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A Technique for *Phytophthora drechsleri* f. sp. *cajani* Sporangia and Zoospores Production

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Phytophthora blight of pigeonpea is caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* (Pal et al.) Kannaiyan et al. The pathogen is a phycomycetous fungus and reproduces asexually by producing sporangia and zoospores. Kannaiyan et al. (1980) have described only briefly the technique for in vitro production of sporangia and zoospores of *Phytophthora drechsleri* f. sp. *cajani* (Pdc). We are enumerating the step-wise procedure for the benefit of other workers who might wish to use this technique.

The technique is based on the method described by Ribeiro (1978) and Kannaiyan et al. (1980) and involves the following steps:

1. Pdc is grown in 90-mm petri dishes, each containing about 12-15 mL of V-8 juice agar medium (V-8 juice 100 mL, calcium carbonate 2 g, agar 20 g, distilled water 900 mL) (Nene et al. 1981).
2. The petri dishes are incubated for 5 days at 30°C.
3. Clear V-8 juice medium is prepared by centrifuging 100 mL of V-8 juice liquid medium (without agar) at 2000 rpm for 15 min. About 75 mL of supernatant is obtained and autoclaved.

4. A total volume of 6 mL of autoclaved clear V-8 juice medium and sterile distilled water in the ratio of 1:5 is pipetted into a 50-mm plastic petri dish. (Alternatively, 75 mL of clarified V-8 juice medium is diluted with 375 mL distilled water in a conical flask and autoclaved. Six mL of this diluted clear V-8 juice medium is pipetted into a 50-mm plastic petri dish.) Several such petri dishes may be prepared, depending on the experimental requirements.
5. Five 5-mm agar discs from the periphery of a 5-day-old colony of Pdc (step 2) are transferred to each petri dish.
6. The petri dishes are incubated for 24 h at 30°C under fluorescent light. Mycelial growth is observed around each agar disc.
7. The medium in each petri dish is decanted and the mycelial discs are rinsed twice with sterile distilled water.
8. Six mL of sterile distilled water is pipetted into each petri dish and incubated at 30°C under fluorescent light.
9. Abundant sporangia are produced in 16 h. About 8-20 zoospores are released from each sporangium. Mature, immature, and empty sporangia, and motile, encysted, and germinating zoospores are seen when the petri dish is observed under the 10 × objective of a light microscope.
10. The water in each petri dish, collected 16-20 h after incubation, is a suspension of about 50 000 zoospores mL⁻¹. The number of zoospores is estimated with a hemocytometer (Nene et al. 1992).

We have been using this technique regularly in our laboratory. A suspension of about 80 000 zoospores mL⁻¹ was obtained by following the above procedure using 90-mm plastic petri dishes, each containing 10 Pdc agar discs in 12 mL of diluted, clear V-8 juice medium. We have also used the technique successfully for mass production of zoospores by transferring a large number of Pdc agar discs to shallow trays containing appropriate volumes of medium. Abundant sporangia and zoospores are also produced using Pdc grown on pigeonpea seed meal agar medium (Sheila et al. 1983). If V-8 juice is not readily available, pigeonpea seed meal agar and diluted clear pigeonpea seed meal liquid medium can be used, and good numbers of sporangia and zoospores can be obtained.

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Entomology

Outbreak of Coccid Pests on Pigeonpea in Tamil Nadu

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More than 200 insect species are associated with pigeonpea at various stages of growth from the time of sowing to harvest and even in storage (Lateef and Reed 1990). When pigeonpea is grown as an annual, sucking pests such as scale insects are not major pests (Reed et al. 1989). With the introduction of short-duration pigeonpea types like Vamban 1 which give economical grain yields in 2-3 flushes, the crop has to be retained in the field for fairly prolonged periods. When these periods coincide with dry spells, sucking pests can cause serious problems. At the National Pulses Research Centre, Vamban Colony, Tamil Nadu, India, field infestation by the scale insect, *Ceroplastodes cajani* Maskell (Coccidae: Homoptera) on Vamban 1 pigeonpea type ranged from 25.6% to 50.5% during Mar-Jun 1993. The infestation by the mealy bug (*Coccidothrips insolitus*) (Green) (Pseudococcidae: Homoptera) was more severe, varying from 75.6% to even 100% on Vamban 1. This is the first field record of an outbreak of these two coccid pests on pigeonpea in Tamil Nadu.

The scale insect infestation was characterized by sickly, wilted, and dried up plants. Dirty white, hemispherical scales were found congregating on stems where these insects feed continuously. Scale insects were found to survive on *Ocimum sanctum*, *Corchorus* sp, *Cyamopsis tetragonaloba*, *Moringa oleigera*, *Dolichos lablab*, *Zizyphus* sp, *Camellia sinensis*, and the common rose (Ayyar 1938, Nair 1986). Patel et al. (1991) have reported similar outbreak of coccids in Gujarat, India.

The mealy bugs feed continuously on leaflets which become chlorotic and drop. Plants turn sick, lose vigor, and stand leafless in the field. While mealy bug nymphs are mobile and greenish-yellow colored, adults are sedentary and have a white waxy coating. The wild relative of pigeonpea, *Cajanus scarabaeoides*, which is extensively used in breeding programs is also heavily infested. As both scale insects and mealy bugs excrete honeydew copiously, ants become common on the infested parts of the plant.

Research on these pests is necessary in order to develop field strategies to combat them.

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