International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 2434-2441

Received: 11-07-2019
Accepted: 15-08-2019

SB Sawant
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

RG Parmar
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
SB Sawant
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

RAPD based molecular variability of different isolates of *Alternaria burnsii* (Uppal, Patel and Kamat) causing blight of cumin

SB Sawant and RG Parmar

Abstract
Molecular variability in cumin blight pathogen (*Alternaria burnsii*) was analyzed using fifteen isolates collected from major cumin growing regions of Gujarat. The genomic DNA extracted from each isolates of *Alternaria burnsii* was subjected to polymerase chain reaction using 15 random decamer primers from OPA and OPE series. Only 10 of the 40 RAPD primers were selected based on repeatability. The 10 RAPD primers produced 622 bands and 84 loci. Among them, 77 loci were found polymorphic with an average of 91.51 per cent polymorphism. The average polymorphic information content value was 0.8473. As much as six primers out of the selected 10 primers viz., OPA 8, OPA 9, OPA 18, OPE 4, OPE 13 and OPE 10 were showed 100% polymorphism. However OPA 10 and OPE 3 showed (83.33%) polymorphism. Primer OPA 13 showed (81.81%) polymorphism. The lowest polymorphism (66.66%) was found in the primer OPE 7. The molecular weight of the amplicons ranged from 139.79 to 2319.40 bp.
Dendrogram formed three clusters namely 'C1', 'C2' and 'C3'. The cluster 'C1' included Ab-15, Ab-13, Ab-11, Ab-9, Ab-5, Ab-4, Ab-2, Ab-7, Ab-3 and Ab-1 isolates. The cluster 'C2' included Ab-8, and Ab-6 while, cluster 'C3' included Ab-12, Ab-10 and Ab-14 isolates. The maximum similarity index value of 0.767 was observed between the isolates Banaskantha (Ab-5) and Morbi (Ab-4) and the least similarity index value of 0.160 was found between the isolates Ahmedabad (Ab-8) and Surendranagar (Ab-14). The molecular characterization of fifteen isolates of *A. burnsii* by RAPD revealed existence of variations. Therefore, the primer OPA 8, OPA 9, OPA 18, OPE 4, OPE 13 and OPE 10 were could be very useful for ascertaining variability among the population of other species of *Alternaria*.

Keywords: Random amplification of polymorphic DNA, molecular variability, *Alternaria burnsii*s

Introduction
Cumin (*Cuminum cyminum* L.) popularly known as Jeera or Jiroo is the most important spice crop in India. Gujarat is second largest producer next to Rajasthan with 50-55% of total production of India. Cumin crop suffers with blight disease caused by *Alternaria burnsii.* Cumin seed is used as culinary for flavouring vegetables, pickles, soups, etc. The seeds contain 17.7 per cent protein, 23.8 per cent fat, 35.5 per cent carbohydrates and 7.7 per cent minerals (Chadha, 2006) [1]. Cumin suffers from important diseases, *viz*., blight (*Alternaria burnsii*), wilt (*Fusarium oxysporum* f.sp. *cumini*) and powdery mildew (*Erysiphe polygoni*) which causes considerable qualitative and quantitative yield losses in the crop. Blight (*A. burnsii)* is of common occurrence in the areas where cumin is grown. Cumin blight was recorded for the first time from Kaira district of Gujarat. The causative agent was reported to be *Alternaria burnsii* by Uppal *et al.* (1938) [16]. Later on, it was reported from Rajasthan by Joshi (1955) [4]. The disease usually appears at flowering stage. The infected plants show small, isolated, whitish necrotic areas on the aerial parts, especially on tips of young leaves (Sharma, 2010). Diseased seed are small, deshaped, shriveled, very light and turn black in colour (Gemawat and Prasad, 1972) [2].
DNA markers have become a powerful tool to study taxonomy and molecular genetics of variety of organisms. The random amplified polymorphic DNA (RAPD) allows quick assessment of genetic variability and has been used to study inter and intra- specific variability amongst the isolates of several fungal species. It shows diversity at genetic level (Singh *et al*., 2016) [13]. Therefore, there is possibility of occurrence of the variability in this pathogen too. The isolates varied widely in the colony colour, colony margin, growth pattern, shape of

conidiophores, their size, number of longitudinal or transverse septa, beak length and sporulation frequency. Although, cultural, morphological and conidial characteristics are valuable in preliminary identification of the plant parasitic fungus, they do not give clear indication of genetic variation or systematic position very closely related isolates. But with the molecular data especially sequence based information is more helpful in deciphering the diversity present among the members / isolates of a particular plant pathogen.

Materials and Methods

The present investigation entitled RAPD based Molecular Variability of Different Isolates of *Alternaria burnsii* (Uppal, Patel and Kamat) Causing Blight of Cumin was carried out at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand.

Alternaria burnsii isolates

The isolates were collected from different cumin growing area of Gujarat and designated as Ab- 10, Ab-13 and Ab-8 for Ahmedabad, Ab-15, Ab-12 and Ab-14 for Surendranagar, Ab-4, Ab-7, Ab-11 for Morbi, Ab-5, Ab-2 and Ab-9 for Banaskantha, Ab-6, Ab-3, and Ab-1 for Patan, respectively.

Isolation of *Alternaria burnsii*

The isolation was done by tissue isolation and seed wash method (Pandey *et al*., 2016) [11] from infected seeds, branches, stems and umbels of cumin plant.

DNA isolation

DNA extraction has been done by CTAB method with slight modification (Niu *et al.*, 2008) [8]. Molecular characterization in respect of RAPD has done according to standard protocol for fungus. DNA amplified with RAPD-PCR technique using random primers (Ozer and Bayraktar, 2015) [10].

Genomic DNA extraction from mycelia

Solutions used for DNA isolation

1. DNA Extraction Buffer
   10 per cent CTAB
   1 M Tris-HCl (pH 8.0)
   0.5 M EDTA (pH 8.0)
   5M NaCl
   Double distilled water
   β- mercaptoethanol
2. Chloroform and iso-amyl alcohol (C: I) solution in the ratio of 24:1
3. DNase-free RNase A (10 mg/ml)
4. 70 per cent Ethanol (Chilled, Absolute)
5. TE buffer (1M Tris-HCl (pH 8.0) and 0.5M EDTA (pH 8.0)

Protocol for isolation of fungal genomic DNA

Exponentially growing fungal culture was harvested in liquid medium (Potato dextrose broth) by shaking, for 5-7 days at 26 ºC. Mycelia collected by filtration through sterilized filter paper and then extensively washed with water. The mycelium dried between the layers of tissue and immediately frozen in liquid nitrogen in foil packets. The tissue gently broken into fine pieces by crushing the foil envelops with a pestle and mortar. Care was taken not to allow the tissue to thaw as this causes lyses and the release of endogenous nuclease.

Protocol for DNA extraction

1. Pre-cooling of a pestle and mortar was done at 4 ºC then grinding of 300 mg mycelium to a fine powder in liquid nitrogen and transferred to a plastic sterile tube by ensuring that the tissue did not thaw.
2. It was added to 10 ml pre washed isolation buffer and incubated for 60 minutes with occasional stirring.
3. It was extracted for 10 minutes with equal volume of chloroform: isoamylalcohal (24:1).
4. Centrifuged at 10,000 rpm for 20 minutes at room temperature (24 ºC).
5. It was separated as the aqueous phase and transferred to a fresh tube.
6. To this aqueous phase, 0.6 volume of ice cold isopropanol and 0.1 volume of sodium acetate were added and incubated at -20 ºC for 30 minute.
7. Centrifugation of the supernatant was done at 10,000 rpm for 10 minutes at 4 ºCand then the aqueous phase was discarded.
8. Again washing of the DNA pellet was obtained with 70 per cent ethanol (5ml) was done.
9. DNA pellet dried and then dissolved in 200 µl of TE buffer.
10. Then RNase treatment was given to the samples where in 6 µl of RNase was added and samples were kept at 37 ºC in hot water bath for 1 hr. and latter at 65 ºC for 10 min.

Spectrophotometric analysis (Nanodrop)

Quality and quantity of isolated genomic DNA was checked through spectroscopic nanodrop. One µl DNA was loaded into the well of nanodrop spectrophotometer and the concentration of DNA and absorbance at 260 nm /280 nm were measured. Quantification and purity of DNA was checked on 0.8% Agarose gel by using 1 x TBE buffer and ethidium bromide (4 µl/ 70 ml) staining. On completion of run, gel was viewed under UV light and observations recorded on gel documentation system.

Random amplification of polymorphic DNA (RAPD) analysis

Decamer primers belonging to OPA and OPE series were screened for RAPD analysis. Of these, A total of 15 decamer primer belonging to OPA and OPE series were screened for RAPD analysis. Of these, 10 primers were selected based on repeatability. The list of RAPD primers were used for the analysis of random amplification of polymorphic DNA to study the polymorphism present in the isolates of *A. burnsii* have been mentioned in Table 1.

Table 1: List of primers used for the RAPD analysis

Sr. No.	Primers	Sequence (5'-3')	Bases
1	OPA-8	GTG ACG TAG G	10
2	OPA-9	GGG TAA CGC C	10
3	OPA-10	GTG ATC GCA G	10
4	OPA-13	CAG CAC CCA C	10
5	OPA-18	AGG TGA CCG T	10
6	OPE-3	CCA GAT GCA C	10
7	OPE-4	GTG ACA TGC C	10
8	OPE-7	AGA TGC AGC A	10
9	OPE-13	CCC GAT TCG G	10
10	OPE-20	AAC GGT GAC C	10
Total			100

PCR protocol

Preparation of reaction mixture

The reaction mixture for RAPD analysis was prepared as under

Millipore Sterilized Water	:	18.2 µl
Taq Buffer A (10 x)	:	2.5 µl
dNTPs (2.5mM each) mix	:	0.5 µl
Taq polymerase (3U/µl)	:	0.3 µl
Primer (10 pmoles/µl)	:	1.0 µl
Template DNA 40 ng/µl)	:	2.5 µl
Total	:	25 µl

The master mix was prepared by adding first millipore sterilized water followed by Taq buffer A 2.5 µl, 0.5 µl dNTPs, 0.3 µl Taq polymerase, 1 µl primer and finally 2.5 µl template DNA was added.

The reagents were mixed thoroughly by slight vortex followed by spinning in micro centrifuge. The tubes were then placed in thermal cycler for cyclic amplification. The conditions for amplification were as follows:

Programming for PCR

Step-1: 94 °C for 4 min. (Initial denaturation)
Step-2: 94 °C for 1 min. (Denaturation after every cycle)
Step-3: 34 °C for 2 min. (Primer annealing)
Step-4: 72 °C for 2 min. (Extension of annealed primer)
Step-5: 72 °C for 10 min. (Final Extension) Step 2, 3 and 4 comprised of one cycle and the total reaction was carried out for 40 cycles.

PCR products were run on electrophoresis with known molecular marker (mol. wt. 100 bp) in 1.5% Agarose gel.

Data scoring

Data was scored on the basis of presence (1) or absence (0) for analysis. If a product was present in a genotype, it was considered as '1' and if absent considered as '0'. The data maintained in the excel sheet format for further analysis. The polymorphism percentage calculated as per the method suggested by Blair and Sangwan (1999) [18].

$$\text{Polymorphism (\%)} = \frac{\text{Total number of bands} - \text{Number of monomorphic bands}}{\text{Total number of bands}} \times 100$$

The molecular weights of the PCR products were estimated by UPGMA software for each primer to analyze alleles range.

Data analysis

The data generated by RAPD was analyzed with the UPGMA method.

Dendrogram

A dendrogram was drawn based on Jaccard's similarity coefficient (1908) by UPGMA (Unweighted Pair Group Method of Arithmetic averages) method.

Results and Discussion

Assay of DNA from the Isolates of *A. burnsii* obtained through Nanodrop.

The spectrophotometric analysis of DNA showed concentration of DNA in the mycelia was 381.00 ng/µl, 135.6 ng/µl, 380.7 ng/µl, 130.5 ng/µl, 375.6 ng/µl, 140.00 ng/µl, 310.2ng/µl, 135.6 ng/µl, 487.7 ng/µl, 487.7 ng/µl, 227.7 ng/µl, 493.0 ng/µl, 487.7 ng/µl, 487.7 ng/µl, 227.7ng/µl for isolate Ab-1, Ab- 2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14 and Ab-15, respectively. The absorbance ratio of DNA at 260/280 wave length was 1.95, 1.83, 1.94, 1.80, 1.90, 1.85, 1.89, 1.83, 1.98, 1.98, 1.87, 1.99, 1.98, 1.98, 1.98 and 1.87 for isolate Ab-1, Ab- 2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14 and Ab-15, respectively (Table 2) and (Plate 1). Extracted DNA was utilized for molecular characterization through RAPD primers.

Table 2: Assay of DNA from the *A. burnsii* isolates obtained through "Nanodrop".

Isolates	Location	(260/280) Wavelength Ratio	Concentration (ng/µl)
Ab-1	Patan	1.95	381.8
Ab-2	Banaskantha	1.83	135.6
Ab-3	Patan	1.94	380.7
Ab-4	Morbi	1.80	130.5
Ab-5	Banaskantha	1.90	375.6
Ab-6	Patan	1.85	140.0
Ab-7	Morbi	1.89	310.2
Ab-8	Ahmedabad	1.83	135.6
Ab-9	Banaskantha	1.98	487.7
Ab-10	Ahmedabad	1.98	487.7
Ab-11	Morbi	1.87	227.7
Ab-12	Surendranagar	1.99	493.0
Ab-13	Ahmedabad	1.98	487.7
Ab-14	Surendranagar	1.98	487.7
Ab-15	Surendranagar	1.87	227.7

Random amplification of polymorphic DNA (RAPD) study

A total of 15 decamer primers belonging to OPA and OPE series were screened for RAPD analysis. Out of these, 10 primers were selected based on repeatability. RAPD markers proved to be very informative and useful in monitoring the genetic diversity in the biological entity. Therefore, this technique was employed to detect variability present in these isolates i.e. Ab-1, Ab- 2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14 and Ab-15 of *A. burnsii*. The fragment sizes were detected by comparing the amplicons with a 100 bp DNA ladder. The purpose of this study was to identify the specific primers which are likely to be efficient in revealing the diversity among the isolates of *A. burnsii*. The results obtained using 10 primers have been presented here as under Plate (2.1-2.10).

A common dendrogram which was generated using the UPGMA software has been collectively discussed for all the 10 primers.

1. Primer OPA-8 (GTG ACG TAG G)
The RAPD primer OPA 8 generated five loci. All the five loci were polymorphic (Plate 8.1). Total 29 bands were observed along with 100% polymorphism. The Polymorphism Information Content value 0.7776 was obtained in this primer. Molecular weight of the amplicons ranged from 461.55 bp to 1363.12 bp.

2. Primer OPA-9 (GGG TAA CGC C)
The RAPD primer OPA 9 generated nine loci. All the nine loci were polymorphic (Plate 8.2). Total 41 bands were observed along with 100% polymorphism. The PIC value 0.87686 was obtained in this primer. Molecular weight of the amplicons ranged from 347.15 bp to 1430.65 bp.

3. Primer OPA-10 (GTG ATC GCA G)
The RAPD primer OPA 10 generated six loci. Out of six loci five were polymorphic (Plate 8.3) giving 83.33% polymorphism. Total 49 bands were observed along with cent percent polymorphism. The PIC value 0.8055 was obtained in this primer. Molecular weight of the amplicons ranged from 316.21 bp to 1727.14 bp.

4. Primer OPA-13 (CAG CAC CCA C)
The RAPD primer OPA 13 generated eleven loci. Out of eleven loci nine were polymorphic (Plate 8.4) giving 81.81% polymorphism. Total 112 bands were observed along with cent percent polymorphism. The PIC value 0.8952 was obtained in this primer. Molecular weight of the amplicons ranged from 240.92 bp to 1985.77 bp.

5. Primer OPA-18 (AGG TGA CCG T)
The RAPD primer OPA 18 generated seven loci. All the seven loci were polymorphic (Plate 8.5). Total 72 bands were observed along with 100% polymorphism. The PIC value 0.8719 was obtained in this primer. Molecular weight of the amplicons ranged from 169.38 bp to 2319.40 bp.

6. Primer OPE-3 (CCA GAT GCA C)
The RAPD primer OPE 3 generated six loci. Out of six loci, five loci were polymorphic (Plate 8.6) giving 83.33% polymorphism. Total 34 bands were observed along with cent percent polymorphism. The PIC value 0.73356 was obtained in this primer. Molecular weight of the amplicons ranged from 760 bp to 930bp.

7. Primer OPE-4 (GTG ACA TGC C)
The RAPD primer OPE 4 generated eight loci. All the eight loci were polymorphic (Plate 8.7) giving 100% polymorphism. Total 69 bands were observed along with cent percent polymorphism. The PIC value 0.8720 was obtained in this primer. Molecular weight of the amplicons ranged from 197.23 bp to 2163.70 bp.

8. Primer OPE-7 (AGA TGC AGC A)
The RAPD primer OPE 7 generated nine loci. Out of nine loci, six loci were polymorphic (Plate 8.8) giving 66.66% polymorphism. Total 93 bands were observed along with cent percent polymorphism.
The PIC value 0.8722 was obtained in this primer. Molecular weight of the amplicons ranged from 139.78 bp to 1927.95bp.

9. Primer OPE-13 (CCC GAT TCG G)
The RAPD primer OPE 13 generated fourteen loci. All the fourteen loci were polymorphic (Plate 8.9) giving 100% polymorphism. Total 73 bands were observed along with cent percent polymorphism. The PIC value 0.9161 was obtained in this primer. Molecular weight of the amplicons ranged from 250.0 bp to 1680.98 bp.

10. Primer OPE-20 (AAC GGT GAC C)
The RAPD primer OPE 20 generated nine loci. All the nine loci were polymorphic (Plate 8.10) giving 100% polymorphism. Total 50 bands were observed along with cent percent polymorphism. The PIC value 0.8536 was obtained in this primer. Molecular weight of the amplicons ranged from 261.89 bp to 1172.13 bp.

Clustering pattern of different RAPD primers
Dendrogram (Fig.1) based on Jaccard's similarity coefficient (1908) (Table 3) by UPGMA method formed three clusters namely 'C1', 'C2' and 'C3'. The cluster 'C1' was subdivided into two clusters C1A and C1B. The cluster C1A was further subdivided into two subclusters C1Aa and C1Ab. The subcluster C1Aa further subdivided into two subclusters C1Aaa and C1Aab which included Surendranagar isolate (Ab-15) and Ahmedabad isolate (Ab-13), respectively. The subcluster C1Ab further divided into two subclusters C1Aba and C1Abb which included Morbi isolates (Ab-11) and Banaskantha isolate (Ab-9), respectively. The cluster C1B was further divided into two subclusters C1Ba and C1Bb. The subcluster C1Bb was further subdivided into C1Bba and C1Bbb which included Patan isolates (Ab-3) and (Ab-1), respectively. The subcluster C1Ba was further divided into two subclusters C1Baa and C1Bab. The subcluster C1Bab included isolate Morbi isolate (Ab-7). The subcluster C1Baa was further divided into two subclusters C1Baaa and C1Baab. The subcluster C1Baab included isolate Banaskantha isolate (Ab-2). The subcluster C1Baaa was further subdivided into C1Baaaa and C1Baaab which included Banaskantha isolate (Ab-5) and Morbi isolate (Ab-4), respectively.
The cluster 'C2' was subdivided into two clusters C2A and C2B which included Ahmedabad isolate (Ab-8) and Patan isolate (Ab-6), respectively.
The cluster C3 was further subdivided into two clusters C3A and C3B. The cluster C3A was further subdivided into two subclusters C3Aa and C3Ab which included surendranagar isolate (Ab-12) and Ahmedabad isolate (Ab-10), respectively. The cluster C3B included Surendranagar isolate (Ab-14).
The maximum similarity index value of 0.767 was observed between the isolates Banaskantha (Ab-5) and Morbi (Ab-4) and the least similarity index value 0.160 was found between the isolates Ahmedabad (Ab-8) and Surendranagar (Ab-14). The average similarity coefficient among isolates was 0.0563 (Table 3).
The RAPD analysis of the fifteen isolates of *A. burnsii* collected from major cumin growing regions of Gujarat and analyzed by 10 random primers produced 84 loci. Out of which, 77 loci were polymorphic. As much as 622 bands were produced by the selected random primers. On an average, 91.51 per cent polymorphism was observed. However, average number of bands and per primer were 62.2. Moreover, average number of polymorphic loci obtained per primer were 8.4. As much as six primers out of the selected 10 primers *viz*., OPA 8, OPA 9, OPA 18, OPE 4, OPE 13 and OPE 10 were showed 100% polymorphism. However OPA 10 and OPE 3 showed (83.33%) polymorphism. Primer OPA 13 showed (81.81%) polymorphism. The lowest polymorphism (66.66%) was found in the primer OPE 7. The molecular

weight of the amplicons ranged from 139.79 to 2319.40 bp (Table 4).

The efficacy of the RAPD primers selected in distinguishing the isolates of *A. burnsii* was apparent by the fact that out of 10 primers selected as many as 6 generated amplicons with 100% polymorphism (Table 4).

Further, the representative RAPD profiles presented in (Plate 2.1-2.10) indicated the extent of genetic differences prevailing among the isolates of *A. burnsii*. In the present study a total of 15 primers comprising of OPA and OPE series were screened against isolates of *A. burnsii*. Out of these, 10 primers were found useful for amplification of DNA of *A. burnsii*. Among the 10 primers, all primers gave 100% polymorphism of the DNA which helped to ascertain variability except, OPA 10 and OPE 3 which showed (83.33%) polymorphism. Primer OPA 13 showed (81.81%) polymorphism and the lowest polymorphism (66.66%) was found in the primer OPE 7. Therefore, these primers would be very useful for ascertaining variability among the population of other species of *Alternaria.*

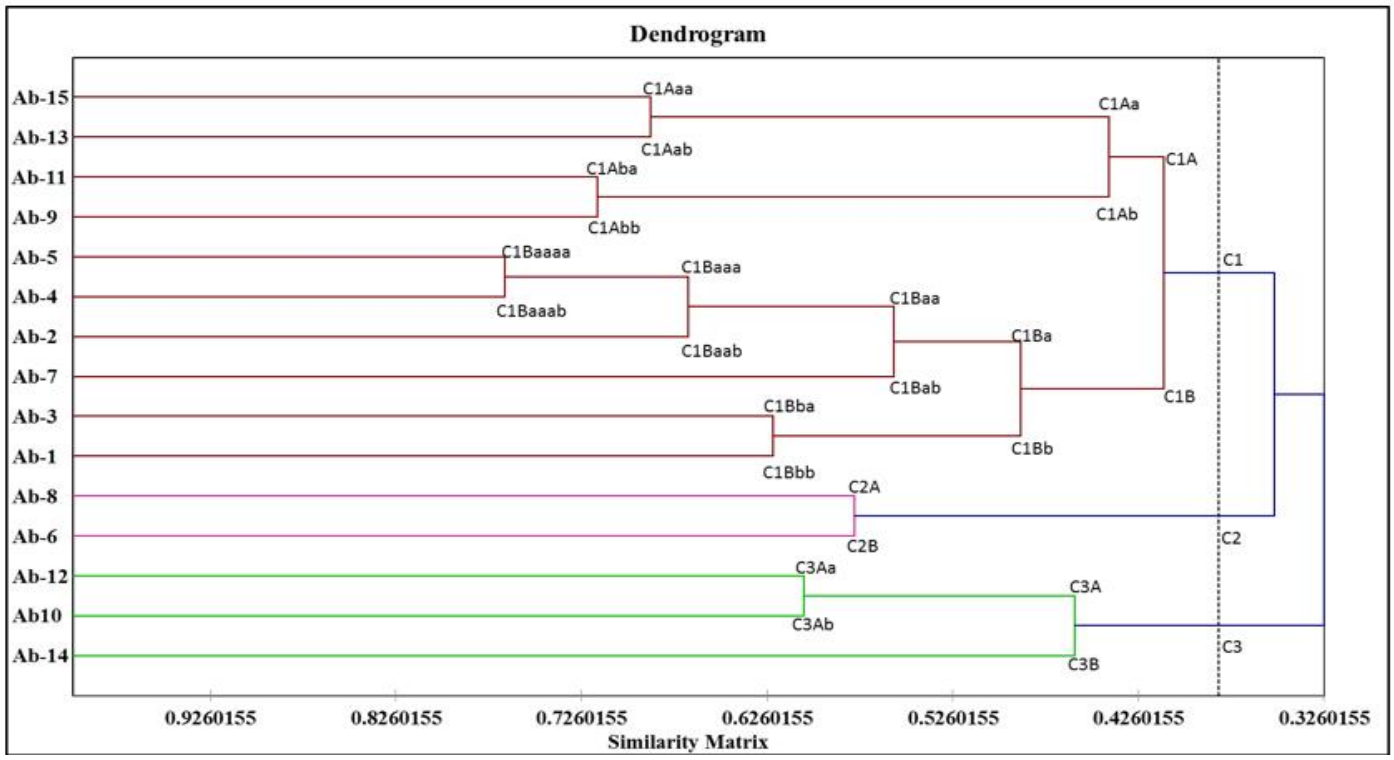

Fig 1: Dendrogram of fifteen isolates of *A. burnsii* Jaccard's similarity coefficient (1908) using UPGMA as the clustering method for RAPD

Table 3: Genetic similarity matrix of RAPD data based on Jaccard's similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1														
2	0.467	1													
3	0.623	0.484	1												
4	0.464	0.726	0.479	1											
5	0.528	0.611	0.500	0.767	1										
6	0.302	0.293	0.259	0.375	0.347	1									
7	0.479	0.521	0.514	0.531	0.622	0.314	1								
8	0.352	0.362	0.328	0.438	0.384	0.579	0.371	1							
9	0.393	0.333	0.439	0.470	0.432	0.383	0.365	0.302	1						
10	0.380	0.271	0.404	0.354	0.329	0.244	0.227	0.304	0.383	1					
11	0.431	0.328	0.554	0.478	0.461	0.346	0.395	0.396	0.717	0.458	1				
12	0.294	0.263	0.321	0.328	0.270	0.262	0.254	0.239	0.378	0.606	0.367	1			
13	0.362	0.393	0.431	0.463	0.427	0.320	0.437	0.321	0.442	0.375	0.429	0.465	1		
14	0.314	0.237	0.291	0.365	0.338	0.227	0.216	0.160	0.465	0.421	0.360	0.500	0.422	1	
15	0.317	0.328	0.431	0.463	0.372	0.467	0.397	0.400	0.442	0.347	0.455	0.432	0.689	0.391	1

Table 4: DNA banding profile of 10 primers in 15 isolates of *A. burnsii*

Sr. No.	Primer	Molecular weight range (bp)	Total bands	No. of loci	No. of Polymorphic Loci	Polymorphism (%)	PIC* value
1	OPA 8	461.55-1363.12	29	5	5	100	0.7776
2	OPA 9	347.15-1430.65	41	9	9	100	0.8768
3	OPA 10	316.21-1727.14	49	6	5	83.33	0.8055
4	OPA 13	240.92-1985.77	112	11	9	81.81	0.8952
5	OPA 18	169.38-2319.40	72	7	7	100	0.8719
6	OPE 3	160.22-1930.52	34	6	5	83.33	0.7335

7	OPE 4	197.23-2163.70	69	8	8	100	0.8720
8	OPE 7	139.78-1927.95	93	9	6	66.66	0.8722
9	OPE 13	250.36-1680.98	73	14	14	100	0.9161
10	OPE 20	261.89-1172.13	50	9	9	100	0.8536
Total	-	-	622	84	77	-	-
Average	-	-	62.2	8.4	7.7	91.513	0.8473

*PIC-Polymorphism Information Content

Studies on RAPD marker against *Alternaria* spp. was done by Kumar *et al.* (2008) [5]; Singh *et al*. (2016) [13]; Mckay *et al*. (1999) [6]; Varma *et al*. (2007) [17]; Jadhav *et al*. (2011) [3]; Naresh *et al*. (2012) [9]; Ozer and Bayraktar, 2015 [10]; Paul *et al*. (2015) [12]; Tiwari *et al*. (2017) [15] by using different primers. In the present study a sizeable number of OPA and OPE primers were screened to find out the variability. The present results are in agreement with above workers.

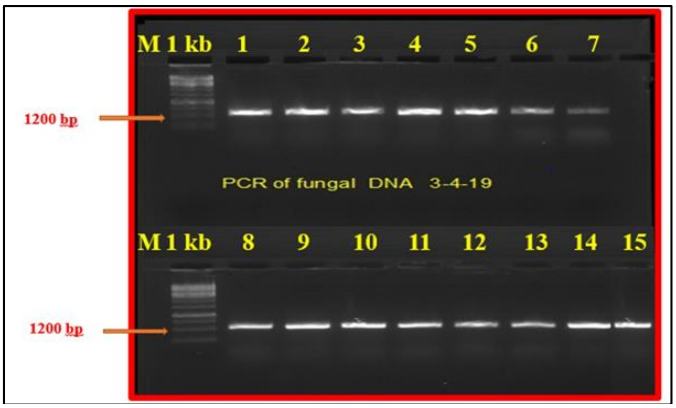

Plate 7: DNA of isolates of Alternaria burnsii M= 1 kb DNA ladder 1- Ab-1=Patan; 2- Ab-2=Banaskantha; 3- Ab-3=Patan; 4- Ab-4=Mnrbi; 5- Ab-5=Banaskantha; 6 -Ab-6=Patan; 7-Ab-7=Morbi; 8-Abe Ahmedabad; 9- Ab-9-Banaskantha; 10- Ab-10- Ahmedabad; 11- Ab-11=Morbi; 12- Ab-2= Surendranagar; 13- Ab-13=Ahmedabad; 14- Ab-14=Surendranagar; 15- Ab-15= Surendranagar

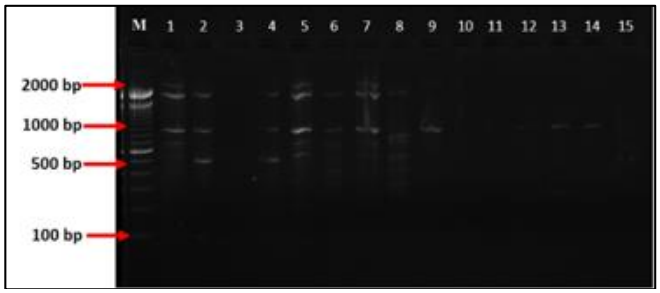

Plate 8.1: RAPD profile of OPA 8

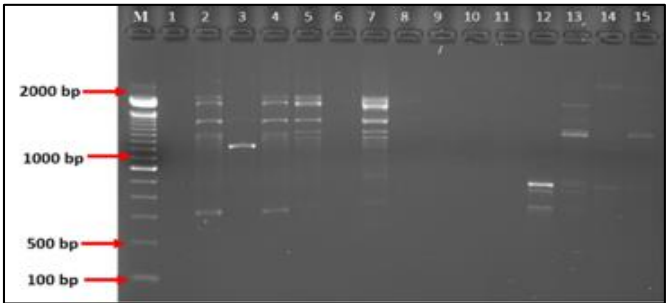

Plate 8.2: RAPD profile of OPA 9

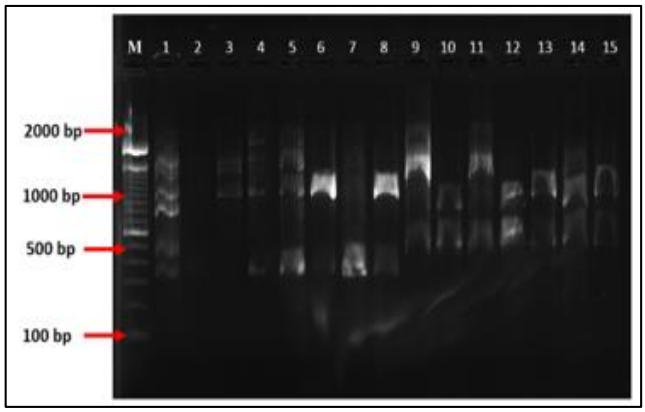

Plate 8.3: RAPD profile of OPA 10 Plate 8.1-8.3 RAPD profile of used primers
M=100 by DNA ladder Ab-I=Patam 2- Ab-2=Banaskamba; Ab-3-Palan; 4- Ab4=Morbi; 5- Ab-5=Ehmaskaatha; 6 -Ab-6=Palan; 7-Ab-7=Morbh 8-Ab-S=Akmedabad; 9- Ab-9=Bammkaalha; 10-Ab-10MAhmedabad;]IAb-11=Morbi; 12- Ab-12=Surendranagam 13-Ab-13= Ahmedabad; 14- Ab-14=Surendranagem 15- Ab-15= Sureadranagar

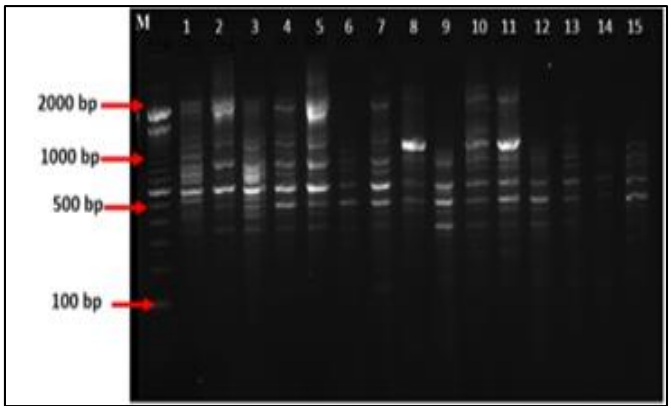

Plate 8.4: RAPD profile of OPA 13

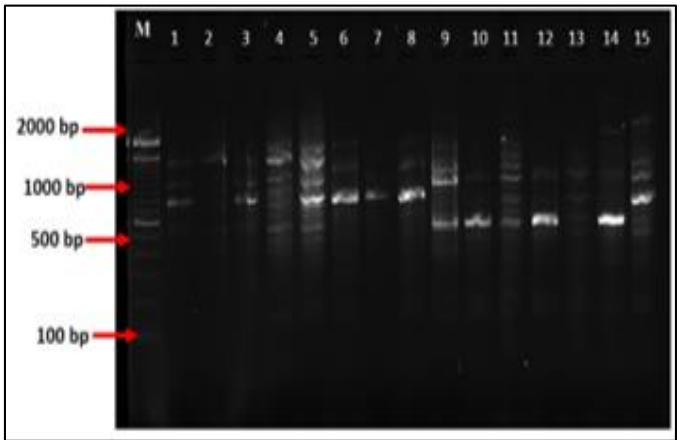

Plate 8.5: RAPD profile of OPA 18

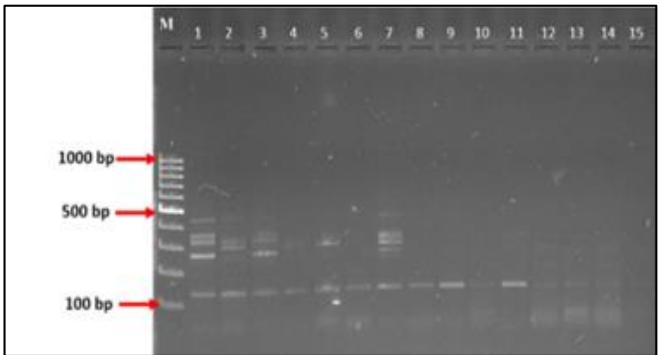

Plate 8.6: RAPD profile of OPE 3 Plate 8.4-8.6 RAPD profile of used primers
M=100 by DNA ladder I- Ah-I =Palau; 2- Ab-2=Banaskantba; 3-A6-3=Patan; 4- Ab-4=31nrbi; 5- Ab-5=Banaskantha; 6 -Ab-6=Patan; 7-A6-7=Morbi; 8- Ab-8=Abmedabad; 9- A6-9=Banaskantha; 10-Abatthmedabad; 11- Ab-11=31orbk 12- Ab-12=Surendranagar; 13-A6-1}= Ahmedabad; 14-.36-14=Surendranagat; 15- A11-15=tiorendranagar

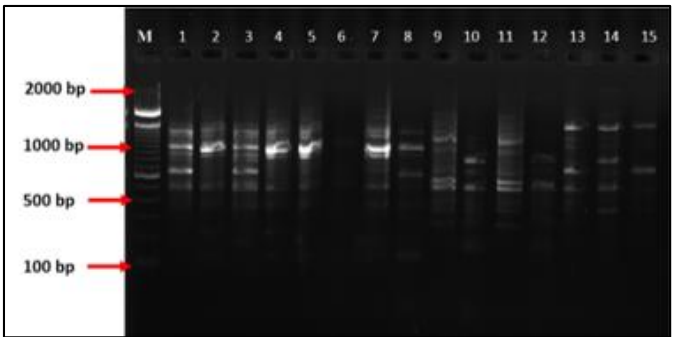

Plate 8.7: RAPD profile of OPE 4

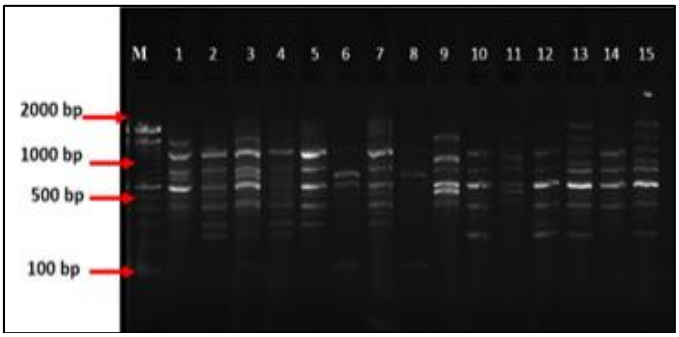

Plate 8.8: RAPD profile of OPE 7

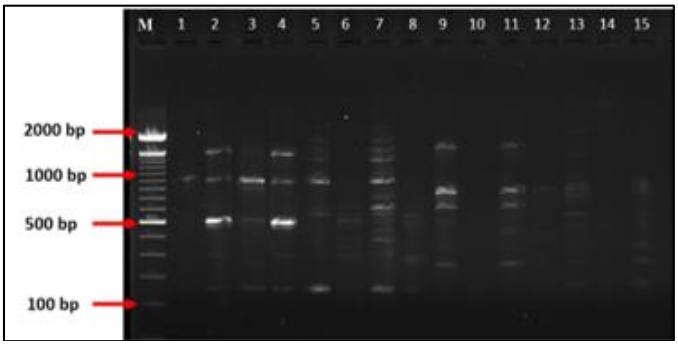

Plate 8.9: RAPD profile of OPE 13 Plate 8.7-8.9 RAPD profile of used primers
M= 100 by DNA ladder I- Ab-I=Patan; 2- Ab-2=Ranaskantha; 3-Ab-3=Patan; 4- Ab-4=Morbi; 5-, th-5=Banaskantba; 6 -Ab-6-Patan; 7-Ab-7=Morbi; Ab-8=Ahmedabad; 9- Ab-9=Baomkantha; 10- Ab-11Ahmedabad; 11- Ab-11=MorM; 12- Ab-12=Surendranagar; 13-Ab-13= Ahmedabad; 14-0,14=Surendranagar; IS- 5= Surendranagar

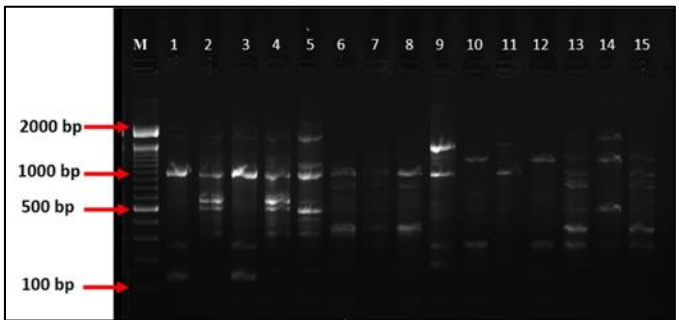

Plate 8.10: RAPD profile of OPE 20 Plate 8.10: RAPD profile of used primers
M=100 by DNA ladder 1- Ab-1=Patan; 2- Ab-2=Banaskantha; 3-Ab-3=Patan; 4- Ab-d=Morbi; 5- Ab-5=Banaskantha; 6 -Ab-6=Patan; 7-Ab-7=Morbi; 8- Ab-8=Ahmedabad; 9- Ah-9=Banaskantha; lo-Ah-10=Ahmedahad; 11- Ab-11=Morhi; 12- Ah-12=Surendranagar; 13- Ah-13= Ahmedabad; 14- Ab-14=Surendranagar; 15- Ah-15= Surendranagar

Conclusion

A large population of *A. burnsii* can be screened by this method to ascertain the races. The virulent races of *A. burnsii* can be ascertained by this method using the OPA 8, OPA 9, OPA 18, OPE 4, OPE 13 and OPE 10 primers. Cumin germplasm can be screened against the virulent races of *A. burnsii* to find out resistant genotype.

Acknowledgement

The authors wish to express their appreciation to Anand Agricultural University, Anand, Gujarat, India for providing best facility to work.

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