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# Phylogeny of *Phytophthora* isolates from West Bengal as inferred from rDNA ITS gene sequences

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*Phytophthora* sp. affecting Betelvine, Brinjal, Guava, Roselle, Pepper, Sesame, Taro, Chilli, Pointed gourd and Papaya were isolated, accessioned, the rDNA ITS1 and ITS2 regions sequenced and deposited in GenBank. The isolate belonged to *P. nicotianae, P. capsici, P. colocasiae, P. melonis,* and *P. palmivora.* Comparison of intra specific phylogenetic distances with representative worldwide isolates and asessment of diversity of these Indian isolates were done by constructing a neighbour-joining (NJ) tree using all the GenBank ITS sequences for the respective species as well as sequence analysis of the rDNA ITS regions. Considerable intraspecific diversity was present among the polyphagous *P. nicotianae* in contrast to *P. capsici.* The respective species (*P. nicotianae, P. capsici, P. colocasiae, P. melonis*) NJ phylogenetic trees throws light for the first time on the deiversity of the Indian isolates *vis a vis* with those around the globe and which will help in formulating strategies for control and quarantine of these devastating phytopathogens.

**Key words**: *P. nicotianae, P. colocasiae, P. melonis, P. capsici, P. palmivora,* India, ITS-phylogenetic tree, Neighbour-joining, sequence analysis.

## INTRODUCTION

*Phytophthora* is one of the most important and desctructive genera of plant pathogens in temperate and tropical regions, causing annual damages of billions of dollars (Drenth and Guest, 2004) due to its high virulence and epidemiological ability to spread rapdily throughout the world. More than US$ 200 million in lost production annually is attributed to *Phytophthora* diseases in Australia alone (Irwin *et al,* 1995) and, in Southeast Asia (Indonesia, Malaysia, Philippines, Thailand and Vietnam), the overall impact on crops (cocoa, durian, rubber, coconut, pepper, potato and citrus) amounts to an average annual loss of US$ 2.3 billion (Drenth and Sendall, 2004). In India too there is considerable damage, but as no concerted study has been takne on this aspect, the reports are fragmentary (Guha Roy, 2007c).

There has been an increasing realization that more knowledge about the genetic structures of plant pathogens is needed to implement effective control strategies and this has led plant pathologists to take a population approach towards study of pathogens in the last two decades. As control strategies must target a population instead of an individual if they are to be effective (Wolfe and Caten, 1987). Defining the genetic structure of a population is a logical first step in studies of fungal population genetics because the genetics because the genetic structure of a population reflects its evolutionary histroy and its potential to evolve: aspects important for formulating disease management strategies.

In the Indian context, even though a third of the *Phytophtohora* species have been reproted from India alone (Mehrotra and Aggarwal, 2001), studies on molecular systematics and diversity of *Phytophthora* species are limited (Virdi and Sachdeva, 2005). An attempt has been made, therefore, to assess the diversity of isolates primarily from West Bengal using ITS sequences in continuation with earlier stuides (Guha Roy, 2007c; Guha Roy *et al.,* 2003; 2007a; 2007b; 2008).

## MATERIALS AND METHODS

### *Culture collection, isolation, identification and maintenance*

The isolates of the pathogen were collected from different farmers' fields (Table 1) from the vegetable growing areas of the lower gangetic Bengal basin. Isolation of the pathogen, checking of sporangium morphology, identification, routine maintenance of culture were done as described elsewhere (Guha Roy *et al.,* 2006; Guah Roy *et al.,* 2007a, b, c; Guha Roy *et al.,* 2008). The voucher specimens were deposited (and accessioned) in the World Phytophthora Collection (WPC), and some additionally in Virginia Polytechnic and State University, USA.

### *DNA extration, ITS PCR amplification and sequencing*

DNA extraction and amplification of ITS region of rDNA were done as described previously (Guha Roy *et al.,* 2006; Guha Roy *et al.,* 2007a, b; Guha Roy *et al.,* 2008). All chemicals were procured from Sanmar Speciality Chemicals, Bangalore, India. The characteristic-banding patterns produced were compared with the PhytID database (Cooke *et al.,* 2006).

For rDNA sequence determination of the twelve isolates (Table 1), the PCR products were sequenced with primers in reverse ITS 7 (AGCGTTCTTCATCGATGTCG) and in forward ITS 8 (GCACATCGATGAAGAACGCT) primers which were located in the 5.8s gene (Cooke *et al.,* 2000a). Sequencing was performed for both strands by a commercial service. (Sanmar Speciality Chemicals, Bangalore, India). The seqeunces were read manually from 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.

### *Seqeunce analysis and Tree construction*

For sequence analysis (Table 2) and phylogenetic tree construction, (Fig. 2) the consensus sequences for each species were aligned with our sequences deposited in GenBank and with each other through CLUSTAL W (www.ebi.ac.uk/clustalw/) to determine variable regions of base sequences unique to each species or isolate. Diversity analysis of the Indian *P. capsici* and *P. nicotianae* isolates were done by comparing both the ITS1 and ITS2 regions of our isolates with the other accessions in GenBank which were collected from India. For the other species in this study, *P. colocasiae, P. melonis* and *P. palmivora* this was not possible as there were no other sequences of indian isolates in the GenBank for comparisions to be made when this study was being undertaken.

Transition/transversion ratio was calculated using MEGA 2.1 (Kumar *et al.,* 2001). Distance matrices between all pairs of sequence from multiple aiignments were calculated using DNADIST and NEIGHBOUR from the phylogenetic inference package (PHYLLIP 3.66) (www.hgmp. mrc.ac.uk) and the unrooted trees were generated by neighbour joining (NJ). Bootstrap analyses of 1000 interactions were performed by SEQBOOT and the best tree selected by CONSENSE routine of the PHYLLIP 3.66. The dendogram was viewed by imparting the calculated values into TREE VIEW (http:/taxonomy-zoology.gla.ac.uk/rod.htm).

## RESULTS

### *Seqeunce analysis and Tree construction*

Sequence analysis of all Indian isolates (ours as well as AY713471 submitted at an earlier date from NBRI, Lucknow and also those submitted later) in GenBank for *P.nicotianae* (Table 2a) showed that ITS1 regions were more variable than ITS2 regions with conserved regions (1-7, 16-32, 34-40, 45-57, 107-114, 122-127, 129-134, 136-141, 150-154, 159-163, 165-177, 182-184, 186-189, 191-195, 197-214) being dispersed in ITS1, however ITS2 regions were by comparisons largely conserved except for the isolate from *Solanum melongena* (DQ075224). Similarly for analysis of *P. capici* all the Indian isolates, ours as well as others which were submitted earlier from other hosts: AF467085, isolate IND44 (Appiah *et al.,* 2004); AF266787, isolate IMI352321 (Cooke *et al.,* 2000a) and DQ464046 were also compared (Table 2b). Among these Indian *P. capsici* isolates, conserved regions were between 1-90, 92-93, 95-102, 104, 123, 125-128, 130-138, 140-142, 144-169, 171-173, in ITS1 regions. Interestingly, in *P.capsici* ITS1 had less

**Table 1.** *Phytophthora* isolates examined.

| Culture Accn. Nos. | Host | Organism | Substrate | Genbank Accession Nos. | Location |
|---|---|---|---|---|---|
| P10984, 33B3 | Brinjal | *P. nicotianae* | Fruit | | Ghoragacha, Nadia |
| P10985, 33B4 | Brinjal | *P. nicotianae* | ,, | | Amdanga, 24PGS(N) |
| | | | ,, | DQ075224, DQ075225 | Madhavpur, 24PGS(N) |
| P10986,33B5 | Brinjal | *P. nicotianae* | | AH015043 | |
| P10987 | Brinjal | *P. nicotianae* | ,, | | Madanpur, Nadia |
| P10988, 33B6 | Brinjal | *P. nicotianae* | ,, | | Bongaon, 24PGS(N) |
| P10989, 33B7 | Brinjal | *P. nicotianae* | ,, | | Mondouri, Nadia |
| 33B8 | Brinjal | *P. nicotianae* | ,, | | Barasat, 24PGS(N) |
| | | | Stem | DQ075220, DQ075221 | Kalyani, Nadia. |
| P10993,33D1 | Sesame | *P. nicotianae* | | AH15041 | |
| P10999 | Sesame | *P. nicotianae* | ,, | | Bijpur, Nadia |
| P11000 | Pepper | *P. nicotianae* | Leaf | DQ910796 | Mondouri, Nadia |
| P10998 | Roselle | *P. nicotianae* | Stem | DQ910797 | Nilgunje, 24PGS(N) |
| P10990,33C6 | Guava | *P. nicotianae* | Fruit | | Mohanpur, Nadia |
| | | | | DQ075218, DQ075219 | Baruipur, 24PGS(S) |
| P10991,33C7 | Guava | *P. nicotianae* | ,, | AH015040 | |
| P10992, 33C8 | Guava | *P. nicotianae* | ,, | | Ghoragacha, Nadia |
| P10978, 33B9 | Betelvine | *P. nicotianae* | Leaf | | Mondouri, Nadia |
| | | | | | Moundouri, Nadia |
| P10979,33C1 | Betelvine | *P. nicotianae* | ,, | DQ075222,DQ075223 | |
| P10980,33C2 | Betelvine | *P. nicotianae* | ,, | AH015042 | Basanti, Nadia |
| | | | ,, | DQ124717,DQ124716 | Kakdip, 24PGS(S) |
| P10981,33C3 | Betelvine | *P. nicotianae* | ,, | AH015112 | |
| P10982,33C4 | Betelvine | *P. nicotianae* | ,, | | Egra, Medinipur |
| P10983,33C5 | Betelvine | *P. nicotianae* | ,, | | Egra, Medinipur |
| | | | ,, | DQ124718,DQ124719 | Simurali, Nadia |
| 35C1 | Betelvine | *P. capsici* | | AH015113 | |
| | | | | DQ124721,DQ124720 | Jaguli, Nadia |
| P10995,35C2 | Chilli | *P. capsici* | Fruit | AH015114 | |
| P10996,35C3 | Chilli | *P. capsici* | ,, | | Jaguli, Nadia |
| | | | ,, | DQ124723,DQ124722 | Jaguli Nadia |
| P10997,35C4 | Chilli | *P. capsici* | | AH015115 | |
| | | | ,, | DQ075216,DQ075217 | Gayeshpur, Nadia |
| P10994,33C9 | Pointed gourd | *P. melonis* | | AH015039 | |
| PG1 | Pointed gourd | *P. melonis* | ,, | | N.C.Pukur, 24PGS(N) |
| PG2 | Pointed gourd | *P. melonis* | ,, | | Mohanpur, Nadia |
| PG3 | Pointed gourd | *P. melonis* | ,, | | Madavpur, 24PGS(N) |
| PG4 | Pointed gourd | *P. melonis* | ,, | | Bongaon, 24PGS(N) |
| PG5 | Pointed gourd | *P. melonis* | ,, | | Basirhat, 24PGS(N) |
| PG5A | Pointed gourd | *P. melonis* | ,, | | Basirhat, 24PGS(N) |
| PG6 | Pointed gourd | *P. melonis* | ,, | | Ramnagar, Medinipur |
| PG7 | Pointed gourd | *P. melonis* | ,, | | Bongaon, 24PGS(N) |
| PG8 | Pointed gourd | *P. melonis* | ,, | | Jalangi, Murshidabad |
| PG10 | Pointed gourd | *P. melonis* | ,, | | Baripada, Orissa |
| 35B9 | Taro | *P. colocasiae* | Leaf | DQ075214,Dq075215 | Mondouri, Nadia |
| | | | | AH015038 | |
| 35B8 | Taro | *P. colocasiae* | ,, | | Kalyani, Nadia |
| 35C5 | Papaya | *P. palmivora* | Stem | DQ910798 | N.C.Pukur, 24PGS(N) |

[a]Accession numbers at World Phytophthora Collection, USA (prefix P and Virginia State University, USA (prefix 33 & 35, others (prefix PG) our reference numbers.

**Table 2a.** Sequence diversity analysis of the Indian *Phytophthora nicotianae* isolates

| | ITS1 region | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DQ822470 *Piper betel* L | 8<br>– | 10<br>A | 14<br>– | 15<br>– | 33<br>T | 41-42<br>A | 44<br>– | 58<br>A | 60-105 | 106<br>G | 115<br>A | 116-121 | 128<br>C | 135<br>G | 142<br>C | 144<br>T | 146-149<br>ATTT | 155<br>T | 156-158<br>CTT | 164<br>C | 178<br>C | 180<br>T | 181<br>T | 185-190<br>A | 196<br>– | 225<br>T | 217-228 | 229-230 | 231<br>– |
| DQ822471 *Piper betel* L | – | . | A | – | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | – – | – |
| DQ822472 *Piper betel* L | – | . | . | A | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | – – | – |
| *DQ910796 *Piper nigrum*L. | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | – | – – | – |
| DQ822473 *Piper betel* L | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | – – | – |
| DQ822474 *Piper betel* L | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | – – | – |
| *DQ075222 *Piper betel* L | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | G | A | . | TA | G |
| *DQ075218 *Psidium guajava* L. | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | G | A | . | . | . |
| *DQ075220 *Sesamum indicum* L. | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | G | A | . | . | . |
| *DQ075224 *Solanum melongena* L. | – | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | G | A | . | . | . |
| AY713471 *Piper betel* L. | A | G | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . |
| *DQ124716 *Piper betel* L. | – | . | . | . | A | T | T | G | – | A | T | – | T | A | T | C | GGCG | C | – | G | T | A | C | T | – | . | . | . | . |

60-105=TTG GCG GCG GCT GCT GGC TTA ATT GTT GGC GGC TGC TGC TGA GTG A, **116-121**= AAA AAA, **217-228** = CTT TTA ACT AGA
Out isolates = *; Identical = . ; Internal deletion = –; Substitution mutations are underlined.

| | ITS2 region | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DQ075220 *Sesamum indicum* L. | 1-7 | 8 A | 9-14 | 15 A | 16-29 | 30 C | 31-50 | 51-108 | 109-195 | 197, 199 G | 200, 04-206 T | 210 T | 211 G | 213 A | 215 T | 217 A | 218 A | 219 C | 225, 226 TT | 227 C | 229 C | 231-233 TTG | 235 T | 237 G | 239 A | 241, 242 AG | 246 T | 249, 250 CG |
| *DQ075222 *Piper betel* L. | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *DQ075218 *Psidium guajava* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *DQ124716 *Piper betel* L | . | – | . | – | . | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *DQ910796 *Piper nigrum* L. | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| DQ822473 *Piper betel* L | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| DQ822474 *Piper betel* L | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| DQ822472 *Piper betel* L | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| DQ822471 *Piper betel* L | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| AY713471 *Piper betel* L | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| DQ822470 *Piper betel* L | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| *DQ075224 *Solanum melongena* L. | – | – | – | – | – | – | – | – | – | C | A | C | T | T | C | C | G | – | CG | – | T | CAA | C | A | T | GT | G | TC |

1-7=ACT GCG A, 9-14-TAC GTA, 16-29= TGC GAT TGC AGG AT, 31-50=TCA GTG AGT CAT CGA AAT TT, 51-108=TGA ACG CAT ATT GC ACTT CCG GGT TAG TCC TGG AGG TAT GCC TGT ATC AGT GTC CGT A, 109-195=CAT TAA ACT TG ACTT TCT TCC TTC CGT GTA GTC GGT GGA GGA GAT GTC AGA TGT GAA GTG TCT TGC GAT TGG TCG TCG GAC CGG CTG

Our isolates=*; Identical= .; Internal deletion = –; Substitution mutations are underlined.

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ITS2 region continued

| | 252 | 253, 254 | 256 | 258, 259 | 261 | 263, 264 | 272-275 | 277, 278 | 279, 280 | 281 | 282 | 289 | 293 | 295 | 296 | 299 300 | 303 | 304 | 307-310 | 312, 315 | 316, 317 | 321, 323 | 325, 326 | 329 | 332, 334 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DQ075220 *Sesamum indicum* L. | T | GC | G | T | T | AA | ATTG | G | CA | A | A | A | G | – | T | TC | C | T | GGCG | TT | 320 G | AT | T | A | CTG |
| *DQ075222 *Piper betel* L. | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| *DQ075218 *Psidium guajava* L. | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| *DQ124716 *Piper betel* L. | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| *DQ910796 *Piper nigrum* | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| DQ822473 *Piper betel* L. | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| DQ822474 *Piper betel* L. | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| DQ822472 *Piper betel* L. | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| DQ822471 *Piper betel* L. | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| AY713471 *Piper betel* L. | A | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| DQ822470 *Piper betel* L. | . | . | . | . | . | . | . | . | . | . | . | . | . | – | . | . | . | . | . | . | . | . | . | . | . |
| *DQ075224 *Solanum melongena* L. | . | AT | T | – | C | GC | GGCT | A | TT | G | – | G | C | G | C | AG | A | – | CCTA | CA | A | GC | A | – | TGC |

Our isolates=*; Identical= .; Internal deletion = –; Substitution mutations are underlined.

[Continuted

ITS2 region continued

| | 338, 339 | 342-344 | 346, 349,350 | 352 | 354-356 | 358, 359 | 361-365 | 367, | 370, 371 | 374-376 | 379, 381 | 383, 384 | 386, 388 | 390, 391 | 393 | 396-400 | 401, 403 | 405, 08,410 | 414-420 | 423, 424 | 431, 33,435 | 436-440 | 441-472 | 473 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DQ075220 *Sesamum indicum* L. | AT | TTG | CCG | C | GAA | A | GCACT | A | G | GTT | TC | GC | GTG | CG | G | GGACT | GT | ACT | | TG | GAT | GCTT | | – |
| *DQ075222 *Piper betel* L. | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| *DQ075218 *Psidium guajava* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| *DQ124716 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T |
| *DQ910796 *Piper nigrum* L. | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| DQ822473 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| DQ822474 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| DQ822472 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| DQ822471 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| AY713471 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| DQ822470 *Piper betel* L | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| *DQ075224 *Solanum melongena* L. | TC | CCT | ATA | – | AGT | T | TTTTC | T | A | CCA | CT | AA | ACT | AA | – | ACGT *G* | TG | GGA | – | CA | ACA | ATAG | – | – |

414-420 = TCG GTG G. 441-472=TGC TGT TGC GAA GTA GGG TGG CAG CTT CGGTT

Our isolates=*; Identical= .; Internal deletion = –; Substitution mutations are underlined.

**Table 2b.** Sequence diversity analysis of the Indian *Phytophthora capsici* isolates

| | ITS1 | | | | | | | | | | | ITS2 | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DQ124722 *Capsicum annuum L.* | 91 | 94 | 103 | 124 | 129 | 130 | 143 | 170 | 174 | 175 | 176 | 74 | 75 | 88 | 92 | 93 | 175 | 215 | 228 | 270 | 318 | 357 | 358 | 362 | 399 | 402 | 404 | 412 | 413 | 427 | 428 |
| | C | G | T | A | T | – | T | G | T | A | G | T | G | G | A | C | – | A | G | A | T | G | A | G | C | T | – | T | T | A | A |
| *DQ1244720 *Capsicum annuum L.* | . | . | . | . | . | A | . | . | . | . | . | . | . | . | G | . | – | . | . | . | . | . | G | . | – | – | – | . | . | . | . |
| *DQ124718 *Piper betel* L. | . | . | . | . | . | – | . | – | . | . | . | . | . | . | G | . | – | . | . | . | . | . | G | C | . | . | G | . | C | . | . |
| AF266787 *Piper nigrum L.* | . | . | . | . | . | – | . | – | – | – | – | . | . | . | . | . | — | . | . | . | . | . | G | . | . | . | . | . | . | . | . |
| AF467085 *Theobroma cacao L.* | T | . | . | T | A | – | C | – | . | . | – | G | T | . | G | T | T | T | A | T | G | A | . | . | . | . | – | . | . | – | – |
| DQ464046 *Areca catechu L.* | T | A | C | T | A | – | . | – | – | – | – | G | T | A | T | . | – | T | . | T | G | . | . | . | G | – | – | C | . | . | . |

Out isolates = *; Identical = . ; Internal deletion = –; Substitution mutations are underlined.

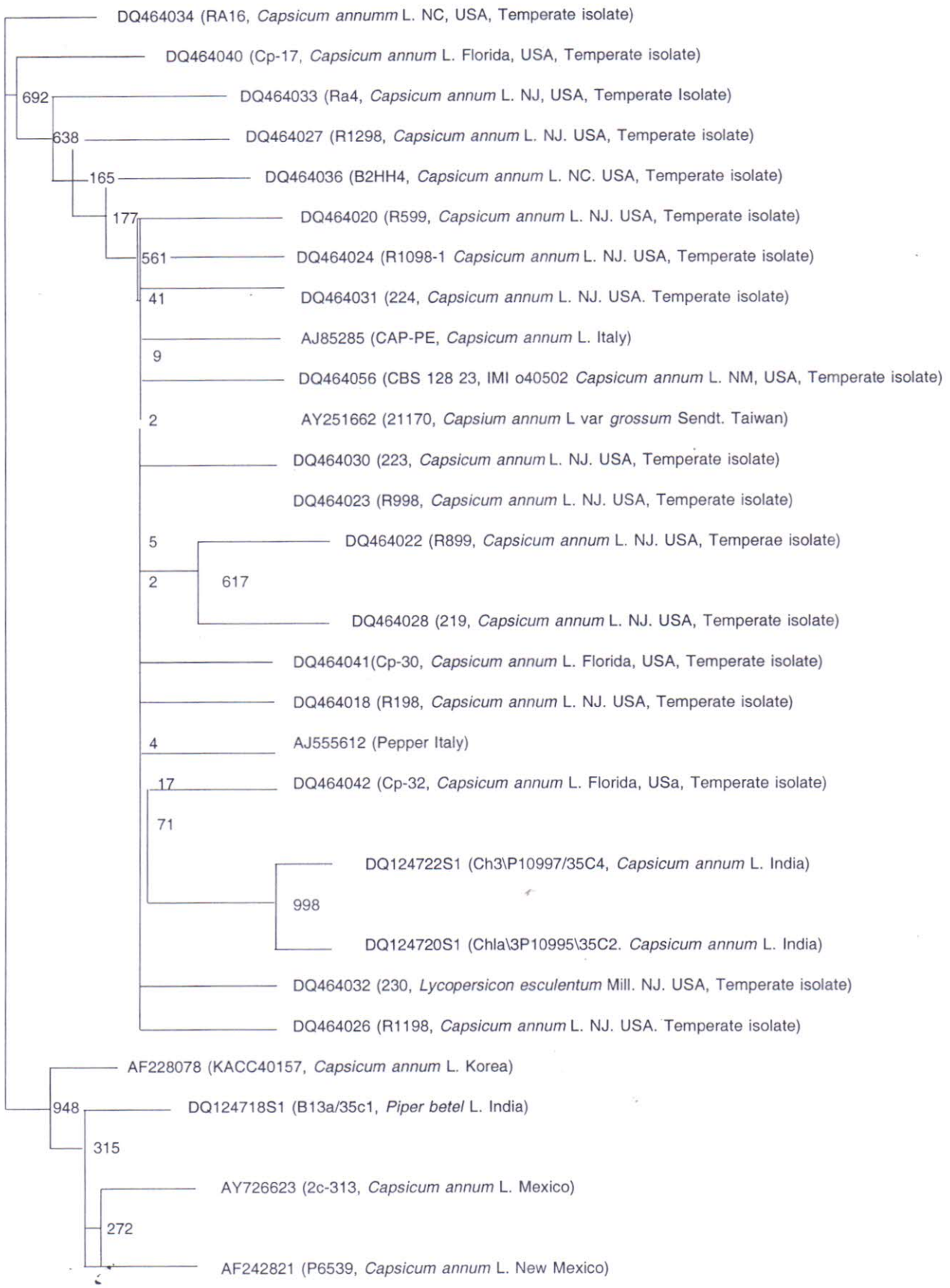


**Fig 2a.** Relationship of *Phytophthora capsici* isolates based on neighbour-joining analysis of rDNA ITS sequences.

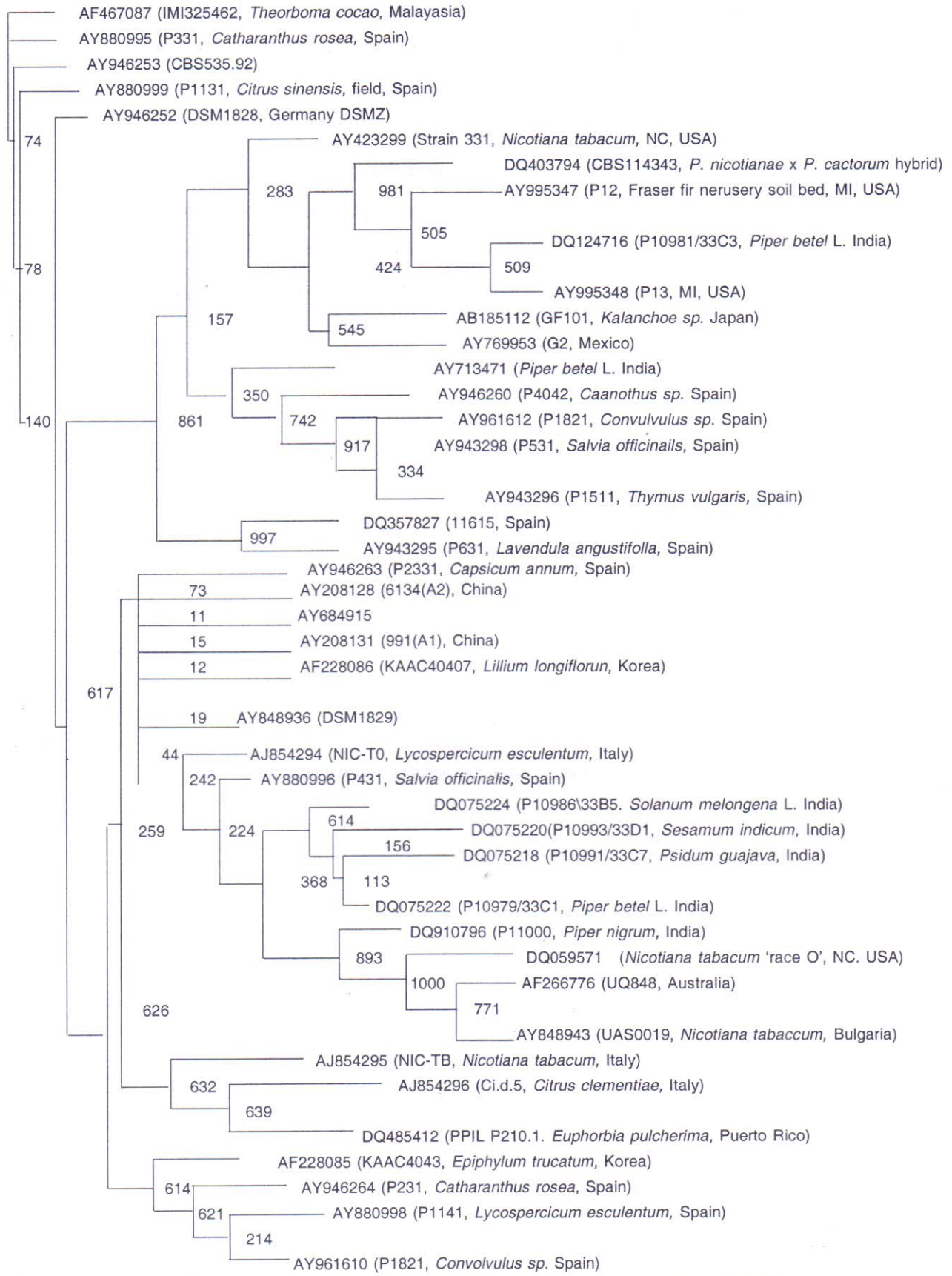


**Fig. 2b**. Relationship of *Phytophthora nicotianiae* isolates based on neighbour-joining analysis of rDNA ITS sequences.

[Continued

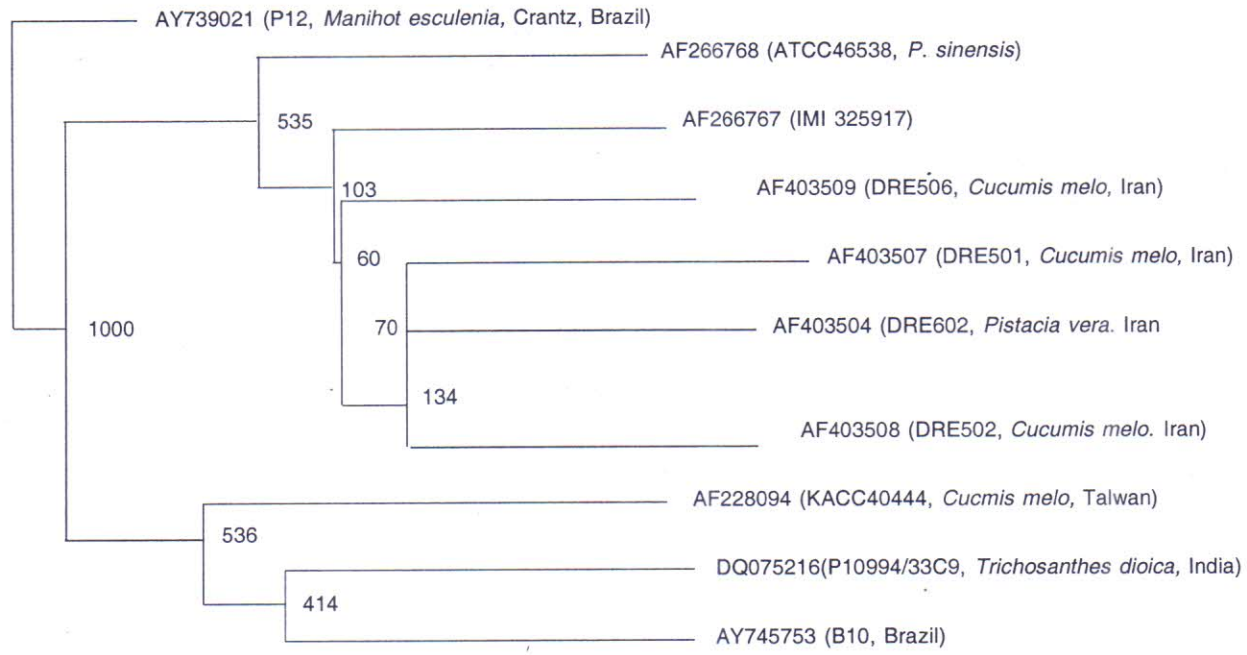


**Fig. 2c.** Relationship of *Phytophthora melonis* isolaes based on neighbour-joining analysis of rDNA ITS sequences.

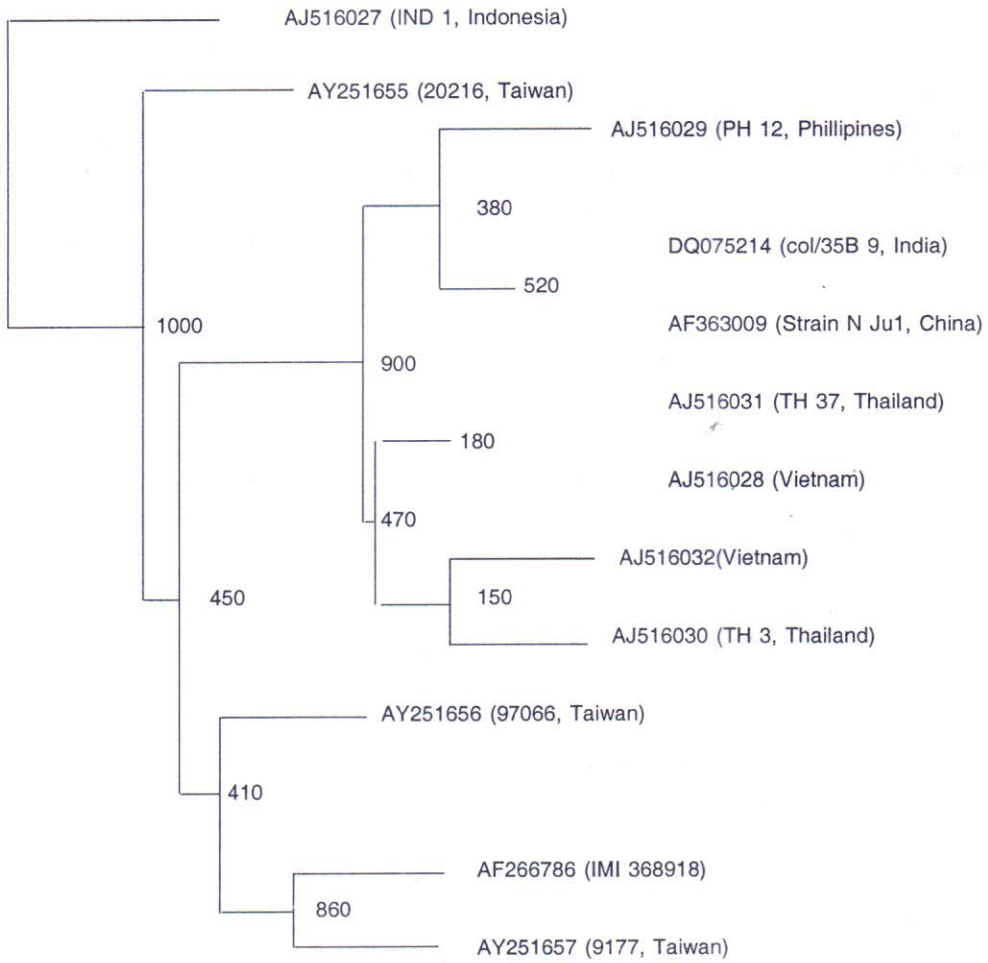


**Fig 2d**. Relationship of *Phytophthora colocasiae* isolates based on neighbour-joining analysis of rDNA ITS sequences.

variable regions than ITS1 of *P. nicotianae* with the converse being true for ITS2 region in these two species.

The unrooted NJ trees of *P. melonis* (Fig. 2c) clustered the isolates into 2 distinct brances (bootstrap value 100%) and our Indian isolate grouped along with the Taiwanese (KAACC40444) and Brazilian isolate (B10). *P. nicotianae* isolates (Fig. 2b) grouped into 3 distinct branches (bootstrap value 62%) with our Indian isolates from guava (P10991/33C7), betelvine (P10979/33C1), sesame (P10993/33D1), brinjal (P10986/33B5) and blackpepper (P11000) were on one branch and the other betelvine isolates [our (P10981/33C3) & the NBRI, Lucknow] on a distinctly separate branch (bootstrap value 86%). *P. capsici* isolates predominantly from capsicum (Fig. 2 a) grouped into 2 distinct branches (bootstrap value 100%) with our Indian isolates from chilli [(P10997/35C4/Ch3) and (P10995/35C2/Ch1a)] on one along with mostly temperate US and Italian isolates (bootstrap value 69%) and our betelvine isolate (35C1/Bt3a) on the other along with Mexican and Korean isolates (bootstrp value 95%). *P.colocasiae* phylogenetic trees (Fig. 2 d) showed that the Indian isolate was distinct from the Taiwanese strains which were in turn closely related to the IMI 368918 type strain. The Indian isolate formed a distinct group (bootstrap values 90%) with the Philippine and Chinese strain with its other close relative being the Vietnamese and Thai strains.

## DISCUSSION

Sequence analysis and the phylogenetic trees showed that there were considerable intraspecific diversity in the polyphagous *P. nicotianae* in contrast to *P. capsici.* Presence and/ or dispersed spatio-temporal introduction of different clonal population in Eastern India in case of *P. nicotianae* could be a possible explanation, in addition if placement of *P. capsici* from different hosts (betelvine and chilli) in different branches of the NJ tree and also placement of *P. nicotianae* betelvine isolates [our (P10981/33C3) & the NBRI, Lucknow) in distinctly different branches (bootstrap value 86%) is taken into consideration, then perhaps a more radical explanation of the presence of some clonal host specific lineages for both *P. capsici* and *P. nicotianae* can be given, or may be both clonal populations as well as local host adapted lineages co-exist, but of course this needs further extensive study.

A very interesting observation was the clustering of *P. nicotianae x P. cactorum* hybrid (CBS114343), similarly the black pepper isolate (P11060) clustered in the same sub branch (bootstrap value 89%) as the 'race 0' US strain from tobacco. Plausible explanations would be possible only after further studies and sufficient samplings are done. This detection of diversity: *P. nicotianae* betelvine isolates from different geographic regions (P10979/ 33C1, P1098/33C3 & isolate from NBRI) are different as also the presence of both *P. capsici* (35C1) and *P. nicotianae* (P10979/33C1) on betelvine in the same geographical region among the Indian *P. nicotianae* and *P. capsici* isolates and the close similarity of *P. colocasiae* isolate (35B9) with other worldwide isolates, *P. melonis* (P10994/ 33C9) isolate with *Cucumis melo* from Taiwan and Brazilian isolates, have important implictions for devising and adopting control strategies used in other geographical locations for this region.

Thus the sequence analysis and NJ phylogenetic trees for the respective species (*P.* nicotianae, *P. capsici, P. colocasiae, P. melonis*) throw light for the first time on the molecular deversity of the Indian isolates in general, and isolates from eastern region of the country in particular, *vis a vis* with those around the globe.

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