

Morph-Cultural Variability among Different Isolates of *Alternaria alternata* Causing Leaf Spot of Ber (*Ziziphus mauritiana* Lamk) in Eastern Uttar Pradesh

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ABSTRACT

Alternaria alternata is one of the most important foliar fungi causing leaf spot of ber. Leaf spot diseased samples were collected from different districts of Uttar Pradesh. Six different isolates of *A. alternata* were obtained in pure culture and designated as A_{a1}, A_{a2}, A_{a3}, A_{a4}, A_{a5} and A_{a6}. The results revealed that there is a greater variation among different isolates of *A. alternata*. The maximum mycelial growth after 10 DAI (days after incubation) was found in A_{a2} (90.10 mm) followed A_{a5} (88.36) and A_{a6} (86.53). Colony

characters like color varied as greenish (A_{a1}), dark olivaceous green (A_{a2}), brown blackish (A_{a3} and A_{a6}), brownish green with black center (A_{a5}), Greenish brown with white center (A_{a4}) and growth was slow (A_{a4} and A_{a5}), medium (A_{a1} and A_{a6}) and fast (A_{a2} and A_{a3}). Growth appearance varied from fluffy (A_{a1}, A_{a4}, A_{a5} and A_{a6}) and compressed (A_{a2} and A_{a3}) with margin regular (A_{a2} and A_{a4}) or irregular margin (A_{a1}, A_{a3}, A_{a5} and A_{a6}). Sporulation was recorded in all six isolates but very good sporulation was observed in A_{a2}, A_{a4} and A_{a5}. Highest average conidial length was recorded in isolates A_{a4} (38.88µm) followed by A_{a3} (30.20µm). Highest average width was recorded in A_{a5} (15.06µm) followed by A_{a3} (14.04 µm). Maximum beak length was observed in A_{a2} (17.28 µm). The number of septa also varied among isolates. Maximum number of transverse septa was found in A_{a5} (4-5). Maximum number of longitudinal septa was found in A_{a5} (2-4).

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INTRODUCTION

Ber (*Ziziphus mauritiana* Lamk) is an important fruit cultivated in arid and semi-arid regions of the world and belongs to the family Rhamnaceae. It is popularly known as “King of arid zone fruits” due to its xerophytic nature and ability to survive under drought condition (Misra *et al.* 2013). It can also be grown on alkali and saline soils too (Kumar *et*

al. 2003). It is very nutritious and rich in Vitamin C, A and B complex nutrients such as iron, calcium and phosphorus, ascorbic acid, carbohydrates and essential minerals (Alam *et al.* 2017). In India, it is commercially cultivated in Haryana, Punjab, Maharashtra, Uttar Pradesh, Rajasthan, Madhya Pradesh, Bihar, Andhra Pradesh and Tamil Nadu. In Uttar Pradesh, ber orchards were mostly cultivated in Varanasi, Ayodhya, Agra and Raebareli (Kumar and Singh 2020). In India, it occupies an area of more than 50,000 ha of land with 5,13,000 MT production (Kaur *et al.* 2020).

A limiting factor in profitable cultivation of ber is the biotic factors mainly fungi which causes an economic yield loss. Ber black fruit spot disease caused by *Alternaria alternata* reduces the fruit quality and also yields of the ber (Manjot *et al.* 2019, Kaur *et al.* 2020). *Alternaria* leaf spot of ber caused by *A. alternata* of ber has played a significant role in causing economical yield losses. Symptoms were observed from margin or tip of leaf which was irregular and light brown in color. In severe condition these spots or lesions gradually increased coalesced and covered the entire surface of leaf results earlier leaf drop (Kumar *et al.* 2016, Mehmood *et al.* 2018). The disease did not have importance due to minor in nature but due to climate change the disease now has economic importance and occurs in moderate to severe form (Chaudhary *et al.* 2021). Continuous variability in the pathogen makes management difficult. So, it is necessary to analyze the variation in plant pathogen population which may provide information about the origin of the pathogen and dispersal as influenced by environmental and human factors. This may suggest the alternate measures to manage the disease. Therefore, keeping in view the importance and seriousness of the disease, present study was conducted to examine the cultural and morphological variability in *A. alternata* populations causing leaf spot of ber.

MATERIALS AND METHODS

Collection, isolation, purification and maintenance of *Alternaria alternata* isolates

Infected leaves showing typical symptom were collected from different districts of Uttar Pradesh

Table 1. Details of isolates of *A. alternata* collected from different districts of Eastern Uttar Pradesh.

Isolates No.	Place of collection	Longitude/Altitude	Plant part used
Aa ₁	MES Horticulture, ANDU-AT, Ayodhya	26°32'29" N, 81°49'54" E	Infected leaves
Aa ₂	Vill-Sonaha, Block-Bahadurpur, Distt.-Basti	25°59'27" N, 82°40'22" E	Infected leaves
Aa ₃	Vill-Kamlapur, Block-Tanda, Distt. -Amedkarnagar	26°32'34" N, 82°40'05" E	Infected leaves
Aa ₄	Vill- Devasani, Block- Mohamdabad, Distt.- Far-rukhabad	25°36'45" N, 83°45'28" E	Infected leaves
Aa ₅	Vill- Maruhar, Block-Lal-ganj, Distt-Pratapgarh	25°57'44" N, 81°29'33" E	Infected leaves
Aa ₆	Vill- Raghupur, Block-Salon (Deva nursery), District-Raebareli	26°01'02" N, 81°29'55" E	Infected leaves

(Table 1). The diseased samples were kept in paper bags, well labeled and brought to the laboratory for isolation of pathogen.

Small pieces of the infected leaves of ber were cut along with some healthy tissues and surface sterilized for 1 minute in 1.0% Sodium hypochlorite (NaOCl₂) solution followed by three washings with sterilized distilled water. Excess moisture was removed by placing these bits on sterilized blotter paper. These bits were transferred aseptically placed in Petri disc containing PDA medium which was supplemented with 100 ppm streptomycin to avoid bacterial contamination and incubated at 25±1°C for 7 days. Sub-culturing from uncontaminated peripheral growth was made on PDA slants. Single spore technique was used for the purification of the fungus. The isolates were designated as A_{a1}, A_{a2}, A_{a3}, A_{a4}, A_{a5} and A_{a6}.

Cultural variability among different isolates of *Alternaria alternata*

Pure cultures of six isolates of the pathogen grown on potato dextrose agar (PDA) medium were individually transferred at the center of the Petri plates

containing PDA and incubated at $25 \pm 1^\circ\text{C}$ temperature in BOD incubator. Each isolate was replicated thrice (Petri dishes) were maintained and followed completely randomized design (CRD). The growth rate was recorded after every 2 days until the growth of the pathogen in petri plate completes. Colony characters viz., zonation, color, growth, margin, appearances and sporulation of isolates were recorded after 10 day of incubation.

Morphological variability among different isolates of *Alternaria 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 10 days old culture at 40X magnification. 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 ocular and stage micrometer (Meena *et al.* 2005).

RESULT AND DISCUSSION

Cultural characterization

A total of six isolates of *A. alternata* were collected from different districts of eastern Uttar Pradesh, and all the culture proved Koch postulates. All the isolates of *A. alternata* produces well defined colony. Colony characters like color varied as greenish (A_{a1}), dark olivaceous green (A_{a2}), brown blackish (A_{a3} and A_{a6}), brownish green with black center (A_{a5}), Greenish brown with white center (A_{a4}) and growth was slow (A_{a4} and A_{a5}), medium (A_{a1} and A_{a6}) and fast (A_{a2} and A_{a3}). Growth appearance varied from fluffy (A_{a1} , A_{a4} , A_{a5} and A_{a6}) and compressed (A_{a2} and A_{a3}) with margin regular (A_{a2} and A_{a4}) or irregular margin (A_{a1} , A_{a3} , A_{a5} and A_{a6}). The zonation was varied from absent (A_{a3} and A_{a5}) and present (A_{a1} , A_{a2} , A_{a4} and A_{a6}). The mycelial growth ranges from slowest radial growth of isolate was recorded (79.20 mm) in A_{a3} and highest radial growth was in (90.10 mm) A_{a2} while, in isolate A_{a1} , A_{a2} , A_{a4} and A_{a5} , it was comparatively less i.e., 85.23 mm, 86.53 mm, 85.23 mm and 88.36 mm respectively on 10th day of incubation under

Table 2. Radial growth of different isolates of *Alternaria alternata* on PDA (Potato Dextrose Agar) medium.

Isolates No.	Radial growth rate (mm) after 10 th day inoculation					Sporulation
	2 th	4 th	6 th	8 th	10 th	
A_{a1}	17.39	34.55	55.62	77.63	85.23	+++
A_{a2}	17.20	31.63	61.12	79.79	90.10	++++
A_{a3}	16.39	29.83	52.41	68.49	79.20	++
A_{a4}	18.52	39.79	57.50	78.69	85.31	++++
A_{a5}	20.22	28.35	51.46	80.28	88.36	++++
A_{a6}	21.65	35.47	59.46	79.77	86.53	++
Mean	22.17	33.27	56.26	77.44	85.78	

uniform environments and medium. Sporulation was recorded in all six isolates but very good sporulation was observed in A_{a2} , A_{a4} and A_{a5} (Tables 2 - 3). The findings are also consistent with those of Verma *et al.* (2007), Raja and Reddy (2007) and Tetarwal *et al.* (2008). Meena *et al.* (2014) reported variation in cultural characters of isolates of *A. alternata* causing leaf blight of isabgol. Bessadat *et al.* (2014) also found colony color olivaceous to dark green of *A. alternata*.

Morphological characterization

Conidia varied in size between isolates, with some being extremely long and narrow. Conidia were borne

Table 3. Cultural characters of different isolates of *Alternaria alternata* on PDA (Potato Dextrose Agar) medium.

Isolates	Growth	Appearances	Margin	Zonation	Colour
A_{a1}	Medium	Fluffy	Irregular	Present	Greenish
A_{a2}	Fast	Compressed	Regular	Present	Dark olivaceous green
A_{a3}	Fast	Compressed	Irregular	Absent	Brown blackish
A_{a4}	Slow	Fluffy	Regular	Present	Greenish brown with white center
A_{a5}	Slow	Fluffy	Irregular	Absent	Brownish green with black center
A_{a6}	Medium	Fluffy	Irregular	Present	Brown blackish

Table 4. Size and septation of conidia in different isolates of *Alternaria alternata*.

Isolates	Conidial morphology of <i>Alternaria alternata</i>									
	Length (μm)		Width (μm)		Beak length (μm)		Septation			
	Range	Mean	Range	Mean	Range	Mean	Transverse septa		Longitudinal septa	
							Range	Mean	Range	Mean
Aa ₁	17.4-31.68	24.54	9.84-13.2	11.52	9.36-16.56	12.96	2-4	3.2	0-1	0.2
Aa ₂	17.4-30.00	23.70	12.24-14.64	13.44	10.36-24.24	17.28	2-5	3.2	1-3	2.2
Aa ₃	20.4-40.00	30.20	10.8-17.28	14.04	9.12-15.12	9.60	1-5	3.4	2-3	2.0
Aa ₄	29.52-48.24	38.88	9.84-17.52	13.68	9.12-15.12	13.56	3-5	4.0	1-3	2.0
Aa ₅	23.52-26.48	25.00	12.72-17.4	15.06	9.84-15.36	12.24	4-5	4.4	2-4	2.8
Aa ₆	21.36-31.68	26.52	7.44-12.48	9.96	7.92-17.52	12.72	1-3	2.2	1-3	1.8

singly or in short chains, and were obpyriform to obclavate, 17.4-48.24 μm length \times 7.44-17.52 μm wide with 1-5 transverse and 0-4 longitudinal septa and the conidial beak length varies between 7.92-24.24 μm (Table 4). Highest average conidial length was recorded in isolates A_{a4} (38.88 μm) followed by A_{a3} (30.20 μm), A_{a6} (26.52 μm), A_{a5} (25.00 μm), Aa1 (24.54 μm) while lowest average conidial length was recorded in isolates A_{a2} (23.70 μm). Highest width was recorded in A_{a5} (15.06 μm) followed by A_{a3} (14.04 μm), A_{a4} (13.68 μm), A_{a2} (13.44 μm), A_{a1} (11.52 μm) while lowest in A_{a6} (9.96 μm). Maximum beak length was observed in A_{a2} (17.28 μm) followed by A_{a4} (13.56 μm), A_{a1} (12.96 μm), A_{a6} (12.72 μm), A_{a5} (12.24 μm) while lowest was found in A_{a3} (9.60 μm). The number of septa also varied among isolates. Maximum number of transverse septa was found in A_{a5} (4-5) and least in A_{a6} (1-3). Maximum number of longitudinal septa was found in A_{a5} (2-4) and lowest in A_{a1} (0-1). Singh *et al.* (2016), Reddy *et al.* (2019), Abbo *et al.* (2018) reported variation in the morphological variability in *A. alternata* isolates of different places of India.

CONCLUSION

Present study clearly indicated that the variation among different isolates of *A. alternata* collected from different districts of Eastern Uttar Pradesh in term of cultural and morphological variation. We conclude that there may be a chance of presence of new races of the pathogen as far as regional occurrence is concern.

Further extensive study is required to identify the variation among *A. alternata* causing leaf spot of ber.

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