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Molecular Identification of *Phytophthora* spp. Affecting some Economically Important Crops in Eastern India through ITS-RFLP and Sequencing of the ITS Region

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Abstract

Molecular identification of the *Phytophthora* spp. affecting betelvine (*Piper betel*), brinjal (*Solanum melongena*), guava (*Psidium guajava*), roselle (*Hibiscus subdariffa*), black pepper (*Piper nigrum*), sesame (*Sesamum indicum*), taro (Colocasia esculenta), chilli (*Capsicum annuum*), pointed gourd (*Trichosanthes dioica*), papaya (*Carica papaya*) was performed through rDNA ITS-RFLP and also additionally by sequencing the Internal Transcriber spacer (ITS) ITS1 and ITS2 regions. *Phytophthora nicotianae*, *Phytophthora capsici*, *Phytophthora colocasiae*, *Phytophthora melonis* and *Phytophthora palmivora* isolates from these 10 different crops were accessioned and the ITS sequences were deposited in Genbank. ITS sequences for *Phytophthora* isolates from most of these crops are being reported here for the first time. In this study, a review of all earlier Indian reports based on morphology from the above crops and their molecular corroboration has been attempted. This study revealed that not only is *P. nicotianae* the most prevalent species but also there is the presence of both *P. nicotianae* and *P. capsici*, but not *P. palmivora* on betelvine; as well as possible first reports of *P. nicotianae* on pepper, *P. capsici* on chilli and *P. palmivora* on papaya from this vegetable growing Eastern region of the country. Mating type assays and RAPD markers were used to assess the genotypic diversity of the population. This detection of diversity is a first and critical step for helping to devise and adopt strategies for control and quarantine of these pathogens in this region.

Introduction

Phytophthora, the 'plant destroyer', is one of the most destructive genera of plant pathogens in temperate and tropical regions causing annual damages of billions of dollars (Drenth and Guest, 2004). There are more than 70 species in the genus *Phytophthora*; most of them plant pathogenic causing diseases on thousands of plant species in a wide range of ecological niches (Erwin and Riberio, 1996). Even though diseases caused by this stramenopile, oomycetous organism have been well studied in the temperate regions mainly due to the historic potato late blight epidemic in Europe during 1845–1847, which in turn provided the impetus for development of plant pathology as a scientific discipline, while in the tropics, due to widespread occurrence on a wide range of agriculturally and economically important crops, the classical taxonomy of the genus is still based on often inconsistent morphological markers (Duncan and Cooke, 2002). Coupled with this is the fact that there is display of considerable morphological plasticity within some taxa limits (Brasier and Griffin, 1979; Erwin and Riberio, 1996; Appiah et al., 2003) and also the need for specialized expertise and time, both of which renders species identification, based on morphological criteria, difficult (Brasier et al., 1981; Erwin and Riberio, 1996). This often leads to misidentification (Hall, 1998) which, in turn, is detrimental to both practical control and clear scientific communication.

Molecular approaches can provide reliable methods for pathogen identification and disease diagnosis and the technology available has sufficient sensitivity to enable detection of variation between organisms at the level of a single base change. It is remarkable that even though about the third of the total established species of *Phytophthora* known so far has been reported from India, excepting a few instances (Chowdappa et al., 2003a,b; Tripathi et al., 2003; Guha Roy et al., 2006), most of the species are yet to be validated by molecular methods. This validation is necessary as various reports on molecular identification of the *Phytophthora*

pathogen based on the nuclear non-coding Internal Transcriber spacer Region (ITS) of rDNA have proved that in the past, new species have been wrongly assigned to current taxa and conversely, morphological variants of the existing taxa incorrectly assigned as new disease threats when the identifications were solely based on morphological criteria (Mirabolfathy et al., 2001; Chowdappa et al., 2003c; Guha Roy et al., 2006). Moreover, some of the earlier Indian reports based solely on morphology has also not been accepted worldwide (Mehrotra and Aggarwal, 2001). A search of the online database of Systemic Botany and Mycology Laboratory (SBML) of USDA (Farr et al., (n.d.) for presence of fungus-host relationship in India, interestingly showed recorded presence of *Phytophthora* on only seven of the 10 hosts selected in this study: *P. nicotianae* on brinjal (*Solanum melongena*), betelvine (*Piper betel*), guava (*Psidium guajava*), black pepper (*Piper nigrum*) and sesame (*Sesamum indicum*); *P. melonis* on pointed gourd (*Trichosanthes dioica*); *P. colocasiae* on taro (Colocasia esculenta). There is no report on the other four hosts: *P. nicotianae* on roselle (*Hibiscus subdariffa*); *P. capsici* on betelvine (*Piper betel*) and on chilli (*Capsicum annuum*); *P. palmivora* on papaya (*Carica papaya*). Moreover, Erwin and Riberio's (1996) in their book '*Phytophthora Diseases Worldwide*' [Table 50.2 Distribution of hosts of *P. nicotianae* (= *P. parasitica*), pp. 397], did not mention the presence of *P. nicotianae* on roselle in India, a pointer perhaps to the urgent need for a molecular validation of the earlier morphological Indian reports.

The identity of the *Phytophthora* species was validated using an ITS region phylogenetic system (Cooke et al., 2000b), which has been reviewed by Martin and Tooley (2003) to be the most comprehensive till date. Although latter reports (Kroon et al., 2004; Villa et al.,2006; Goker et al., 2007) differ in their opinion, presently, only the online resources (http://www.PhytID.org) hosted by CABI allows easy access for comparison of species-specific ITS-RFLP profile against a comprehensive global reference collection making identification unambiguous from any part of the globe. The molecular identification of *Phytophthora* species followed in this study was based on species-specific characteristic ITS-RFLP profile (Cooke et al., 2000a) and additionally supported by ITS sequence data (Crawford et al., 1996; Cooke and Duncan, 1997; Cooke et al., 2000b) for unambiguous interpretation (Appiah et al., 2004). The present objective of this study was therefore to unambiguously present the Indian reports of *Phytophthora* species from these different crop hosts based on molecular methods for the first time and assess the diversity present there in.

Materials and Methods

Culture collection, isolation maintenance and sporangium morphology

The pathogen isolates were collected from different farmers' fields (Table 1) from the vegetable growing areas of the lower Gangetic Bengal basin. Isolation of the pathogen was done as described elsewhere (Guha Roy et al., 2006). Sporangium morphology was checked for by 'agar-disk-in-water' technique (Erwin and Riberio, 1996) and also on V8JA media following (Appiah et al., 2003). Identification of the isolates was done following the key of Stamps et al., (1990). The cultures were then routinely maintained on V_8 JA medium and the isolates were deposited in the World Phytophthora Collection (WPC) and some additionally in Virginia Polytechnic and State University, USA.

DNA extraction, ITS PCR amplification, ITS-RFLP and sequencing

DNA was extracted from the mycelium, which was scrapped directly from the modified P_{10}ARP V_8JA amended medium under aseptic conditions, washed in sterilized demineralized water, freeze dried and stored in −20°C freezer for future extraction. DNA extraction was performed according to Cooke et al. (2000a).

Amplification of ITS region of rDNA and ITS-RFLP was carried out as described previously (Guha Roy et al., 2006). (All chemicals used were procured from Sanmar Speciality Chemicals, Bangalore, India.) The characteristic-banding patterns produced were compared with the PhytID database (Cooke et al., 2000a).

For rDNA sequence determination of the eleven isolates (Table 1), the PCR products were sequenced with primers in reverse ITS 7(AGCGTTCTTCATCGATGTGC) and in forward ITS 8(GCACATCGATGAAGAACGCT) primers, which were located in the 5.8s gene (Cooke et al., 2000b). Sequencing was performed for both strands by a commercial service, (Sanmar Speciality Chemicals, Bangalore, India). The sequences generated were again compared manually with the supplied Electrophenogram data. A BLAST search was performed to compare the ITS 1 and ITS 2 sequences generated with those available in GenBank and identify the isolates. The sequences were then deposited in GenBank. (Table 1).

Determination of mating type

Mating or compatibility type of isolates was determined by the single unknown isolate method (Kaosiri et al., 1980). A plug of 0.5cm^2 from each isolate was pared with a known A1 and A2 tester on V_8 agar plates 20–25 mm from each other. Dr C. X. Hong (Virginia Polytechnic and State University, VA, USA), kindly tested a few amongst some of our isolates accessioned there, subsequently, these isolates were used as testers for further studies here in our laboratory. The plates were sealed and incubated in the dark at 25°C for 4 weeks. Slides were prepared from the line produced at the junction of the two colonies and examined by microscopy. Presence of gametangia (oogonia and antheridia) indicated that the unknown isolate was of the opposite mating type to that of the tester. Control pairings involving selfed testers were included.

Table 1
Phytophthora isolates examined in this study

Culture accn. nos.[a]	Host	Organism	Substrate	Genbank accession nos.	Mating type	Colony morphology type[b]
P10984, 33B3	Brinjal	*P.nicotianae*	Fruit		A2	III
P10985, 33B4	Brinjal	*P.nicotianae*	”		A1	IIB
P10986, 33B5	Brinjal	*P.nicotianae*	”	DQ075224, DQ075225 AH015043	A1	IIB
P10987	Brinjal	*P.nicotianae*	”		A1	IIB
P10988, 33B6	Brinjal	*P.nicotianae*	”		A1	IIB
P10989, 33B7	Brinjal	*P.nicotianae*	”		A1	IIB
33B8	Brinjal	*P.nicotianae*	”		A1A2	IIB
P10993, 33D1	Sesame	*P.nicotianae*	Stem	DQ075220, DQ075221 AH15041	A2	IIB
P10999	Sesame	*P.nicotianae*	”		A2	IIB
P11000	Pepper	*P.nicotianae*	Leaf	DQ910796	A1	IID
P10998	Roselle	*P.nicotianae*	Stem	DQ910797	A1	IIBB
P10990, 33C6	Guava	*P.nicotianae*	Fruit		A1	IIBB
P10991, 33C7	Guava	*P.nicotianae*	”	DQ075218, DQ075219 AH015040	A1	IIBB
P10992, 33C8	Guava	*P.nicotianae*	”		A1A2	IIBB
P10978, 33B9	Betelvine	*P.nicotianae*	Leaf		A2	IIC
P10979, 33C1	Betelvine	*P.nicotianae*	”	DQ075222, DQ075223 AH015042	A2	IIA
P10980, 33C2	Betelvine	*P.nicotianae*	”		A2	IIA
P10981, 33C3	Betelvine	*P.nicotianae*	”	DQ124717, DQ124716 AH015112	A2	IIC
P10982, 33C4	Betelvine	*P.nicotianae*	”		A2	IIC
P10983, 33C5	Betelvine	*P.nicotianae*	”		A2	IIA
35C1	Betelvine	*P.capsici*	”	DQ124718, DQ124719 AH015113	A2	IA
P10995, 35C2	Chilli	*P.capsici*	Fruit	DQ124721, DQ124720 AH015114	A2	IA
P10996, 35C3	Chilli	*P.capsici*	”		A2	IA
P10997, 35C4	Chilli	*P.capsici*	”	DQ124723, DQ124722 AH015115	A2	IA
P10994, 33C9	Pointed gourd	*P.melonis*	”	DQ075216, DQ075217 AH015039	A2	IIB
PG4	Pointed gourd	*P.melonis*	”		A2	IIB
PG6	Pointed gourd	*P.melonis*	”		A2	IIB
PG7	Pointed gourd	*P.melonis*	”		A2	IIB
PG10	Pointed gourd	*P.melonis*	”		A2	IIB
35B9	Taro	*P. colocasiae*	Leaf	DQ075214, DQ075215 AH015038	A2	IA
35B8	Taro	*P. colocasiae*	”		A2	IA
35C5	Papaya	*P. palmivora*	Stem	DQ910798	A2	1A

[a]Accession numbers at World Phytophthora Collection, USA (prefix P) and Virginia State University, USA (prefix 33 and 35), others (prefix PG) our reference numbers.
[b]IA – stellate striated pattern,IB – stellate striated pattern with aerial mycelium, IC – slightly stellate pattern, IIA – cottony mycelium no pattern, IIB – dense cottony mycelium no pattern, IIBB – fluffy cottony mycelium no pattern, IIC – fluffy cottony mycelium and cottony with slightly striated pattern, IID – cottony with slightly pettaloid pattern, III – cottony mycelium with slightly stellate pattern, IV – pettaloid pattern, VA – dense rosette pattern, VB – light rosette pattern (modified after Appiah et al., 2003)].

RAPD and its analysis using SAHN trees

The DNA extracted earlier from each isolate as mentioned before was used for carrying out PCR–RAPD amplifications. The PCR amplification cycle consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles with conditions of 94°C for 30 s (denaturation), 36°C for 30 s (annealing), 72°C for 1 min (extension) and a final extension at 72°C for 7 min. The PCR reaction mixture (25 μl) contained 20 ng template DNA, 2.5 mM dNTPs, 2.5 μl 10× PCR buffer, 100 ng of decamer primer, 1.5 units, *Taq* DNA polymerase (Chromous Biotech, Bangalore, India). Ten primers (Table 3) were selected out of 35 primers based on repeatability of DNA band profile. The amplification products were electrophoresed at a constant voltage (60 V) on ethidium bromide stained horizontal agarose gel (1.5%) in 1× TBE buffer. The bands were compared with DNA markers and documented using Kodak Digital Science Electrophoresis Documentation and Analysis Systems, Gel logic 200 (Kodak, Rochester, NY, USA). RAPD products were scored for presence or absence of bands using a 1, 0 matrix for analysis with NTYS-PC 2.1® software (Exeter Software, Setauket, NY, USA). A similarity matrix tree Sequential Agglomerative Hierarchical and Nested cluster (SAHN) was constructed using the tree plot option in the software.

Results

Sporangium morphology

Morpho-cultural characteristics of the isolates were assessed. Other responses *viz*. response to commercial fungicides and biocontrol agent of these isolates were also checked as reported elsewhere (Guha Roy et al., 2003, 2007a,b; Guha Roy et al., 2007c). Intra-specific diversity in colony morphology type was observed only amongst *P. nicotianae* isolates (Table 1). As it is now actually more accepted that a combination of both morphological and molecular identification is necessary, ITS-RFLP studies and sequencing of the ITS1 and ITS2 regions were undertaken to unambiguously assign taxa especially in the Indian context for reasons discussed earlier.

rDNA ITS-RFLP and its sequencing

Representative ITS-RFLP profiles of the isolates of five different species were obtained and their sizes (base pairs) were characteristic of the species. Two distinct

types of RFLP pattern were seen for isolates from betelvine host. While isolate P10979, 33C1 is *P. nicotianae*, isolate 35C1 is *P. capsici* as evidenced by ITS-RFLP and sequencing. All other isolates of betelvine host showed identical ITS-RFLP for *P. nicotianae*. ITS-RFLP patterns and their sequences from brinjal, guava, sesame, rosella and the black pepper isolates respectively were similar suggesting there by that the identity of all the isolates from the different hosts are similar i.e. *P. nicotianae*. Taro isolates (35B8, 35B9), chilli isolates (P10995/35C2, P10996/35C3, P10997/35C4), pointed gourd isolates and the papaya isolate showed typical *P. colocasiae*, *P. capsici*, *P. melonis* and *P. palmivora* ITS-RFLP patterns respectively.

Sequence analysis of all our isolates showed that the identity of the isolates to be same as that inferred from ITS-RFLP patterns.

Mating type

Mating type tests showed that *P. nicotianae* betelvine isolates were all of A2 mating type and brinjal isolates were all of A1 type excepting one (P10984/33B3). *P. capsici*, *P. colocasiae* and *P. melonis* isolates were uniformly of A2 type (Table 1). The A1A2 type was designated for those cultures, which exhibited sexual structures in single culture and was found for two *P. nicotianae* isolates; one from brinjal (33B8) and the other from guava (P10979/33C8), similar to the phenomenon noted in earlier reports (Savage et al., 1968; Smart et al., 1998; Goodwin and Drenth, 1997).

Thus, it can be seen that both mating types are present within *P. nicotianae* in the eastern regions (Table 2) as well as in other parts of the country and similarly, also in *P. capsici* (Oudemans and Coffey, 1991; Mchau and Coffey, 1995; Chowdappa and Chandramohanan, 1997; Appiah et al., 2003) as well as in *P. colocasiae* ((Narula and Mehrotra, 1980; Tyson and Fullerton, 2007). However, in case of *P. colocasiae*, the isolates tested previously were from northern India. This study also indicates the presence of A2 among *P. colocasiae* populations of eastern India. The A2 type is being reported here for the first time from betelvine, sesame and brinjal in case of *P. nicotianae* and chilli in case of *P. capsici*; whereas in *P. melonis* and *P. palmivora* (Oudemans and Coffey, 1991; Mchau and Coffey, 1994a) perhaps for want of studies in other regions, only A2 mating type has been found till date. This presence of both mating types in close proximity is significant on epidemiological grounds and warrants further study to ascertain if pairing is occurring to form oospores *in planta* and also perhaps whether hybrids are arising in nature. While the rise of A2 mating types has been well documented in *Phytophthora infestans* from potato in India and worldwide and its occurrence has been explained by migration hypothesis (Spielman et al., 1991; Goodwin and Drenth, 1997); for other *Phytophthora* species, such data is lacking, one cannot therefore ascertain whether the presence of both mating types documented here for the first time on comparison with earlier

Table 2
Mating types of *Phytophthora* species detected in India till date

Phytophthora sp.	Mating type	Host	References
P. infestans	A1 and A2	Potato (*Solanum tuberosum*)	(Singh et al., 1994)
P. capsici	A1 and A2	Cocoa (*Theobroma cacao*)	(Chowdappa and Chandramohanan, 1997)
	A2	”	(Mchau and Coffey, 1995)
	A1	”	(Appiah et al., 2003)
	A2	Chilli (*Capsicum annuum*)	Present Study
	A2	Betelvine (*Piper betel*)	Present Study
	A1 and A2	”	(Mchau and Coffey, 1995)
P. nicotianae	A1	Black Pepper (*Piper nigrum*)	(Oudemans and Coffey, 1991)
		”	(Mchau and Coffey, 1995)
		”	Present Study
	A2	Brinjal (*Solanum melangena*)	(Mchau and Coffey, 1995)
	A1 and A2	”	Present Study
	A2	*Dolichos lablab*	(Mchau and Coffey, 1995)
	A1	*Ailanthus exelsa*	(Mchau and Coffey, 1995)
	A2	Betelvine (*Piper betel*)	Present Study
	A1	Guava (*Psidium guajava*)	Present Study
	A2	Seasame (*Sesamum indicum*)	Present Study
	A1	Roselle (*Hibiscus subdariffa*)	Present Study
P. colocasiae	A1	Taro (*Colocasia esculenta*)	(Narula and Mehrotra, 1980)
	A2		Present Study (Tyson and Fullerton, 2007)
P. melonis	A2	Pointed gourd (*Trichosanthes dioica*)	Present Study
P. meadii	A1 and A2	Rubber (*Hevea brasiliensis*)	(Liyanage and Wheeler, 1989)
		”	(Oudemans and Coffey, 1991)
	A1	Cardamum (*Elletaria cardamomum*)	(Oudemans and Coffey, 1991)
P. areacae	A2	Betelnut (*Areca catechu*)	(Mchau and Coffey, 1994a)
		”	(Oudemans and Coffey, 1991)
P. dreschleri f. sp. *cajani*	A1	*Cajanus cajan*	(Mills et al., 1991)
P. citrophthora	A2	Tube Rose (*Tabernaemontana coronaria*)	(Mchau and Coffey, 1994b)
P. palmivora	A2	*Palmarya* sp.	(Oudemans and Coffey, 1991)
	A2	Coconut (*Cocos nucifera*)	(Mchau and Coffey, 1994a)
	A2	Papaya (*Carica papaya*)	Present study

reports (Liyanage and Wheeler, 1989; Mills et al., 1991; Oudemans and Coffey, 1991; Mchau and Coffey, 1994a,b, 1995; Chowdappa and Chandramohanan, 1997; Appiah et al., 2003) has arisen newly or were present earlier, but not detected or presumably, that, these shifts in mating type was due to effect of application of fungicides as previously thought for *P. infestans* (Ko, 1981; Ko et al., 1986; Ann and Ko, 1989) and/or by induced selfing due to use of antagonistic fungi, especially *Trichoderma* sp. (which is used to control *Phytophthora* spp.), as there have been various reports, which show that A2 isolates when in close proximity and even without physical contact have been known to induce oospores (Brasier, 1971, 1972, 1975a,b; Pratt et al., 1972; Reeves and Jackson, 1972; Reeves, 1975; Jahangirdar and Siddaramaiah, 2001).

Whatever the cause, the detection for the first time from this study in *P. capsici* of the presence of A2 mating type from chilli and likewise in *P. nicotianae* of both A1 and A2 from brinjal, A2 types from betelvine and sesame along with previously known presence of A1 types for both species respectively (Oudemans and Coffey, 1991; Mchau and Coffey, 1995; Chowdappa and Chandramohanan, 1997; Appiah et al., 2003) in this vegetable growing region, is a cause for concern.

RAPD and its analysis using SAHN trees

RAPD analysis showed that there is considerable intra-specific diversity within *P. nicotianae* isolates collected from this region. The isolates did not cluster either according to host and geographical origin or mating type. Five distinct clusters could be identified. (Fig. 1a).The betelvine isolates were the most diverse and were found in three clusters with isolates P10981/33C3 and P10980/33C2 being distinctly different from the rest of the population. Conversely, guava isolates were the most homogenous clustering in a single group. The isolates from sesame (P10999) and from black pepper (P11000) were most dissimilar to the isolate from betelvine (P10980/33C2) with a simi-

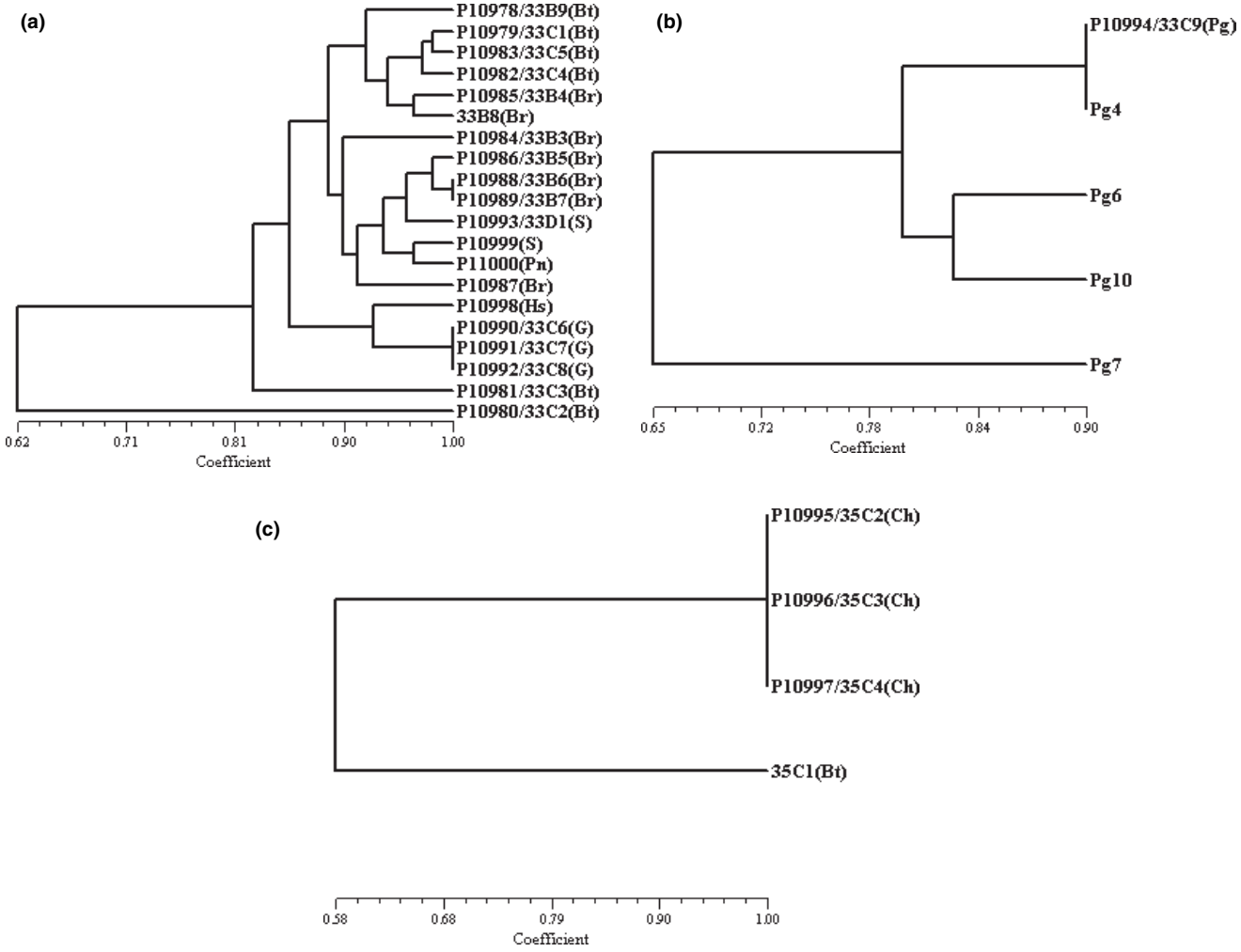

Fig. 1 SAHN tree showing intra-species relatedness of the Indian *Phytophthora* isolates from different crop hosts using RAPD data, *P. nicotianae* (a), *P. melonis* (b) and *P. capsici* (c). The numbers are isolate accession numbers at World Phytophthora Collection, USA (prefix P) and Virginia State University, USA (prefix 33 and 35), others (prefix PG) our reference numbers. The alphabets in parenthesis refer to the host from which isolated: Br, Brinjal; Bt, Betelvine; S, Sesame; Pn, *Piper nigrum* (Black pepper); Hs, *Hibiscus subdariffa* (Roselle); Pg, Pointed gourd; Ch, Chilli; G, Guava

Table 3
Sequences of the RAPD primers used

Primer	Sequence
P	5′-GTGTGCCCCA-3′
R	5′-GGTCTACACC-3′
O	5′-TTGGCACGGG-3′
W	5′-ACAAGCGCGA-3′
H	5′-CCCGTAGCAC-3′
K	5′-GGACCCAACC-3′
E	5′-CTCCCTGAGC-3′
F	5′-GGCGGTTGTC-3′
J11	5′-CTGACCAGCC-3′
S	5′-ACCCGGTCAC-3′

larity (SM) coefficient of 0.5690. Amongst all *P. nicotianae* isolates, brinjal isolates, P10988/36B6, P10989/33B7 and P10986 were most similar (SM coefficient = 0.9828).

Intra-specific diversity within *P. melonis* isolates from pointed gourd is present as evidenced by clustering of the isolates into three distinct groups (Fig. 1b). P10994/33C9 was most similar to Pg4 (SM coefficient = 0.9038) and most dissimilar to Pg7 (SM coefficient = 0.5769). *P. capsici* isolates clustered into two distinct groups based on host origin; chilli and betelvine (Fig. 1c). RAPD profiles (data not shown) of the *P. colocasiae* isolates (which were only two in number) showed them to be homogenous.

Discussion

Taxonomic studies based on morphology attribute the casual organism of foot and leaf rot disease of betelvine in India variously to *P. parasitica* var. *piperina* from the Central provinces (Dastur, 1927), from South India to *P. capsici*. Leonian (Rao, 1996) and *P. palmivora* MF 4 (Marimuthu, 1991), from the Eastern region to *P. nicotianae* var. *piperina* (McRae, 1934) and *P. palmivora* (Maiti and Sen, 1979). Molecular corroboration for *P. nicotianae* (=*P. parasitica* var. *piperina*) as one of the causal organisms in India has been established (Tripathi et al., 2003). As *P. palmivora* MF 4 has been redesignated as *P. capsici* as an amended description of this species, this study also confirms *P. capsici* as another causal organism of this disease. However, there was no evidence of *P. palmivora* in this region. A report based on morphology from Central India (Madhya Pradesh) also indicates the same (Aggarwal and Mehrotra, 1995). The fact that both the species have been isolated from the same region may point to the existence of a 'disease complex', which has important ramifications for disease control.

The observation about the presence of *P. nicotianae* through ITS-RFLP co-relates well with the earlier Indian reports of the pathogen on brinjal (Padwick, 1938; Jain et al., 1982) and also in postharvest condition (Guha Roy et al., 1986). The pathogen on Guava has been reported from all over India; from Eastern region (Mitra, 1929), Western (Patel et al., 1949; Rao, 1970), Northern (Singh et al., 1976; Gupta et al., 1977) and Southern India (Sohi and Sridhar, 1971) and also in postharvest condition (Guha Roy 1996). *Phytophthora* on sesame has been reported from Eastern India (Butler, 1918) and from Western India (Kale and Prasad, 1957) and on roselle from Eastern India (McRae, 1932; Kar and Saha, 1943). Even though *P. nicotianae* var. *nicotianae*, *P. nicotianae* var. *parasitica*, *P. nicotianae* var. *subdariffa* and *P. parasitica* have been variously named in these reports as the causal organisms for the above mentioned host, it is now been well established through ITS fingerprinting that these are all synonyms of *P. nicotianae* (Cooke et al., 2000b).

The identity of the casual organism of foot and leaf rot disease of black pepper in India has been established as *P. capsici* both morphologically (Sarma et al., 1991) as well as at molecular level (Chowdappa et al., 2003c), additionally, report of *P. palmivora* causing rapid wilt of *P. nigrum* from Karnataka is also there (Sastry and Hegde, 1991). The P11000 isolate is a first molecular report of *P. nicotianae* infecting black pepper and evidence of its presence in the Eastern part of the country, which was earlier reported only from tracts of rubber plantations in South India (Thankama, 1983).

Analysis of the taro isolates 35B8 and 35B9 showed it to be *P. colocasiae*, which is in perfect conformity with earlier Indian morphological reports (Sydow and Butler, 1907; Butler and Kulkarni, 1913) and earlier molecular corroboration in 2003 from analysis of *CoxI* and *CoxII* genes (AY129187).

All the chilli isolates showed typical *P. capsici* ITS-RFLP patterns, which was also confirmed by ITS sequences. This co-relates well with the earlier morphological reports. Presence of *P. capsici* on chilli is a possible first report for the Eastern region. However, there are also morphological reports of *P. nicotianae* var. *nicotianae* infecting chilli, both in field (Sharma and Bharadwaj, 1976) and in postharvest condition (Guha Roy 1996), which could not be found amongst the isolates in this study.

Pointed gourd isolates showed typical *P. melonis* patterns as reported earlier (Guha Roy et al., 2006) as well as sequences and similarly papaya isolates to that of *P. palmivora* corroborating earlier morphological reports (Balakrishnan, 1947), however, both in postharvest condition has been reported to be affected by *P. nicotianae* (Guha Roy 1996).

RAPD analysis showed that there was considerable intra-specific diversity in the polyphagous *P. nicotianae* in contrast to *P. colocasiae*. However, in case of the latter due to extremely small sample size, the conclusion cannot be generalized. Presence and/or dispersed spatio-temporal introduction of different clonal population in Eastern India in case of *P. nicotianae* could be a possible explanation for the intra-specific diversity encountered among and between the different hosts. A similar conclusion may be drawn for *P. melonis*, which showed considerable diversity even with a small sample size. The extremely homogenous populations in

case of *P. nicotianae* guava isolates and *P. capsici* chilli and betelvine isolates could perhaps be explained if presence of some clonal host-specific lineages for both *P. capsici* and *P. nicotianae* is considered or may be both clonal populations as well as local host adapted lineages co-exist. Plausible explanations would be possible only after further extensive studies with sufficient sampling are done. This detection of diversity has important implications for devising and adopting control strategies for this region.

Thus, our findings represent the first evidence for presence and molecular identification of *P. capsici* on betelvine along with *P. nicotianae*, *P. nicotianae* on pepper, *P. palmivora* on papaya and *P. capsici* on chilli in the Eastern region of the country. It also reports for the first time molecular identification and corroboration of *Phytophthora* species on brinjal, guava, sesame, roselle, chilli, black pepper, papaya, (with presence on betelvine and taro being reported earlier) and the rDNA ITS sequences of five *Phytophthora* species affecting these important crops in India. Except for the presence of *P. palmivora* on betelvine, which could not be corroborated despite extensive sampling, our data indicate that the molecular identification of the *Phytophthora* species in this region in the other instances corresponds well with identification by traditional methods and the above reports can therefore be unambiguously documented. The detection of some new mating types and intra-specific diversities of the respective species (*P. nicotianae*, *P. capsici*, *P. colocasiae*, *P. melonis*) throw light for the first time on the diversity of the Indian *Phytophthora* isolates on some economically important crops grown in this region.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. RAPD profile of the *P. capsici* isolates with E, H, F, K and P primers.

Figure S2. RAPD profile of the *P. capsici* isolates with J11 and R primers.

Figure S3. RAPD profile of the *P. capsici* isolates with O primer.

Figure S4. RAPD profile of the *P. capsici* isolates with S and W primers.

Figure S5. RAPD profile of the *P. colocasiae* isolates with E and H primers.

Figure S6. RAPD profile of the *P. colocasiae* isolates with F and O primers.

Figure S7. RAPD profile of the *P. colocasiae* isolates with K, P and W primers.

Figure S8. RAPD profile of the *P. colocasiae* isolates with R, S and J11 primers.

Figure S9. RAPD profile of the *P. melonis* isolates with E, F and R primers.

Figure S10. RAPD profile of the *P. melonis* isolates with H primer.

Figure S11. RAPD profile of the *P. melonis* isolates with J11 primer.

Figure S12. RAPD profile of the *P. melonis* isolates with K primer.

Figure S13. RAPD profile of the *P. melonis* isolates with P, J10 and TC1 primers.

Figure S14. RAPD profile of the *P. melonis* isolates with W, O and S primers.

Figure S15. RAPD profile of the *P. nicotianae* isolates with R primer.

Figure S16. RAPD profile of the *P. nicotianae* isolates with E primer.

Figure S17. RAPD profile of the *P. nicotianae* isolates with F primer.

Figure S18. RAPD profile of the *P. nicotianae* isolates with H primer.

Figure S19. RAPD profile of the *P. nicotianae* isolates with J11 primer.

Figure S20. RAPD profile of the *P. nicotianae* isolates with K primer.

Figure S21. RAPD profile of the *P. nicotianae* isolates with O primer.

Figure S22. RAPD profile of the *P. nicotianae* isolates with P primer.

Figure S23. RAPD profile of the *P. nicotianae* isolates with S primer.

Figure S24. RAPD profile of the *P. nicotianae* isolates with W primer.

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