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Collection of different isolates of *Alternaria solani* in Bhind, Morena and Gwalior districts of Madhya Pradesh

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Abstract

Collection of diseased samples from different locations of Bhind, Morena and Gwalior districts of Madhya Pradesh for determining variability among collected fifteen isolates of *Alternaria solani*, the causal agent of early blight of tomato based on conidial morphology, cultural variability (color of mycelium, growth of mycelium) on PDA media. Out of fifteen isolates, five isolates were found brownish black, four isolates were found greenish black and three isolate were found reddish black and dark black colour of mycelium. On the basis of type of mycelial growth the isolates were categorized, seven isolates grew with irregular margin and rough surfaced colony, five isolates grew with circular margin and smooth surface, two isolates grew with irregular margin and smooth surface and one having circular margin and rough surface. The maximum conidial length was occurred in isolate MRN-2 and conidial width was in the isolate MRN-2. Maximum number of horizontal septa was associated with the isolate MRN-2 and least septation was observed in isolate GWL-2. The number of vertical septa was higher in the isolate MRN-3 and least was noticed in the isolate GWL-1. The maximum beak length was associated with the isolate BND-2 and least beak length was observed in the isolate GWL-4.

Keywords: *Alternaria solani*, Cultural and morphological variability, tomato, isolates.

1. Introduction

Early blight incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is an economically important and widely distributed disease throughout the world on tomato crop. The main symptom of the disease is dark brown to black concentric rings, which produce target board on leaves, stem and other foliar part of plant. Variability is well known phenomenon in *A. solani* and variability in morphological, cultural and biochemical criteria indicate the existence of different pathotypes (Marak *et al.*, 2014) [13]. The existence of the high level of variability was reported by many workers (Castro *et al.*, 2000; Pryor and Gilbertson, 2002; Pryor and Michailides, 2002; Quayyum *et al.*, 2005; Kumar *et al.*, 2008) [2, 10, 11, 12, 6]. The variants within population may affect the rate of disease development and induce infection in more host lines, which might have implication for stability of cultivar resistance (Naik *et al.*, 2010) [8]. However, little is known about the variability of *A. solani* isolates in India as well as in Madhya Pradesh. Analysis of pathogenic variation within pathogen populations is helpful in understanding host-pathogen coevolution, epidemiology and developing strategies for resistance management (Leung *et al.*, 1993) [7]. Understanding the pathogen variability will help in developing the effective management strategies. So, the present investigation was carried out to find a comprehensive understanding of this causal organism with reference to morphological and cultural variability within the fifteen isolates collected from three districts of Madhya Pradesh region.

2. Methods and Materials

2.1 Sample collection: Early blight infected tomato samples were collected from different districts of Madhya Pradesh during 2014-15. The isolates were coded given in table-1. The samples obtained were surface sterilized with 0.1% mercuric chloride and then washed thrice in sterile water. Leaf bits of approximately 5-7mm were excised from the lesion edges and plated on potato dextrose agar (PDA) medium and incubated at 25±1°C. *A. solani* cultures were identified based on morphological characteristics.

Table 1: Collection of diseased plants samples of early blight of tomato, collected from different districts of Northern Madhya Pradesh

S. no.	Isolate code	Districts	Villages
1	GWL 1	Gwalior	Research farm
2	GWL 2	Gwalior	Ekehara
3	GWL 3	Gwalior	Saujana
4	GWL 4	Gwalior	Shyawari
5	GWL 5	Gwalior	Milavali
6	BND 1	Bhind	Daboha
7	BND 2	Bhind	Jamana
8	BND 3	Bhind	Mehgaon
9	BND 4	Bhind	Gigirkhi
10	BND 5	Bhind	Gormi
11	MRN 1	Morena	Ambah
12	MRN 2	Morena	Dimni
13	MRN 3	Morena	Sirmorkapura
14	MRN 4	Morena	Bharatpura
15	MRN 5	Morena	Ranpur

2.2 Morphological variability: The slides of 15 isolates were prepared in lactophenol from 10 days old culture. For morphological studies, 10 observations for size of conidia, number of septation and length of beak per microscopic field belonging to each isolates were taken under high power (40X) microscopic field. These isolates were categorized in various groups according to size of conidia, number of septa, and length of beak.

2.3 Cultural variability: The cultural characters of isolates of *A. solani* recorded from culture grown on PDA. Twenty ml of sterilized PDA was poured in each of previously sterilized petri plates. Five mm discs were cut through sterilized cork borer from the margin of seven days old colony of the fungal culture grown in petri plates. One disc was placed in the center of each plate and incubated at $25 \pm 1^\circ\text{C}$ for seven days. The differences between observations regarding colony color, growth rate and type of mycelial growth of each isolate were taken.

3. Results

3.1 Cultural characters

3.1.1 Color of mycelium: Isolates of *A. solani* depicted great variability in pigment production on PDA medium. Details of color of mycelium for all 15 isolates after 7th day of incubation are given in Table-2.

Table 2: Colour of the mycelial growth of various isolates of *Alternaria solani*.

Colour of the mycelial growth	Isolate No.	Total	Per cent
Brownish black	1,4,8,11,15	5	33.33
Greenish black	2,3,10,13	4	26.66
Reddish black	5,6,14	3	20.00
Dark black	7,9,12	3	20.00
Total		15	100

Color of mycelium was observed and categorized into four groups as brownish black, greenish black, reddish black and dark black. Five isolates (1,4,8,11,15) produced brownish black, four (2,3,10,13) greenish black pigment, three isolates (5,6,14) were reddish black pigmented while remaining three isolates (7,9,12) dark black pigmented on PDA after 7 days of inoculation at $25 \pm 2^\circ\text{C}$. These isolates were showed variation for their pigmentation.

Table 3: Type of mycelial growth and average mycelial growth of various isolates of *A. solani* on potato dextrose agar medium at 7 days after inoculation

Isolates No.	Type of mycelial growth		Average mycelial growth (7days) mm
	Mycelial margin	Colony character	
1	Irregular	Rough	35.50
2	Circular	Smooth	34.00
3	Circular	Smooth	35.00
4	Irregular	Rough	33.75
5	Irregular	Rough	30.75
6	Circular	Rough	31.75
7	Circular	Smooth	32.75
8	Irregular	Smooth	31.50
9	Irregular	Rough	33.25
10	Irregular	Rough	33.00
11	Circular	Smooth	29.50
12	Irregular	Rough	31.00
13	Irregular	Rough	33.75
14	Circular	Smooth	34.00
15	Irregular	Smooth	30.00

*Average of four replications.

Radial growth observed for 15 isolates presented in Table-3 were showed minor difference. Among all the isolates, the isolate no. 1 belong to research farm Gwalior had maximum average mycelial growth 35.50 mm. On the basis of average mycelial growth the data denotes that the slowest average mycelial growth (29.50 mm) had been obtained in isolates 11. On the basis of type of mycelial growth the isolates were categorized in four groups as irregular-rough, irregular-smooth, circular-smooth and circular-rough margin type mycelial growth.

3.1.2 Morphological characters of different isolates of *A. solani*

Table 4: Morphological characters of various isolate of *Alternaria solani*

S. No.	Isolate code	Size of conidia (μm)		Septa in conidia		Length of beak (μm)
		Length	Breadth	Horizontal	Vertical	
1	GWL-1	101.6	12.4	3-4	0-1	55.8
2	GWL-2	112.5	14.2	2-3	0-1	57.6
3	GWL-3	260.5	22.4	6-7	1-2	105.2
4	GWL-4	146.5	16.2	3-4	1-2	43.4
5	GWL-5	219.0	17.2	4-5	0-2	99.0
6	BND-1	206.4	16.4	5-6	1-2	98.5
7	BND-2	266.4	23.2	5-7	1-2	148.5
8	BND-3	133.1	14.0	3-4	0-1	58.2
9	BND-4	110.5	12.2	2-3	1-2	48.8
10	BND-5	195.1	18.2	3-4	0-2	112.3
11	MRN-1	240.5	19.8	6-7	1-2	133.1

12	MRN-2	285.0	24.4	8-10	2-3	108.1
13	MRN-3	257.5	21.2	7-8	3-4	140.0
14	MRN-4	237.5	19.2	5-6	2-3	132.8
15	MRN-5	159.1	15.4	4-6	1-3	94.6

*Average of 10 observations.

Studies on morphological characters of 15 isolates of *A. solani* have shown variation with respect to conidial size, beak length and septation (Table-4). The isolates varied among themselves with regard to size of conidia and septation. The average conidial length was maximum in isolate MRN-2 (285.0 μm) followed by isolate BND-2 (266.4 μm), GWL-3 (260.5 μm), MRN-3 (257.5 μm), while, conidial width was maximum in the isolate MRN-2 (24.4 μm) followed by BND-2 (23.2 μm). The number of septa also varied among the isolates. Maximum number of horizontal septa were associated with the isolate MRN-2 (8-10) followed by MRN-3 (7-8) and least septation was observed in isolate GWL-2 (2-3) and BND-4 (2-3). The number of vertical septa was higher in the isolate MRN-3 (3-4) and least number of vertical septa were noticed in the isolate GWL-1, GWL-2, BND-3 (0-1). The maximum beak length was associated with the isolate BND-2 (148.5 μm) followed by MRN-3 (140.1 μm) and least beak length was observed in the isolate GWL-4 (43.4 μm). Diversity in cultural characters such as colony colour, its margins and topography were noticed among the isolates of *A. solani*. Several workers (Tong-Yunhui *et al.*, 1994; Perez and Martinez, 1997; Babu *et al.*, 2000) [14, 9, 1] also observed diversity in cultural characteristics such as growth rate, type of growth, colony colour and sporulation among different isolates of *A. solani*. The conidial morphology of *A. solani* isolates are in accordance with those described by Ellis and Ellis (1985) [3]. However, Kaul and Saxena (1989) concluded that spore dimensions were not useful in distinguishing *A. solani* strains. Gour *et al.* (2012) showed variations in different isolates of *Alternaria alternata* in symptoms, growth characteristics, colony diameter, pattern of sporulation, size of conidia and mean number of septa.

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