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Abstracts of the Research by the Winners of the Young Scientist Award

Studies on Physiological Specialization of Downy Mildew of Crucifers Caused by *Peronospora parasitica*†

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Peronospora parasitica (Persoon : Fries) Fries, the cause of downy mildew of Cruciferae, is an obligate parasite. In Japan, no study has appeared on physiological specialization of this fungus since Hiura and Kanegae in 1934. The present study made a new advance in the field of specialization of the fungus by establishing a preservation technique for conidia to easily handle many isolates at one time without cross contamination. Thereby, physiological specialization could be studied using single-spore isolation for the downy mildew fungus.

1. Preservation of conidia

Conidia of *P. parasitica* from broccoli lost germinability after 6 months storage at −20°C. The germination rate also markedly decreased when conidia were directly subjected to −80°C for 1 hr. On the other hand, conidia were well preserved for at least 12 months when suspended in protective substances : 10% dimethyl sulfoxide +5% skim milk, or 10% dimethyl sulfoxide +10% skim milk. Preliminary freezing at −20°C for 24 hr prior to −80°C preservation improved conidial longevity in combination with the protective substances. Conidia recovered from the preservation were still highly pathogenic to broccoli seedlings.

2. Parasitism of single-spore isolates

A study of the host range of several single-spore isolates of *P. parasitica* obtained from cruciferous vegetables indicated the differentiation of three categories of pathogenicity among isolates, *i.e.*, from *Brassica oleracea* (cabbage and broccoli), *B. campestris* (Chinese cabbage and turnip) and *Raphanus sativus* (Japanese radish). Isolates obtained from *B. oleracea* were mostly pathogenic to *B. oleracea* cultivars inoculated, but were not pathogenic to cultivars of *B. campestris* and *R. sativus*. Two cultivars of cabbage, however, were resistant to these isolates, suggesting the presence of resistance genes. Pathogenicity of isolates from *B. oleracea* was fairly uniform on all cruciferous cultivars tested. Each isolate from *B. campestris* varied in pathogenicity ; they were pathogenic to most *B. campestris* cultivars and to some *B. oleracea* cultivars, whereas all isolates were nonpathogenic to cultivars of *R. sativus*. The third group of isolates, obtained from *R. sativus*, also varied in pathogenicity ; they were parasitic to most cultivars of both *B. oleracea* and *R. sativus*, but not to cultivars of *B. campestris*.

3. RFLPs (restriction fragment length polymorphisms)

Total DNA was extracted from conidia of broccoli downy mildew fungus for cloning. Among the clones obtained, one clone was specified to hybridize with DNAs of the fungus isolated from broccoli, turnip and Japanese radish. The clone did not react with other fungal pathogens of crucifers or with cruciferous hosts. The specified clone was used as a probe after digesting total DNAs of the fungal isolates from broccoli, turnip or Japanese radish, with restriction enzymes ; RFLPs were subsequently recognized for the three isolates. RFLPs and pathogenic specialization were subsequently recognized for the three isolates. The probe can be used for further molecular diagnosis, *e.g.*, to detect of infection on host plants and to monitor infection sites within the host tissues.

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