
**INFLUENCE OF NITROGEN ADDITION AND SODIUM CHLORIDE IN
IRRIGATION WATER ON GROWTH OF LETTUCE**

Idress A. Al Gehani* and Niseia B. Mjawer

Department of Plant Production, Faculty of Agriculture, University of Benghazi, Benghazi, Libya.

Tel.: +218 91 4014212; P.O.Box: 17401 Benghazi-Libya.

ABSTRACT

Plants of lettuce (*Lactuca sativa* L. cv Great Lakes 118) were irrigated by different levels of NO_3NH_4 as source of nitrogen and NaCl as source of salinity to investigate the effect of addition nitrogen on plant growth under salinity condition. Three levels of Nitrogen (N): 0, 50 and 100 ppm were prepared from 0, 145 and 285 ppm of NO_3NH_4 . Four levels of Salinity: 500 (fresh water), 700, 900 and 1100 ppm were prepared by adding 0, 200, 400 and 600 ppm of NaCl in fresh water. The leaf numbers (LN), leaf area (LA), specific leaf weight (SLW), head fresh weight (HFW), head dry weight (HDW) and water content of leaves (WC) were measured for each treatments at the end of the experiment. The results indicate that the lettuce used did not appear to be significantly affected by the high level of salinity applied in this experiment. On the other hand, the addition of nitrogen in the form of NO_3NH_4 had a positive effect on the growth of plants under salinity conditions, and appears to prevent the toxicity of the plant from Na^+ and Cl^- . We conclude that lettuce plants have shown a reasonable degree of tolerance to irrigation water salinity when specific levels of nitrogen fertilization are available.

Keywords: *Lactuca sativa* L.; NaCl; Nitrogen; Salinity

INTRODUCTION

Many plants are subjected to various adverse environmental conditions throughout their lifetimes and caused many types of stress. The nonliving components, or abiotic factors, include a variety of naturally occurring chemical compounds and environmental influences. Salinity is one of the most important aspects of the abiotic stress of higher plants, which's 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). Lettuce has been classified as a little tolerance or sensitive plant to salinity (Chenping and Beiquan, 2015), biomass values and photosynthetic rate were decreased as the salinity levels increased with lettuce (Zhilong, *et al.*, 2004).

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 saline soil (Lopez and Satti, 1996). Salinity has been previously shown to be a major factor responsible of low nitrogen availability (Debouba *et al.*, 2006). Addition of nitrogen fertilizer via the soil solution had beneficial effects in plants under salinity conditions (Gimeno *et al.*, 2009). Nitrogen fertilizer not only promotes plant growth, it may also reduce salinity effects (Flores *et al.*, 2001). Nitrate addition lead to increase plant tolerance or avoiding the injurious effects of NaCl stress (Figueira and Caldeira, 2005). 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).

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). Nutrient imbalances may caused by the competition of Na^+ and Cl^- with nutrient ions 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 nitrogen levels by addition NO_3NH_4 in irrigation water to investigate what extent that increase the NaCl tolerance in the lettuce plants, evaluating the effects of saline water on growth and development.

MATERIALS AND METHODS

The experiment was conducted at the Faculty of Agriculture, University of Benghazi, Libya. Seeds of Lettuce (*Lactuca sativa* L. cv Great Lakes 118) were germinated in seedling trays filled by compost. Seedlings were transferred to 3L plastic pots filled by soil after 30 days, in each pot was had three seedlings. The plants were gradually thinned to one plant in each pot. Plants were grown under plastic cover to prevent the rainfall reach to place of experiment, photoperiod was at 10h, and photosynthetic active radiation reached a daytime peak value of $800 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, the temperature and relative humidity ranged to 18/10°C and 65/80% during day/night periods, respectively.

Plants were daily irrigated after two weeks from transplantation by different levels of NO_3NH_4 as source of nitrogen and NaCl as source of salinity (Table 1.). Three levels of Nitrogen (N): 0, 50

and 100 ppm were prepared from 0, 145 and 285 ppm of NO_3NH_4 . Four levels of Salinity: 500 (fresh water), 700, 900 and 1100 ppm were prepared by adding 0, 200, 400 and 600 ppm of NaCl in fresh water. Treatments were continued for four weeks until appearance of leaves burn status on untreated plants.

Table 1. Represent of irrigation water concentrations (ppm) of different combination treatments of NO_3NH_4 and NaCl.

NO_3NH_4	NaCl			
	0	200	400	600
0	500(fresh water)	700	900	1100
145 (50 unit of N)	645	845	1045	1245
285 (100 unit of N)	785	985	1185	1385

Plants were harvested at the end of the experiment; the leaf numbers (LN), leaf area (LA), specific leaf weight (SLW) was determined by measuring the disks weight of leaf (total area of disks was 38 cm^2), head fresh weight (HFW), head dry weight (HDW) and water content of leaves (WC) were measured for each treatment. The WC was calculated as $[(\text{FW}-\text{DW}) \times (\text{FW})^{-1}] \times 100$ according to Morgan (1984).

The study was conducted in three replicates (three plants in each replicate), using factorial experimental 3×4 in completely randomized design (CRD), with the treatments of nitrogen and salinity. 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

There is no significant difference among treatments on the values of leaf numbers (LN) (Table 2.). Leaf area (LA) was not affected by salinity (NaCl) treatments, but it was increased in response to the both nitrogen supplement treatments compared with other treatments, on the other hand LA values were increased by nitrogen treatments in presence of NaCl treatments more than values in NaCl treatments alone (Table 2.). However, specific leaf weight (SLW) was just increased with 600 ppm of salinity and it was more increased in response to the both nitrogen treatments, but the NaCl treatments with nitrogen have no significant effect on SLW with respect exception that gives significant increase compared with 0 nitrogen treatment (Table 2.).

Table 2. Effect of addition the salinity (NaCl) and the nitrogen (NO₃NH₄) with irrigation water on leaf number (LN), leaf area (LA) and specific leaf weight (SLW) of lettuce plants.

Concentrations (ppm)		Measurements		
NO ₃ NH ₄	NaCl	LN	LA (m ²)	SLW (g/38cm ²)
0	0	10.3 ^a	0.22 ^a	1.38 ^a
	200	9.6 ^a	0.24 ^{ab}	1.44 ^{ab}
	400	10.0 ^a	0.26 ^{abc}	1.50 ^{ab}
	600	9.2 ^a	0.22 ^a	1.58 ^{bc}
145	0	9.4 ^a	0.25 ^{abc}	1.73 ^{cd}
	200	11.1 ^a	0.28 ^{bcde}	2.17 ^g
	400	10.4 ^a	0.27 ^{bcd}	2.04 ^{fg}
	600	11.3 ^a	0.30 ^{cdef}	1.97 ^{ef}
285	0	11.1 ^a	0.41 ^g	1.85 ^{def}
	200	9.9 ^a	0.32 ^{ef}	1.82 ^{de}
	400	11.7 ^a	0.31 ^{def}	1.81 ^{de}
	600	11.0 ^a	0.33 ^f	1.92 ^{def}

Each value represents mean of three replicates (3 plants in each replicate). Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level. Control treatment was irrigated by fresh water (500 ppm).

The treatments of salinity by NaCl have no effect on head fresh weight (HFW) of lettuce, whereas nitrogen treatments lead to obviously increase in heads which reached to 100% at 285 ppm NO₃NH₄ treatment (Table 3.), there is significant effect in the interaction between salinity and nitrogen treatments on HFW compared with no addition of nitrogen, this effect was positive toward increasing heads growth (Table 3). The salinity treatments had no significant difference on values of head dry weight (HDW), while nitrogen treatment with 285 ppm was increasing HDW value about 50% compared to control treatment (Table 3.). Significant effects have been appeared at interaction between salinity and both levels of nitrogen treatments, such the increasing values of HDW were accompanied with upgraded of salinity (NaCl) concentrations in both nitrogen treatments (Table 3.). There is no significant difference among treatments of salinity on the values of water contents (WC) either from nitrogen treatments, whereas the

interaction effect of both treatments was clear in maximal value of WC (up to 97%) at level of 600 ppm NaCl and both levels of nitrogen treatments (Table 3).

Table 3. Effect of addition the salinity (NaCl) and the nitrogen (NO₃NH₄) with irrigation water on head fresh weight (HFW), head dry weight (HDW) and water content (WC) of lettuce plants.

Concentrations (ppm)		Measurements		
NO ₃ NH ₄	NaCl	HFW (g)	HDW (g)	WC (%)
0	0	169 ^a	10.2 ^a	93.4 ^a
	200	171 ^a	11.3 ^b	93.2 ^a
	400	172 ^a	11.0 ^{ab}	93.0 ^a
	600	159 ^a	11.2 ^{ab}	93.1 ^a
145	0	220 ^b	10.8 ^{ab}	93.2 ^a
	200	239 ^{bc}	12.6 ^c	95.1 ^b
	400	258 ^{cd}	14.4 ^d	96.7 ^c
	600	248 ^c	16.8 ^{ef}	97.0 ^c
285	0	341 ^c	16.0 ^e	93.3 ^a
	200	251 ^{cd}	16.5 ^{ef}	95.2 ^b
	400	253 ^{cd}	18.3 ^g	94.8 ^b
	600	266 ^d	17.3 ^f	97.3 ^c

Each value represents mean of three replicates (3 plants in each replicate). Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level. Control treatment was irrigated by fresh water (500 ppm).

DISCUSSION

The results presented in Table 2 and 3 indicate that salinity levels of NaCl used in this experiment did not affect the growth measurements presented with one exception at the highest salinity level treatment (600 ppm), which led to an increase in the SLW compared to other levels. It is evident from the results that the variety of lettuce used show salinity tolerance reached 1100

ppm as mention by Chenping and BeiQuan (2015), and also has a high degree of tolerance to the toxic effect of increased levels of ions of Cl^- and Na^+ to 600 ppm NaCl. On the other hand, an increase in the value of SLW at the highest salinity level indicates the susceptibility of plants to specific changes at the leaf morphology aspect, which is interpreted into increasing the thickness of the leaf (Al Gehani and Ismail, 2016) (Table 2). Fageria *et al.* (2006) pointed out that the plant reduces the amount of water lost in transpiration by increasing the thickness of the cuticle and by consequence compensating the deficit in water absorbed by root, because of the high water potential caused by increasing the level of salinity in the soil, also to the possibility of an osmotic adjusting.

The results show that the addition of nitrogen in the form of NH_4NO_3 has increased the growth of the plant in various aspects. We observed the increase in the values of different measurements such as the LA, SLW, HFW and HDW of plants (Table 2. and 3.) as indicated by many researchers that the increase in nitrogen lead to increased plant growth regardless of the level of salinity in the soil (Maas, 1990). In a different way, the addition of nitrogen did not cause a change in the WC of the plant (Table 3.), which means that an increase in growth led to an increase in plant size while maintaining the same water ratio for biomass. It is obviously that the addition of nitrogen to salt water has led to an increase in the HDW and WC (Table 2.) as well as some cases in SLW (Table 3.).

The presence of nitrogen in the form of NH_4^+ and NO_3^- may have a positive effect in increasing growth on the one hand and reducing the effect of toxicity on the other. The researchers often pointed out that the plants that feed on nitrogen in the form of NO_3^- give greater growth than which feeds on other forms, We found an application of this theory in our experiment where the lettuce plant showed good growth measurements with this type of fertilization making it more able to resist with some conditions such as abiotic stresses, such saline stress. Also, there can be a competition between ions when the root absorption of the soil solution between Na^+ and NH_4^+ on the one hand and Cl^- and NO_3^- on the other. In this regard, Grattan and Grieve (1992), Cerezo *et al.* (1999) pointed to the effect of absorption of NO_3^- due to increased Cl^- ions. This competition reduces the amount of decomposing toxic ions of NaCl from the mass flow to the plant tissues and is partially replaced by dissolved ions of NH_4NO_3 (Cant *et al.*, 2007). Increasing plant growth under salinity conditions will undoubtedly increase its salinity tolerance and become less sensitive to the toxicity effect of ions.

In this experiment, the increase of nitrogen in the form of NO_3^- for plants growing under certain levels of salinity had an effect on increased growth in the form of dry matter accumulation (HDW) (Table 3.), also because the competition between NH_4^+ and Na^+ has a role in reducing toxicity. Consequently, we can understand that a cumulative effect of both nitrogen and salinity factors toward improving plant tolerance have emerged. It is important to consider with interest the possibility of increasing the salinity level to a higher limit than what has been applied in this experiment, in order to determine the result of the overlap of the addition of nitrogen to irrigation water that may be more saline, and in a similar way with other varieties of lettuce more diverse.

CONCLUSIONS

The positive effect of increasing growth due to the addition of nitrogen in the form of NH_4NO_3 under the conditions of increasing the level of salinity in the form of NaCl would be an interesting achievement in addressing the problem of salinity. This could be the emergence of a strategy to mitigate undesirable impacts on different crops such as growing lettuce under specific environments, this effort will also be an attempt to develop a reasonable solution to overcome the problem of salinity and the consequent damage to the quantity and quality of crops.

ACKNOWLEDGEMENTS

The authors thank Salim Saaed for assistance in the field and laboratory, Dr. Saaed Helmi for analysis of the samples.

REFERENCES

1. Al Gehani, I.A. and Ismail, T. M., (2016). Effect of Soil Amendment on Growth and Physiological Processes of Rocket (*Eruca sativa* L.) Grown Under Salinity Conditions. *Aust. J. Basic & Appl. Sci.*, 10(1): 15-20, 2016
2. Cerezo, M., Garcia-Agustin, P., Primo-Millo, E., (1999). Influence of Chloride and Transpiration on Net NO_3^- Uptake Rate by *Citrus* Roots. *Annals of Botany*. 84, 117-120.
3. Chenping, X. and Beiqun, M., (2015). Evaluation of lettuce genotypes for salinity tolerance. *HortScience* 50(10):1441–1446.
4. Cuartero, J. and R. Fernandez-Munoz, (1999). Tomato and salinity . *Sci. Hortic.*, 78: 83-125.
5. Debouba, M., Gouia, H., Suzuki, A. and Ghorbel, M.H., (2006). NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato "*Lycopersicon esculentum*" seedlings. *J. Plant Physiol.*, 163: 1247-1258.
6. Fageria, N.K., Baligar, V.C., Clark, R.B., (2006). *Physiology of crop production*. Haworth Press, Inc. p. 171-174.
7. Figueira, E.M. and Caldeira, G.C., (2005). Effect of nitrogen nutrition on salt tolerance of *Pisum sativum* during vegetative growth. *J. Plant Nutr. Soil Sci.* 168:359-363.
8. Flores, P., Carvajal, M., Cerda, A., Martinez, V., (2001). Salinity and ammonium/nitrate interactions on tomato plant development, nutrition, and metabolites. *J. Plant Nutr.* 24, 1561-1573.
9. Gimeno, V., Syvertsen, J.P., Nieves, M., Simon, I., Martinez, V., Garcia-Sanchez, F., (2009). Additional nitrogen fertilization affects salt tolerance of lemon trees on different rootstocks. *Sci. Hortic.* 121, 298-305.

10. Grattan, S.R. and Grieve, C.M., (1992). Mineral element acquisition and growth response of plants grown in saline environments. *Agri., Ecosy. & Enviro.* 38(4):275-300.
11. Hajer, A.S., Malibari, A.A., Al-Zahrani, H.S., Almaghrabi, O.A., (2006). Responses of three tomato cultivars to sea water salinity. 1. Effect of salinity on the seedling growth. *Afr. J. Biotechnol.* 5(10), 855-861.
12. Kant, S., Kant, P., Lips, H., Barak, S., (2007). Partial substitution of NO_3^- by NH_4^+ fertilization increases ammonium assimilating enzyme activities and reduces the deleterious effects of salinity on the growth of barley. *J. Plant Physiol.* 164, 303-311.
13. Lea-Cox, J.D. and Syvertsen, J.P., (1993). Salinity reduces water use and nitrate-N-use efficiency of citrus. *Annals of Botany.* 72, 47-54.
14. Little, T.M. and Hills, F.J., (1978). *Agricultural experimentation design and analysis.* John Wiley and Sons. USA.
15. Lopez, M.V. and Satti, S.M.E. (1996). Calcium and potassium-enhanced growth and yield of tomato under sodium chloride stress. *Plant Sci.* 114:19-27.
16. Maas, E.V., (1990) Crop salt tolerance, *Agricultural Salinity Assessment and Management*, (K.K. Tanji, ed.), Am. Soc. Civil Eng. ASCE Manuals and reports on Engineering Practice No. 71, ASCE, New York, pp. 262-304.
17. Maggio, A., De Pascale, S., Angelino, G., Ruggiero, C., Barbieri, G., (2004). Physiological response of tomato to saline irrigation in long-term salinized soils. *Eur. J. Agron.* 21, 149-159.
18. Marschner, H., (1995). Functions of mineral nutrients: macronutrients. In: *Mineral nutrition of higher plants.* 2nd ed. San Diego, CA, USA: Academic Press. p. 229-299.
19. Morgan, J.M., (1984). Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol.* 35, 299-319.
20. Romero-Aranda, R., Moya, J.L., Tadeo, F.R., Legaz, F., Primo-Millo, E., Talon, M., (1998). Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. *Plant Cell Environ.* 21, 1243-1253.
21. Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy P., McDonald, G.K., (2011). Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. *J. Exp. Bot.* 62 (6), 2189-2203.
22. Zhilong, B.; Tadashi, I.; Yutaka, S. (2004). Effects of sodium sulfate and sodium bicarbonate on the growth, gas exchange and mineral composition of lettuce. *Sci. Horti.* 99:215-224.