

# Variation Among Some *Fusarium oxysporum* f. sp. *melonis* Isolates as Measured by Their Effect on Muskmelon Plant Growth and Wilt Severity

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## ABSTRACT

*Fusarium* wilt of muskmelon (*Cucumis melo* L.), caused by *Fusarium oxysporum* f. sp. *melonis*, is one of major constraints in melon-growing regions of Tunisia. Necrosis, gum exudates, stunting, vine death and light to dark brown vascular discoloration were recorded on diseased plants late in the season. Eight isolates of the vascular wilt pathogen obtained from different fields were identified on the basis of morphologic and cultural criteria and pathogenicity. All isolates tested showed incidence of the disease on cv. 'Ananas d'Amérique' plants, estimated by the disease index, height and fresh weight. A significant negative effect was observed on all the measured characteristics revealing the negative influence of *Fusarium* wilt on the growth of muskmelon.

**Keywords:** *Cucumis melo* L., *Fusarium* wilt, growth limitation, incidence, leaf damage

## INTRODUCTION

Muskmelon (*Cucumis melo* L.) has become the most important crop among the cucurbits grown in many temperate and sub-tropical countries. In Tunisia, over 10,000 ha of muskmelon are grown annually, and nearly 116,000 tons were produced with a yield of over 25 tons/ha (Anonymous 2004). This cucurbit is cultivated in glasshouses, mainly in Tunisian Sahel regions (Monastir, Sousse and Mahdia), in low tunnels and also in the open in different regions of Tunisia (Ariana, Bizerte, Sfax, Bèjà and Jendouba) (Jebari *et al.* 2004). The cultivated areas have gradually decreased during the last decades because of the impact of several fungal diseases, such as *Verticillium* and *Fusarium* wilts (Martyn 1983; El Mahjoub and Ben Khedher 1987; El Mahjoub *et al.* 1987; Ayed *et al.* 2007; Jabnoun-Khiareddine *et al.* 2007).

*Fusarium* wilt of muskmelon, caused by *Fusarium oxysporum* f. sp. *melonis*, has become one of the most critical problems in many parts of the world (Netzer and Weintall 1979; Zink and Gubler 1985; Jacobson and Gordon 1990; Shreuder *et al.* 2000). This pathogen, including several physiological races (0, 1, 2, 1-2 y and 1-2 w), can cause extensive losses of up to 90% (Martyn and Amador 1987; Champaco *et al.* 1993; Katan *et al.* 1994). It infects plants through roots via direct penetration, after which the xylem vascular tissue of the plants is colonized, causing stunting, vascular wilting and ultimately plant death (El Mahjoub and le Picard 1985).

In Tunisia, during 2005 and 2006 growing seasons, wilt and sudden plant collapse were observed in some farms at the beginning of melon fructification and progressing until harvest. The pathogen was subsequently identified as *Fusarium oxysporum*. The objectives of this study were to describe the disease symptoms, identify the *formae speciales* and to determinate the effect of *F. oxysporum* f. sp. *melonis* on seedling development.

## MATERIALS AND METHODS

### Pathogen isolation

During 2005, surveys for *Fusarium* wilt were carried out in the Tunisian muskmelon-growing areas of Bizerte, Jendouba, Bèjà and Kairouan. Melon plants showing symptoms of *Fusarium* wilt were collected from open fields. Isolations were made from root, crown and stem tissues and from fruits collected from infected muskmelon plants.

Stems and roots were washed under a fine spray of tap water and cut into 0.5-1 cm pieces. After surface-disinfesting in sodium hypochlorite (10%) for 3 min, the plant pieces were rinsed three times in sterile distilled water and air-dried under a laminar flow bench. Each fragment was placed on Potato Dextrose Agar (PDA) containing 300 mg/l of streptomycin sulfate and incubated at 25°C for 4-5 days in the dark. Single conidial isolates of *F. oxysporum* were selected and identified according to Messiaen and Cassini (1968) and Tivoli (1988).

Eight isolates obtained from diseased muskmelon in 2005 were included in this study (Table 1). For plant inoculation, mycelium was taken from the edge colony of each isolate and placed in Erlenmeyer flasks containing 150 ml of Potato Dextrose Broth (PDB) and incubated at 25°C for 5 days in a rotary incubator (120 rpm). The liquid culture was filtered and the conidial suspension

**Table 1** Origin of *Fusarium oxysporum* isolates collected from field-grown muskmelon in Tunisia during 2005.

Isolate	Site of origin	Cultivars
I <sub>2</sub>	Bizerte	Blanco
I <sub>4</sub>	Bizerte	Blanco
I <sub>5</sub>	Bizerte	Gold Mayen
I <sub>7</sub>	Bizerte	Ananas d'Amérique
I <sub>10</sub>	Jendouba	Jaune Canari
I <sub>12</sub>	Bèjà	Lobnani
I <sub>15</sub>	Kairouan	Ananas d'Amérique
I <sub>20</sub>	Gabès	Lobnani

was adjusted to  $10^7$  conidia/ml by a Malassez cystometer (Namiki *et al.* 1998).

### Plant material

Seeds of the muskmelon cultivar ‘Ananas d’Amérique’, susceptible to *Fusarium* wilt, were disinfected by immersion for 2 min in fresh 10% ethanol. They were then rinsed in sterile water and placed on moistened cotton wool in Petri dishes in the dark at  $27 \pm 2^\circ\text{C}$  for 3 days. The germinated seeds were planted in peat, previously sterilized at  $105^\circ\text{C}$  for 1 h. The trays, after inoculation at the expansion of the first-true-leaf stage, were placed in a growth chamber at  $26 \pm 1^\circ\text{C}$  during the day and  $20 \pm 1^\circ\text{C}$  at night with a 14 h photoperiod ( $64.75 \mu\text{mol}/\text{m}^2/\text{s}$ ).

### Pathogenicity tests

After approximately 10 to 14 days, seedlings were uprooted at the expansion of the first-true-leaf stage (Latin and Snell 1986). Roots were washed in tap water, pruned to  $\sim 2.5$  cm and dipped for 1 min in the inoculum suspension adjusted at  $10^7$  conidia/ml (Jacobson and Gordon 1988; Lorenzini *et al.* 1997). Each isolate was used to inoculate five seedlings. Controls were immersed in sterile distilled water. Inoculated and control seedlings were transplanted to plastic pots (0.7 l) filled with an autoclaved mixture of peat and perlite (3:1) and placed in a growth chamber at  $26 \pm 1^\circ\text{C}$  during the day and  $20 \pm 1^\circ\text{C}$  at night with 14 h photoperiod. They were irrigated regularly and fertilized with the nutrient solution used by El Mahjoub (1985) (per liter): 0.225 g of  $\text{KNO}_3$ , 0.3 g of  $\text{KH}_2\text{PO}_4$ , 0.225 g of  $\text{NH}_4\text{Cl}$ , 0.45 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.3 g of  $(\text{NH}_4)_2\text{SO}_4$ , 1.2 g of  $\text{Ca}(\text{NO}_3)_2$ , 0.045 g of EDTA Na, Fe and other oligo-elements.

Wilt symptoms, as described by Jacobson and Gordon (1988) (stunting, chlorosis, necrosis and death), were first observed 15-25

days after inoculation. If none of these symptoms were present, such isolates were categorized as non-pathogenic to muskmelon. Reisolations were made, at the end of the bioassay, from diseased plants in order to confirm incrimination of *F. oxysporum* in the induced symptoms.

### Disease incidence on muskmelon development

The effect of *F. oxysporum* f. sp. *melonis* on seedling development was assessed through the index of disease severity using a scale of 0-4 every 3-4 days where: 0 = asymptomatic leaf, 1 = leaf wilted, 2 = leaf with hemiplegic yellowing, 3 = leaf with necrosis, 4 = dead leaf. Isolate virulence was expressed as an index of leaf damage (I.L.D.) which is calculated for each muskmelon plant (Béye and Lafay 1985):

$$\text{I.L.D.} = \frac{\sum \text{notes}}{\text{max}}$$

where

$$\sum \text{notes} = \text{total notes.}$$

Max = 4 times the number of developed leaves.

Plant height and fresh weight were recorded 43 days after inoculation. Pathogen isolations were done, at the end of the bioassay, to confirm its incrimination in the disease development.

### Statistical analyses

Five plants were inoculated for each treatment (isolates and control). Analyses of variance were conducted for each dependant variable to determine the effect of isolates on plant development. Data are arranged by completely randomized design where treatments (seedlings inoculated by each of eight *Fusarium oxysporum* isolates and control seedlings) represented the only fixed factor. They were analyzed using SPSS and subjected to analysis of variance and Fisher's least significant difference test LSD (at  $P \leq 0.05$ ).



**Fig. 1** Development of *Fusarium* wilt of field-grown muskmelon. (A) Plages having a *Fusarium* wilt symptoms in muskmelon field in Kalaat El Andalous in Bizerte, (B) Yellowing and death of the lower leaves of muskmelon plant, (C) Death of some branches, (D) Death of entire muskmelon plant, (E) Brown lesions with dark gum exudates, (F) White mycelial growth on fruit surface.

**Table 2** Evolution of the Leaf Damage Index (I.L.D.) for muskmelon plants, cv. “Ananas d’Amérique”, inoculated by *F. oxysporum* f. sp. *melonis* isolates observed 15 days after inoculation.

Days after inoculation	Index of Leaf Damage								
	Control	I <sub>2</sub>	I <sub>4</sub>	I <sub>5</sub>	I <sub>7</sub>	I <sub>10</sub>	I <sub>12</sub>	I <sub>15</sub>	I <sub>20</sub>
15	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0.133 a	0 b
19	0 b	0 b	0 b	0 b	0.075 b	0 b	0.0125 b	2.35 a	0.075 b
23	0 b	0 b	0 b	0.03 b	0.1372 b	0.05 b	0.05 b	2.8 a	0.0833 b
27	0 b	0.0374 b	0.0262 b	0.1746 b	0.2239 b	0.1884 b	0.2174 b	2.8 a	0.3598 b
31	0 c	0.0765 bc	0.041 c	0.2388 bc	0.4974 b	0.4095 bc	0.4024 bc	2.8 a	0.44 b
35	0.1099 d	0.5518 cd	0.54 cd	1.059 bc	1.5549 b	0.9791 bc	1.0424 bc	2.8 a	1.202 b
39	0.0556 d	0.9258 cd	0.9248 cd	3.5584 a	1.864 abcd	1.45 bcd	1.4082 bcd	-	3.2334 ab
43	0.1414 c	1.3518 bc	3.0566 abc	3.9534 ab	3.0168 ac	1.466 bc	2.129 bc	-	5.175 a

I<sub>n</sub>: *F. o. f. sp. melonis* Isolates.<sup>2</sup> Within lines, means followed by the same letters are not significantly different ( $P \leq 0.05$ ) according to S.N.K.test.

## RESULTS

### Symptom development

During 2005, muskmelon showing wilt symptoms were collected from field plantings in major Tunisian production areas (Fig. 1A). The first symptoms of this disease were a yellowing and a death of some leaves (Fig. 1B) followed by a death of one or more lateral vines, starting at the tip with runners dying back towards the crown (Fig. 1C) and of all plant (Fig. 1D), respectively. Some runners may remain healthy. Reddish-brown lesions were observed at the base of the plant (Fig. 1E) developing along branches. They could have dark-brown gum exudates (Fig. 1E). Furthermore, this soil-borne pathogen could also colonize fruit infecting their tissues and developing above it a white mycelium (Fig. 1F).

### Pathogenicity tests of *F. oxysporum* isolates

Typical symptoms of a *Fusarium* wilt disease were induced by all *F. oxysporum* isolates and they appeared 15 to 25 days after inoculation. Inoculated muskmelon plants showed hemiplegic yellowing at the lower leaves, browning in the vascular region especially in the stem and ascending wilt symptoms. These plants wilted in the end of the bioassay comparatively to the control plants.

### Disease incidence on the development of muskmelon plants

#### Effect of *Fusarium* wilt on leaf damage

All isolates, used to inoculate seedlings by the root dip method, caused wilt on muskmelon cv. ‘Ananas d’Amérique’ showing a significant difference between healthy and inoculated plants (Table 2).

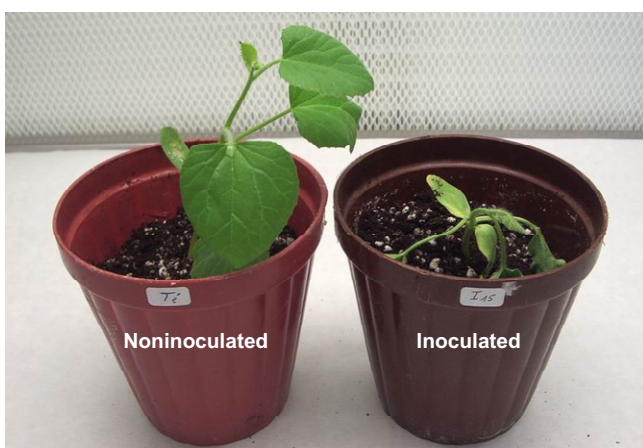


Fig. 2 Comparison between a healthy muskmelon plant, cv. “Ananas d’Amérique” (left), and a plant inoculated with isolate I15 of *F. oxysporum* f. sp. *melonis* (right) 15 days after inoculation.

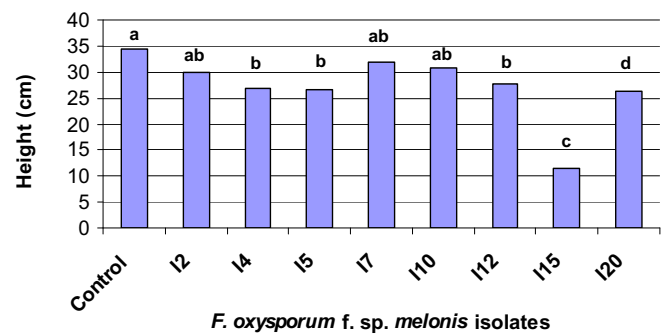


Fig. 3 Effect of inoculation by different *F. oxysporum* f. sp. *melonis* isolates on muskmelon plant height (cv. “Ananas d’Amérique”), observed 43 days after inoculation.

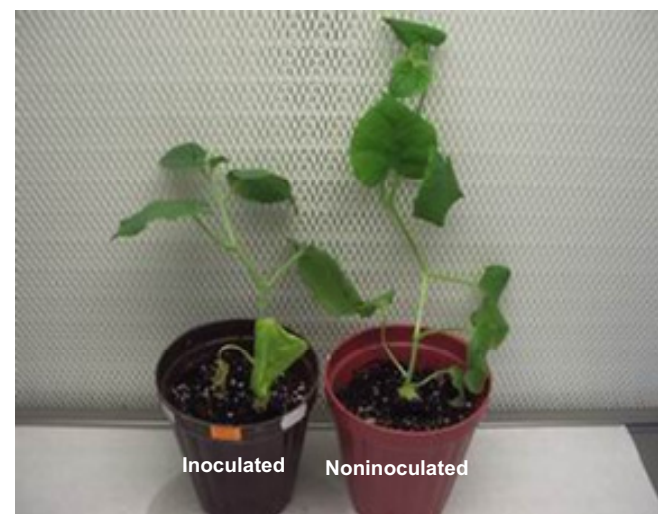


Fig. 4 Effect of inoculation by a *F. oxysporum* f. sp. *melonis* isolate on muskmelon plant, cv. ‘Ananas d’Amérique’, comparatively to the noninoculated control observed 25 days after inoculation.

Plants developed disease symptoms, 15-23 days after inoculation, which increased differently during the bioassay. The isolate I<sub>15</sub> is the most virulent showing higher disease severity noted at different evaluation periods and causing the premature death of plants, 27 days after inoculation (Fig. 2). The other isolates such as I<sub>5</sub>, I<sub>7</sub> and I<sub>20</sub> also caused premature and important wilt symptoms. Their leaf damage index reached 3.9534, 3.0168 and 5.175, respectively. However, I<sub>2</sub> and I<sub>10</sub> were lesser wilt pathogens than the other wilt isolates showing 1.3518 and 1.466 in the end of the bioassay respectively and inducing a lower disease incidence on plants (Table 2).

#### Effect of *Fusarium* wilt on plant height

Regardless of leaf damage, disease incidence was evaluated via plant growth. Indeed, these eight *F. oxysporum* f.

sp. *melonis* isolates tested caused significant reductions in muskmelon height (Fig. 3). The most important height reduction, 66.3%, was induced by the isolate I<sub>15</sub> comparatively to non-inoculated plants. In the same way, isolates I<sub>2</sub>, I<sub>4</sub>, I<sub>5</sub> and I<sub>20</sub> reduced clearly plant length by 13.4 to 23.3% (Figs. 3, 4).

### Effect of *Fusarium wilt* on plant fresh weight

*Fusarium* wilt effect on plant growth was proved by reducing plant fresh weight comparatively to non-inoculated plants with a significant difference between isolates (Fig. 5). Isolate I<sub>15</sub> seemed to be the most aggressive, I<sub>5</sub> and I<sub>20</sub> also showed an important effect in reducing fresh weight by 76.3 and 78.8%, respectively.

## DISCUSSION

*Fusarium* wilt disease has become a serious threat for muskmelon crop in Tunisia in recent years. It has been reported in North America, Europe, India, East Asia and the Middle East (Dutky *et al.* 1986; Jacobson and Gordon 1988; Abou-Jawdah and Al-Khoury 1996; de Cara *et al.* 2004). *Fusarium* symptoms, observed under field conditions, such as wilting, necrosis, gum exudates, stunting and discoloration, differed from those observed under greenhouse conditions, such as wilting, root rot and a yellowish-orange discoloration on the crown and lower stem. Discoloration was first observed in the vascular tissues and was later visible externally as the pathogen spread into the cortical tissues. The same symptoms were observed by Punja *et al.* (2001) and Kurt *et al.* (2002).

On the basis of their ability to cause disease on muskmelon, *F. oxysporum* isolates are grouped into *formae speciales melonis* (Armstrong and Armstrong 1981). These eight strains showed different levels of virulence on muskmelon plants affecting leaf alteration, height and fresh weight. The negative effects on melon growth were reported by Bletsos and Thanassouloupoulos (2000).

This soil-borne pathogen penetrates and invades plant tissue, overcomes host defenses and optimizes growth in the plant. To perform these tasks correctly, the fungus must perceive chemical and physical signals from the host and respond with the appropriate metabolic and morphogenetic changes required for pathogenic development. Such changes include direct hyphal growth, adhesion to the plant surface, occlusion of colonised vessels by gels, gums and tyloses, differentiation of specialized infection structures and secretion of lytic enzymes and phytotoxins (El Mahjoub *et al.* 1984; El Mahjoub 1985; di Pietro *et al.* 2001). In this respect, Martinez *et al.* (1991) reported that the ability of the pathogen to cause wilt symptom is correlated with high pectic activities such as exopolysaccharuronase, endopolysaccharuronase and lyases.

In the same way, Lorenzini *et al.* (1997) reported that xylem colonisation by *F. oxysporum* increases resistance to water flow within the plant, thus resulting in leaf water deficits that might lead to reductions in leaf photosynthetic and transpiration rates, leaf longevity and consequently plant fresh weight. Furthermore, some genes encode cell wall-degrading enzymes and plant saponin-detoxifying enzymes, which have been implicated as possible key factors in pathogenicity or virulence (di Pietro and Roncero 1998; Inoue *et al.* 2001).

Being one of the limiting factors for melon production, the prevalence and distribution of *F. oxysporum* f. sp. *melonis* races in Tunisia revealed necessary in order to develop commercial melon cultivars carrying resistance to these races.

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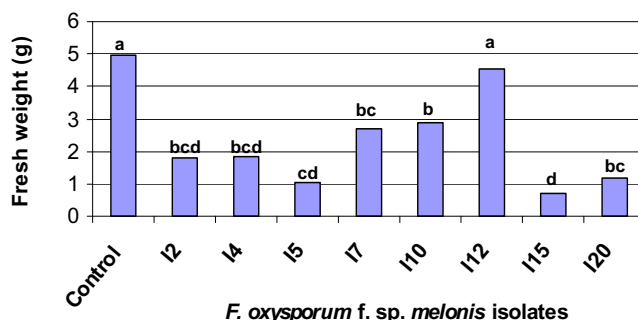


Fig. 5 Effect of inoculation by different *F. oxysporum* f. sp. *melonis* isolates on muskmelon plant fresh-weight (cv. 'Ananas d'Amérique'), observed 43 days after inoculation.

assistance.

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