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EFFECT OF SALINITY ON RECOVERY OF *RHIZOCTONIA SOLANI* FROM INFECTED TOMATO (*SOLANUM LYCOPERSICON* MILL)

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Abstract

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The effect of salinity levels in irrigation water on recovery of *Rhizoctonia solani* from tap roots of tomato plants was investigated in two experiments conducted under greenhouse conditions. Potted soil was infested with *R. solani* and subjected to four salinity levels of 0 (control), 3, 8, and 10-mS cm^{-1}. Recovery of *R. solani* from segments of tomato tap roots was evaluated. Dry weight of above ground plant parts, fruit number, plant height, and several plant physiological parameters were measured. Response of *R. solani* to salinity was variable. However, little or no significant differences were found among treatments with respect to all the variables measured.

Key words: salinity; tomato; *Rhizoctonia solani*; physiological parameters; growth parameters

Introduction

Tomato (*Solanum lycopersicon* Mill.) is one of the most valuable vegetables grown worldwide. The estimated production of tomato was about 161.8 million tonnes in 2012 with a value of about 58 billion U.S. dollars (FAO, 2012). The crop faces several destructive pathogens (Fakhro et al., 2010) such as *Rhizoctonia solani*, a fungal microorganism that causes damping-off of seedlings. *Rhizoctonia solani* is a widely distributed soilborne plant pathogen with a wide host range (Nelson et al., 1996; Al-Hammouri et al., 2013). A combination of environmental factors (Wharton et al., 2007) and salinity (Singh et al., 2012) has been linked to the prevalence of the pathogen.

Salinity is one of the most constraining abiotic factors that limit vegetable production. In Jordan, salinity is a significant constraint to the production of many crops including vegetables (Al-Karaki et al., 2001).Tomato is moderately sensitive to salinity (Singh et al., 2012), and large genetic variation of tolerance to salt level exists among tomato genotypes. Physiological responses of tomato to salinity stress have been documented widely (Mitchell et al., 1991; Niedziela et al., 1993). Salinity stress was reported to reduce water potential and cause ion imbalance and toxicity in vegetables (De la Peña and Hughes, 2007). In addition, salinity stress affects the level and the speed of seed germination, root/shoot dry weight, and Na^+/K^+ ratio in root and shoot (Parida and Das, 2005).

A major concern in any production system is the interaction among factors that affect production. Salinity and soilborne pathogens may interact with negative impacts on crops. It was found that roots of chile grown under salinity stress were vulnerable to infection by *Phytophthora* (Snapp and Shennan, 1994). Similarly, chile plants grown under salinity

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showed increased severity of *Phytophthora capsici* (Sanogo, 2004). Salinity also affects pathogenicity and virulence of *R. solani* through interference with the activity of cell wall degrading enzymes (Abyad et al., 1988; Abyad et al., 1992). It was found that germination of *R. solani* sclerotia was inhibited under salinity stress. Also, germination of *Fusarium oxysporum* and other soilborne fungi was inhibited under salinity conditions. It was found that salinity stimulated mycelial growth of *R. solani* and inhibited mycelial growth of other plant pathogenic fungi (Abyad et al., 1988). Likewise, salinity stress increased Phytophthora on root and crown of rhododendron (Blaker and Mcdonald, 1981). A wide range of salinity effect was shown on charcoal rot (*Macrophomina phaseolina*) of melon. Therefore, it is important to manage salinity to decrease disease severity (Cuartero et al., 2006). It was shown that salinity affected colonization of cotton roots by *R. solani* and *Fusarium* sp., and the colonization percentage was higher for *Fusarium* sp. (Abdul Rauf et al., 2014). However, the effect of salinity stress on root colonization by soilborne pathogens is not well-studied in tomato (Swiecki and Macdonald, 1991) and in other vegetables. In this study, we investigated the effect of three different salinity levels on i) recovery of *R. solani* from infected tomato plants and ii) several physiological and growth parameters of tomato.

Materials and Methods

Inoculum Preparation

The *Rhizoctonia solani* isolate used was originally recovered from infected tomato plants, and maintained in the laboratory inside a refrigerator for one week. In order to assure pathogenicity, tomato plants were occasionally inoculated, and the pathogen was recovered from symptomatic plants. The recovered pathogen was grown on potato dextrose agar (PDA) for 10 days in 9-cm diameter petri plates. Mycelium plugs (1-cm diameter) were taken from the PDA culture of *R. solani* to infested potted soil.

Plant Production and Inoculation

A large composite sample of silty clay soil was collected from the top 15-cm of a field at the Hashemite University, Zarqa, Jordan. The soil was thoroughly mixed and stored until use. Round plastic pots (10-cm radius and soil depth of 16-cm) were filled with soil (4-kg). On April1, 2013 in both experiment 1 and experiment 2, five tomato seeds of the cultivar, ace55vf, were planted in each pot. The pots were irrigated immediately after planting and maintained in a greenhouse with a minimum air temperature ranging from 14 to 21°C and maximum air temperature ranging from 35 to 39°C. Two weeks later, tomato plants were thinned to one seedling per pot. Plants were inoculated when seedlings were at the 7-8 leaf growth stage. Three equidistant holes were made to a depth of 2.5-cm and approximately 1-cm from the seedlings to facilitate inoculation. One mycelium plug (1-cm diameter) of *R. solani* grown on PDA was placed into each hole, and covered with soil (Al-Hammouri et al., 2013). All seedlings were irrigated with normal tap water after inoculation. Water used in irrigation was stored in plastic containers to bring irrigation water temperature to greenhouse temperatures.

Salinity Treatments

Tap water was dispensed into a large plastic container. Then, calcium carbonate and sodium chloride salts were added at a ratio of 3:1, respectively. The salts were dissolved into the tap water, while rod of the electrical conductivity meter was immersed inside the solution to monitor salinity level of the irrigation water. Three stock solutions of 3, 8, and 10-mS cm^{-1} salinity levels were stored in the greenhouse, each in a large plastic container, until the time of treatment application. A plastic container filled only with tap water was also stored under the same conditions. The EC of tap water was 0.0043-mS cm^{-1}.

During the first week following inoculation, plants assigned to salinity treatments were irrigated with a tap water of salinity level of 3-mS cm^{-1} to avoid salinity shock to the plants. In the second week after inoculation, plants assigned to salinity levels of 8 and 10-mS cm^{-1} were irrigated with tap water of salinity 8-mS cm^{-1}. In the third week following inoculation, all plants were irrigated periodically twice a week according to the assigned treatment.

Rhizoctonia solani Assessment

Tap root segments were collected for *R. solani* assessment and were stored in a refrigerator until plating for one week. The tap root segment for each plant was soaked for two minutes in a 10% sodium hypochlorite solution, and rinsed for two minutes in deionized water. The tap root was clipped into top, middle and bottom portions. The middle portion of the tap root was then clipped into eight segments (1-cm long) under aseptic conditions, and four segments were placed equidistantly on PDA medium in each of two 9-cm diameter Petri plates. All plates were then incubated at room temperature (22 to 25°C). Two weeks after incubation, the number of tap root segments from which colonies of *R. solani* emerged to provide the frequency of recovery of *R. solani* based on 8 tap root segments. Non-*Rhizoctonia* colonies emerging from the segments were also counted.

Plant Physiological Measurements and Growth Parameters

Leaf gas exchange measurements were taken from the top 30-cm of each plant on July 3, 2013 about one week

before harvesting in both experiments. Leaf gas exchange measurements comprised of net photosynthesis (P_N), stomatal conductance (g_s), transpiration (E), vapor pressure deficit (VPD) and leaf temperature (T_L) were measured on 6-cm^2 leaf area using an infrared gas analyzer-based photosynthesis system (LI-1600, Li-Cor Inc., Lincoln, NE, USA). All measurements were taken at an internal photon flux of 1000-µmol m^{-2} s^{-1}, using a built-in LED light source, flow rate of 400-µmol s^{-1} and an internal CO_2 concentration of 400-µmol mol^{-1}. Plant height and fruit number were recorded two days before harvesting. Then, dry weights of the above-ground plant parts were taken after oven drying at 68°C for 24 hours.

Statistical analysis

Each experiment was conducted in a randomized complete block design with five replications for each of the four treatments: control + *R. solani*, salinity level of 3 mS cm $^{-1}$ + *R. solani*, salinity level of 8 mS cm $^{-1}$ + *R. solani*, and salinity level of 10mS cm $^{-1}$ + *R. solani*. All data were analyzed using analysis of variance using the Proc GLM procedure in SAS version 9.2.0.8031 (SAS institute, Cary, NC). Proc GLM was used to calculate F statistics for the overall treatment effect. Least significant difference (LSD) was used to carry out pairwise comparisons to separate treatment differences. All statistical tests were assessed at 5% significance level.

Results

No significant differences (P > 0.05) were detected among treatments (Figure 1 and Figure 2).

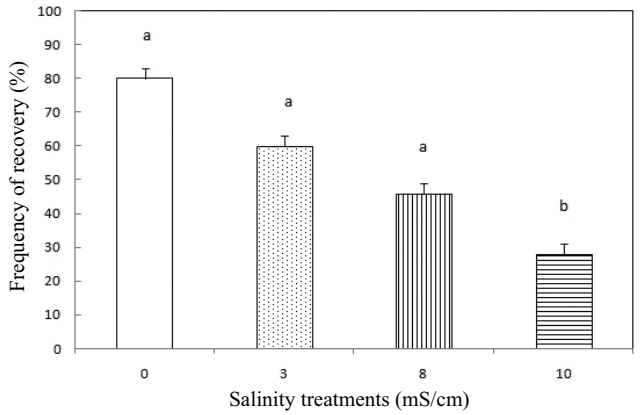

Fig. 1. Frequency of recovery (%) of *Rhizoctonia solani* from tap roots of tomato in experiment 1

Each column represents the mean of five replications. Bars on columns represent standard deviation (SD), and bars with different letters are significantly different (p < 0.05)

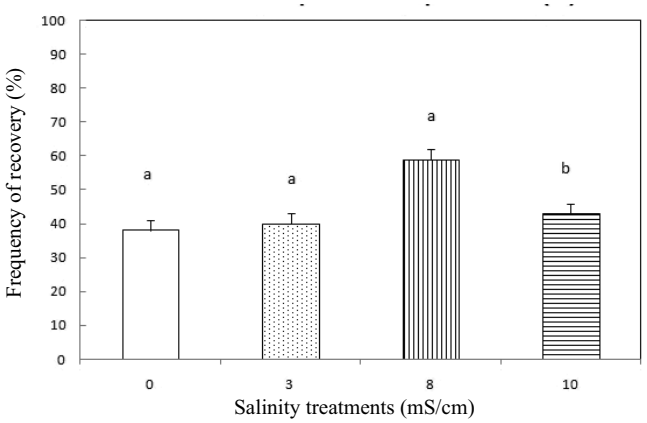

Fig. 2. Frequency of recovery (%) of *Rhizoctonia solani* from tap roots of tomato in experiment 2

Each column represents the mean of five replications. Bars on columns represent standard deviation (SD), and bars with different letters are significantly different (p < 0.05)

Similarly, no significant differences were found with respect to measured physiological parameters (Figure 5). However, in one of the experiments, we could not take physiological measurements because plants were not suitable for measurements due the fact they were exposed, beyond our control, to high greenhouse temperature and their leaves became dried. Although plant growth parameters showed little apparent differences among treatments (Figure 3 and Figure 4), the apparent differences were not statistically significant.

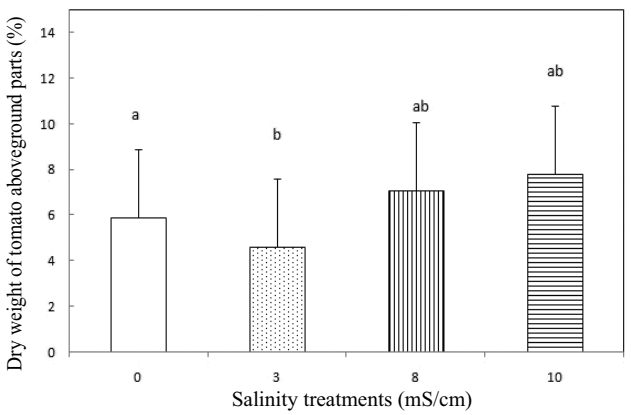

Fig. 3. Dry weight of aboveground parts of tomato plants in experiment 1

Each column represents the mean of five replications. Bars on columns represent standard deviation (SD), and bars with different letters are significantly different (p < 0.05)

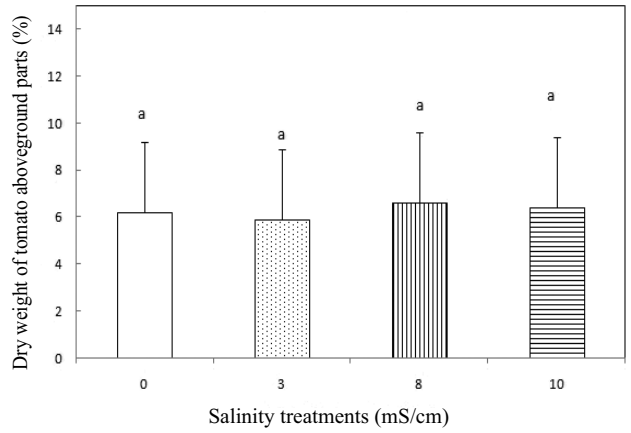

Fig. 4. Dry weight of aboveground parts of tomato plants in experiment 2

Each column represents the mean of five replications. Bars on columns represent standard deviation (SD), and bars with different letters are significantly different ($p < 0.05$)

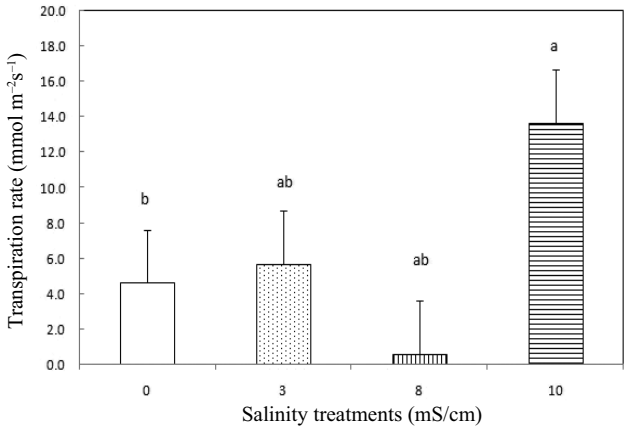

Fig. 5. Transpiration rate of tomato plants in experiment 1

Each column represents the mean of five replications. Bars on columns represent standard deviation (SD), and bars with different letters are significantly different ($p < 0.05$)

Discussion

Tomato plants, like the cultivar used in this experiment, are moderately sensitive to salinity (Al-Karaki, 2000; Singh et al., 2012), and their response to *R. solani* under saline conditions is sporadic (El-Abyad et al., 1988). Moreover, the interaction between salinity, *R. solani*, and plant did not cause significant differences in the percentage of recovery of *R. solani*, dry weight of plant aboveground parts, plant height, fruit number, and physiological parameters. Our findings are in agreement with results from other studies, which found that, in some genotypes, the response of tomato plants was not affected by salinity and soilborne pathogens (Snapp and Shennan, 1992). We used the ace55vfcultivar, which is widely grown. But, there are cultivars that also grown that may need to be evaluated. Thus, further experiments should investigate a wider range of the interaction between salinity and *R. solani* recovery on several plant genotypes.

Conclusion

Varied responses of *R. solani* to salinity conditions had been documented in several studies. However, we found that the three salinity levels in our study had little influence on recovery of *R. solani* from tap roots of tomato. Also, plant growth responses were not significantly affected by *R. solani* and salinity. Under arid and semi-arid regions, it is important to study the interaction between salinity and *R. solani* on vegetable crops such as tomato. No or little work has been conducted on the effect of the interaction of salinity and *R. solani* on tomato in Jordan. This study provides a pedestal for further research on understanding the interaction between salinity and soilborne pathogens on tomato and other vegetable crops in Jordan.

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