

Morphological and Molecular Variability in *Alternaria burnsii* and *Alternaria alternata* Causing Blight Disease of Cummin (*Cuminum cyminum* L.)

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Abstract

A. burnsii and *A. alternata* showed considerable variations in growth pattern, colony diameter, rate of sporulation and in conidial morphology. Among the isolates of *A. burnsii* maximum colony diameter was observed in UDPAb-1 and least in JSL Ab-3 while in *A. alternata* maximum colony diameter was observed in isolate JLR Aa-2 and least in JSL Aa-3 after 7 days of incubation. Maximum number of spores (15.2×10^3 conidia mm⁻²) was produced by isolate UDP Ab-1 in *A. burnsii* and among *A. alternata* it was maximum in isolate JLR Aa-2 and least (9.2×10^3 conidia mm⁻²) by JSL Aa-3. Largest conidial size 88-111×26-32 µm (with beak) and 30-38×14-18 µm (without beak) was observed in isolates UDP Ab-1 among isolates of *A. burnsii* and smallest conidial size 70-88×25-32 µm (with beak) and 31-39×15-19 µm (without beak) was observed in isolate JLR Ab-1. Whereas, among *A. alternata* isolates, largest conidial size 38-48×9-11 µm (with beak) and 30-38×11-14 µm (without beak) was observed in isolates JSL Aa-2. Further, molecular variability was assessed using nine RAPD primers and all these 9 primers showed polymorphism. Primers OPB-20, OPC-2 and OPG-17 generated maximum 10 bands with PIC value of 0.31, 0.34 and 0.33 respectively. A total number of 76 amplified bands were obtained and out of them 41 were polymorphic. The maximum and minimum percentage of polymorphism is observed in primers OPC-2 (80%) and OPA-19 (25%) respectively, these results exhibits the importance of RAPD markers for diversity analysis.

1. Introduction

Cultivation of cummin (*Cuminum cyminum* L.) ranked first among all spices with an acreage of around 594 thousand hectares, total production of 394 thousand mt and productivity of 0.7 mt ha⁻¹ in India (Annonymus, 2016). Cummin is mainly cultivated in various parts of Rajasthan, Gujarat and Maharashtra, during winter season with suitable agronomic practices to achieve highest production. But its sustainable cultivation is continuously challenged by various diseases like blight (*Alternaria burnsii* Uppal, Patel and Kamat), wilt (*Fusarium oxysporum* f.sp. *cumini* Patel, Prasad, Mathur and Mathur) and powdery mildew (*Erysiphe polygoni* DC ex. St.-Am.) causing quantitative and qualitative yield loss (Holliday, 1980). Among these, *Alternaria* blight is considered to be the most devastating to cummin and is quite prevalent. It appears in very destructive form as it affects all above ground plant parts including seed, thus, causing havoc yield loss. Rastogi (1993) isolated *A. brunsii* from contaminated black colored

seeds of cummin in 47 samples out of 105 samples collected from 16 districts of Rajasthan, India. Losses up to 70% have been reported (Holliday, 1980). Mixed inoculum of *A. brunsii* and *A. alternata* causes higher reduction in yield up to 54.8% (Surendra et al., 2015).

Variability studies are important to document the changes occurring in populations and individuals as variability indicate the existence of different pathotypes. Dubey et al. (2016) observed variability in physical and chemical properties of cummin seed samples collected from cummin growing areas located in the 7 districts of Rajasthan and 5 districts of Gujarat classified under Agro-Ecological Sub Regions (AESR) of India. The variability is a well known phenomenon in genus *Alternaria* and may be noticed as changes in spore shape and size, growth and sporulation, pathogenicity, etc. Pathogen population structure and mechanisms by which variation arises within a population is of paramount importance for devising a successful disease management strategy. This requires continuous monitoring of the development of pathogen



variability more so for the breeding programme aimed at developing resistant genotypes to the given set of pathogenic races. Variation in pathogen populations can generally be detected with methods like cultural, morphological, pathogenic and molecular variability. Hence, the present investigations were carried out to study the variability in *Alternaria burnsii* (Uppal) and *Alternaria alternata* (Kessler) causing blight disease.

2. Materials and Methods

The study was carried out at Department of Plant Pathology, Rajasthan College of Agriculture (RCA), Udaipur, Rajasthan (India) during 2012–13 and 2013–14. Disease samples of blighted cumin plants were collected during *rabi* 2011 from Jalore (2011), RCA Udaipur (2011) and Jaisalmer (2011) of Rajasthan all from local land races. The main aim was to explore possibility of existence of different spp. and/or variables of *Alternaria* blight pathogen. The infected aerial parts of the diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory. Isolations of the pathogen were attempted from all samples. These infected aerial parts were thoroughly washed in running tap water to remove the adhering soil. These were then cut into small pieces with the help of a sterilized scalpel, washed in sterilized water, surface sterilized by dipping in 0.1% mercuric chloride (HgCl₂) solution for two minutes rinsed thrice in sterilized distilled water and transferred on potato dextrose agar (PDA) medium in Petri plates. The plates were incubated at 28±1 °C for growth. Sub cultures were made from the periphery of the mycelial growth which appeared after 3–4 days. The list of the isolates recovered is given in Table 1.

2.1. Cultural and morphological characters of isolates of *Alternaria burnsii* and *Alternaria alternata*

The nine isolates of *A. burnsii* and nine isolates of *A. alternata* were grown on Potato Dextrose Agar (PDA) and Richard's agar medium. The autoclaved medium was dispensed in plate and allowed to solidify. Five mm disc of the individual isolate of *A. burnsii* and *A. alternata* removed from the periphery of seven day old culture and was aseptically placed in the centre of the PDA and Richards's agar plate, keeping five plates as five replications for each isolate. The plates were incubated at 28±1 °C. The variations in growth pattern and colony diameter in all isolates were recorded. Spore production by each isolate was determined by removing agar-plugs (5 mm diameter) from three liner spots across the centre of the colony, which were suspended in 10 ml sterile water in glass test tube and agitated twice for about 10 seconds each time on a vortex shaker to dislodge conidia. The number of conidia in the resultant suspensions was determined using a haemocytometer and expressed as number of conidia mm⁻² of medium. For spore

Table 1: Isolates of *Alternaria burnsii* and *Alternaria alternata* recovered from samples collected from fields in cumin growing areas of Rajasthan

Sl. No.	Place of collection	Code	Pathogens isolated	Isolate designation
1.	RCA, Udaipur	UDP	<i>A. burnsii</i>	UDP Ab-1
				UDP Ab-2
				UDP Ab-3
			<i>A. alternata</i>	UDP Aa-1
				UDP Aa-2
				UDP Aa-3
			<i>A. burnsii</i>	JSL Ab-1
				JSL Ab-2
				JSL Ab-3
2.	Jaisalmer	JSL	<i>A. burnsii</i>	JSL Ab-1
				JSL Ab-2
				JSL Ab-3
			<i>A. alternata</i>	JSL Aa-1
				JSL Aa-2
				JSL Aa-3
			<i>A. burnsii</i>	JLR Ab-1
				JLR Ab-2
				JLR Ab-3
3.	Jalore	JLR	<i>A. burnsii</i>	JLR Ab-1
				JLR Ab-2
				JLR Ab-3
			<i>A. alternata</i>	JLR Aa-1
				JLR Aa-2
				JLR Aa-3

size (length and width) mounts were prepared in aniline-blue lacto-phenol and measurements were taken by measuring 50 spores of each isolates using stage and ocular micrometer.

2.2. Molecular variability (RAPD)

For molecular variability nine RAPD primers were used to assess nine isolates of *Alternaria burnsii* and nine isolates of *Alternaria alternata*. These isolates were obtained from Rajasthan College of Agriculture farm Udaipur, Jaisalmer and Jalore and further named as (UDP Ab-1), (UDP Ab-2), (UDP Ab-3), (JSL Ab-1), (JSL Ab-2), (JSL Ab-3), (JLR Ab-1), (JLR Ab-2), (JLR Ab-3) and (UDP Aa-1), (UDP Aa-2), (UDP Aa-3), (JSL Aa-1), (JSL Aa-2), (JSL Aa-3), (JLR Aa-1), (JLR Aa-2), (JLR Aa-3) respectively for convenience. Molecular work was carried out at Escherichia Genomics Research Laboratories in New Delhi. DNA was extracted from fungal mycelium using CTAB method as described by Doyle and Doyle (1987) with slight modifications. DNA was amplified with RAPD primer (Table 1a) using PCR technique. The bands were scored for their presence and absence.

2.3. Statistical analysis for similarity coefficient

The scores (0 or 1) for each band obtained from photograph were entered in the form of a rectangular data matrix (qualitative data matrix). The pair-wise association coefficients



Table 1a: List of primers used for the RAPD analysis

Sl. No.	Primer	Sequence (5'-3')	Bases
1.	OPA-05	5'-AGGGGTCTTG3'	10
2.	OPA-19	5'-CAAACGTCGG-3'	10
3.	OPB-07	5'-GGTGACGCAG-3'	10
4.	OPB-13	5'-TTCCCCCGCT-3'	10
5.	OPB-19	5'-ACCCCCGAAG-3'	10
6.	OPB-20	5'-GGACCCTTAC-3'	10
7.	OPC- 2	5'- GTGAGGCGTC-3'	10
8.	OPG-16	5'-AGCGTCCTCC-3'	10
9.	OPG-17	5'-ACGACCGACA3'	10

were calculated from qualitative data matrix using Jaccard's similarity coefficient. The equation for calculating Jaccard's similarity coefficients 'F' between two samples A and B is:

$$F = n_{xy} / (n_1 - n_z)$$

Where,

n_{xy} = No. of bands common to sample A and sample B

n_1 = Total number of bands present in all samples

n_z = No. of bands not present in sample A or B but found in other samples.

Cluster analysis for the genetic distance was then carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the clones using computer program NTSYS pc version 2.02 (Rohlf, 1998).

3. Results and Discussion

Researcher are continue trying to control Alternaria blight by using chemicals, but due to the variability in the pathogen strain, this disease is still not completely removed from farmer field. Due to the dispersion of this pathogen in all form viz., air, seed and soil born it is serious challenge to plant pathologist as well as others. Thus the accurate identification of strain or races is essential to know the mechanism of survival of pathogen. Assessment of variability is essential for correct prediction of pathogen to survival, dissemination and development of the disease. Thus, in present study total 18 diverse isolates were used to check the variability and further research on field application using morphological and molecular markers.

3.1. Cultural and morphological character of isolates

Nine isolates of each of *A. burnsii* and *A. alternata* collected from three different locations viz., Udaipur, Jaisalmer and Jalore, exhibited variations in colony diameter, size, colour of the colony and rate of sporulation on PDA medium at 28±1 °C after 7 days of incubation. Among the species of

A. burnsii isolates collected from Udaipur shows maximum colony diameter 89.4 mm i.e., UDP Ab-1, followed by 88.2 mm in UDP Ab-2, 86.6 mm in UDP Ab-3. Among Jaisalmer isolates 80.4 mm colony diameter was recorded in JSL Ab-1, followed by 79.4 mm in JSL Ab-2 and 77.0 mm was observed with JSL Ab-3, Among the Jalore isolates JLR Ab-1 showed 87.1 mm colony diameter, followed by 85.9 mm in JLR Ab-2, 83.1 mm in JLR Ab-3. (Table 2)

In the isolates of *A. burnsii* Udaipur isolates showing the maximum number of conidia 15.2×10³ conidia mm⁻² in UDP Ab-1, followed by 14.2×10³ conidia mm⁻² by UDP Ab-2, 12.9×10³ conidia mm⁻² UDP Ab-3. In isolates of Jaisalmer 11.2×10³ conidia mm⁻² in JSL Ab-1, followed by 10.4×10³ conidia mm⁻² in JSL Ab-2, 10.9×10³ conidia mm⁻² in JSL Ab-3. Similarly in isolates of Jalore 13.3×10³ conidia mm⁻² in JLR Ab-1, followed by 12.0×10³ conidia mm⁻² JLR Ab-2, 11.9×10³ conidia mm⁻² in JLR Ab-3, (Table 3).

Among the *A. alternata* isolates collected from Udaipur maximum colony diameter 85.1 mm in UDP Aa-1, followed by 83.9 mm in UDP Aa-2, 82.3 mm in UDP Aa-3. However, the isolates of Jaisalmer 76.2 mm in JSL Aa-1, followed by 75.0 mm in JSL Aa-2, 73.3 mm was observed with JSL Aa-3, whereas, the isolates of Jalore 85.2 mm was observed with JLR Aa-2, followed by 83.6 mm in JLR Aa-1, 83.5 mm in JLR Aa-3 (Table 4). Moreover, the isolates of *A. alternata* collected from Udaipur the maximum number of conidia 13.3×10³ conidia/mm² were produced in isolate UDP Aa-1, followed by 12.1×10³ conidia mm⁻² by UDP Aa-2, 11.0×10³ conidia mm⁻² in UDP Aa-3, Among the isolates of Jaisalmer 11.0×10³ conidia mm⁻² in JSL Aa-1, followed by 10.1×10³ conidia mm⁻² in JSL Aa-2, 9.2×10³ conidia mm⁻² in JSL Aa-3. Further, the isolates of Jalore 14.0×10³ conidia mm⁻² in JLR Aa-2, followed by 12.0×10³ conidia mm⁻² in JLR Aa-1, 11.6×10³ conidia mm⁻² in JLR Aa-3, sporulation was recorded (Table 5). Colony characters i.e. margin/shape and colour of the cultures of individual isolate of *A. burnsii* are given in Table 2.

3.2. Growth on Richards agar media

The nine isolates of *A. burnsii* and nine isolates of *A. alternata* collected from three different locations viz., Udaipur, Jaisalmer and Jalore, three isolates of each species collected from each location exhibited variations in colony diameter, size, colour of the colony and rate of sporulation on Richards Agar medium at 28±1 °C after 7 days of inoculation.

Among the species of *A. burnsii* isolates collected from Udaipur, maximum colony diameter 88.7 mm was observed with UDP Ab-1, followed by 87.4 mm in UDP Ab-2, 85.2 mm in UDP Ab-3. In Jaisalmer, isolates 81.8 mm in JSL Ab-3, followed by 80.4 mm in JSL Ab-2, 78.8 mm was observed with JSL Ab-1, Among the Jalore isolates JLR Ab-1 showed



Table 2: Radial growth and cultural characters of nine isolates of *A. burnsii* on PDA

Sl. No.	Isolates	Diameter* (mm) 7 th days	Sporulation** ($\times 10^3$ mm ⁻² medium)	Growth characters and colony colour
1.	UDPAb-1	89.4	15.2	Cottony felty aerial growth, without zonation, white regular margin.
2.	UDPAb-2	88.2	14.2	Cottony submerged growth, dark greyish, without zonation with white regular margin.
3.	UDPAb-3	86.6	12.9	Felty cottony mycelia growth grey coloured, zonation present with white regular margin.
4.	JSL Ab-1	80.4	11.2	Velvety submerged growth, without zonation, white regular margin, steel grey in centre.
5.	JSL Ab-2	79.4	10.4	Velvety submerged growth, without zonation, white regular margin, steel grey in centre.
6.	JSL Ab-3	77.0	10.1	Cottony velvety suppressed growth, light zonation present with off white and kight grey in centre, white regular margin.
7.	JLR Ab-1	87.1	13.3	Felty suppressed olivaceous uniform growth, light zonation present, off white regular margin pale grey in centre.
8.	JLR Ab-2	85.9	12.0	Cottony felty suppressed white and grayish mycelia growth with white regular margin.
9.	JLR Ab-3	83.1	11.9	Aerial felty flobose light olivaceous green to dull black, white regular margin.
	SEm \pm	1.23	0.39	
	CD ($p \leq 0.05$)	3.68	1.19	
	CV%	2.53	5.55	

*Mean of three replications; **Mean of three replications, three from each of the plate

Table 3: Variation in conidial morphology of nine isolates of *A. burnsii* on PDA

Isolate	Conidial morphology with beak (μ m)				Conidial morphology without beak (μ m)			
	Length		Width		Length		Width	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
UDPAb-1	99.6 \pm 4.61	88–111	29.3 \pm 1.35	26–32	34.6 \pm 1.60	30–38	16.5 \pm 0.76	14–18
UDPAb-2	96.5 \pm 5.14	85–108	30.0 \pm 1.59	26–33	36.6 \pm 1.9	32–40	17.2 \pm 0.91	15–19
UDPAb-3	93.4 \pm 4.85	83–104	32.4 \pm 1.68	28–36	35.2 \pm 1.82	31–39	15.2 \pm 0.78	13–17
JSL Ab-1	88.9 \pm 4.62	79–99	36.0 \pm 1.87	32–40	37.5 \pm 1.94	33–42	17.0 \pm 0.88	15–19
JSL Ab-2	83.4 \pm 4.35	74–93	35.1 \pm 1.83	31–39	38.2 \pm 1.99	34–42	16.0 \pm 0.83	14–17
JSL Ab-3	86.4 \pm 4.00	76–96	37.1 \pm 1.72	33–41	36.0 \pm 1.66	32–40	15.4 \pm 0.71	13–17
JLR Ab-1	78.8 \pm 3.65	70–88	29.0 \pm 1.34	25–32	34.8 \pm 1.61	31–39	17.1 \pm 0.79	15–19
JLR Ab-2	84.0 \pm 4.47	74–94	28.0 \pm 1.49	24–31	34.0 \pm 1.81	30–38	19.0 \pm 1.01	16–21
JLR Ab-3	82.8 \pm 4.30	73–92	27.6 \pm 1.43	24–30	37.0 \pm 1.92	32–41	16.2 \pm 0.84	14–18
SEm \pm	0.23		0.09		0.11		0.05	
CD ($p \leq 0.05$)	0.75		0.27		0.30		0.14	
CV%	2.19		2.22		2.17		2.19	

*Mean no. of 50 conidia and \pm S.D. of mean value

85.9 mm colony diameter, followed by 84.0 mm in JLR Ab-2, 83.0 mm in JLR Ab-3 (Table 6).

Among the isolates of *A. burnsii* collected from Udaipur the

maximum no. of conidia 15.0×10^3 mm² were produced by isolate UDP Ab-1, followed by 14.3×10^3 conidia mm² by UDP Ab-2, 12.6×10^3 conidia mm² UDP Ab-3. Among the isolates



Table 4: Radial growth and cultural characters of nine isolates of *A. alternata* on PDA

Sl. No.	Isolate	Diameter* (mm) 7 th days	Sporulation** ($\times 10^3$ mm ⁻² medium)	Growth characters and colony colour
1.	UDPAa-1	85.1	13.3	Aerial light olivaceous uniform growth, light zonation present, white regular margin.
2.	UDPAa-2	83.9	12.1	Cottony light grey to olive green in centre, clear zonation present with regular white margin.
3.	UDPAa-3	82.3	11.0	Aerial felty mycelial growth, zonation present, steel grey in centre with white regular margin.
4.	JSL Aa-1	76.2	11.0	Felty velvety suppressed growth, dull black or dark grey in centre, clear zonation present with white regular margin
5.	JSL Aa-2	75.0	10.1	Cottony velvety submerged growth, white or dark grey in colour, without zonation, white regular margin but sometimes irregular.
6.	JSL Aa-3	73.3	9.2	Felty velvety cottony and grayish mycelia growth, light zonation present, sometimes dull white towards margin.
7.	JLR Aa-1	83.6	12.0	Aerial velvety cottony pure white uniform mycelial growth, without zonation, light grey in centre.
8.	JLR Aa-2	85.2	14.0	Cottony velvety submerged uniform mycelial growth, pure white colour, without zonation
9.	JLR Aa-3	83.5	11.6	Aerial cottony submerged growth, white in centre and sometimes light brown at periphery.
SEm \pm		1.23	0.19	
CD ($p \leq 0.05$)		3.70	1.39	
CV%		2.65	6.95	

*: Mean of three replications; **: Mean of three replications, three from each of the plate

Table 5: Variation in conidial morphology of nine isolates of *A. alternata* on PDA

Sl. No.	Isolate	Conidial morphology with beak (μ m)				Conidial morphology without beak (μ m)			
		Length		Width		Length		Width	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	UDP Aa-1	27.1 \pm 1.41	24–30	13.1 \pm 0.68	11–14	20.5 \pm 1.07	18–22	13.4 \pm 0.70	11–15
2.	UDP Aa-2	33.5 \pm 1.55	29–37	11.1 \pm 0.51	9–12	28.6 \pm 1.32	25–32	11.5 \pm 0.53	10–12
3.	UDP Aa-3	37.5 \pm 2.00	33–42	12.0 \pm 0.64	10–13	24.7 \pm 2.30	15–34	12.3 \pm 0.65	10–13
4.	JSL Aa-1	36.9 \pm 1.97	32–41	11.8 \pm 0.63	10–13	24.7 \pm 1.31	22–27	11.4 \pm 0.61	10–12
5.	JSL Aa-2	43.4 \pm 2.25	38–48	10.6 \pm 0.55	9–11	34.5 \pm 1.79	30–38	12.5 \pm 0.65	11–14
6.	JSL Aa-3	26.3 \pm 1.37	23–29	12.7 \pm 0.66	11–14	29.8 \pm 1.55	26–33	13.5 \pm 0.70	12–15
7.	JSL Aa-1	25.9 \pm 1.20	23–29	12.6 \pm 0.63	11–14	19.0 \pm 0.88	16–21	12.8 \pm 0.59	11–14
8.	JSL Aa-2	32.0 \pm 1.70	28–35	10.6 \pm 0.55	9–11	27.0 \pm 1.43	24–30	10.0 \pm 0.53	8–11
9.	JSL Aa-3	35.8 \pm 1.86	31–40	11.1 \pm 0.66	9–12	23.6 \pm 1.22	21–26	11.5 \pm 0.60	10–12
SEm \pm		0.10		0.03		0.11		0.03	
CD ($p \leq 0.05$)		0.28		0.09		0.33		0.09	
CV%		2.18		2.04		0.25		2.05	

*Mean number of 50 conidia and \pm S.D. of mean value

Table 6: Radial growth and cultural characters of nine isolates of *A. burnsii* on RAM

Sl. No.	Isolate	Diameter* (mm) 7 th days	Sporulation** (×10 ³ mm ⁻² medium)	Growth characters and colony colour
1.	UDPAb-1	88.7	15.0	Cottony velvety suppressed mycelia growth, zonation present, pure white in centre with irregular margin.
2.	UDPAb-2	87.4	14.3	Felty aerial suppressed light brown olivaceous sometimes dull white, highly suppressed at margin, regular white margin.
3.	UDPAb-3	85.2	12.6	Velvety cottony suppressed growth, without zonation, light brown in centre with dull white regular margin.
4.	JSL Ab-1	78.8	10.3	Felty aerial cottony mycelia growth light zonation, dull white irregular margin and light grey or brown in centre.
5.	JSL Ab-2	80.4	11.1	Velvety aerial at centre, submerged suppressed mycelia growth, clear zonation present, dark grey in centre later turned in to dull black with irregular off white margin.
6.	JSL Ab-3	81.8	11.2	Aerial cottony pure white in centre, light zonation present, off white regular margin while light brown at centre.
7.	JLR Ab-1	85.9	13.8	Aerial felty mycelia growth, zonation present, dull black in centre with white regular margin.
8.	JLR Ab-2	84.0	12.0	Aerial cottony, submerged uniform growth, sometime pale olive brown at periphery with pure white in centre without zonation
9.	JLR Ab-3	83.0	11.9	Cottony velvety submerged growth, clear zonation present, dull black and dark grey in centre with irregular margin and little white near margin.
SEm±		1.45	0.41	
CD ($p \leq 0.05$)		4.35	1.24	
CV%		3.0	5.74	

*: Mean of three replications; **: Mean of three replications, three from each of the plate

of Jaisalmer 11.2×10^3 conidia mm⁻² in JSL Ab-1, followed by 11.1×10^3 conidia mm⁻² in JSL Ab-2, 10.3×10^3 conidia mm⁻² in JSL Ab-1. Among the isolates of Jalore 13.8×10^3 conidia mm⁻² in JLR Ab-1, followed by 12.0×10^3 conidia mm⁻² JLR Ab-2, 11.9×10^3 conidia mm⁻² in JLR Ab-3 (Table 7).

Among the species of *A. alternata* isolates collected from Udaipur maximum colony diameter 87.7 mm in UDP Aa-3, followed by 86.3 mm in UDP Aa-2, 84.3 mm in UDP Aa-3. Among the isolates of Jaisalmer 75.5 mm in JSL Aa-1, followed by 74.2 mm in JSL Aa-2, 72.9 mm was observed with JSL Aa-3, among the isolates of Jalore 84.7 mm was observed with JLR Aa-3, followed by 84.0 mm in JLR Aa-2, 82.3 mm in JLR Aa-3 (Table 8).

Among the isolates of *A. alternata* collected from Udaipur the maximum number of conidia 14.2×10^3 mm⁻² were produced in isolate UDP Aa-3, followed by 13.7×10^3 conidia mm⁻² by UDP Aa-2, 10.8×10^3 conidia mm⁻² in UDP Aa-1, Among the isolates of Jaisalmer 10.2×10^3 conidia mm⁻² in JSL Aa-1, followed by 10.0×10^3 conidia mm⁻² in JSL Aa-2, 9.8×10^3 conidia mm⁻² in JSL Aa-3. Among the isolates of Jalore 13.4×10^3 conidia mm⁻² in JLR Aa-3, followed by 10.7×10^3 conidia mm⁻² in JLR

Aa-2, 10.2×10^3 conidia mm⁻² in JLR Aa-1, sporulation was recorded (Table 9).

Colony characters i.e. margin/shape and colour of the culture of individual isolates of *A. burnsii* are shown in table-8. Morphological characters of nine isolates of *A. burnsii* were similar to standard description given by Holliday (1980). The spore characteristics of individual isolates are presented in Table 7.

Colony characters i.e. margin/shape and colour of the culture of individual isolates of *A. alternata* are shown in Table-8. The morphological characters of nine isolates of *A. alternata* were similar to standard description given by Holliday (1980). Pryor and Michailides (2002) reported similar culture conditions for characterization of colony morphology and sporulation habit among small-spored catenulate *Alternaria spp.* The spore characteristics of individual isolates are given in Table 9. Our characters are similar with Pandey et al. (2005) where, they concluded that variability in *Alternaria solani* exists only with respect to cultural characters. Eleven isolates of *A. solani* causing early blight of tomato showed cultural variability in respect of radial growth, growth rate day⁻¹ and pigmentation on potato dextrose agar. These results are also in similarity with

Table 7: Variation in conidial morphology of nine isolates of *A. burnsii* on RAM

Sl. No.	Isolate	Conidial morphology with beak (μm)				Conidial morphology without beak (μm)			
		Length		Width		Length		Width	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	UDPAa-1	99.1 \pm 4.59	88–111	30.9 \pm 1.43	27–34	36.2 \pm 1.6	32–40	17.8 \pm 0.82	15–20
2.	UDPAa-2	95.4 \pm 5.08	84–106	33.0 \pm 1.75	29–36	32.0 \pm 1.70	28–35	19.4 \pm 1.03	17–21
3.	UDPAa-3	99.3 \pm 5.16	88–111	31.2 \pm 1.62	27–35	35.1 \pm 1.82	31–39	16.0 \pm 0.83	14–17
4.	JSL Aa-1	89.8 \pm 4.66	79–100	37.8 \pm 1.96	33–42	37.5 \pm 1.95	33–42	17.9 \pm 0.93	16–20
5.	JSL Aa-2	87.0 \pm 4.54	77–97	36.2 \pm 1.89	32–40	33.0 \pm 1.72	29–36	16.0 \pm 0.83	14–17
6.	JSL Aa-3	82.3 \pm 3.81	73–92	34.0 \pm 1.57	30–38	39.6 \pm 2.54	32–49	15.6 \pm 0.72	13–17
7.	JSL Aa-1	78.5 \pm 3.63	69–87	28.7 \pm 1.33	25–32	31.4 \pm 1.45	27–35	19.0 \pm 0.88	16–21
8.	JSL Aa-2	80.0 \pm 4.26	71–89	25.0 \pm 1.33	22–28	38.5 \pm 2.05	34–43	17.0 \pm 0.90	15–19
9.	JSL Aa-3	82.1 \pm 4.26	73–91	26.4 \pm 1.37	23–29	35.3 \pm 1.38	31–39	17.9 \pm 0.93	16–20
	SEm \pm	0.27		0.10		0.13		0.05	
	CD	0.76		0.27		0.37		0.15	
	($p \leq 0.05$)								
	CV%	2.20		2.25		2.68		2.19	

*Mean number of 50 conidia and \pm S.D. of mean valueTable 8: Radial growth and cultural characters of nine isolates of *A. alternata* on RAM

Sl. No.	Isolate	Diame-ter* (mm)	Sporulation** ($\times 10^3 \text{ mm}^{-2}$ medium)	Growth characters and colony colour
		7 th days		
1.	UDPAa-1	84.3	10.8	Aerial at centre, cottony suppressed near margin with clear zonation, off white and dull grey in centre, white regular margin.
2.	UDPAa-2	86.3	13.7	Felty cottony velvety mycelia growth, submerged uniform off white regular margin with light grey and white in centre, without zonation.
3.	UDPAa-3	87.7	14.2	Cottony felty off white uniform mycelia growth at centre, dull grey and brown at periphery with off white regular margin.
4.	JSL Aa-1	75.5	10.2	Aerial, felty, velvety, suppressed mycelial growth, dull black or light brown in centre, light zonation present, sometimes dull white near off white irregular margin.
5.	JSL Aa-2	74.2	10.0	Aerial cottony velvety suppressed growth, zonation present, light black or dull grey in centre, sometimes off white with margin.
6.	JSL Aa-3	72.9	9.8	Aerial felty, suppressed mycelial growth, dull black or dark grey in centre, sometimes off white around centre with irregular margin.
7.	JLRAa-1	82.3	10.6	Velvety cottony submerged growth without zonation, pure white in centre with regular margin.
8.	JLR Aa-2	84.0	10.7	Felty cottony mycelium growth, olivaceous brown and velvety at centre, light zonation present with regular white margin.
9.	JLR Aa-3	84.7	9.8	Cottony velvety mycelium growth, pure white in centre with irregular off white margin, light zonation present around centre and sometimes light grey near margin.
	SEm \pm	1.37	0.30	
	CD ($p \leq 0.05$)	4.12	0.89	
	CV%	2.93	4.52	

*: Mean of three replications; **: Mean of three replications, three from each of the plate



Table 9: Variation in conidial morphology of nine isolates of *A. alternata* on RAM

Sl. No.	Isolate	Conidial morphology with beak (μm)				Conidial morphology without beak (μm)			
		Length		Width		Length		Width	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	UDPAa-1	27.4 \pm 1.43	24–30	13.4 \pm 0.70	11–15	18.2 \pm 0.95	16–20	11.0 \pm 0.57	9–12
2.	UDPAa-2	34.0 \pm 1.86	30–40	11.5 \pm 0.53	10–12	29.1 \pm 1.34	25–32	12.2 \pm 0.56	10–13
3.	UDPAa-3	38.0 \pm 2.02	33–42	12.1 \pm 0.64	10–13	25.1 \pm 1.34	22–28	13.5 \pm 0.72	12–15
4.	JSL Aa-1	37.3 \pm 1.98	33–41	11.0 \pm 0.58	9–12	24.7 \pm 1.31	22–27	11.8 \pm 0.63	10–13
5.	JSL Aa-2	44.0 \pm 2.28	39–49	12.3 \pm 0.64	11–13	35.3 \pm 2.12	30–40	12.6 \pm 0.65	11–14
6.	JSL Aa-3	26.5 \pm 1.38	23–29	13.3 \pm 0.69	11–14	20.2 \pm 1.05	17–22	13.9 \pm 0.73	12–15
7.	JLRAa-1	26.3 \pm 1.22	23–29	10.3 \pm 0.47	9–11	19.9 \pm 0.92	16–24	12.9 \pm 0.60	11–14
8.	JLR Aa-2	32.8 \pm 1.74	29–36	11.4 \pm 0.60	10–12	28.0 \pm 1.49	24–31	9.8 \pm 0.52	8–11
9.	JLR Aa-3	36.1 \pm 1.87	32–40	13.0 \pm 0.67	11–14	23.9 \pm 1.24	21–26	11.4 \pm 0.59	10–11
	SEm \pm	0.11		0.03		0.09		0.03	
	CD	0.31		0.09		0.26		0.09	
	($p \leq 0.05$)								
	CV%	2.39		2.04		2.65		2.08	

*Mean number of 50 conidia and \pm S.D. of mean value

the results obtained by Verma et al. (2007), Raja and Reddy (2007) and Tatarwal et al. (2008).

3.3. Molecular variability analysis using RAPD markers

A total of 9 random decamer primers (RAPD) were used for RAPD analysis, to generate DNA fingerprint profile of 18 isolates of *A. burnsii* and *A. alternata* with a view to detect polymorphism and access molecular diversity amount isolates.

3.4. Detection of polymorphism by RAPD markers

Nine RAPD primers were used for the present investigation; all the primers showed amplification. All these 9 primers

showed polymorphism whereas; primers OPB-20, OPC-2 and OPG-17 generated maximum 10 bands with PIC 0.31, 0.34 and 0.33 respectively. A total of 76 amplified bands were obtained and 41 were polymorphic and maximum percentage of polymorphism observed in primer OPC-2 (80%) and minimum percentage observed in OPA-19 (25%), these results exhibits the importance of RAPD markers for diversity analysis. The DNA amplification and polymorphism generated among various genotypes of *A. burnsii* and *A. alternata* using RAPD primers are presented in Table 10.

The total no. of bands observed for every primer was recorded

Table 10: DNA polymorphism generated using RAPD primers

Name of primer	Sequences	GC (%)	TM ($^{\circ}\text{C}$)	No. of amplicons	Polymorphic Amplicons	Per cent polymorphisms (%)	Band-ing pattern	Primer discriminatory power (%)	PIC values
OPA-05	5'-AGGGGTCTTG3'	60	32	9	5	55	10	0.92	0.34
OPA-19	5'-CAAACGTCGG-3'	60	32	8	2	25	3	0.58	0.33
OPB-07	5'-GGTGACGCAG-3'	70	34	7	5	71	9	0.92	0.40
OPB-13	5'-TTCCCCCGCT-3'	70	34	6	2	33	4	0.70	0.41
OPB-19	5'-ACCCCCGAAG-3'	70	34	8	5	62	9	0.89	0.30
OPB-20	5'-GGACCCTTAC-3'	60	32	10	5	50	10	0.92	0.31
OPC-2	5'-GTGAGGCGTC-3'	70	34	10	8	80	13	0.95	0.34
OPG-16	5'-AGCGTCCTCC-3'	70	34	8	3	37	10	0.89	0.45
OPG-17	5'-ACGACCGACA3'	60	32	10	6	60	11	0.95	0.33
Total= 9				Avg.8.44	Avg.4.5	Avg.52			0.36



separately and polymorphic bands percentage was calculated subsequently. Average polymorphism across the all 18 isolates was found to be 52% and overall size of PCR amplified products ranged between 88 bp to 1500 bp.

3.5. Estimation of genetic similarity coefficient and clustering of *Alternaria* strains based on RAPD profile

Based on RAPD similarity matrix data (Table 10), the value of similarity coefficient ranged from 0.65 to 0.91 i.e. 65–91%. The average similarity across all the genotypes was found out to be 0.80 showing that the genotypes were highly genetically diverse. Maximum similarity value of 0.91 was observed between isolate Aa-2 JLR and Aa3-JLR. Isolate Aa-3JSL and Ab-1 UDP was found to be genetically diverse with minimum similarity value of 0.65.

The dendrogram based on RAPD analysis has generated two groups (Figure 1). However, two isolates i.e. AA-3JSL and AB-1 UDP which belongs to Jaisalmer and Udaipur did not cluster with the group. The group consisted five isolates i.e.

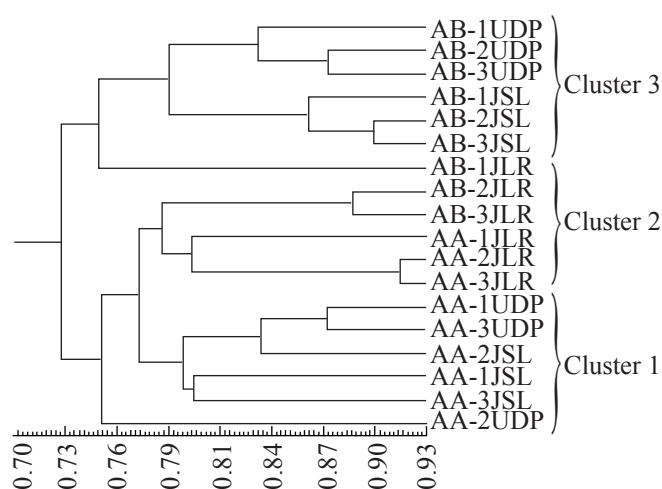
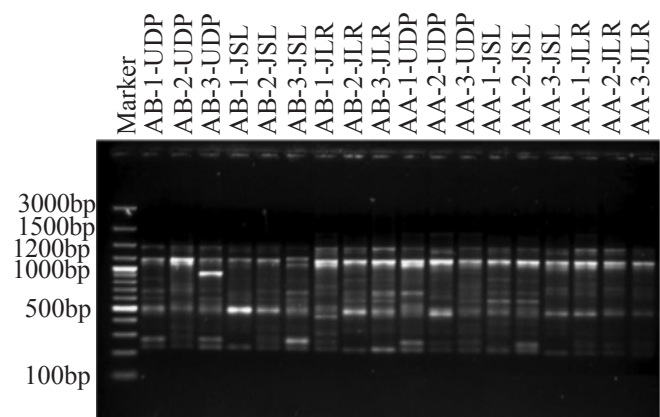


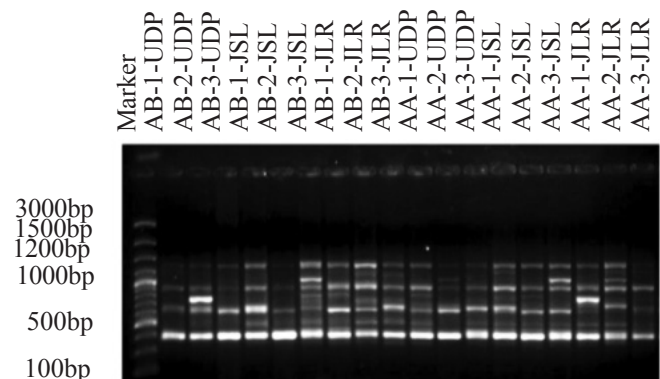
Figure 1: Dendrogram generated through 9 RAPD primer in 18 accession of Fungi *Alternaria brunssii* (9 accession) and *Alternaria alterarta* (9 accession)

AB-2 JLR, AB-3 JLR, AA-1 JLR, AA-2 JLR and AA-3 JLRA had higher group similarity of 78.00%. The primers OPC-2 and OPB-07 gave 80% and 71% polymorphism of the DNA respectively which helped to ascertain variability. Therefore, these primers could be very useful for ascertaining variability among the population of other species of *Alternaria*. Tigno et al. (2003) studied the genetic variability of 22 isolates of *A. alternata* and *A. cassiae*, obtained from *Senna angustifolia*, based on RAPD analysis. A total of 491 scorable bands were produced with the use of 28 primers and cluster analysis based on similarities computed through RAPD markers showed 2 distinct genetic groups of isolates related to both species. RAPD analysis proved to be an efficient method for detecting genetic variability of *Alternaria alternata* and *Alternaria*

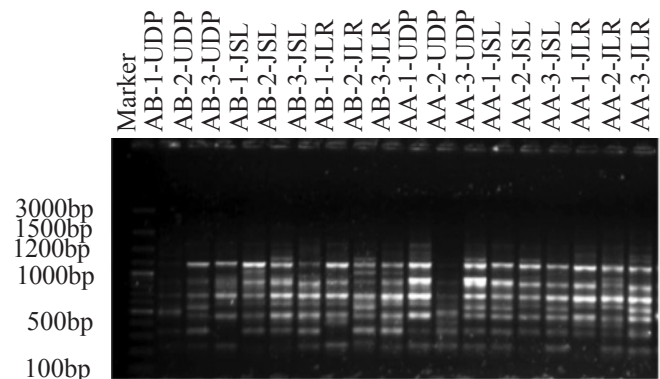
cassia isolates occurring in *Senna angustifolia*, and also for distinguishing *Alternaria* species. Analysis of RAPD fragment patterns confirmed that when *Alternaria* isolates are cultured and observed under defined conditions, their phenotypic plasticity is minimized and valid taxonomic separations can be made upon morphological characteristics (Roberts et al., 2000). The genetic variability based on these studies supports the present results distinctly as shown in dendrogram.



(A) OPA-5: RAPD profile generated for eighteen *A. brunssii* & *A. alternata* isolates (M=Marker)



(B) OPB-20: RAPD profile generated for eighteen *A. brunssii* & *A. alternata* isolates (M=Marker)



(C) OPG-17: RAPD profile generated for eighteen *A. brunssii* & *A. alternata* isolates (M=Marker)

Plate 10: Molecular characterization of *A. brunssii* and *A. alternata* isolates by RAPD marker (OPB-20, OPA-5 and OPG-17)

4. Conclusion

Total 18 diverse isolates were used to check the variability and further research on field application using morphological and molecular markers. All isolates of each of *A. burnsii* and *A. alternata* collected from different locations exhibited variations in colony diameter, size, colour of the colony and rate of sporulation on PDA and Richards medium at $28\pm 1^\circ\text{C}$ after 7 days of incubation. Nine RAPD primers were used for the present investigation; average polymorphism across the all 18 isolates was found to be 52% and overall size of PCR amplified products ranged between 88 bp to 1500 bp. Based on RAPD similarity matrix the value of similarity coefficient ranged from 0.65 to 0.91 i.e. 65–91%.

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