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Pigmentation and growth-temperature response of *Phytophthora meadii* and *P. nicotianae* var. *nicotianae* infecting cardamom (*Elettaria cardamomum* Maton)

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Abstract

Capsule rot ('Azhukal') and leaf blight are two major *Phytophthora* diseases of small cardamom (*Elettaria cardamom* Maton). Variability among the *Phytophthora* isolates causing these diseases were studied using *Phytophthora* cultures isolated from rot affected plant parts such as capsules, rhizomes, leaf and also from blight affected leaves collected from different locations. Detailed studies on growth of these cultures in different media and at different temperatures showed significant difference between these isolates. *P. meadii* did not cause any pigmentation in casein hydrolysate tyrosine agar (Timmer's medium) and the fungus did not grow at high temperature while *P. nicotianae* var. *nicotianae* showed chocolate reddish brown pigmentation in casein hydrolysate tyrosine agar and did grew at high temperature. Therefore pigmentation in casein hydrolysate tyrosine agar and growth at high temperature were found ideal for differentiating *Phytophthora* isolates infecting cardamom.

Key words: fungal morphology, leaf blight, pigmentation, *Phytophthora nicotianae* var. *nicotianae*, *Phytophthora meadii*

Cardamom (*Elettaria cardamom* Maton) is susceptible to a number of fungal diseases, of which capsule rot prevalent during the monsoon season and leaf blight commonly seen during the post monsoon season are of great importance. Earlier studies revealed the association of three species of *Phytophthora* viz. *P. nicotianae* var. *nicotianae*; *P. meadii* and *P. palmivora* with capsule rot disease (Liyanage *et al.* 1983). Later, detailed studies confirmed that capsule rot of cardamom is mainly caused by *P. meadii* McRae of A2 mating type (Anonymous 1986) and was also found to be caused by *Phytophthora* species (Suseela Bhai 1998). Though these two diseases are of *Phytophthora* origin, they differ greatly in their period of occurrence and nature of infection. The variability of the species reported earlier lead the author to study the variation among the isolates of *Phytophthora* obtained from these two types of diseases.

Thirty four samples of rot affected capsules (16), leaves (14), pseudo stem (4 numbers) were collected from 25 different locations during the monsoon and post monsoon seasons. Surface sterilized infected tissues were plated in *Phytophthora* specific medium namely PVPH (Tsao & Guy 1977) to obtain pure cultures. The pure cultures thus obtained were maintained in corn meal agar medium for further studies. For convenience the isolates were grouped into three viz. capsule, leaf and pseudostem isolates.

Timmer's medium (Timmer *et al* 1970; Ho 1981) containing casein hydrolysate and tyrosine was used for pigment production. The medium was sterilized at 121°C at 15 lbs pressure for 20 min. After sterilization, the medium was poured into petridishes and inoculated. Simultaneously agar slopes were also inoculated with different isolates and incubated in the dark at 24±1°C for 21 days. The observations were recorded after 21 days of inoculation (Kennedy & Duncan 1995)

Chocolate brown pigmentation in Timmer's medium was observed with eight isolates. Among the 16 isolates from capsules, only one isolate (CPC 275) showed the chocolate brown pigmentation in the medium whereas out of the four isolates from pseudostem only two (CPP 244 and CPP 350) showed pigmentation.

Pigment production was first used to differentiate within progeny of crosses of A1 and A2 isolates of *P. dreschleri* (Galindo & Zentmyer 1964). Timmer *et al.* (1970) reported variation in production of melanin pigment among single oospore cultures of *P. capsici*. Erwin (1983) concluded that pigment production might be a poor taxonomic criterion but might be useful as a primary screening technique. This was supported by the investigations carried out by Dantanarayana *et al.* (1984). Pigment production in casein hydrolysate tyrosine medium was first used as a diagnostic character by Ho (1981). Accordingly pigment production, *i.e.* visible brown pigmentation was found to be the characteristic of several *Phytophthora* species like as *P. cactorum, P. cryptogea, P. cambivora, P. capsici, P. citricola, P. citrophthora, P. dreschleri* var. *dreschleri , P. erithroseptica,* var. *erithroseptica, P. infestans, P. megasperma, P. palmivora* MF1, *P. parasitica,* var. *parasitica, P. parasitica* var. *nicotianae, P. nicotianae* var. *nicotianae,* and *P. vignae*. Kennedy & Duncan (1995) used casein hydrolysate tyrosine agar for pigment production to compare papillate *Phytophthora* species from raspberry. Therefore in this study the pigment producing isolates were identified as *P. nicotianae* var. *nicotianae* whereas the non-pigment producers were identified as *P. meadii*.

Carrot agar medium was used for the temperature studies. The vegetative growth at temperatures 5, 10, 15, 20, 24, 28, 32, 36 and 40°C was studied. Inoculum discs of 3 mm diameter were cut out from the margin of 72 h old culture and inoculated in the centre of 9 mm petri dishes containing 15 ml of the medium. Plates were incubated in BOD incubators adjusted to the required temperatures and radial growth of the fungus was recorded at 24 h interval for 96 h and the average growth was calculated.

All the isolates grew between 10-32°C but failed to grow at 5°C and at 40°C. Irrespective of the source of isolation, the highest growth (8-9 mm day^{-1}) occurred at 20-28°C. At 32°C the growth rate was 1-6 mm day^{-1} where the isolate from capsules showed the minimum growth (1.89 mm day^{-1}) as compared to isolate from leaf (5.91 mm day^{-1}) and isolates from pseudostem (4.54 mm day^{-1}). Minimum growth was observed at 10°C (1-1.73 mm day^{-1}) for all the three groups. Among the 34 isolates, eight of the leaf isolates and three of the pseudostem isolates were found to be growing at 36°C. But only 6.25% of the capsule isolates were found to be growing at this temperature (Table 1).

Among the 57 isolates of *P. nicotianae* var. *parasitica* studied by Tucker (1931), seven isolates grew well at 35°C. Minimum temperature for the growth of *P. meadii* was reported as 10°C, 28-30°C as optimum for mycelial growth and 25-30°C or maximum 35°C for sporangia production (Waterhouse 1974). Lim & Chang (1986) studied fruit rot of durian caused by *P. palmivora* and explained the failure of these isolates to grow around 36°C and distinguished them from *P. nicotianae* and *P. nicotianae* var. *parasitica* which could grow well at temperature above 35°C.

Based on the preliminary characterization studies, two basic groups could be recognized: Group I producing pigment in casein hydrolysate tyrosine medium and Group II without pigment production. Each group could again be divided into two depending upon the formation of chlamydospores. All the pigment forming isolates were not found to be producing chlamydospores. So also the group I was found to grow at high temperature (>35°C).

Table 1. Effect of temperature on growth of *Phytophthora* isolates from cardamom

Source of isolate	Growth rate (mm day $^{-1}$) at temperature (°C)							
	10	15	20	24	28	32	36	40
Capsule (16 samples)	1.73±0.20	4.67±0.17	7.93±0.22	8.73±0.28	8.86±0.24	1.89±0.54	0.73±0.53	0.0
Leaf (14 samples)	1.53±0.19	3.97±0.27	7.94±0.47	8.45±0.52	9.30±0.48	5.91±1.54	3.26±0.59	0.0
Pseudostem (4 samples)	1.10±0.12	4.25±0.42	8.79±0.66	8.68±0.50	9.22±0.5	4.54±1.63	2.85±0.47	0.0

*Data are means of three replications

According to the key of Ho (1981) the Group I having pigmentation, chlamydospore formation and growth at >35°C with occasional hyphal swellings were identified as *P. parasitica* group. Hyphal swellings spherical with radiating hyphae in agar or in water were characteristic of *P. nicotianae* var. *nicotianae*. Likewise analogous radial projections as outgrowth of the wall that occur as a unique character in sporangia of many isolates of *P. nicotianae* var. *nicotianae*.

The non-chlamydospore forming isolates without pigmentation are invariably *P. meadii*. The non-production of chlamydospores in the medium is a specific character of *P. meadii*, which distinguished it from other species of *Phytophthora* (Rebeiro 1978). In the present study among the 34 isolates, 14 isolates did not produce chlamydospores under any condition, is truly agreeing with the above statement and could be identified as *P. meadii*. Intermediate pedicel length with caducous sporangia and swelling at the sporangiophore also distinguished them as *P. meadii*. Thankamma & Pillai (1973) and Nair (1979) isolated *P. nicotianae* var. *nicotiane* from infected fruits and leaves of cardamom. Association of *P. nicotianae* in cardamom was reported from Sri Lanka also (Liyanage *et al.* 1983). Hence, the present study leads to an authentic conclusion that more than one species of *Phytophthora* are associated with diseases of cardamom of which *P. meadii* played the major role in capsule rot while *P. nicotianae* var. *nicotianae* is involved as the predominant species in causing leaf blight disease of cardamom. However there is little amount of overlapping as evidenced from the production of pigmentation by some of the capsule isolates.

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