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Molecular characterization of *Pestalotiopsis* spp. associated with tea (*Camellia sinensis*) in southern India using RAPD and ISSR markers

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The genus *Pestalotiopsis* is second most important fungal pathogen causing grey blight disease in tea plant (*Camellia sinensis*). Due to grey blight disease, total tea crop loss is estimated to be 17% in southern India. In the present study, 42 *Pestalotiopsis* isolates were collected from five different regions in southern India. Among them, 22 isolates showed diverse morphological characters like colour, size and length of conidia and virulence. Genetic diversity was studied for these 22 isolates using two molecular marker systems (RAPD and ISSR). In RAPD, a total of 255 loci were generated and all were polymorphic in nature and the band size ranged from 0.2 to 3.0 kb. In ISSR, 194 amplified loci were observed and all were polymorphic as like RAPD and the band size ranged from 0.25 to 3.2 kb. Using Jaccard's similarity coefficient matrix, highest similarity of 95.9% and 92.6% was observed between AP-8 and AP-9 isolates in both RAPD and ISSR markers, respectively. Lowest similarity was observed between AP-14 and NP-5 (8.8%) in RAPD matrix but in ISSR matrix lowest similarity (18.5%) was between AP-14 and EN-5. The UPGMA clustering of both methods was comparable. The results indicate that, within southern India, the diversity of *Pestalotiopsis* was high both morphologically and genetically.

Keywords: *Camellia sinensis*; *Pestalotiopsis,* RAPD, ISSR, phylogenetic, PCR, tea

Introduction

The genus *Pestalotiopsis* is cosmopolitan in distribution. It is a weak pathogen, and also occurs as an endophyte. Its species infect many economically important plants such as mango (*Pestalotiopsis mangiferae*), coconut (*P. palmarum*), rice (*P. versicolor*) and tea (*P. theae*), etc. *P. microspora* is a common endophyte isolated from many tropical and sub-tropical plants[1]. Agnihothrudu[2] reported five species of *Pestalotiopsis* in tea; of which *P. theae* and *P. longista* are the major species causing grey blight disease in tea. The crop loss due to grey blight was estimated to be 10-20% in Japan[3] and 17.0% in southern India[4].

Classification of this genus is a difficult task. Guha[5] and Nag Raj[6] classified the genus based on the morphology of conidia and on conidiogenesis. Steyaert[7] divided the genus into different sections based on the number of apical appendages. Sutton[1] reviewed the history of the genus *Pestalotia* and its relationship with *Pestalotiopsis* Stey. and *Truncatella* Stey. Molecular characterization has been proposed as a complementary tool to the morphological identification of microbial strains. Large number of molecular markers has enabled precise characterization of fungal strains. Among these, random amplified polymorphic DNA (RAPD) technique based on polymerase chain reaction is the simplest and fastest[8].

DNA polymorphism in fungus has been widely studied following the methods like restriction fragment length polymophisum (RFLP)[9], DNA sequencing[10] and large subunit rRNA sequencing[11]. In many cases, RAPD and inter simple sequence repeat (ISSR) techniques have been used for the identification of individuals in different populations and distinguishing isolates of various fungi[12-19]. As these techniques are simple, inexpensive and do not require any sequence information, they are widely followed in molecular studies[20]. In the present study, attempts were made to investigate the morphological variation and difference in virulence and genetic diversity among the isolates of *Pestalotiopsis* obtained from different tea growing regions of southern India.

Materials and Methods

Isolation of Pestalotiopsis

Grey blight infected leaves of tea were collected from different agro-climatic zones of southern India and from these *Pestalotiopsis* spp were isolated and purified in PDA medium[21]. Endophytic *Pestalotiopsis* spp were isolated from uninfected brown stalks of

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pencil thickness collected from healthy tea plants. The stalk segments were repeatedly washed and surface sterilized[22]. The disinfected segments were placed in plates containing water agar supplemented with streptomycin sulphate (100 mg L^{-1}). The plates were incubated for 10-15 d at 23°C, and the growing mycelial tips were sub-cultured and purified in PDA medium. The purified cultures were stored in PDA slants at 4°C and sub-cultured every three months.

Morphological Observations

Purified fungal isolates were grown on PDA plates at 23°C for 15-20 d. Conidia from conidiomata were separated and used for morphological studies. Water mount conidia were observed under microscope and morphological characters like, colour of median cells, length and width of conidia and length of apical appendages were recorded. About 50 conidia were observed for each isolate and their mean values were calculated (Table 1).

Table 1—Morphometric characters and virulence of *Pestalotiopsis* isolates

Code/region	Colour of median cells	Length of conidia (μ) [a]	Width (μ) [a]	Length of apical appendages (μ)[a]	Diameter of lesion (mm)[b]	Virulence
AP 1 (Anamallais)	Concolorus	22.2 - 27.2 (25.0)	4.8 - 7.0 (6.0)	12.5 - 18.0 (16.1)	12.2±1.5	HV
AP 2 (Anamallais)	Versicolorus	21.2 - 22.5 (22.1)	6.2 - 8.0 (7.5)	20.5 - 25.0 (23.3)	4.8±0.9	MV
AP 8 (Anamallais)	Concolorus	25.0 - 27.5 (25.7)	6.0 - 6.5 (6.2)	10.0 - 12.5 (11.3)	11.3±1.2	HV
AP 9 (Anamallais)	Versicolorus	30.0 - 31.0 (30.5)	6.5 - 7.5 (6.9)	25.0 - 26.0 (25.3)	10.9±1.2	HV
AP 14 (Anamallais)	Concolorus	29.5 - 31.5 (30.5)	5.5 - 6.5 (5.9)	25.0 - 27.5 (26.2)	10.4±1.0	HV
NP 2 (Nilgiris)	Versicolorus	32.5 - 35.0 (34.0)	6.0 - 7.5 (7.0)	25.5 - 30.5 (24.1)	6.2±1.2	V
NP 3 (Nilgiris)	Concolorus	28.0 - 32.5 (30.3)	5.0 - 6.2 (5.4)	21.0 - 25.0 (22.8)	4.2±1.2	MV
NP 5 (Nilgiris)	Concolorus	23.5 - 24.6 (24.1)	8.2 - 9.0 (8.6)	25.6 - 28.5 (27.1)	4.6±0.8	MV
NP 6 (Nilgiris)	Versicolorus	28.0 - 30.5 (29.3)	6.0 - 7.0 (6.2)	18.0 - 30.5 (25.8)	5.5±1.6	V
GUD 1 (Gudalur)	Versicolorus	22.0 - 25.0 (24.0)	6.0 - 7.0 (6.4)	22.5 - 25.0 (24.3)	6.2±1.2	V
GUD 3 (Gudalur)	Concolorus	24.0 - 27.5 (26.1)	5.5 - 7.0 (6.4)	28.5 - 30.5 (29.5)	7.4±1.4	V
GUD 5 (Gudalur)	Versicolorus	25.0 - 27.5 (22.0)	5.0 - 5.5 (5.2)	20.0 - 22.5 (21.1)	6.3±1.3	V
WP 1 (Wayanad)	Concolorus	35.0 - 41.0 (38.2)	6.8 - 7.5 (7.1)	27.0 - 35.0 (31.2)	8.2±1.7	V
WP 2 (Wayanad)	Concolorus	34.5 - 38.0 (36.6)	4.0 - 7.5 (6.3)	30.5 - 37.0 (32.8)	7.6±1.2	V
WP 3 (Wayanad)	Concolorus	32.6 - 39.5 (35.3)	6.5 - 7.5 (7.2)	21.8 - 25.0 (23.7)	5.4±1.3	V
VP 1 (Vandiperiyar)	Versicolorus	29.0 - 32.5 (30.0)	6.2 - 7.5 (6.7)	23.0 - 25.0 (23.7)	7.2±1.0	V
VP 3 (Vandiperiyar)	Versicolorus	28.5 - 32.5 (30.3)	7.5 - 8.5 (7.9)	25.5 - 30.5 (28.1)	7.8±1.0	V
VP 4 (Vandiperiyar)	Versicolorus	25.2 - 27.0 (25.4)	7.4 - 8.9 (8.2)	20.0 - 22.5 (21.3)	7.1±1.2	V
VP 6 (Vandiperiyar)	Versicolorus	28.5 - 31.0 (30.1)	5.0 - 6.5 (5.9)	35.0 - 38.0 (36.3)	8.2±1.2	V
En 1 (Vandiperiyar)	Concolorus	24.7 - 27.0 (25.6)	4.5 - 5.0 (4.8)	12.0 - 16.5 (14.2)	4.4±1.3	MV
En 4 (Vandiperiyar)	Versicolorus	23.5 - 27.5 (26.0)	7.0 - 8.5 (7.8)	28.0 - 30.5 (29.4)	4.8±1.0	MV
En 5 (Vandiperiyar)	Versicolorus	21.0 - 23.0 (21.9)	6.5 - 8.0 (7.3)	20.0 - 22.5 (21.1)	4.3±1.0	MV

a – range with mean value in parenthesis, b - ± SD, about fifty conidia were observed for each isolate.
V-virulent, MV-mildly virulent and HV-highly virulent.

Testing for Virulence of the Pathogen

Virulence of the isolates was tested on field grown tea clone UPASI-9. Mature healthy leaves of same age were wounded (3 mm) with a sterile scalpel and artificially inoculated with spore suspension in water (1×10^5 spores mL^{-1}) of *Pestalotiopsis* isolates. One hundred leaves were inoculated for each isolate. Twenty days after inoculation, diameter of the lesions was measured. Based on the size of lesion, the isolates were categorized as mildly virulent (<5 mm), virulent (5-10 mm) and highly virulent (>10 mm).

Extraction of Genomic DNA

The *Pestalotiopsis* isolates were grown on PDA medium for 5 d and the mycelia were scraped off from the surface of the medium for DNA extraction. Genomic DNA was extracted following a modified method of Doyle and Doyle[23]. Two hundred mg of the mycelia were ground with liquid nitrogen and 600 µL of cetyl trimethyl ammonium bromide (CTAB) extraction buffer (2% w/v CTAB, 100 m*M* Tris-HCl, 1.4 *M* $NaCl_2$, 20 m*M* EDTA, *p*H 8.0) in a 1.5 mL Eppendorf tube. The contents were incubated at 65°C in a water bath for 40 min with occasional swirling. Protein contamination was removed with ice-cold chloroform:isoamyl alcohol (24:1, V/V). After centrifugation at 12,000 rpm for 10 min at 4°C, the DNA was precipitated by adding 0.7 volume of iso-propanol and DNA pellet was collected by centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant was discarded and the DNA pellet was re-suspended by adding 200 µL of TE buffer (10 m*M* Tris-HCl and 1 m*M* EDTA, *p*H 8) followed by treating with 5 µL of RNase-A (10 mg/mL) and incubated at 37°C for 1 h. Then equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, V/V) mixture was added, mixed gently by inverting the tube and centrifuged at 12,000 rpm for 10 min at 4°C. The aqueous phase was transferred to a new Eppendorf tube; finally DNA was precipitated by adding 2.5 volumes of chilled absolute ethanol and incubated at –20°C for 30 min. The DNA pellet was collected by centrifugation at 12,000 rpm for 10 min at 4°C. The DNA pellet was washed two times with 500 µL of 70% ethanol and air-dried. The pellet was re-suspended in 100 µL of sterile distilled water and stored at –20°C till use. The quality and quantity of extracted genomic DNA was checked in spectrophotometer and 0.9 % agarose gel.

RAPD

RAPD amplification was generally followed by the method of Williams *et al*[24] in peltier thermal cycler (PTC-200, MJ Research, Inc., USA) using 10 decamer random primers (Table 2) with the following minor modifications. Each 25 µL reaction mixture contained 1 unit of *Taq* DNA polymerase, 0.2 m*M* each dNTP, 1X PCR buffer, 3 m*M* $MgCl_2$ (Bangalore Genei Pvt. Ltd. India), 10 pmole of primer (OPERON-Qiagen Company, USA) and approximately 50 ng of template

Table 2—List of primers used in the study along with their sequences and some characteristics of the amplification products in *Pestalotiopsis* isolates

Sl. No.	Primer code	Sequence (5' to 3')	Total No. of PCR Products	Size of PCR Product (bp)
1	OPA-01	CAGGCCCTTC	105	260 to 2300
2	OPA-02	TGCCGAGCTG	192	265 to 2000
3	OPA-03	AGTCAGCCAC	193	280 to 2200
4	OPA-04	AATCGGGCTG	117	350 to 2000
5	OPA-05	AGGGGTCTTG	181	254 to 3000
6	OPA-06	GGTCCCTGAC	77	423 to 1505
7	OPA-07	GAAACGGGTG	153	204 to 2340
8	OPA-08	GTGACGTAGG	152	210 to 1834
9	OPA-09	GGGTAACGCC	196	212 to 3012
10	OPA-10	GTGACGTAGG	211	302 to 2689
11	ISSR-01	(CT)8G	215	263 to 2213
12	ISSR-02	(GA)8C	295	250 to 1910
13	ISSR-03	(AC)8YT	222	271 to 2503
14	ISSR-04	(AC)8YG	196	268 to 2400
15	ISSR-05	(CT)8RG	165	286 to 2682
16	ISSR-06	(GT)8YR	77	607 to 1843
17	ISSR-07	(CT)8TG	125	287 to 2534
18	ISSR-08	(CT)8AC	114	350 to 2431
19	ISSR-09	(CT)8GC	112	252 to 2341
20	ISSR-10	(CA)7AC	121	283 to 3212

Y = C+T, R = A+G

genomic DNA. PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 45 cycles of denaturation at 94°C for 45 sec, annealing at 36°C for 1 min and extension at 72°C for 2 min followed by final extension at 72°C for 10 min. The amplified products were separated on 2% agarose gel using 0.5X TBE buffer followed by staining in ethidium bromide solution (1 µg/mL) and documentation was carried out by placing the stained gel on UV-Tran illuminator.

ISSR

Polymerase chain reaction was carried out in a peltier thermal cycler (PTC-200, MJ Research, Inc., USA) with 10 primers having dinucleotide repeat along with different anchor sequence at 3′ end (Table 2). Each 25 µL reaction mixture contained 1 unit of *Taq* DNA polymerase, 0.2 m*M* each dNTP, 1X PCR buffer, 2 m*M* $MgCl_2$ (Bangalore Genei Pvt. Ltd. India), 50 pmole of primer (Sigma, USA) and approximately 100 ng of template genomic DNA. Same PCR conditions were applied as described in the previous section except the annealing temperature, which was maintained at 45°C for 1 min. The amplified products were separated on 2% agarose gel using 0.5X TBE buffer followed by staining in ethidium bromide solution (1 µg/mL) and documentation was carried out by placing of the stained gel on UV-Tran illuminator.

Data Analysis

DNA bands obtained from both the marker systems were scored as present (1) or absent (0) in the entire sample. Only reproducible bands were scored. Molecular weight of each band was estimated using 1 kb DNA ladder (Fermentas Life Science, Germany) as a standard. Similarity coefficient matrix was constructed by calculating Jaccard's similarity coefficient values for each pairwise comparison between samples[25]. A dendrogram was generated from this matrix following unweighted pair group method for arithmetic average analysis (UPGMA).

Results and Discussion

A total of 42 isolates of *Pestalotiopsis* was obtained from different agroclimatic zones of southern India. Among these, 22 isolates having distinct colony characteristics and spore morphology were selected for detailed study. This included three endophytes (EN1, EN4 and EN5). The spore morphology of selected isolates is given in Table 1. The size of the spores widely varied between the isolates. The length varied from 21.85 µ to 38.2 µ and width from 4.76 µ to 8.6 µ. In general, spores from Gudalur were smaller in size and those from Wayanad were bigger. The length of apical appendages was as small as 11. 3µ in AP-8 (Anamallais) and as long as 36.3 µ in VP-6 (Vandiperiyar). Colour of the cells varied from concolourus to versicolorus (Table 1).

Field screening of *Pestalotiopsis* isolates for their virulence indicated that the pathogenic isolates were more virulent than the endophytes. The initial symptom with the purple ring formation around the inoculated region was noticed after 8 d. Characteristic grey blight symptoms with concentric zonations could be noticed within 12-15 d after inoculation. In general, the Anamallais isolates were more virulent compared to others (Table 1). The size of lesion produced on artificially inoculated leaves was highest in AP1 followed by AP8, AP9 and AP14. All these isolates were grouped under highly virulent category. On the other hand, lesion size was minimum (<5 mm) with endophytic isolates and thus grouped them under mildly virulent. Isolates from Wayanad, Gudalur and Vandiperiyar were categorized as virulent (Table 1). Nilgiris isolates also were mild in their virulence. The virulence study indicated that Anamallais region is most vulnerable to this disease followed by Wayanad, Gudalur, Vandiperiyar and Nilgiris. There was no correlation between the morphological variation among the isolates and their virulence. The variation exhibited by the isolates on their virulence could be due to the emergence of region specific virulent strains. Repeated attack by the pathogen in certain areas could be due to the emergence of virulent strains.

The 10 RAPD primers amplified 1517 bands. The RAPD profile generated with primer OPA-07 is presented in Fig.1a. All the bands were polymorphic in nature. The band size varied from 204 to 3000 bp, with an average of 151.7 bands per primer. There was no monomorphic band among the isolates, but when the isolates were differentiated on the basis of region there were many monomorphic bands. In the Jaccard's similarity coefficient matrix, a wide range (0.088 to 0.959) of similarity was noticed among them. The highest similarity was observed between AP-8 and AP-9, both the isolates were from the same region (Anamallais) and lowest similarity was between AP-14 and NP-5, which were isolated from different regions (Anamallais and Nilgiris).

In the case of ISSR, 1642 bands were amplified with 10 primers. The ISSR profile generated with primer

ISSR-05 is presented in Fig. 1b. All the bands were polymorphic in nature. The band size ranged from 250 to 3212 bp, with an average of 164.2 bands per primer. Like RAPD, there was no monomorphic band among the isolates, but when isolates were differentiated regionwise, there were many monomorphic bands. As per Jaccard's similarity coefficient, a wide range (0.185 to 0.926) of similarity was exhibited among the isolates. The highest similarity was observed between AP-8 and AP-9, similar to that of RAPD result. The lowest similarity was observed between AP-14 and EN-5, which were isolated from Anamallais and Vandiperiyar, respectively.

Clustering of these 22 isolates was carried out based on UPGMA dendrograms, which were generated on the basis of Jaccard's similarity coefficients matrix. In the RAPD based dendrogram (Fig. 1c), 19 isolates were grouped into three clusters (CL-1, CL-2 & CL-3) and remaining 3 isolates (AP-1, AP-14 & NP-5) were not included in any of the clusters (Fig. 1c). These 3 isolates were from Anamallais (AP-1 & AP-14) and Nilgiris (NP-5). CL-1 comprised of 14 isolates (AP-2, NP-3, NP-6, EN-5, GUD-1, GUD-3, GUD-5, NP-2, VP-4, EN-1, EN-4, VP-6, VP-1 & VP-3) where highest similarity (0.92) was found between EN-1 and EN-4, both of them were endophytes and from the same region (Vandiperiyar). The lowest similarity (0.29) was between VP-3 and EN-5, which were collected from different regions (Vandiperiyar & Gudalur, respectively). CL-2 comprised of 2 isolates AP-8 and AP-9, both were isolated from the same region. These 2 isolates expressed the highest similarity

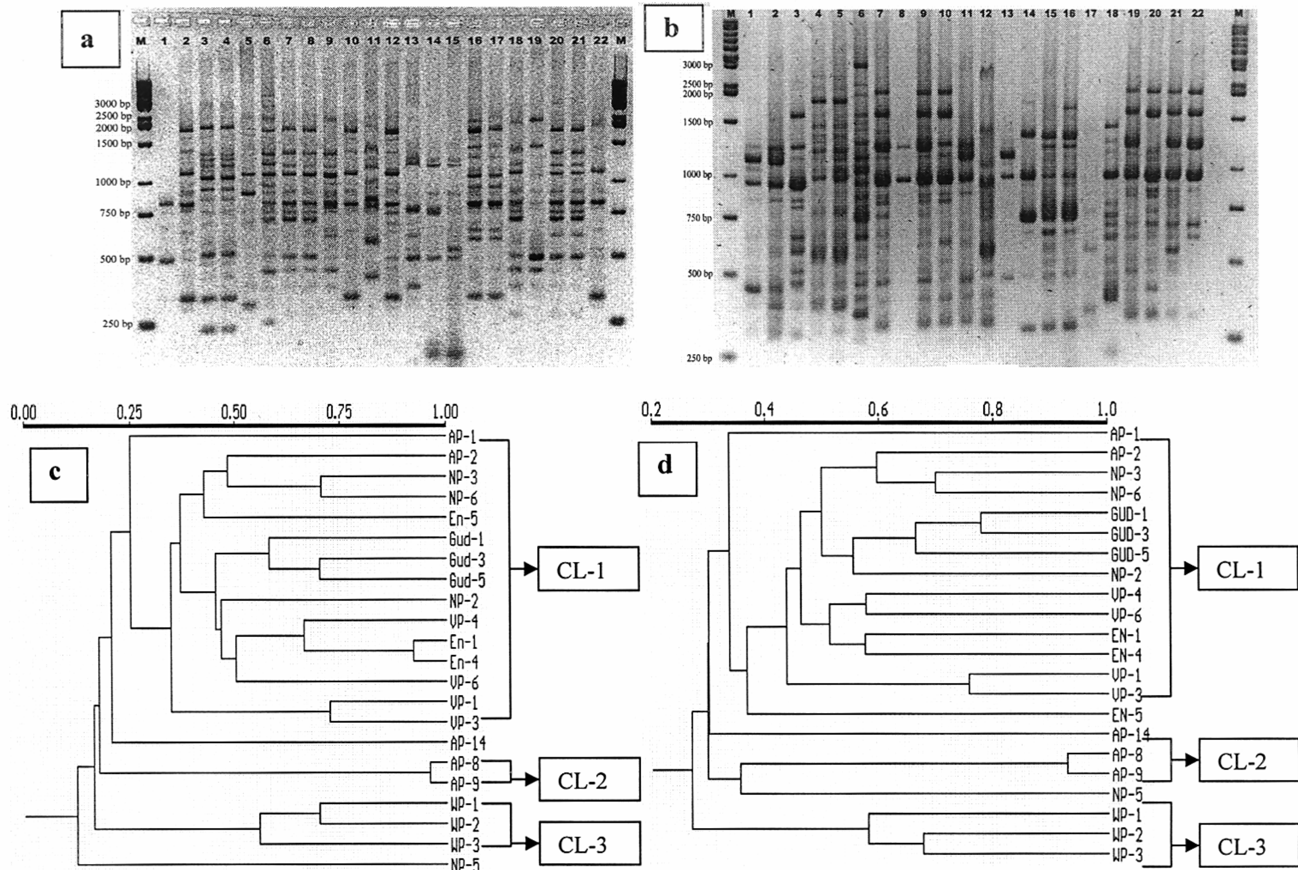

Fig. 1a —RAPD profile of 22 *Pestalotiopsis* isolates generated by primer OPA-07. Lanes 1 and 24: Molecular weight marker (1 kb, Fermantas), lanes 2-23: isolates AP1, AP2, AP8, AP9, AP14, NP2, NP3, NP5, NP6, GUD1, GUD3, GUD5, WP1, WP2, WP3, VP1, VP3, VP4, VP6, En1, En4 and En5, respectively; b ISSR profile of 22 of *Pestalotiopsis* isolates generated by primer ISSR-05. Lanes 1 and 24: Molecular weight marker (1 kb), lanes 2-23: isolates AP1, AP2, AP8, AP9, AP14, NP2, NP3, NP5, NP6, GUD1, GUD3, GUD5, WP1, WP2, WP3, VP1, VP3, VP4, VP6, En1, En4 and En5, respectively; c Dendrogram generated using UPGMA analysis demonstrating the relationship among *Pestalotiopsis* isolates (AP1, AP2, AP8, AP9, AP14, NP2, NP3, NP5, NP6, GUD1, GUD3, GUD5, WP1, WP2, WP3, VP1, VP3, VP4, VP6, En1, En4 & En5, respectively) based on RAPD data; & d Dendrogram generated using UPGMA analysis demonstrating the relationship among *Pestalotiopsis* isolates (AP1, AP2, AP8, AP9, AP14, NP2, NP3, NP5, NP6, GUD1, GUD3, GUD5, WP1, WP2, WP3, VP1, VP3, VP4, VP6, En1, En4 & En5, respectively) based on ISSR data.

(0.92) among all the isolates. CL-3 comprised of 3 isolates WP-1, WP-2 and WP-3, which were isolated from the same region (Wayanad). The highest similarity (0.7) was between WP-1 and WP-2 isolates and lowest (0.51) between WP-1 and WP-3.

In the ISSR based dendrogram (Fig. 1d), 19 isolates were grouped into three clusters (CL-1, CL-2 & CL-3) and remaining three isolates (AP-1, AP-14 & NP-5) were out grouped (Fig. 1d). CL-1 comprised of the 14 isolates (AP-2, NP-3, NP-6, GUD-1, GUD-3, GUD-5, NP-2, VP-4, VP-6, EN-1, EN-4, VP-1 & VP-3), where the highest similarity (0.78) was between GUD-1 and GUD-3. The lowest similarity (0.29) was between EN-5 and VP-3. CL-2 comprised of 2 isolates, AP-8 and AP-9. They exhibited the highest similarity (0.92), among all the clusters similar to that of the result from RAPD. CL-3 comprised of 3 isolates (WP-1, WP-2 & WP-3), which were isolated from the same region. The highest similarity (0.67) was between WP-1 and WP-2 isolates and the lowest similarity (0.51) was between WP-1 and WP-3. From the results it is clear that the clustering pattern is identical in both RAPD and ISSR.

The dendrograms revealed a fairly high degree of polymorphism among the samples collected from different regions, while those from same region showed more similarity. When the samples were compared regionwise, the 3 isolates of Anamallais were distributed in two different clusters and remaining 2 isolates (AP-1 & AP-14) stood independently in both RAPD and ISSR dendrograms. Likewise, out of 4 isolates of Nilgiris, 3 were grouped in one cluster and 1 isolate remained apart in both the dendrograms. All the isolates from Gudalur and Vandiperiyar were grouped in one cluster along with isolates from Anamallais and Nilgiris while all the isolates from Wayanad formed a separate group in both the dendrograms. Among the 3 endophytic isolates 2 (EN-1 & EN-4) were grouped in one cluster and remaining one (EN-5) grouped with a different cluster in both the cases.

In this study, a moderate degree of genetic diversity was noticed among the samples of *Pestalotiopsis* collected from different tea growing regions of southern India. More genetic diversity was noticed among the regions than within the regions. Similar observations on genetic diversity have been reported in many other fungi. Li *et al*[26] have studied the genetic diversity of western gall rust fungus (*Endocronartium harknesii*) collected across western and central Canada from two host pine species. Most of the genetic variation was found between the two host species. Within the host species, variation was more among the geographically distant locations than within location. Similarly, Saleh *et al*[27] observed genetic variability in the population of *Cephalosporium maydis* which was more with lineage and geographic region. Diversity among pathotypes had a correlation with the cultivars[28]. Increased variation in pathogens is attributed to the diversified agroecosystem. In the present study, there was no correlation between the morphological characteristics of the spores and genetic variability. Samples, which were grouped together on morphological characters, were grouped in different clusters on genetic analysis. This indicated that the spore size/morphology has little or no influence on grouping of samples on genetic analysis. Similarly, Khalil *et al*[29] could not find any clear cut relationship between RAPD profile and pathogenicity and geographic origin for *Fusarium* spp.

Advent of DNA based molecular methods helped to study inter- and intra-specific variation in filamentous fungi. In most of the phylogenetic studies of fungal systems only single method was followed[12-19] of which the RAPD method was the most popular. In higher plants, RAPD and ISSR methods were used for phylogenetic study[30]. In the present study, we have used these two methods for validating the techniques in the phylogenetic studies in fungal system. Clustering of isolates in both the methods was comparable. Absence of monomorphic bands indicated wide genetic difference between regions. This genetic diversity may have developed due to simple mutation, genetic drift or selection. Other mechanisms like horizontal gene transfer between *Pestalotiopsis* and its host and evolution resulting from natural and stress induced transposition may also be possible. Similar mechanism had been suggested for genetic diversity of other pathogenic fungi[19,31,32]. On the basis of the present study, it is concluded that moderate diversity of *Pestalotiopsis* present in the south Indian tea growing regions, which are genetically heterogeneous and special attention is required to contain this disease in Anamallais region, either to develop tolerant clone or to develop potential chemical/biological control agent, where all the isolates are highly virulent.

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