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Cultural, morphological and pathogenic variability in isolates of *Alternaria alternata* causing Alternaria Blight of Tomato

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Alternaria blight disease caused by *Alternaria alternata* has been a serious problem in tomato growing areas particularly at temperate subtropical, humid tropical regions of the world. To know the variability among the isolates of *Alternaria alternata* diseased plants of tomato were collected from four districts (Jaipur, Alwar, Dausa and Tonk) of Rajasthan. All the isolates of *Alternaria alternata* differed in their colony characters and colony diameter. All the isolates produced initially white mycelium later turned grey, dark grey and black or remained in white colour with regular or irregular margins. Most of the isolates were black to grey in colour. Isolates (AltJp1 and AltJp2) from Jaipur district showed maximum mycelial growth with a colony diameter 90.00 mm and 88.00 mm, respectively. Morphological observations recorded on each isolate revealed that isolates differ in their conidial length and width. In general, conidial length and width were observed in between 22.69 to 36.15 μ m and 9.92 to 18.65 μ m, respectively. Number of horizontal and vertical septa varied between 3 to 4 and 1 to 3, respectively. All the isolates were pathogenic to tomato and produced characteristics symptoms of disease. The per cent disease intensity was with Jaipur isolates AltJp1 (70.25 %) and AltJp2 (67.42 %).

Key words: *Alternaria alternata*, tomato, isolates, cultural, morphological and pathogenic variability.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular, highly remunerative and widely grown vegetable in the world. In India it is far from being produced in the world due to several biotic and abiotic stresses. The crop suffers from many fungal, viral, bacterial and nematode diseases which cause reduction in the yield and quality of produce. The diseases caused by fungi such as wilt and foliar diseases cause considerable losses. Among the foliar diseases, *Alternaria* blight disease caused by *Alternaria alternata* is highly destructive both under field and post-harvest stage. Under field conditions, it leads to leaf and stem blight which causes defoliation of leaves resulting in drastic reduction in fruit yield, while fruit infection in the field and after harvest results in rotting of tomato fruits in addition to affecting marketable quality. In recent years, the disease has been assumed as serious problem in Rajasthan state.

In India, tomato crop is mainly grown in the states of Odisha, Andhra Pradesh, Madhya Pradesh,

Karnataka, West Bengal, Chhattisgarh, Telangana, Bihar, Gujarat, Rajasthan and Uttar Pradesh etc. In Rajasthan, tomato crop is mainly grown in Jaipur, Dausa, Alwar, Tonk, Dholpur, Bharatpur and Chittorgarh districts etc. In Rajasthan, tomato is cultivated over an area of 85.00 million hectares with an annual production of 21.00 million tonnes (Anonymous, 2017).

Tomato is one of the most important "protective foods" because of its special nutritive value. Tomato is grown for its edible fruits, which can be consumed either fresh or in processed form and is a very good source of vitamin A, B, C and minerals as well as lycopene-natural antioxidant. It has niacin 0.712 mg, calcium 31 mg and water 94.28 g per hundred g weight. So tomatoes are called as 'Poor man's fruit'. The crop is grown from almost MSL to an altitude of 1500 m in tropical and subtropical regions, with an annual rainfall of 60-150 cm. Well drained sandy loam soil with high level of organic contents is suitable for tomato cultivation. Soil with high acidity is not suitable for tomato cultivation. Tomato is highly sensitive to abiotic stresses especially extreme temperature, salinity, drought, excessive moisture and

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environmental pollution and biotic stresses. Tomato plants are suffered with large number of biotic stresses including insect pests and diseases from the time of emergence to harvest. It suffers with various diseases incited by fungi, bacteria, viruses, nematodes etc. in several countries. Large number of fungal diseases such as Alternaria blight (*Alternaria alternata*), Early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), Powdery mildew (*Oidiopsis taurica*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Collar rot (*Sclerotium rolfsii*), and Damping off (*Pythium* sp.) are causes severe losses in tomato. Among the fungal diseases, early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop. Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani*. Alternaria blight caused by *A. alternata* (Fr.) Keissleris is one of the most catastrophic disease. It is very difficult to manage Alternaria blight because the pathogen has wide host range, extreme variability in pathogenic isolates and prolonged active phase of the disease cycle. The causal organism is air borne and soil inhabiting and is responsible for early blight, collar rot and fruit rot of tomato.

MATERIAL AND METHODS

The experimental details, methodology followed and criteria adopted for evaluation of different treatments during the course of investigations was described in this chapter.

Experimental site

During the course of present investigation, all the experiments were carried out during zaid 2017 and 2018 in the Department of Plant Pathology and also at the Horticulture Research Farm, S.K.N. College of Agriculture, Jobner, Sri Karan Narendra Agriculture University, Jobner, Jaipur (Rajasthan). Jobner is situated at latitude 26°5' N, longitude of

75°20' E and altitude of 427 meters above MSL (mean sea level). The region falls under semi-arid eastern plain (Agro Climatic Zone- III A) of Rajasthan. The details of the methodology adopted in experimentation are described here.

Variability among the isolates of *Alternaria alternata*

To know the variability among the isolates of *Alternaria alternata* diseased plants of tomato were collected from four districts of Rajasthan. From each district, two isolates were maintained. The pathogen was isolated on PDA medium and cultural, morphological and pathogenic studies were conducted.

Cultural and morphological variability

Single spore cultures of different isolates established and maintained on Potato Dextrose Agar (PDA) were studied for their cultural and morphological characters. Seven days old culture of each isolate was inoculated (5 mm diameter disc) separately on PDA and incubated at 25±1 °C. After 7 day of incubation, radial growth of fungal mycelium, colony characters of each isolate such as colony growth and colony colour were recorded. The measurements of size (length and width) of conidia and septation were measured with the help of florescence microscopy.

Pathogenic variability

To test the pathogenic variability among the isolates, spore suspension of each isolate were prepared in sterilized distilled water separately by blending 7 days old fungal culture in pestle and mortar and filtered through cheese cloth, Spore suspension was further diluted to 1x10⁵ spores/ml and 15 days old tomato plant were separately inoculated with each isolate and observations on disease intensity were recorded after 60 days of inoculation by using following formula

$$PDI = \frac{\text{Sum of the individual disease ratings}}{\frac{\text{Number of fruits/leaves observed X Maximum disease grade}}{}} \times 100$$

RESULTS AND DISCUSSION

Variability among the isolates of *Alternaria alternata*

Alternaria blight infected plant of tomato samples were collected from two villages of each four

The coding for each isolates were given as under

District	Tehsil	Village	Isolate no.
Jaipur	Amer	Nangal-ladi	AltJp1
Jaipur	Amer	Shubrampura	AltJp2
Alwar	Bansur	Nimuchana	AltAw1
Alwar	Bansur	Chatrapura	AltAw2
Dausa	Lalsot	Didwana	AltDs1
Dausa	Lalsot	Salempura	AltDs2
Tonk	Peeplu	Dhundhiya	AltTk1
Tonk	Peeplu	Nathri	AltTk2

districts of Rajasthan viz., Jaipur, Alwar, Dausa and Tonk for variability studies. Cultural, morphological and pathogenic variability among eight isolates of *Alternaria alternata* were studied.

The results showed that isolates of *Alternaria alternata* differ in their colony characters, colony colour, colony texture and colony diameter (Table 1, Fig.1,3). Out of eight isolates, isolate AltJp1 and

Table 1: Cultural and morphological variability in different isolates of *Alternaria alternata* at 7th day of incubation

Isolate No.	Colony characters		Colony diameter (mm)	Length x width of conidia (μ m)	Septation	
	Colony color	Colony texture			Horizontal	Vertical
AltJp1	Black	Smooth	90.00	36.15x18.65	4	3
AltJp2	Black and gray	Rough	88.00	34.67x11.56	3	2
AltAw1	Gray	Smooth	84.00	29.31x14.13	4	1
AltAw2	Black	Smooth	79.00	31.70x16.50	3	2
AltDs1	Black and greenish periphery	Centrally raised and smooth periphery	80.00	22.69x 9.92	3	1
AltDs2	Dark gray	Raised and smooth periphery	70.00	25.61x10.50	4	1
AltTk1	Grayish	Smooth	80.00	30.14x12.50	3	2
AltTk2	Black and greenish periphery	Smooth	65.00	26.85x13.26	3	2

Table 2: Pathogenic variability in different isolates of *Alternaria alternata*

Isolate No.	District	Tehsil	Village	Disease intensity (%)
AltJp1	Jaipur	Amer	Nangal-ladi	70.25
AltJp2	Jaipur	Amer	Shubrampura	67.42
AltAw1	Alwar	Bansur	Nimuchana	66.00
AltAw2	Alwar	Bansur	Chatrapura	62.46
AltDs1	Duasa	Lalsot	Didwana	59.33
AltDs2	Duasa	Lalsot	Salempura	57.55
AltTk1	Tonk	Peeplu	Dhundhiya	54.80
AltTk2	Tonk	Peeplu	Nathri	52.46
CD (p=0.05)	4.25	3.99	4.12	

*Average of three replications

Cultural and morphological variability

The cultural and morphological variability such as shape, colour and size of colony, texture of colony, size of conidia and septation were recorded for different isolates of *Alternaria alternata* with the help of fluorescence microscopy.

AltJp2 showed maximum colony diameter, 90.00 and 88.00 mm respectively, followed by AltAw1 (84.00 mm) and minimum colony diameter by isolate AltTk2 (65.00 mm).

Morphological observations revealed that all the isolates vary in their spore length and width. In general, spore length and width was found in

between 36.15 to 22.69 μm and 18.65 to 9.92 μm , respectively. Whereas number of horizontal and vertical septa varied between 3 to 4 and 1 to 3, respectively (Table 1). The AltJp1 isolate showed maximum length and width of conidia 36.15 μm and 18.65 μm and maximum horizontal and vertical septation of 4 and 3 septa, respectively. Minimum length and width of conidia 22.69 μm and 9.92 μm

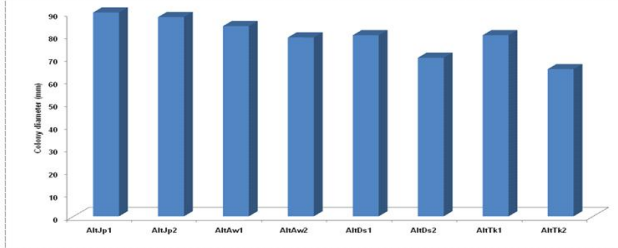


Fig. 1: Cultural and morphological variability in different isolates of *Alternaria alternata* at 7th day of incubation

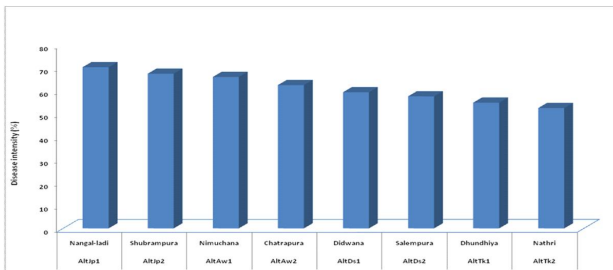


Fig. 2: Pathogenic variability in different isolates of *Alternaria alternata*

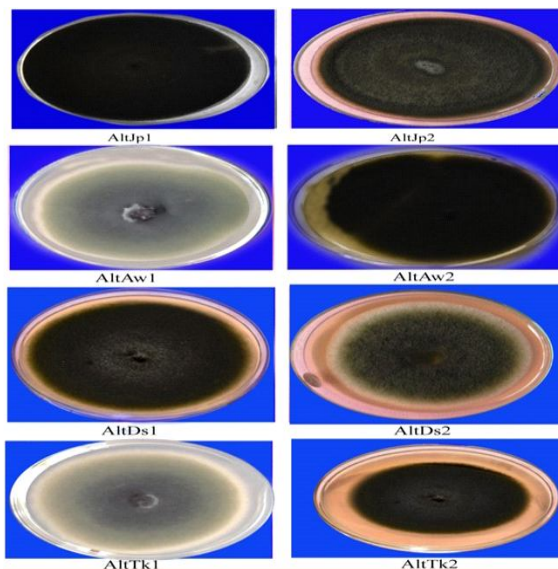


Fig. 3: Cultural variability in different isolates of *Alternaria alternata* and minimum horizontal and vertical septation of 3 and 1 showed in isolate AltDs1.

Pathogenic variability

Results presented in Table 2 and Fig. 2 revealed that all the isolates were pathogenic to tomato and produced characteristic symptoms of the disease.

Among these isolates AltJp1 was observed to be most virulent and produced maximum (70.25 %) disease intensity followed by AltJp2 isolate (67.42 %), whereas AltTk2 isolate was found least virulent with minimum disease intensity (52.46 %).

Conidiophores of *A. solani* are dark or olivaceous brown, thick-walled, straight to flexuous, septate; arise singly or in small groups, up to 110 μm in length and 6–10 μm in diameter. Naik *et al.* (2010) reported the morphological characteristics of four isolates of *A. solani*, and reported that conidiophores of the fungus were formed singly or in groups, straight or flexuous, brown to olivaceous brown in colour. The conidia were solitary, straight or slightly flexuous, muriform and ellipsoidal tapering to a beak, pale or olivaceous brown, 19.5 -70 μm in length and 6-22 μm width with 2 -7 transverse septa and 1-4 longitudinal septa. Alhussaen (2012) studied morphological characteristic of the selective four isolates of *Alternaria solani* obtained from tomato in Jerash, Jordan and observed that the conidiophores were formed singly or in groups or flexuous and brown to olivaceous brown in colour. The conidia were solitary straight or slightly flexuous or muriform or ellipsoidal tapering to beak, pale and sometimes branched. The conidia were 35-75 μm in length and 10-20 μm in width. There were 2-7 transverse septa and 1-4 longitudinal septa. The mycelia width ranged from 0.8-1.5 μm .

Variability in reference to cultural and morphological characteristics was recorded with different isolates of *A. alternata* under present investigation. Some isolates showed profuse mycelial growth whereas; others produced poor growth of mycelium on culture medium. The result obtained in the present study agreed with the findings of Alhussaen (2012) who reported that the mycelia width between 0.8-1.5 μm and conidia are 35-75 μm in length and 10-20 μm in width and 2-7 transverse septa and 1-4 longitudinal septa. Similar result was also obtained by Naik *et al.* (2010) who described *Alternaria solani* isolate from tomato plant and those results were agreed with the result presented in this study. Similar type result was also obtained by Marak *et al.* (2014).

Variability in colony diameter, colony colour and colony texture of mycelium were observed among the isolates of *Alternaria alternata*. On the basis of cultural variability it is concluded that the isolate

AltJp1 (Jaipur) showed maximum mycelial growth and isolate AltTk2 (Tonk) showed minimum mycelial growth. The isolates of *Alternaria alternata* showed, morphological variability. All the isolates differed in their conidial length and width. In general conidial length and width was observed in between 22.69 to 36.15 μm and 9.92 to 18.65 μm , respectively.

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