Brown-blotted Root Rot of Carrots in Japan

(2) Culture and Identification

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Abstract

A *Pythium* species (isolates A-1 and A-4) obtained from diseased carrot roots with water-soaked brown blot-like symptoms in Chiba Prefecture is slow in growth rate and it has specific pathogenicity only limited to carrot roots. It is morphologically characterized by formation of globose, subglobose or peanut-shaped hyphal swellings and predominantly single monoclinal clavate, crook-necked or various-shaped antheridia with transverse furrows and folds. This fungus was identified as *P. sulcatum* Pratt and Mitchell based on morphological and physiological characteristics.

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Key words : *Pythium sulcatum*, carrots, brown-blotted root rot, identification.

Introduction

No reports had been published on *Pythium* disease of carrots (*Daucus carota* L.) in Japan before Nagai *et al.* (1984)³⁾ who first demonstrated the pathogenicity of *Pythium* species isolated from the diseased roots with blot-like symptoms occurred in Chiba Prefecture and named this *Pythium* disease as brown-blotted root rot, Shimigusare-byo in Japanese.

This is another paper of two continued papers⁴⁾ with the same title, mainly concerned with culture, morphology and identification of the causal fungus, *Pythium sulcatum* Pratt and Mitchell, of brown-blotted root rot of carrots in Japan.

A preliminary report has been published⁹⁾.

Materials and Methods

Isolates. Two isolates, A-1 and A-4, representing an identical morphological group of 55 isolates obtained in the previous study⁴⁾ were used throughout this study.

Cultures. The fungus was cultured on various media including Difco PDA (Plate I-1), Difco corn meal agar, Difco oatmeal agar and V-8 juice agar.

Temperature responses of the fungus was studied by determining the mycelial growth rates (colony radius, mm/24 hr) of the fungus on PDA and corn meal agar in sterile 9-cm plastic Petri dishes incubated at 12 different temperature levels from 7 to 40 C with

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3 C interval, using 6 plates/treatment in two separate experiments.

Morphological study. Morphology of the fungus was studied on cultures of various media. Glass-blade cultures^{5,10)} and several other cultures were tested to induce zoospore discharge using various soaking solutions including Petri salt solution, soil extract, pond water and tapwater under various temperature conditions.

Results

Culture

The fungus grows on various media tested, but it is slow in growth rate. For example, the colony radius reaches nearly 8 mm on PDA or corn meal agar media after 24-h incubation at the optimum temperature of 28 C. It does not grow below 10 or above 34 C on PDA (Fig. 1).

Mycelia are homogeneous with or without slight aerial hyphae on PDA (Plate I-1) or corn meal agar, respectively. Hyphal swellings are formed easily on agar media, but sporangia and zoospores have not been observed by various methods tested. Appressoria are formed at 22 C in grass-blade cultures. Mature oospores are rarely observed in V-8 juice agar and other rich media, although sexual organs are easily formed on any medium tested.

Morphology

Hyphae usually 2.2–6.3 μm diam. Appressoria often catenulate, sickle- or sausage-shaped, usually $25 \times 7.5 \mu\text{m}$ for each. Hyphal swellings terminal or intercalary, globose (Plate I-2; -7; -10), subglobose, ovate, peanut-shaped (Plate I-3) or various in shape, single (Plate I-2) or catenulate (Plate I-3), occasionally formed on antheridial (Plate I-7)

or oogonial stalks (Plate I-8; -10), usually 20–30 μm diam. Oogonia (Plate I-4 to 2-13) predominantly terminal, globose, 15–28 μm diam. Antheridia usually 1–2, rarely 3–4 (Plate I-10) per oogonium, borne on antheridial stalks with or without a few septa, predominantly monoclinal (Plate I-4; -5; -10), occasionally diclinal (Plate I-6), globose (Plate I-8), clavate (Plate I-7), crook-necked (Plate I-11; -13), hypha-like, characteristically with furrows or folds (Plate I-7; -8; -11 to -13), often encircling the oogonium (Plate I-5; -8; -13). Oospores (Plate I-14; -15) aplerotic, globose, 13.7–21.3 μm , with a central reserve globule; oospore wall less than 1 μm wide.

Fig. 1. Radial mycelial growth rates of *Pythium sulcatum* (isolates A-1, A-4) after incubation for 24 hr on PDA under different temperature conditions.

Discussion

Carrot was listed as a host of 11 *Pythium* species including *P. aphanidermatum* (Edson) Fitz., *P. coloratum* Vaartaja, *P. debaryanum* Hesse, *P. irregulare* Buisman, *P. oligandrum* Drechsler, *P. paroecandrum* Drechsler, *P. polymastum* Drechsler, *P. spinosum* Sawada, *P. sulcatum* Pratt and Mitchell, *P. sylvaticum* Campbell and Hendrix and *P. ultimum* Trow^{1,2,7,8)}.

Our *Pythium* species is very characteristic because of slow growth rate and pathogenicity very specific to carrot roots as demonstrated in the previous paper⁴⁾. It is very similar to *P. sulcatum* morphologically and physiologically among the above 11 species, because our carrot *Pythium* and *P. sulcatum* form hyphal swellings, especially peanut-shaped ones, and predominantly single monoclinal furrowed antheridia, and both are slow in growth rate, and specific in pathogenicity probably only limited to carrot roots. In addition, our fungus is classified into the category II B in the Key to *Pythium* Pringsheim by Waterhouse¹⁰⁾ in which zoospores are rarely or never produced. Among 12 species in this category, 5 species form spherical or elliptical vegetative hyphal bodies and aplerotic oospores. They are *P. irregulare*, *P. paroecandrum*, *P. ultimum* var. *ultimum*, *P. vexans* de Bary and *P. violae* Chesters and Hickman. Our fungus is differentiated from these fungi because *P. irregulare* usually forms one to several number of conical or finger-like projections on oogonial wall, *P. paroecandrum*, mostly intercalary, often catenulate oogonia, *P. ultimum* var. *ultimum*, predominantly single antheridia originating immediately adjacent to the oogonium and thick-walled oospores, *P. vexans*, single monoclinal bell-shaped antheridial cells and *P. violae*, large oogonia (av. 29.5 μ m diam.) and oospores (av. 27 μ m diam.)⁵⁾. Furthermore, our fungus is similar to *P. sylvaticum* and *P. heterothallicum* Campbell and Hendrix according to Plaats-Niterink⁵⁾, but different from these species because of heterothallism of the latter species. Even homothallic isolates of *P. sylvaticum*⁶⁾ have a characteristically branched antheridium forming two large inflated antheridial cells (usually 2-4 antheridia per oogonium). Therefore, our fungus was identified as *P. sulcatum* Pratt and Mitchell based on morphological and physiological characteristics, although zoosporangia and zoospores were not observed in this study.

This is the first report on *P. sulcatum* in Japan.

Literature cited

1. Middleton, J. T. (1943). Mem. Torrey Bot. Club 20 : 1-171.
2. Mildenhall, J. P., Pratt, R. G., Williams, P. H. and Mitchell, J. E. (1971). Plant Dis. Rep. 55 : 536-540.
3. Nagai, Y., Fukami, M. and Watanabe, T. (1984). Ann. Phytopath. Soc. Japan 50 : 393 (Abstr. in Japanese).
4. Nagai, Y., Fukami, M., Murata, A. and Watanabe, T. (1986). Ann. Phytopath. Soc. Japan 52 : 278-286.
5. Plaats-Niterink, A. J. Van Der. (1981). Monograph of the genus *Pythium*. Centraalbureau voor Schimmelcultures, Baarn. Studies in Mycol. No. 21.
6. Pratt, R. G. and Green, R. J., Jr. (1971). Can. J. Bot. 49 : 273-279.
7. Pratt, R. G. and Mitchell, J. E. (1973). Can. J. Bot. 51 : 333-339.

8. Sutton, J. C. (1975). Can. J. Plant Sci. 55 : 139-143.
9. Watanabe, T., Nagai, Y. and Fukami, M. (1984). Ann. Phytopath. Soc. Japan 50 : 393-394 (Abstr. in Japanese).
10. Waterhouse, G. M. (1967). Commonw. Mycol. Inst., Mycol. No. 109.

和 文 摘 要

渡辺恒雄・長井雄治・深見正信：日本で発生したニンジンしみ腐病 (2) 培養と同定

千葉県産のニンジンしみ腐病罹病株から同一とみられる多数の *Pythium* 菌が分離された。その代表の2菌株 (A-1 と A-4) は、ニンジンの根だけに病原性を有し、菌糸の生育速度は非常に遅い。PDA やコーンミール培地上でのコロニー半径 (1日当り) は最適温度の28℃下で 8 mm にも達しない。胞子のう (hyphal swellings) は球形、亜球形あるいは落花生形で、PDA ほか各種培地上でたやすく形成するが、遊走子のうや遊走子は観察できなかった。雌雄同菌糸性がほとんどで、雄精器は通常 1~2 個、まれに 3~4 個が各藏卵器に付着する。雄精器細胞は、棍棒状、かぎ型など様々な形をし、外壁の一部に切れこみやくぼみを有する。卵胞子は未充満で、卵胞子膜は薄い。本菌は、生理的および形態的特徴から、*Pythium sulcatum* Pratt and Mitchell と同定した。

Explanation of plate

Plate I

Pythium sulcatum (isolates A-1, A-4) in culture.

1. 12-day-old colony on PDA in 9-cm plastic Petri dish at 28 C.
- 2, 3, 7, 8, 10. Hyphal swellings, single (2) or catenulate (3), formed terminally (2, 7) and intercalarily (2) in hyphae or sometimes in the antheridial (7) and oogonial stalks (8, 10).
- 4-13. Oogonia and antheridia. Note predominantly one (4-7), two (8, 9), or rarely 3 (10), monoclinal (4, 5) or diclinal (6) antheridia often branched (9) or encircling the oogonium (5, 8, 13). Antheridia are clavate (7) or crook-necked (11, 13) with furrows or folds (7, 8, 11-13).
- 14-15. Oospores. Note oospores aplerotic and with a central reserve globule.
Scales: 2 = 20 μ m, applicable to 3-14; 15 = 5 μ m.

Plate I