

Occurrence and distribution of mating types of *Phytophthora* species causing black pod disease of cocoa

P. CHOWDAPPA and R. CHANDRAMOHANAN

Central Plantation Crops Research Institute, Regional Station, Vittal 574 243

ABSTRACT : The mating types of isolates of *Phytophthora palmivora*, *Phytophthora capsici* and *Phytophthora citrophthora* causing black pod disease of cocoa were determined by pairing them with known A₁ and A₂ mating types of *P. palmivora*, *P. capsici* and *P. meadii*. Isolates of *P. palmivora* and *P. capsici* produced cospores when paired intra- and inter-specifically with either A₁ and A₂ mating types of *P. palmivora*, *P. capsici* and *P. meadii*, whereas the isolates of *P. citrophthora* did not produce oospores. Both A₁ and A₂ mating types were found among the isolates of *P. palmivora* and *P. capsici*. A₂ mating type was predominant in *P. palmivora* and A₁ in *P. capsici*. A₂ mating types of *P. palmivora* was predominant in all the districts of Kerala and Karnataka states. Both A₁ and A₂ mating types of *P. palmivora* and A₁ mating types of *P. capsici* were found in certain localities of Kerala state from where *P. citrophthora* isolates were collected. The occurrence of both mating types of different species in same locality reveals the possibility of intra- and inter-specific hybridization in nature and formation of new strains. This is the first report of occurrence of mating types of *Phytophthora* species causing black pod disease of cocoa in India.

Keywords : *Phytophthora palmivora*, *Phytophthora capsici*, *Phytophthora citrophthora*, mating types

Cocoa (*Theobroma cacao* L.) is mainly grown as mixed crop in the existing arecanut (*Areca catechu* L.) and coconut (*Cocos nucifera* L.) gardens in Karnataka and Kerala states of India. Black pod is a major disease of cocoa in this country. The causal organism of this disease was identified as a species of *Phytophthora*, when it was first reported from India (Ramakrishnan and Thankappan, 1965). Later, while studying the *Phytophthoras* associated with cocoa, Chandra Mohanan *et al.* (1979) identified the species causing black pod disease as *P. palmivora* (Butl.) Butl. Recent systematic studies on taxonomic complex of *Phytophthora* associated with black pod disease

of cocoa revealed the occurrence of *P. capsici* (Chowdappa *et al.*, 1993) and *P. citrophthora* (Chowdappa and Chandra Mohanan, 1996) on cocoa in India besides *P. palmivora*. These studies revealed that *P. palmivora* was predominant species causing black pod disease in India, and *P. capsici* and *P. citrophthora* occurred only in few localities in Kerala. The objective of the present study was to determine mating types of these three species of *Phytophthora* and their distribution in India.

MATERIALS AND METHODS

The mating types of 120 isolates of *P. palmivora*, five isolates each of *P. capsici* and *P. citrophthora* isolated from black pod affected co-

Received for publication December 2, 1996.

*CPCRI Contribution No : 934

cocoa pods collected from various localities of Kerala and Karnataka states, major cocoa growing region, were determined by pairing each of them with known A_1 and A_2 mating types of *P. palmivora*. The isolates were maintained on carrot agar slopes at $24 \pm 1^\circ\text{C}$ by periodical subculturing. Disks of five mm diam cut from the advancing margin of three-day old cultures were used for pairing the isolates. The mycelial disks of *P. palmivora* (A_1 or A_2) and *Phytophthora* isolate to be tested were placed 50 mm apart on CA simultaneously. Inoculated plates were incubated at 20°C in dark for 10-12 days. The plates were then examined for the presence of sex organs and oospores. An isolate was considered A_1 mating type when it produced oospores with A_2 and not with A_1 . Similarly, a culture was considered as A_2 mating type when it produced oospores with A_1 type and not with A_2 . The representative isolates belonging to three species of *Phytophthora* viz., *P. palmivora*, *P. capsici* and *P. citrophthora* were also crossed intra- and inter-specifically with *P. palmivora* A_1 and A_2 , *P. capsici* A_1 and A_2 and *P. meadii* A_1 and A_2 .

RESULTS

All the 130 isolates of *Phytophthora* spp. failed to produce oospores in single cultures on CA. However, isolates of *P. palmivora* and *P. capsici* readily produced abundant oospores when they were paired with either A_1 or A_2 mating types of *P. palmivora*, *P. capsici* and *P. meadii*. No oospores were formed when isolates of *P. citrophthora* were paired with either A_1 or A_2 mating types of *P. palmivora*, *P. capsici* and *P. meadii*. Of the 120 isolates of *P. palmivora*, 22 isolates were identified as A_1 mating types and the remaining 98 isolates as A_2 mating types. Similarly, out of five isolates of *P. capsici*, one isolate (I-CP/23) was identified as A_2 and four isolates (I-CP/25, I-CP/27, I-CP/76 and I-CP/89) as A_1 mating types. Thus, it was found that A_2 mating type was predominant in *P. palmivora* population and A_1 in *P. capsici*. The distribution of A_1 and A_2 mating types of *P. palmivora* and *P. capsici* in

different districts of Kerala and Karnataka states is shown in Fig. 1. The A_2 isolates of *P. palmivora* were predominant in all districts of Kerala and Karnataka states. *Phytophthora* isolates collected from Kasaragod (3) and Ernakulam (4) districts of Kerala and Dakshina Kannada (12), Shimoga (3) and Kodagu (4) districts of Karnataka were only A_2 mating type of *P. palmivora* whereas A_1 mating of *P. palmivora* was also present in Thottapally (1) and Chengannur (1) of Alappuzha district, Thodupuzha (1) of Idukki district, Changancherry (7), Nedumkunnam (1) and Meenachil (1) of Kottayam district, Kozhikode (2) of Kozhikode district, Kozhencherry (1) and Mallappally (1) of Pathanamthitta district, Nedumangad (1) and Thiruvananthapuram (1) of Thiruvananthapuram district, Thrissur (1) of Thrissur district, Vaithiri (1) and Wynad (1) of Wynad district and in Salkani (1) of Uttara Kannada district. It is evident from Fig. 1 that both A_1 and A_2 mating types of *P. palmivora* and A_1 mating type of *P. capsici* were present in Thrissur, Pathanamthitta and Idukki districts from where *P. citrophthora* isolates were collected.

DISCUSSION

The occurrence of A_1 and A_2 mating types in the population of *P. palmivora* and *P. capsici* causing black pod disease of cocoa in India has been established for the first time. A_2 was found to be predominant mating type in *P. palmivora* population in all the districts of Kerala and Karnataka states. In *P. capsici*, A_1 and A_2 mating types occurred in the ratio of 4:1 indicating the predominance of A_1 even though only five samples collected from Kerala yielded *P. capsici*. *P. palmivora* A_2 has been reported as the predominant mating type of cocoa worldwide except in Jamaica where A_1 was predominant (Brasier and Griffin, 1979; Zentmyer, 1988). Both A_1 and A_2 mating types were common in *P. capsici* (Zentmyer, 1988). The occurrence of both A_1 and A_2 mating types within a heterothallic species of *Phytophthora* is a universal phenomenon (Savage *et al.*, 1968; Brasier and Griffin, 1979).

Isolates of *P. citrophthora* failed to produce oospores when they were paired with either A₁ or A₂ mating types of *P. palmivora*, *P. capsici* and *P. meadii*, indicating they are sterile. Based on the studies on intra- and inter-specific pairings of *P. citrophthora*, earlier researchers have reported both sterility (Leonian, 1925; Tucker, 1931; Kellam and Zentmyer, 1986a) and heterothallism (Haasis and Nelson, 1963; Savage *et al.*, 1968); Ferguson, 1976; Riberio *et al.*, 1975; Liyanage and Wheeler,

1989). This has led to some confusion. Detailed studies on a world wide collection of *P. citrophthora* isolates from 30 different hosts species by Mchau and Coffey (1994) showed that 70 per cent of the isolates were sexually sterile.

In the present study, both A₁ and A₂ mating types of *P. palmivora* and *P. capsici* were found occurring in Thrissur, Idukki and Pathanamthitta districts of Kerala state where *P. citrophthora* was also present. The occurrence of two mating types

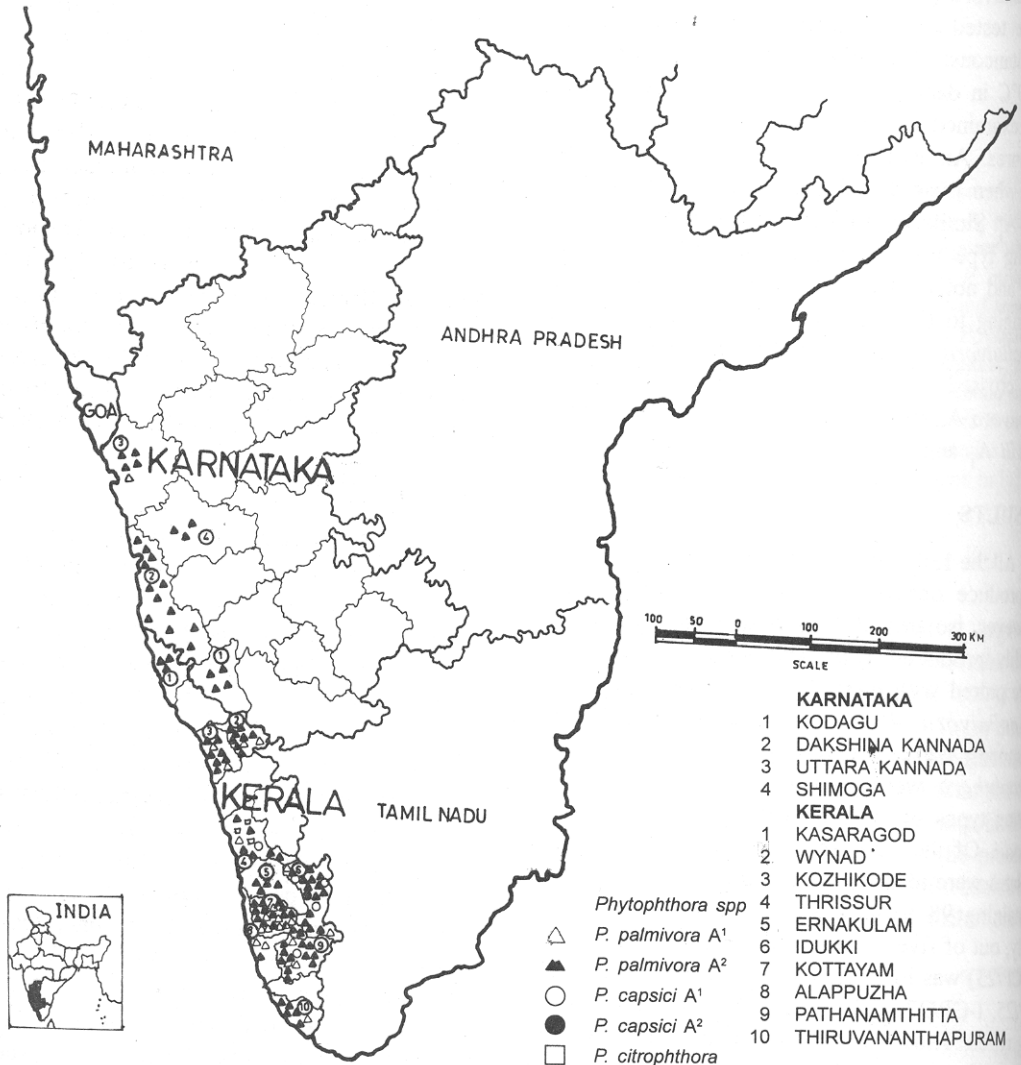


Fig. 1. Distribution of different species and mating types of *Phytophthora* causing black pod disease of cocoa in India.

of different species in same locality in India raises the possibility of intra- and inter-specific hybridization between them in nature and formation of new strains. This possibility is further supported by the observations on oospore formation in nature on infected cocoa pods and their germination in Brazil (Kellam and Zentmyer, 1986 b). Braiser (1992) hypothesized *P. citrophthora*, a sterile species, might have evolved through interspecific hybridization between *P. nicotianae* of citrus and *P. capsici* of cocoa or between *P. palmivora* and *P. capsici* of cocoa as *P. citrophthora* was often found associated with these species in nature.

Species of *Phytophthora* cause severe diseases like 'fruit rot' of arecanut, 'black pod' of cocoa, 'capsule rot' of cardamom, 'foot rot' of black pepper and 'abnormal leaf fall' of rubber in the plantation of South India where all these crops are grown either in the high density multispecies cropping systems or in the vicinity of each other as pure plantations. These isolates are cross inoculable in most cases. In the present study, *P. palmivora* and *P. capsici* isolates readily produced oospores when they were paired intra- and inter-specifically with *P. palmivora*, *P. capsici* and *P. meadii* suggesting that there is possibility of intra- and inter-specific hybridization among *Phytophthora* species affecting plantation crops and formation of new strains. Sastry and Hedge (1987) reported the existence of A₁ and A₂ mating types of *P. palmivora* causing 'wilt' of black pepper and A₁ type of *P. meadii* causing fruit rot of arecanut in Uttara Kannada district of Karnataka and inter-specific hybridization between them. Recent increase in the incidence of 'fruit rot' of arecanut and severe outbreak of 'wilt' of black pepper might be due to development of new strains through hybridization.

REFERENCES

- Brasier, C.M. and Griffin, M.J. (1979). Taxonomy of *Phytophthora palmivora* on cocoa. *Trans. Br. mycol. Soc.* **72**: 111-143.
- Brasier, C.M. (1992). Evolutionary biology of *Phytophthora*. Part I Genetic system, sexuality and the generation of variation. *Annu. Rev. Phytopathol.* **30**: 153-171.
- Chandramohan, R., Anandaraj, M. and Joshi, Y. (1979). Studies on *Phytophthora* diseases of cocoa occurring in India. *PLACROSYM II* : 335-341.
- Chowdappa, P. Chandramohan, R. and Ramanujam, B. (1993). Occurrence of *Phytophthora capsici* on cocoa in Kerala. *Indian Phytopath.* **46**: 92-93.
- Chowdappa, P. and Chandramohan, R. (1996). *Phytophthora citrophthora* from cocoa in India. *Trop. Agric. (Trin.)* **73** : 158-160.
- Ferguson, M.W. (1976). Variation in isolates of the fungus *Phytophthora citrophthora* (Smith & Smith) Leonian. M.S. thesis, California Polytechnic University, Pomona, USA. 48pp.
- Haasis, F.A. and Nelson, R.R. (1963). Studies on the biological relationship of species of *Phytophthora* as measured by oospore formation in intra- and inter-specific crosses. *Plant Dis. Repr.* **47**: 705-709.
- Kellam, M.K. and Zentmyer, G.A. (1986a). Morphological, physiological, ecological, and pathological comparisons of *Phytophthora* species isolated from *Theobroma cacao*. *Phytopathology* **76**: 159-164.
- Kellam, M.K. and Zentmyer, G.A. (1986b). Comparisons of single oospore isolates of *Phytophthora* species from naturally infected cocoa pods in Brazil. *Mycologia* **78**: 351-358.
- Leonian, L.H. (1925). Physiological studies on the genus *Phytophthora*. *Amer. J. Bot.* **12**: 444-498.
- Liyanaige, N.I.S. and Wheeler, B.E.J. (1989). Comparative morphology of *Phytophthora* species on rubber. *Plant Pathology* **38**: 592-597.
- Mchau, G.R.A. and Coffey, M.D. (1994). An integrated study of morphological and isozyme patterns found within a world wide collection of *Phytophthora citrophthora* and a redescription of the species. *Mycol. Res.* **98**: 1291-1299.
- Ramakrishnan, K. and Thankappan, M. (1965). First report of black pod disease of cocoa in India. *S. Indian Hort.* **13**: 33-34.
- Ribeiro, O.K., Erwin, D.C. and Zentmyer, G.A. (1975). An improved synthetic medium for oospore

- production and germination of several *Phytophthora* species. *Mycologica* 67: 1012-1019.
- Sastry, M.N.L. and Hedge, R.K. (1987). Distribution of mating types and their role in perpetuation of *Phytophthora palmivora* and *P. meadii*. *Indian Phytopath.* 40: 370-373.
- Savage, E.J., Clayton, C.W., Hunter, J.H., Brenneman, J.A., Laviola, C. and Gallegly, M.E. (1968). Homothallism, heterothallism and inter-specific hybridization in the genus *Phytophthora*. *Phytopathology* 58: 1004-1021.
- Tucker, C.M. (1931). Taxonomy of the genus *Phytophthora* de Bary. University of Missouri Agricultural Experiment Station Research Bulletin 153: 208 pp.
- Zentmyer, G.A. (1988). Taxonomic relationships and distribution of species of *Phytophthora* causing black pod of cacao. *10th International Cocoa Research Conference Proceedings*. 17-23 May 1987. Santo Domingo, Dominican Republic, pp. 391-399.