American Journal of

Plant Physiology

ISSN 1557-4539

Academic
Journals Inc.

www.academicjournals.com

American Journal of Plant Physiology 8 (1): 32-40, 2013
ISSN 1557-4539 / DOI: 10.3923/ajpp.2013.32.40

Nutritional Status and Growth of Wheat Plants Grown under Salinity Stress Conditions as Responded to Different Nitrogen Sources

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ABSTRACT

Nitrogen fertilization under salinity stress is of main concern to be investigated, especially in the arid and semiarid regions. Pot experiment was conducted in the greenhouse of the "Micronutrient Project", Fertilization Technology Department, National Research Centre, Dokki, Cairo, Egypt with Wheat (*Triticum aestivum* L. var. Gemieza9). Plants were grown on sandy soil under salinity stress to study their response to soil application of different nitrogen sources compared to those irrigated with tap water. Nitrogen fertilization was added to the pots in the rate of 168 kg N ha^{-1} as urea, ammonium nitrate, calcium nitrate or ammonium sulfate. Fifteen days after sowing, half of the pots of every treatment were irrigated regularly with tap water (S1) and the other half was irrigated with saline water (S2). Results showed that salinity stress of irrigation water significantly inhibited growth parameters in terms of plant height, fresh weight and dry weight accumulation. Nitrogen form significantly interacted with salinity stress of irrigation water. The highest reduction in all determined parameters was recorded with urea, while very slight or none with calcium nitrate. Macro- and micro-nutrient concentration and uptake by wheat shoots were significantly affected by salinity stress of irrigation water. The best uptake of almost all determined nutrients was achieved by calcium nitrate fertilizer, especially under salinity stress conditions. Thus among the used N- forms, calcium nitrate is the best nitrogen form can alleviate the harmful effects of saline ions and realizing good plant growth under salinity stress of irrigation water.

Key words: Wheat cultivation, growth parameters, nutritional status, abiotic stress, nitrogen forms

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important commodity in the Egyptian food market. Although wheat production in Egypt increased to 8.4 million tons in the year 2011, it is still insufficient for the population needs (FAO, 2011). Using of brackish water (3-8 dS m^{-1}) in irrigation was increased in the last decade, especially in the new reclaimed areas. Land use statistics showed that about 25% of the irrigated land in the arid and semiarid regions is salinity affect, resulting in substantially or partially unproductive lands. Salinity affects plant growth in three ways including accumulation of ions to toxic levels, shortage in water uptake and reduction of nutrient availability (Ashraf, 1994). Only few plant species can develop mechanisms to coop with salinity stress effects (Munns, 2002).

Most of studies on N-deficient soils showed that addition of N improved growth and/or yield of barley (Dregne and Mojallali, 1969) and wheat (Nathawat *et al.*, 2005) when the degree of salinity was not severe. Some of these studies, however, suggested that applied N decreased plant salt tolerance. Other studies showed an increase in crop yield under saline conditions when N was applied above optimal level (Ravikovitch and Yoles, 1971a, b; Selassie and Wagenct, 1981; Akram *et al.*, 2010) suggesting that additional N increased the salt tolerance. The form of N uptake found also to influence growth. Application of mixed NO_3^-/NH_4^+ was reported to produce higher yields under normal and saline conditions (Botella *et al.*, 1997; Drihem and Pilbeam, 2002).

This study aims at studying response of wheat plants grown under salinity stress of irrigation water to different nitrogen sources compared to plants irrigated with fresh water.

MATERIALS AND METHODS

Pot experiment was conducted in the greenhouse of the "Micronutrient Project", Fertilization Technology Department, National Research Centre, Dokki, Cairo, Egypt with Wheat (*Triticum aestivum* L. var. Gemieza9) grown on sandy soil. The main object of the study was to investigate response of the plants grown under salinity stress of irrigation water to soil application of different nitrogen sources compared to those irrigated with tap water. Physical and chemical characteristics of the used soil are shown in Table 1.

The experiment followed the Randomized Complete Block Design (RCBD) with four treatments in three replicates. The plants were sown on Mid-November, 2011 in Mitscherlich pots contained 7.0 kg sandy soil. Before sowing, each pot received 1.40 g super mono-phosphate (15.5% P_2O_5) and 0.35 g potassium sulphate (48-50% K_2O) as basic fertilization. At the seedling age, the plants were thinned to leave 6 plants per pot.

Treatments: Nitrogen fertilization were added to the pots in the rate of 168 kg N ha^{-1}, which equal 1.05 g pot^{-1} urea, 1.47 g pot^{-1} ammonium nitrate 3.15 g pot^{-1} calcium nitrate and 2.38 g pot^{-1} ammonium sulfate. Fifteen days after sowing, half of the pots of every treatment were irrigated

Table 1: Chemical and physical characteristics of the used soil

Physical characteristics	Nutrient concentration
pH	8.6^{VH}
EC (dS m^{-1})	1.23^{L}
$CaCO_3$ (%)	1.38^{L}
Organic matter (%)	0.98^{VL}
Sand (%)	82.8
Silt (%)	8.1
Clay (%)	9.1
Texture sand	Exchangeable macronutrients (mg 100 g^{-1} soil)
P	1.2^{M}
K	9.4^{VL}
Ca	151^{M}
Mg	9.3^{VL}
Available micronutrients (mg kg^{-1} soil)	
Fe	3.1^{VL}
Mn	6.2^{L}
Zn	1.3^{L}
Cu	0.5^{L}

EC: Electric conductivity of the soil solution, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Fe: Iron, Mn: Manganese, Zn: Zinc, Cu: copper, ^{VH}Very high, ^{H}High, ^{M}Medium, ^{L}Low, ^{VLL}Very low (Ankerman and Large, 1974) regularly with tap water (S1) and the other half were irrigated with saline water (S2). The used saline water was a mixture of sea and fresh water, Electric Conductivity (EC) = 3.0 dS m^{-1}.

Harvest: Three months after sowing, the plants were harvested to measure plant height and determine fresh and dry weights. The plant tissues, then analyzed for nutrient concentrations and nutrient uptake was calculated.

Determinations and measurements

Soil: A representative soil sample before fertilization was air-dried and passed through 2-mm sieve pores. Soil fractions were determined using the hydrometer method (Bauyoucos, 1954). E.C. and pH were determined in a soil/water extract (1:2.5) according to Jackson (1973). The $CaCO_3$ content was determined using the calcimeter method according to Black (1965). Organic matter was determined using the potassium dichromate method according to Walkely and Black (1934). Soil P was extracted using sodium bicarbonate ($NaHCO_3$) (Olsen *et al.*, 1954). Magnesium and potassium were extracted using ammonium acetate ($C_3H_3O_2NH_4$) (Chapman and Pratt, 1978). Chloride was water extracted. Iron, Mn, Zn and Cu were extracted using DTPA-solution (Lindsay and Norvell, 1978). Soil sample was also taken from the experimental units after plant harvest to determine soil salinity and the concentrations of alkaline ions Na, Cl, Mg and Ca.

Vegetative tissue: Arial parts of wheat plants were washed with tap water, 0.01 N HCl and bi-distilled water, respectively, dried at 70°C for 24 h, weighed and ground. A part of the dry material was wet-digested according to the method of Chapman and Pratt (1978).

Nutrient measurements: Nitrogen was determined using Micro-Kjeldahl method digestion and titration method (Ma and Zuazage, 1942). Phosphorus was photometrical determined using the molybdate-vanadate method. Potassium was measured using Dr. Lang -M8D Flame-photometer. Chloride was determined using potassium chromate (K_2CrO_4) and silver nitrate ($AgNO_3$) according to Richards (1954). Magnesium, Fe, Mn, Zn and Cu were determined using the Atomic Absorption Spectrophotometer (Perkin-Elmer 100 B).

Evaluation of the nutrient status: Soil nutrient concentrations were evaluated according to the tentative values of Ankerman and Large (1974).

Statistical analysis: Data were subjected to statistical analysis as specified by Snedecor and Cochran (1990). Treatment means were calculated and subjected to the two-ways ANOVA analysis and Student-Newman Keuls (SNK) and LSD ($p = 0.05$) tests-multiple comparison of means, using Costate 2 Program (Cohort software) for different treatments.

RESULTS AND DISCUSSION

Effect of N-source on nutrient concentrations and uptake: Macronutrient concentration (Table 2) and uptake (Table 3) by wheat shoots were significantly affected by salinity stress of irrigation water. Nitrogen, P, K, Ca and Mg concentrations and uptake were reduced, while Na and Cl uptake and concentrations were increased as the plants irrigated with saline water. Similar results were found by Alyemeni (1997) with *Vigna ambacensis*. The same trend was also found with micronutrients. Iron, Mn, Zn and Cu concentrations (Table 4) and uptake (Table 5) were also

Table 2: Macronutrients concentration in wheat shoot tissues as affected by salinity of irrigation water and application of different N-sources

	Dry weight basis (%)								
	Nitrogen			Phosphorus			Potassium		
Treatment	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Urea	2.22	1.89	2.06	0.24	0.21	0.23	2.87	2.50	2.69
Ammonium nitrate	2.96	1.91	2.44	0.22	0.21	0.22	2.70	2.77	2.74
Calcium nitrate	1.80	1.82	1.81	0.20	0.20	0.20	2.67	2.46	2.56
Ammonium sulfate	2.15	1.94	2.05	0.33	0.21	0.28	2.70	2.36	2.53
Mean	2.28	1.89	-	0.25	0.21	-	2.74	2.52	-
LSD $_{5\%}$									
Salinity	0.04			0.01			0.08		
N source	0.06			0.01			0.11		
Salinity X N-source	0.08			0.02			0.16		

	Dry weight basis (%)											
	Magnesium			Calcium			Sodium			Chloride		
Treatment	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Urea	0.248	0.235	0.242	0.67	0.62	0.65	0.364	0.614	0.489	0.893	1.380	1.137
Ammonium nitrate	0.272	0.252	0.262	0.66	0.61	0.64	0.538	0.750	0.644	0.790	1.210	1.000
Calcium nitrate	0.238	0.225	0.232	0.73	0.75	0.74	0.431	0.536	0.484	1.167	0.960	1.064
Ammonium sulfate	0.239	0.233	0.236	0.70	0.72	0.71	0.461	0.611	0.536	0.760	0.783	0.772
Mean	0.249	0.236	-	0.69	0.68	-	0.449	0.628	-	0.903	1.083	-
LSD $_{5\%}$												
Salinity	0.003			0.01			0.015			0.056		
N source	0.005			0.02			0.021			0.079		
Salinity X N-source	0.007			0.03			0.030			0.112		

S1: Fresh irrigation water, S2: Saline irrigation water

reduced as the plants subjected to salinity stress of irrigation water. Concentration reduction was 12, 8, 12.8 and 9.7% for iron, manganese, zinc and copper, respectively. As affected by salinity stress of irrigation water, iron uptake was also reduced by 24.4% while uptake of manganese, zinc and copper were reduced by 18, 18.1 and 17%, respectively. Salinity effect on the reduction of nutrient concentrations can be attributed to the increase of the root osmotic pressure due to saline ions. Seawater found to cause noticeable increase in osmotic adjustment, organic solutes such as Total Soluble Solids (TSS), Total Soluble Nitrogen (TSN), proline, organic acids and glycerol (Aldesuquy *et al.*, 2012) which may limit the uptake and translocation of nutrients within the plant tissues.

The best N-source achieved the higher concentrations of the basic fertilizers (NPK) was ammonium nitrate which recorded 2.44 mg for N, 0.22 mg for p and 2.74 mg for K (Table 2). However, the best uptake of almost all determined nutrients was achieved by calcium nitrate fertilizer, especially under salinity stress of irrigation water, where N, P and K-uptake reached 97.7, 10.8 and 132.7 mg kg^{-1} D.W, respectively compared to lesser values for the other forms (Table 3). Data analysis of the interaction between N-source and salinity stress of irrigation water on nutrient concentrations and uptake showed significant effect of the form of N-fertilizer on nutrient concentrations and uptake of both macronutrients (Table 2, 3) and micronutrients

Table 3: Macronutrient uptake by wheat shoot tissues as affected by salinity of irrigation water and application of different N-sources

	Dry weight basis (mg kg^{-1})								
	Nitrogen			Phosphorus			Potassium		
Treatment	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Urea	93.9	58.3	76.1	10.0	6.4	8.2	121.1	76.8	99.0
Ammonium nitrate	115.5	69.2	92.4	9.3	7.6	8.5	116.1	100.2	108.2
Calcium nitrate	96.3	97.7	97.0	10.9	10.8	10.9	142.6	132.7	137.7
Ammonium sulfate	83.9	60.8	76.9	12.9	7.3	10.1	105.4	83.9	94.7
Mean	97.4	73.8	-	10.8	8.0	-	121.3	98.4	-
LSD $_{5\%}$									
Salinity	2.5			0.4			4.5		
N- source	3.5			0.6			6.3		
Salinity X N-source	5.0			0.9			9.0		

	Dry weight basis (mg kg^{-1})											
	Magnesium			Calcium			Sodium			Chloride		
Treatment	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Urea	10.5	7.2	8.9	28.2	20.4	24.3	16.6	18.9	17.8	37.7	42.4	40.1
Ammonium nitrate	11.7	9.1	10.4	27.0	22.1	24.6	23.2	27.2	25.2	33.9	43.8	38.9
Calcium nitrate	12.8	12.1	12.5	39.4	40.5	40.0	23.1	28.8	26.0	62.5	51.7	57.1
Ammonium sulfate	9.3	8.2	8.8	27.5	25.5	26.5	18.0	21.4	19.7	29.6	27.8	28.7
Mean	11.1	9.2	-	30.5	27.1	-	20.2	24.1	-	40.9	41.4	-
LSD$_{5\%}$												
Salinity	0.3			1.6			1.3			NS		
N- source	0.5			2.2			1.8			4.0		
Salinity X N-source	0.7			3.1			2.6			5.6		

S1: Fresh irrigation water, S2: Saline irrigation water

Table 4: Micronutrient concentration in wheat shoot tissues as affected by salinity of irrigation water and application of different N-sources

	Dry weight basis (mg kg^{-1})											
	Iron			Manganese			Zinc			Copper		
Treatment	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Urea	126	108	117	27	24	26	72	59	66	9	7	8.0
Ammonium nitrate	104	88	96	24	23	24	74	65	70	10	9	9.5
Calcium nitrate	77	72	75	22	22	22	67	65	66	11	11	11.0
Ammonium sulfate	89	78	84	27	24	26	74	61	66	11	10	10.5
Mean	99	87	-	25	23	-	72	63	-	10.3	9.3	-
Mean reduction (%)	12			8			12.8			9.7		
LSD$_{5\%}$												
Salinity	3			1			2			0.4		
N source	4			1			NS			0.5		
Salinity X N source	5			NS			4			0.7		

S1: Fresh irrigation water, S2: Saline irrigation water, NS: Not significant

(Table 4, 5) under salinity stress of irrigation water. Ammonium nitrate fertilizer caused the highest reduction of N-concentration and uptake by shoot tissues followed by urea and ammonium sulphate, while there was no reduction with calcium nitrate fertilizer. Phosphorus, k, Ca and Mg showed undistinguished behaviors with different N-forms. However, sodium and chloride concentrations and uptake were lowest with calcium nitrate fertilizer compared to other N-forms. The lowest reduction of micronutrients concentrations and uptake was also achieved by calcium nitrate fertilizer, while other N-forms showed different reductions in micronutrients concentrations and uptake. Irshad *et al.* (2002) reported that the concentration of cation was higher in nitrate-treated plants than in other forms of N and ammonium-N and urea-N tended to inhibit the uptake of cations compared to nitrate-N under saline conditions. On the other hand, Hawkins and Lewis (1993) concluded that Ca can partially ameliorate NaCl mediated inhibition of NO_3 uptake by the wheat tissues.

Effect of N-source on growth parameters: Salinity stress of irrigation water significantly inhibited growth parameters in terms of plant height (Fig. 1), fresh weight (Fig. 2) and dry weight accumulation (Fig. 3). Botella *et al.* (1997) reported that salinity decreased dry matter production

Table 5: Micronutrient uptake by wheat shoot tissues as affected by salinity of irrigation water and application of different N-sources

	Dry weight basis (μg kg^{-1})											
	Iron			Manganese			Zinc			Copper		
Treatment	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Urea	534	332	433	114	75	95	304	182	243	38	21	30
Ammonium nitrate	449	318	384	103	85	95	517	237	277	47	33	40
Calcium nitrate	414	388	401	118	117	118	294	351	323	57	61	59
Ammonium sulfate	349	281	315	107	86	97	290	215	253	44	37	41
Mean	437	330	-	111	91	-	301	246	-	47	39	-
Mean reduction %	24.4			18			18.1			17		
$LSD_{5\%}$												
Salinity	17			5			22			2		
N-source	24			7			31			4		
Salinity X N	34			10			44			5		

S1: Fresh irrigation water, S2: Saline irrigation water

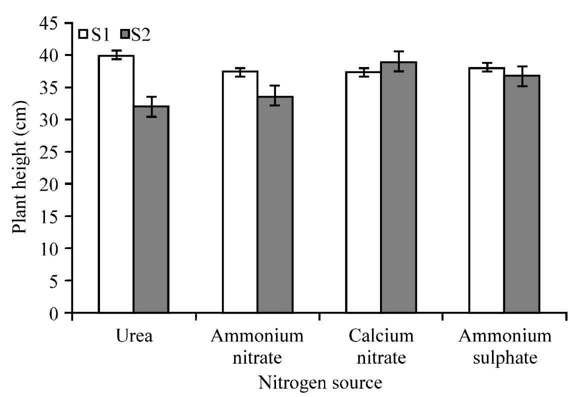

Fig. 1: Wheat plant height (cm) as affected by salinity of irrigation water and application of different N-sources, $LSD_{0.05}$, Salinity = 0.9, N-source = 1.2, Salinity x N-source = 1.72

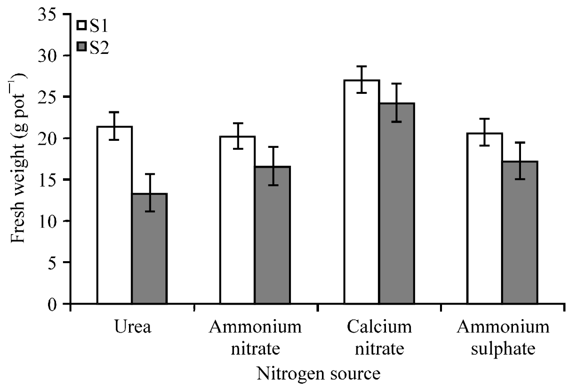

Fig. 2: Fresh weight of wheat plants as affected by salinity of irrigation water and application of different N-sources, $LSD_{0.05}$, Salinity = 0.6, N-source = 0.9, Salinity x N-source = 1.27

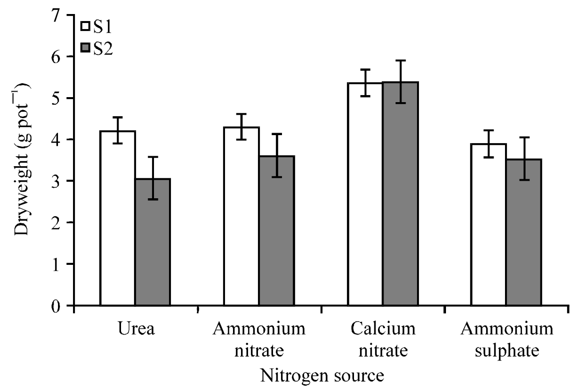

Fig. 3: Dry weight of wheat plants as affected by salinity of irrigation water and application of different N-sources, $LSD_{0.05}$, Salinity = 0.11, N-source = 0.15, Salinity x N-source = 0.21

of shoot and root of wheat seedlings grown under controlled conditions in a growth chamber. Irshad *et al.* (2002) concluded that salinity decreased dry matter production of shoot and root of wheat plants grown under medium or high salinity stress.

N-form significantly affected wheat plant height (Fig. 1). The tallest plants were achieved by urea using fresh water in irrigation (average of 39 cm), while calcium nitrate was the best achieved an average pant height of 37 cm using saline irrigation water. On the other hand, fresh and dry weights (Fig. 2, 3, respectively) of wheat plants were significantly affected by nitrogen source. Using saline water in irrigation, the highest fresh and dry weights were achieved by calcium nitrate form. Fresh weight achieved by calcium nitrate was 24.3 g pot^{-1} compared to 13.4, 16.7 and 17.3 g pot^{-1} achieved by urea, ammonium nitrate and ammonium sulphate, respectively. In the mean time, dry weight achieved by calcium nitrate under saline irrigation water stress was 5.4 g pot^{-1} compared to 4.2 g pot^{-1} achieved by urea, 3.6 g pot^{-1} by ammonium nitrate and 3.5 g pot^{-1} by ammonium sulphate. Nitrogen form was also observed to significantly interact with salinity stress of irrigation water regarding growth parameters. The highest reduction in all determined parameters was recorded with urea, while very slight or none with calcium nitrate form. Lewis *et al.* (1989) reported that in nitrate-fed wheat, raising the calcium concentration from 2 to 12 mM in the presence of 60 mM salinity produced 11% increase in growth. He attributed this effect to the improvement of nitrate uptake due to calcium protection of the nitrate transporter which was not evident in ammonium-grown wheat. Houdusse *et al.* (2008) reported that the beneficial action of NO_3^- alleviating the negative effects of NH_4^+ nutrition on plant growth seems to be mediated by a complementary action. He suggested that this action decrease the pool of total putrescine within the plant and increase putrescine conjugation, which accompanied by a decrease in the value of the free putrescine ratio associated with a better plant growth under saline stress conditions. Nathawat *et al.* (2005) declared that the activity of nitrate-assimilating enzymes (nitrate reductase and nitrite reductase) was maximum with nitrate fertilizer and minimum with the ammoniac form in the Indian mustard (*Brassica juncea* L.) irrigated with saline water.

CONCLUSION

From the present study, it can be concluded that medium salinity of irrigation water can restrict nutrient uptake by wheat roots and consequently lower their concentrations in the shoot tissues and this followed by a growth retardant. The form of nitrogen feeding is the most important to determine nutrient uptake and growth under salinity stress conditions. According to the results of this study, among the common nitrogen fertilizers (urea, ammonium nitrate, ammonium sulphates and calcium nitrate), calcium nitrate is the best nitrogen form can alleviate the harmful effects of saline ions and realizing good plant growth under salinity stress of irrigation water.

ACKNOWLEDGMENTS

This study was conducted as a part of the Egypt-German Project “Micronutrients and Other Plant Nutrition Problems” executed by the National Research Centre (NRC), Fertilization Technology Department (Coordinator, Prof. Dr. M.M. El-Fouly) and the Institute for Plant Nutrition, Technical University, Munich (Prof. Dr. A. Amberger). The Egyptian Academy of Scientific Research and Technology (ASRT) and the German Federal Ministry of Technical Cooperation (BMZ) through the German Agency for Technical Cooperation (GTZ), supported the project.

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