Plant Pathol. J. 21(4) : 391-394 (2005)

The Plant Pathology Journal

Influence of Soil Salinity on the Interaction between Tomato and Broomrape plant (*Orobanche cernua*)

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(Received on June 29, 2005; Accepted on November 12, 2005)

Tomato seedlings (20- days old) were transplanted to infested soil with *Orobanche cernua* and non-infested soils. All plants were maintained under 0, 25, 50 and 75 mM NaCl soil salinity levels throughout their growing period under greenhouse conditions. Plants grown in *O. cernua* infested soil infiltrated with 0, 25, and 50 mM NaCl solution for salinity showed significant reduction in their growth as well as their total soluble carbohydrate and protein contents in compared with those grown in non-infested soil. However, under 75 mM NaCl salinity level all plants showed similar growth values whether they were grown in *O. cernua* infested or non-infested soil. Starting at the fifth and through out the eighth week after transplantation there was a significant increase in plant height in the 0, 25 and 50 mM NaCl irrigated plants over other treatments. Irrigation with 50 mM NaCl significantly reduced the emergence of *O. cernua* (2/plant) and the number of attachments (4.4 attachments) on roots of tomato. Furthermore, irrigation with 75 mM NaCl resulted in complete elimination of *O. cernua* emergence.

Keywords : interaction, *Orobanche cernua*, salinity, tomato growth

Broomrape plant (*Orobanche cernua)* is a root parasite, lacks chlorophyll and takes all its nourishment from the host plant. It is spread by tiny (less than 0.5 mm) seeds which are easily carried by farm and construction equipment, water, wind, or animal droppings. Broomrape plant lives directly on their hosts by attaching strong haustoria to their roots, penetrating the tissues, and absorbing the food gathered by the host plants for their own development (Alkhateeb et al., 2003). The first flowers appear just three days after the plant breaks ground, and seed pods mature in just 14 days. Each plant may produce 50,000 or more seeds. The seeds are long lived, some surviving in the soil for more than 10 years. Only a portion of the viable *Orobanche* seed in the soil will germinate each year.

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Growth and development of *Orobanche* occurs at the expense host plant. (Morozov et al., 2000). Therefore, *Orobanche* depletes photosynthesis process produced materials (Hibberd et al., 1996), lowering soluble carbohydrate and protein content (Al-Khesraji et al., 1987). Nandula et al. (1998) also showed that *O. aegyptiaca* can cause a decreased total amino acid content of the carrot leaves. Borg and Van Ast (1991) reported that at early stages of *O. crenata* development on *Vicia faba* there was an increase in the host dry weight. It was anticipated that *Orobanche* may acts as an extra sink, which stimulate the photosynthesis process in the host.

Mediterranean region is the main area of *Orobanche* distribution. It's also present in other regions of similar climatic conditions such as California, Western Australia, and Cuba (Miller, 1994). Those regions, in general, are characterized by semiarid, light soil with low salinity levels.

In Jordan, Abu Irmaileh (1998) observed that no *Orobanche* infections were found in the area around the Dead Sea and the southern Jordan valley where soil salinity may reach 16.4 ds/m on tomato.

There is no previous investigation on the effect of salinity on *Orobanche cernua* infection on tomato plants. Therefore, in this study, the effect of different levels of soil salinity on infection of *O. cernua* to tomato will be investigated.

Materials and Methods

Plants materials and inoculationTomato seedlings, 20- days old, uniform in size were transplanted into 5 L plastic pots by three plants per pot, and utilized as experimental unit. Pots were filled with silty clay soil supplemented with sand (soil:sand = 2:1, v/v). Some plants were inoculated with 5 mg seeds of *O. cernua.* The seeds were uniformly dispersed upon the root system at the time of transplantation.

Salinity treatment. Four salt levels were maintained by daily irrigation with 0 (tap water), 25, 50, and 75 mM NaCl solutions for four weeks after tranplantation. The soil in each treatment was gradually salinized in order to avoid

osmotic shock. Accumulation of salts was avoided by flooding each pots with excessive salt solutions and allowing it to drain. At the time when 50% and above of the pots in the control treatments were showing *Orobanche* emerged above the soil surface, the incidence and severity of *Orobanche* infection were rated. The tomato plants were harvested eight weeks after transplanting. Vegetative growth of tomato was observed and subjected to biochemical analysis. Root system was carefully washed, assessed tomato biomass and the severity of *Orobanche* infection was weighed in number of attachment per plant root system.

Total soluble carbohydrate (CH_2O): Extracts of frozen tomato leaves (500 mg) obtained by grinding in 5 ml distilled water, and filtrated through filterpaper (Whatman, 9 cm diam) was analyzed for its total soluble carbohydrate (CH_2O) using Anthron chemical test according to procedure adapted by Abu-Qamar (2000). Aliquots of 0.2 ml of a ten folds diluted extract were mixed with 2 ml of the Anthrone solution (0.2 w/v Anthrone with concentrated sulfuric acid) inside large test tube. Samples placed for 10 min. in a boiling water bath. Optical density of the solution was measured using a spectrophotometer (Cecil Ce1010, Cambridge. England) at wavelength of 620 nm.

Total soluble proteins: Tomato leaves from all treatments and control trials were analyzed for their total protein content according to Lowry assay (Harborne, 1984). Five hundred mg of frozen tomato leaves were homogenized in 5 ml of 0.1 M Na-phosphate buffer (pH = 7.0) and centrifuged at 16,000 × for 30 min. Aliquots of 0.2 ml from 10 folded diluted extracts were mixed with 2 ml Lowry C, 10 min later 0.2 ml of folin-Ciocalteus reagent was added to the mixture and left at room temperature for 30 min. Optical density of the solution was measured using spectrophotometer (Cecil Ce1010, Cambridge, England) at wavelength of 750 nm.

Statistical analyses: All pots were arranged in randomized complete blocked design (RCBD) with five replications. Data were subjected to analysis of variance (ANOVA) by using SAS (Ver. 9.02) to determine if significant differences were present among treatments. Means were separated according to the least significant differences (LSD) at 0.05 level of probability (Steel and Torrie, 1980).

Results and Discussion

Salinity affected progressive change in heights of tomato plant under both soil treatments (infested with broomrape plant and non-infested soil) throughout 8 weeks. Significant differences in plant height between infested and non-infested soil treatments were appeared 5 and 6 weeks after transplantation in control (tap water only) and 25 mM NaCl irrigated plants, respectivily. For tomato plants irrigated with 50 mM NaCl solution, a significant difference started to be evidence at the seveneth week between infested and non-infested soil treatments. On the other hand, the height of the tomato plants irrigated with 75 mM NaCl solution didn't different under infested and non-infested soil throughout the 8 weeks (Table 1). In treatment with 0, 25, and 50 mM NaCl, tomato plants grown in *Orobanche* infested soil showed significantly lower values of plant height, shoot dry weight and root dry weight than those grown in non-infested soil. However, under 75 mM NaCl, there was no significant differences between *Orobanche* infested and non-infested soil.

Increasing salt level caused significant decrease in plant height, shoot dry weight and root dry weight in the *Orobanche* infested and non-infested soil as well (Table 2). These results indicated that the adverse effect of salinity and *Orobanche* infection was not of additive nature. At certain level(s), salinity might have counteracted the adverse effect of the parasite, possibly by curtailing its

Table 1. Effect of salinity on the height of tomato plants transplanted in soil infested with *Orobanche cernua* seeds and non-infested soil

	Plant Height (cm)							
	0 mM NaCl		25 mM NaCl		50 mM NaCl		75 mM NaCl	
Week No.	Infested	Noninfested	Infested	Noninfested	Infested	Noninfested	Infested	Noninfested
1	9	9	9	9	9	10	9	10
2	12	12	11	13	12	14	11	12
3	16	17	17	16	15	16	15	15
4	21	23	24	22	20	21	19	18
5	23	29	26	27	24	26	24	23
6	25	34	28	34	27	29	27	28
7	26	38	29	38	29	32	30	31
8	28	43	29	41	30	34	32	32
LSD	3.9		2.3		3.4		2.6	

Table 2. Plant height, root and shoot dry weight of tomato plants grown in *Orobanche cernua* seeds infested and non-infested soil under 0, 25, 50, and 75 mM NaCl levels

NaCl level (mM)	Root dry weight (g)		Shoot dry weight (g)	
	Infested	Noninfested	Infested	Noninfested
0	2.44	3.43	4.92	9.15
25	2.51	3.19	4.99	9.29
50	2.42	2.80	5.81	6.96
75	2.37	2.42	7.28	7.17
LSD	0.11		0.81	

Table 3. Carbohydrate and protein content (mg/g dry weight) of tomato plants grown in *Orobanche cernua* seeds infested and non-infested soil under 0, 25, 50, and 75 mM NaCl levels

NaCl level (mM)	Carbohydrate		Protein	
	Infested	Noninfested	Infested	Noninfested
0	11.8	20.2	13.6	26.0
25	16.6	20.8	15.2	30.6
50	15.4	15.2	26.0	35.2
75	15.6	15.8	32.0	32.6
LSD	1.4		1.95	

Table 4. Number of *Orobanche cernua* shoots, attachments and dry weight (g) per tomato plant grown under 0, 25, 50, and 75 mM NaCl levels

Treatment	No. of Shoots	No. of Attachments	Dry weight (g)
0 mM	4.8	11.0	7.5
25 mM	5.2	11.2	5.9
50 mM	2.0	4.4	1.9
75 mM	0	0	0
LSD	1.76	1.83	0.43

infection. This could be ascertained via infection incidence and severity coupled with different salinity levels and plant biomass accumulation (Table 4).

Results of the chemical analysis showed that plants grown in *Orobanche* infested soil at 0 and 25 mM NaCl levels contained significantly lower soluble CH_2O than plants in non-infested soil. Also the total soluble proteins in infested soil was significantly lowered at 0, 25 and 50 mM NaCl treatments of the same plants than non-infested soil. On the other hand, no significant differences in protein and CH_2O were founded in plants irrigated with 75 mM NaCl, whether they were in infested or non-infested soil (Table 3). Broomrape plant infection was consistently associated with growth reduction and lower carbohydrate and protein content of tomato plants infected with *Orobanche cernua*. However, in presence of salts in the irrigation solution at 50 and 75 mM NaCl, the magnitude of that effect was drastically reduced, which indicate certain degree of *Orobanche* elimination. Such findings indicate certain degree of eliminating the anticipated adverse effect of *Orobanche*. Results confirmed that irrigation of tomato plants with tap water (control) and 25 mM NaCl solution didn't significantly affect the number of emerged *Orobnache* shoots and number of attachments per tomato plant. However, irrigation with 50 mM NaCl significantly reduced the number of emerged *Orobnache* shoots and number of attachments per tomato plant. Furthermore, irrigation with 75 mM NaCl resulted in complete elimination of *Orobnache* emergence and attachment in all of the replicates of this treatment (Table 4). Increasing salinity levels also significantly decreased *Orobnache* dry weight for of the three treatments (7.5, 5.9 and 1.9 g of *Orobanche* per tomato plant for 0, 25, and 50 mM NaCl respectively) (Table 4). Results of the present study supported the hypothesis that salinity have an adverse effect on *O. cernua. This could be shown by* the complete absence of the parasite on tomato seedlings under 75 mM NaCl, and the reduction of its infection incidence by 64% under 50 mM NaCl. Under those conditions, salinity may had directly affected *Orobanche* infection through a direct effect of salinity on *Orobanche* seeds (Al-Khateeb et al., 2003), either by preventing germination and/or by establishing infections on tomato roots. Salinity also may have affected anatomical and biochemical characteristics of plant root itself. Thus, preventing the attachment process to take place. Dunlap and Binzel (1996) reported a decrease in IAA levels in tomato roots grown under saline conditions (50-150 mM NaCl). Furthermore, Harb (2002) found that number of *Orobanche ramosa* attachments was lowered as a result of reduction in IAA level in the root system of tomato plant which was also lowered by salinity. Salinity also might affect root exudation, of chemicals required for *Orobanche* seed germination. Hence, seed germination may not happened.

Acknowledgements

Authors would like to thank the Deanship of Research at Jordan University of Science and Technology for supporting this study, Project #80/01.

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