

## Characterization of *Alternaria* Leaf Blight of Cotton

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Cotton (*Gossypium* spp.) is one of the most important fiber crops playing a key role in economic and social scenario of the globe. *Alternaria* blight is the predominant disease causing economic loss to the cotton. The study was conducted for characterization of *Alternaria* blight of cotton under south Gujarat condition and morphological, pathogenic and cultural study was conducted along with histopathological study. Among morphological study, *Alternaria macrospora* of Bharuch isolates recorded the maximum mean mycelial width (7.95  $\mu$ m), conidia size (70.11 x 21.845  $\mu$ m), length of beak (7.58  $\mu$ m), no. of transverse septa (3 to 7) and longitudinal septa (0 to 3), in cultural sporulation was maximum in Bharuch isolate with smooth colony margin and grayish color while maximum (85mm) radial growth was observed in Narmada. Digvijay, G.Cot25 and G.Cot23 showed disease free reaction.

**Keywords:** *Alternaria macrospora*, *Alternaria* blight, cotton, morphological study

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### INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important cash crop belongs to Malvaceae family which played a dominant role in the industrial and agricultural economy of India. It provides employment and sustenance to a population of nearly 42 Million people, who are involved directly or indirectly in cotton production, processing, textiles and related activities (Manickam, 2013). India is leader in cotton cultivation and production in world since last one decade. India ranks first place in area and second place in cotton production after USA in the world. India is the largest cotton growing country in the world with an area of around 105 lakh/ha with production of 351 lakh bales and productivity 568 kg/ha (Anonymous, 2017). In India, cotton is cultivated on a large scale in Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh, Punjab, Rajasthan, Haryana, Tamil Nadu and Uttar Pradesh. In Gujarat, cultivated area of cotton is 24.00 lakh with production of 95 lakh bales and productivity 673 kg/ha (Anonymous, 2017).

*Alternaria* species have a wide host range and they are reported on leaves, stem and fruits of the plant. The fungus *Alternaria* causes the leaf spot and blight symptoms on different plant parts. *Alternaria* leaf blight of cotton is essentially a disease complex caused by two pathogens, *A. macrospora* and *A. alternata*. The pathogen *A. macrospora* is the most prevalent pathogen during the early growing season and *A. alternata* towards the end of the growing season (Bhuiyan *et al.* 2007). The present investigation was done to characterize the *Alternaria* spp. infecting to cotton leaf under south Gujarat condition and comprehensive understanding of the causal organism.

### MATERIALS AND METHODS

#### *Collection, isolation and purification*

The naturally infected diseased leaves of cotton plant showing the typical characteristics symptoms of *Alternaria* blight were collected from farmers field of Bharuch, Navsari, Surat and Narmada district of south Gujarat region during *kharif* 2017-18. Small pieces of diseased tissues along with

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adjoining healthy tissues were cut and surface sterilized by dipping in (0.1%) NaOCl<sub>2</sub> solution for one minute followed by three successive washings with distilled sterile water. The sterilized pieces were then transferred aseptically in sterilized Petri plates containing Potato Dextrose Agar (PDA) medium. The Petri plates incubated at 27 ± 1°C in BOD incubator for seven days. The mycelium isolated and purified by serial dilution method and it was microscopically examined for the identification. The well-isolated single spore was marked and then transferred to PDA slants separately under aseptic conditions for purification and further investigation.

#### **Variability of *Alternaria* spp. infecting cotton leaves Morphological**

The seven days old isolates of *Alternaria* culture grown on PDA plates were used to study the morphological characters like width of mycelium, size of conidia and number of transverse as well as longitudinal septa and beak length. The size of conidia and mycelium width measured under light microscope at 40X using software Scope photo 3.0. Twenty observations were recorded randomly for conidial measurement, beak length, number of transverse as well as longitudinal septa and for mycelium measurement and mean values were calculated.

#### **Pathogenic**

The difference in virulence among the four isolates was studied by inoculating cotton seedlings with spore and mycelium suspension of the fungus as per Bashan and Levanony (1991). A total of ten various entries/genotypes were screened along with seedlings of cotton variety LRA-5166 raised in earthen pots fill with sterilized soil. Two seedlings were maintained in each earthen pot. The twenty five days old seedlings were inoculated in the evening spore suspension of (5.4 x 10<sup>6</sup> spores/ml) by using atomizer. Before inoculation of plants, leaves were rubbed with sand paper to have small injuries for easy infection. The four replication of each isolate was maintained. One control pot was maintained with spraying sterilize water for each isolate. All the pots were kept in shade net for development of the disease symptoms. The

observations on the intensity of foliar diseases were recorded after initiation of lesion development upto 90 and 120 DAS. The experiment was conducted in CRD. The disease intensity was recorded in 0 to 4 scale given by Raj (1988).

$$\text{Diseases intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves} \times \text{maximum rating value}} \times 100$$

#### **Cultural**

The cultural characters of four isolates of *Alternaria* were recorded from culture grown on PDA. Five mm disc was cut through sterilize cork borer from the seven days old fungal culture and placed in the center of each plate and incubated at 27 ± 1°C for seven days. The differences between observations regarding colony color, sporulation, type of margin, radial growth and type of mycelial growth of four isolates were recorded 7 days after inoculation.

#### **Histopathological study of cotton leaf blight infected by *Alternaria* spp.**

The host pathogen relationship was studied in infected leaves. Fresh green leaves on healthy plants of cotton were inoculated with viable spores of *A. macrospora* and covered with polythene bags to maintain humidity for 24 h. With the initiation of symptoms, infected leaves were collected and fixed. The mature lesions were also fixed for subsequent examination. Cross sections from the fixed material were examined microscope regularly.

### **RESULTS AND DISCUSSION**

#### **Morphological**

The mean mycelial width (7.95 ìm), conidia size (70.11 x 21.845 ìm), length of beak (7.58 ìm), no. of transverse septa (3 to 7) and longitudinal septa (0 to 3) was maximum in Bharuch isolate and least mean mycelial width (5.09 ìm), conidia size (42.46 x 10.66 ìm), length of beak (5.02 ìm), no. of transverse septa (3 to 4) and longitudinal septa (0 to 1) was observed in Navsari isolate. In Surat isolate mean mycelial width (46.514 ìm), conidia

Grade	Reaction	Description
1	Disease free or Immune	No infection
2	Resistant	Few < 2 mm, scattered, brown and 6-20% leaf area
3	MR	Spots bigger, 3 mm, not coalescing, brown and 6 -20% leaf area covered
4	MS	Spots 3 -5 mm, irregular in shape -coalescing, 21 -40% leaf area covered
5	S	Spots coalescing to form bigger lesions, irregular > 40% leaf area

**Table 1:** Morphological variability of *A. macrospora*

Isolate name	Mycelial width ( $\mu$ m)	Conidia size ( $\mu$ m)		Beak length ( $\mu$ m)	No. of transverse septa	No. of longitudinal septa
		Length	Width			
Navsari	5.09	42.46	10.66	5.02	3-4	0-1
Surat	4.33	46.51	12.88	5.73	3-8	0-2
Bharuch	7.95	70.11	21.84	7.58	3-7	0-3
Narmada	5.48	48.51	12.90	5.79	3-5	0-1

**Table 2:** The grade and reaction of cotton entries/genotypes against Alternaria blight in pot condition

Entries/ Genotypes	LRA5166	Digvijay	Mohini	Ankur	BGDS 1063	DHH1251
Reaction	MR	DF	R	R	MR	MR
Grade	3	1	2	2	3	3
Entries/ Genotypes	G.Cot25	G.Cot23	GSHH2595	GSHH2599	GCot16	HJHV511
Reaction	DF	DF	R	MR	MR	MS
Grade	1	1	2	3	3	4

**Table 3 :** Cultural variability of *A. macrospora* infecting leaf spot of cotton

Isolate name	Cultural Variability				
	Colony color	Sporulation	Type of margin	Radial growth (mm)	Type of mycelial growth
Navsari	Grayish white	++	Irregular	71	Medium raised
Surat	Gray	+++	Smooth	84	Raised
Bharuch	Grayish with sector	++++	Smooth	78	Raised
Narmada	Grayish black with sector	+++	Smooth	85	Raised

++++ Excellent sporulation, +++ Good sporulation, ++ Moderate sporulation, + Poor sporulation



Fig. 1 : Pathogenic variability of *Alternaria* leaf spot of cotton

size (46.51 x 12.88  $\mu\text{m}$ ), length of beak (5.73  $\mu\text{m}$ ), no. of transverse septa (3 to 8) and longitudinal septa (0 to 2) and mean mycelial width (5.48  $\mu\text{m}$ ), conidia size (48.51 x 12.90  $\mu\text{m}$ ), length of beak (5.79  $\mu\text{m}$ ), no. of transverse septa (3 to 5) and longitudinal septa (0 to 1) observed in Narmada isolate as mentioned in Table 1.

Similar result to the present investigation was reported by Kaur and Aggarwal (2015) who reported that size of conidia ranged from 25-57.5 x 12.5-25  $\mu\text{m}$  with 1 to 6 transverse and 0-2 longitudinal septa with beak length ranged between 5-15 x 5-7.5  $\mu\text{m}$  in pathogen *A. macrospora* infecting cotton. Venkatesh and Darvin (2016) found similar result to the present study on morphological characteristics of *A. macrospora* and recorded conidia size ranged between 43.4 x 13.3  $\mu\text{m}$  with 1 to 8 transverse septa. Sangeetha *et al.* (2016) recorded maximum conidial length with 90-180 ( $\mu\text{m}$ ), conidia recorded 1-5 vertical and 4-9 horizontal septa and beak length was equal or twice of the length of conidia.

### Pathogenic

The seedlings of susceptible cotton variety LRA-5166 were used during studies on pathogenic variability of *A. macrospora* isolates. Total ten entries/genotype along with LRA-5166 was screened in glasshouse by artificial inoculation with the spore suspension of *Alternaria* isolates under pot condition during *Kharif* 2017-18. Digvijay,

G.Cot25 and G.Cot23 were showed disease free reaction, Mohini, Ankur and GSHH2595 showed resistance reaction while HJHV511 recorded moderately susceptible reaction and rest were moderately resistance (Table 2). The intensity of *Alternaria* blight disease was recorded on 90 and 120 days after sowing by using 0-4 scale (Raj, 1988). On the basis of per cent disease intensity recorded in LRA-5166 after 120 days this variety showed moderately resistant reacting in all *A. macrospora* isolates in pot condition (Fig. 1). The highest disease intensity was recorded in Bharuch (18.67%) followed by Narmada (15.33 %) and Surat (12.67 %) isolate. The least disease intensity was recorded in Navsari isolate (11.33 %). Analogous result was observed by the Hosagoudar *et al.* (2008) who found both Bt and Non Bt cotton genotypes were susceptible to foliar diseases at Dharwad. The LRA-5166 found resistant reaction against *Alternaria* leaf spot disease under field condition. Further, 19 genotypes showed moderately resistant reaction and 7 genotypes showed susceptible reaction while, 2 genotypes viz., LRA 5166 and GSIV 218 were highly susceptible to the *Alternaria* blight (Suryawanshi, 2013).

### Cultural

It was clear from the present study that colonies varied in respect to their color (gray to grayish white; grayish black with sector), sporulation (Excellent sporulation >30 spores/microscopic field (40X), good sporulation 21-30 spores/microscopic field (40X) and moderate sporulation 11-20 spores/microscopic field (40X), poor sporulation 6-8 spores/microscopic field (40X), type of margin irregular to smooth, radial growth ranged between 71 to 85 mm with medium raised to raised mycelial growth as per (Table 3). The colony color of Navsari, Surat, Bharuch and Narmada was grayish white, gray, grayish with sector and grayish black with sector, respectively. The excellent sporulation was observed in Bharuch isolate followed by Surat and Narmada. The poor sporulation was recorded in Navsari isolate. The highest radial growth (85 mm) was recorded in Narmada isolate followed by Surat isolate (84 mm), Bharuch isolate (78 mm) and lowest in Navsari isolate (71 mm). The Navsari colony showed medium raised colony growth while

other isolates showed raised pattern of mycelial growth. The similar result to the present investigation reported by Sangeetha and Ashtaputre (2016) observed that colony color of *A. macrospora* varied from gray to black with white to black colony margin with irregular or smooth and mycelial growth with sectoring. Perane *et al.* (2015) recorded similar result to the present study and indicated that growth characteristics of *A. macrospora* exhibited 72 mm colony diameter at 7 days of incubation. Hosagoudar (2012) studied on cultural variability of seventeen isolates of *Alternaria* spp. and recorded colony diameter upto 60-80 mm, colony color gray to blackish, smooth irregular margin with flat to medium raised mycelial growth.

### Histopathological studies

The histopathology study of *Alternaria* leaf blight done under controlled condition by pin pricking method and development of fungus was observed regularly. Spore germinated and development of mycelium was observed after 12 h, while appressorium formation after 24 h. Mostly, appressoria were single globose and produced incurably on hyphal branches. The host epidermal cell disruption by mycelium was observed after 48 h and development of mycelium structure inside the host tissue started after that. The branching of mycelium was irregular at acute angle. The conidiophores of the fungus were brown to dark brown, simple and septate. Conidia were light to dark brown, solitary or sometimes in short chain in acropetal manner. The conidia were obclavate but few were oval or pyriform.

### CONCLUSION

The morphological characterization of *Alternaria macrospora* found that Bharuch isolates has maximum mean mycelial width (7.95  $\mu$ m), conidia size (70.11 x 21.845  $\mu$ m), length of beak (7.58  $\mu$ m),

no. of transverse septa (3 to 7) and longitudinal septa (0 to 3), in cultural sporulation was maximum in Bharuch isolate while maximum (85mm) radial growth was observed in Narmada. The highest disease intensity was recorded in Bharuch (18.67%) isolate in LRA5166 as compared to other isolates.

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