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Research Article
Comparative Assessment of RAPD and ISSR Markers to Study Genetic Polymorphism in *Colletotrichum gloeosorioides* Isolates of Mango

[1]Archana Sornakili, [2]Prabukarthikeyan Karthikeyan Rathinam, [1]Raguchander Thiruvengadum and [1]Prabakar Kuppusamy

[1]Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, 641003 Coimbatore, Tamil Nadu, India
[2]Division of Crop Protection, National Rice Research Institute (NRRI), Cuttack, Odisha, India

Abstract

Background and Objective: Morphological traits of the genus *Colletotrichum* are extremely variable and hosts might be infected by a single or multiple species of the pathogen, hence development of species-specific primers has provided a powerful tool for the detection of plant pathogens. The objective of the study was to assess efficiency of Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) primers against *C. gloeosporioides* in terms of genetic variability. **Materials and Methods:** Random amplified polymorphic DNA and inter simple sequence repeat primers are simple PCR-based assays targeting microsatellite regions of the genome. Intra and interspecific polymorphisms among the twentysix isolates of *C. gloeosporioides*, causing anthracnose on mango were evaluated by these markers. **Results:** In the present research, RAPD primers generated 519 amplicans with 82 alleles of which 77 were polymorphic with 7.4 assay efficiency index; ISSR markers produced 1469 amplicans with 189 amplification products, out of which 180 were polymorphic with 11.81 assay efficiency index. Although both the techniques were efficient and reproducible, ISSR indicated higher genetic variability in terms of percent polymorphism, polymorphism information content and effective multiplex ratio compared to RAPD analysis. Among 16 ISSR primers $(CAG)_5$, $(TGTC)_4$, $(AGG)_5$, $(TCC)_5$, $(CAG)_3$, $(AG)_8$ T, $(GA)_8$ T, $(TG)_8$ A, $(GA)_8$ YG, $(GT)_8$ YC recorded highest percent polymorphism and highest PIC value of 0.97 by $(GA)_8$ T and EMR of 23.04 by $(GACA)_4$ was recorded. **Conclusion:** It is concluded that well-chosen ISSR primers could result in quick estimate in terms of their efficiency in detecting polymorphisms among the isolates.

Key words: *C. gloeosporioides*, diversity in polymorphism,genetic markers, ISSR, RAPD primers

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Corresponding Author: Archana Sornakili, Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, 641003 Coimbatore, Tamil Nadu, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colletotrichum gloeosporioides, anthracnose diseases of mango is more prevalent and severe in humid areas, affecting mango tress with maximum disease incidence. Genome sequencing has virtually opened a door for characterization and evaluation of genetic diversity within and between species and populations using molecular markers. It has been showed that different markers might reveal various classes of diversity[1,2]. It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay[3]. Though, genetic variability among the fungal population limited by the proteins and isozymes, correlation of molecular markers to morphological, cultural and virulence characters provide us requisite landmarks for the elucidation of genetic variation. An ideal molecular marker should be highly polymorphic in nature with consistent distribution throughout the genome, also provide adequate resolution of genetic differences, have linkage to distinct phenotypes and without necessitate evidence about the genome of an organism[4,5].

The RAPD and ISSR markers does not require radioactive compounds and it could be revealed a high degree of polymorphism. In the recent era, the efficiency of different molecular marker systems has been studied in assessing genetic diversity using various statistical parameters[6].

By using these markers, the correlation between different polymorphism assays may vary between as well as within species. Therefore, careful evaluation is needed for each PCR based marker system before being wellgrouped[7]. In addition, primers with more than 50% GC content are desired because during polymerization process, it could be strong enough to form a duplex. Kumar *et al*.[8] compared three marker system viz., Random Amplified Polymorphic DNA (RAPD), Universal Rice Primers (URP) and Inter Simple Sequence Repeat (ISSR) markers to characterize twenty *C. falcatum* isolates and among three markers, URP markers could successfully assess genetic diversity in *C. falcatum* as it produced more number of total bands amplified (TB) and Polymorphic Bands (PB). In addition, the parameters measuring the efficiency of markers viz., expected heterozygosity (Hn), Effective Multiplex Ratio (EMR), resolving power (Rp) and Marker Index (MI) were more in URP markers. Studies by Tymon and Pell[9] clearly depicted that usage of ISSR, ERIC and RAPD markers for the estimation of genetic diversity of 30 isolates of the entomopathogenic fungus *Pandora neoaphidis* of different geographic origins produced larger number of polymorphic bands obtained from ISSR. In addition, ISSR marker is a simple and rapid technique, requires no sequence information and using a single primer for detection and random amplification of the DNA. Hence, comparing RAPD and ISSR amplification will be clearly depicted the polymorphism among the *C. gloeosporioides* isolates of mango separately by each primer, which was further used for genetic variability.

Mirmajlessi *et al*.[10] assessed the genetic diversity, by comparing RAPD and ISSR markers in twenty three isolates of *Rhizoctonia solani* from root, crown and rhizosphere of cucumber, pumpkin, watermelon and melon plants and reported that ISSR profiles showed the highest levels of polymorphism (0.37) in *R. solani* AG4, while the lowest levels of polymorphism (0.32) was detected with RAPD profiles. A comparative analysis of genetic variation from different places of Tamil Nadu will help explain the overall structure of the *C. gloeosporioides* population and the diversity within each location will better define against potentially damaging strains. The present study objectives were designed to analyse the genetic diversity of *C. gloeosporioides* pathogen of mango anthracnose disease using two molecular markers viz., RAPD and ISSR.

MATERIALS AND METHODS

Isolation of *C. gloeosporioides*: Twenty six isolates of *C. gloeosporioides* were isolated from anthracnose disease infected mango samples during the period of 2011-2012 from different districts of Tamil Nadu, India and their pathogenecity, morphological variation and virulence of all isolates were previously studied by Archana *et al*.[11].

Molecular identification: Genomic DNA was extracted from the mycelial mat of *C. gloeosporioides* isolates by Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Knapp and Chandlee[12]. The ITS1-5.8S-ITS2 region of ribosomal DNA from twenty six isolates of *C. gloeosporioides* was amplified with ITS1 (5' -TCCGTAGGTGAACCTGCGG-3') and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers and further it confirmed with the specific primer (CgInt) (5′-GGCCTCCCGCCTCCGGGCGG-3′) coupled with ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′).

Comparison of marker systems: A set of 10 RAPD (OPB 07, OPF 11, OPF14, OPF 07, OPL 12, OPL 05, OPD 07, OPA 09, OPG 16, OPC 08) and 16 ISSR ($(CAG)_5$, $(GACA)_4$, $(GACAC)_3$, $(TGTC)_4$, $(AGG)_5$, $(ACTG)_4$, $(TCC)_5$, $(GTG)_5$, $(CAG)_3$, $(CAC)_5$, $(AG)_8$ T, $(AG)_8$ C, $(GA)_8$ T, $(TG)_8$ A, $(GA)_8$ YG, $(GT)_8$ YC) primers (Chromous Biotech

Pvt. Ltd., Bangalore) were used to identify the molecular variability of twenty six *C. gloeosporioides* isolates.

Data analysis: For data analysis, each band with a different electrophoretic mobility was assigned a position number and a mark of 1 or 0 based on the presence or absence of the band. Only reproducible bands were considered for analysis. Bands common to all isolates were incorporated into the analysis. The parameters of banding pattern viz., total number of bands, number of monomorphic, polymorphic bands per primer and percent polymorphism were calculating by counting the number of bands produced by each primer in all the twenty six isolates. In addition, assay efficiency index (AEI = Polymorphic bands/Total number of primers) of RAPD and ISSR markers were documented.

Polymorphism Information Content (PIC) was calculated using the formula developed by Anderson *et al.*[13]. A PIC value of each locus was calculated as:

$$PIC_j = 1 - \sum_{l = 1 \text{ to } L} P^2_{lj} \quad (1)$$

where, P_{lj} is the relative frequency if the l^{th} allele for the locus j and is summed across all the alleles (L) over all lines. The PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed but also the relative frequencies of those alleles. The PIC values may range from 0 (monomorphic) to 1 (very highly discriminative), with many alleles in equal frequencies.

Genotypic gene diversity was calculated as described by Mariette *et al.*[14]:

$$H_g = 1 - (p_i^2 - q_i^2) \quad (2)$$

where, p_i^2-q_i^2 are the frequencies of the dominant and null alleles, respectively. Here, allele frequencies were calculated based on the frequency of the null allele (i.e., the number of individuals without the band). Where, p_i ($p_i = 1-q_i$) represents the frequency of the dominant allele and q_i represents the frequency of the null allele:

$$q_i = \left\{ \frac{\text{No. of individuals for which the band was NOT present}}{\text{Total no. of individuals surveyed}} \right\}^{1/2}$$

Marker Index (MI) was determined as the product of PIC and the number of polymorphic bands per assay unit and Effective Multiplex Ratio (EMR) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay:

$$EMR\ (E) = n_p\ (n_p/n) \quad (3)$$

where, n_p is the number of polymorphic loci and n is the total number of loci[1].

Statistical analysis: The banding patterns were scored for RAPD and ISSR primers in each *C. gloeosporioides* isolate starting from the small size fragment to large sized one. Presence and absence of each band in each isolate was coded as 1 and 0 respectively. The scores were used to create a data matrix to analyse genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) described by Rohlf [15].

RESULTS AND DISCUSSION

In the present study analysis of the ITS sequence of the ribosomal DNA, all the twenty six isolates amplified with the primer pairs of ITS1 and ITS4 and CgInt and ITS4, confirming that they pertained to *C. gloeosporioides* by producing the amplicons at 560 and 450 bp, respectively.

To compare the utility of the two marker systems, twenty six isolates of *C. gloeosporioides* were analyzed with 10 RAPD and sixteen ISSR primers. Various parameters viz., total number of alleles, number of polymorphic bands per assay unit, mean percentage of polymorphism per assay, number of monomorphic bands per assay, Polymorphic Information Content (PIC) value, genotypic gene diversity, Marker Index (MI), Effective Multiplex Ratio (EMR) and Assay Efficiency Index (AEI) were recorded as criteria to differentiate their efficacy.

RAPD analysis: All the twenty six *C. gloeosporioides* isolates had polymorphic fragments which were generated by 10 oligonucleotides decamers. The selection of primers was based on clear, scorable and reproducible amplified banding patterns. The number of amplification products obtained in all isolates was specific to each primer and the size was varied from 100 to 2000 bp. Of the 10 primers used, six primers viz., OPB 07, OPF 14, OPF 07, OPL 05, OPD 07 and OPG 16 were found to show 100 per cent polymorphism which is presented in (Table 1, Fig. 1). Of the 82 total alleles observed, 77 alleles were polymorphic and maximum numbers of 14 alleles were obtained with primer OPL 12, followed by primer OPF 14 with 12 alleles. Minimum numbers of 5 alleles were generated with primer OPL 05. Thus, amplifications varied across the primer employed. Among the 10 RAPD primers, the Polymorphism Information Content (PIC) was in the range of 0.69 to 0.90 and the Marker Index (MI) were 3.47 to 10.82. In addition, the Effective Multiplex Ratio (EMR) was in the range of 4.17-12.00.

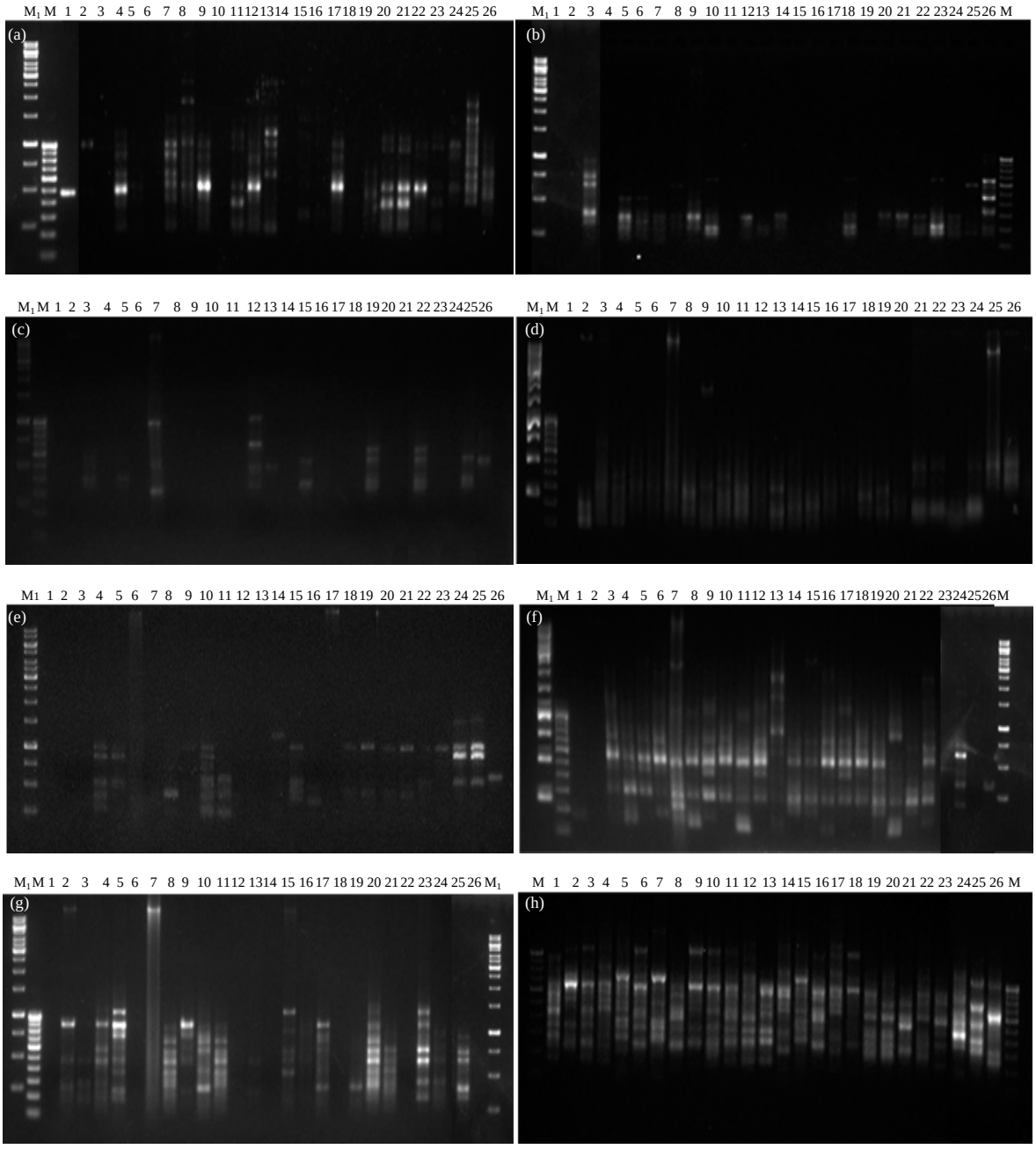

Fig. 1(a-h): RAPD fingerprints of *C. gloeosporioides* obtained by arbitrarily selected RAPD primers (a) OPB 07, (b) OPF 07, (c) OPL 06, (d) OPD 07, (e) OPA 09, (f) OPF 14, (g) OPG 16 and (h) OPL 12

1: MCG 1, 2: MCG 2, 3: MCG 3, 4: MCG 4, 5: MCG 5, 6: MCG 6, 7: MCG 7, 8: MCG 8, 9: MCG 9, 10: MCG 10, 11: MCG 11, 12: MCG 12, 13: MCG 13, 14: MCG 14, 15: MCG 15, 16: MCG 16, 17: MCG 17, 18: MCG 18, 19: MCG 19, 20: MCG 20, 21: MCG 21, 22: MCG 22, 23: MCG 23, 24: MCG 24, 25: MCG 25, 26: MCG 26, M: Marker (100 bp) and M_1: Marker (1 kb)

The minimum PIC value, MI and EMR was observed in the primer OPA 09. By using RAPD markers a total of 519 amplicons were observed and the Assay Efficiency Index (AEI) was 7.4. By analyzing these parameters, the most informative RAPD primers were OPL 12 and OPF 14.

ISSR analysis: Sixteen ISSR markers that generated 189 alleles were used to estimate the genetic diversity of twenty six *C. gloeosporioides* isolates, among that 180 were polymorphic bands and 9 were monomorphic bands. Clearly detectable amplified ISSR fragments of allele ranged from

Table 1: Polymorphism detected by RAPD markers

Primers	Total alleles	MB	PB	MM (%)	PM (%)	Total amplicans	Allele range	PIC	Genotypic gene diversity	MI	EMR	AEI
OPB 07	8	0	8	0.00	100.00	56	100-1000	0.74	1.86	5.94	8.00	7.4
OPF11	6	1	5	16.67	83.33	69	100-800	0.80	1.81	3.98	4.17	
OPF14	12	0	12	0.00	100.00	26	100-1000	0.90	1.71	10.79	12.00	
OPF 07	8	0	8	0.00	100.00	59	100-1400	0.87	1.88	6.96	8.00	
OPL 12	14	2	12	14.29	85.71	114	300-2000	0.90	1.66	10.82	10.29	
OPL 05	5	0	5	0.00	100.00	22	150-700	0.76	1.76	3.82	5.00	
OPD 07	6	0	6	0.00	100.00	87	400-1500	0.85	1.84	5.07	6.00	
OPA 09	6	1	5	16.67	83.33	37	100-400	0.69	1.91	3.47	4.17	
OPF 06	7	1	6	14.29	85.71	18	300-1500	0.80	1.88	4.81	5.14	
OPG 16	10	0	10	0.00	100.00	31	200-1400	0.75	1.71	7.55	10.00	
Total	82	5	77	61.92	938.08	519		8.06	18.02	63.21	72.76	
Mean	8.20	0.50	7.70	6.19	93.81	51.90		0.81	1.80	6.32	7.28	

MB: No. of monomorphic bands, PB: No. of polymorphic bands, MM (%): Monomorphism percentage, PM (%): Polymorphism percentage, PIC: Polymorphism information content, MI: Marker index, EMR: Effective multiplex ratio, AEI: Assay efficiency index

Table 2: Polymorphism detected by ISSR markers

Primers	Total alleles	MB	PB	MM (%)	PM (%)	Allele range	Total amplicans	PIC	Genotypic gene diversity	MI	EMR	AEI
$(CAG)_5$	16	0	16	0.00	100.00	100-1500	139	0.93	1.58	14.81	16.00	11.81
$(GACA)_4$	25	1	24	4.00	96.00	100-2000	250	0.95	0.95	22.85	23.04	
$(GACAC)_3$	20	2	18	10.00	90.00	100-1000	182	0.94	0.96	16.87	16.20	
$(TGTC)_4$	4	0	4	0.00	100.00	100-600	41	0.69	0.95	2.78	4.00	
$(AGG)_5$	13	0	13	0.00	100.00	100-1000	71	0.89	0.99	11.59	13.00	
$(ACTG)_4$	17	2	15	11.76	88.24	100-2500	35	0.89	0.99	13.28	13.24	
$(TCC)_5$	4	0	4	0.00	100.00	600-2500	24	0.74	0.98	2.96	4.00	
$(GTG)_5$	23	2	21	8.70	91.30	150-1500	161	0.94	0.98	19.67	19.17	
$(CAG)_3$	10	0	10	0.00	100.00	100-1000	88	0.88	0.97	8.79	10.00	
$(CAC)_5$	17	1	16	5.88	94.12	100-1500	159	0.94	0.96	14.96	15.06	
$(AG)_8$ T	3	0	3	0.00	100.00	100-500	18	0.66	0.98	1.98	3.00	
$(AG)_8$ C	11	1	10	9.09	90.91	100-800	91	0.86	0.97	8.62	9.09	
$(GA)_8$ T	4	0	4	0.00	100.00	100-400	8	0.97	0.98	3.89	4.00	
$(TG)_8$ A	10	0	10	0.00	100.00	100-1000	80	0.94	0.99	9.40	10.00	
$(GA)_8$ YG	7	0	7	0.00	100.00	100-750	65	0.81	0.96	5.74	7.00	
$(GT)_8$ YC	5	0	5	0.00	100.00	200-800	57	0.79	0.94	3.92	5.00	
Total	189	9	180	49.43	1550.57		1469	13.82	16.13	162.11	171.80	
Mean	11.81	0.56	11.25	3.09	96.91		91.81	0.86	1.01	10.13	10.74	

MB: No. of monomorphic bands, PB: No. of Polymorphic bands, MM (%): Monomorphism percentage, PM (%): Polymorphism percentage, PIC: Polymorphism information content, MI: Marker index, EMR: Effective multiplex ratio, AEI: Assay efficiency index

100-2500 bp in size (Table 2, Fig. 2). The number of alleles revealed by each marker ranged from 3 alleles in $(AG)_8$ T to 25 alleles in $(GACA)_4$, with an average of 11.81 alleles per locus. With the average of 96.91 per cent polymorphism produced by sixteen ISSR primers, percent polymorphism was detected by the primers $(CAG)_5$, $(TGTC)_4$, $(AGG)_5$, $(TCC)_5$, $(CAG)_3$, $(AG)_8$ T, $(GA)_8$ T, $(TG)_8$ A, $(GA)_8$ YG and $(GT)_8$ YC. Polymorphism Information Content (PIC), a measure of gene diversity was an average of 0.86 with a range of 0.66 by $(AG)_8$ T to 0.97 by $(GA)_8$ T primer. A convenient estimate of marker utility may therefore, be devised from the product of information as measured by PIC and the number of polymorphic bands per assay unit. The maximum marker index value of 22.85 was observed in the primer $(GACA)_4$ and the minimum marker index of 1.98 was observed in the primer $(AG)_8$ T. Among the sixteen ISSR primers used, $(GACA)_4$ produced highest EMR of 23.04 and the primer $(AG)_8$ T produced lowest EMR of 3.00.

Comparison of RAPD and ISSR marker systems for their efficacy in assessing the genetic diversity of *C. gloeosporioides* isolates: The mean number of allele per assay unit, number of polymorphic and monomorphic bands per assay unit in ISSR analysis was 11.81, 11.25 and 0.56 respectively, which were superior over RAPD primers accounting 8.20, 7.70 and 0.50 alleles (Table 3). The ISSR marker index (10.13) indicative of marker utility and mean polymorphic information content per assay (0.86) was greater than the value of RAPD, which has 6.32 and 0.81 respectively, is due to ISSR's higher effective multiplex ratio (10.74). The mean genotypic gene diversity was 1.80 for RAPD analysis, while for ISSR it was 1.01, despite the higher multiplex ratio and marker index. Further, the higher percentage of polymorphic bands obtained from ISSR analysis (96.91%) compared to RAPD (93.81%).

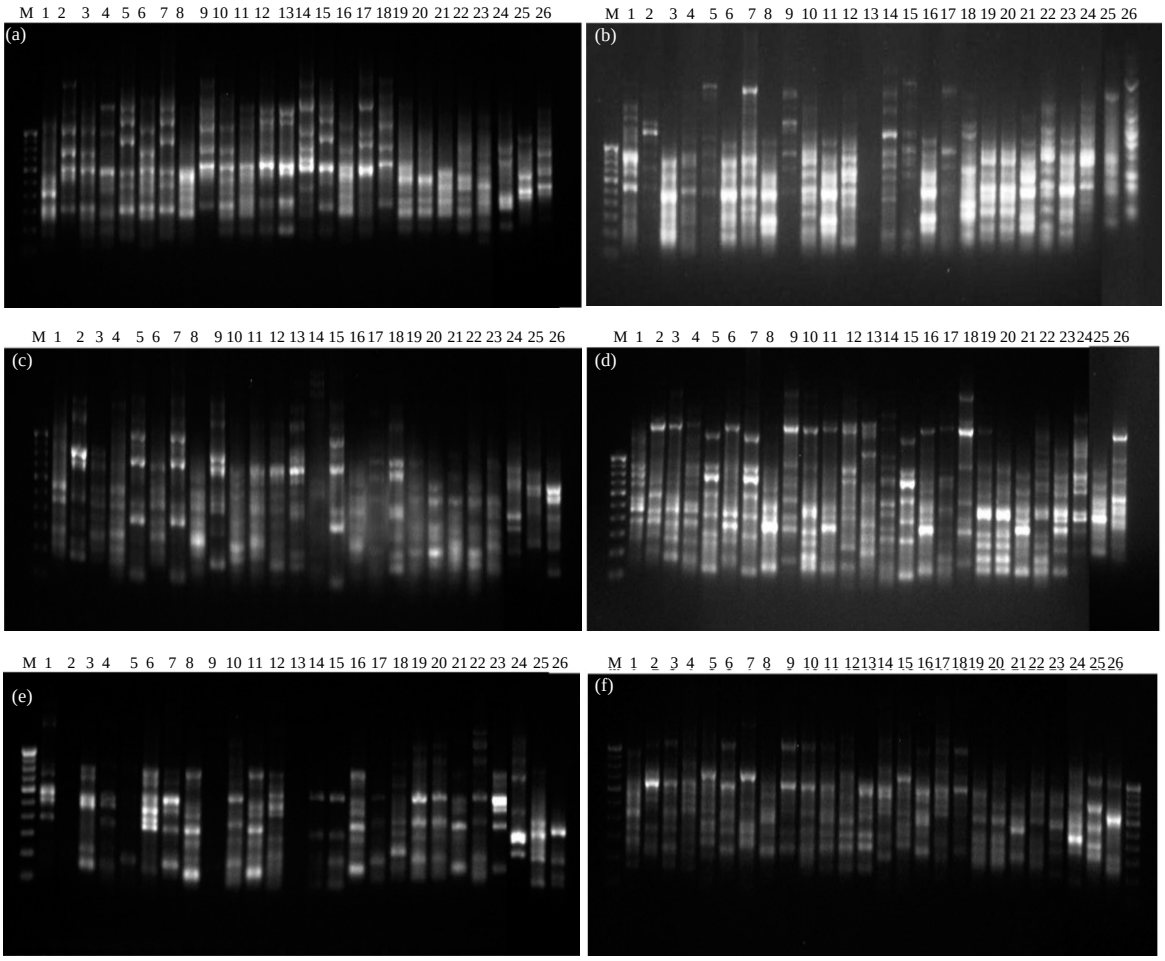

Fig. 2(a-f): DNA fingerprint of *C. gloeosporioides* isolates by ISSR primers (a) $(GACA)_3$, (b) $(GTG)_5$, (c) $(CAC)_5$, (d) $(GACA)_4$, (e) $(CAG)_5$ and (f) $(ACTG)_4$

M: Marker (100 bp), 1: MCG 1, 2: MCG 2, 3: MCG 3, 4: MCG 4, 5: MCG 5, 6: MCG 6, 7: MCG 7, 8: MCG 8, 9: MCG 9, 10: MCG 10, 11: MCG 11, 12: MCG 12, 13: MCG 13, 14: MCG 14, 15: MCG 15, 16: MCG 16, 17: MCG 17, 18: MCG 18, 19: MCG 19, 20: MCG 20, 21: MCG 21, 22: MCG 22, 23: MCG 23, 24: MCG 24, 25: MCG 25 and 26: MCG 26

Table 3: Comparative analysis of banding patterns generated by RAPD and ISSR

Components	RAPD	ISSR
Number of assay units	10.00	16.00
Total amplicans	519.00	1469.00
Total No. of alleles	82.00	189.00
Mean No. of allele per assay unit	8.20	11.81
No. of polymorphic bands per assay unit	7.70	11.25
Mean (%) polymorphism per assay	93.81	96.91
Number of monomorphic bands per assay unit	0.50	0.56
Mean PIC per assay	0.81	0.86
Mean genotypic gene diversity	1.80	1.01
Mean marker index (MI)	6.32	10.13
Effective multiplex ratio	7.28	10.74
Assay efficiency index	7.40	11.81

RAPD: Random amplified polymorphic DNA, ISSR: Inter simple sequence repeats

The ISSR marker system outperformed in all marker parameters i.e., higher allele number, total amplified bands, EMR, MI as well as PIC except the mean genotypic gene diversity revealed best by the RAPD marker system. Considering these parameters, it confirmed that the ISSR technique is very potent to evaluate genetic diversity among the *C. gloeosporioides* isolates, also it could be highly polymorphic and have a significant discriminative power when compared to RAPD. Further, from the studies it was observed that ISSR primers could clearly distinguish the isolates according to their virulent nature but it was not so in RAPD. Thus, microsatellites are ideal markers for assessing the genetic diversity of *C. gloeosporioides* isolates.

The present study was aimed to analyse the molecular diversity of *C. gloeosporioides* isolates, causing anthracnose disease of mango from various places of Tamil Nadu, India. Variability in terms of morphological, physiological and genetical was important to study the biology of the pathogen

in an endemic area. Hence, in this paper, RAPD primers and highly efficient microsatellite ISSR primers are used for the genetic diversity of *C. gloeosporioides* with aim of identifying the ideal marker.

Random amplified polymorphic DNA (RAPD): Molecular methods have been employed successfully to differentiate between populations of *Colletotrichum* from many hosts in general, according to the study of Agwanda *et al.*[16]. Random Amplified Polymorphic DNA (RAPD) markers, in which short oligonucleotides of random sequence are used as primers to arbitrarily amplify segments of a target genome, are used widely to detect genetic variation[4].

In this study, the suitability of RAPD technique for rapid molecular characterization of *C. gloeosporioides* isolates was assessed. Based on the results it was observed that six primers (OPB 07, OPF 14, OPF 07, OPL 05, OPD 07 and OPG 16) produced 100 per cent polymorphism with the allele range of 100-2000 bp, although majority was below 1000 bp. It was observed that there was a variation in the number of alleles and their intensity, number of monomorphic (5) and polymorphic bands (77), polymorphism information content (80.06), marker index (63.21) and effective multiplex ratio (72.76) among the ten RAPD primers used. The EMR was ranged from 4.17 (OPA 09) to 12.00 (OPF 14). This research was similar to results of Kumar *et al.*[8], where the EMR ranged from 7 (OPA07) to 12 (OPA04) in *Colletotrichum falcatum* isolates. Application of RAPD markers to fungal isolates would be useful in providing information regarding polymorphisms within the reference isolates of *C. gloeosporioides* and established DNA fingerprints which was useful for race characterization[17].

Inter simple sequence repeat (ISSR): The ISSR primers are based on di-, tri-, tetra- or pentanucleotide repeats with 5' or 3' anchored base(s). To access the variation around the diverse microsatellite regions, Inter Simple Sequence Repeat (ISSR) markers are powerful tools which can be utilized as molecular tools[5] for the characterization of genetic variations within fungi[18]. In the present study, all the 16 primers generated amplification products of *C. gloeosporioides* isolates for a total of 189 allelles, 180 of which were polymorphic with the allele range of 100-2500 bp. Out of sixteen ISSR primers, ten primers viz., $(CAG)_5$, $(TGTC)_4$, $(AGG)_5$, $(TCC)_5$, $(CAG)_3$, $(AG)_8$ T, $(GA)_8$ T, $(TG)_8$ A, $(GA)_8$ YG, $(GT)_8$ YC were shown percent polymorphism. The distribution of different microsatellite sequences in all the genomes determines the possibility of using this method for DNA fingerprinting. This study was in accordance with Mahmodi *et al.*[19] where he reported the ISSR primers viz., UBC 808, UBC 810, UBC 820, UBC 834, UBC 841, UBC 864 and UBC112 produced 16.5 average numbers of bands per primer which ranged in size from 300-2600 bp with 100% polymorphism in *Colletotrichum* spp., obtained from cowpea. Further, Kumar *et al.*[8] reported, two ISSR markers, ISSR 02 ($ACTG_4$) and ISSR10 (CAC_5) differentiated twenty five *C. falcatum* isolates with alleles ranging from 250-3500 bp witheight average number of bands per primer (100% polymorphism). In the current research, primer $(GACA)_4$ produced highest total number of alleles (25 alleles), total number amplicons (250), effective multiplex ratio (23.04) and marker index (22.85) revealed more heterozygosity as tetranucleotide repeats are more abundant in the genome and was thus better able to characterize the polymorphism in *Colletotrichum* isolates. The overall eficiency of a primer can be judged by the higher value of marker index. This study was in accordance with the findings of Abadio *et al.*[20]. Further it produced higher mean PIC value as 0.86 with 16 primers, it was supported by Chadha and Gopalakrishna[21], where the mean PIC value was 0.27 with 17 ISSR primers in Magnaporthe grisea.

Comparison of markers: Molecular markers are useful for assessing the genetic variation rapidly within and among species[22]. In this study, comparison of two different molecular marker systems RAPD and ISSR was carried out to define genetic relationships and polymorphism among the twenty six isolates of *C. gloeosporioides* causing mango anthracnose and to investigate which marker system can be more effectively used. The foregoing study compared two marker systems by estimating discriminatory power of matrices viz., number of total allele, allele range, per cent polymorphism, Polymorphism Information Content (PIC), Marker Index (MI), Effective Multiplex Ratio (EMR) and Assay Efficiency Index (AEI).

Among the sixteen ISSR markers assessed across the *C. gloeosporioides* isolates, ten markers possessed more than ten alleles indicating better resolving power of the ISSR markers. This is because of polyallelic nature of ISSR markers. Comparison of PIC values for two marker systems (a parameter associated with the discriminating power of markers) indicated that the range of PIC values for RAPD primers was from 0.69-0.90 with an average of 0.81 and for ISSR primers it was from 0.66 to 0.97 with an average of 0.86. Marker Index (MI) is the marker attribute used to calculate the overall utility of a marker system and the mean of marker index was higher in ISSR (10.13) than RAPD (6.32) markers. In this study, a comparison of the PIC values and MI between the RAPD and ISSR data clearly demonstrated the stronger discriminatory

power of ISSR primers. Mean of effective multiplex ratio for each of these marker systems in this study (7.28 for RAPD and 10.74 for ISSR) suggested that ISSR marker systems were effective in determining polymorphism. It may be due to highly polymorphic, abundant nature of the microsattelites due to slippage in DNA replication[23]. The ISSR technique has specific and higher levels of polymorphism with more reproducibility than RAPD technique due to the use of longer oligonucleotide sequences, allowing more stringent annealing conditions in PCR amplification[24].

Cluster analysis was carried out on two sets of marker profiling data based on RAPD and ISSR. The results based on the two DNA marker profiles broadly grouped twenty six isolates into two clusters. However, formation of subclusters within the main cluster varied between RAPD and ISSR. The similarity coefficients of *C. gloeosporioides* based on 10 RAPD markers and sixteen ISSR markers ranged from 65.00-88.00 and 59.50-78.00%, respectively. The cluster formation was observed at the minimum of 59% similarity value, indicating the presence of considerable divergence between the isolates. The cluster analysis of the RAPD data confirmed the presence of high diversity at molecular level among the *C. gloeosporioides* isolates under the study. The results of ISSR analysis demonstrated, not only high diversity among studied isolates but also ISSR markers can be highly polymorphic.

Comparison of the DNA profile performance of current experiment indicated that ISSR technique was more informative in detecting genetic diversity in *C. gloeosporioides* than RAPD technique and it was supported by Mirmajlessi *et al.*[10] in *Rhizoctonia solani*. Similar results have been found by Mahmodi *et al.*[19], that high level of polymorphism detected by ISSR-PCR technique, suitable for the discrimination of *Pseudocercospora griseola* causing angular leaf spot of bean. The utility of a marker system is a balance between the level of polymorphism detected and the extent of an assay which can identify multiple polymorphisms[25]. Comparative studies in different species using various marker systems were successfully conducted by other researchers and concluded that ISSR would be a better tool than RAPD for phylogenetic studies[26]. While considering all, the results of the present study clearly depicted that the ISSR primer can be well adopted for the molecular variability studies.

CONCLUSION

The diversity of 26 isolates of *C. gloeosporioides* isolates were analyzed using the primers of RAPD and ISSR using the molecular parameters. The valuing parameters clearly showed that the polymorphism percentage, assay efficiency index and marker utility was higher in ISSR primers.

SIGNIFICANCE STATEMENT

Analyzing morphological and molecular diversity of the pathogen *C. gloeosporioides*, causing mango anthracnose disease is important to control the disease. Here, this study revealed that ISSR was the best choice to study the genetic diversity compare to RAPD primer.

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