an unidentified *Pythium* sp. were isolated (Table 3). The morphology of each *Pythium* spp. were as follows.

P. paroecandrum (C-32) formed sessile, monoclinal or diclinal antheridia, smooth oogonia (14.8-24.6 μ m in diameter) including aplerotic oospores (12.9-19.7 μ m in diam-

Table 2. Isolation of *Pythium* spp. from three varieties of corn seeds

Soil	Varieties					
	Heigenwase		Wasehomare		Daiheigen	
	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm
Naganuma	5/15 ^{a)}	7/17	2/5	3/10	—	—
Memuro	25/25	23/25	19/25	18/25	24/25	21/25

a) No. of embryos or endosperms yielding *Pythium* spp./no. of embryos or endosperms tested.

Table 3. Frequency of isolation of *Pythium* spp. from corn seeds planted in two corn monoculture soils

<i>Pythium</i> species	Soil	
	Naganuma	Memuro
<i>P. paroecandrum</i>	0 ^{a)}	25
<i>P. spinosum</i>	0	30
<i>P. sylvaticum</i>	92	5
<i>P. ultimum</i>	8	20
<i>Pythium</i> sp.	0	20

a) Percent corn seeds infected with the designated *Pythium* species.

Table 4. Pathogenicity of *Pythium* spp. to corn

<i>Pythium</i> species (Isolate) ^{a)}	% Germination of corn	
	Soakage	Soil inoculation
<i>P. paroecandrum</i> (C-32)	47.4 a ^{b)}	56.7 a
<i>P. spinosum</i> (C-36)	16.0 b	60.0 a
<i>P. sylvaticum</i> (Co-25)	16.5 b	53.3 a
<i>P. ultimum</i> (Co-8)	0 b	56.7 a
<i>Pythium</i> sp. (C-5)	10.0 b	40.0 a
Control	90.0 c	100.0 b

a) Co-8 and Co-25 were isolated from Naganuma soil and C-5, C-32 and C-36 isolated from Memuro soil.

b) Numbers in each column followed by the same letter do not differ significantly ($P=0.05$) according to Duncan's multiple range test.

eter), and spherical of subspherical sporangia (10.0–24.6 μm in diameter) that did not discharge zoospores.

P. spinosum (C-36) formed slender digitate protuberances (6.0–8.4 μm long) on oogonia, plerotic oospores (13.4–25.3 μm in diameter), and sporangia (14.0–25.0 μm in diameter). The antheridia were typically monoclinal although sometimes diclinous.

P. sylvaticum (Co-25) formed mostly spherical sporangia (9.8–24.6 μm in diameter), terminally or intercalary. By the mating test, smooth oogonia including aplerotic oospores were formed.

P. ultimum (Co-8) formed sporangia (11.2–25.2 μm in diameter) and smooth oogonia (15.2–24.8 μm in diameter) including aplerotic oospores (12.6–20.0 μm in diameter) together with hypogenous of monoclinal, sessile, single swollen antheridia.

Pythium sp. (C-5) did not form sexual organs. Spherical sporangia (14.8–27.1 μm in diameter) were formed.

Pathogenicity of *Pythium* spp. to corn seeds

All five species of *Pythium* (Co-8, Co-25, C-5, C-32, C-36) were pathogenic to three corn cultivars reducing the germination percentage equally under the low temperature (10 C) condition (Table 4).

The height of the seedlings inoculated

decreased 20% as compared with the uninoculated control. The endosperm of ungerminated corn seeds was rotted. All *Pythium* spp. used for inoculation were reisola-

ted from these seeds.

Discussion

In the Hokkaido district of Japan seed rot of corn was observed many years ago⁴⁾ and *Fusarium*, *Penicillium*, and *Pythium* spp. have been considered to be the cause of seed rot or seedling blight⁵⁾. Recently, the frequency of isolation of *Pythium* spp. from ungerminated corn seeds was so high that additional studies were conducted⁷⁾. The most important causal organisms of seed rot were previously reported to be *Pythium* species, including *P. irregulare*, *P. debaryanum*, *P. splendens*, *P. vexans*, *P. rostratum*, *P. paroecandrum* and *P. ultimum*⁶⁾. In this study *P. paroecandrum* and *P. ultimum* also were important, but *P. spinosum*, *P. sylvaticum* and an unidentified *Pythium* sp. were found to cause seed rot under a low temperature condition. Although *Fusarium* spp. were also isolated from corn seeds in this study, these isolates did not cause seed rot. Therefore, it is considered that *Pythium* spp. are primarily responsible for the occurrence of seed rot in Hokkaido when a low temperature condition continues for several days after planting.

Control of seed rot caused by *Pythium* species is likely to be difficult. At low temperatures unfavorable for germination of seeds, the corn plant apparently has little resistance to these pathogens. Rotation is not likely to be beneficial because *Pythium* species are known to have a very wide host range and to remain viable in soil for many years²⁾. Also, most soils contain some *Pythium* species⁸⁾ so planting in uninfested soils may not be possible. Treatment of seeds with fungicides active against *Pythium* species has been helpful in some cases with other crops, and it may probably offer the best opportunity to minimize the damage caused by these pathogens.

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和 文 摘 要

近藤則夫・児玉不二雄・赤井 純：低温下の不発芽トウモロコシ種子から分離された *Pythium* 属菌の病原性

北海道のトウモロコシ連作土を用いて、低温条件下（10℃）でトウモロコシを栽培すると発芽率が著しく低

くなり、不発芽種子からは5種の *Pythium* 属菌 (*P. paroecandrum*, *P. spinosum*, *P. sylvaticum*, *P. ultimum*, 未同定の *Pythium* 属菌) が分離された。接種試験の結果、これら5種の *Pythium* 属菌はすべてトウモロコシに対し病原性を示した。北海道においてはトウモロコシ苗立枯病は主として *Pythium* 属菌が起こすと考えられる。