



## Characterization of *Phytophthora palmivora* isolates inciting bud rot and nut rot in coconut

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### Abstract

Cultural and morphological characters of 35 isolates of the *Phytophthora palmivora* collected from rotting bud and nut of coconut were characterized. Basically the colonies were stellate or striate type. Rate of growth in carrot agar varied significantly among the isolates. Rate of growth was fast in Pp22 and Pp23 and slow in isolate Pp14. The sporangia of all the 35 isolates were found to be caducous and were shed with short, broad and occluded pedicel. All isolates of *Phytophthora* collected in the present study were found to be heterothallic and were of A2 mating type which produced oospores when paired with A1 isolates. Oospore formation in all isolates was noticed only after 15 days of pairing with an A1 isolate. From the above characteristics, all the 35 isolates were confirmed as *Phytophthora palmivora* Butl. Maximum growth of both bud rot and nut fall isolates of *P. palmivora* were observed at pH 6.5 (Mean dry weight, 62.36 mg). A temperature range of 25 and 27°C supported maximum growth in both nut and bud rot isolates. Among organic Nitrogen sources, glutamine showed maximum dry weight of mycelium both in case of nut fall (58.27 mg) and bud rot isolates (57.00 mg) followed by those supplemented with L-asparagine.

**Keywords:** Bud rot, coconut, Kerala, nut fall, *Phytophthora palmivora*

### Introduction

The productivity of coconut in different coconut growing areas of the country varies considerably due to varying agro-climatic conditions, management practices and occurrence of pests and diseases. Loss due to diseases is one of the key factors for low productivity in several areas. Coconut palm, in spite of its hardy nature is affected by a number of diseases, some of which not only reduce the yield but also kill the palms. Bud rot is a fatal disease of the coconut palm, characterized by the rotting of the single terminal bud and surrounding tissues. Even though it affects palms of all ages, young palms in low lying and moist situations are more susceptible to the disease. Incidence of the disease has been reported from almost all coconut growing countries of the world. Butler (1906) first reported bud rot disease from India, where it is quite common along the West and East Coast tracts. Radha and Joseph (1974) reported a disease incidence of 1.2 to 10.9 per cent in Kerala, and 35 to 40 per cent in gardens having large number of palms

in certain locations. Studies have clearly shown the existence of two dominant *Phytophthora* spp. viz., *P. palmivora* Butl. and *P. katsurae* Ko & Chang. causing bud rot disease of coconut. The occurrence of *P. katsurae* causing bud rot has been reported also from Hawaii and Ivory Coast (Uchida *et al.*, 1992). Veena *et al.* (1997) reported *P. katsurae* from Kuttiady area in Kerala. However, the information obtained by these earlier workers was rather limited. Hence, in the present study physical, physiological and biochemical characterization of the coconut *Phytophthora palmivora* isolates was undertaken.

### Materials and Methods

Survey was conducted in different coconut growing tracts of Kasaragod, Kannur, Calicut, Thrissur, Malappuram, Ernakulam, Wynad and Palakkad Districts of Kerala and Dakshina Kannada District of Karnataka based on the information collected from respective Krishi Bhavans. A total of 172 samples were collected from

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bud rot and nut fall affected coconut gardens of Kerala and Karnataka State for the isolation of the bud rot causal organism. These samples consisted of spear leaves, nuts, petioles, organic debris from crown and soil. The samples were collected in fresh polythene bags and brought to the laboratory for the isolation of the causal organism of bud rot.

### Isolation of the causal organism

Coconut tissues showing typical symptom of bud rot were thoroughly washed in running water. Tissues from the lesions showing initial stages of infection were used for the isolation of the fungus. Pieces of 5x5mm size, of infected tissues were cut with sterile scalpel from the advancing margin of the lesion and surface sterilized by 70 % ethyl alcohol and inoculated on 90 mm dia. petriplates containing P<sub>10</sub>ARP medium (Kannwischer and Mitchell, 1978). These plates were incubated in the dark at 24±1°C. Characteristic growth of *Phytophthora* obtained on the third day was sub cultured and maintained on carrot agar slopes.

A higher isolation rate was obtained from freshly infected tissues that had not yet been affected by secondary microbes. For avoiding these inconveniences, baiting was done in the field itself by using, unopened fresh male flower buds which are easily available in the coconut gardens.

For isolation of *Phytophthora* spp. from soil, samples were taken at 10–15 cm depth, at 50 cm distance from the bole. Samples of about 100 g were taken in sterile polythene bags and moistened with sterile distilled water (Thevenin, 1992). Trapping was done using unopened male flower buds, spear leaf discs and two month old Chowghat Orange Dwarf (COD) nuts. These were monitored daily for the development of lesions and isolations were carried out in P<sub>10</sub>ARP medium.

The organic debris collected from bud rot affected coconut gardens were moistened with sterile distilled water and baiting was done with two month old COD nuts. These samples with baits were incubated at 24±1°C for 3-8 days. These baits were regularly observed for lesion development. Isolation from these lesions were carried out in P<sub>10</sub>ARP medium. Identification of the fungus was done based on sporangial morphology (Waterhouse, 1970).

### Studies on the variability of morphological characteristics

Cultural and morphological characters of 35 isolates of the fungus collected from rotting bud and nut were studied for characterization. Petri plates of 90 mm

dia. containing 15 ml of carrot agar (CA) were used for all the studies on cultural and morphological characters.

All the isolates were inoculated centrally on CA plates and incubated in the dark at 24±1°C for three days. Three replicates were maintained for each isolate. Colony morphology was examined against black background.

Carrot agar plates were inoculated at the centre with a 5mm dia. mycelial disc taken from the periphery of a three day old culture on CA and incubated in the dark at 24±1°C (Brasier, 1972; Brasier *et al.*, 1993). Colony diameter was measured three days after incubation.

To produce sporangia all the fungal isolates were grown on CA for first three days at 24±1°C. Subsequently these cultures were incubated under continuous light for three days. Three replicate plates were maintained for each isolate. Sporangium caducity was studied using 5x5mm mycelial discs cut from the highly sporulating part of the culture and gently immersing the upper surface of the disc 10 times in a drop of cotton blue lacto - phenol stain on a glass slide (Tsao *et al.*, 1985).

The compatibility of 35 isolates of *Phytophthora* were determined by pairing them with A<sub>1</sub> and A<sub>2</sub> isolates of *Phytophthora* received from IISR Calicut. Discs of 5mm dia. cut from the advancing margin of three day old cultures were used for pairing the isolates. The mycelial discs of *Phytophthora* A<sub>1</sub> and A<sub>2</sub> isolates and the *Phytophthora* isolate to be tested were incubated at 20°C in the dark for 10-20 days (Brasier *et al.*, 1993). The plates were then examined for the presence of sex organs and oospores. An isolate was considered A<sub>1</sub> mating type when it produced oospores with A<sub>2</sub> and not with A<sub>1</sub>. Similarly, a culture was considered A<sub>2</sub> mating type when it produced oospores with A<sub>1</sub> type and not with A<sub>2</sub>.

To study the sex organ formation in single cultures, discs of 5 mm dia. cut from the advancing margins of three day old culture were inoculated in the center of 90 mm petri dishes containing CA and incubated at 20°C for 10-20 days and observations were made at five day intervals for 30 days.

### Physiological and biochemical characterization

One isolate each from bud rot and nut fall sample (Pp1 and Pp10) were studied for the effect of different pH levels on growth. Carrot broth was used as the basal medium. These isolates were grown at different pH levels (3.5- 8.0) to find out the optimum pH for its growth. Different pH levels were adjusted with 0.1N HCl and 0.1N NaOH using pH meter. All cultures were incubated

for three weeks in the dark at  $24\pm 1^\circ\text{C}$  (Mahendra Pal and Grewal, 1975a). After three weeks of growth the mycelial mats were filtered on weighed Whatman No. 42 filter papers. They were washed thoroughly with distilled water and dried at  $60^\circ\text{C}$  to a constant weight.

One isolate each from bud rot and nut fall samples (Pp1 and Pp10) were tested to find the effect of different temperature levels on growth. Carrot broth was used as basal medium. The different temperatures tested were 10, 15, 20, 22, 25, 27, 30, 35 and  $40^\circ\text{C}$ . All the cultures were incubated for three weeks in dark (Mahendra Pal and Grewal, 1975a). After three weeks of growth the mycelial mats were filtered on weighed Whatman No. 42 filter papers. They were washed thoroughly with distilled water and dried at  $60^\circ\text{C}$  to a constant weight.

The amount of Nitrogen present in the basal medium (Bartnicki Garcia liquid medium) was calculated and replaced with equivalent amount of Nitrogen from different inorganic and organic nitrogenous compounds calculated on the basis of their structural formulae. One isolate each from bud rot and nut fall samples (Pp1 and Pp10) were tested to assess the effect of Nitrogen sources on growth of *Phytophthora* isolates. The mycelial discs cut from three day old cultures were thoroughly washed with sterile distilled water and blotted to remove excess water (Mahendra Pal and Grewal, 1975b). One mycelial disc was used for inoculating one flask. The inoculated flasks were incubated at  $24\pm 1^\circ\text{C}$ . After three weeks of growth mycelial mats were washed thoroughly with distilled water and filtered on weighed Whatman No. 42 filter papers. They were dried up to a constant weight at  $60^\circ\text{C}$ .

### Results and Discussion

Isolation of the causal organism from bud rot and nut fall samples yielded 35 *Phytophthora* isolates of which 17 were from Kasaragod District, 7 from Kannur District, 5 from Calicut District, 2 from Wayanad and 1 isolate each from Thrissur, Palakkad and Malappuram Districts of Kerala, and Dakshina Kannada District of Karnataka State. Out of the 35 isolates collected, 13 were from young petioles, 9 were from spear leaf, 5 from nut, 4 from crown debris (organic debris) from the leaf axils, and 2 isolates each from crown tissue and soil (Table 1).

Unopened male flower buds, easily available in the coconut gardens were found to be useful baits for the isolation of *Phytophthora* propagules from the diseased tissue. Chowghat Orange Dwarf (COD) nuts were found to be a good bait for the isolation of the *Phytophthora* propagules from the crown debris. The

rate of isolation from soil and crown debris was found to be high in endemic gardens compared to areas having sparse incidence of the disease.

### Morphological characterization

The isolates exhibited variation in colony morphology; they were stellate or striate type. Rate of growth in carrot agar varied significantly among the isolates. Rate of growth was fast for Pp22 and Pp23 and slow for isolate Pp14. Among the nut isolates, Pp22 showed higher growth rate and Pp18 showed slow growth rate. Isolates Pp23 and Pp14 showed fast and slow growth rate respectively among bud rot isolates (Table 1).

Sporangia of all 35 isolates were ovoid to ellipsoid in shape with a round base and conspicuous papilla. Both single and double papillate sporangia were present in the isolates. Based on the above characteristics, all the 35 isolates were confirmed as *Phytophthora* spp. Sporangial morphology of the 35 fungal isolates from bud rot and nut fall samples showed significant variation in length (L), breadth (B), L/B ratio and pedicel length. The sporangia of all the 35 isolates were found to be caducous and were shed with short, broad and occluded pedicel. Pedicel length of all the 30 bud rot isolates was  $< 5\ \mu\text{m}$ . In bud rot isolates, the average pedicel length ranged from  $3.19\text{--}4.91\ \mu\text{m}$  and L/B ratio ranged 1.51-1.89. In 5 nut isolates the average pedicel length was  $< 5\ \mu\text{m}$  and ranged from  $3.19\ \mu\text{m}\text{--}4.25\ \mu\text{m}$ . L/B ratio of the nut isolates ranged from 1.60-1.89. The nut fall isolates ranged between  $45.91\text{--}61.38\ \mu\text{m}$  in length and  $28.58\text{--}32.48\ \mu\text{m}$  in breadth. Bud rot isolates ranged between  $47.58\text{--}61.57\ \mu\text{m}$  in length and  $28.71\text{--}40.60\ \mu\text{m}$  in breadth (Table 2).

All isolates of *Phytophthora* collected in the present study were found to be heterothallic and were of  $A_2$  mating type which produced oospores when paired with  $A_1$  isolates. Oospore formation in all isolates was noticed only after 15 days of pairing with an  $A_1$  isolate. After 20 days, all the isolates produced oospores. Oospore formation was found to be inhibited by light. *P. palmivora* forms oogonia and oospores when  $A_1$  and  $A_2$  strains are paired (Ashby, 1929; Brasier and Griffin, 1979).

Identification of species of fungi is primarily based on morphology criterion. Variability in *Phytophthora* spp. have been reported by many workers (Waterhouse, 1974; Zentmyer, 1974). Brasier and Griffin (1979) who studied cultural characters and taxonomy of *P. palmivora* on cacao, reported the existence of variation within isolates. Tucker (1931) reported that sporangia of coconut isolates

**Table 1. Details of *P. palmivora* isolates of coconut - Growth rate and sporangial morphology**

Isolate No.	Source of isolation	Length (µm)		Breadth (µm)		Length/ Breadth		Pedicel length (µm)		Growth rate (mm/day)
		Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	
Pp1	Soil	53.26	15.26	34.02	11.82	1.56	13.92	3.37	32.76	18.2
Pp2	Soil	50.07	20.70	30.94	11.50	1.61	17.48	3.28	30.50	17.5
Pp3	Petiole	55.10	14.88	34.22	18.40	1.61	15.82	4.85	31.51	17.3
Pp4	Petiole	59.90	12.42	32.70	10.02	1.83	16.70	3.60	35.08	17.3
Pp5	Petiole	53.40	21.55	32.38	9.72	1.64	16.24	4.45	37.29	17.4
Pp6	Petiole	56.01	16.39	30.54	10.79	1.84	20.16	3.28	30.50	17.1
Pp7	Petiole	53.42	20.06	32.48	12.92	1.64	18.04	4.25	34.59	15.3
Pp8	Spear leaf	56.25	13.84	36.05	11.11	1.56	14.23	3.96	48.92	16.6
Pp9	Petiole	57.73	12.10	34.12	10.39	1.70	15.02	3.57	34.88	17.5
Pp10	Nut	54.08	19.39	32.47	16.82	1.66	20.89	3.28	45.35	18.6
Pp11	Petiole	58.09	11.16	35.47	12.45	1.64	12.31	4.54	39.96	15.2
Pp12	Spear leaf	58.87	14.47	31.99	8.41	1.84	14.86	3.19	27.73	17.5
Pp13	Spear leaf	59.50	12.41	34.41	10.55	1.73	14.54	5.02	31.15	17.7
Pp14	Spear leaf	61.57	13.53	40.60	10.18	1.51	16.16	3.18	17.20	14.2
Pp15	Spear leaf	55.10	14.82	31.61	11.13	1.74	12.67	3.38	32.54	17.9
Pp16	Nut	47.57	11.51	28.71	8.93	1.65	11.36	3.19	27.73	17.2
Pp17	Spear leaf	57.62	12.29	34.20	10.47	1.68	13.92	3.60	35.08	17.7
Pp18	Nut	45.91	17.94	28.58	8.85	1.60	11.95	3.28	38.30	16.4
Pp19	Petiole	58.19	15.03	37.89	17.04	1.55	17.36	4.93	46.65	17.2
Pp20	Spear leaf	58.96	12.41	33.48	21.11	1.73	14.28	4.91	28.82	15.6
Pp21	Petiole	48.94	12.16	29.19	10.73	1.68	11.92	3.57	34.88	18.4
Pp22	Nut	50.07	17.65	30.93	9.95	1.60	13.90	4.25	34.63	18.7
Pp23	Spear leaf	53.50	15.30	32.70	10.57	1.63	12.53	3.30	30.83	18.7
Pp24	Nut	61.38	18.44	32.48	11.58	1.89	18.95	3.67	35.50	17
Pp25	Petiole	51.12	10.42	31.72	27.16	1.61	13.92	3.86	41.00	16.7
Pp26	Crown debris	53.55	13.62	31.80	10.1	1.68	14.37	4.15	47.36	17.3
Pp27	Crown tissue	52.40	11.97	31.72	27.16	1.65	13.90	3.57	34.84	17.5
Pp28	Crown tissue	53.26	15.16	31.61	24.25	1.68	21.13	4.64	38.84	17.3
Pp29	Spear leaf	59.50	12.41	34.12	10.39	1.74	15.30	3.28	38.30	18.3
Pp30	Petiole	52.64	21.01	28.71	8.93	1.85	22.22	3.28	30.50	15.5
Pp31	Crown debris	53.36	20.16	28.80	8.74	1.83	20.82	3.38	32.49	16.3
Pp32	Crown debris	56.30	15.82	36.73	15.28	1.55	20.28	3.27	30.87	17.3
Pp33	Petiole	60.99	12.51	32.72	27.16	1.86	14.89	2.99	17.66	17.1
Pp34	Crown debris	47.58	11.50	28.90	10.05	1.64	18.37	3.86	34.96	17.5
Pp35	Petiole	58.19	15.03	30.68	22.58	1.89	12.56	3.57	34.88	17.4

CV=1.31 CD for isolates (P=0.05) = 0.364

were variable in size with an average length of 26 to 88-mm and 18 to 41-mm in breadth with L/B ratio of 1. 7:1.

Joseph and Radha (1975) reported *P. palmivora* as the causal organism of coconut bud rot occurring in Kerala. *P. katsurae* was also reported as an incitant of coconut bud rot from Kuttiady area in Calicut District of Kerala (Veena *et al.*, 1997). However, the detailed survey and isolation in Kuttiady area resulted in the isolation of *P. palmivora*. This may be because of the presence of *P. katsurae* in a limited pocket which could have escaped the attention in the present survey.

### Physiological and biochemical characterization

The study on effect of pH on growth revealed that variation due to pH was significant for the growth of *Phytophthora* isolates from coconut. Both bud rot and

**Table 2. Growth of *P. palmivora* isolates from coconut at different pH levels**

pH	Average dry weight of mycelium (mg)		
	Bud rot sample	Nut fall sample	Mean
3.5	29.93	31.86	30.90
4.0	32.36	32.23	32.30
4.5	33.96	34.36	34.16
5.0	35.00	35.16	35.08
5.5	46.13	45.33	45.73
6.0	57.70	58.56	58.13
6.5	62.43	62.30	62.36
7.0	56.56	54.90	55.73
7.5	51.36	52.10	51.73
8.00	41.90	41.43	41.66

C.V% = 2.34 CD (P= 0.05) for pH = 1.22

nut fall isolates behaved in a similar fashion. Maximum growth of both bud rot and nut fall isolates of *P. palmivora* were observed at pH 6.5 (Mean dry weight, 62.36 mg) followed by pH 6.0 (58.13 mg) (Table 3). Dry weight of mycelium was significantly reduced when grown at pH levels above and below this range both in the case of bud rot and nut fall isolates.

**Table 3.** Growth of *P. palmivora* isolates from coconut at different temperature levels

pH	Average dry weight of mycelium (mg)		
	Bud rot sample	Nut fall sample	Mean
10	3.76	3.63	3.70
15	16.40	16.26	16.33
20	42.26	42.63	42.45
22	47.60	46.53	47.06
25	55.30	57.70	56.50
27	55.43	55.53	55.48
30	53.40	54.23	53.81
35	43.50	43.63	43.56
40	1.66	1.96	1.81

C.V% = 2.1  
CD (P = 0.05) for temperature = 1.01

The study on the effect of temperature on the growth of *P. palmivora* revealed that a temperature range of 25 to 27°C supported maximum growth in both nut and bud rot isolates. Even though nut fall isolate had acquired slightly higher dry weight (57.7mg and 55.50mg respectively at 25 and 27°C) than bud rot isolate (55.30 mg and 55.43mg respectively at 25 and 27°C) the differences were not statistically significant. The mean dry weights of both bud rot and nut fall isolates were high at 25°C (Table 4). The minimum temperature that supported the growth of *P. palmivora* was 11°C and maximum was at 35°C (Waterhouse, 1974). The present study shows that a temperature range between 25-27°C is optimum for the growth of *P. palmivora* from coconut and a temperature below and above this range had an adverse effect.

Among the various organic and inorganic Nitrogen sources tested, the inorganic Nitrogen source, Ammonium nitrate elicited maximum dry weight of mycelium both in nut fall (59.13 mg) and bud rot isolates (58.23 mg), compared to other organic and inorganic Nitrogen sources tested (Table 5). It was observed that from among the inorganic Nitrogen sources Ammonium nitrate proved to be the best followed by Ammonium sulphate, Potassium nitrate, Sodium nitrate and Ammonium chloride and the minimum was observed in

Calcium nitrate. Both bud rot and nut fall isolates behaved similarly and the differences between them was not significant. Among organic Nitrogen sources, Glutamine promoted maximum dry weight of mycelium both in case of bud rot (58.27 mg) and nut fall isolates (57.00 mg) followed by L-Asparagine. The requirement for Nitrogen sources differ with species of *Phytophthora* (Hohl, 1983). Isolates of *P. infestans* varied in their growth requirements for inorganic nitrate, Ammonium and amino acids (Hohl, 1983). Development of strategies to manage bud rot disease in coconut requires a thorough understanding of the pathogen of the disease. So the current study will help to evolve better management practices to combat the bud rot disease.

**Table 4.** Utilization of nitrogen sources by *P. palmivora*

Type	Nitrogen source	Dry weight of mycelium (mg)		
		Nut-isolate	Bud-rot isolate	Mean
Inorganic	Ammonium nitrate	59.13	58.23	58.68
	Ammonium sulphate	49.11	48.83	48.97
	Potassium nitrate	46.27	46.45	46.36
	Ammonium chloride	42.65	41.96	42.31
	Calcium nitrate	33.13	32.83	32.98
	Sodium nitrate	43.65	42.86	43.26
	L - Proline	36.24	35.39	35.81
	L - Isoleucine	15.34	14.12	14.73
	L - Alanine	44.10	43.36	43.73
	L - Cysteine	22.54	21.90	22.22
Organic	L - Tyrosine	15.86	15.70	15.78
	L - Histidine	35.12	34.86	34.99
	L -Asparagine	53.33	53.66	53.50
	Glutamine	57.00	58.27	57.63
	L - Phenylalanine	26.68	25.23	25.94
	L- Lysine	24.03	22.96	23.50
	Glycine	36.29	34.40	35.34
	Control	0.54	0.54	0.54

C.V% = 2.37  
CD (P = 0.05) for nitrogen sources = 0.96

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