Salinity Effects on Fusarium Wilt Severity and Tomato Growth

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INTRODUCTION

In the past, salt stress had little importance as it was specific of coastal regions or occurring in particular environments with evaporation of salt-rich waters (Rivero et al. 2003). Thus, due to the low-quality and high-saline irrigation water originating from the contamination of the groundwater, agriculture in coastal regions may be adversely affected (FAO 1995). In addition to these seawater salinity effects, climatic changes including increased temperatures, reduced irrigation water availability, flooding and salinity, are also considered as limiting factors in sustaining and increasing vegetable productivity (FAO 2001). Hence, environmental stress was found to be the primary cause of crop losses worldwide, reducing average yields for most major crops by more than 50% (Boyer 1982; Bray 1997). Salinity, in particular, is one of the major factors that limit crop productivity in arid and semi-arid countries. Almost 1000 million ha of cultivated lands are affected by high salinity (Szabolcs 1994) which has become more problematic due to the increase in irrigation around the world (Debouba et al. 2007) and due to its impact on soil fertility (Abd-Alla and Omar 1998). Moreover, secondary salinization, occurring via inadequate irrigation management, was reported to affect 20% of irrigated lands worldwide (Ghassemi et al. 1995). Indeed, particularly in irrigated croplands, vegetable production, which provides 40% of the world’s food, is threatened by increased soil salinity (FAO 2001).

Amongst vegetable crops, tomatoes are the most important horticultural crop worldwide and are grown on over 4 million ha of land area (FAO 2006) and more than 30% of world production comes from the Mediterranean countries (FAO 1995).

In Tunisia, tomato, which is a strategic crop shown to be moderately sensitive to salinity (Ayers and Westcot 1985; Katerji et al. 2000), is adversely affected by irrigation with saline water at levels exceeding 1.7 g/l of total salts. However, the majority of water available for plant irrigation is brackish (Davet 1967; El Mahjoub et al. 1979), especially in coastal zones where tomato is intensively cultivated under greenhouse or field conditions. This situation is more evident in the Sahel region where salt concentration reached 7 g/l and 50% of surface wells available for irrigation presented a salinity level higher than 4 g/l.

Salinity has been reported to increase the susceptibility of some crops to soil-borne fungus-like microorganisms such as Phytophthora spp. (Bouchibi et al. 1990; Snapp et al. 1991; Sanogo 2004) and Pythium ultimum (Martin and Hancock 1981; Rasmussen and Stanghellini 1988) and fungi including Alternaria solani, Verticillium dahliae and V. albo-atrum (Livescu et al. 1990; Nachmias et al. 1993; Howell et al. 1994). However, the interaction of Fusarium oxysporum with salt stress varied depending on formae speciales (f. sp.) and host-plants involved. In fact, increased disease incidence following irrigation with high-salinity water had been reported in several pathosystems including a variety of F. oxysporum f. sp., including vasinfectum (Ragazzi et al. 1994; Ragazzi and Moricca 1995; Turco et al. 2002), radicis-lycopersici (Woltz et al. 1992; Jones et al. 1993; Triky-Dotan et al. 2005) and lycopersici (Besri 1980).

In tomato, numerous investigations have been reported on the effects of salt stress on growth reduction (Minamide and Ho 1986; Atta-Aly et al. 1992; Al-Rawahy et al. 1992; Knight et al. 1992), but the interactive effects of abiotic and biotic stresses on tomato diseases were limited to those

ABSTRACT

The aim of the present study was to elucidate the effects of water salinity and Fusarium oxysporum f. sp. lycopersici (FOL) on tomato (cv. ‘Ventura’) growth, wilt severity and subsequent yield loss. The six salt treatments tested showed no significant effect on pathogen mycelial growth in vitro but increased sporulation was recorded with the highest NaCl doses (8 and 10 g/l). Increasing salinity stress (2 to 10 g of NaCl/l) to the inoculated tomato plants enhanced the severity of Fusarium wilt disease and resulted in a significant increase in the leaf damage index (LDI) recorded from 35 to 62 days post-planting (DPP). Moreover, the LDI noted on plants under highest salt stress (8 and 10 g of NaCl/l) was increased by 55 and 60%, respectively compared with the LDI recorded on unimpacted plants. Salinity treatment in watering tomato plants (inoculated or not with FOL), from 15 DPP until the end of the assay (i.e., 62 DPP), decreased plant height by 9.7 and 35% when the salinity level varied from 2 to 10 g/l. A similar effect was noted after inoculation with FOL. The fresh and dry weights of the aerial part were generally lower with the increase in NaCl dose; these parameters were reduced by 45-50% and by 34-41% with higher salt treatments tested (8 and 10 g of NaCl/l), respectively, compared to the non-saline water. The range of decrease in root fresh and dry weights was 12-74 and 13-70%, respectively, when salinity levels were 2 and 10 g of NaCl/l, compared to the unimpaired plants. Fruit fresh weight was also adversely affected by the highest NaCl doses tested (8 and 10 g/l) and was reduced by 40 and 78%, respectively compared to non-saline water.

Keywords: disease incidence, Fusarium oxysporum f. sp. lycopersici, Lycopersicon esculentum Mill., leaf damage, mycelial growth, salt stress, yield

In Tunisia, Fusarium wilt is the most severe disease of tomato cultivated in summer (Davet 1967) which is mainly monitored with genetic resistance and cultural practices. However, little information is available on the effects of the concomitant occurrence of water salinity and *F. oxysporum f. sp. lycopersici* (FOL) on tomato growth associated with wilt severity. Furthermore, in the aforementioned Besri (1980, 1981a) studies, only pathogen development and survival *in vitro* and disease severity with regard to soil and irrigation water quality were considered. Thus, the major objective of the present work was to study the action of NaCl on FOL development *in vitro*, particularly the salinity × pathogen interaction, and the *in vivo* effects of salinity and pathogen on several tomato growth parameters as well as wilt severity.

**MATERIALS AND METHODS**

**Plant material**

Tomato seeds (*Lycopersicon esculentum* Mill. cv. ‘Ventura’) used were resistant to FOL race 1. They were gratefully provided by the laboratory of seed and plant control of the General Direction of the Protection and Control of the Agricultural Product Quality, Tunisia. Tomato seeds were superficially disinfected by immersion in absolute ethanol for 2 min, followed by extensive rinsing in sterile distilled water. Seeds were then sowed in alveolus plates filled with previously sterilized peat. Seedlings were grown in a greenhouse and watered daily until use. Experiments were performed with 4-week-old tomato plants.

**Pathogen**

FOL tested in the present study was isolated from tomato plants cv. ‘Riogrande’ (resistant to FOL race 1) showing typical Fusarium wilt symptoms and vascular discoloration. Monospore FOL isolates were cultured at 25°C on Potato Dextrose Agar (PDA) medium added with 300 mg/l of streptomycin sulphate (Pharmadrug Production Gmbh, Hamburg, Germany) and their pathogenicity was previously verified on cv. ‘Riogrande’ tomato plants (unpublished data).

Liquid cultures used for substrate inoculation were prepared on Potato Dextrose Broth (PDB) medium and incubated at 25°C under continuous agitation at 150 rpm during 4 to 5 days. The sporulation concentration used was adjusted to 10⁵ spores/ml by a Malassez cytometer. For their long-term preservation, FOL isolates were stored up to 12 months at -20°C in a 20% glycerol solution.

**Effect of different salinity levels on FOL mycelial growth**

Osmotic potential of PDA was modified by the addition of different quantities of NaCl (Chemi-Pharma, Tunisia) into Erlenmeyer flasks containing 200 ml of PDA just before pouring it in Petri dishes (9 cm diameter). Agar plugs (diameter 4 mm), removed from the leading edges of colonies on 10 days-old pathogen cultures, were plated in the center of each treated Petri dish. The mean diameter of the pathogen developing colonies was noted after 7 days of incubation at 25°C. These *in vitro* FOL × NaCl dose interactions were completed by several macroscopic and microscopic observations of pathogen colonies.

Statistical analyses were performed following a completely randomized design where salt treatments represented the sole fixed factor. Six replicates (six Petri plates) were used per salinity level and means were separated using Fisher’s protected LSD test (at p≤0.05). All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) version 11.

**Effect of different salinity levels on tomato Fusarium wilt severity and plant growth**

Seedling inoculation was performed according to the root-dip method (Hibar et al. 2006). Seedlings at two- to three-leaf stages were uprooted, their roots washed with tap water, rinsed in sterile distilled water and dipped during 30 min in pathogen spore suspension (10⁵ spores/ml). Seedlings dipped in only sterile distilled water served as uninoculated control.

All the seedlings were transplanted, immediately after inoculation, to pots (16 cm diameter) filled with a mixture of peat and perlite (75%: 25%), previously sterilized at 107°C for 1 h, and maintained under unheated greenhouse conditions where the temperature ranged between 9 and 23°C (minimum and maximum, respectively).

During the first 15 days following inoculation, plants were watered regularly with tap water to favor revival following transplanting. Thereafter, pots were watered, every 2 days, with water amended or not with NaCl. The added quantity of water was adequate to replenish the water level in the culture substrate to pot capacity. Six NaCl doses were tested: 0, 2, 4, 6, 8 and 10 g/l. Tomato plants uninoculated with FOL were included in the experiment to elucidate the effect of salinity alone. Five plants were used per elementary treatment.

Fusarium wilt severity was estimated 62 days post-planting (DPP), via the leaf damage index (LDI) and according to a 0-4 scale which depends on symptom severity on leaves (Daami-Remadi et al. 2007).

Plant height and LDI were recorded weekly. At the end of the assay, the dry and fresh weights of roots and stems, and the fruit weight were noted for all tomato plants.

Statistical analyses were performed, for all measured parameters, following a completely randomized factorial design where FOL inoculation and NaCl dose were the fixed factors. Five replicates per elementary treatment were used and means were separated according to Fisher’s protected LSD test (at p≤0.05).

**RESULTS**

**Effect of NaCl on FOL mycelial growth *in vitro***

The effects of six NaCl doses added to PDA on the mean diameter of FOL colonies, formed after 7 days of incubation at 25°C, was shown to be insignificant at p<0.05. Otherwise, pathogen colony development under diverse salt stresses is statistically comparable to those of the untreated control (Fig. 1). Microscopic observations showed that increasing the NaCl dose in the growth medium did not also negatively affect pathogen sporulation. Moreover, with highest NaCl doses tested such as 8 and 10 g/l, FOL colonies showed abundant sporulation associated with an important differentiation of sporogenous phialide cells (i.e. enhanced sporulating process) compared to the unamended control colonies.

**Effect of NaCl on tomato Fusarium wilt severity**

The effects of salinity on the severity of tomato Fusarium wilt caused by FOL were investigated. Since uninoculated control plants were symptomless under all stress conditions, only data of inoculated plants are presented. Fig. 2 showed that increasing the salinity stress (2 to 10 g of NaCl/l) to the inoculated tomato plants enhanced the progression of Fusarium wilt disease and resulted in a significant increase in the LDI recorded from 35 to 62 DPP. Hence, a higher LDI was noted at 8 and 10 g/l salinity levels.

At the end of the assay i.e. 62 days post-inoculation, the LDI recorded depended on the fungal pathogen inoculation and the NaCl dose tested; a significant interaction was obtained between both fixed factors at p≤0.05. Fig. 3 shows that wilt severity was significantly enhanced in inoculated plants watered with increasing water salinity compared to plants watered with non-saline water. LDI of plants under highest salt stress (8 and 10 g of NaCl/l) increased by 55 and 60%, respectively compared to the LDI recorded at 0
Salt stress and Fusarium wilt. Daami-Remadi et al.

Nevertheless, NaCl at 2, 4 and 6 g/l showed significantly comparable LDI with the unstressed control.

Fig. 1 Effect of different NaCl-based treatments on *Fusarium oxysporum* f. sp. *lycopersici* radial growth noted on PDA medium after 7 days of incubation at 25°C. Bars with the same letter are not significantly different according to Fisher’s protected least significant difference LSD test (p≤0.05).

Fig. 2 Effect of increasing water salinity on the disease severity of tomato cv. ‘Ventura’ plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* recorded 35 to 62 days post-planting.

Fig. 3 Effect of increasing water salinity on the disease severity of tomato cv. ‘Ventura’ plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* recorded 62 days post-planting. Bars with the same letter are not significantly different according to Fisher’s protected least significant difference LSD test (p≤0.05).
Effect of NaCl on tomato growth and production

1. Plant height

Plant height increase, from 21 to 62 DPP, was independently related to NaCl dose and fungal pathogen inoculation, as the interaction between both fixed factors was statistically insignificant (at p<0.05). In fact, salinity treatment in watering tomato plants from 15 DPP until the end of the assay negatively affect plant growth as height decreased with increasing salinity (Fig. 4). The increase in plant height over time was greatest in the case of plants irrigated with non-saline water. In contrast, under highest salt treatments (8 and 10 g of NaCl/l), plant growth was strongly reduced compared to unstressed plants (0 g/l). The increase in plant height related to the fungal pathogen inoculation (FOL treatment) was slowed down compared with the un inoculated control (Fig. 5). The same observation was noted with saline water irrigation.

Plant height, noted 62 DPP, depended significantly on NaCl dose (Fig. 6). This growth parameter decrease ranged between 9.7 and 35% when salinity level increased from 2 to 10 g/l, respectively. Also, plant height decreased after inoculation by the fungus and was about 18% less that the un inoculated control (Fig. 7).

2. Aerial part weight

The fresh and dry weights of the aerial part, noted 62 DPP, depended significantly (at p<0.05) on salinity levels tested only as fungal pathogen inoculations were shown to have an insignificant effect on both these growth parameters.

The pathogen × salinity interaction was evaluated in vitro via radial growth, which is a reliable parameter for the assessment of the effect of an environmental stress on a given pathogen (Subbarao et al. 1993), and which may reflect the pathogen behavior in vivo, inside plant tissues (Cook 1973). The present results showed that six salinity levels tested did not affect negatively the in vitro development of FOL. Moreover, at the highest NaCl doses, FOL showed enhanced sporulation compared with the unstressed control. These findings are in accordance with those of Besri (1981a) who found that irrigation and soil salinity have a big effect on FOL population and that this pathogen tolerates very low water potentials (~144 bars) and sporulates profusely in saline environments. Similarly, El-Abayed et al. (1988) and Ragazzi et al. (1994) reported that mycelial growth and sporulation of different Fusarium species, including F. oxysporum, were motivated under salt stress conditions. In the case of V. dahliae (Triky-Dotan et al. 2004), it was reported that F. oxysporum f. sp. radicis-lycopersici growth in culture or survival of conidia, when added to soil, was not affected by saline water. Moreover, neither growth in culture nor conidial germination was affected by water salinity at 100 mM NaCl, a high level of salinity at which even crop growth is damaged. Convergent results were reported in in vitro V. dahliae × salinity interactions (Besri 1980; Regragui et al. 2003; Daami-Remadi et al. 2009). Tolerance of soil-borne fungus-like microorganisms and fungi to high salinity levels has been also demonstrated in several other studies (MacDonald 1982; Blaker and MacDonald 1985; Rasmussen and Stanghellini 1988; Ragazzi and Vecchio 1992; Ragazzi et al. 1994; Ragazzi and Morica 1995).

The present study showed that by increasing the salinity stress from 2 to 10 g of NaCl/l to inoculated ‘Ventura’ tomato plants enhanced the progression of Fusarium wilt disease and that the highest LD50 were noted at 8 and 10 g/l salinity levels. In a previous work (Daami-Remadi et al. 2009), under similar environmental conditions and salt stress, increased Verticillium wilt severity was also observed on tomato cv. ‘Ventura’ plants watered with saline water. In Moroccan Atlantic coastal tomato-growing areas, known by their high salinity levels, Fusarium and Verticillium wilts of tomatoes are reported to be the most serious

DISCUSSION

A plant disease is the result of the interactions between three major components: the host plant, the pathogen and the biotic and abiotic environment. Any external factor introduced into this system can positively or negatively affect some or all of these components, leading to a decrease, increase or no effect on disease incidence and severity. In fact, salt stress was shown to induce various types of physical and chemical stress in plants, causing complex responses involving changes in plant morphology, physiology and metabolism (Cheeseman 1988; Cheeseman and Bortciov 1991; Yamaguchi and Blumwald 2005). This environmental stress may lead to a decrease in other nutrient ions in plant tissues (Bernstein and Kafkafi 2002; Parida and Das 2005), including K+, which is frequently related with resistance to pathogens. However, a particular mineral nutrient may be associated with an increase in severity of a certain disease in one crop, but with a suppression of the disease in another crop or have simply no effect. In fact, high salinity may increase the incidence and severity of Fusarium wilt of cotton (Turco et al. 2002) and Fusarium crown and root rot of tomato (Triky-Dotan et al. 2005) and may suppress Fusarium wilt of date palm (Brac de la Perriere et al. 1995), Fusarium crown and root rot of asparagus (Elmer 2003), and Fusarium wilt of cyclamen (Elmer 2002).

Each interaction is specific for a particular mineral nutrient, plant and pathogen. Furthermore, salinity levels, pathogen populations (pathogen physiological races) and plant material (cultivars) vary between countries, and between areas within a country; the above-mentioned interactions may be also site-specific. Thus, the present study is the first one to describe the in vivo multiple effects of the tripartite complex salinity × FOL × tomato on plant growth, wilt severity and subsequent yield loss.

The pathogen × salinity interaction was evaluated in vitro via radial growth, which is a reliable parameter for the assessment of the effect of an environmental stress on a given pathogen (Subbarao et al. 1993), and which may reflect the pathogen behavior in vivo, inside plant tissues (Cook 1973). The present results showed that six salinity levels tested did not affect negatively the in vitro development of FOL. Moreover, at the highest NaCl doses, FOL showed enhanced sporulation compared with the unstressed control. These findings are in accordance with those of Besri (1981a) who found that irrigation and soil salinity have a big effect on FOL population and that this pathogen tolerates very low water potentials (~144 bars) and sporulates profusely in saline environments. Similarly, El-Abayed et al. (1988) and Ragazzi et al. (1994) reported that mycelial growth and sporulation of different Fusarium species, including F. oxysporum, were motivated under salt stress conditions. In the case of V. dahliae (Triky-Dotan et al. 2004), it was reported that F. oxysporum f. sp. radicis-lycopersici growth in culture or survival of conidia, when added to soil, was not affected by saline water. Moreover, neither growth in culture nor conidial germination was affected by water salinity at 100 mM NaCl, a high level of salinity at which even crop growth is damaged. Convergent results were reported in in vitro V. dahliae × salinity interactions (Besri 1980; Regragui et al. 2003; Daami-Remadi et al. 2009). Tolerance of soil-borne fungus-like microorganisms and fungi to high salinity levels has been also demonstrated in several other studies (MacDonald 1982; Blaker and MacDonald 1985; Rasmussen and Stanghellini 1988; Ragazzi and Vecchio 1992; Ragazzi et al. 1994; Ragazzi and Morica 1995).
Fig. 4 Effect of increasing water salinity on the plant height of tomato cv. ‘Ventura’ plants recorded 21 to 62 days post-planting. Six NaCl doses were tested: 0, 2, 4, 6, 8 and 10 g/l.

Fig. 5 Plant height of tomato cv. ‘Ventura’ plants inoculated or not with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) recorded 21 to 62 days post-planting.

Fig. 6 Effect of increasing water salinity on the plant height of tomato cv. ‘Ventura’ plants recorded 62 days post-planting. Bars with the same letter are not significantly different according to Fisher’s protected least significant difference LSD test (p<0.05).
diseases (Besri 1981b). This enhanced wilt severity is due to plant osmotic stress, to lowered water potential, and to the water deficit imposed by salt stress (Bernstein and Kafkafi 2002; Munns 2002). Salt water in the root zone induces osmotic changes and interferes with nutrient uptake (Cor nellon and Palloix 1997; Halperin et al. 2003; Ben-Gal et al. 2009). Furthermore, the effect of salinity on root exudates is considered as a crucial factor in the early stages of infection by soil-borne pathogens (Bernstein and Kafkafi 2002). In addition to the osmotic stress and nutritional disruption caused by salinity, the above-mentioned increased FOL sporulation under saline conditions seems to also be involved...
in successful plant infection. These findings are also in agreement with those of Triky-Dotan et al. (2005). In fact, irrigation with saline water significantly increased disease severity in tomato transplants inoculated with *F. oxysporum* f. sp. *radicis-lycopersici*. Disease onset was shown to occur earlier and more severely in plots irrigated with saline water than those preirrigated with tap water. Similarly, increased Fusarium wilt severity of cotton under salt stress, estimated...
via the percentage of infected leaf area and the date of onset of wilt, was reported by Ragazzi and Moricca (1995).

Even though plant sensitivity to salt stress is generally expressed by loss of turgor, growth reduction, wilting, leaf curling and epinasty, leaf abscission, decreased photosynthesis, respiratory changes, loss of cellular integrity, tissue necrosis, and potentially death of the plant (Jones 1986; Cheseman 1988), Gour et al. (1990) suggested the involvement of fungal infection in wilting and yellowing of plants at high salinity levels.

The present results showed that the majority of growth parameters were negatively affected by water salinity levels. Only the highest NaCl concentrations affected all of the considered parameters and caused a significant reduction in root and shoot growth. These findings joined those of several authors who found that salinity adversely affected the vegetative growth of tomato and reduced plant height, dry weight (Adler and Wilcor 1987), fresh weight, shoot weight and root weight (Omar et al. 1982; Shannon et al. 1987; Hajer et al. 2006). Increasing NaCl concentration in nutrient solution adversely affected tomato shoot and roots and plant height (Al-Karaki 2000). Furthermore, the leaf and stem dry weights of tomato were also significantly reduced in plants irrigated with saline nutrient solution in contrast with control plants of Al-Yahay (1995). Similarly, Satti et al. (1994) noted that both stem height and leaf growth were the most affected growth parameters in salt-stressed tomato. It is supposed that leaves are the most sensitive plant tissue to high salinity, while root growth is only moderately affected (Muñns and Termaat 1986). However, Cruz and Cuartero (1990), cited by Cuartero and Fernández-Muñoz (1999), mentioned that in spite of the negative effect of salt on roots, root growth in tomato appears to be less affected by salt than shoot growth and so the root/ shoot dry weight ratio is higher in plants grown under salt stress than in control plants at all their stages of development.

The fruit fresh weight was adversely affected, as the other growth parameters, by higher NaCl doses tested (8 and 10 g/l). Similarly, Triky-Dotan et al. (2005) found that the earlier disease onset and the increased disease severity, recorded on plants irrigated with saline water, were associated with lower yield. Also, greater yield losses were caused by salinity stress submitted to alfalfa plants previously inoculated with F. albo-atrum (Howell et al. 1994).

Plant responses to salinity depend upon various factors, such as the duration and degree of the stress and the growth stage at stress exposure (Triky-Dotan et al. 2005). Consequently, the reduced plant growth and the enhanced wilt severity reported in the present work are expected to be more pronounced under natural conditions due to the duration of exposure to salt stress, and to the greater soil inoculum. Moreover, salt stress may weaken the genetic resistance of plants to disease by inducing changes in susceptibility of root tissue to pathogens (MacDonald et al. 1984). Ragazzi and Moricca (1995) reported that even cotton variety 'GSC 20', which is normally moderately resistant to F. oxysporum f. sp. vassinfestum, exhibited a fairly high percentage of infection after the lowest post-inoculation water potentials. A similar phenomenon was noted on potato where disease expression and colonization levels of the fungus were found to be more severe in V. dahlie-tolerant cultivars irrigated with saline water compared with plots irrigated with fresh water (Nachmas et al. 1993). Thus, the assessment in the future of the behavior of the most cultivated tomato cultivars against FOL under salt stress may give additional information on the stability of the genetic resistance to this pathogen which will be useful for future breeding programs.

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