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Morphological and Physiological Characterization of *Alternaria solani* Isolated from Tomato in Jordan Valley

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Abstract: *Alternaria solani* is known economically important and the casual agent of early blight on potato and tomato. Identification of plant pathogens is very important in helping to find effective disease control or management methods. Morphology and physiology characteristics of *Alternaria solani* were investigated for identification and variability. The optimum pH levels of *Alternaria solani* grow *in vitro* were 6-7 and the optimum growing temperatures of the isolates recovery in this study was 25 and 30°C. The mycelial width between 0.8-1.5 μm and the conidia are 35-75 μm in length and 10-20 μm in width and 2-7 transverse septa and 1-4 longitudinal septa. This study pointed that there was a variation in the population of *Alternaria solani* isolated from Jordan valley based on morphology and physiology characteristics.

Key words: *Alternaria solani*, early blight, morphology and physiology characteristics, tomato, Jordan valley, disease control

INTRODUCTION

The species of *Alternaria solani* (Ellis and G. Martin) Jones and Grout was first recorded in 1882 in New Jersey, USA on potato plants (Bose and Som, 1986). The genus of *Alternaria* is indigenous to soil and many of their species are pathogenic to plants including *Alternaria solani* which is known economically important and the casual agent of early blight on potato and tomato.

Early blight of tomato is an important and widely distributed disease throughout the world resulting economic yield losses. Symptoms of early blight on tomato plant start on lower, old and mature leaves which become chlorotic and abscise prematurely spots. These spots may enlarge until they are one-half inch in diameter. Spots have concentric rings or ridges that form a target-like pattern and are often surrounded by a yellow halo. Moreover, these symptoms affect stem and fruits as well (Barksdale and Stoner, 1977; Agrios, 2005).

The classification of *Alternaria solani* is belong to the phylum Ascomycota, class Othideomycetes, order Pleosporales and to family Pleosporaceae (Simmons, 2007). According to morphological characters and phylogenetic analyses, *Alternaria solani* belong to larg, long-beaked and noncatenated spores group of the genus *Alternaria* (Simmons, 2000). The mycelium consisted of septate, branched, light brown hyphae which turned darker with age. The conidiophores were short, 50- 90 μm and dark coloured. Conidia were 120-296×12-20 μm in size, beaked, muriform dark coloured and borne singly. However in culture, they formed short chains. Singh (1987a, b) report that the conidia contained 5-10 transverse septa and 1-5 longitudinal septa.

In most pathogens, there is a variation between the populations from different areas. Moreover, it is well known that the variation in populations of plant pathogens directly affects disease control, especially when the method related to the development of resistant cultivars and fungicide usage. *Alternaria solani* found to be a highly variable pathogen (Castro *et al*., 2000; Pryor and Michailides, 2002).

Various researches have been characterized *Alternaria solani* in different part of the word (Petrunak and Christ, 1992; Martinez *et al*., 2004; Lourenco *et al*., 2009). However in Jordan, a few researches have been investigated *Alternaria solani* and most of them about control the disease (Al-Mughrabi, 2004; Goussous *et al*., 2010; Abu-El-Samen and Al-Shudifat, 2011). This study is designed to characterize the fungus of *Alternaria solani* isolated from tomato plants grown in Jordan valley based on morphology and physiology features.

MATERIALS AND METHODS

Isolation: Tomato leaves showing typical early blight symptoms were collected from different farms in the

Jordan valley in early 2012. The infected leaves were cut into small bits measuring about 5 mm and surface sterilized in 1% sodium hypochlorite solution for 1 min, rinsed with sterile distilled water. Pieces were then placed on Potato Dextrose Agar (PDA) and incubated under 12 h light and 12 h dark at 25±1°C according to Naik *et al*. (2010). Pure culture of the fungus was obtained by Hyphal Tip Isolation Method.

Physiological studies

Effect of pH levels: Four isolates were selected based on colony to study the effect of pH on growth *in vitro* for *A. solani* and examined on PDA. The pH of the medium was adjusted to various levels 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 by adding 0.1 N sodium hydroxide and 0.1 N hydrochloric acid. About 5 mm discs taken from 7 days old culture were inoculated and incubated under 12 h light and 12 h dark at 25±1°C for 7 days. Three replications were used for each isolate and treatment. The diameter of the colony growth was measured and recorded after 7 days. General Linear Model (GLM) ANOVA was used to differences ($p \leq 0.05$) between treatments mean (SPSS Ver. 10).

Effect of temperature: Eight temperatures from 5-40°C (5°C intervals) were used to incubate PDA Petri dish cultures of the four *Alternaria solani* isolates selected to find out the optimum temperature as well as the lowest and the highest temperatures at which fungal and growth occurred. All incubation was carried out under 12 h light and 12 h dark for 7 days at 25±1°C. Three replicates were used for each isolate at each temperature. Growth was measured after 7 days using two diameter measurements perpendicular to each other. General Linear Model (GLM) ANOVA was used to differences ($p \leq 0.05$) between treatments mean (SPSS Ver. 10).

Morphological characterization: The morphological characters of representative isolates of the four *Alternaria solani* isolates including conidia size (length and width), length of beak and hyphal width and number of septa in conidia were measured under power objective 40X using light microscope. The *Alternaria solani* cultures were 7 days old grown on PDA.

RESULTS

Isolation: All isolates recovery from tomato leaves showing typical early blight symptoms collected from Jordan valley area were identified as *Alternaria solani* based on the morphological characteristics according to Alternaria identification manual (Simmons, 2007).

Physiological studies

Effect of pH levels: The optimum pH level of the four isolates of *Alternaria solani* tested was 7. Moreover, all isolate tested grew very well at pH levels of 6 and 6.5. At pH levels of 4.5, 5, 5.5 and 7.5, all four isolates were grew well. However, slightly growth was appeared at pH levels of 4 and 8 for all isolates tested (Table 1).

There were significant differences in colony growth between the four *Alternaria solani* isolates tested at each pH levels ($p = 0.01$). Moreover, there were significant differences between the colony growth at different pH levels for each isolate ($p = 0.00$). However, there were no significant differences in colony growth for each isolate between pH levels of 6, 6.5 and 7 (Table 1).

Effect of temperature: Isolates of *Alternaria solani* have an optimum growth temperature of 30°C. They also grew well at temperature of 30°C. At temperature 20°C, *Alternaria solani* grew slightly (mean). Limited growth was occurred at temperatures of 10 and 40°C but no growth was appeared at temperature of 5°C (Table 2).

There were significant differences in colony growth between the four *Alternaria solani* isolates tested at each

Table 1: Mean growth (mm) of 4 isolates of *Alternaria solani* incubated on PDA with nine pH levels (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8) in 12 h light and 12 h dark. Growth was measured after 7 days

	Mycelial diameter growth (mm) of different isolates			
pH level	Isolate 1	Isolate 2	Isolate 3	Isolate 4
4.0	3.9^{b}	3.3^{b}	3.5^{b}	3.3^{b}
4.5	5.3^{c}	5.1^{c}	5.4^{c}	5.0^{c}
5.0	6.8^{d}	7.0^{d}	6.9^{d}	6.6^{d}
5.5	6.7^{d}	6.6^{d}	6.8^{d}	6.8^{d}
6.0	8.1^{e}	7.9^{d}	8.3^{e}	8.1^{e}
6.5	8.6^{e}	8.7^{e}	8.5^{e}	8.5^{e}
7.0	8.8^{e}	8.9^{e}	8.6^{e}	8.7^{e}
7.5	5.4^{c}	5.0^{c}	5.2^{c}	5.3^{c}
8.0	2.1^{a}	2.0^{a}	2.1^{a}	2.0^{a}

Means followed by the same letter are not significantly different from each other at $p \leq 0.05$; n = 3 for each isolate at each pH level

Table 2: Mean growth (mm) of 4 isolates of *Alternaria solani* incubated at eight different temperatures from 5-40°C on PDA in 12 h light and 12 h dark. Growth was measured after 7 days

	Mycelial diameter growth (mm) of different isolates			
Temperature (°C)	Isolate 1	Isolate 2	Isolate 3	Isolate 4
05	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
10	0.1^{b}	0.2^{b}	0.2^{b}	0.1^{b}
15	1.1^{c}	1.9^{c}	1.2^{c}	1.0^{c}
20	2.1^{d}	2.8^{d}	2.4^{d}	2.8^{d}
25	8.6^{e}	8.8^{e}	8.8^{e}	8.5^{e}
30	8.8^{e}	8.7^{e}	8.9^{e}	8.3^{e}
35	2.9^{d}	3.5^{d}	3.1^{d}	3.5^{d}
40	0.2^{b}	0.1^{b}	0.1^{b}	0.1^{b}

Means followed by the same letter are not significantly different from each other at $p \leq 0.01$; n = 3 for each isolate at each temperature

temperature (p = 0.01). Moreover, there were significant differences between the colony growth at different temperatures for each isolate (p = 0.00). Nevertheless, there were no significant differences in colony growth for each isolate between temperatures of 25 and 30°C (Table 2).

Morphological characterization: The morphology characterization of the selective four isolates of *Alternaria solani* recovery from Jordan valley area, indicated the conidiophores were formed singly or in groups or flexuous brown to olivaceous brown. 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 (Table 3). There were 2-7 transverse septa and 1-4 longitudinal septa. The mycelial width was 0.8-1.5 μm.

DISCUSSION

This is the first study to characterize *Alternaria solani*, the casual agent of early blight on tomato isolated from Jordan valley. A scarcity of studies in Jordan on *Alternaria solani* on disease management were done and fungicides sensitivity (Al-Mughrabi, 2004; Abu-El-Samen and Al-Shudifat, 2011). Variation in populations of plant pathogens is important to control strategies. This study demonstrates that there was a variation in the population of *Alternaria solani* isolated from Jordan valley based on morphology and physiology characteristics.

Alternaria solani isolated in this study was identified based on morphology and physiology characteristics according to the *Alternaria* identification manual (Simmons, 2007). Four isolates were selected to represent all recovery isolates based on colony characteristics in this study for the morphology and physiology characteristics. The results of this study pointed that the optimum pH level of *Alternaria solani* grow *in vitro* were 6-7. Moreover, the optimum growing temperature of the isolates recovery in this study was 25 and 30°C. These results are contestant with Ibrahim *et al*. (2009) who found that the optimum conditions for *Alternaria solani* isolated from North of Jordan were 25°C and pH 7 when they study the ability of *Alternaria solani* to utilize the polyester-polyurethane. Moreover, Naik *et al*. (2010) reported that the optimum growth and the maximum sporulation temperatures were 25 and 30°C. Furthermore, other research found that *Alternaria solani* has an optimum growth temperature of 25°C and pH level of 6.5 (Tatiana *et al*., 2010).

Table 3: Morphological characteristics of Alternaria solani isolated from tomato

		Conidia size (μm)			Septa in conidia	
Isolates	Mycelial width (μm)	Length	Width	Length of beak (μm)	Horizontal	Vertical
1	0.8-1.2	25-50	10-15	8-10	3-5	1-4
2	1.1-1.5	35-65	10-20	10-14	2-5	1-3
3	0.9-1.3	45-75	15-20	12-17	4-7	1-3
4	1.0-1.4	40-65	10-15	11-15	3-6	2-4

Morphology features of *Alternaria solani* described in this study were found to have mycelial width between 0.8-1.5 μm. Moreover, the conidia are 35-75 μm in length and 10-20 μm in width and 2-7 transverse septa and 1-4 longitudinal septa. Naik *et al*. (2010) describe *Alternaria solani* isolated from tomato plants and those results were agreed with the results presented in this study. However, other research found that the conidia were solitary straight or slightly flexuous, oblong or ellipsoidal tapering to a beak, smooth, 150-300 μm in length, 13-20 μm thick in the broadest part with 8-10 transverse and 1-4 longitudinal septa (Arunakumara, 2006). Furthermore, Kumar *et al*. (2008) reported that the width of conidiogenous hyphae were 1.17-9.56 micro.

CONCLUSION

The results of this research found that there were variations in the population of *Alternaria solani* isolated from Jordan valley based on morphology and physiology characterization. In Jordan, there was no study look into the population of *Alternaria solani* based on morphology and physiology characterization. However, there were a few study look into the variation of *Alternaria solani* of fungicides sensitivity (Al-Mughrabi, 2004; Abu-El-Samen and Al-Shudifat, 2011). In the world, various studies look into the population of *Alternaria solani* based on both morphology and molecular characteristics (Petrunak and Christ, 1992; Weir *et al*., 1998; Van der Waals *et al*., 2004; Lourenco *et al*., 2009). Other studies found there were a variation in the population of *Alternaria solani* based on the pathogenicity (Kumar *et al*., 2008; Naik *et al*., 2010). Further studies should investigate the population of *Alternaria solani* in Jordan based on genetic variation.

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