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Characterization of *Phytophthora colocasiae* Raci. Isolates Causing Blight of Colocasia in North-Western Himalayas

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***Abstract*:** Leaf blight and corm rot incited by *Phytophthora colocasiae* is the most devastating disease of colocasia. Twenty isolates of *P. colocasiae* were isolated from the disease samples collected from five districts of Himachal Pradesh and characterized based on morpho-cultural variability, pathogenic variability, chlamydospore formation and mating type. Among different media tested carrot agar was best medium for mycelial growth and sporulation of *P. colocasiae*. On the basis of morpho-cultural characters and pathogenic variability, twenty isolates were categorised into six and five groups, respectively. Only four isolates formed chlamydospores abundantly under dark condition at pH 6.0 in carrot broth incubated at 18°C. Out of twenty isolates 18 were of A_1 mating type whereas, 2 were of mating type A_2. Maximum oospore formation frequency was on carrot agar media at 25°C and was favored by dark conditions.

Keywords: Colocasia blight, *Phytophthora colocasiae*, Colocasia, Oospore, Chlamydospore, Variability

Colocasia (*Colocasia esculenta* (L.) Schott) is a major root crop belonging to family Araceae grown widely for its edible corms and leaves. Colocasia is an important crop of Asian, Pacific, African, American and Caribbean countries (Rao et al 2010). The crop is attacked by several pathogens but colocasia blight incited by *Phytophthora colocasiae* is the most serious disease causing huge yield losses (Misra et al 2008, Adomako et al 2017, Bhandhari et al 2021). The disease was first reported from Java in 1900 (Raciborski 1900). Its occurrence from India was reported by Butler and Kulkarni (1913) whereas Luthra (1938) reported its occurrence from Kangra Valley of Himachal Pradesh. In India very few studies have been conducted on the variability of *P. coloacsiae* (Misra et al 2011, Nath et al 2015, Padmaja et al 2015). The pathogen is known to persist as sporangia in vegetative parts under unfavourable conditions for a few days to two weeks (Fullerton and Tyson 2003) or overwinters as oospores and chlamydospores in infected plant tissue or soil (Nelson et al 2011). There is need to identify critical to investigate the factors that influence the formation of these structures. Sexual reproduction is the primary cause of variation, which may result in the generation of more virulent phenotypes (Goodwin et al 1995). Since, *P. coloacsiae* is heterothallic, it requires both both A_1 and A_2 mating types to produce oospores. However, there is no information on the mating type distribution of *P. colocasiae* in the North Western Himalayas. Current study aimed to characterize the pathogen population in terms of variability, chlamydospore formation, and mating types.

MATERIAL AND METHODS

Collection, Isolation and identification of the pathogen: Different colocasia growing districts of Himachal Pradesh *i.e.* Kangra, Hamirpur, Bilaspur, Mandi and Sirmaur were surveyed during *kharif* 2019 and the leaves showing characteristic colocasia blight symptoms were collected, placed in paper bags and brought to the laboratory (Table 1). The pathogen was isolated from diseased colocasia leaves on potato dextrose agar (PDA) medium. Bits of 2-3 mm size having half portion of diseased and healthy tissue were cut with the help of a sterilized surgical blade. These bits were surface sterilized by dipping in 1 per cent sodium hypochlorite solution for 10-15 seconds and were subsequently washed 3-4 times with sterilized distilled water under laminar air flow. The bits were then placed between two folds of sterilized blotting sheets to remove excess moisture and transferred to PDA Petri plates under aseptic conditions and incubated at 24±1°C in Bio-oxygen demand (BOD) incubator. The culture was purified by hyphal tip method. The identity of the pathogen associated with colocasia blight was established by studying the morpho-cultural traits for the pathogen culture raised on PDA slants by following standard keys (Waterhouse 1963). All isolates were maintained by periodical sub-culturing and after third sub-culture each isolate was inoculated on healthy host and then re-isolated to avoid loss of virulence.

Pathogenicity test: Pathogenicity test was conducted on a Green Stalked variety of colocasia both under net house and laboratory condition as a whole plant assay and detached

leaf method, respectively. Inoculum prepared from ten days old culture with zoospore concentration was adjusted to 6×10^4 spore per ml with the help of hemocytometer was sprayed using hand atomizer. As colocasia have waxy leaves tween 20 (0.5%) was added to the inoculum to prevent inoculum run off.

Whole plant bioassay: Colocasia plant at 3-5 leaf stage was sprayed with test pathogen inoculum. After inoculation pots were covered with polyethylene bags and sprayed three times a day with water for three consecutive days to maintain high relative humidity.

Detached leaf assay: Healthy freshly plucked colocasia leaves were placed in Petri plates bedded with cotton and surfaced with moist blotting sheet. Pathogen inoculum was sprayed on leaves and incubated at 24±1°C. The Petri plates were maintained moist by spraying sterilized distilled water at periodical interval of 6-8 hrs to maintain high relative humidity (>90%). Leaves were periodically observed for initiation of symptoms. The experiment was repeated four times for accuracy.

Evaluation of culture media: Five solid media *viz*., potato dextrose agar (PDA), V-8 juice agar (V8), carrot agar (CA), oat meal agar (OMA) and lima bean agar (LBA) were tested to find the best medium for the growth and sporulation of *P. colocasiae*. Media were prepared and sterilized by autoclaving at 1.05 kg/cm^3 (121.6°C) for 20 minutes. Twenty ml of each medium was poured in Petri plates (90 mm), allowed to solidify and inoculated with 5 mm culture discs taken from actively growing margin of 7 days old culture of the *P. colocasiae*. Each media was quadruplicated and incubated at 24±1°C. Radial growth of the obtained colonies was recorded after 3, 5 and 7 days after inoculation (dai). Sporulation was observed on each medium 10 days after inoculation and the number of spores was counted with the help of hemocytometer.

Variability Studies

Cultural variability: Twenty of *P. colocasiae* isolates collected from different areas were used to study cultural variability. Cultural characters of each isolate were studied on CA media. Mycelial bits of 5 mm diameter were cut with the help of cork borer from the margins of the actively growing colony and placed in the centre of the media plates. Inoculated plates were then incubated at 24±1°C in BOD incubator. Each isolate was replicated thrice. Observations were recorded on colony diameter, colour, shape and type of growth seven days after inoculation.

Morphological variability: For morphological studies, mycelial bits of each isolate were taken from seven days old culture. Morphological characteristics such as shape of sporangia, position of sporangia on sporangiophore,

Table 1. *Phytophthora colocasiae* isolates collected from different locations of Himachal Pradesh

District	Location	Variety	Isolate
Kangra	Palampur (32.1109°N, 76.5363°E)	Local	Pc-1
	Palampur (32.1109°N, 76.5363°E)	Sarkaghat Local	Pc-2
	Palampur (32.1109°N, 76.5363°E)	Una Gagret	Pc-3
	Palampur (32.1109°N, 76.5363°E)	Kangra Malan	Pc-4
	Palampur (32.1109°N, 76.5363°E)	Sirmour Nahan	Pc-5
	Palampur (32.1109°N, 76.5363°E)	Ponta Sahib	Pc-6
	Gaggal (32.1586°N 76.2063°E)	Local	Pc-7
	Rakkar (32.1992°N 76.3677°E)	Local	Pc-8
	Baijnath (32.0521°N, 76.6493°E)	Local	Pc-9
Mandi	Joginder Nagar (31.9912°N, 76.7899°E)	Local	Pc-10
	Bharol (31.9341° N, 76.7055° E)	Local	Pc-11
	Bir (32.0456° N, 76.7236° E)	Local	Pc-12
	Ropru (31.7196°N, 76.8519°E)	Local	Pc-13
	Balotu (=32.0053°N 76.4405°E)	Local	Pc-14
Hamirpur	Nadaun (31.7785°N, 76.3445°E)	Local	Pc-15
	Barsar (31.5255°N, 76.4606°E)	Local	Pc-16
	Kohli (*31.7524*°N, *76.34292*°E)	Local	Pc-17
Bilaspur	Barla (31.5480°N, 76.5336°E)	Local	Pc-18
	Ghumarwin (31.4491°N, 76.7048°E)	Local	Pc-19
Sirmaur	Dhaula Kuan (30.4620° N, 77.4817° E)	Local	Pc-20

presence or absence of papillae were recorded. Data on dimensions of sporangia *i.e.* length, breadth and pedicel length were taken by examining 25 randomly chosen sporangia under compound microscope using stage and ocular meter. Length breadth ratio (L:B ratio) was also calculated.

Size (µm) = Number of ocular division × Calibration factor

Where

$$\text{Calibration factor} = \frac{\text{Number of ocular division}}{\text{Number of division of ocular}} \times 100$$

Pathogenic variability: Pathogenic variability among all the isolates was determined by detached leaf method (Dohroo et al 2012). Colocasia leaves of same age group were collected from plants of three local colocasia strains *viz*., Violet Stalked, Green Stalked collected from Palampur and Sarkaghat Local collected from Sarkaghat. Leaves were cut to 4 cm length and placed on moist blotting sheet kept in Petri plates. Spore suspension of 10 µl was inoculated on each leaf disc for each isolate. Leaf disc inoculated with distilled water served as control for each variety and three replications were kept for each treatment. The inoculated leaves were incubated at a temperature of 24±1°C. The leaves were kept moist and inspected daily for the development of symptoms. Subsequently, data on the incubation period and latent period were recorded. The disease score was also calculated using a 0–6-point scale (Little and Hills 1978). Colocasia strains with a disease score between 0 and 2 were categorized as resistant, whereas those with a disease score between 3 and 6 were regarded as susceptible. Further, different isolates were classified as pathotypes based on either of the two reaction types *i.e.* either resistant (R) or susceptible (S) assigned.

Chlamydospore formation: All the isolates of *P. colocasiae* were cultured on modified cleared V-8 $CaCO_3$ liquid medium under submerged condition (Tsao 1971). Mycelial agar plug (1 cm diameter) of each isolate from actively growing margins of 7 days old culture was transferred to a conical flask (250 ml) containing 25 ml cleared V-8 $CaCO_3$ liquid medium. Two replications were kept for each isolate. The flasks were then incubated vertically at 25°C in darkness for 24 hrs and were shaken to fragment hyphae grown out of inoculum plug and incubated further under same conditions. After 6 days, 100 ml of sterile distilled water was added to each flask, mycelial mat sank to bottom of liquid within few seconds and were further incubated vertically at 18°C in darkness for 10 weeks. Chlamydospores were examined for its formation under light microscope, four samples were taken from each isolate randomly. Fifteen arbitrarily selected chlamydospores were measured for diameter and wall thickness for each isolate showing chlamydospore formation.

Mating type: Isolates were paired in order to determine their mating types. Firstly, isolates from different geographic locations were paired to determine A_1 and A_2 mating types. The identified A_1 and A_2 mating types were used as a tester. The type cultures of A_1 and A_2 were paired individually with other test isolates. The mycelial plugs (5 mm diameter) from actively growing margins of 7 days old culture were placed 3 cm apart on carrot agar media at 24±1°C in darkness for 2 weeks. Absence of oospore at the interface of two isolates indicated same mating type and *vice-versa*. Positive control was a cross between two testers. Three replications were kept for each paired culture. Diameter of oospores was measured.

Factors effecting survival spore formation: The isolate that produced abundant survival spore (chlamydospores and oospores) was used to evaluate the effect of different factors *viz*., culture media, light regimes, temperatures and pH. Chlamydospore and oospore formation were evaluated at different temperatures, *viz.,* 15°C, 18°C, 20°C, 24°C, and 30°C. Each experiment was replicated four times. Different liquid media (potato dextrose broth, V-8 broth, carrot broth, oat meal broth, and lima bean broth) for chlamydospore formation and solid media for oospore development were incubated at three different light regimes, *i.e.* light, dark, and intermittent light and dark at 18°C and 24°C, respectively for 10 weeks. Effect of pH on chlamydospore formation was also evaluated at three different pH (6, 7 and 8), which were replicated five times. In each experiment, the total spore formed were recorded by counting the number of spores visualized per microscopic field at 10X.

Statistical analysis: All data obtained were subjected to statistical analysis using OPSTAT software at 5% level of significance. Before analysis the data was transformed wherever found necessary.

RESULTS AND DISCUSSION

Isolation of pathogen and pathogenicity test: Twenty isolates of pathogen were established as pure culture and identified as *Phytophthora colocasiae* Raci. on the basis of morphological characteristics (Fig.2a). Pathogenicity was proved on Green Stalked variety of colocasia with pure culture of all the isolates and maintained for further studies. Symptoms of the disease began as small light brown water-soaked lesion which enlarged rapidly to form large dark brown lesions. Characteristic symptoms of the disease were produced after third and fourth days of inoculation, respectively under laboratory (Fig. 2b) and net house conditions (Fig. 2c). Isolates from different geographical locations were designated as “Pc-1 to Pc-20” (Fig. 1; Table 1).

Evaluation of culture media: CA medium supported the

maximum mycelial growth and sporulation of the pathogen (Fig 2d; Fig. 3). Padmaja et al (2015) have also reported that CA medium supported maximum growth in comparison to PDA and other tested media. Tsopmbeng et al (2012) did not find PDA as the best medium for the mycelial growth and sporulation of *P. colocasiae*. As the CA medium was determined to be the best, it was utilised in subsequent studies.

Cultural variability: Colony colour of this oomycetous pathogen was observed either white or dull white with circular or irregular colony shape. Majority *i.e.* fourteen isolates showed circular colony while six showed irregular colony. Out of 20 isolates, thirteen produced white colonies and seven showed dull white colony colour. On the basis of mycelial growth pattern, the pathogen isolates were categorised into six groups *viz*., cottony with concentric ring, cottony with dense mycelial growth, cottony with sparse mycelial growth, regular colony, stellate colony and rosaceous colony (Table 2). Maximum growth (mm per day) was observed in Pc-2 (11.3 mm per day) and minimum in Pc-15 (6.7 mm per day). Growth per day also differed within morphological groups. Misra et al (2011) divided *P. colocasiae* isolates to four morphological groups on the basis of mycelial growth namely cottony, petaloid, rosaceous and stellate. However, Nath et al (2015) also characterized *P. colocasiae* isolates into different morphological groups on the basis of colony appearance on potato dextrose agar medium namely cottony, stellate, cottony with concentric rings, plain with irregular concentric rings, irregular pattern, plain, uniform with concentric rings, uniform without pattern and flat with concentric rings. Adomako et al (2018) divided isolates into four morpho-group (MG1 to MG4) on the basis of colony growth pattern *viz*., stellate, petal shaped, sparse cottony and uniform cottony type.

Morphological variability: Sporangia in all isolates were semi-papillate produced terminally with mean pedicel length ranged from 3.5-8.7 µm and ovoid shape sporangia in majority of isolates except few (Pc-3, Pc-10, Pc-12, and Pc-14) which had globose shape. The mean sporangial length and breadth varied from 32-80 µm and 19-47.5 µm, respectively while mean L:B ratio ranged from 1.6-2.2 (Table 1). Isolates Pc-3 and Pc-8 exhibited maximum average length while Pc-10 and Pc-18 had minimum average length. Bandyopadhyay et al (2011) observed sporangia of *P. colocasiae* to be hyaline, papillate and measuring 25 to 55 µm and 15 to 30 µm in length and breadth, respectively. Omane et al (2012) reported that sporangia were ovoid, hyaline, papillate, caducous, 30 to 60 µm × 17 to 28 µm in size having 3.5 to 10 µm pedicel length. Misra et al (2011) mentioned that sporangia of all isolates were semi-papillate and caducous on long pedicel with mean length and breadth of 38 to 60 µm and 18 to 36 µm, respectively. Length breadth ratio ranged from 1.4 to 1.6 and pedicel length from 40-70 µm. Nath et al (2015) observed that sporangia shape of all fifty isolates of *P. colocasiae* varied from ovoid to ellipsoidal with an average length of 32.7 to 52.7 µm and average width of 18.6 to 32.2 µm. Adomako et al (2018) observed sporangium shape of isolates varied from ovoid to globose. However, in the present investigation sporangia of Pc-1, Pc-3, Pc-4, Pc-6, Pc-7, Pc-8, Pc-13, Pc-14, Pc-15 and Pc-19 isolates were exceptionally bigger than reported earlier in literature while sporangia of isolates Pc-10 and Pc-18 were smaller in size. These variations may be attributed to geographical adaptation or indicate speciation. On the basis of morpho-cultural variability 20 isolates were categorized into six morpho groups (Table 4).

Pathogenic variability: The pathogenic behavior of twenty isolates was tested on three local varieties of colocasia *i.e.* Violet Stalked, Green Stalked and Sarkaghat Local by detached leaf method. Most isolates showed lesion initiation 2-3 days after inoculation at 24±1°C (Fig. 5). Initially the inoculated leaves showed yellowish to light brown water-soaked lesions which later turned into dark brown at site of infection, white sporulation of the pathogen was often visible on the lesion after 4-5 days of incubation. The disease severity was calculated in terms disease score and disease reaction was assigned to all the isolates on different varieties (Table 5).

On the basis of disease reaction, various isolates were categorized into five pathogroups *viz*., PcPG1 to PcPG5 (Table 4). Pathogroup PcPG2 was the most virulent and was able to infect all the three colocasia varieties, whereas pathogroup PcPG4 was least virulent. In general, for majority of the isolates of different pathogroup did not show any correlation with the morpho-cultural group but isolate Pc-2 is distinct (PcPG2) and showing relation with the morpho-cultural group (PcG2) also. Nath et al (2015) studied the pathogenic variability of *P. colocasiae* isolates on single susceptible variety and found that majority of isolates had incubation period of 2-3 days and there was no correlation between results obtained from the virulence assay and morphology or geographical origin of the isolates. Adomako et al (2017) studied aggressiveness of *P. colocasiae* associated with colocasia blight in Ghana and reported that mean incubation period of the isolates ranged from 2 to 4 days, irrespective of the morpho-group.

Chlamydospore formation Variable chlamydospore formation was observed in *P. colocasiae*. Out of twenty isolates tested, only four isolates produced chlamydospores (Table 6). Chlamydospores were circular, single, hyaline to pale yellow in colour and were formed both terminally and

Table 2. Morpho-cultural variability of *Phytophthora colocasiae*

Isolate	Cultural characteristics				Morphological characteristics				
	Shape of colony	Mycelial growth pattern	Colony colour	Colony diameter (mm/day)	Shape of sporangia	Length	Breadth	L:B ratio	Pedicel length
Pc-1	Circular	Regular with sparse mycelial growth	Dull-white	8.0	Ovoid	73.0 (62-90.4)	39 (32-46.2)	1.8	3.5 (3.8-6.6)
Pc-2	Circular	Slightly stellate with dense mycelial growth	Dull-white	11.3	Ovoid	61.3 (46.2-77)	32 (15.4-38.5)	1.9	5.1 (4.4-9.6)
Pc-3	Circular	Regular with sparse mycelial growth	Dull-white	8.3	Globose	80.0 (70-85)	47 (40-55)	1.6	4.0 (3.5-6.1)
Pc-4	Circular	Cottony with dense mycelial growth	White	7.4	Ovoid	72.0 (75-85)	36 (29.1-46)	2.0	7.7 (5.9-6.7)
Pc-5	Circular	Cottony with dense mycelial growth	White	7.2	Ovoid	65.0 (55-89)	33.8 (28-37)	1.9	6.3 (5.4-6.7)
Pc-6	Irregular	Cottony with sparse mycelial growth	White	7.5	Ovoid	77.0 (75-87)	39 (27-45)	1.9	6.3 (5.7-7.9)
Pc-7	Circular	Regular with sparse mycelial growth	Dull-white	7.9	Ovoid	75.0 (60-92)	42 (30-45)	1.8	4.2 (4-5.4)
Pc-8	Circular	Cottony with dense mycelial growth	White	7.1	Ovoid	80.0 (57-87)	36.4 (32-45)	2.2	4.3 (3.8-4.9)
Pc-9	Circular	Rosaceous with dense mycelial growth	Dull-white	8.9	Ovoid	45.2 (30-54)	25.0 (20-35)	1.8	8.2 (6-8.7)
Pc-10	Irregular	Cottony with sparse mycelial growth	White	7.1	Globose	32.0 (23-35)	20.0 (18.4-23)	1.6	5.5 (4.5-6.9)
Pc-11	Irregular	Cottony with sparse mycelial growth	White	7.8	Ovoid	55.7 (45-64.4)	29.2 (23-40)	1.8	6.1 (5.2-8.8)
Pc-12	Circular	Regular with sparse mycelial growth	Dull-white	8.1	Globose	62.0 (60-85)	38.0 (32-40)	1.6	3.5 (3.3-5.5)
Pc-13	Irregular	Cottony with sparse mycelial growth	White	7.2	Ovoid	79.0 (67-99)	46.0 (39-50)	1.7	5.9 (3.3-6.6)
Pc-14	Irregular	Cottony with sparse mycelial growth	White	7.0	Globose	78.7 (65-95)	47.5 (40-55)	1.6	5.7 (4.3-6.9)
Pc-15	Irregular	Cottony with sparse mycelial growth	White	6.7	Ovoid	77.0 (61-92.4)	43.2 (30.8-46.2)	1.8	5.4 (3.7-5.9)
Pc-16	Circular	Cottony with concentric ring	White	7.6	Ovoid	59.4 (48-70)	27.0 (24-30.8)	2.1	7.1 (6.2-9)
Pc-17	Circular	Cottony with dense mycelial growth	White	7.1	Ovoid	57.0 (48-69)	25.0 (24-31)	2.2	5.5 (4-6.5)
Pc-18	Circular	Rosaceous with dense mycelial growth	Dull-white	8.5	Ovoid	33.0 (23-38)	19.0 (16-27)	1.7	8.7 (7.5-9.9)
Pc-19	Circular	Cottony with dense mycelial growth	White	7.3	Ovoid	73.0 (62-90.4)	31.0 (28-40)	2.0	5.5 (3.9-5.9)
Pc-20	Circular	Cottony with concentric ring	White	7.9	Ovoid	61.0 (46.2-77)	39.0 (35-50)	2.0	7.5 (6.8-9.3)

intercalary in mycelium (Fig. 5a, b). Chlamydospores were formed abundantly in isolate Pc-18, Pc-19 and Pc-20 while rarely in Pc-15. The diameter of chlamydospores ranged from 26.3-30 µm while wall thickness of chlamydospores ranged from 1.9-2.8 µm. On the basis of chlamydospore formation twenty isolates were divided into 2 groups *i.e.*

Table 3. Grouping of *Phytophthora colocasiae* isolates on the basis of morpho-cultural characteristics

Group	Isolates	Morpho-cultural characteristics
PcG1	Pc-1, Pc-3, Pc-7 and Pc-12	Circular, dull-white colonies with regular sparse mycelial growth pattern. The L:B ratio of the isolates ranged from 1.6-1.8, sporangial length and breadth ranging from 60-92 and 30-55 µm, respectively and pedicel length varied from 3.3 to 6.6 µm. Colony growth per day ranged from 7.9 to 8.3 mm.
PcG2	Pc-2	Circular, dull-white colonies with slightly stellate dense mycelial growth pattern. The L:B ratio of isolate is 1.9, sporangial length and breadth ranging from 46.2-77 and 15.4-38.5 µm, respectively with pedicel length varied 4.4 to 9.6 µm. Colony growth per day was 11.3 mm.
PcG3	Pc-4, Pc-5, Pc-8, Pc-17 and Pc-19	Circular, white colonies with cottony dense mycelial growth pattern. The L:B ratio of isolates ranged from 1.9-2.2 sporangial length and breadth of 48-90.4 and 24-46 µm, respectively with pedicel length varied from 3.8-6.7 µm. Colony growth per day was 7.1-7.4 mm.
PcG4	Pc-6, Pc-10, Pc-11, Pc-13, Pc-14 and Pc-15	Irregular, white colonies with cottony sparse mycelial growth pattern. The L:B ratio of the isolates ranged from 1.6-1.9, sporangial length and breadth of 23-99 and 18.4-55 µm, respectively with pedicel length varied from 3.3-8.8 µm. Colony growth per day was 6.7-7.8 mm.
PcG5	Pc-9 and Pc-18	Circular, dull white colonies with rosaceous dense mycelial growth pattern. The L:B ratio of the isolates ranged from 1.7-1.8, sporangial length and breadth of 23-54 and 16-35 µm, respectively with pedicel length varied from 6-9.9 µm. Colony growth per day was 8.5-8.9 mm.
PcG6	Pc-16 and Pc-20	Circular, white colonies with cottony concentric ring mycelial growth pattern. The L:B ratio of isolates ranged from 2.0-2.1, sporangial length and breadth of 46.2-77 and 24-50 µm, respectively with pedicel length varied from 6.2-9.3 µm. Colony growth per day was 7.6-7.9 mm.

Table 4. Disease reaction of different isolates of *Phytophthora colocasiae* on local strains of colocasia

Isolate	Violet stalked				Green stalked				Sarkaghat local					
	Incubation period*	Latent period	Disease score	Disease reaction	Incubation period	Latent period	Disease score	Disease reaction	Incubation period	Latent period	Incubation period	Latent period	Disease score	Disease reaction
Pc-1	4	5	2	R	3	4	3	S	-	-	-	-	0	R
Pc-2	2	3	3	S	2	3	4	S	3	4	3	4	3	S
Pc-3	-	-	0	R	2	4	3	S	-	-	-	-	0	R
Pc-4	3	4	3	S	2	3	3	S	-	-	-	-	0	R
Pc-5	3	4	3	S	2	3	3	S	-	-	-	-	0	R
Pc-6	4	5	2	R	3	4	2	R	-	-	-	-	0	R
Pc-7	4	5	3	S	4	5	3	S	-	-	-	-	0	R
Pc-8	3	5	3	S	2	4	4	S	-	-	-	-	0	R
Pc-9	3	4	3	S	2	3	4	S	-	-	-	-	0	R
Pc-10	2	3	3	S	2	3	4	S	-	-	-	-	0	R
Pc-11	2	3	4	S	2	3	4	S	-	-	-	-	0	R
Pc-12	2	3	3	S	2	4	3	S	-	-	-	-	0	R
Pc-13	3	5	2	R	2	4	3	S	-	-	-	-	0	R
Pc-14	3	5	2	R	2	4	3	S	-	-	-	-	0	R
Pc-15	2	4	3	S	3	4	4	S	-	-	-	-	0	R
Pc-16	2	4	3	S	2	3	3	S	-	-	-	-	0	R
Pc-17	3	5	3	S	3	5	3	S	-	-	-	-	0	R
Pc-18	3	4	3	S	3	4	3	S	-	-	-	-	0	R
Pc-19	3	4	3	S	3	4	4	S	-	-	-	-	0	R
Pc-20	3	5	3	S	4	5	2	R	-	-	-	-	0	R

* Incubation period and latent period are measured in dai (days after inoculation)

PcC1 include 4 isolates which produced chlamydospore and PcC2 include 16 isolates which did not produce chlamydospore. Misra et al (2011) reported chlamydospores formation in *P. colocasiae.* Out of 14 isolates, 6 isolates were able to produce chlamydospores. Chlamydospores were single, terminal or intercalary and pale yellow in colour with diameter ranging from 18.0 to 34 µm and wall thickness of chlamydospore ranged from 2.0 to 2.5 µm.

Mating type: Mating types were determined for all 20 isolates by pairing isolates in different combination and the cross combination in which oospore were of opposite mating type. Solo cultures were used as control. Combination which formed oospore *i.e.* Pc-18 and Pc-20 and were therefore used as tester. Oospores were amphigynous (Fig. 5c, d) with mean oospore diameter ranging from 23.2 to 29.1 µm. As the oospore diameter of Pc-18 was greater than Pc-20 so, Pc-18 was considered to be A_1 and Pc-20 was considered to be A_2 mating type. These mating types obtained were then crossed separately with other 19 isolates to determine their mating type on carrot agar media. A_1 and A_2 mating types identified as represented in table 6. On the basis of oospore formation isolates were divided into two mating types A_1 types (Pc-1, Pc-2, Pc-3, Pc-4, Pc-5, Pc-6, Pc-7, Pc-8, Pc-10, Pc-11, Pc-12, Pc-13, Pc-14, Pc-16, Pc-17, Pc-18 and Pc-19) and A_2 mating type (Pc-9 and Pc-20). Tyson et al (2007) concluded that out of 54 isolates collected from pacific region, India and South East Asia only 40 isolates were A_2 mating type and 14 did not form oospore with either mating type thus no A_1 or self-fertile isolates were found. Misra et al (2011) reported that all 14 isolates they collected were heterothallic and twelve out which were A_1 mating type while only two isolates were A_2 mating type. Mean diameter of oospores ranged from 18.1 to 30.4 µm and diameter of A_2 mating type isolates were lesser than A_1 mating type. Lin et al (2014) concluded that all 218 isolates collected from islands of Oahu, Kavai, Molokai and Big Island of Hawaii were of A_2 mating type. Mellow et al (2018) reported in Upolu, Samoa that out of 54 isolates 50 isolates were found to be of A_2 and 4 of A_0 type and none of A_1 type when paired with tester *P. nicotianae*.

Factors effecting survival spore formation: The effect of different growth conditions on chlamydospore formation was studied by using isolate Pc-19, a prolific chlamydospore producer among all the isolates. The maximum number of chlamydospores and oospores formation was observed at 18°C and 25°C, respectively (Fig. 6). Among the different media, the maximum number of chlamydospores were formed in carrot broth, followed by oat meal broth, V-8 broth, lima bean broth, and potato dextrose. Among the different media, the maximum number of chlamydospores were formed in carrot broth, followed by oat meal broth, V-8 broth, lima bean broth, and potato dextrose broth. Maximum chlamydospores per microscopic field were recorded in dark conditions, followed by light conditions and intermittent light and dark regimes, and this was the same for each medium. Likewise, maximum oospore formation was recorded in CA medium under dark condition and minimum oospore formation was recorded in PDA medium at intermittent dark and light regimes (Table 8). The highest and lowest numbers of chlamydospore formation were observed at pH 6 and 8, respectively (Fig. 7).

Table 5. Grouping of *Phytophthora colocasiae* isolates on the basis of disease reaction on three colocasia lines

Isolate	Violet stalked	Green stalked	Sarkaghat local	Pathogroups
Pc-1, Pc-3, Pc-13 & Pc-14	R	S	R	PcPG1
Pc-2	S	S	S	PcPG2
Pc-4, Pc-5, Pc-7, Pc-8, Pc-9, Pc-10, Pc 11, Pc-12, Pc-15, Pc-16, Pc-17, Pc-18 & Pc-19	S	S	R	PcPG3
Pc-6	R	R	R	PcPG4
Pc-20	S	R	R	PcPG5

Table 6. Chlamydospore formation in different isolates of *Phytophthora colocasiae*

Isolate	Chlamydospore formation	Diameter (µm)*	Wall thickness (µm)*
Pc-1 to Pc-14, Pc-16 & Pc-17	-	-	-
Pc-15	+	27.0 (26.3-28.1)	2.3 (2.1-2.3)
Pc-18	+	28.0 (27.3-29.6)	2.1 (1.9-2.2)
Pc-19	+	28.0 (27.1-28.7)	2.2 (2-2.4)
Pc-20	+	29.0 (27.8-30)	2.5 (2.3-2.8)

*Average of 15 chlamydospore
+ Chlamydospore formed
- Chlamydospore not formed

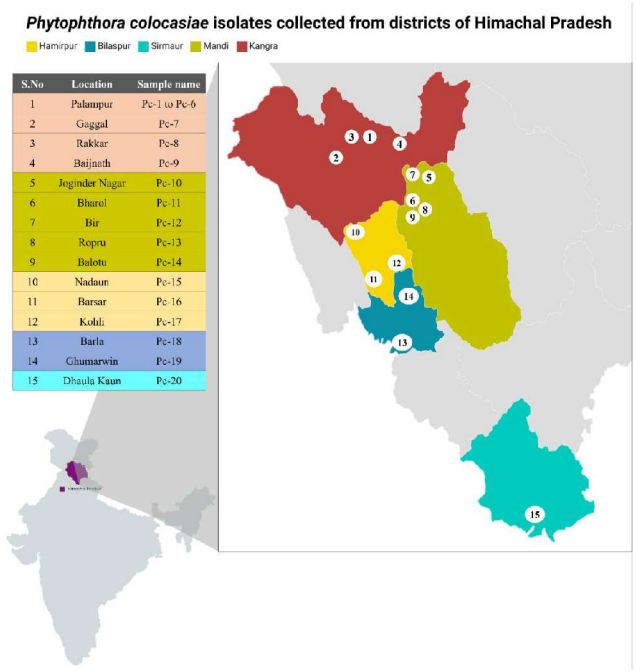


Fig. 1. *Phytophthora colocasiae* isolates collected from different districts of Himachal Pradesh

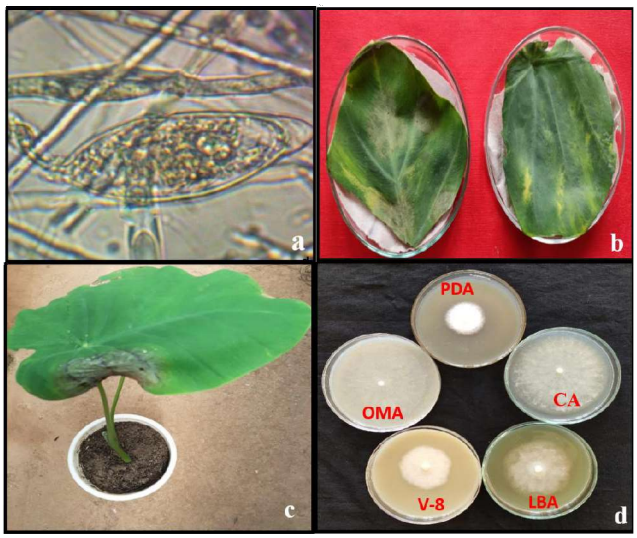


Fig. 2. Pathogenicity test and media study of *Phytophthora colocasiae* a) Hyaline, semi-papillate sporangia of *Phytophthora colocasiae* b) Pathogenicity test of *Phytophthora colocasiae* in laboratory by detached leaf method and c) under net house condition d) Mycelial growth on different media

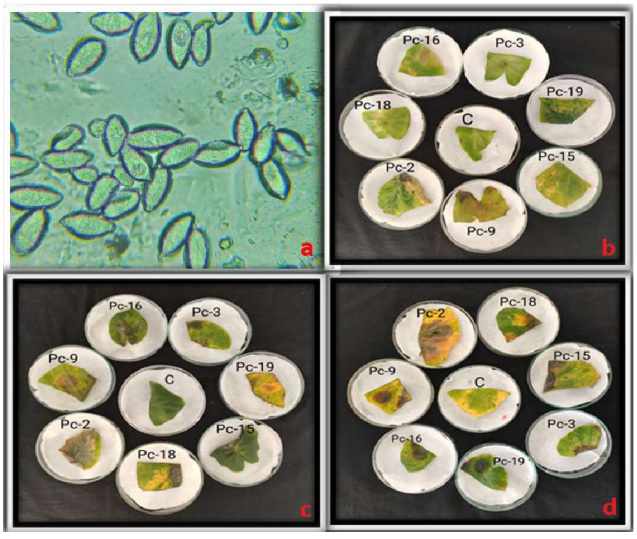


Fig. 4. Pathogenic variability of *Phytophthora colocasiae* a) Microscopic view of sporulation on infected diseased leaves b) Pathogenic variability of isolate Pc-2, Pc-18, Pc-15, Pc-3, Pc-19, Pc-16 and Pc-19 on Sarkaghat Local, c) Green Stalked and d) Violet Stalked colocasia strains

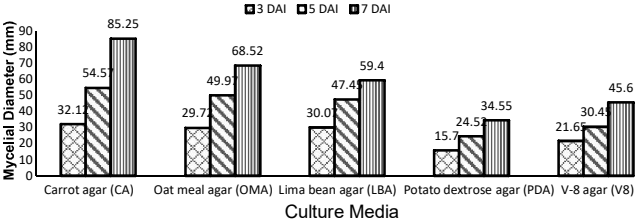


Fig. 3a. Effect of different media on mycelial growth of *Phytophthora colocasiae*

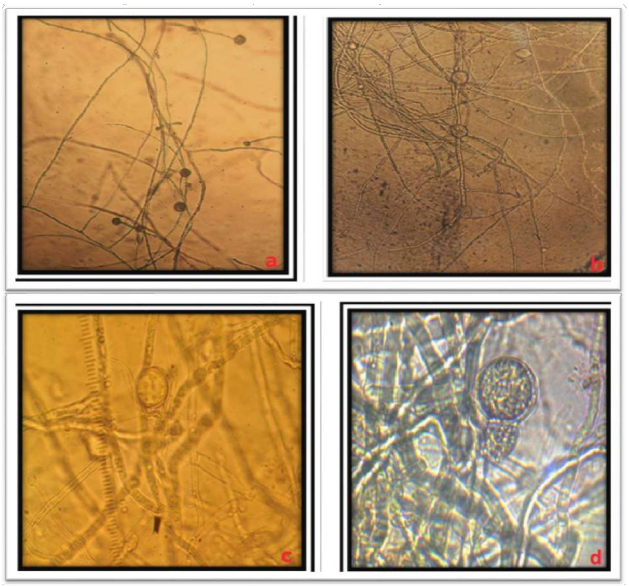
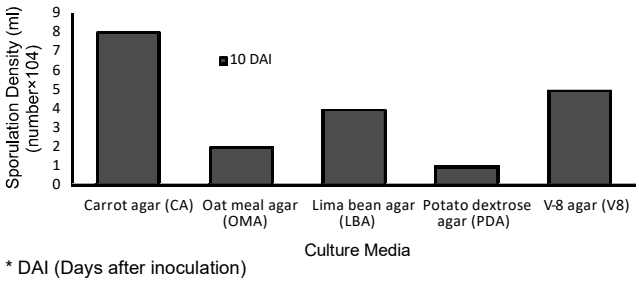


Fig. 5. Chlamydospore and oospore of *Phytophthora colocasiae* Chlamydospore of a) Pc-18 and b) Pc-19 c, d) Oospore under 40X microscopic field



* DAI (Days after inoculation)

Fig. 3b. Effect of different media on mycelial growth and sporulation of *Phytophthora colocasiae*

Table 7. Characterization of mating type of different isolates of *Phytophthora colocasiae*

Isolate	Mating type	Oospore diameter (µm)*
Pc-1	A_1	28.3 (27-29.5)
Pc-2	A_1	28.0 (26-28.7)
Pc-3	A_1	29.0 (27.2-30)
Pc-4	A_1	28.4 (26.5-29)
Pc-5	A_1	27.8 (26.4-28)
Pc-6	A_1	28.0 (25-28.3)
Pc-7	A_1	27.5 (26.1-27.6)
Pc-8	A_1	29.1 (25.6-30)
Pc-9	A_2	23.5 (22-24)
Pc-10	A_1	28.2 (25-28.5)
Pc-11	A_1	29.0 (26-29.4)
Pc-12	A_1	29.0 (25.5-30)
Pc-13	A_1	27.9 (26.3-29.3)
Pc-14	A_1	28.4 (27-29.8)
Pc-15	A_1	28.0 (26-28.3)
Pc-16	A_1	28.1 (27.6-30)
Pc-17	A_1	29.0 (26.8-29)
Pc-18	A_1	29.1 (28.2-30)
Pc-19	A_1	27.8 (27-30)
Pc-20	A_2	23.2 (22.8-25)

*Average of 10 oospore

CONCLUSION

The present study confirms carrot agar as the best medium for mycelial growth and sporulation. It demonstrates that not only the isolates that belonged to different geographical origins but also those obtained from the same area but different colocasia varieties varied with respect to morpho-cultural characters, aggressiveness, and biology. However, some isolates showed few characteristics in common and were classified into six groups on the basis of morpho-cultural variability and five groups on the basis of pathogenic variability. Among the different mycelial growth patterns observed, isolates with a stellate and rosaceous pattern showed faster growth, while isolates showing cottony with sparse and dense mycelium morphology showed slower growth. Isolate Pc-2 was unique with its stellate growth pattern, maximum growth per day, and highest virulence. Twenty per cent of the isolates formed chlamydospores under *in-vitro* conditions, which emphasizes the role of chlamydospores as a survival structure; additionally, their formation was abundant at 18°C, favoring a slightly acidic pH, and in complete darkness. The presence of 18 A_1 and 2 A_2 mating types in Himachal Pradesh highlights the chances of sexual reproduction leading to recombination and hence variability in the pathogen. Oospore formation was seen at its maximum on carrot agar media at 25°C and was favored by dark conditions.

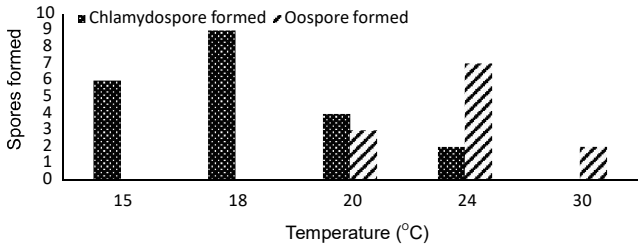


Fig. 6. Effect of temperature on survival spore formation in *Phytophthora colocasiae*

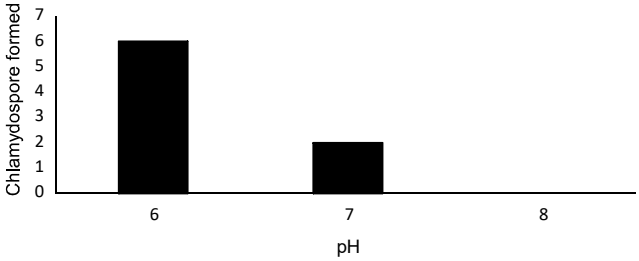


Fig. 7. Effect of pH on chlamydospore formation in *Phytophthora colocasiae*

Table 8. Effect of different media and light regimes on survival spore formation in *Phytophthora colocasiae*

Media	Chlamydospore formation (Broth)			Oospore formation (Solid media)		
	Dark	Light	Intermittent light and dark	Dark	Light	Intermittent light and dark
Potato dextrose	2	1	0	2	0	1
Oat meal	9	8	6	5	1	2
V-8	6	3	2	6	2	4
Carrot	15	6	4	9	5	7
Lima bean	3	1	1	4	0	0
CD (p=0.05)	0.708	0.589	0.708	0.76	0.735	0.589

* Mean of 15 microscopic field at 10X

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