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ORIGINAL ARTICLE

Morphological and molecular diversity of endophytic *Colletotrichum gloeosporioides* from tea plant, *Camellia sinensis* (L.) O. Kuntze of Assam, India

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Abstract Thirty isolates of endophytic fungi were isolated from healthy asymptomatic leaves of tea plant (*Camellia sinensis*) and identified morphologically based on colony morphology, spore shape and size, growth and sporulation rate. Internal transcribed spacer r-DNA sequence analysis supported for molecular identification of all the isolates. Based on morphological and molecular characteristics the isolates were identified as *Colletotrichum gloeosporioides*. Variations on colony morphology which included the production of conidial masses, led to divide the isolates into different groups. Variations on spore size, growth rate and sporulation rate were exhibited by all the isolates. With RAPD molecular markers, genetic variations among the thirty isolates were observed. Genetic variations and relatedness among the thirty isolates were analyzed with UPGMA phylogram using NTSYS program. Two major groups were obtained among the thirty isolates. Group I comprised of 16 isolates which included three sub groups (Ia, Ib and Ic) and Group II constituted fourteen isolates and it also had three sub groups (IIa, IIb and IIc). A partial co-relationship among the isolates was established on the basis of morphological and molecular based clustering.

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1. Introduction

More than 700 species of *Colletotrichum* comprise the genus *Colletotrichum corda* [36] having similar morphological characteristics which may be endophytic, pathogenic and saprophytic in nature [16,30,32]. Tea plant *Camellia sinensis* (L.) O. Kuntze is a cash crop in North-East India and is reported to harbor by different sp of endophytic fungi. *Colletotrichum* sp., which is a well known pathogen causing serious disease 'anthracnose' in some crops like cereals, coffee and legumes [2,17] and on tropical fruits like banana, avocado, papaya, coffee, passion fruits and others [24] and is very frequently isolated as endophytic fungus from the tissues of healthy leaves and branches of tea plants [34,35]. Apart from tea plant, *Colletotrichum gloeosporioides* was also commonly isolated as endophytic fungus from a range of plant species [38,4,6,30,10,29].

Initially *Colletotrichum* species were identified based on morphological features like optimal temperature for growth of the fungi, growth rate, colony color, size and shape of conidia, presence or absence setae [51,46]. Sole morphological criteria are not adequate for proper identification of *Colletotrichum* species because of environmental influences on morphological stability and existence of intermediate form [30], which led to focus on molecular techniques that provided alternative method for taxonomic studies, and, are important tools in solving the problems for species delimitation [20]. With the help of morphology and multilocus phylogeny many *Colletotrichum* species have been accurately identified [44,31,55]. For successful identification of the *Colletotrichum* species, molecular phylogeny with combination of morphological and cultural characteristics, physiology and pathogenicity tests were suggested [7,12]. Internal transcribed spacer (ITS) region which is present between the smaller and larger subunits of ribosomal DNA (rDNA) is recently used as an identification and detection tool for the fungi [33,11] and for *Colletotrichum* sp. [9].

Genetic variability within the species or among the isolates of a species has been studied since past decades with different molecular markers like Restriction Fragment Length Polymorphism [3], simple sequence repeat [18], inter simple sequence repeat [56], amplified fragment length polymorphism [52] and Random Amplified Polymorphic DNA [40]. In our studies we also focused our attention on genetic diversity of endophytic *Colletotrichum* sp. which exhibited distinct morphological characteristics. Due to some advantages of RAPD marker like simple handling nature, experimental conditions are very simple, do not require species specific probe libraries or hybridization step [50] and its rapid, quick and accurate result, we selected RAPD markers in our research experiment.

The objective of our study was to isolate the endophytic fungi from tea plant, to study their morphological characteristics and variations, to identify them and to analyze their genetic variations with molecular marker.

2. Materials and methods

2.1. Isolation of endophytic fungi

For isolation of endophytic fungi four districts of Assam, North East India were selected. Isolation was done from healthy and mature leaves of *C. sinensis*. The samples were processed within 24 h of collection and isolation was done according to Petrini and Dreyfuss [27,28]. The leaves were cut into small pieces (1 cm^2), washed in running tap water followed by 96% alcohol treatment for 30 s, washed with sterile distilled water for 30 s followed by sodium hypochloride NaOCl (15%)–distilled water (1:3) treatment for 5 min, treated with 96% alcohol for 30 s and finally washed with sterile water for 1–2 min followed by drying. Surface sterilization was followed as per the protocol described by Petrini and Dreyfuss [27,28]. It was then kept in Petriplates containing PDA media and kept in incubator at 28 °C at inverted position for 5–6 days. As the hyphal growth appeared, it was finally transferred to PDA slant for further processing and PDA slant is prepared in test tube which helps to store the fungi for a long period.

2.2. Morphological and cultural characteristics of endophytic fungi

The isolates were inoculated on PDA (media) containing plates and allowed to grow in an incubator at 28 °C for 6–8 days. Morphological studies were carried out when the mycelium of each isolate occupied the whole Petri plate. Morphological characteristics included macroscopic and microscopic characteristics. Macroscopic characteristics included the colony morphology and growth rate of the fungal isolates and microscopic characteristics included the conidial shape and size and rate of sporulation. Sporulation rate was studied by preparing spore suspension. Spores were counted by taking 9 µl cell suspensions in hemocytometer which was placed under a microscope.

2.3. Amplification of ITS-rDNA for molecular identification

Genomic DNA of all the isolates of endophytic fungi was extracted using Nucleopore gDNA Fungal Bacterial Mini Kit (Genetix, India) as per the manufacturer's instructions. For extraction of genomic DNA, each fungal isolate was grown on Potato Dextrose Broth which is a liquid medium and genomic DNA was isolated from the harvested mycelium. For amplification of ITS-rDNA, the whole genomic DNA was amplified in a thermocycler (Gen Amp 9700, Applied Biosystem, US) using primer pair ITS1 (TCCGTAGGTGAACCT GCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [53] which allow to amplify the ITS region of the fungal species. The conditions for Polymerase Chain Reaction for ITS-rDNA amplification was 95 °C for 5 min, 35 cycles of 94 °C for 45 s, 60 °C for 30 s, 72 °C for 45 s and final 72 °C for 5 min. The product size was approximately 575 bp which was visible in 1.2% agarose gel under UV light. The PCR product of each isolate was purified using PCR purification kit (Fermentas, Lithuania) as per the provided protocol. Sequencing of the PCR product was done by a Sanger's Dideoxy method on applied Biosystem 3730XL (Bioloink, New Delhi, India). The sequence of each isolate was subjected to BLAST search (http://wwwncbinlmgov/BLAST) with NCBI database [1]. All the sequences were aligned with representative sequences in the NCBI database using CLUSTAL W [48] and employing MEGA 52 software, the phylogenetic analysis of the alignment was performed with Maximum Likelihood Method.

2.4. RAPD analysis of the isolates

Twenty RAPD primers of OPC decamer series (OPC1–OPC20) were screened to obtain informative and reproducible RAPD primers for the 30 isolates of tested endophytes. Amplification reaction was carried out in a total volume of 25 µl reaction mixtures containing 1 µl DNA (23–25 ng), 10× buffer (2.5 µl), 25 mM $Mgcl_2$ (2.5 µl), 10 mM dNTPs (0.5 µl), 10 pm/µl RAPD primer (2.5 µl) and 1 µM Taq DNA polymerase (Fermentas, Lithuania). The conditions for Polymerase Chain Reaction for RAPD amplification was 95 °C for 5 min, 45 cycles of 95 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min and final 72 °C for 8 min. The amplified products were separated in 1.5% agarose gel at constant 80 V in 1× TAE buffer. A binary matrix was generated for all the bands present in each strain, using '1' for the presence of an amplicon, and '0' for its absence. The computer program numerical taxonomy and multivariate analysis system NTSYS-PC (version 170) was used to analyze the binary matrix of all the 30 isolates of the tested endophytes. Jaccard Similarity Coefficient was clustered and unweighted pair-group method with arithmetic average (UPGMA) was selected to generate a dendogram using the SAHN clustering program.

3. Result

3.1. Isolation and identification of endophytic fungi

30 endophytic isolates were obtained from healthy and mature leaves of *C. sinensis* of Assam, India. Based on colony morphology, conidial shape and size, all the 30 isolates were identified as *C. gloeosporioides* [30]. Distinct morphological characteristics were observed among the isolates. All the 30 isolates were broadly classified into two groups based on the production of conidial masses on the fungal colony (Table 1). Group I contained 18 isolates (CgloTIN01, CgloTIN02, CgloTIN05, CgloTIN06, CgloTIN07, CgloTIN08, CgloTIN09, CgloTIN10, CgloTIN11, CgloTIN14, CgloTIN15, CgloTIN17, CgloTIN18, CgloTIN20, CgloTIN22, CgloTIN23, CgloTIN25 and CgloTIN26) which had white mycelium, white to black mycelium or white to pale grey mycelium without any conidial masses on the fungal colony. Group II contained 12 isolates (CgloTIN03, CgloTIN04, CgloTIN12, CgloTIN13, CgloTIN16, CgloTIN19, CgloTIN21, CgloTIN24, CgloTIN27, CgloTIN28, CgloTIN29 and CgloTIN30) which also had white mycelium, white to black mycelium or white to pale grey mycelium with orange conidial masses near the inoculums point or around the fungal colony.

Each isolate of the two groups had the spore of cylindrical shape. The size of the spore was almost similar for every isolate of the two groups. Isolates of the two groups can't be differentiated based on growth rate and sporulation rate. Growth rate for Group I *C. gloeosporioides* isolates ranged from 64 mm/d to 13 mm/d. In Group II *C. gloeosporioides* isolates, the growth rate also ranged from 54 mm/d to 13 mm/d. Rate of sporulation of each isolate was also quite varied for the two groups (Table 1).

3.2. Analysis of rDNA internal transcribed spacer (ITS) sequence

With molecular identification tool like analysis of ITS1-58S-ITS2 sequence and partial sequence of 18S and 28S rDNA, all the thirty isolates were further confirmed as *C. gloeosporioides*. The total size of ITS region for every isolate was varied from 570 to 600 bp and the size of amplified fragment was compared with the marker which was 100 bp. The amplified products of ITS regions were sequenced and analyzed. The sequenced isolates with accession number along with morphological designation are listed in Table 2. The evolutionary history was inferred using the Neighbor-Joining method [41]. 1000 maximum bootstrap replicates were performed and the tree was drawn to scale, with branch lengths in the same unit

Table 1 Cultural characteristics of thirty endophytic isolates of *Colletotrichum gloeosporioides*.

Group I (mycelium without conidial masses)				Group II (mycelium with conidial masses)			
Isolates	Size of spore (µm)	Growth rate (days)	Sporulation rate (spore/ml)	Isolates	Size of spore (µm)	Growth rate (days)	Sporulation rate (spore/ml)
CgloTIN01	14.4–3.6	11	23×10^4	CgloTIN03	10.05–2.57	13	102×10^4
CgloTIN02	13.11–4.3	10	570×10^4	CgloTIN04	12.8–3.7	9.2	131×10^4
CgloTIN05	13.59–3.92	12.5	30×10^4	CgloTIN12	15.01–4.25	6	72×10^4
CgloTIN06	15.45–4.09	12.5	9×10^4	CgloTIN13	12.4–2.96	5.4	1052×10^4
CgloTIN07	13.2–4.8	12.5	85×10^4	CgloTIN16	13.86–4.16	7	1159×10^4
CgloTIN08	10.5–3.62	12.5	3×10^4	CgloTIN19	13.11–3.61	12	1277×10^4
CgloTIN09	12.35–4.5	10.7	139×10^4	CgloTIN21	10–4.1	13	41×10^4
CgloTIN10	15.7–4.4	6.4	154×10^4	CgloTIN24	15.6–3.3	13	622×10^4
CgloTIN11	13.1–3.3	10	252×10^4	CgloTIN27	12.02–3.62	11	1104×10^4
CgloTIN14	11.52–4.44	7	12×10^4	CgloTIN28	12.03–3.66	12.5	1480×10^4
CgloTIN15	13.9–3.2	8	10×10^4	CgloTIN29	10.5–3.9	10	7×10^4
CgloTIN17	13.8–3.7	10	1350×10^4	CgloTIN30	12.21–2.98	12.5	525×10^4
CgloTIN18	14.4–4.7	10	182×10^4				
CgloTIN20	11.34–3.62	7	885×10^4				
CgloTIN22	12.5–3.5	7	1512×10^4				
CgloTIN23	14.2–3.3	11	43×10^4				
CgloTIN25	13.9–4.6	7.8	13×10^4				
CgloTIN26	13.74–3.32	10	321×10^4				

Table 2 Isolates of *Colletotrichum gloeosporioides* and their NCBI, GenBank accession number.

Isolates ID	GenBank accession number
CgloTIN01	KF053197
CgloTIN02	KF053198
CgloTIN03	KF053199
CgloTIN04	KF053200
CgloTIN05	KF053201
CgloTIN06	KF053202
CgloTIN07	KJ676453
CgloTIN08	JX131348
CgloTIN09	KJ676454
CgloTIN10	KJ676455
CgloTIN11	JX131349
CgloTIN12	KJ676456
CgloTIN13	KJ777826
CgloTIN14	KJ676457
CgloTIN15	KJ777828
CgloTIN16	KJ777829
CgloTIN17	KJ676458
CgloTIN18	KJ777831
CgloTIN19	KJ676459
CgloTIN20	KJ777830
CgloTIN21	KJ676460
CgloTIN22	KJ777827
CgloTIN23	KJ676461
CgloTIN24	KJ676462
CgloTIN25	KJ676463
CgloTIN26	KJ777825
CgloTIN27	KJ676464
CgloTIN28	KJ676465
CgloTIN29	KJ777823
CgloTIN30	KJ777824

as those of the evolutionary distances used to infer the phylogenetic tree. The phylogenetic tree was constructed using Kimura 2-parameter [15] method and the analysis involved 33 nucleotide sequences.

The thirty endophytic isolates of *Colletotrichum* sp. showed 98–100% similarity with the representative reference *C. gloeosporioides* isolates published in NCBI GenBank database. The phylogenetic analysis of these isolates divided them into two groups. Group I constituted 23 isolates, which were clustered with the reference *C. gloeosporioides* isolates (accession No. GQ424104 and GQ424105) from NCBI GenBank with high bootstrap support (100%). Group II comprised of seven isolates and clustered with *Colletotrichum kahawae* (accession No. JN715845) from NCBI GenBank (Fig. 1).

3.3. Molecular characterization through RAPD analysis

Using RAPD markers, genetic variation was detected among the thirty isolates of *C gloeosporioides*. Only six primers of OPC decamer series which included OPC2, OPC5, OPC6, OPC8, OPC11 and OPC13 were selected as they produced informative and specific RAPD profiles among the 20 primers used in RAPD analysis. Sufficient numbers of polymorphic bands were obtained from all the six primers, although they produced some common bands among the isolates. A total of 84 amplicons were generated from thirty isolates of *C. gloeosporioides* and an average of 12 amplicons were produced by each primer. The amplified fragments were compared with Lambda DNA/EcoRI + HindIII marker and a range of amplicons were found to be 125–2027 bp.

UPGMA phylogram was constructed using NTSYS and the dendogram was used to analyze the relatedness and genetic variations among the 30 isolates which were isolated from the leaves of *C. sinensis*. Genetic similarity coefficient ranged from 066 to 1 (Fig. 2). Two major groups were obtained among the isolates (Fig. 3). Group 1 subdivided into three subgroups (Ia, Ib and Ic) which included 16 isolates and showed 68–72% similarity. Two isolates (CgloTIN05 and CgloTIN13) within subgroup IIb showed 100% similarity. Group II also categorized into three sub groups (IIa, IIb and IIc) which comprised of 14 isolates and exhibited 70–80% similarity.

4. Discussion

The result from our studies revealed that the *C. gloeosporioides* harbor as endophytic fungi on the leaves of tea plant. Previous report also revealed that *C. gloeosporioides* was also frequently isolated as endophytic fungi from tissues of healthy leaves and branches of tea plant [34]. Though the leaves of tea plant were only the sole source of isolation tremendous variations on morphological characteristics, i.e., colony color, growth rate and rate of sporulation were observed among the isolates. A previous research on *C. gloeosporioides* indicated that they were also classified into three groups based on morphological and cultural characteristics which were isolated from banana, ginger and *Eupatorium thymifolia* and sub grouping of those isolates indicated the complexity of the species [30]. We also divided our isolates into two groups based on the production of conidial masses on the fungal colony and we did not find any difference on spore size, growth rate and sporulation rate of the isolate of the two groups. Hence, it can be concluded that spore size, growth rate and sporulation rate are not dependent on the production of conidial masses on the fungal colony.

Analysis of ITS-rDNA sequences divided all the thirty isolates into two groups. One group contained twenty-three isolates which were clustered with two r-DNA ITS sequence of *C. gloeosporioides* (GQ424104 and GQ414205) published in NCBI GenBank database. Another group contained seven isolates and clustered with *C. kahawae* (JN715845) published in NCBI GenBank database. Previous studies reported a close genetic relationship between *C. gloeosporioides* and *C. kahawae* when sequences of ITS r-DNA were analyzed which differed from each other only for two to three bases, i.e., 98.8–98.2% [30]. *C. gloeosporioides*, *C. kahawae* and *Colletotrichum fragariae* were not suggested to be considered as separate species due to their closeness on molecular analysis [9,5,22]. High degrees of molecular similarities were found among *C. gloeosporioides*, *C. fragariae* and *C. kahawae* isolates upon analyses of ribosomal and mitochondrial sequences using Restriction Fragment Length Polymorphism Tool [45]. We also agree with the above previous research and would like to conclude that all the isolates of our studies belong to *C. gloeosporioides* on the basis of morphological characteristics and molecular analysis.

The purpose of our research was to study the morphological and genetic variability among all the isolates of *C. gloeosporioides* and to establish possible relationship of

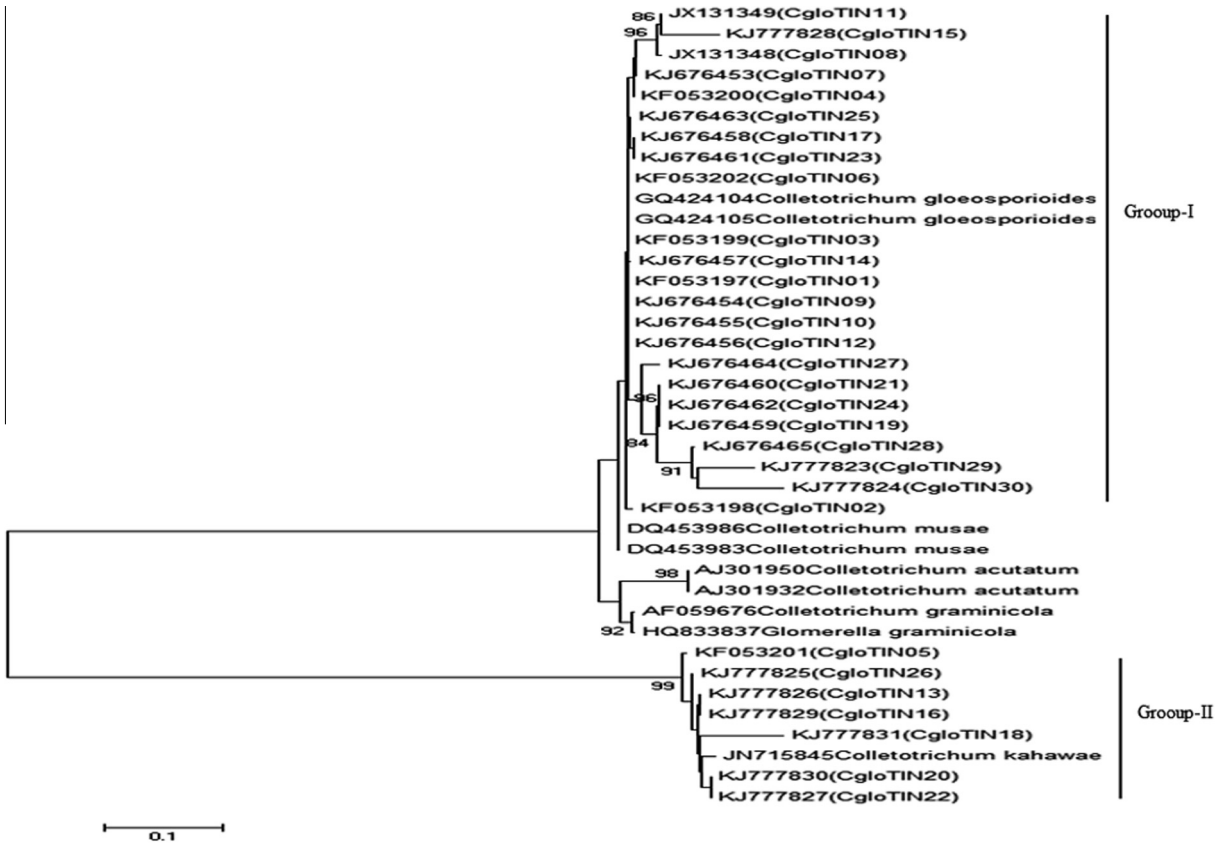

Figure 1 The phylogenetic tree of ITS sequences of 30 isolates of *C. gloeosporioides* along with different isolates of *Colletotrichum* sp. from NCBI was inferred using the Neighbor-Joining method (MEGA 5.2). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown below the branches. The evolutionary distances were computed using the Kimura-2 parameter method.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30
C1	1.00																													
C2	0.68	1.00																												
C3	0.75	0.77	1.00																											
C4	0.56	0.67	0.71	1.00																										
C5	0.69	0.67	0.73	0.73	1.00																									
C6	0.67	0.69	0.76	0.71	0.71	1.00																								
C7	0.67	0.69	0.71	0.65	0.76	0.63	1.00																							
C8	0.71	0.73	0.72	0.67	0.82	0.64	0.90	1.00																						
C9	0.67	0.67	0.71	0.68	0.81	0.65	0.89	0.90	1.00																					
C10	0.61	0.61	0.65	0.68	0.71	0.60	0.81	0.77	0.84	1.00																				
C11	0.64	0.61	0.65	0.65	0.76	0.55	0.76	0.82	0.84	0.86	1.00																			
C12	0.67	0.59	0.71	0.65	0.84	0.65	0.73	0.77	0.78	0.76	0.81	1.00																		
C13	0.69	0.67	0.73	0.73	1.00	0.71	0.76	0.82	0.81	0.71	0.76	0.84	1.00																	
C14	0.69	0.67	0.68	0.63	0.78	0.60	0.73	0.77	0.73	0.68	0.73	0.71	0.78	1.00																
C15	0.65	0.68	0.69	0.69	0.82	0.67	0.80	0.81	0.80	0.80	0.80	0.72	0.82	0.82	1.00															
C16	0.61	0.61	0.71	0.65	0.71	0.71	0.68	0.67	0.65	0.68	0.63	0.65	0.71	0.76	0.77	1.00														
C17	0.60	0.60	0.61	0.61	0.67	0.67	0.69	0.71	0.72	0.69	0.61	0.64	0.67	0.61	0.73	0.72	1.00													
C18	0.69	0.67	0.73	0.73	0.97	0.71	0.73	0.80	0.78	0.68	0.76	0.84	0.97	0.78	0.80	0.68	0.64	1.00												
C19	0.72	0.75	0.71	0.60	0.65	0.81	0.60	0.61	0.60	0.57	0.52	0.60	0.65	0.63	0.64	0.63	0.67	0.65	1.00											
C20	0.64	0.61	0.73	0.68	0.68	0.71	0.68	0.67	0.65	0.68	0.63	0.65	0.68	0.73	0.75	0.97	0.72	0.65	0.60	1.00										
C21	0.69	0.80	0.76	0.68	0.68	0.71	0.63	0.64	0.63	0.55	0.57	0.60	0.68	0.65	0.67	0.60	0.69	0.68	0.81	0.57	1.00									
C22	0.68	0.65	0.72	0.72	0.98	0.69	0.75	0.81	0.80	0.69	0.75	0.82	0.98	0.77	0.81	0.69	0.65	0.96	0.64	0.67	0.67	1.00								
C23	0.65	0.76	0.82	0.61	0.69	0.67	0.72	0.73	0.72	0.67	0.64	0.64	0.69	0.72	0.71	0.67	0.65	0.67	0.69	0.64	0.72	0.71	1.00							
C24	0.65	0.76	0.85	0.72	0.69	0.69	0.67	0.71	0.72	0.61	0.61	0.69	0.61	0.68	0.64	0.68	0.67	0.67	0.67	0.75	0.68	0.78	1.00							
C25	0.59	0.72	0.84	0.71	0.76	0.65	0.71	0.72	0.71	0.63	0.63	0.68	0.76	0.65	0.75	0.65	0.69	0.73	0.63	0.65	0.73	0.75	0.80	0.88	1.00					
C26	0.57	0.73	0.80	0.82	0.67	0.72	0.64	0.65	0.67	0.61	0.59	0.61	0.67	0.61	0.63	0.59	0.63	0.69	0.67	0.61	0.69	0.65	0.71	0.81	0.80	1.00				
C27	0.67	0.61	0.71	0.60	0.68	0.65	0.60	0.64	0.68	0.60	0.63	0.65	0.68	0.52	0.67	0.55	0.67	0.68	0.63	0.55	0.65	0.67	0.64	0.72	0.68	0.61	1.00			
C28	0.65	0.73	0.77	0.77	0.69	0.77	0.69	0.71	0.75	0.61	0.61	0.69	0.69	0.59	0.65	0.53	0.63	0.69	0.72	0.56	0.77	0.68	0.68	0.76	0.75	0.78	0.75	1.00		
C29	0.63	0.71	0.77	0.72	0.67	0.72	0.64	0.68	0.69	0.59	0.61	0.67	0.67	0.56	0.60	0.56	0.63	0.67	0.69	0.59	0.75	0.65	0.68	0.76	0.69	0.76	0.72	0.86	1.00	
C30	0.63	0.71	0.75	0.82	0.72	0.77	0.67	0.68	0.72	0.61	0.59	0.67	0.72	0.59	0.63	0.56	0.65	0.69	0.72	0.59	0.77	0.71	0.68	0.76	0.69	0.81	0.69	0.89	0.84	1.00

Figure 2 Genetic similarity co-efficient of 30 isolates of *C. gloeosporioides* which ranged from 0.66 to 1.0.

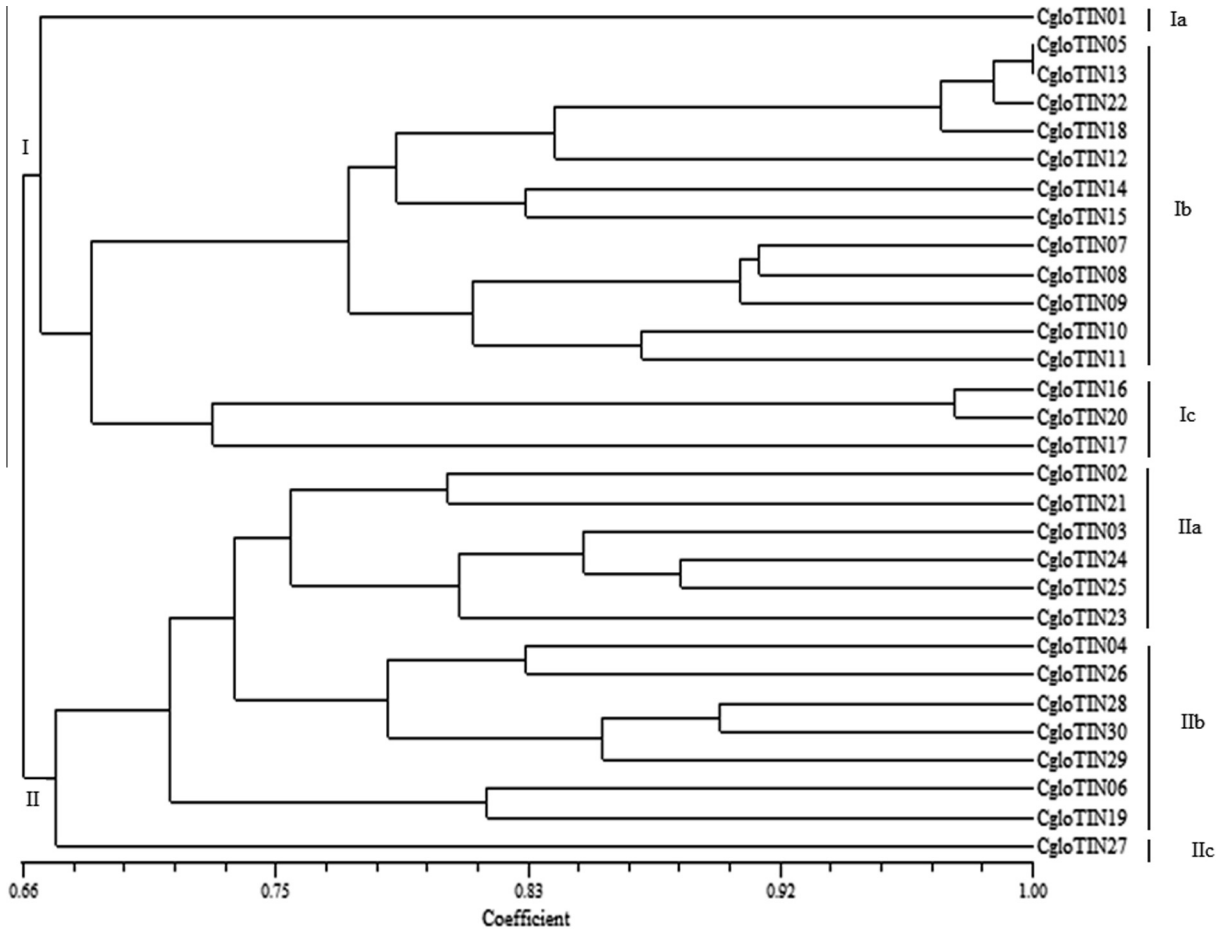

Figure 3 UPGMA dendogram of *Colletotrichum gloeosporioides* isolates from tea plant based on RAPD markers using NTSYS pc (version 1.70) using the SAHN Clustering program.

these variations. In our studies, clustering of thirty isolates into two groups indicated the genetic variability among the isolates. Both interspecies and intraspecies genetic diversity of *Colletotrichum* species has been studied with RAPD markers since a long period. In interspecies genetic diversity the species formed different clusters in RAPD dendogram and each cluster represented one species [21,42]. Following *Colletotrichum* species showed intraspecies genetic diversity within the isolates of same species while analyzed with RAPD markers and the species included *Colletotrichum acutatum* [54], *Colletotrichum lindemuthianum* [26], *Colletotrichum falcatum* [14], *Colletotrichum capsici* [13] and *C. gloeosporioides* [47,25,43,8]. To differentiate the isolates of *C. gloeosporioides*, RAPD markers may be a reliable or quick method for the new isolates which could not be differentiated from wild types [37]. Different endophytic *Colletotrichum* sp. also exhibited a high level of molecular diversity [19,23,39] when they were analyzed with different molecular markers. The isolates of *C. gloeosporioides* of our studies also exhibited genetic variation and formed two main groups on RAPD dendogram. Again we categorized the isolates into two groups on the basis of morphological characteristics. We found partial co-relationship between morphological and RAPD based grouping. All the 16 isolates of Group I in RAPD dendogram were those isolates which did not produce conidial masses on the mycelium on morphological studies except three isolates which were CgloTIN12, CgloTIN13 and CgloTIN16. Similarly the other group obtained from RAPD dendogram also consisted of those isolates which produced conidial masses on the mycelium during culturing except CgloTIN02, CgloTIN06, CgloTIN23, CgloTIN25 and CgloTIN26. Thottappilly et al. [49] also reported that with RAPD markers 51 isolates of *C. gloeosporioides* were classified into four groups, which were initially categorized with morphological and virulence characteristics which indicated a correlationship among morphological, virulence and molecular characteristics. Isolates of *C. gloeosporioides* formed two groups in RAPD dendogram and did not find any correlation regarding locations [43]. Figueiredo et al. [8] also found two groups of *C. gloeosporioides* when they were analyzed with RAPD markers though the source of isolation was only the leaves of cashew plant. However, more studies are required to analyze the genetic variation among the isolates of a species with different markers and to establish a proper relationship between the morphological variation and genetic variation.

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