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RESPONSE OF CHILE PEPPER (*Capsicum annuum* L.) TO SALT STRESS AND ORGANIC AND INORGANIC NITROGEN SOURCES: II. NITROGEN AND WATER USE EFFICIENCIES, AND SALT TOLERANCE

[RESPUESTA DE PLANTAS DE CHILE (Capsicum annuum L.) AL ESTRÉS SALINO Y FUENTES ORGANICAS E INORGANICAS DE NITROGENO: II. EFICIENCIAS EN EL USO DEL AGUA Y DE NITROGENO Y TOLERANCIA A LAS SALES]

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SUMMARY

The response to two nitrogen sources on water and nitrogen use efficiencies, and tolerance of salt-stressed chile pepper plants (*Capsicum annuum* L.) cv. Sandia was investigated in a greenhouse experiment. Low, moderate and high (1.5, 4.5, and 6.5 dS m^{-1}) salinity levels, and two rates of organic-N fertilizer (120 and 200 kg ha^{-1}) and 120 kg ha^{-1} of inorganic fertilizer as ammonium nitrate were arranged in randomized complete block designs replicated four times. The liquid organic-N source was an organic, extracted with water from grass clippings. Water use decreased about 19 and 30% in moderate and high salt-stressed plants. Water use efficiency decreased only in high salt-stressed plants. Nitrogen use efficiency decreased either by increased salinity or increased N rates. An apparent increase in salt tolerance was noted when plants were fertilized with organic-N source compared to that of inorganic-N source.

Key words: green pepper; soil salinity; organic fertilizer; nitrogen use efficiency; water use efficiency; salt tolerance.

RESUMEN

La respuesta a dos fuentes de nitrógeno sobre las eficiencias en el uso de agua y nitrógeno de plantas de chile (*Capsicum annuum* L.) cv. Sandia bajo estrés salino fue investigado en un experimento de invernadero. Niveles de salinidad bajo, moderado y alto (1.5, 4.5 y 6.5 dS m^{-1}) y dos dosis de un fertilizante orgánico (120 y 200 kg ha^{-1}) y 120 kg ha^{-1} de nitrato de amonio como fertilizante inorgánico fueron arreglados en un diseño experimental de bloques completos al azar con cuatro repeticiones. La fuente orgánica de fertilizante líquido fue extraída con agua de residuos de zacates. El uso de agua disminuyo entre el 19 y 30% en plantas sujetas a moderada y alta salinidad. La eficiencia en el uso del agua disminuyo solamente en plantas altamente estresadas por salinidad. La eficiencia en el uso de nitrógeno disminuyo ya sea por incremento en la salinidad o incremento en la dosis de N. Un aparente incremento en la tolerancia a la salinidad fue observado cuando las plantas fueron fertilizadas con la fuente orgánica de N comparado a aquella de la fuente inorgánica de N.

Palabras clave: Chile verde; suelos salinos; fertilizante orgánico; uso eficiente de nitrógeno; uso eficiente del agua; tolerancia a la salinidad.

INTRODUCTION

Salinity represents one of the major factors limiting crop production in arid and semi-arid regions. The inhibitory effects of salinity on plant growth is caused by both lowering of the water potential of the root environment (and hence restricted water and ion uptake by plants) and the accumulation of ions in plant tissues at concentrations that may be toxic or to give rise to nutritional imbalances (Greenway and Munns, 1980; Alam, 1994; Grattan and Grieve, 1994; Munns *et al*., 2005).

In addition to salinity, nutrient deficiencies are major factors reducing plant productivity. Among the essential nutrients, nitrogen (N) is usually the most important growth limiting plant nutrient in saline or non-saline soils (Amonkar and Karnakar, 1995; Irshad *et al*., 2002). The supplementation with N usually enhances plant growth and yield regardless of whether the plant is salt-stressed or not (Grattan and Grieve, 1999). Investigations showed that application of fertilizers in saline soils might result in increased, decreased or unchanged plant salt tolerance. In other words, plant response to fertilizers depends on severity of salt stress in the root zone (Maas & Grattan, 1999). Under low salinity stress, nutrient deficiency limits plant growth more than salinity and a positive interaction or an increased salt tolerance response occurs. While under moderate and high salinity, the limiting effect of salinity also affects plant growth (Grattan & Grieve, 1999).

Inorganic N fertilizers are one of the expensive inputs used by farmers to achieve desired crop yields. The production and use of inorganic fertilizer has enhanced environmental problems associated with the nitrate pollution caused by agricultural practices (Kramer *et al*., 2006). On the other hand, the overwhelming environmental concerns regarding animal waste utilization have been focused on nutrient accumulation in the arable soil profile, contamination of surface and groundwater and ammonia emission into the atmosphere (Ceotto, 2004). An alternative to inorganic and solid organic fertilizers for producing vegetables is to extract the nutrients from plant wastes such as that produced from grass clippings from the greens of golf courses (Saha, 2002). The liquid fertilizer is dissolved in water and is easily available for plant uptake contrary to composted material where only a fraction of the nutrient is available for plant uptake and often needs to be supplemented with mineral fertilizer.

Besides affecting plant growth, salinity reduces yield potential (Fernandez-García *et al*. 2004; Maas and Hoffman, 1977; Maas, 1996; Tadesse *et al*., 1999; Chartzoulakis and Klapaki, 2000). Usually, this reduction in yield at increasing salinity has been evaluated using the traditional model proposed by Mass and Hoffman (1977) where the salt tolerance of crop plants usually has been expressed as the yield decrease for a given level of soluble salts in the root medium compared with yields under nonsaline conditions. Maas and Hoffman (1977) described the salt tolerance as a function with two parameters: a threshold value (the maximum salinity level at which yield begin to be decreased) and the slope (the percentage of yield expected to be reduced for each unit of added salinity above the threshold value).

Pepper (*Capsicum annuum* L.) is one of the three important solanaceous vegetable crops grown for their fruits, which are consumed, either fresh or dried (Hedge, 1997). It is classified as moderately sensitive to salinity (Maas and Hoffman, 1977), and some adverse effects of salinity have been reported (Cornillon and Palloix, 1997; Gomez *et al*., 1996; Günes *et al*., 1996; Tadesse *et al*., 1999; Chartzoulakis and Klapaki, 2000; De Pascale *et al*., 2003; Navarro *et al*., 2003; Villa-Castorena *et al*., 2003).

Considering the benefits of N fertilization on crop productivity and that some researchers have hypothesized that N fertilizer additions mitigate the detrimental effect of salinity on plants (Gomez *et al*., 1996; Grattan and Grieve, 1999; Kaya and Higgs, 2002; Kaya and Higgs, 2003), the present work was carried out to compare the salt tolerance of chile pepper plants to organic or inorganic fertilization. The effects of salinity and nitrogen source on the nitrogen and water use efficiencies of chile pepper plants were also investigated.

MATERIALS AND METHODS

Seedlings of chile pepper cv. "Sandia" were transplanted to plastic pots filled with 15 kg of a non saline (ECe = 0.9 dS m^{-1}), air dried soil passed through a 2-mm sieve. Brazito sandy loam (Mixed thermic Typic Torripsamment) (USDA, 1980) soil was used in the experiment performed in the New Mexico State University. A greenhouse experiment was arranged in a randomized complete block design where each salinity level, and N-fertilizer source and rate combination was replicated four times. Three salinity levels (Low: 1.5 dS m^{-1}, Moderate: 4.5 dS m^{-1}, and High: 6.5 dS m^{-1}) were prepared by adding solutions of a mixture of NaCl and $CaCl_2$ salts on a 1:1 equivalent weight ratio. The amount of each salt (mg) to add to the solution was calculated according to Villa-Castorena *et al*. (2003) as follow:

$$\text{Solution} = \frac{10\,\text{ECe}}{2} \times \text{EW} \times \text{SSV} \qquad [1]$$

where EW is the equivalent weight of each salt in mg meq^{-1}, and SSV is the soil saturation volume of the pot in L. The constant 10 is an empirical factor to convert ECe in dS m^{-1} to total dissolved salt in the soil saturated paste extract in meq L^{-1}, and this value is divided by 2 to consider the contribution of each salt to ECe. Two sources of nitrogen (inorganic or organic fertilizer) were combined to each salinity level. Inorganic fertilizer was added at rate of 120 kg ha^{-1}. Ammonium nitrate was used as the inorganic source. Organic fertilizer was added at two rates (120 and 200 kg ha^{-1}). The organic liquid fertilizer was obtained from grass clippings, whose nutrients were extracted with water through a bioleaching process under anaerobic conditions (Saha, 2002). A brief description of this process is explained by Samani (2010). The N concentration (total Kjeldahl nitrogen, TKN) of this liquid fertilizer was 0.70 %. Each fertilizer rate was split in four equal doses and applied at transplanting, twenty days after transplanting, at flowering and after the first harvest. Three non- fertilized pepper plants were left apart in which only the fresh fruit weight were taken.

The plants were hand irrigated with reverse osmosis water (EC < 0.015 dS m^{-1}) and the plant water used during the plant growth period was determined by measuring the water volume added to the pots by weighing every day each pot to restore the soil moisture about field capacity. Chile pepper fruits were handpicked five times. Fruit yields are the means of the fruits harvested from four plants of each treatment and reported in grams per plant.

The evaluated parameters were: water use, water use efficiency, nitrogen use efficiency and salt tolerance. The water use efficiency (WUE, g L^{-1}) was calculated as the ratio between fruit yield per plant and total water use per plant during the growing season. Nitrogen use efficiency (NUE, kg kg^{-1}) was calculated using the following formula (Baligar *et al*., 2001):

$$\mathrm{NUE} = \frac{Y_F - Y_C}{N_A} \quad [2]$$

where Y_F and Y_C are the fruit yields (kg ha^{-1}) of the plants with fertilizer and without fertilizer, respectively. N_A is the quantity of N applied (kg ha^{-1}).

Finally, chile pepper fruit yield response to soil salinity was analyzed using a model similar to the traditional model proposed by Mass and Hoffman (1977). This model is a piecewise linear response function proposed by van Genuchten (1983), given as:

$$Y = \begin{cases} Y_m & 0 < ECe \le EC_0 \\ Y_m - Y_m\, S\,(ECe - EC_t) & EC_t < ECe \le EC_0 \\ 0 & ECe > EC_0 \end{cases} \quad [3]$$

where Y = absolute fruit yield; Y_m = absolute fruit yield in nonsaline conditions; S = slope of the response function (fruit yield decrease per unit soil salinity increase ECe); ECe = soil salinity, EC dS m^{-1} exceeding the threshold; EC_t = EC at which fruit yield starts to decrease; and EC_0 = EC at which fruit yield equals zero. Furthermore, the soil salinity at which the fruit yield was reduced by 50% (EC_{50}) was calculated as:

$$Y = \frac{Y_m}{1 + \left(\frac{ECe}{EC_{50}}\right)^p} \quad [4]$$

where p is an empirical constant that depend of the form of the S-shaped function between yield and soil salinity. Van Genuchten and Gupta (1993) reported that the value of p in equation 4 is close to 3 for most crops. The computer "SALT" program (van Genuchten, 1983) was used to determine simultaneously Y_m, S and EC_t by choosing the option 5. The EC_{50} and p parameters were determined with the same "SALT" program selecting the option 12.

Water use, and both N and water use efficiencies data were analyzed using the SAS package software for analysis of variance (ANOVA) to determine the effect of each treatment. Multiple mean comparisons were performed using Duncan's Multiple Range Test at the 0.05 level of probability.

RESULTS AND DISCUSSIONS

Water use and water use efficiency (WUE)

Water use

Increased soil salinity resulted in significant decreases in plant water use by chile pepper plants (Table 1). Similar results were observed by Tadesse et al. (1999) who reported that water use decreased about 50% in pepper plants grown at 8.0 dS m^{-1} compared to those grown at 2.0 dS m^{-1}. In our study, analysis of variance (data not shown) on plant water use indicated that low salt-stressed plants consumed 46.31 L per plant, which was significantly reduced 19% and 29% in moderate and high salt-stressed plants, respectively. The variation in water use by fertilizer effect was not significant and averaged 38.79 L $plant^{-1}$. In agreement with these results, Papadopoulos and Rendig (1983) also found that increase in N rates increased water use in tomato plants grown at 1 dS m^{-1}, but these N rates did not affect water use in salt-stressed tomato plants grown at 5 and 9 dS m^{-1}.

Water use efficiency (WUE)

Water use efficiency was influenced by salinity as well as fertilizer treatments (Table 1). Taking the average values for each salinity treatment, the ANOVA results indicated that the differences in WUE between low (12.78 g L^{-1}) and moderate (11.04 g L^{-1}) salt-stressed plants were not significant, but both were different from that of high salt-stressed plants (8.59 g L^{-1}). Katerji *et al*. (1998) found that WUE decreased 20% and 50% in tomato plants grown at soil salinities of 4.5 and 6.4 dS m^{-1} respectively compared to those grown at 0.8 dS m^{-1}.

Table 1. Water used (WU), water use efficiency (WUE), and Nitrogen use efficiency (NUE), in chile pepper plants as affected by salinity and fertilizer source and rate and analysis of variance (ANOVA).

Treatment		Parameter		
Salinity	Fertilizer	WU	WUE	NUE
(dS m^{-1})	(kg ha^{-1})	L	g L^{-1}	kg kg^{-1}
1.5	120 IF	45.21 a	12.79 a	165.7 a[§]
1.5	120 OF	45.50 a	12.22 a	158.4 a
1.5	200 OF	48.21 a	13.36 a	110.6 b
4.5	120 IF	37.48 bc	9.51 ab	99.5 bc
4.5	120 OF	35.11 bcd	11.52 a	112.1 b
4.5	200 OF	39.37 b	12.09 a	81.0 bc
6.5	120 IF	32.88 cd	6.98 b	60.3 c
6.5	120 OF	33.53 cd	7.40 b	64.0 c
6.5	200 OF	31.86 d	11.41 a	59.9 c
			ANOVA	
Source of variation				
Block		NS	NS	* *
Salinity level (S)		* *	* *	* *
Fertilization (F)		*	*	* *
S x F		NS	NS	NS

[§] Within columns, means followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($\alpha \leq 0.05$). NS indicates no significant at the > 0.05 probability level, and * indicates significant at the $0.01 < P \leq 0.05$ probability level, and * * indicates significant at the ≤ 0.01 probability level. IF = inorganic fertilizer; OF = organic, plant-based fertilizer

Our data showed that mean WUE values of high salt-stressed plants fertilized with 120 kg ha^{-1} of any N source were significantly different from those of low and moderate salt-stressed plants. These findings are in agreement with those results observed by Kütük *et al*. (2004) who found that increase in salinity levels decreased WUE in tomato plants grown at 12 dS m^{-1} which was statistically lower than WUE of tomato plants grown at 3, 6, and 9 dS m^{-1}. They also found that tomato plants fertilized with 240 mg N kg^{-1} had significantly higher WUE than plants fertilized with 0, 80, and 160 mg N kg^{-1}. On the other hand, the obtained results show that the use of organic fertilizer increased WUE. Results in Table 1 revealed that the addition of organic fertilizer with the highest rate of application up to 200 kg ha^{-1} gave the best values of WUE. These results are in agreement with those reported by Saleh *et al*. (2003) who indicated that the use of saline water to irrigation decreased the yield of onion plants and the application of organic manure significantly increased onion yield.

Nitrogen use efficiency (NUE)

The results of our experiments showed that NUE was significantly affected by salinity and fertilizer treatments (Table 1). NUE significantly decreased to 97.7 (32%) and 61.8 kg kg^{-1} (57%) in moderate and high salt-stressed plants from that of low salt-stressed plants. Nitrogen use efficiency of pepper plants fertilized with 120 kg N of any N source were not different (average of 110.1 kg kg^{-1}) but they were statistically greater than plants fertilized with the high N rate of 200 kg ha^{-1} organic, plant-based fertilizer (84.1 kg kg^{-1}).

Salt tolerance

The application of the "SALT" program by van Genuchten (1983) to the mean values of fruit yield and their respective soil salinities (ECe, dS m^{-1}) resulted in threshold EC_t values of 1.44, 2.62 and 2.05 dS m^{-1} and percentages of decrease in fruit yield for each unit of increase in the ECe exceeding the threshold EC_t (S) of 12, 16 and 10% for chile pepper plants fertilized with 120 kg ha^{-1} inorganic fertilizer, and 120 and 200 kg ha^{-1} organic, plant-based fertilizer, respectively (Figure 1). The EC_t (1.44 dS m^{-1}) and slope (12%) of plants fertilized with 120 kg ha^{-1} inorganic fertilizer (Figure 1A) were consistent with those set by Mass and Hoffman (1977) for pepper with a 14% decrease for each unit increase in ECe from the threshold EC_t of 1.5 dS m^{-1}, and with those reported by Chartzoulakis and Klapaki (2000) for two pepper hybrids which presented the same threshold EC_t of 1.8 dS m^{-1} but different rate of the yield reduction as ECe increased beyond EC_t (8.4 and 11.7%). Threshold EC_t values from plants fertilized with 120 (Figure 1B) and 200 kg ha^{-1} organic, plant-based fertilizer (Figure 1C) were 54 and 20% respectively greater than that reported by Maas and Hoffman (1977), which indicates that fertilization of chile pepper plants with the organic source decreased the negative effect of salinity in some degree. Regardless of the application of organic fertilization increased EC_t up to 2.62 dS m^{-1} for the 120 kg ha^{-1} N rate compared to the same N rate of inorganic fertilizer, the soil salinity EC_{50} at which the yield was reduced by 50% was about 5.5 dS m^{-1} for both inorganic and organic sources. This value was consistent with the EC_{50} of 6.0 dS m^{-1} reported by De Pascale et al. (2003) for pepper plants. On the other hand, pepper plants fertilized with 200 kg ha^{-1} organic, plant-based fertilizer had an EC_{50} of 6.7 dS m^{-1}, value also close to 6.0 dS m^{-1} also reported by De Pascale et al. (2003).

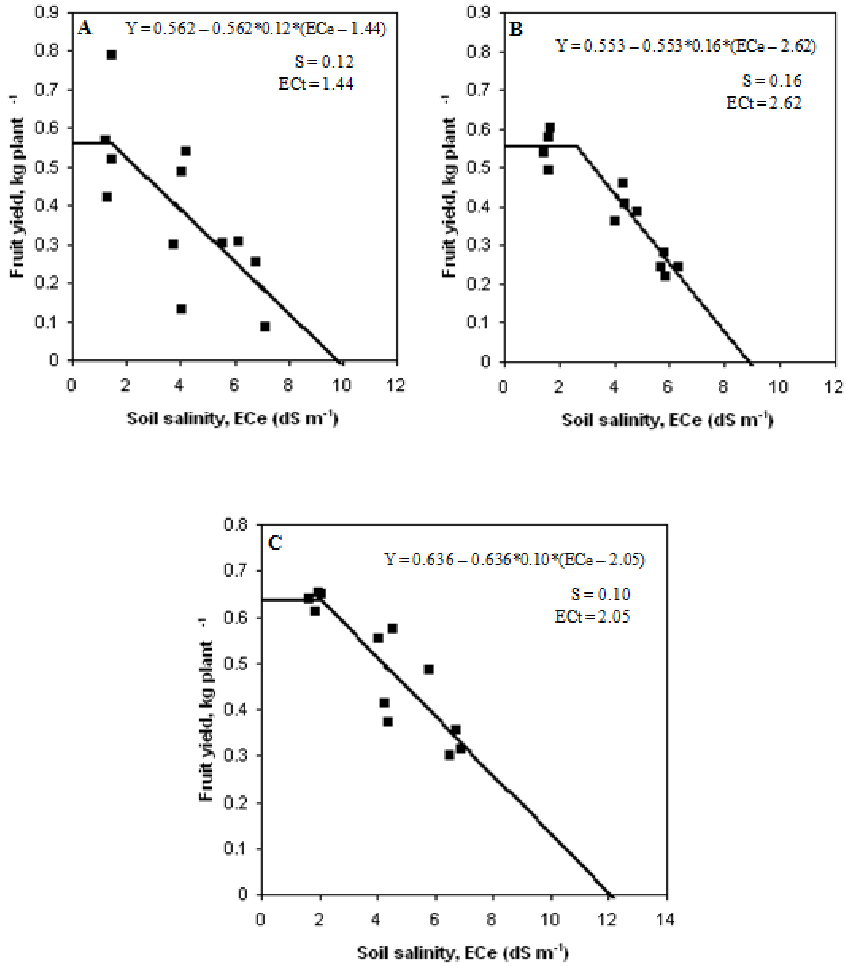

Figure 1. Observed (■) and fitted (▬) salt tolerance response for chile pepper plants grown under greenhouse conditions and fertilized with 120 kg ha^{-1} inorganic fertilizer (A), 120 kg ha^{-1} organic, plant-based fertilizer (B), and 200 kg ha^{-1} organic, plant-based fertilizer (C).

CONCLUSIONS

Statistical significances in almost all parameters analyzed showed the individual effects of salinity and fertilization (N source and rate).

Increased salinity decreased water use by pepper plants. The variation in water use was not affected by fertilization.

Increased salinity also decreased WUE. Although WUE of plants fertilized with 120 kg ha^{-1} of any N source was not different, the high N rate organic, plant-based fertilizer increased WUE.

NUE decreased significantly at increased salinity. Similarly, high rate of N-organic also decreased NUE with respect to low N rates of both sources.

Even though the absolute yield of pepper fertilized with low doses of both N sources were similar, the salt tolerance was higher with the application of organic fertilizer. The absolute yield of pepper fertilized with the high N-organic rate was superior. Also the salt tolerance was greater of pepper fertilized with the low N-inorganic rate but smaller of that fertilized with the low N-organic rate.

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