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Short Communication

Department of Mycology and Plant Pathology,
Institute of Agricultural Sciences, Banaras Hindu University,
Varanasi, India

Oospore Formation in *Phytophthora drechsleri* f. sp. *cajani*

U. P. SINGH and V. B. CHAUHAN

Authors' address: U. P. SINGH and V. B. CHAUHAN, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 (India).

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Abstract

Phytophthora drechsleri f. sp. *cajani* (Pal *et al.*) Kannaiyan *et al.* causes stem and leaf blight in pigeon pea (*Cajanus cajan* [L.] Millsp.) in India. The asexual phase occurs in artificial culture as well as on the host tissue. Sparse oospore formation has been observed in old cultures. A technique has been evolved in which abundant mature oospores are formed on the leaflets of pigeon pea and also on glass slides using zoospores and mycelial discs on the former but only mycelial discs on the glass slides. The largest number of oospores was formed after incubation for 36 h at 25 °C.

Zusammenfassung

Oosporenbildung bei *Phytophthora drechsleri* f. sp. *cajani*

In Indien wird die Stengel- und Blattfäule der Straucherbse (*Cajanus cajan* [L.] Millsp.) durch *Phytophthora drechsleri* f. sp. *cajani* (Pal *et al.*) Kannaiyan *et al.* verursacht. In Kulturen auf künstlichen Nährmedien wie auch auf Wirtspflanzengewebe wird die asexuelle Phase ausgebildet. Nur eine spärliche Oosporenbildung wurde in älteren Kulturen beobachtet. Eine Methode wurde ausgearbeitet, bei der Oosporen an Straucherbsenblättchen und an Glasobjektträgern gebildet werden. An den Blättchen können Zoosporen und Myzelscheiben, an den Objektträgern nur Myzelscheiben eingesetzt werden. Die höchste Anzahl an Oosporen wurde nach 36 h Inkubation bei 25 °C erreicht.

Phytophthora drechsleri f. sp. *cajani* (Pal *et al.*) Kannaiyan *et al.* causes a serious disease of pigeon pea (*Cajanus cajan* [L.] Millsp.) in several parts of India.

The pathogen occurs mostly in its asexual phase both *in vitro* and *in vivo*. Oospores, however, are formed in senescent cultures but this occurs very rarely. During the course of developmental studies of the fungus in leaflets of pigeon pea, it was observed that the fungus forms oospores on the leaflets of the susceptible cv. ICP-7179. In this paper the results of repeated experimentation investigating the conditions for maximum oospore production in the laboratory are reported.

Materials and Methods

The fungus was isolated from freshly infected stems and leaflets of pigeon pea on to potato dextrose agar (PDA), established in axenic culture on PDA by the transfer of hyphal tips and maintained on PDA slants at 25 °C.

Mycelial discs (5 mm diam) cut from the peripheral growth of a 3—4-day old culture of *P. drechsleri* f. sp. *cajani* grown on PDA were transferred to Petri dishes (5 cm diameter) containing 5 ml diluted tomato juice broth (200 g tomato + 2 g calcium carbonate + 1000 ml distilled water). The Petri dishes were incubated for 24 h at 25—30 °C and this medium was replaced by sterilized distilled water. Abundant sporangia were produced when cultures were incubated for 12 h under white fluorescent light (65 lux) followed by darkness at 25 °C. Zoospore production and discharge was then induced in these cultures by incubating the sporangia in Petri dishes at 4 °C for 30 min and further incubation at 24 °C for 2 h. Aqueous zoospore suspensions (8×10^3 ml^{-1}) were used as inoculum.

Detached leaflets of susceptible cv. ICP-7119 were floated on 5 % sucrose solution in Petri dishes (YARWOOD 1946). Drops (0.1 ml) of zoospore suspension were placed at four places equidistantly on each leaflet and a single drop on each glass slide. Sterile distilled water drops were placed as controls on leaflets as well as on glass slides. Four (5 mm diameter) mycelial discs taken from the growing colonies of the fungus were similarly used as inoculum on leaflets. Mycelial discs of the same size were also put in a drop of sterile water on glass slides with a control consisting only of sterile distilled water. The inoculated leaflets, the glass slides bearing the mycelial discs or zoospore suspension and the controls were then incubated in complete darkness at 15, 20, 25, 30 or 35 °C and observed for oospore formation at 6 h intervals. The observations of oospores in inoculated leaflets were made following clearing in 10 ml acid-alcohol solution (1 part glacial acetic acid : 1 part 95 % ethanol) at 60 °C for about 10 min, followed by simmering until the leaflets became translucent. The leaflets were cooled and cleared in saturated chloral hydrate solution for 30 min. They were stained in 0.05 % cotton blue in lactophenol and mounted on clean glass slides (LATCH and HANSON 1962). The slides containing mycelial discs or zoospores, along with the cleared leaflets as well as the controls, were examined for oospore formation. All the experiments were conducted in triplicate and repeated several times.

Results and Discussion

On the leaflets at the end of 30 h the zoospores had germinated, developed mycelium and started to form oospores. The mycelial discs likewise grew slightly and formed oospores in the same period. On the glass slides the oospores were observed only in the mycelial discs and not with the zoospores as the latter are highly ephemeral. Abundant oospores were formed after 36 h at 25 °C. Oospores were not formed in control sets. Mature oospores, 22—35 μm diameter, were spherical to globose, smooth, purple yellow to brown, thick walled (1.2 μm) and morphologically similar to those formed in culture. The reason for oospore formation on the detached pigeon pea leaflets, glass slides and not in the pigeon pea plants under field conditions needs further investigation. Since oospores of *P.*

drechsleri f. sp. *cajani* are normally not formed in artificial cultures (CHAUHAN 1985, KANNAIYAN *et al.* 1980), or in the host tissues, this technique will be useful in studies on the biology and control of this fungus.

Literature

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