

Cultural and morphological variability among the Isolates of *Alternaria alternata* (Fr.) Keissler, incitant of fruit rot of chilli

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ABSTRACT

Cultural and morphological variability studies on seven different media viz., potato dextrose agar, host leaf extract agar, host fruit extract agar, oatmeal agar, Richards' agar, Czapek's Dox agar and Rose Bengal agar revealed considerable variation among the isolates of *A. alternata* indicated the existence of variability in the pathogen. Moreover, Oatmeal agar and potato dextrose agar were found as an excellent media to support the growth and spore formation of isolates of *A. alternata*, respectively. In case of isolates, Ahmedabad isolate (Aa-7) (61.90 mm) and Rajkot isolate (Aa-8) (61.71 mm) were at par in supporting the mycelium growth and Anand isolate (Aa-1) supports the sporulation abundantly. Among eight isolates of *A. alternata*, distinct differences in terms of conidial length, breadth, beak length and number of septa were recorded. The average conidial length varied from 16.93 to 59.24 μm and breadth ranges from 6.90 to 14.98 μm with beak length of 3.25 to 44.07 μm . The transverse and longitudinal septa varied from 2 to 10 and 0 to 4, respectively. In the present studies, glaring differences in conidial size were noticed among the isolates even when same medium was used for the growth of the isolates. It can be assumed that variation in the isolates may be inherent since isolates were collected from diverse agroclimatic zones of Gujarat. Hence, these variations in the conidial size indicated the existence of variability in this pathogen.

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INTRODUCTION

Chilli (*Capsicum annum* L.) crop is vulnerable to many diseases and pests due to its extreme delicacy and succulence. Diseases caused by fungi, bacteria and viruses are major constraints to chilli production. In India, the first report of *Alternaria* sp. was made from Delhi by Dutt in 1937. Mathur and Agnihotri (1961) reported fruit rot of chilli caused by *Alternaria tenuis* Nees from Rajasthan. Sreekantiah *et al.* (1973) reported a virulent strain of *A. alternata* causing leaf spot and fruit rot of chilli from Mysore, while from Maharashtra, Khodke and Gahukar (1993) also reported *Alternaria* sp.

associated with chilli. Bhatt *et al.* (2000) recorded *A. alternata* causing fruit rot on chilli from Kumaon hills of Uttar Pradesh, India. Narain *et al.* (2000) reported *Alternaria alternata* causing fruit rot of chilli on fruits, as initially small blackish brown, circular to elongated water soaked depressed lesions are formed on the pericarp of fruits, which leads to rotting of fruits in later stage. The characteristic lesions observed at semi ripe stage of chilli fruits. Fruit rot is a major constraint in chilli causing several losses in terms of quality and quantity (Sreekantiah *et al.*, 1973). Mathur and Agnihotri (1961) and Singh (1987) reported 5-85 per cent yield losses due to this

Isolate designation	Location of isolates	Variety	Characters/ symptoms on fruits
Aa-1	Anand	GVC-101	Brown spot with dark rings
Aa-2	Kheda	GVC-112	Reddish brown spot
Aa-3	Bharuch	Jwala	Large blackish brown lesions
Aa-4	Navsari	GVC-111	Brown spot with yellow dry lesions
Aa-5	Vadodara	Sitara	Large yellowish lesions with blackish border
Aa-6	Surat	S-49	Reddish brown spot
Aa-7	Ahmedabad	Jwala	Blackish brown spot
Aa-8	Rajkot	Resam patto	Large yellowish brown lesions

Sr. No.	Rate of sporulation	No. of spores / microscopic field	Sporulation category
1.	Abundant	> 30	4
2.	Good	21 – 30	3
3.	Moderate	10 – 20	2
4.	Scanty	< 10	1
5.	Nil	0	0

disease. An attempt was made to study the cultural and morphological variability among the isolates of *Alternaria alternata* (Fr.) Keissler, incitant of fruit rot of chilli.

MATERIAL AND METHODS

Cultural variability among the isolates of *A. alternata* :

The fruit rot infected samples were collected from major chilli growing areas of Gujarat and isolations were made to study the cultural and morphological variations among the different isolates (Table A).

Growth characters on solid media :

The growth characters of different isolates of *Alternaria alternata* were studied on seven different solid media viz., Potato dextrose agar, Host leaf extract agar, Host fruit extract agar, Oatmeal agar, Richards' agar, Czapek's Dox agar and Rose Bengal agar media.

Twenty ml of each of the medium was poured into each of sterilized Petriplates. Inoculation was made by transferring the five mm disc of mycelial mat, taken from the periphery of 15 days old culture of different isolates. Each treatment was replicated thrice. The plates were incubated at $27\pm 1^\circ\text{C}$ for 15 days. Observation on fungal radial growth was recorded when the maximum growth was attained in any one of the media tested. Other cultural characters viz., type of margin, topography of colony, colour of colony and sporulation were also recorded.

Sporulation :

The sporulation of each isolates on different media were assessed by microscopic observations. A loopful of one-week-old culture was transferred to a clean glass slide and mixed

well with lactophenol and place cover slip on it. The rate of sporulation (Table B) was recorded in five different microscopic fields (Pandey and Vishwakarma, 1998).

Morphological variability among the isolates of *A. alternata* :

The morphological characters of different isolates of *A. alternata* including size of conidia (length and breadth), beak length and number of septa (transverse and longitudinal) were measured from 15 days old culture under high power magnification (45X). The photomicrographs were taken by using camera attachment binocular microscope to show the typical spore morphology of the isolates. The conidial measurements of different isolates were done by using 'SImage 2013 Beta' software.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads :

Cultural variability among the isolates of *A. alternata* :

The study on cultural characteristics of isolates of *A. alternata* was carried out on seven different solid media as described in 'Materials and Methods'. These isolates showed significant differences in cultural characters viz., colony colour, topography of colony and colony margin, colony diameter and sporulation of the isolates of *A. alternata* on seven different solid media (Table 1 to 3).

Growth characteristics of isolates of *A. alternata* on different solid media :

The result presented in Table 1 revealed that there was a considerable variations among the colony characteristics of

the different isolates on seven different solid media. These observations are in conformity with the findings of earlier workers (Pandey *et al.*, 2005; Pipaliya and Jadeja, 2008 and Ramegowda and Naik, 2008). Diversity in cultural characters such as colony colour, its margins and topography were noticed among the isolates of *A. alternata*.

Several workers (Varma *et al.*, 2007; Tatarwal *et al.*, 2008 and Naik *et al.*, 2010) also observed diversity in cultural characteristics such as growth rate, type of growth, colony colour and sporulation among the different isolates of *Alternaria* spp.

Colony diameter and sporulation of isolates of *A. alternata* on different media :

Colony diameter (mm) :

The result presented in Table 2 revealed significant differences between isolates and media and also interaction. Among the seven different media tested, maximum colony diameter (76.58 mm) was recorded in Oat meal agar (OMA). The next best media was Host leaf extract agar (HLEA) (75.21 mm) followed by Potato dextrose agar (PDA) and Host fruit extract agar (HFEA) having colony diameter of 73.67 and 72.17 mm, respectively. The moderate growth was recorded in Richards' agar (RA) (69.17 mm). Rose bengal agar (RBA) and

Table 1 : Mean colony diameter of isolates of *A. alternata* on different media

Isolates/ Media	Colony diameter (mm)							Mean
	PDA	HLEA	HFEA	OMA	RA	CzDA	RBA	
Aa-1	75.00	77.67	76.00	77.00	70.67	11.33	23.00	58.67
Aa-2	77.67	74.00	63.67	78.00	69.67	12.00	20.00	56.43
Aa-3	78.00	72.67	72.67	78.00	69.00	12.67	18.33	57.33
Aa-4	65.67	71.33	72.67	74.00	70.00	10.67	24.33	55.52
Aa-5	66.00	75.33	74.33	73.67	69.00	10.00	15.67	54.86
Aa-6	70.67	74.33	75.33	77.67	63.33	12.67	47.67	60.24
Aa-7	78.00	77.67	72.00	77.00	70.00	10.33	48.33	61.90
Aa-8	78.33	78.67	70.67	77.33	71.67	12.67	42.67	61.72
Mean	73.67	75.21	72.17	76.58	69.17	11.54	30.00	
			S.E. ±		C.D. (P = 0.05)		C.V. (%)	
Media			0.36		1.01			
Isolates			0.39		1.08		3.03	
Isolates × Media			1.02		2.87			

Table 2 : Relative amount of sporulation of isolates of *A. alternata* on different media

Isolates/ Media	Sporulation on media							Mean
	PDA	HLEA	HFEA	OMA	RA	CzDA	RBA	
Aa-1	4	4	4	4	1	1	1	2.71
Aa-2	4	4	4	4	0	1	1	2.57
Aa-3	4	4	4	4	1	1	0	2.57
Aa-4	4	4	4	3	1	1	0	2.43
Aa-5	4	3	4	4	1	1	1	2.57
Aa-6	4	2	3	2	1	1	1	2.00
Aa-7	4	2	2	4	1	1	0	2.00
Aa-8	4	1	2	4	1	1	0	1.86
Mean	4.00	3.00	3.38	3.63	0.88	1.00	0.50	

Note: Degree and categories of sporulation

Rate of sporulation	No. of spores / microscopic field (45X)	Sporulation category
Abundant	> 30	4
Good	20 – 30	3
Moderate	10 – 20	2
Scanty	< 10	1
Nil	0	0

Table 3 : Colony characteristics (colony colour, colony margin and topography of colony) of isolates of *A. alternata* on different media

Isolates/ Media	PDA	HLEA	HFEA	OMA	RA	CzDA	RBA
Aa-1	Whitish green colour Irregular margin Fluffy mycelial growth	Whitish green colour Smooth margin Medium fluffy growth	Black colour Smooth margin Flat mycelial growth	Blackish green colour Irregular margin Medium fluffy growth	Brown colour margin Flat hairy mycelium growth	Whitish green colour Smooth margin Raised mycelial growth	White colour Irregular margin Raised fluffy growth
Aa-2	Whitish green colour Smooth margin Medium fluffy with circular rings	Whitish green colour Smooth margin Flat mycelium with circular rings	Blackish green colour Irregular margin Medium fluffy growth	Whitish green colour Smooth margin Flat mycelium with circular rings	Brown colour margin Flat hairy mycelium growth	Greenish black colour Smooth margin Raised mycelial growth	White colour Smooth margin Raised fluffy growth
Aa-3	Whitish green colour Smooth margin Medium fluffy with circular rings	Whitish green colour Irregular margin Flat mycelium with irregular rings	Black colour Smooth margin Flat mycelial growth	Whitish green colour Smooth margin Medium fluffy growth	Brown black colour Smooth margin Flat hairy mycelium growth	Whitish green colour Smooth margin Raised fluffy growth	White colour Irregular margin Raised fluffy growth
Aa-4	Blackish green colour Smooth margin Medium fluffy growth	Whitish green colour Irregular margin Flat mycelium with irregular rings	Black colour Smooth margin Flat mycelial growth	Blackish green colour Irregular margin Medium fluffy growth	Brown black colour Irregular margin Flat hairy mycelium growth	Whitish green colour Smooth margin Flat mycelial growth	White colour Smooth margin Raised fluffy growth
Aa-5	Blackish green colour Irregular margin Medium fluffy with circular rings	Blackish green colour Smooth margin Flat mycelium with circular rings	Black colour Smooth margin Flat mycelial growth	Blackish green colour Smooth margin Flat mycelial growth	Brown colour margin Flat hairy mycelium growth	Greenish black colour Smooth margin Flat mycelial growth	White colour Smooth margin Raised fluffy growth
Aa-6	Whitish green colour Irregular margin Medium fluffy with irregular rings	Black colour Smooth margin Fluffy mycelial growth	Black colour Smooth margin Flat mycelial growth	Blackish green colour Smooth margin Medium fluffy growth	Brown colour margin Flat hairy mycelium growth	Whitish green colour Smooth margin Raised mycelial growth	White colour Smooth margin Raised fluffy growth
Aa-7	Whitish green colour Smooth margin Raised fluffy growth	Whitish green colour Smooth margin Flat mycelium with circular rings	Black colour Irregular margin Flat mycelial growth	Blackish green colour Smooth margin Flat mycelial growth	Brown colour margin Flat hairy mycelium growth	Greenish black colour Smooth margin Raised mycelial growth	White colour Irregular margin Medium fluffy growth
Aa-8	Black colour Smooth margin Raised fluffy growth	Black colour Smooth margin Flat mycelial growth	Black colour Smooth margin Flat mycelial growth	Black colour Smooth margin Flat mycelial growth	Brown colour margin Flat hairy mycelium growth	Greenish black colour Smooth margin Flat mycelial growth	White colour Smooth margin Medium fluffy growth

Czapek's dox agar (CzDA) did not support the growth of *A. alternata* as evident from growth diameter of 30.00 and 11.54 mm, respectively.

In case of different isolates, maximum colony diameter (61.90 mm) was recorded in Ahmedabad isolate (Aa-7), which was at par with Rajkot isolate (Aa-8) (61.71 mm). The next best was the Surat isolate (Aa-6) (60.24 mm) followed by Anand isolate (Aa-1) (58.67 mm), Bharuch isolate (Aa-3) (57.33 mm) and Kheda isolate (Aa-2) (56.42 mm). The moderate growth was recorded in Navsari isolate (Aa-4) (55.52 mm) and Vadodara isolate (Aa-5) (54.86 mm).

The interaction between media and isolates was found significant, which indicated the variation among the isolates in utilizing the media. Among them, maximum colony diameter (78.67 mm) was recorded in Rajkot isolate (Aa-8) on HLEA media followed by same isolate on PDA media (78.33 mm).

The least colony diameter of 10.00 mm was recorded in Vadodara isolate (Aa-5) on CzDA media. Overall, the excellent colony growth was recorded on OMA, HLEA, PDA and HFEA media as compared to others. The moderate growth was recorded on RAM and least growth was recorded in CzDM and RBA media.

Sporulation :

With regard to sporulation, the result presented in Table 3 revealed that abundant sporulation was noticed in PDA media (4.00) having more than 50 spores in single microscopic field at 45X followed by OMA (3.63). The next best was HFEA (3.38) followed by HLEA (3.00). The sporulation was recorded scanty in CzDA (1.00), RA (0.88) and RBA (0.50) having less than 10 spores in single microscopic field.

In case of isolates, good sporulation (2.71) was recorded

Isolates/ media	Conidial length (µm)							Mean
	PDA	HLEA	HFEA	OMA	RA	CzDA	RBA	
Aa 1	40.26	43.43	30.60	28.22	33.99	26.81	24.73	32.58
Aa 2	48.85	44.03	34.48	33.72	19.80	40.26	21.02	34.59
Aa 3	59.21	41.79	53.35	40.00	35.25	36.19	19.34	40.73
Aa 4	46.46	40.11	44.74	36.67	27.11	25.53	16.93	33.93
Aa 5	48.10	48.84	40.97	28.24	35.01	30.10	24.74	36.57
Aa 6	38.38	36.87	59.24	32.99	37.19	34.71	23.24	37.52
Aa 7	46.06	38.62	41.75	47.48	32.63	35.19	24.61	38.05
Aa 8	37.76	39.54	48.23	45.69	40.77	29.87	23.60	37.92
Mean	45.64	41.65	44.17	36.63	32.72	32.33	22.28	
				S.E. ±		C.D. (P = 0.05)		C.V. (%)
Media				0.48		1.34		6.43
Isolates				0.51		1.43		
Isolates × Media				1.35		3.79		

Isolates/ media	Conidial breadth (µm)							Mean
	PDA	HLEA	HFEA	OMA	RA	CzDA	RBA	
Aa 1	11.55	12.97	11.41	10.37	9.73	10.59	8.28	10.70
Aa 2	9.66	10.54	11.52	10.44	7.46	13.83	8.59	10.29
Aa 3	13.64	11.33	8.64	8.56	13.07	12.16	8.03	10.78
Aa 4	11.67	10.72	10.62	11.53	8.97	8.13	6.90	9.79
Aa 5	10.41	9.23	12.63	10.10	12.04	10.50	7.38	10.33
Aa 6	11.26	10.61	14.58	12.09	15.73	11.16	7.42	11.83
Aa 7	11.92	12.56	10.69	12.15	9.69	12.03	10.20	11.32
Aa 8	11.46	12.27	8.61	11.55	10.91	8.35	10.65	10.54
Mean	11.45	11.28	11.09	10.85	10.95	10.84	8.43	
				S.E. ±		C.D. (P = 0.05)		C.V. (%)
Media				0.17		0.48		7.89
Isolates				0.18		0.51		
Isolates × Media				0.49		1.37		

in Anand isolate (Aa-1) followed by Kheda isolate (Aa-2) (2.57), Bharuch isolate (Aa-3) (2.57) and Vadodara isolate (Aa-5) (2.57). The moderate sporulation was recorded in Navsari isolate (Aa-4) (2.43), Surat isolate (Aa-6) (2.00) and Ahmedabad isolate (Aa-7) (2.00). The sporulation was recorded scanty in Rajkot isolate (Aa-8) (1.86).

Based on above results, Oatmeal agar (OMA) and Potato dextrose agar (PDA) were found as an excellent media to support the growth and spore formation of isolates of *A. alternata*, respectively.

Morphological variability among the isolates of *A. alternata* :

In all the isolates, the conidia were light to dark brown solitary but few were in short chain in acropetal manner, obclavate and some were oval in shape, broadly rounded base with 0-4 vertical and 1-10 horizontal septa. However, the size of conidia varied among the isolates, some of them were very long and narrow, while some were fairly board.

The result presented in Table 4-7 revealed that there were distinct variations among the morphological characters

viz., length and breadth of conidia, conidial beak length and conidial septation of the isolates of *A. alternata* on seven different solid media.

Conidial length (μm) :

The result presented in Table 4 revealed significant differences between isolates and media and also interaction. Among the seven different media tested, maximum conidial length (45.64 μm) was noticed in PDA, followed by HFEA (44.17 μm) and HLEA (41.65 μm). While, the minimum conidial length (22.28 μm) was noticed in RBA.

In case of different isolates, maximum (40.73 μm) and minimum (32.58 μm) conidial length was noticed in Bharuch isolate (Aa-3) and Anand isolate (Aa-1), respectively. The interaction between media and isolates was found significant, which indicated the variation among the isolates in utilizing the media. Among them, maximum (59.24 μm) conidial length was recorded in Surat isolate (Aa-6) in HFEA media, which was at par with Bharuch isolate (Aa-3) (59.21 μm) in PDA media. The minimum conidial length was noticed in Navsari

Table 6 : Mean beak length of conidia of isolates of *A. alternata* on different media

Isolates/ media	Conidial beak length (μm)							Mean
	PDA	HLEA	HFEA	OMA	RA	CzDA	RBA	
Aa 1	19.31	7.89	5.20	5.20	7.14	4.54	4.37	7.67
Aa 2	28.55	9.59	7.08	6.21	8.00	15.14	5.42	11.43
Aa 3	44.07	9.40	7.07	16.61	9.91	8.60	5.30	14.42
Aa 4	34.42	11.63	8.56	7.36	9.39	4.52	3.25	11.31
Aa 5	16.71	16.61	5.59	7.39	7.04	9.48	4.47	9.61
Aa 6	19.09	4.39	9.73	7.93	8.54	5.32	6.06	8.72
Aa 7	18.40	5.22	9.80	16.77	9.49	7.91	5.88	10.49
Aa 8	13.37	7.58	24.40	8.58	15.60	9.18	4.05	11.82
Mean	24.24	9.04	9.68	9.51	9.39	8.09	4.85	
				S.E. \pm		C.D. (P = 0.05)		C.V. (%)
Media				0.19		0.54		8.77
Isolates				0.20		0.57		
Isolates \times Media				0.54		1.52		

Table 7 : Conidial septation of isolates of *A. alternata* on different media

Isolates/ media	Conidial septation (No.)													
	PDA		HLEA		HFEA		OMA		RA		CzDA		RBA	
	T*	L*	T	L	T	L	T	L	T	L	T	L	T	L
Aa-1	4-6	0-3	3-7	0-1	4-6	0-1	3-4	0-1	3-4	0-2	3-6	0-2	3-4	0-1
Aa-2	6-8	0-2	3-6	0-1	3-6	0-1	3-6	0-2	3-4	0-1	3-7	0-1	1-2	0
Aa-3	2-5	0-3	3-9	0-1	4-6	0-3	3-6	0-2	2-3	0-2	2-5	0-1	2-4	0-1
Aa-4	3-5	0-2	4-6	0-2	4-7	0-1	3-5	0-1	3-4	0-2	2-4	0-1	2-3	0-1
Aa-5	4-6	0-2	4-10	0-4	4-5	0-1	3-4	0-1	3-4	0-2	3-5	0-1	3-4	0-1
Aa-6	3-5	0-4	4-6	0-3	4-8	0-2	3-4	0-1	2-3	0-2	2-4	0-3	3-4	0-1
Aa-7	4-5	0-2	4-6	1-3	2-4	0-2	4-6	0-1	2-3	0-2	3-4	0-1	3-4	0-1
Aa-8	4-6	0-1	3-5	0-3	4-7	0-3	4-5	0-2	3-4	0-2	3-4	0	2-3	0-1

*T= Transverse, L= Longitudinal

isolate (Aa-4) (16.93 μm) and Bharuch isolate (Aa-3) (19.34 μm) in RBA media.

Conidial breadth (μm) :

The result presented in Table 5 revealed significant differences between isolates and media and also interaction. Among the seven different media tested, maximum conidial breadth (11.45 μm) was noticed in PDA, followed by HLEA (11.28 μm) and HFEA (11.09 μm). While, the minimum conidial breadth (8.43 μm) was noticed in RBA.

With respect to different isolates, maximum (11.83 μm) and minimum (9.79 μm) conidial breadth was noticed in Surat isolate (Aa-6) and Navsari isolate (Aa-4), respectively. The interaction between media and isolates was found significant. Among them, maximum (14.58 μm) conidial breadth was recorded in Surat isolate (Aa-6) on HFEA media, which was at par with Bharuch isolate (Aa-3) (13.64 μm) in PDA media. The minimum conidial breadth was noticed in Navsari isolate (Aa-4) (6.90 μm) and Vadodara isolate (Aa-5) (7.38 μm) in RBA media.

Conidial beak length (μm) :

The result presented in Table 6 revealed significant differences between isolates, media and their interaction. Among the seven different media tested, longest conidial beak (24.24 μm) was noticed in PDA, followed by HFEA (9.68 μm) and OMA (9.51 μm). While, the smallest (4.85 μm) conidial beak length was recorded in RBA.

In case of different isolates, longest (14.42 μm) and smallest (7.67 μm) conidial beak length was noticed in Bharuch isolate (Aa-3) and Anand isolate (Aa-1), respectively. The interaction between media and isolates was found significant. Among them, longest (44.07 μm) conidial beak length was recorded in Bharuch isolate (Aa-3) in PDA media. The smallest conidial beak length was noticed in Navsari isolate (Aa-4) (3.25 μm) and Rajkot isolate (Aa-8) (4.05 μm) in RBA media.

Conidial septation (Number) :

The result presented in Table 7 revealed significant differences among the isolates with respect to transverse and longitudinal septation of conidia in different media.

The maximum numbers of transverse septa were associated with the Vadodara isolate (Aa-5) in the range of 4 to 10 in HLEA media and least septation was noticed in Kheda isolate (Aa-2) in the range of 1 to 2 in RBA media. The number of longitudinal septa was higher in the Vadodara isolate (Aa-5) and Surat isolate (Aa-6) in the range of 0-4 in HLEA and PDA media, respectively. The isolates of Kheda (Aa-2) and Rajkot (Aa-8) did not show any longitudinal septa in RBA and CzDA media, respectively. Overall, the average number of septation among the isolates varied from 2 to 10 transverse and 0 to 4 longitudinal septa (Table 7).

The conidial morphology of isolates of *A. alternata* are in accordance with those described by Kumar *et al.* (2003) and Mehta *et al.* (2003) who reported differences among the isolates of *A. brassicae* in terms of conidial length, breadth and number of septation.

Tetarwal *et al.* (2008) described similar results in the isolates of *A. alternata* and Shahnaz *et al.* (2013) in the isolates of *A. porri*. Thus, the present findings tallied with the studies carried out by earlier workers.

Morphological variation *i.e.* conidial size in the isolates of *A. alternata* could be due to nutrition rather than a characteristic pathological variation. However, in the present studies glaring differences in conidial size were noticed among the isolates even when same medium was used for the growth of the isolates. It can be assumed that variation in the isolates may be inherent since isolates were collected from diverse agroclimatic zones of Gujarat. Hence, these variations in the conidial size indicated the existence of variability in this pathogen.

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