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Genetic Diversity of *Phytophthora colocasiae* Causing Taro Leaf Blight: Analysis Using Start Codon Targeted (SCoT) Polymorphism

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Abstract

The Oomycetous fungus *Phytophthora colocasiae* that causes taro leaf blight is one of the most devastating diseases of taro widely distributed in India. Cultural and molecular techniques were employed for assessing the genetic variability among 30 isolates of *P. colocasiae* obtained from different geographical origins of India. Cultural characters like pathogenicity assay and mating type showed variation among isolates. Eight Start Codon Targeted Polymorphism (SCoT) markers produced 121 reproducible fragments with 100% polymorphism. The average value of the number of observed alleles, the number of effective alleles, mean Nei's genetic diversity and Shannon's information index were 2.00 ± 0.00, 1.58 ± 0.30, 0.34 ± 0.13 and 0.51 ± 0.16, respectively. Analysis of molecular variance (AMOVA) showed that 89% of the diversity was present within population of *P. colocasiae*. Dendrograms based on the molecular data using the unweighted pair group method with arithmetic mean (UPGMA) classified the *P. colocasiae* isolates into two major clusters. Cophenetic correlation coefficient between dendrogram and original similarity matrix were significant for SCoT marker (r = 0.904). The results of this study displayed a high level of genetic variation among the isolates irrespective of the geographical origin. The possible mechanisms and implications of this genetic variation are discussed.

Key words: Taro, *Phytophthora colocasiae*, leaf blight, SCoT marker, genetic diversity, disease management

Introduction

Taro (*Colocasia esculenta* (L.) Schott), a member of the Araceae family, is an important tropical tuber crop used as a staple food or subsistence food by millions of people in the developing countries in Asia, Africa and Central America (Lebot and Aradhya, 1991). In terms of production, it ranks fourteenth among staple crops and fifth among root crops, after potato, cassava, sweet potato and yams. In India, it is grown throughout the country occupying an area of 0.2 m ha with an annual production of 2.0 m t. The corms, leaves and petioles are used as vegetables. The taro plant is a rich source of carbohydrates, proteins, minerals and vitamins and has medicinal properties against tuberculosis, ulcers, pulmonary congestion and fungal infection (Sharma et al., 2008; Misra and Sriram, 2002). Besides the medicinal properties, taro corms are utilized in various industries for the preparation of high fructose syrup and alcohols (Misra et al., 2008).

Taro leaf blight caused by *Phytophthora colocasiae* Raciborski, is the most destructive disease of taro. It

was first reported from Java in 1900 (Raciborski, 1900). Leaf blight has become a limiting factor in all taro growing countries including India causing yield loss of up to 50% (Gollifer and Brown, 1974; Misra and Chowdhury, 1997). The disease is characterized by the formation of brownish water-soaked circular spots on young and mature leaves (Fig. 1). As the infection progresses, the spots enlarge to form patches and, as the disease spreads, the whole leaf rots (Lebot et al., 2003). Under favourable conditions (intermittent rainy weather and temperature around 28°C), *P. colocasiae* is capable of devastating a field in less than two weeks. In India, this disease is more prominent in northern and eastern zones, which are potential areas of taro production. In South India, this disease appears occasionally but in serious proportions (Misra and Chowdhury, 1997). In addition, *P. colocasiae* also causes serious post-harvest decay of corms.

Several strategies are being advocated to combat the disease. Cultural practices like crop rotation is a simple and promising strategy, but may not be practicable because of the longevity of the pathogen in infested soil. Application of metalaxyl based fungicides is another alternative but the presence of waxy coating on the leaf lamina makes it ineffective (Misra, 1999) and uneconomical as large quantities of fungicides and repeated applications are needed. Also, the effect of fungicide treatment is not always consistent and development of resistance to fungicide is another major threat (Cohen and Coffey, 1986; Nath et al., 2012). Although, host resistance is the most economically viable and environmentally sound practice to manage this disease, popularization of resistant cultivars is limited due to lack of other desirable economic and market value traits.

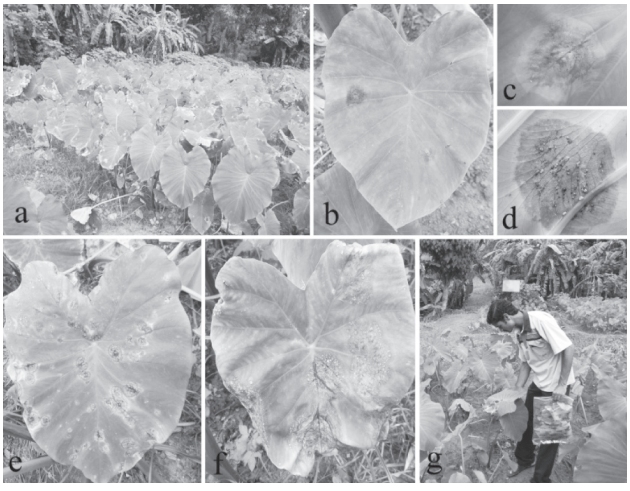

Fig. 1. Symptoms of taro leaf blight disease in India; a. Field view of taro leaf blight; b. Early stage of leaf blight; c. Closer view of the symptom adaxial side; d. Abaxial side; e. At a later stage spots spread across the leaf; f. Coalesce and entire leaf is destroyed; g. First author inspecting and collecting leaf samples

Genetic analysis of plant pathogen population is fundamental to the understanding of the epidemiology, host-pathogen coevolution and resistance management (Milgroom and Fary, 1997). The knowledge of the pathogenic composition of population is essential for efficient management of taro leaf blight and for initiating suitable breeding programs for the development of resistant cultivars of taro. Despite the huge economic loss associated with taro leaf blight, little attention has been paid on the biology of Indian isolates of *P. colocasiae*. Specific studies to evaluate inter and intraspecific genetic variation and to establish the possible pathways by which the pathogen has been introduced and distributed to new areas are quite limited. Molecular markers are useful tools in the analysis of genetic variation in population of phytopathogenic fungi. The advent of the Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990) and Amplified Fragment Length Polyporphism (AFLP) (Vos et al., 1995) markers have permitted the study of the population structure of many organisms that have fewer discernible morphological characters or are otherwise difficult to characterize using more traditional markers (Demeke et al., 1992; Lynch and Milligan, 1994). Variation among *P. colocasiae* isolates in phenotypic characters such as growth rate, colony morphology, metalaxyl resistance and virulence were recognized in old population (Misra et al., 2011). Significant genetic diversity in *P. colocasiae* isolates from Asia and South Pacific regions has been previously described through RAPD and isozyme markers (Lebot et al., 2003). However, the RAPD technique despite its simplicity is largely questioned in literature for its lack of reproducibility and reliability in assessing genetic diversity of phytopathogens. While AFLP proves to be reproducible, it suffers the drawback of being extremely laborious in standardizing the assay for the organism of interest. In quest of a novel marker which is reproducible and easy to perform, we have come across a recently described marker Start Codon Targeted Polymorphism

(SCoT) originally described in plants (Collard and Mackill, 2009).

The objectives of the present study was (1) to evaluate the utility of SCoT markers in assessing genetic diversity of *P. colocasiae* isolates obtained from diverse geographical origins (2) to assess how this genetic diversity is distributed among *P. colocasiae* isolates (3) to examine the genetic differentiation by cluster analysis based on allele frequencies of gene loci. It is expected that the knowledge of the population genetics of *P. colocasiae* may eventually contribute to the development of more durable disease management strategies.

Materials and Methods

Sampling and isolation of pathogen

Leaves and stem tissues from both susceptible and tolerant cultivars of taro showing typical symptoms of blight were collected from various geographical boundaries of India (Table 1). Regions showing a high degree of disease incidence were given preference for sample collection. Plants were kept in between moistened filter paper to minimize tissue deterioration during the transportation of the samples from the survey site to the laboratory.

For *Phytophthora* isolation, plant samples were washed under running tap water to remove surface debris. After washing, tissue segments of 1–2 cm from the dead-live margins of the leaf blight infected area were excised. The segments were sterilized in 70% ethanol for 1 min, followed by 1% sodium hypochlorite for 2 min. The fragments were then rinsed thrice with sterile distilled water. Tissue segments were blotted dry on sterile Whatman filter paper in a laminar flow hood and placed onto *Phytophthora*-selective media, i.e. rye agar amended with 20 mg l^{-1} rifamycin, 200 mg l^{-1} vancomycin, 200 mg l^{-1} ampicillin, 68 mg l^{-1} pentachloronitrobenzene and 50 mg l^{-1} 50% benlate (Erwin and Ribeiro, 1996). Segments were incubated in petri dishes for 4–5 days at 28°C. Hyphal tips from developing *Phytophthora* colonies were transferred to potato dextrose agar medium (PDA: 250 mg l^{-1} potato, 20 mg l^{-1} dextrose and 20 mg l^{-1} agar). Each isolate was stored at -20°C in 50% glycerol (long-term storage) and at 15°C on potato dextrose agar (PDA) slants in the dark (short-term storage). The isolates were identified as *P. colocasiae* based on morphological features like colony morphology and sporangial characteristics by comparing with the authentic isolates of *P. colocasiae* maintained by Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, India.

Pathogenicity assay

For pathogenicity assay, a floating disc method was used (Nath et al., 2013). Five leaf discs (5 x 5 cm) of taro (cv. Sree Kiran, leaf blight susceptible) were floated in sterile distilled water in 200 mm glass petri plates and inoculated with a mycelial disc excised from the margins of actively growing cultures of *P. colocasiae*. Leaf pieces with sterile mycelial plugs served as control treatments. The leaf discs were incubated at 25°C in dark and daily examined for disease symptoms. Subsequently, the lesion diameter was recorded 4 days after inoculation (d.a.i.). Re-isolation was made from all resulting lesions according to Koch's postulate. The assay was repeated twice.

Mating type determination

The mating type of isolates was determined by pairing each unknown isolate with the isolate of a known A1 (98-111) and A2 (98-35a) (IISR culture collection) mating type on carrot agar (CA) medium (250 g l^{-1} carrot juice and 20 g l^{-1} agar) at 5 cm apart. After incubation at 28°C in darkness for 4 weeks, agar blocks were examined microscopically. The absence of oospores at the interface between colonies indicated the same mating type, whereas the presence of oospores indicated opposite mating type. The solo culture of each isolate was examined for oospore formation as a control. The positive control was a cross between two tester isolates of opposite mating types. Three replicates were used for each isolate at similar conditions mentioned previously.

DNA isolation

For DNA isolation, *P. colocasiae* isolates were grown in potato dextrose broth medium (PDB: 250 g l^{-1} potato, 20 g l^{-1} dextrose) at 28°C with 50 rpm. After 5-7 days depending on the growth of the isolate, mycelium was harvested by filtration through cheesecloth, blotted dry with sterile paper towels and used immediately for DNA isolation. DNA was extracted using a Genomic DNA purification kit (Fermentas, EU) according to manufacturer's instructions. The nucleic acid obtained was dissolved in TE buffer (100 μl; pH = 8.0). The DNA was treated with 3 μl of RNase A (10 mg ml^{-1}),

incubated at 37°C for 1 h and stored at -20°C until required for further use.

SCoT analysis

SCoT analysis was performed as described by Collard and Mackill (2009). Eight pre-screened primers that produced distinct, reproducible bands with high polymorphism were selected for the analysis (Table 2). Each 25 μl of PCR reaction consisted of 50 ng of template DNA, 100 μM each deoxynucleotide triphosphate, 20 ng of primer, 1.5 mM $MgCl_2$, 2.5 μl Taq buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl, 0.01 % gelatin), 1 U of Taq DNA polymerase (Merck Genei, Bangalore, India). Standard PCR cycling parameters were used: an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 1 min at 50°C and 2 min at 72°C. The cycles were followed by 5 min at 72°C for final extension. Amplified products were resolved on a 1.8 % agarose gel containing 0.5 μg ml^{-1} ethidium bromide and visualized under UV light. Gel photographs were scanned through a Gel Doc System (Alpha Imager, Alpha Innotech, California, USA). At least two replicates of the amplification assay were performed with template DNA from two different DNA extractions to ensure the consistency of each band.

Data analysis

All clearly detectable bands were scored for their presence (1) or absence (0) by visual observation. In order to ensure credibility only reproducible and well defined bands were scored. Polymorphic and monomorphic bands were determined for each SCoT primer, but only polymorphic bands were included in the analysis. Bands were assumed to be independent, and those of identical size were assumed to have identical sequences. A dendrogram was constructed using genetic similarity matrices to display relationships between isolates using the Nei and Li (1979) distance according to the unweighted pair group mean algorithm using the TREECON software package version 1.3 (Van de peer and Dewachter, 1994). The relative support for the different groups and stability of the dendrogram was assessed by bootstrap analysis (2000 replicates). The cophenetic correlation coefficient was calculated to provide statistical support for the dendrogram obtained and Mantel's test (Mantel, 1967) was performed to check the goodness-of-fit of the cluster analysis of the matrix on which it was based (1000 permutations). When the value of a cophenetic correlation coefficient was ≥ 0.8, the data within a cluster were most likely to be highly reliable (Rohlf, 1993).

The similarity matrix was also used to perform a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al.,1992) by using FAMD Software version 1.25 (Schluter and Harris, 2006). This analysis enables partitioning of the total SCoT variation into within and among geographical region variation components. This provides a measure of inter-region genetic distances as the proportion of the total SCoT variation residing between *P. colocasiae* of any two regions (called Phi statistics).

Allelic frequencies of SCoT marker were used separately to estimate the percentage of polymorphic loci (*P*), observed number of alleles (*NA*), effective number of alleles (*NE*), Nei's gene diversity (*H*) and Shannon index (*I*) using the software POPGENE 32, version 1.31 (Yeh et al., 1997).

Statistical analysis

The data on pathogenicity assay of *P. colocasiae* were analyzed by analysis of variance (ANOVA) and the means were compared by Duncan's Multiple Range Test (DMRT) using SPSS (version17. 0; SPSS, Inc., Chicago, IL, USA). Statistical significance was defined as $P = 0.05$.

Results and Discussion

The current study is a part of a megaproject aimed at investigating phenotypic and molecular diversity of *P. colocasiae* isolates associated with taro leaf blight disease collected from various taro growing regions of India. In the present study, a combination of cultural and molecular techniques was employed to gain insights into the nature of *P. colocasiae* from India. Our results indicate that *P. colocasiae* population in India form an extremely diverse group with a remarkable amount of molecular diversity existing among them.

Isolation of pathogen

During the course of this study (2007 - 2012), a total of 30 isolates of *P. colocasiae* were isolated from diseased samples collected from different regions of India (Table 1). In some instance, the severely infected samples were used as inoculum to produce fresh symptoms on

detached taro leaves to enable successful isolation. Isolation was not successful from rotten or decayed samples.

Pathogenicity assay

The results of the pathogenicity test are summarized in Table 1. The majority of the isolates were able to produce disease symptoms on taro leaf discs. There was a significant difference in the lesion length among the isolates ($P = 0.05$). No lesions were produced on the control leaf discs. The isolates initiated lesion development 2-3 d.a.i. which progressed in a circular fashion from the inoculation point. The inoculated sites showed yellow to brown water-soaked lesions at the beginning which turned dark brown upon the progression of the disease (Fig. 2). Variation in lesion length as observed in this study has also been reported in other *Phytophthora* spp. (Granke et al., 2011; Costamilan et al., 2012) and in other plant pathogens (Baskarathevan et al. 2012; Mahto et al., 2012). The

Table 1. Details of *Phytophthora colocasiae* isolates used in the study along with their place of collection, mating types and pathogenicity lesion diameter

Sl. No.	Isolate code	Location	District/ sampling site	Year of collection	Mating type	Pathogenicity assay (lesion diameter in cm)*
1	P16	Andhra Pradesh	Veerwada	2010	A1	1.26±0.05^{c}
2	P26	Andhra Pradesh	East Godawari	2011	A1	2.60±0.10^{g}
3	P29	Andhra Pradesh	Parudin pallam	2010	A1	1.76±0.05^{e}
4	P27	Andhra Pradesh	Veerwada	2011	A1	2.53±0.05^{g}
5	P19	Assam	Nellie Road	2007	A1	0.00±0.00^{a}
6	P30	Assam	Nellie Road	2010	A2	1.26±0.05^{c}
7	P1	Kerala	Block 2, CTCRI field	2010	A1	1.26±0.05^{c}
8	P3	Kerala	Block 1, CTCRI field	2010	A1	2.20±0.10^{f}
9	P21	Kerala	Farm, CTCRI	2011	A1	3.20±0.10^{i}
10	P9	Kerala	Thiruvananthapuram	2008	A1	0.00±0.00^{a}
11	P4	Kerala	Aleppey	2011	A1	3.86±0.05^{j}
12	P7	Kerala	Pathanamthitta	2011	A1	3.10±0.10^{h}
13	P6	Kerala	Kottayam	2011	A1	3.86±0.05^{j}
14	P23	Kerala	Kollam	2010	A1	1.46±0.05^{d}
15	P15	Kerala	Haripad	2012	A1	4.53±0.05
16	P28	Kerala	Idukki	2010	A1	1.26±0.05^{c}
17	P11	Kerala	Calicut	2007	A2	0.00±0.00^{a}
18	P22	Kerala	Calicut	2008	A1	0.00±0.00^{a}
19	P8	Meghalaya	Ribhoi	2009	A1	0.83±0.05^{b}
20	P20	Meghalaya	Nongpoh	2010	A1	1.46±0.05^{d}
21	P17	New Delhi	New Delhi	2010	A1	0.83±0.05^{b}
22	P5	Odisha	Nayagarh	2007	A1	0.00±0.00^{a}
23	P12	Odisha	Khandapara	2007	A1	0.00±0.00^{a}
24	P2	Odisha	Regional Centre, CTCRI	2008	A1	0.00±0.00^{a}
25	P13	Odisha	Salepur	2008	A1	0.83±0.05^{b}
26	P24	Odisha	Puri	2007	A1	0.00±0.00^{a}
27	P25	Odisha	Puri	2007	A1	0.00±0.00^{a}
28	P14	Odisha	Malikpur	2007	A1	0.00±0.00^{a}
29	P18	Tripura	West Tripura	2010	A1	2.20±0.10^{f}
30	P10	West Bengal	Nadia	2009	A1	1.76±0.05^{e}

*Values with the same superscript in a colomn are not significantly different

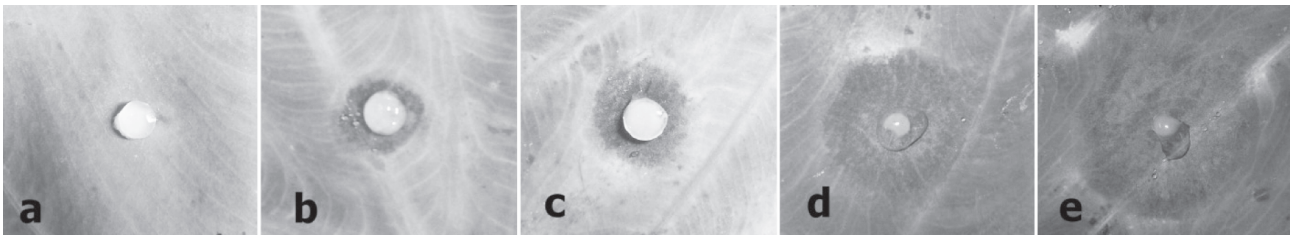

Fig. 2. Pathogenicity assay on taro leaf discs; a. Control leaf disc; b. 2 d.a.i; c. 3 d.a.i; d. 4 d.a.i; e. 5 d.a.i.

variable lesion lengths produced by the *P. colocasiae* isolates reflects the high degree of genetic diversity present among them. It is worth commenting that previous study by Misra et al. (2011) have shown isolates of *P. colocasiae* from Kerala to be less pathogenic, but in our study they were equally aggressive as those from other parts of the country. It can be said that the results of previous studies may be skewed by the low number of isolates used for the study. Nevertheless, an increase in aggressiveness of the isolates should be treated with caution as this indirectly suggests the evolutionary potential of the isolates.

Determination of mating type

The majority of the isolates (28) of *P. colocasiae* tested were of A1 mating type. Only two isolates were found to be of A2 mating type (Table 1). Coexistence of compatible mating types in the same field/region could not be detected in our study. Similar results were reported by Misra et al. (2011), who also observed the lack of compatible mating types (A1 and A2) in India. Recently, a study by Tyson and Fullerton (2007) found only one mating type of *P. colocasiae* (A2) throughout the Pacific region, including Guam, Hawaii, Indonesia, Philippines, Papua New Guinea and Samoa. Oospores of *P. colocasiae* have not been often detected in naturally infested soil or in association with naturally infected host tissues (Ko, 1979). From the results obtained, it can be commented that sexual reproduction may not be playing a direct role in disease epidemics of taro in India.

SCoT analysis

This is the first time that Start Codon Targeted Polymorphism (SCoT) markers have been used for assessing the genetic diversity of Indian *P. colocasiae* isolates. SCoT markers are generally reproducible and it is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer (Collard and Mackill, 2009). The eight primers amplified 121 reproducible fragments ranging in size from 150 to 1200 bp, of which 121 (100 %) were polymorphic (Table 2). When fingerprints of these isolates were compared, some bands common to the majority of isolates were observed, while others were unique to one or few isolates. The

Table 2. Summary statistics for *Phytophthora colocasiae* isolates from different taro growing regions of India based on SCoT marker

Marker	Primer code	Sequence5´- 3´	Number of bands	Number of polymorphic bands	Mean number of bands	Polymorphism (%)
SCoT	SCoT 1	CAACAATGGCTACCACCA	22	22	3.5	100
	SCoT 2	CAACAATGGCTACCACCC	17	17	5.1	100
	SCoT 11	AAGCAATGGCTACCACCA	9	9	2.3	100
	SCoT 12	ACGACATGGCGACCAACG	18	18	6.5	100
	SCoT 14	ACGACATGGCGACCACGC	15	15	5.5	100
	SCoT 20	ACCATGGCTACCACCGCG	18	18	4.4	100
	SCoT 21	ACGACATGGCGACCCACA	12	12	4.1	100
	SCoT 61	CAACAATGGCTACCACCG	10	10	3.5	100
		Total	121	121	35.1	
		Average	26.89	26.89	7.80	100

highest number of amplification products (22) was obtained with the primer SCoT 1, while the lowest was with SCoT 11 (9); the average number of bands among the eight primers was 26.8. The number of polymorphic fragments detected by each primer varied from 9 to 22, with an average of 26.8. The highest number of polymorphic bands (22) was produced by the primer SCoT 1, whereas the primer SCoT 11 generated the lowest number of polymorphic bands (9). Example of SCoT polymorphism is shown in Fig. 3.

Based on an UPGMA clustering algorithm, the isolates were grouped into two major clusters (Fig. 4) with high bootstrap values. Cluster I formed the major group in 28 isolates, while cluster II had only two isolates. The clustering of isolates was not found to be associated with their geographical origin or phenotypic characters which were in agreement with previous reports on genetic diversity analysis in *P. colocasiae* (Lebot et al., 2003; Mishra et al., 2010; Nath et al., 2013).

The cophenetic correlation coefficient between dendrogram and the original similarity matrix were significant for SCoT marker ($r = 0.904$).

Analysis of genetic diversity

Population genetic parameters were calculated with the assistance of POPGENE software.

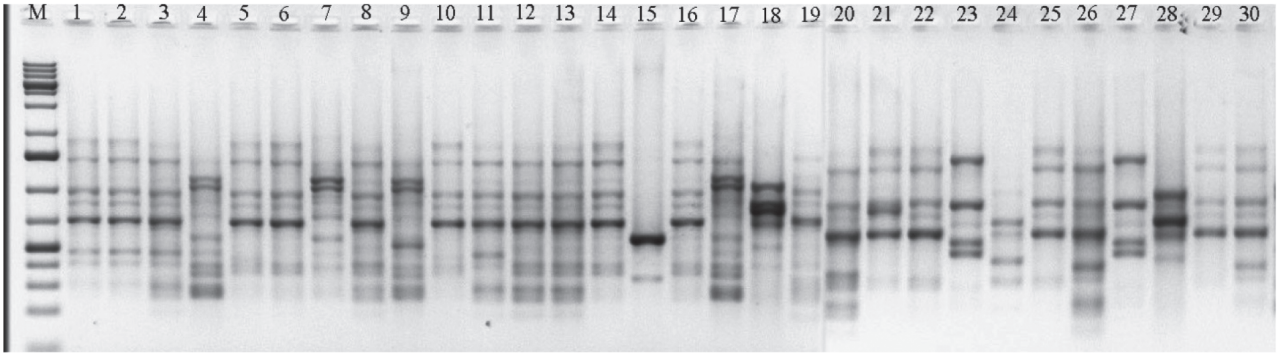

Fig. 3. SCoT amplification profile for SCoT 1 primer. 1-30 refers isolates listed in Table 1

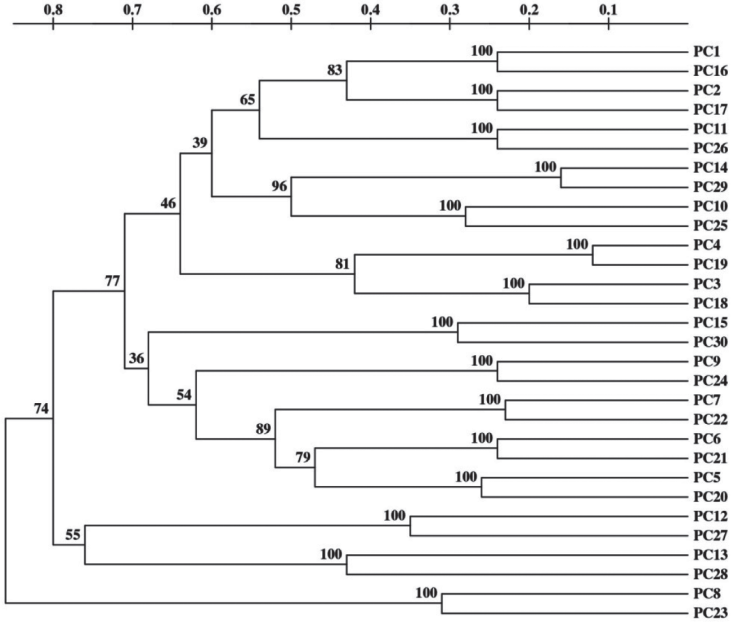

Fig. 4. UPGMA dendrogram depicting genetic relatedness of 30 *Phytophthora colocasiae* isolates based on ScoT markers. Numbers at nodes represents bootstrap values (2000 replicates)

The isolates were divided into sub-population based on their geographic origin to project their diversity at the regional level. The observed number of alleles (*NA*), effective number of alleles (*NE*) and Nei's gene diversity (*H*) varied among population (Table 3).

Analysis of molecular variance (AMOVA) showed that a high percentage (89.16%) of the total genetic diversity of *P. colocasiae* population in this study was distributed within population and only 10.83% among population (Table 4). The coefficient of genetic differentiation among population (*GST*) was 0.1896, which supports the AMOVA analysis indicating only limited genetic diversity among population and high diversity within population. The estimate of gene flow (*Nm*) among population was 2.13 migrants per generation, obtained from the GST value.

Table 3. Population genetic parameters for *Phytophthora colocasiae* isolates from taro based on molecular marker and geographical origins

Marker	Population code	Polymorphic bands	PPB (%)	*NA*	*NE*	*H*	*I*
ScoT	Kerala	115	95.04	1.95±0.21	1.57±0.32	0.33±0.14	0.49±0.19
	Andhra Pradesh	82	67.77	1.67±0.46	1.45±0.37	0.26±0.19	0.39±0.28
	Odisha	101	83.47	1.83±0.37	1.53±0.35	0.30±0.17	0.46±0.23
	Assam	67	55.37	1.55±0.49	1.44±0.39	0.24±0.22	0.35±0.31
	West Bengal	80	66.12	1.66±0.47	1.49±0.39	0.27±0.20	0.40±0.29
	Total	121	100	2.00±0.00	1.58±0.30	0.34±0.13	0.51±0.16

PPB: percentage of polymorphic bands; NA: observed number of alleles; NE: effective number of alleles; H: Nei's gene diversity; E: Shannon's information index

Table 4. Analysis of molecular variance (AMOVA) of 30 isolates of *Phytophthora colocasiae* using ScoT marker

Marker	Source	df	SSD	ϕ statistics	Variance components	Proportion of variation components (%)
SCoT	Among populations	5	1.60	0.108	0.027	10.83
	Within populations	25	5.60		0.226	89.16
	Total	30	7.2		0.254	

df: degrees of freedom; SSD: sums of squared deviations

Several reasons could be attributed to the high intra-zonal diversity detected in the present investigation. Other than sexual recombination, the genetic variation seen within the population may have arisen via asexual mechanisms, such as mitotic recombination. *P. colocasiae* is known to rapidly reproduce asexually through the formation of large numbers of sporangia, which either germinate directly or differentiate into motile zoospores. Evidence of mitotic recombination in *Phytophthora* species has been previously reported (Goodwin, 1997; Fry and Goodwin, 1995; Abu-El Samen et al., 2003). According to Goodwin (1997), mutation is thought to be the primary source of genetic variation in oomycetes. These mutations in most cases can be neutral and may not cause any observable changes in phenotype, but it is not impossible that at least a part of the genotype variation might have been the result of spontaneous mutation (Silvar et al., 2006). Alternatively, other mechanisms such as translocations, chromosome deletions and duplications may occur in this asexually reproducing oomycetes fungus that could be the possible means for such variation. Variability in the pathogen could also be elucidated by the fact that the isolates were collected from different agroclimates, although the number of isolates used in the study would not allow for a robust inference to be made about the influence of climate on the variability in the pathogen population.

In conclusion, a polyphasic approach allowed us to efficiently characterize *P. colocasiae* isolates from India. The high level of genetic diversity shows that the *P. colocasiae* population could respond rapidly to selection exerted by newly introduced host resistance genes or fungicides, underlining the importance of relying on integrated disease management. Disease management programmes should focus on local scale than on a regional level since it is likely that effective packages at one particular location may not prove so in other regions. The findings presented here will significantly enhance our understanding of this patho-system and provide baseline information for developing management strategies for mitigating taro leaf blight disease in India

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