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EFFECT OF NITROGEN FERTILIZATION AND SALINITY ON GROWTH AND PHYSIOLOGICAL ATTRIBUTES OF TOMATO SEEDLINGS

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill, cv. Rio Grand) seedlings grown in pots at greenhouse were subjected to different concentrations of NO_3NH_4 as a source of nitrogen and NaCl as a source of salinity, with nutrient solution (NS), for 3 weeks. Three levels of Nitrogen: (i) NS alone containing 15 mM N, (ii) 22.5 mM N and (iii) 30 mM N, and four levels of salinity: 0, 20, 35 and 50 mM NaCl were used. The results showed that salinity led to a decrease of dry weight (DW) and relative water content (RWC), while specific leaf weight (SLW) was increased. There were no significant differences in chlorophyll fluorescence parameters of stressed plants. Increasing nitrogen level with salinity led to a significant decrease of seedlings DW, whereas the ratio of shoot:root was increased. The salinity enhanced the decrease in the total N and K^+ accumulation in plant with additional nitrogen treatments. While, the addition of nitrogen showed a reduce in Na^+ and induce in Cl^- accumulation under salinity treatments. However, decreasing the growth under NaCl treatment can be attributed to an uptake competition between Cl^- and NO_3^- with respect of toxicity from NaCl. Despite the parameters indicate to an osmotic adjustment favour to growth, the plant growth was reduced. Our overall results indicated that the supplemental nitrogen would ameliorate the ability of plant from inhibitory effects of NaCl stress in tomato seedlings by reducing Na^+ accumulation thereby enhancing plant tolerance.

Keywords: Lycopersicon esculentum; Salinity; NaCl; Nitrogen.

INTRODUCTION

Salinity is known as a major problem for agricultural production in the arid and semi-arid regions. The salinity of water is due to water quality and uncontrolled use of irrigation, especially in costal lands where irrigation water often has high salinity from NaCl.

Excessive amounts of salts have adverse effects on soil properties and therefore alterations induced in plant growth, yield and quality. Shoot and root biomass values decreased as the salinity levels increased, as largely reported in the literature (Shannon et al., 1987; Cuartero and Fernandez-Munoz, 1999; Maggio et al., 2004; Hajer et al., 2006).

To overcome this problem, many efforts have been directed by plant breeders and physiologists toward developing cultivars and agro-management techniques to improve growth and yield of crops under saline condition. Fertilizer applications under saline conditions have been tried to alleviate or neutralize growth inhibition due to salinity, and to increase the productivity of salinized soils (Lopez and Satti, 1996). Salinity has been previously shown to be a major factor responsible of low nitrogen availability (Debouba et al., 2006). Additional nitrogen fertilization via the soil solution had beneficial effects in salinized plants (Gimeno et al., 2009). Nitrogen fertilization not only promotes plant growth, it may also reduce salinity effects (Flores et al., 2001). Nitrogen is required in large quantities by plants and is taken up in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Marschner, 1995). The biomass production of ammonium-fed plants was lower than that of nitrate-fed plants. (Misra and Gupta, 2006).

Nutrient uptake by plants can be disrupted by the osmotic effects of the saline solutions, which decrease transpiration and reduce the mass flow of ions to the root

either via direct ionic competition between ions and ionic toxicity, or a combination of these factors (Lea-Cox and Syvertsen, 1993; Tavakkoli, et al., 2011).

K^+ levels decreased with increased NaCl in tomato leaves (Al-Rawahy et al., 1992). On the other hand, K^+ concentration declined as NO_3NH_4 concentration increased, and it is known to play a major role in osmotic adjustment and can therefore benefit the plant by increasing the driving force for water uptake (Lauchli and Pflugler, 1978). Hence, it regulates plant water content. In fact, the loading of NO_3^- in xylem depends closely on the xylem ionic composition particularly on the K^+ content, which functions as the major charge-balancing cation (Pilbeamtd and Kirkby, 1990). Na^+ and Cl^- accumulations were correlated with a decline of K^+ and NO_3^- in the leaves and roots (Debouba et al., 2006). Nutrient imbalances may caused by the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{++}, Mg^{++} and NO_3^- acting on biophysical and/or metabolic components of plant growth (Romero-Aranda et al., 1998).

The objective of this study was to find out the effects of supplementing nitrogen levels by addition NO_3NH_4 to investigate what extent that increase the salt tolerance in the tomato seedlings, evaluating the effects of saline water on growth and physiological parameters.

MATERIALS AND METHODS

Plant material, treatments, and growth conditions

The experiment was conducted at the Faculty of Agriculture, University of Benghazi, in 2009 and 2010. Seeds of tomato (*Lycopersicon esculentum* Mill. cv Rio Grand) were germinated in seedling trays filled with compost. Seedlings were

transferred to pots filled with washed sand culture, each pot had one seedling. Plants were grown in greenhouse at 14h photoperiod, photosynthetic active radiation reached a daytime peak value of 1200 µmol.m^{-2}.s^{-1}, the temperature and relative humidity ranged to 30°/18°C and 40/75% during day/night periods, respectively. Treatments were initiated when the plants reached the first leaf stage with a height of about 5 cm. Plants were irrigated daily by modified Hoagland Solution as a complete nutrient solution (NS). The full NS contains (in mmol/L) 5 KNO_3, 5 $Ca(NO_3)_2 \cdot 4H_2O$, 2 $MgSO_4 \cdot 7H_2O$, 1 KH_2PO_4, mixture of 0.02 $FeSO_4 \cdot 7\ H_2O$; 0.02 Na_2-EDTA; 2 H_2O; 0.045 H_3BO_3; 0.01 $MnCl_2 \cdot 4\ H_2O$, and (in µmol/L) 0.8 $ZnSO_4 \cdot 7\ H_2O$, 0.4 $Na_2MoO_4 \cdot 2\ H_2O$, and 0.3 $CuSO_4 \cdot 5\ H_2O$. Different levels of NO_3NH_4 as source of nitrogen and NaCl as source of salinity were added to NS. Three levels of Nitrogen: (i) NS alone containing 15 mM N, (ii) 22.5 mM N and (iii) 30 mM N; four levels of salt: 0, 20, 35 and 50 mM NaCl.Treatments were continued for 3 weeks.

Growth measurements

At the end of the experiment, plants were harvested and divided into shoot and root parts. Fresh weights (FW) were measured for each treatment. For determination of dry weight (DW) these tissue parts were dried three days in an oven at 70 °C (until there was no decrease in weight).

Relative water content and specific leaf weight

The midday relative water content (RWC) was measured using leaves, which immediately weighed to obtain a leaf fresh weight. Leaves were placed in a beaker with the petioles submerged in water overnight in the dark at 4°C, so leaves could become fully hydrated. Leaves were reweighed to obtain turgid weight and dried at 70°C for 3 days to obtain dry weight. RWC was calculated as [(*FW–DW*) × (*TW–DW*)$^{-}$

^{1}]×100 according to Morgan (1984). Where *FW* is the leaf fresh weight; *TW* is the turgid weight; and *DW* is the dry weight. The specific leaf weight (SLW) was determined by dividing leaf dry weight by leaf area.

Ion analysis

Shoots of four plants per replicate were washed with distilled water to remove dust and other residues, and dried in an oven at 70°C for 3 days to determine dry weights. The dried tissues were finely ground and stored in paper bags. The total content of nitrogen (N) was determined by the micro-Kjeldahl method. After digestion of ground tissue with H_2SO_4 and $HClO_4$, Na^+ and K^+ contents were measured in the DW by flame photometer, while those of Cl^- were determined by titration with $AgNO_3$ in the presence of NaCl, according to A.O.A.C., 1985.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters (presented by the quantum yield of PSII "F_v/F_m") were measured in vivo 0.5 h after darkness adaptation of the leaves, using a portable fluorometer plant efficiency analyzer (Hansatech Instruments, King's Lynn, Norfolk, United Kingdom). Where F_v is a variable fluorescence and F_m is a maximal fluorescence.

Experimental design and statistical analysis

The data presented are representative of two independent experiments. The study was conducted in four replicates (three plants in each replicate), using factorial experimental 3×4 in completely randomized design, with the following treatments of Nitrogen: control (NS alone containing 15 mM N), 22.5 mM N and 30 mM N as the

first factor, and the following treatments of salinity: 0, 20, 35 and 50 mM NaCl as the second factor. Data were subjected to analysis of variance using a two-way ANOVA (Little and Hills, 1978). Differences among means of treatments were compared by Duncan's multiple range test at the 0.05 confidence level (SPSS statistical package, Chicago, IL).

RESULTS

Plant growth

The shoot and root fresh weight (FW) were decreased by about 17 and 65% respectively with increasing external salinity (Table 1). The increasing of NO_3NH_4 concentration led to more shoot FW reduction with all salt stress treatments (by 20 to 43% relative to the control). In contrast, the more of NO_3NH_4 addition showed higher root FW levels with salinity treatments. On the other hand, shoot and root FW were increased by 16 and 42% respectively with increasing NO_3NH_4 concentration at 0 mM NaCl treatment. The shoot and root dry weight (DW) were decreased under salinity treatments, while, it was not affected at higher salt stress treatment (50 mM NaCl), relative to the control (Table 1). At all salt stress treatment, shoot and root DW showed a significant decrease (40 and 56% reduction relative to the control) as NO_3NH_4 concentration increased. The ratio of shoot:root showed higher values with increasing NO_3NH_4 concentration, whereas those was slightly noted with all salinity treatments (Table 1). The results also indicated that shoot growth of tomato seedlings was more response to NO_3NH_4 addition than was root growth.

Table 1. Effect of salinity (NaCl) and nitrogen addition (NO_3NH_4) on fresh weight (FW) and dry weight (DW) (g/plant) of shoot and root in tomato seedlings.

Treatments		Shoot		Root		Shoot:Root ratio
Nitrogen level (mM N)	Salt level (mM NaCl)	FW	DW	FW	DW	DW
15 (NS)	0	14.1[a]	1.59[a]	2.87[a]	0.58[a]	2.78[a]
	20	12.0[b]	1.28[b]	2.10[b]	0.45[b]	2.75[a]
	35	12.3[b]	1.21[b]	1.87[b]	0.38[c]	2.90[a]
	50	11.7[b]	1.61[a]	1.00[c]	0.55[a]	2.95[a]
22.5	0	14.7[a]	1.60[a]	3.50[d]	0.50[ab]	3.25[b]
	20	11.5[b]	1.30[b]	2.86[a]	0.44[b]	3.31[b]
	35	11.2[b]	1.02[c]	2.68[a]	0.28[d]	3.60[c]
	50	10.0[c]	1.31[b]	2.55[a]	0.37[c]	3.70[c]
30	0	16.8[d]	1.70[a]	4.97[e]	0.43[b]	3.96[d]
	20	9.6[c]	1.03[c]	3.73[d]	0.25[d]	4.03[d]
	35	9.5[c]	1.05[c]	3.49[d]	0.24[d]	4.11[d]
	50	6.6[e]	0.96[c]	3.33[d]	0.24[d]	4.30[e]

Each value represents mean of four replicates. Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level. NS: Nutrient solution (control of the nitrogen treatments).

Table 2. Effect of salinity (NaCl) and nitrogen addition (NO_3NH_4) on Specific leaf weight (SLW), Relative water content (RWC) and Chlorophyll Fluorescence (F_v/F_m) in tomato seedlings.

Treatments		SLW (g/cm^2)	RWC (%)	Chlorophyll Fluorescence (F_v/F_m)
Nitrogen level (mM N)	Salt level (mM NaCl)			
15 (NS)	0	0.057[a]	89.0[a]	0.851[a]
	20	0.065[b]	86.5[b]	0.845[a]
	35	0.074[c]	84.8[c]	0.841[a]
	50	0.086[d]	83.1[d]	0.849[a]
22.5	0	0.059[a]	89.6[a]	0.831[a]
	20	0.066[b]	87.0[b]	0.839[a]
	35	0.076[c]	85.0[c]	0.850[a]
	50	0.089[d]	82.5[d]	0.840[a]
30	0	0.057[a]	89.3[a]	0.831[a]
	20	0.068[b]	87.3[b]	0.838[a]
	35	0.077[c]	85.8[c]	0.848[a]
	50	0.088[d]	82.0[d]	0.855[a]

Each value represents mean of four replicates (10 samples of each replicate). Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level. NS: Nutrient solution (control treatment of the nitrogen treatments).

Specific weight of leaf, relative water content, and chlorophyll fluorescence

Specific leaf weight (SLW) was increased in response to the NaCl treatments but not by increasing NO_3NH_4 concentration (Table 2). However, relative water content (RWC) of leaves were reduced with salinized treatments, while there were no significant differences due to more NO_3NH_4 addition compared to control treatment (Table 2). There was not significant differences in the chlorophyll fluorescence parameters, presented in the ratio of F_v /F_m were noted among treatments (Table 2).

Ion concentration

The total nitrogen content (N total) was highly increased in the shoots with increasing NO_3NH_4 concentrations to 40% under fresh water treatment, relative to the control, while it was showed similar accumulation levels at all saline stress treatments (20, 35 and 50 mM of NaCl) (Figure 1). In contrast, N total levels were progressively decreased in the shoots with increasing NO_3NH_4 concentrations to 46% under saline water treatments, relative to the control. However, K^+ content was significantly and progressively decreased in the shoots by about 10% by increasing NO_3NH_4 concentration under higher salt stress treatment (Figure 1). Shoot Na^+ content was obviously increased with salinity and regardless the NO_3NH_4 concentrations, while Na^+ content was reduced by 30-50% in leaves when the NO_3NH_4 concentration increased (Figure 1). Cl^- accumulated in the shoots was not changed with salinity treatments (Figure 1). Under the fresh water treatment (0 mM NaCl), Cl^- content in shoots was significantly decreased by 18 to 25% as the NO_3NH_4 concentration increased. In contrast, the amount of Cl^- was significantly increased with more salinity treatments (by 15 to 30%) with increasing NO_3NH_4 concentrations, relative to the control.

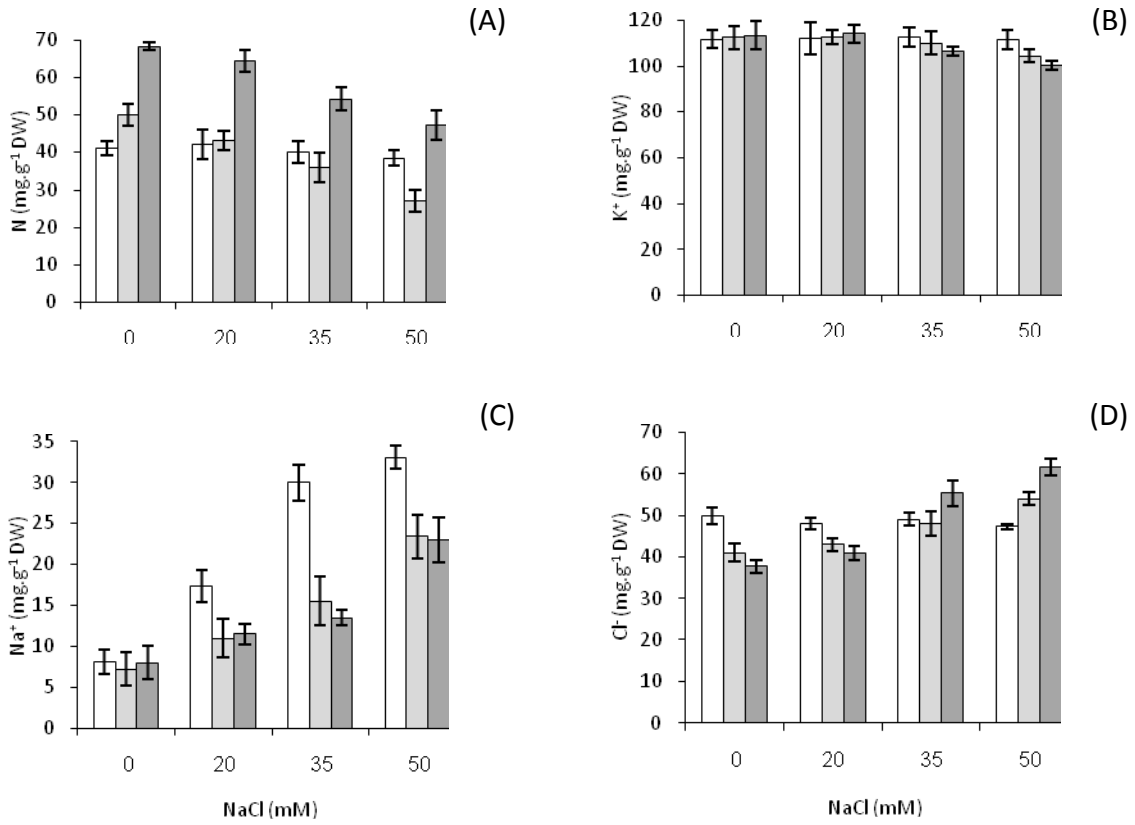

Fig. 1. Effect of salinity (NaCl) and nitrogen addition (NO_3NH_4) on: (A) N total, (B) K^+, (C) Na^+ and (D) Cl^- content ($mg.g^{-1}$ DW) in tomato seedlings. Data are means of four replicates ±SE. Duncan's multiple range test at 5% level. NS: Nutrient solution.

DISCUSSION

Irrigation of glycophytic plants with saline water causes reduced plant growth and can thus have a severe effect on plant development (Debouba et al., 2006). In this experiment on tomato seedlings, symptoms of salt stress were achieved despite using relatively low levels of water salinity (the maximum of EC_e value was about of 5.47 dS m^{-1}). In the previous studies, the decline in growth of plants caused by salinity may be due to three principle mechanisms: osmotic stress, nutritional disruption and ion toxicity (Caines and Shennan, 1999).

The symptoms of salt stress were reflected in reduction of biomass and dry matter of shoots and roots. On the other hand, the results showed that the chlorophyll fluorescence was not the physiological parameter that has correlation with salinity tolerance, as has been noted by Mekkaoui et al. (1989) and Monneveux et al. (1990).

There was an apparently greater detrimental effect of 50 mM NaCl treatment on FW compared to that on DW. The seedlings were showed an osmotic adjustment which reflected in restore the growth (Fageria et al., 2006).

The results were showed a decrease in leaf RWC with increasing NaCl concentrations, thus, to be indicating that shoot tissues were under effect of osmotic stress. In addition, an increased SLW was rather common response in stressed plants and it's positively related to RWC reduction under saline stress condition. It means that less water is taken up by the root and transported into the shoot. Consequently, less water is available for normal growth and development.

Based on the results, a gradually decrease in S:R ratio was found with decreasing supply of water in the plant. The reduction response of S:R ratio by saline stress has been observed previously in glycophyte plants (Maggio et al., 2007). Root plays an important role in plant tolerance to unfavorable conditions, since it is the first organ exposed to salinity stress. On the other hand, the decrease of tomato S:R ratio was due to the higher salt sensitivity of the shoots compared to the roots. It's assumed that the marked shoot sensitivity was related to the higher Na^+ and Cl^- accumulation into their tissues. The decrease of S:R ratio supports the hypothesis that the tomato allocated more DW to roots to maximize capacities for nutrient and water

absorption. This may also indicate that NaCl induced nutritional deficiencies (Shangguan et al., 2004). Zhang (1995) showed that decreasing S:R ratio reduced shoot growth, yield, and water and nutrient use efficiencies.

Undoubtedly, the increase of the added nitrogen in NS should increase plant growth under absence of NaCl. In contrarily, root DW was declined compared to their FW with 0 NaCl treatment, this was widely reported, that plant growth would be oriented to the shoot rather than to the root with increasing of nitrogen fertilization (Gebauer et al., 1987). Also the same effect has been noted on root DW values from the interaction effect between all different levels of salinity and nitrogen treatments.

The results showed that no evidence of added nitrogen to ameliorate plant growth measurements under salinity stress. Therefore, we suppose that the effects of supplementing nitrogen under saline conditions may be appear in other phenomenon, such as the competition between ions.

The range of NaCl concentrations in this study was not affected the N content in leaves. Therefore, the reduction of seedling growth by salinity can be not attributed to N deficiency in tissues of plant. It is possible, that the growth reduction was related to increase of Na^+ and Cl^- levels in tissues under salinity. On the other hand, the supplementing N had a limit effect on the accumulation of Na^+ in leaves under salinity. Many studies were suggested that with other species, competition between NH_4^+ and Na^+ for root uptake sites reduces Na^+ uptake and transport from roots to shoots under NH_4^+ nutrition source, thereby minimizing the Na^+ accumulation in leaves (Sagi et al., 1997; Kant et al., 2007).

The results have suggests that this is also the case with tomato since Na^+ content in leaves was reduced by 50% with the supplemented N in NS compared to NS alone under salinity stress. On the contrary, the supplementing N had a promote effect on the accumulation of Cl^- in the leaves under salinity, accompanied by reduction in the total N content. With respect the competition between ions, Cl^- may inhibit the uptake of NO_3^- into the root and their transport into the shoot through the xylem, eventually leading to decreasing N level in the tissue (Debouba et al., 2006).

However, despite these antagonistic effects between NH_4^+/NO_3^- and Na^+/Cl^- ions, the total N content in leaves was at levels of similar pattern of accumulation in nitrogen-supplemented and none supplemented seedlings under salinity treatments. Much attention has been devoted to understand the adverse effects of NaCl on NH_4^+ and NO_3^- uptake by roots. Similarly finding with barley has been explained that the detrimental effects of salinity can be reduced by partial substitution of NO_3^- with NH_4^+ and that this is due to the power energy cost of N assimilation with NH_4^+ as opposed to NO_3^- nutrition and an increase in total plant N content (Kant et al., 2007). However, the beneficial effect of supplemented nitrogen (by NH_4NO_3) could be contributed to prevent ions toxicity by reducing Na^+ uptake in roots.

There is the competition between Na^+ and K^+ at the intracellular level, as the two ions could be transported by a common protein (Niu et al., 1995). In addition, when Na^+ is absorbed and reaches the plasma membrane, it causes a membrane depolarization and leads to a loss of K^+ (Shabala, 2000). This study showed that lower K^+ content in leaves was not associated with increasing Na^+ accumulation under salinity treatments; this would be expected under the conditions of the experiment of a moderate salinity

condition in root environment. Since, the reduction of K^+ content was only observed when the salinity treatments supplemented by more nitrogen concentration. Obviously, the ions competition between K^+ and NH_4^+ explained the reduction of K^+ content in leaves under supplemented nitrogen treatments, regardless the total N content which favored by NO_3^- nutrition.

Indeed, at 0 NaCl with supplemented nitrogen treatments, leaves showed a negative correlation between Cl^- and total N content, since total N content increased against Cl^- content decreased, whereas, gradually increased in Cl^- content against gradually decrease in total N content under salinity applied by increasing NaCl in nutrient solution. The reduction in seedling growth upon NaCl treatment can be enhanced to an uptake competition between Cl^- and NO_3^-. Similar finding has been reported by Cerezo et al., 1999. On the other hand, the increase of total N content can be attributed to a higher rate supplied of NO_3^- in the rooting medium.

The effects of supplemented nitrogen under salinity by NaCl have been contributed to decrease Na^+ content and in parallel increase Cl^- content in leaf tissue. Although the Na^+ accumulated by lesser amount at high level of salinity under supplemented nitrogen compared to none supplemented nitrogen treatments, but still in higher level compared to without salinity treatment. Salinity tolerance of a plant species depends on its ability to limit Na^+ absorption by the roots and to maintain low levels of Na^+ in the leaves (Tadano, 1983; Yamanoouchi, 1995; Murillo-Amador et al., 2006).

There was no evidence of Cl^- antagonism of NO_3^- uptake, but the supplemented nitrogen treatments led to increase the concentration of NO_3^- in nutrient solution and favor the competence uptake of NO_3^- ions. Lea-Cox

and Syvertsen (1993) reported that NO_3^- uptake was positively correlated with whole plant transpiration. Thus, it appears that reductions in plant growth are more strongly related to reduce water uptake than to Cl^- antagonism from salt stress. The reduction in growth was associated with low RWC under salinity treatments.

Besides the effects of osmotic stress and competition between ions to plant growth, effect of toxicity are caused by the accumulation of Na^+ and Cl^- ions (Bernstein, 1975; Tavakkoli, et al., 2011) which cannot be overlooked in this study.

Mori et al. (2008) reported that nitrogen fertilization could help tomato plants to increase tolerance to salinity. This is not confirmed at high levels of water EC used in this study, but under such conditions the reduction of Na^+ content with supplemented nitrogen (NH_4NO_3) may be functioned to enhance plant tolerance to a moderate salinity. More work at the role of NO_3^- regime will improve understanding of how nitrogen fertilization increase tolerance to NaCl stress in tomato plants.

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الملخص العربي

تأثير التسميد النيتروجيني والملوحة على النمو والصفات الفسيولوجية لشتلات الطماطم

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تم ري شتلات طماطم (*Lycopersicon esculentum* Mill.) صنف Rio Grand النامية في أصص داخل صوبة بلاستيكية بمحلول مغذي مضاف إليه تركيزات مختلفة من نترات الأمونيوم كمصدر للنيتروجين وكلوريد الصوديوم كمصدر للملوحة وذلك لمدة ثلاثة أسابيع. تضمنت المعاملات على ثلاث مستويات من النيتروجين في المحلول المغذي: 15، 22.5 و 30 ملي مولر نيتروجين، وأربع مستويات من الملوحة: 0، 20، 35 و 50 ملي مولر كلوريد الصوديوم. أوضحت النتائج أن الملوحة أدت إلى انخفاض الوزن الجاف ومحتوى الماء النسبي للنباتات في حين أدت إلى زيادة وزن الورقة النوعي. لم تَظهر النتائج أي فروق معنوية في قياسات توهج الكلوروفيل للنباتات المجهدة مقارنةً بالكنترول. إن زيادة مستوى النيتروجين مع الملوحة أدى إلى حدوث انخفاض معنوي في الوزن الجاف للشتلات في حين ازدادت نسبة المجموع الخضري إلى المجموع الجذري. في المقابل أدت الملوحة إلى انخفاض تراكم النيتروجين الكلي وأيون البوتاسيوم في النبات عند إضافة النيتروجين. أيضاً إضافة النيتروجين مع زيادة الملوحة أدى إلى انخفاض أيون الصوديوم وزيادة تراكم أيون الكلور. يظهر أن انخفاض النمو عند المعاملة بكلوريد الصوديوم قد يُعزى إلى التنافس بين أيون الكلور وأيون النترات عند امتصاص النبات لهما مع الأخذ في الاعتبار التأثير السام لكلوريد الصوديوم. بالرغم من أن نتائج القياسات أشارت إلى حدوث تعديل أسموزي مفيد إلا أنه حدث اختزال في النمو. مما سبق تشير نتائج التجربة إلى أن زيادة كمية النيتروجين المضافة أدت إلى تحسين مقدرة شتلات الطماطم على تحمل الإجهاد الناجم عن التأثيرات الثبيطية لكلوريد الصوديوم وذلك عن طريق تخفيض تراكم أيون الصوديوم.

الكلمات المفتاحية: شتلات الطماطم، الملوحة، كلوريد الصوديوم، النيتروجين.