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AWARDS AND HONOURS

Dastur Memorial Lecture Award*

Bio-diversity of *Phytophthora* species in India

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Well, I have been asked to deliver the Dastur Memorial Lecture by Indian Phytopathological Society, New Delhi for which I express my gratefulness to the society. Before I actually talk with regard to biodiversity of *Phytophthora* species in India, I would like to pay my tribute to Late Professor Jahangir Fardunji Dastur (1886-1971). Dastur received world wide recognition on his monumental work in the discovery of a new species of *Phytophthora* and named by him as *Phytophthora parasitica*. Professor Dastur was an outstanding plant pathologist and was the first elected President of the Indian Phytopathological Society. In consonance of Professor Dastur's immense contribution on *Phytophthora parasitica* responsible for blight of castor oil plants and foot rot and leaf rot of *Piper betle* L. caused by *Phytophthora parasitica* var. *piperina*, I have decided to talk about the bio-diversity of *Phytophthora* species in India. In all, around 34 species and varieties of *Phytophthora* have been reported from India so far (Table 1).

The genus *Phytophthora* (Gr. *Phyton*, a plant, *phthora*, destroyer) was established by deBary (1876) with *P. infestans* de Bary as the type species. He recognized that the fungus causing late blight of potato in Europe in 1840's previously identified as *Botrytis infestans* Montagne, later *Peronospora infestans* (Mont.) Caspary, had unique characteristics like branching of the sporangiophore (at first monopodial, secondary branches sympodial), the shedding of the sporangia, the formation of zoospores in the sporangium and the germination of the sporangium by zoospores or by tube, which he considered distinct enough to be assigned a new genus name. The genus *Phytophthora* contains a wide range of species, from primitive to advanced, from soil to soil-aerial habitats. Because of its economic importance, the taxonomy, biology and pathology of *Phytophthora* species has been extensively studied and reviewed by several workers.

The classification of *Phytophthora* has always been difficult and confusing due to variability among species. In this review, a critical evaluation of the recent approaches using different parameters used in *Phytophthora* taxonomy is presented.

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Dastur's new species of *Phytopthora*

Dastur (1913) described a new species of *Phytophthora* as *P. parasitica*. Until 1963, the name *P. parasitica* Dastur (1913), the cause of a seedling blight of castor bean (*Ricinus communis* L.) had been accepted world wide rather than *P. nicotianae* as the proper name for this species because the latter had been inadequately described by Breda de Haan (1896). Ho and Jong (1989), stated that if nomenclaure of *Phytophthora* species is subject to International nomenclature, the name *P. nicotianae* legalistically should prevail. However, in the opinion of Erwin and Ribeiro (1996), *P. parasitica* is preferable.

Important Pytophthora diseases in India and their pathogens

Koleroga of arecanut: *Phytophthora* of Areca nut fruit rot and bud rot is called locally as 'Koleroga' or 'Mahali' disease caused by *P. arecae* and *P. meadii. P. arecae* differs from *P. meadii* in having sporangia without distortions, without swellings at the base of sporangia and with a small pedicel, 2.4 µm or not exceeding 6µm. Both species are predominantly heterothallic but formation of oospores in single culture or naturally infected nuts have been reported.

Bud rot of coconut and palmyra palm: It is caused by *P. palmivora* which produces oospores in complementary strains.

***Phytophthora* associated with cocoa:** It causes black pod, stem canker, seedling dieback, chupon blight, twig blight, (dieback) caused by *P. palmivora*, *P. capsici* and *P. citrophthora*.

***Phytophthora* disease of rubber**: It causes abnormal leaf fall, shoot rot, patch, canker and bark rot during rains. Four species of *Phytophthora* viz. *P. palmivora, P. meadii, P. nicotianae* var. *parasitica* and *P. botryose* are involved. *P. meadii* of both the mating types have been reported.

Foot rot and leaf rot of black pepper: It is caused by *P. capsici* and *P. palmivora.*

***Phytophthora* on small cardamom:** It is caused by *P. nicotianae* var. *nicotianae*, *P. palmivora* and *P. meadii*. The isolates are heterothallic.

Table 1. Species of *Phytophthora* reported from India for the first time

Species	References
Phytophthora arecae	Coleman (1910), Butler (1918)
P.boehmeriae	Nema (2000)
P. botryosa	Thankamma *et al.* (1968)
P. cactorum	Singh, U.B. (1943)
P. cajani	Amin *et al.* (1978)
P. cambivora	Ramakrishnan & Soumini (1948)
P. capsici	Chowdappa *et al.* (1993)
P. cinnamomi	Ramakrishnan (1951)
P. citrophthora	Naqvi (1988)
P. colocasiae	Butler & Kulkarni (1913)
P. citricola	Rana & Gupta (1984)
P. cyperi bulbosi	Seethalakshmi & Ramakrishnan (1953)
P. cyperi-rotundati[1]	Seethalakshmi (1953)
P. drechsleri f.sp.cajani	Pal *et al.* (1970), Kannaiyan *et al.* (1980)
P. fragariae	Bharadwaj & Sharma (2000)
P. gonapodyides	Bhargava & Singh (1965)
P. himalayensis[2]	Dastur (1948)
P. infestans	Butler (1903)
P. irritabilis[3]	Mantri & Deshpande (1966)
P. katsurae	Veena, Peethambram & Sarma (1997)
P. macrospora[4]	Thirumalachar *et al.* (1953)
P. meadii	McRae (1919)
P. megasperma	Suryanarayana & Pathak (1968)
P. megasperma var.sojae	Singh & Pavgi (1970)
P. melonis	Guha Roy *et al.* (2006)
P. parasitica[5]	Dastur (1913)
P. parasitica var. piperina	Dastur (1935)
P. parasitica var. macrospora	Rao *et al.* (1963)
P. parasitica var. nicotianae	Mundkur (1938)
P. parasitica var. sesami	Kale & Prasad (1957)
P. palmivora	Butler (1910, 1917)
P. pini var. antirhini[6]	Sundaraman & Ramakrishnan (1928)
P. rubra[7]	Mantri & Deshpande (1966)
P. vignae	Nirwan & Upadhyay (1972)

(Modified from Mehrotra and Aggarwal, 2001)

Betelvine foot rot and leaf rot: Different regions have different pathogens and in some areas more than one species are involved. In Uttar Pradesh and Madhya Pradesh it is mainly caused by *P. parasitica*, in West Bengal it may be *P. palmivora* and *P. parasitica* or even *P. capsici*, in South India, *P. capsici*, *P. palmivora* are mainly involved.

***Phytophthora* disease of citrus:** It is caused by *P. nicotianae* var. *parasitica, P. palmivora* and *P. citrophthora* in different parts of the country. *P. nicotianae* is primarily heterothallic.

Collar rot of apple: It is caused by *P. cactorum*, a heterothallic fungus.

Late blight of potato: It is caused by *P. infestans* which is a heterothallic fungus.

***Phytophthora* leaf blight of taro:** It is caused by *P. colocasiae*, a heterothallic fungus.

Criteria for classification of *Phytophthora* species

Morphological parameters

The first key by Rosenbaum (1917) included eleven species and was entirely based on morphological characteristics. He differentiated 11 species based on antheridial position, shape and size of sporangia, chlamydospores and oospores. Tucker (1931) studied 150 isolates of *Phytophthora* and the diagnostic characters used were mycelial growth on agar medium, temperature growth relationship, antheridial type, sporangial dimensions, size of oospores and pathogenicity. Leonian (1925, 1934) doubted the reliability of morphology, temperature-growth relationship and pathogenicity as stable taxonomic characters and emphasized the physiological characters, for example the ability to grow and produce sporgania, oogonia and chalmydospores at different temperatures and the influence of different chemicals such as malachite green, tartaric acid and potassium carbonate.

Waterhouse (1963) used papillation and proliferation of sporangia, antheridial form and size, oogonial diameter and ornamentation, cultural patterns, host specificity and cardinal temperature for growth of various *Phytophthora* species.

Newhook *et al.* (1978) gave a tabular key for the identification and description of 43 species and 4 varieties of *Phytophthora* in six groups on the basis of morphological and physiological features.

Ho (1981) gave synoptic keys to 38 species of *Phytophthora* as an alternative key to the traditional dichotomous keys and presented three synoptic keys respectively, for the plant pathogenic species (terrestrial), in the plant pathogenic species known only on hosts and the aquatic species.

Gerrettson-Cornell (1985, 1989) proposed a working key based on morphological and physiological characters. The key included 39 species of *Phytophthora* and were divided into eight groups mainly on the basis of the ability to form sexual organs, surface morphology of oogonium, type of antheridium and sporangial formation on the solid media. In 1989, he published "A compendium and classification of the species of the genus *Phytophthora* de Bary" by the canons of the traditional taxonomy, described 41 species of *Phytophthora* of major importance in forestry and agriculture and brought forward most of the available, taxonomically useful information on each of 41 species of *Phytophthora*.

A revised updated key of Stamps *et al.* (1990), lists 67 species and the varieties including 19 new ones described since, 1978. The division of species into groups was on the basis of sporangial papillation and the arrangement of sex organs and other characters.

Zentmyer *et al.* (1977) and Al-Hedaithy and Tsao (1979) emphasized the importance of sporgangium caducity, pedicel length and ontogeny in relation to classification of species of *Phytophthora*. In the non-caducous species which include all non-papillate species (Waterhouse, 1963), shedding does not occur in any isolate.

Brasier and Griffin's (1979) summary of the pertinent literature shows that many isolates previously considered to be *P. palmivora* were not actually *P. palmivora*. After a comprehensive study with 950 isolates identified by various research workers as *P. palmivora* from different regions of the world, they redefined the species *P. palmivora*, described the new species *P. megakarya* (MF3 of Griffin, 1977) and concluded that another species existed (MF4), which may be similar to *P. capsici*. Brasier and Griffin (1979) no longer supported the existence of MF2 emphasizing the MF1, MF2, MF3 and MF4 designations should be discontinued to avoid further confusion and concluded that Griffin's isolates constituted the valid *P. palmivora* species with n = 9-12 chromosomes, papillate sporangia varying from near spherical to ovate elongate, a short pedicel (2-5 µm) and worldwide distribution. MF3 was re-described as the new species, *P. megakarya*, found only in West Africa, characterized by n=5-6 large chromosomes, papillate sporangia and a pedicel of medium length (10-30 µm). The MF4 type has elongated sporangial stalk (20-150 µm) and was found in Central and South America and West Indies and considered to be closely related to *P. capsici.* Tsao *et al.* (1981) stated that the sporangial pedicel of typical *P.capsici* isolate was shorter than MF4. The *Piper nigrum* (black pepper) isolates of Tsao and Tummakate (1977) considered to be MF4 were later considered as *P. capsici (P. palmivora* MF4) by Sarma *et al.* (1982). Chee (1969) studied 260 isolates from rubber with abnormal leaf fall symptoms and divided them into those he considered typical of *P. palmivora* and another group that he considered as *P. botryosa*. *P. botryosa* grew more slowly than *P. palmivora*, has a lower temperature, produce chalmydospores sparsely, sporangia in clumps and oospores in both heterothallic and homothallic manner, whereas *P. palmivora* isolates were all heterothallic. Lengths of pedicels of *P. botryosa* were within intermediate range (5-20µm).

Cytological parameter

Of late, chromosomes have been utilized for the identification of species of *Phytophthora*. About 9 to 10 or 12 chromosomes are present in many species, but *P. magasperma* var. *megasperma* has twice the usual number, whereas *P. megasperma* var. *sojae* has 1-15. At one time *P. palmivora* was considered to be the only causal organism of black pod of cocao, although some typical isolates were recognized. At an international conference held in the United Kingdom, information was pooled and isolates were tentatively designated by morphological forms (MF). The morphological forms includes the typical *P. palmivora* MF1 the "S": chromosome type (n = 9-12 bivalents of "S" type short type): M3, the "L" chromosome type (n=5-6 bivalents) (Large chromosome type); and described a new species of *Phytophthora*, *P. megakarya*; and MF4, subsequently re-described as part of the established species *P. capsici* (Tsao and Alizadeh, 1988; Tsao, 1991) an MF2 was proposed but later considered invalid.

The discovery of *P. megakarya* as a new species from the cytological study of the chromosome morphology of A1 and A2 mating types of *Phytophthora* that had been isolated from cocoa from Nigeria is interesting.

L type with 5-6 large chromosomes at metaphase and S type with 9-12 much smaller chromosomes. The L type was described *as P. megakarya* because of its large chromosomes size and other specific features. A new approach to Karyotype analysis called pulse field gel electrophoresis of chromosomal DNA promotes greatly to extend our information. Hansen *et al.* (1986) have demonstrated four karyotypes. K-I to K-IV ranging from n= about 10 to n=30. Earlier distinct polyploidy karyotypes were shown to be associated with two varieties of *P. megasperma*.

Biochemical and molecular parameters

Perhaps the most promising of these methods is the determination of protein pattern of different *Phytophthora* mycelia by gel electrophoresis. Clare and Zentmyer (1966) found that the protein patterns of *P. cinnamomi, P. citrophthora* and *P. palmivora* were distinctly different and that patterns of different isolates were identical or similar regardless of the geographic or host source using polyacrylamide gel electrophoresis (Hall *et. al.* 1969). Similar results were obtained with *P.cactorum, P.cinnamomi* and *P. palmivora* but *P. palmivora* isolates were different quantitatively and identical qualitatively. It was further demonstrated that while protein patterns in electrophoresis differed markedly between species they were very similar or identical within a seven species, regardless of the date of isolation, host or geographic locality and mating type. It has been used alongwith other conventional criteria for precise identification of *P. citricola* from Avocado (Zentmyer *et al.,* 1974), *P. drechsleri* from cucumber (Ann & Ko, 1985) and *P. cinnamomi* from Taiwan (Ho *et al.* 1984) and for establishment of new taxas in *P. pseudotsugae* and *P. humicola*. In most cases, the electrophoresis pattern correlated with the morphological characters. However, Erselius and Vallavieille (1984) pointed out that whereas the intraspecific variation of protein patterns of *P. cactorum, P. capsici, P. citrophthora, P. cryptogea* and *P. nicotianae* were negligible, the two isolates identified as *P. citricola* on morphological grounds had a protein pattern similar to that of *P. citrophthora*. Hamm and Hansen (1983) also found lack of consistency in protein bands of isolates of *P. cactorum*. At least distinct group of *P. megasperma* could be distinguished based on protein pattern.

Chowdappa and Chandramohnan (1995) described three species of *Phytophthora* viz. *P. palmivora, P. capsici and P. citrophthora* causing black pod disease of cocoa in India. The gel isolates of the same species were successfully distinguished both qualitatively by visual similarity in banding patterns and qualitatively by calculating similarity coefficients. Aggarwal *et al.* (2001) indientified a species of *Phytophthora* from *Chukrasia tabularis* as *P. nicotianae* on the basis of electrophoretic patterns of mycelial protein.

Chowdappa *et al.* (2003) identified *P. capsici, P. nicotianae, P. areacae* (P. *palmivora*) and *P. meadii* at Central Horticultural Experimental Station, Hirehalli in South India by using RFLP of PCR - amplified internal transcribed spacer regions of ribosomal RNA. Tripathi *et al.* (2003) confirmed that the isolates from betelvine in Uttar Pradesh were *P. nicotianae* and not *P. capsici* and it was done by genomic extraction of DNA of the pathogen, polymerase chain reaction (PCR), southern hybridization and restriction length polymorphism (RFLP) analysis of the PCR products to establish the identity of the Indian and other isolates of *Phytophthora.* Guha Roy *et al.* (2007) identified *P. melonis*, *P. nicotianae, P. colocasiae and P. capsici,* both through morphological as well as molecular methods like restriction fragment length polymorphism (RFLP) of the Internal Transcriber Spacer regions of DNA, sequencing of the ITS region. Guha Roy and Bhattacharya (2009) further reported the phylogeny of *Phytophthora* isolates from West Bengal as inferred from DNA ITS gene sequences. Considerable intraspecific diversity was present among the polyphagous *P. nicotianae* in contrast to *P. capsici.*

Races and sexuality of *P. infestans* and resistance to fungicides

Late Blight of potato has remained a challenge for plant pathologists even after more than 150 years of Irish famine due to this disease. Yield losses ranging from 15-100 percent have been reported depending upon the cultivar used, weather conditions and crop protection measures adopted (Thind and Mohan, 1998). Polycyclic nature of *Phytophthora infestans* and its ever-changing racial pattern due to diploidy have posed a serious challenge to the plant breeders for developing commercial varieties with durable resistance. Oosporers of the pathogen have been reported in hilly areas (Singh *et al.,* 1994). Role of tuber infection in causing initial infection foci has been well worked out in India (Thind *et al.,* 2008).

CPRI has developed a good number of varieties such as Kufri Badshah, Kufri Jyoti, Kufri Chipsona etc. that have field resistance to late blight. However, these varieties also succumb to the disease pressure under continuous favourable weather conditions. In the absence of durable resistance in the commercial varieties, fungicides play an important role in managing Late Blight. Several fungicides belonging to different groups are now available which have remarkable activity against late blight.

Apart from phenylamides (Metalaxyl, Benalaxyl, Ofurace, Oxadixyl), cyanoacetamides (cymoxanil), cinnamic acid derivates (dimethomorph) which have been commonly used for controlling this disease, some recently developed fungicides with novel modes of action such as fenamidone, iprovalicarb, famoxadone, mandipropamid have a good potential to control potato late blight (Thind *et al*., 2004). However, due to the specificity in their mode of action, there are chances of development of resistance in *P. infestans*. Resistance development to Metalaxyl is well documented in several countries including India (Kaur *et al.,* 2010). To check build up of resistance, these fungicides are generally used in combination with multisite fungicides such as dithiocarbamates and copper based compounds in pre-packed mixtures. A forecasting model based on 7 day moving rainfall has been developed for hilly regions (Bhattacharyya *et al.* 1983). Web-based decision support systems are now available which guide the farmers in a particular region in timing fungicide applications depending on weather conditions.

National Network Project on *Phytophthora* diseases of horticultural crops (PHYTONET)

In view of heavy crop loss due to *Phytophthora* Dr. Y.R. Sarma (Sarma, 2000) conceptualized the holistic view of the *Phytophthora* programmes of the horticultural crops in India based on expertise he developed. This led to the establishment of National Network of *Phytophthora* diseases of Horticultural Crops (PHYTONET) with nine centres throughout India, covering plantation crops and spices, fruit crops (citrus and apple) vegetables (tomato and potato) and floriculture (crossandra and gerbera) with headquarters at Calicut. This ensured better interaction of Phytophthora workers in India to develop effective disease management and thus reduce crop loss. Several important findings emanated from the project both in basic and applied aspects are of great relevance for India.

A National Repository of *Phytophthora* was set up at IISR, Calicut that is the biggest in Asia and characterization of various species has been initiated to study the variability through conventional taxonomical and molecular techniques. Database on *Phytophthora* was established and would continue and National Directory of *Phytophthora* workers was compiled and released.

Since space will not permit the present author to discuss more on the biodiversity of *Phytophthora* species, it can be concluded that more concerted efforts are to be made on the taxonomy of *Phytophthora* species especially using the modern biochemical and molecular techniques for the correct identification of *Phytophthora* species in conjunction with traditional morphological criteria.

Finally, this paper is a tribute to the dedication of 'Phytophthorologists' that they continue their investigations with different species of the genus *Phytophthora*. Despite many recent advances in taxonomy and biology of this pathogen, working with *Phytophthora* still requires a special dedication.

Some important contributions/publicatioins by Indian workers on *Phytophthora*

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