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Effects of salinity and nitrogen on cotton growth in arid environment

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Abstract The influences of different N fertilization rates and soil salinity levels on the growth and nitrogen uptake of cotton was evaluated with a pot experiment under greenhouse conditions. Results showed that cotton growth measured as plant height was significantly affected by the soil salinity and N-salinity interaction, but not by N alone. Cotton was more sensitive to salinity during the emergence and early growth stages than the later developmental stages. At low to moderate soil salinity, the growth inhibition could be alleviated by fertilizer application. Soil salinity was a dominated factor affecting cotton's above-ground dry mass and root development. Dry mass of seed was reduced by 22%, 52%, and 84% respectively, when the soil salinity level increased from control level of 2.4 dS m^{-1} to 7.7 dS m^{-1}, 12.5 dS m^{-1} and to 17.1 dS m^{-1}, respectively. N uptake increased with N fertilization at adequate rates at both low and medium soil salinities but was not influenced by over N fertilization. At higher salinities, N uptake was independent of N rates and mainly influenced by soil salinity. The uptake of K decreased with soil salinity. The concentration of Na, Cl and Ca in plant tissues increased with soil salinity with highest concentrations in the cotton leaf.

Keywords Soil salinity · Nitrogen · Fertigation · Cotton

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Introduction

Soil salinity adversely affects crop productivity and agricultural sustainability in many areas of the world, especially in arid and semi-arid regions (Mohammed et al. 1998; Feng et al. 2005; Endris and Mohammed 2007). Plant growth can be inhibited by high salt concentrations through osmotic stress, nutritional imbalance, and specific ion toxicity (Alam 1994; Jacoby 1994; Munns and Tester 2008). It is known that the growth inhibition and the adverse effects induced by salinity can be alleviated by proper use of fertilizer and water management, depending on plant species, salinity level, and environmental conditions (Shen et al. 1994; Soliman et al. 1994; Albassam 2001; Flores et al. 2001; Tuna et al. 2007). Nonetheless, over

fertilization with N may contribute to soil salinization and increase the negative effects of soil salinity on plant performance (Villa-Castorena et al. 2003). In addition, the potential for NO_3 leaching may increase where moderate to high amounts of salts are present in the soils because plants under salt stress can not absorb and or utilize the applied N as efficiently as the plants not subjected to salt stress (Ward et al. 1986; Pessarakli and Tucker 1988; Bowman et al. 2006). Further, as the salinity of irrigation water rises, the leaching fraction must increase to control root zone salinity. Higher leaching fractions combined with lower N use efficiencies represent a worst case with regard to groundwater pollution (Devitt and Morris 1990; Leskys et al. 1999). Therefore, judicious fertilizer and water management is essential in salt-affected soils to sustain yields and to minimize the degradation of soil and groundwater.

In the arid Xinjiang province of China, cotton is being grown widely because of its high salinity tolerance. In these regions, water sources are frequently brackish and high-quality water for agricultural purposes is increasingly scarce due to rising demands from urban areas (Wang et al. 2008). As a result, the proportion of crop production under deficit irrigation with poorer quality water is increasing. Drip irrigation is thought to be the most efficient irrigation method (Mohammad 2004). With fertigation (combined irrigation and fertilization), the application of fertilizer can be controlled to match the plant needs at each physiological growth stage, which can enhance plant growth and increase fertilizer and water use efficiency while minimizing environmental pollution.

Plant responses to salinity change with plant age, plant development, and growth stages (Maas 1993). It is important to study plant growth response to N and soil salinity during the whole plant life cycle to reveal whether the amount of N applied alleviates or aggravates the detrimental effects of salinity during a specific growth stages (Villa-Castorena et al. 2003). In addition, examining plant growth during the whole growing season provides information about crop salt tolerance over time. The objective of this work was to determine the influence of different soil salinity levels and N fertilization rates on the cotton growth, including the root development, plant height, and above-ground mass. The uptake of N, K, Ca, Na and Cl were measured to understand the combined effects of N and salinity on cotton growth.

Materials and methods

Experimental design

The experiments were conducted in a greenhouse from May to November at an agricultural experimental station at Shihezi University, Xinjiang, China (86°02′E, 44°18′N). Cotton (*Gossypium hirsutum* L. cv. Xinluzao 13) was grown in plastic pots (height 0.55 m, top diameter 0.48 m, bottom diameter 0.40 m) with a volume of 84 L. The minimum and maximum air temperature was 17°C and 32°C, respectively. The relative humidity ranged from 40–62%. A clay loam soil taken from the station field was passed through a 2-mm sieve and packed in the plastic pots with 0.1 m increments to 0.5 m. Each pot was filled with 96 kg air dry soil. The bulk density of the packed soil was 1.2 g cm^{-3} and the gravimetric water content of the saturated paste of the soil was 45.6%. Selected physical and chemical properties of the soil are presented in Table 1.

The experimental design was a 4×4 factorial with four salinity treatments and four levels of nitrogen. Soil salinity was created with applying NaCl and $CaCl_2$ (1:1 weight ratio) to the soil before the experiment. The resulting ion compositions in the treated soil were similar to those observed in the local saline soils. The soil salinity levels (EC_e, electrical conductivity of a saturated-paste extract) were 2.4, 7.7, 12.5, and 17.1 dS m^{-1}, referred to as non-saline (S0), low (SL), medium (SM), and high (SH) saline, respectively. The low and high salt treatments were set based on the 100% and 50% yield threshold values for cotton, respectively (Maas and Grattan 1999). The amount of N was estimated with a

Table 1 Selected physical and chemical properties of the soil used

Characteristic	Value
Clay (%)	18.4
Silt (%)	40.5
Sand (%)	41.1
EC_e (dS m^{-1})	2.4
pH (H_2O)	7.5
Organic matter (OM, g kg^{-1})	15.3
Total nitrogen (N, g kg^{-1})	1.1
Available phosphorus (Olsen-P, mg kg^{-1})	19.0
Available potassium (K, mg kg^{-1})	194

population density of 221,000 plants ha^{-1} according to common field practices used by local farmers. The N application rates were 0, 135, 270, and 405 kg ha^{-1}, which correspond to 0, 2.65, 5.30, and 7.95 g N per pot in terms of plant population density, referred to as no (N0), low (NL), medium (NM), and high (NH) fertilization. The medium fertilization level was set based on the common field practices used by local farmers. The experiments followed a completely randomized block design with four replications for each treatment. Each block included 16 treatments.

Water was applied through drip irrigation units with a discharge rate of 1.1 L h^{-1}. Drip laterals were installed on the top of the pots, and the emitter was fixed in the centre. Each pot was irrigated by one emitter. Cotton seeds were planted at 5 cm distance from the emitter in each pot on 28 May, and then the top of the pot was covered with a polyethylene film to reduce evaporation. Each pot received 9 L of water to help germination and seedling establishment. At the three-true-leaves stage (32–35 days after planting, DAP), the crop was thinned to four plants per pot, yielding a population of 221,000 plants ha^{-1}. Drip irrigation were implemented from the squaring stage (50 DAP) to the boll opening stage (150 DAP). Fresh water (EC of 0.33 dS m^{-1}) was used for all treatments. Two pots for each treatment were weighed every 2 days to keep the soil water content between 50% and 80% of the field capacity during the growing season. For each pot, the amount of water to be applied was determined by gathering water discharged from the emitter close to the pot. There was no drainage or leaching through the pots.

Triple superphosphate and potassium sulfate were applied as base fertilizers when filling the pots, while nitrogen fertilizer was applied through the drip irrigation system during the cotton growth period. Urea was used as the N source and applied in five equal splits at 50, 65, 78, 90 and 108 DAP, according to common field practices used by local farmers. The fertilizer solution was stored in a plastic container of 100 L volume and pumped into the irrigation system. The experiment was terminated at the boll-opening stage (160 DAP).

Sampling and measurement methods

Plant height was measured weekly during the growth season. The shed (leaf, bud, flower, and boll) were collected daily for each pot. At the end of the experiment, plants were cut at the soil surface and separated into leaf, stem, bur, and seed. Roots were collected after the soil was passed through a 0.5-mm sieve with the aid of a water jet. Debris, weeds and dead roots were sorted by hand from the 'live' roots during washing, based on visual observation that the 'live' roots appeared light in colors (Oikeh et al. 1999; Qu et al. 2003; Ruiz et al. 2005). Each plant component was washed with distilled water, dried in an oven at 70°C for 72 h, and weighed. Dry masses of each component were measured and samples were grounded to pass through a 1-mm sieve. Soil samples were taken with a 0.03 m diameter tube sampler from three randomly chosen replicates of each treatment, at distances of 0−0.05, 0.05−0.1, 0.1−0.15 and more than 0.15 m from the emitter. The soil cores were divided into 0.1 m increments to a depth of 0.5 m, soil cores from the same treatment and depth were pooled, air dried, and a sub-sample was fine-ground with a mortar and pestle.

The gravimetric water content of the soil was measured via drying in an oven at 105°C for 48 h. The EC of soil saturated-paste extract solution was determined with a DDS-308A conductivity meter (Shanghai Precision & Scientific Instrument Inc., Shanghai, China). The plant samples were extracted with concentrated H_2SO_4 + H_2O_2 before element analysis. Potassium and Na were determined by flame emission method (FP640, Shanghai Precision & Scientific Instrument Inc., Shanghai, China), and Ca by atomic absorption spectrophotometry (AA6200, Shimadzu Corp., Kyoto, Japan). Chloride was extracted with boiling water and determined with an Orion ion specific electrode. Nitrogen content was analyzed using an Auto-Kjeldahl Unit (B-339, Buchi Labortechnik AG, Switzerland).

Plant water use was determined by summing water loss from the pots (Martin and Thorsteson 1988). Water use efficiency (WUE) was calculated as total dry mass (aboveground and root) divided by water use. Nitrogen uptake was calculated as the product of N content of each plant component (leaf, stem, bur, seed, shed, and root) and corresponding dry mass. Total N uptake was the sum of all plant parts. The apparent N recovery (ANR, %) of different N application rates under the same salinity treatments was calculated as follows (Zemenchik and Albrecht 2002; Li et al. 2007):

$$ANR(\%) = 100(a - b)/R$$

where a is total N uptake in cotton (g pot^{-1}) receiving N fertilizer; b is total N uptake in non-fertilized cotton (g pot^{-1}); R is the total amount of N applied (g pot^{-1}).

Data analysis

The data were analyzed using the SPSS statistical program (version 11.5, SPSS Inc, 2003) with a two-way ANOVA at a significance level of 0.05, with salinity and nitrogen as the independent variables. A Duncan multiple range test was carried out to determine if significant ($p<0.05$) differences occurred between individual treatments.

Results

Plant height

During the early growth stage (planting to emergence, and to squaring), growth was significantly affected by the soil salinity level. The measured plant heights at 46 DAP decreased in proportion to the soil salinity level (Fig. 1). There was no significant difference ($P<0.05$) between different N treatments. The average plant height decreased from 30.3 cm, to 19.7 cm, 13.8 cm and to 6.0 cm as the soil salinity level

increased from 2.4 dS m^{-1} to 7.7 dS m^{-1}, 12.5 dS m^{-1} and to 17.1 dS m^{-1}, respectively. After the first fertigation (50 DAP), the plants went into a rapid growth period. The differences between treatment of S0, SL and SM were gradually reduced during this growth stage (squaring to full bloom). The full bloom appeared about 81 DAP under these three treatments. After that, the cotton height approached a plateau (Fig. 1). Under the SH treatment, the vegetative growth of cotton was significantly inhibited in the earlier growth stage in comparision with other salinity treatment. The inhibition was released by the irrigation and N application. After the first fertigation, the plant height rapidly increased from below 6 cm to above 60 cm at 88 DAP and then slowly reached a higher plateau than the other three treatments (on average of four N application rates).

The two-way ANOVA results showed that cotton height was significantly affected by salinity and N interaction, but not by N alone (Table 2). The influences of soil salinity and N application rate on cotton height at harvest are illustrated in Fig. 2. Under the treatments of S0, height increased with N application rates: under the NH treatment, the height was 17.5% and 14.1% higher than those under the N0 treatment and the NL treatment, respectively. Under

Fig. 1 Changes of cotton height with time under different soil salinity (ECe) levels: 2.4 dS m^{-1} (S0), 7.7 dS m^{-1} (SL), 12.5 dS m^{-1} (SM), and 17.1 dS m^{-1} (SH) and N application rates: 0 kg ha^{-1} (a), 135 kg ha^{-1} (b), 270 kg ha^{-1} (c), and 405 kg ha^{-1} (d). The error bars refer to one standard deviation
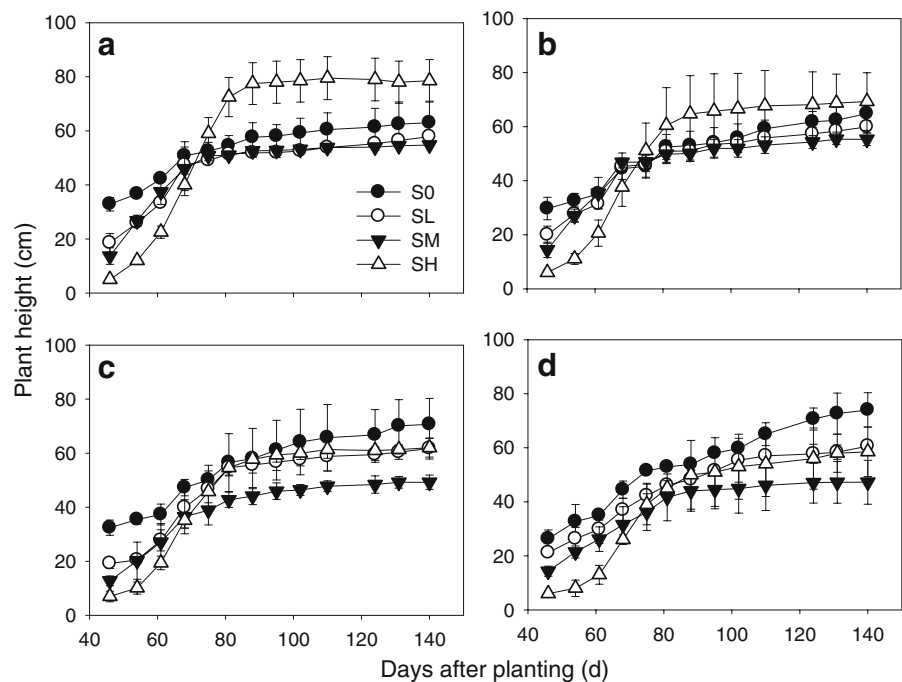
Table 2 Results of a two-way ANOVA of plant characteristics by salinity (S) and N treatments and their interactions

Dependent variable	S	N	S × N
Plant height (cm)	33.4 ***	1.4 n.s.	5.6 ***
Leaf dry weight (g pot^{-1})	4.8 **	2.2 n.s.	2.6 *
Stem dry weight (g pot^{-1})	32.3 ***	1.3 n.s.	6.7 ***
Bur dry weight (g pot^{-1})	54.3 ***	13.4 ***	5.7 ***
Seed dry weight (g pot^{-1})	150.4 ***	13.9 ***	2.5 *
Shed dry weight (g pot^{-1})	9.1***	0.6 n.s.	0.7 n.s.
Aboveground dry weight (g pot^{-1})	312.8 ***	15.6 ***	9.2 ***
Root dry weight (g pot^{-1})	50.1 ***	0.4 n.s.	0.6 n.s.
Water use efficiency (WUE, g l^{-1})	92.4 ***	14.9 ***	6.9 ***
N content in leaf(g pot^{-1})	17.9 ***	12.4 ***	4.3 **
N content in stem (g pot^{-1})	16.5 ***	1.5 n.s.	5.0 ***
N content in bur (g pot^{-1})	34.4 ***	21.8 ***	6.5 ***
N content in seed (g pot^{-1})	273.8 ***	34.1 ***	4.2 **
N content in shed (g pot^{-1})	8.4 ***	0.8 n.s.	1.4 n.s.
N content in root (g pot^{-1})	57.1 ***	2.4 n.s.	1.7 n.s.
Total N uptake (g pot^{-1})	152.5 ***	25.7 ***	3.6 **
Apparent N recovery (ANR, %)	44.7 ***	121.0***	3.3 *
K concentration in leaf (g kg^{-1})	36.6 ***	0.9 n.s.	1.0 n.s.
K concentration in stem (g kg^{-1})	15.0 ***	2.7 n.s.	1.9 n.s.
K concentration in bur (g kg^{-1})	10.7 ***	0.9 n.s.	2.3 n.s.
K concentration in seed (g kg^{-1})	1.6 n.s.	0.3 n.s.	1.0 n.s.
Na concentration in leaf (g kg^{-1})	472.6 ***	1.0 n.s.	0.5 n.s.
Na concentration in stem(g kg^{-1})	421.2 ***	2.4 n.s.	1.5 n.s.
Na concentration in bur (g kg^{-1})	255.1 ***	0.7 n.s.	2.1 n.s.
Na concentration in seed (g kg^{-1})	2.4 n.s.	2.3 n.s.	2.1 n.s.
Ca concentration in leaf (g kg^{-1})	171.8 ***	10.7 ***	1.6 n.s.
Ca concentration in stem (g kg^{-1})	25.2 ***	11.8 ***	1.2 n.s.
Ca concentration in bur (g kg^{-1})	24.9 ***	14.0 **	1.3 n.s.
Ca concentration in seed (g kg^{-1})	2.1 n.s.	1.3 n.s.	1.8 n.s.
Cl concentration in leaf (g kg^{-1})	211.9 ***	2.8 n.s.	0.8 n.s.
Cl concentration in stem (g kg^{-1})	99.8 ***	2.1 n.s.	0.4 n.s.
Cl concentration in bur (g kg^{-1})	76.5 ***	1.6 n.s.	0.7 n.s.
Cl concentration in seed (g kg^{-1})	0.6 n.s.	1.7 n.s.	0.5 n.s.

Numbers represent F-values. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; n.s., non-significant

the treatments of SL, N application rates had no significant effects on the cotton growth, while with the treatments of SM and SH, plant height decreased with N application rate. The height under the treatment of N0 was about 20% higher than that under the treatment of NH. With treatments of N0 and NL, height under the high soil salinity treatment (SH treatment) was significantly higher than under the other three treatments. With treatments of NM and NL, height under different salinity treatment followed a decreasing of S0 > SL and SH > SM.

Above-ground biomass

The effects of salt and N treatments on dry mass of cotton plant parts (leaf, stem, bur, seed and shed) are summarized in Table 3. The dry mass of different cotton plant parts were all significantly affected by the soil salinity levels (Table 2). The dry mass of cotton leaf and stem in the 2.4 dS m^{-1} (S0) treatment was significantly greater by about 12% and 30% than under the other three treatments, respectively. The dry mass of cotton bur decreased linearly from 51.5 g pot^{-1}, to 43.7 g pot^{-1}, to 34.0 g pot^{-1}, and to 28.3 g pot^{-1} as the soil salinity level increased from 2.4 dS m^{-1}, to 7.7 dS m^{-1}, to 12.5 dS m^{-1}, and to 17.1 dS m^{-1}, respectively. For the seed, the effect of soil salinity was even more pronounced, especially under the high soil salinity treatment. The dry mass of seed was reduced by 22%, 108%, and 514% respectively, when the soil salinity level increased from the control level of 2.4 dS m^{-1} to 7.7 dS m^{-1}, 12.5 dS m^{-1}
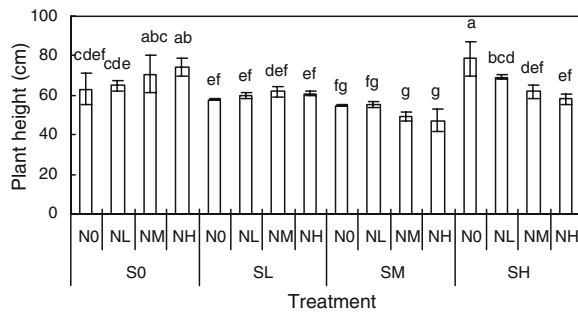
Fig. 2 Effect of soil salinity and N levels on plant height of cotton in full development stage. Bars represent mean ± SD. Different letters indicate significant difference ($P<0.05$). The symbols, S0, SL, SM, and SH, are corresponding to the soil salinity (ECe) of 2.4, 7.7, 12.5 and 17.1 dS m^{-1}, respectively. The symbols, N0, NL, NM, and NH are corresponding to the N application of 0, 135, 270, and 405 kg ha^{-1}, respectively. The symbols (S0, SL, SM, and SH; N0, NL, NM, and NH) in the following tables and figures are the same as those in this figure

and to 17.1 dS m^{-1}, respectively. The dry mass of shed under soil salinity treatments of SM and SH were significantly lower than those under lower soil salinity treatments. But, the average ratio of shed to total biomass under the high soil salinity treatment (0.16) was much higher than the other treatments (0.09–0.12).

The effects of N application rates on dry mass of different cotton plant tissues were insignificant except for cotton bur and seed (Table 2). The average dry

mass of cotton bur under different N treatments followed a decreasing order of NM (45.0 g pot^{-1}) > NH (41.6 g pot^{-1}) > NL (37.4 g pot^{-1}) > N0 (33.3 g pot^{-1}). The average dry mass of seed followed in decreasing order of NL (50.2 g pot^{-1}) > NM (48.5 g pot^{-1}) > NH (38.9 g pot^{-1}) > N0 (33.8 g pot^{-1}). The average dry mass of seed under the N application rate of 135 and 270 kg N ha^{-1} was significantly higher than those under the N application rates of 0 and 405 kg N ha^{-1}.

The total aboveground dry mass of cotton was significantly affected by salinity, N, and N × salinity interaction (Table 2). The total dry mass under the N application rate of 135 kg N ha^{-1} was the highest, which was about 14% greater the treatment without N application (Table 3). There was no significant difference of the total dry mass under the N application rate of 135, 270 and 405 kg N ha^{-1}. The total dry mass decreased linearly as the soil salinity increased. On average, the total dry mass under the treatment of S0 was 23, 60, and 84% higher than those under the soil salinity treatments of SL, SM and SH, respectively.

Root biomass and distribution

Statistical analysis showed that the cotton root mass was significantly affected by the soil salinity

Table 3 Aboveground dry mass (g pot^{-1}) under different soil salinity and N treatments

Salinity level	N level	Leaf	Stem	Bur	Seed	Shed	Total
S0	N0	52.9±0.9 bc	38.9±0.1 cd	46.9±1.1 b	51.4±2.6 cd	29.8±3.5 abc	219.9±1.0 cd
	NL	48.8±0.4 bc	41.1±1.8 c	40.5±2.6 bcd	81.0±3.2 a	35.5±4.2 a	246.9±0.6 b
	NM	58.0±0.7 b	51.1±9.1 b	59.9±1.2 a	78.3±4.9 a	33.7±9.5 ab	281.0±3.7 a
	NH	68.2±14.0a	62.4±6.7 a	58.5±11.0 a	72.5±2.7 ab	33.0±7.8 ab	294.6±26.6 a
SL	N0	51.8±1.4 bc	37.6±2.5 cde	41.5±3.8 bc	42.6±3.2 d	20.5±0.9 abcd	194.0±3.8 ef
	NL	55.2±5.5 b	36.0±5.0 cdef	42.7±0.3 bc	68.5±14.2 ab	28.2±10.1 abc	230.5±6.8 bc
	NM	47.6±4.8 bc	36.3±3.6 cdef	43.2±5.3 bc	61.4±4.0 bc	24.1±6.3 abcd	212.7±11.3 cd
	NH	49.1±2.0 bc	38.9±4.5 cd	47.3±1.8 b	47.6±2.4 d	23.9±4.5 abcd	206.9±5.2 de
SM	N0	44.0±0.6 a	33.7±1.9 cdefg	32.3±0.3 de	29.1±6.7 e	16.1±0.6 bcd	155.3±6.4 hi
	NL	52.1±7.7 bc	37.6±2.9 cde	36.4±5.7 cde	40.0±1.4 de	13.8±2.4 cd	179.8±15.3 fg
	NM	47.8±0.6 bc	29.0±2.3 efg	36.8±1.2 cde	39.1±0.5 de	10.3±8.3 d	162.9±14.9 gh
	NH	51.5±7.0 bc	28.4±6.9 fg	30.3±0.2 e	28.0±6.1 e	14.8±1.9 cd	153.0±9.8 hi
SH	N0	52.8±1.1 bc	33.0±1.0 cdefg	12.4±2.1 f	12.1±0.3 f	26.3±6.3 abcd	136.6±6.6 i
	NL	50.7±0.4 bc	30.5±0.5 defg	30.1±3.5 e	11.4±0.1 f	18.0±1.7 abcd	140.8±0.8 i
	NM	48.0±9.0 bc	37.3±7.8 cdef	40.2±8.0 cde	15.1±11.8f	10.0±7.2 d	150.6±12.3hi
	NH	52.6±6.1 bc	26.1±5.7 g	30.4±9.1 e	7.5±1.9 f	22.7±12.7 abcd	139.3±0.2 i

Mean values ± SD are shown. Different letters in the same column indicate significant difference ($P<0.05$). The symbols represent as in Fig. 2

($p<0.001$), while the effects of the N application ($p>$ 0.05) and N × salinity interaction ($p>0.05$) on root mass were quite limited (Table 2). In general, the root mass decreased with soil salinity level under the same N application rate (Table 4). The average root masses under the salinity level of S0, SL and SM were about 1.6, 1.4 and 1.2 times, respectively, higher than that under the salinity level of SH.

The root distribution followed a similar pattern under different soil salinity levels, namely, the amounts of root decreased with soil depth and the majority of the roots (80–90%) were distributed in the top 20 cm (Table 4). Under the high soil salinity treatment (SH), the root distribution ratio in the top 10 cm layer was significantly lower than those under low soil salinity treatment (S0 and SL). Compared to the treatment of S0, the root distribution ratio in the top 10 cm layer under treatment of SH was reduced by about 4%. There was no significant difference of the root distribution ratio in the 10–30 cm layer under different salinity treatments. As the soil salinity increased, more roots were distributed in 30–50 cm soil layers. Compared to the treatment of S0, the root distribution ratio in the bottom 30–50 cm layer under treatment of SH increased by about 4%.

Water use efficiency

Water use efficiency (WUE) of cotton was significantly affected by salinity, N, and N × salinity interaction (Table 2, Fig. 3). Generally, WUE of cotton decreased as the soil salinity level increased. Under treatment of S0, WUE of cotton significantly increased with N application rate. WUE under N application rate of 405 kg N ha^{-1}(NH treatment) was 37% and 20% greater than that under N application rate of 0 and 135 kg N ha^{-1} (N0 and NL treatment), respectively, but was not significantly different from that under the NM treatment. Under the low and medium salinity conditions (SL and SM treatments), the highest WUE was found under the treatment of NL. Under the high salinity condition (SH treatment), there was no significant difference of WUE between different N application rates.

Nitrogen uptake and apparent N recovery

The effects of salt and N treatments on uptake of N are summarized in Tables 2 and 5. Under the four different N application rates, N uptake of all decreased as soil salinity increased. On average, the

Table 4 Distribution of root dry mass (g pot^{-1}) at different soil depths and total root dry mass (g pot^{-1}) under different soil salinity and N treatments

Salinity level	N level	Soil depth (m)					Total root mass
		0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	
S0	N0	15.87±1.12 ab	3.99±0.28 a	1.30±0.09 ab	0.83±0.06 ab	0.43±0.03 d	22.43±1.58 a
	NL	17.03±1.54 a	3.70±0.33 a	1.29±0.12 abc	0.85±0.08 ab	0.53±0.05 bcd	23.39±2.11 a
	NM	16.10±0.37 a	3.71±0.08 a	1.46±0.03 a	0.84±0.02 ab	0.49±0.01 cd	22.61±0.52 a
	NH	16.30±1.68 a	3.76±0.39 a	1.48±0.15 a	0.85±0.09 ab	0.50±0.05 bcd	22.89±2.36 a
SL	N0	15.38±0.77 ab	2.95±0.15 b	1.23±0.06 bc	0.96±0.05 a	0.61±0.03 ab	21.13±1.06 ab
	NL	13.73±1.04 bc	3.02±0.23 b	1.16±0.09 bcd	0.81±0.06 bc	0.54±0.04 bc	19.26±1.46 bc
	NM	13.69±0.61 bc	2.00±0.09 d	1.33±0.06 ab	0.77±0.03 bcd	0.53±0.02 bcd	18.31±0.82 bc
	NH	13.66±0.69 bc	1.99±0.10 d	1.33±0.07 ab	0.77±0.04 bcd	0.53±0.03 bcd	18.28±0.92 bc
SM	N0	10.68±0.58 de	2.93±0.16 b	0.85±0.05 f	0.85±0.05 ab	0.67±0.04 a	15.98±0.87 cde
	NL	11.25±1.01 de	2.53±0.23 bcd	0.94±0.08 ef	0.75±0.07 bcd	0.60±0.05 abc	16.07±1.44 cde
	NM	12.10±0.14 cd	2.68±0.03 bc	1.01±0.01 def	0.68±0.01 cd	0.60±0.01 abc	17.06±0.20 cd
	NH	11.64±0.46 cde	2.58±0.10 bcd	0.97±0.04 def	0.65±0.03 d	0.57±0.02 abc	16.41±0.64 cde
SH	N0	9.85±0.92 de	2.13±0.20 de	1.09±0.10 cde	0.83±0.08 ab	0.65±0.06 a	14.55±1.36 de
	NL	9.35±0.49 e	2.02±0.11 d	0.86±0.04 f	0.71±0.04 bcd	0.59±0.03 abc	13.53±0.70 e
	NM	9.28±0.60 cde	2.29±0.15 cd	0.90±0.06 ef	0.70±0.05 bcd	0.65±0.04 a	13.82±0.89 de
	NH	9.55±0.91 cde	2.36±0.22 cd	0.93±0.09 ef	0.72±0.07 bcd	0.67±0.06 a	14.22±1.35 de

Mean values ± SD are shown. Different letters in the same column indicate significant difference ($P<0.05$). The symbols represent as in Fig. 2
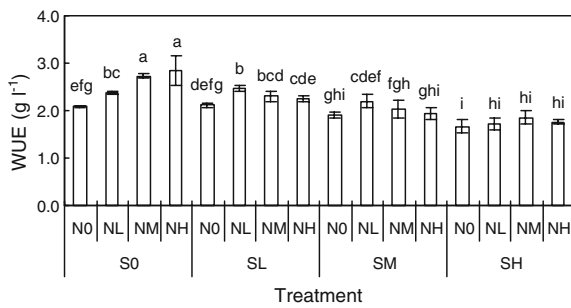
Fig. 3 Effect of soil salinity and N levels on water use efficiency (WUE, g l^{-1}) of cotton. Bars represent mean ± SD. Different letters indicate significant difference (P<0.05). The symbols are the same as in Fig. 2

total N uptake decreased from 6.39 g pot^{-1}, to 4.85 g pot^{-1}, 3.77 g pot^{-1} and to 3.05 g pot^{-1}, respectively, as the soil salinity level progressively increased. Under the treatment of S0, the total N uptake increased with the N application rates. The total N uptake under N application rate of 405 kg N ha^{-1} was about 46% greater than that under the treatment without N application. Under the treatment of SL and SM, the total N uptake under N application rates of 135 kg ha^{-1} and 270 kg ha^{-1} was significantly greater than that under the two higher N treatments.

Under the treatment of SH, there was no significant difference of total N uptake between different N application rates. Overall, the total N uptake under the different N application rates followed in a decreasing order of NM (5.04 g pot^{-1}) > NL (4.76 g pot^{-1}) > NH (4.59 g pot^{-1}) > N0 (3.67 g pot^{-1}).

Apparent N recovery (ANR) of cotton was significantly affected by salinity (p<0.001), N (p<0.001), and N × salinity interaction (p<0.05) (Table 2). In general, ANR decreased with N application rate (Fig. 4). On average, ANR decreased from 41%, to 26%, and to 12%, respectively, as the N application rate increased from 135, to 270, and to 405 kg ha^{-1}. The ANR also was significantly reduced by soil salinity. The highest ANR was found in S0 treatment and the lowest in SH treatment, but there was no significant difference between NL and NM treatments (Fig. 4).

Salt ion uptake

Effects of N application rate and salinity on concentrations of K, Na, Ca and Cl in different plant parts after harvest are presented in Table 2. Except for seed, the concentrations of these four ions in plant parts were significantly affected by the soil salinity. The

Table 5 N uptake (g pot^{-1}) as influenced by soil salinity and N treatments

Salinity level	N level	Leaf	Stem	Bur	Seed	Shed	Root	Total
S0	N0	1.23±0.12 cd	0.58±0.14 cde	0.88±0.12 cd	1.45±0.07 d	0.42±0.05 abc	0.33±0.03 b	4.90±0.57 cd
	NL	1.38±0.11 bc	0.70±0.11 bc	0.77±0.05 cde	2.46±0.10 ab	0.46±0.05 ab	0.41±0.05 a	6.17±0.35 b
	NM	1.48±0.12 ab	0.76±0.13 bc	1.44±0.13 a	2.62±0.16 a	0.61±0.17 a	0.43±0.09 a	7.33±0.69 a
	NH	1.64±0.34 a	1.03±0.21 a	1.13±0.21 b	2.29±0.09 b	0.59±0.14 a	0.48±0.07 a	7.16±0.76 a
SL	N0	1.08±0.03 d	0.62±0.08 bcd	0.77±0.07 cde	1.15±0.07 de	0.23±0.01 cd	0.26±0.04 bcde	4.11±0.35 ef
	NL	1.25±0.12 cd	0.65±0.09 bcd	0.86±0.11 cd	1.80±0.37 c	0.37±0.13 abc	0.30±0.03 bcd	5.23±0.31 c
	NM	1.09±0.11 d	0.62±0.06 bcd	0.84±0.10 cd	1.97±0.13 c	0.50±0.13 ab	0.32±0.02 bc	5.34±0.27 c
	NH	1.05±0.04 d	0.65±0.08 bcd	0.91±0.13 cd	1.41±0.07 d	0.42±0.08 abc	0.30±0.05 bcd	4.73±0.43 cde
SM	N0	0.79±0.06 e	0.47±0.07 de	0.55±0.07 e	0.73±0.17 g	0.27±0.01 bc	0.24±0.03 de	3.05±0.24 gh
	NL	1.50±0.14 ab	0.48±0.06 de	0.68±0.09 de	1.08±0.04 ef	0.23±0.04 bc	0.24±0.01 bcde	4.21±0.42 def
	NM	1.11±0.11 d	0.46±0.04 de	0.95±0.13 bc	1.29±0.02 de	0.15±0.15 c	0.24±0.02 cde	4.21±0.25 def
	NH	1.12±0.15 d	0.45±0.12 de	0.70±0.08 de	0.80±0.17 fg	0.29±0.04 bc	0.22±0.04 de	3.59±0.38 fg
SH	N0	1.07±0.02 d	0.61±0.02 bcd	0.21±0.05 f	0.14±0.00 h	0.38±0.09 abc	0.19±0.03 e	2.61±0.12 h
	NL	1.26±0.08 cd	0.56±0.06 cde	0.82±0.12 cd	0.27±0.00 h	0.34±0.03 abc	0.18±0.03 e	3.44±0.43 fg
	NM	1.10±0.21 d	0.80±0.17 b	0.73±0.14 de	0.32±0.47 h	0.15±0.12 c	0.19±0.05 e	3.29±0.12 gh
	NH	1.11±0.13 d	0.41±0.09 e	0.57±0.17 e	0.22±0.05 h	0.37±0.14 abc	0.19±0.05 e	2.87±0.19 gh

Mean values ± SD are shown. Different letters in the same column indicate significant difference (P<0.05). The symbols represent as in Fig. 2
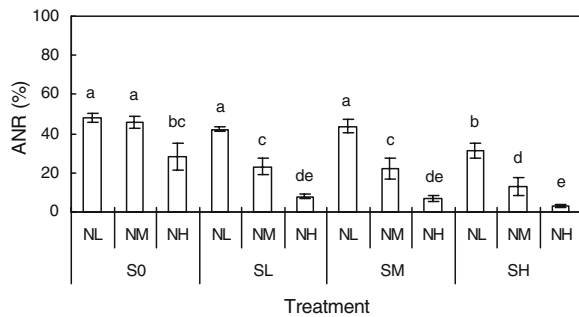
Fig. 4 Effect of soil salinity and N levels on apparent N recovery (ANR, %) of cotton. Bars represent mean ± SD. Different letters indicate significant difference ($P<0.05$). The symbols are the same as in Fig. 2

effect of N application on concentrations of these four ions was limited except for Ca. No N × salinity interaction was observed. As the soil salinity level increased, the K concentration of cotton leaf, stem and bur significantly decreased. Conversely, the Ca, Na and Cl concentration of cotton leaf, stem and bur was significantly increased (Table 6). There was no significant difference of K, Ca, Na, and Cl concentrations of seed under different soil salinity treatments. Uptake of Ca was also affected by the N application rates. The Ca concentration of cotton leaf, stem and bur increased as N application rate increased (data was not presented).

Discussion

Understanding salinity-fertilizer relationships is of great economic importance and many studies have been conducted to evaluate N and other nutrients uptake by plants under saline conditions (Papadopulos and Rendig 1983; Feigin 1985; Villa-Castorena et al. 2003; Kutuk et al. 2004). Differences in salt tolerance among plant species have been long recognized; however, the role that salt tolerance plays in causing differences in nutrient uptake at different growth stages is still not fully understood (Pessarakli 1991; Kutuk et al. 2004).

Our experimental results indicated that cotton was more sensitive to salt during the emergence and early growth stages than at later stages. In addition to the vegetative growth, the reproductive growth was also significantly affected by high soil salinity. In our study, under the high soil salinity treatment (SH), the three-true-leave and the peak blooming stages were delayed about 4 days and 1 week, respectively, in comparison with other treatments. There were fewer bolls per plant and a greater chance of shedding under the high soil salinity treatment (SH), which might be attributed to the nutrient imbalance caused by high soil salinity. Gouia et al. (1994) reported that high salt stress results in slower growth and even death of seeding cotton, lower dry matter accumulation, less

Table 6 Ion concentrations (g kg^{-1} dry weight) in cotton plant parts (leaf, stem, bur, and seed) as affected by soil salinity treatments

Plant part	Salinity level	K	Na	Ca	Cl
Leaf	S0	21.33±2.52 a	1.04±0.11 d	13.01±1.20 d	10.76±1.18 d
	SL	18.89±1.75 b	3.54±0.33 c	26.94±4.62 c	27.83±3.05 c
	SM	15.85±1.13 c	4.54±0.34 b	33.15±3.94 b	36.89±4.75 b
	SH	14.44±1.37 c	6.07±0.42 a	39.39±4.99 a	50.39±5.92 a
Stem	S0	19.58±2.00 a	1.12±0.12 d	8.22±1.15 c	8.02±0.56 d
	SL	18.59±1.76 ab	2.43±0.29 c	9.96±1.51 b	11.87±1.43 c
	SM	16.92±2.32 b	3.45±0.32 b	11.35±2.49 b	14.66±1.30 b
	SH	14.82±2.21 c	4.62±0.32 a	13.63±2.83 a	18.79±2.34 a
Bur	S0	32.90±4.99 a	0.83±0.12 d	9.02±1.71 c	7.68±0.80 d
	SL	34.08±3.17 a	1.33±0.15 c	9.84±1.68 bc	10.70±1.38 c
	SM	31.01±2.87 a	1.65±0.13 b	10.85±1.33 b	12.87±1.24 b
	SH	27.20±2.87 b	2.28±0.17 a	12.69±1.52 a	15.83±1.81 a
Seed	S0	10.13±1.04 a	0.29±0.12 a	4.63±0.59 a	4.70±0.44 a
	SL	10.30±0.99 a	0.29±0.06 a	4.36±0.58 a	4.74±0.48 a
	SM	10.55±0.79 a	0.38±0.14 a	4.40±0.66 a	4.45±0.63 a
	SH	9.67±1.11 a	0.30±0.10 a	4.87±0.57 a	4.62±0.63 a

Mean values ± SD are shown. Different letters in the same column indicate significant difference ($P<0.05$). The symbols represent as in Fig. 2
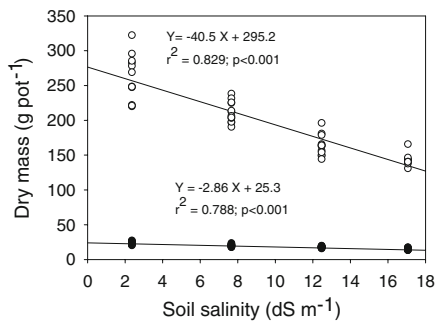
Fig. 5 Dry mass of aboveground and root as a function of soil salinity. The results presented are averages of soil salinity treatments for all N levels. Lines represent linear correlation

roots, softer and darker leaves, shorter period of blooming, and even death of seedling cotton. Ashraf (2002) showed that under high salt stress, the growth rate of cotton was significantly reduced with less fruiting branches. The appearance of square, flowers and boll was significantly retarded; the blooming stage was shorter and the shedding also increased.

Nevertheless, our experimental results also indicated that the adverse effects of soil salinity on cotton growth could be alleviated by fertilizer application, but over fertilization with N might contribute to soil salinization and increase the negative effects of soil salinity on plant performance. Villa-Castorena et al. (2003) concluded that over fertilization during the early plant development might contribute to higher soil salinity and decreased pod yield of chile pepper, while salt-stressed chile performed well when they were adequately fertilized. Fertilizer should be applied in amounts that increase with plant need over the growing season. Similarly, Kaya and Higgs (2003) reported that supplementary urea could overcome the effects of high salinity on fruit yield and whole plant biomass in pepper plants.

The effects of soil salinity and N application on cotton above-ground dry mass were dominated by the soil salinity and could be correlated with root development. The root dry mass also decreased linearly with soil salinity, but at a less pronounced rate (Fig. 5). Reduction in dry matter production with increasing salinity level was noticed by a number of researchers (Abd-Ella and Shalaby 1993; Leidi and Saiz 1997; Qadir and Shams 1997; Ashraf and Ahmad 1999; Basal et al. 2006).

The effect of soil salinity on the root distribution may be closely related to the dynamic salt distribution

corresponding to the irrigation and root water uptake (Malash et al. 2008). During an irrigation, the salts around the emitters were transported with water to farther distance and to deeper soil layers. During the intervals between irrigations, the salts may be transported toward the soil surface and roots due to the root water uptake and evaporation. Our measurements showed that the lowest salinity in the soil profile was around the emitter and the highest salinity in the soil profile was found in the top 0–10 cm soil layer 15–20 cm from the emitter. The average soil salinity at different depths is illustrated in Fig. 6. Under the low soil salinity treatment of S0, the soil salinity in the 30–50 cm soil layers was significantly higher than that in the 0–20 cm soil layers. In contrast, under the high soil salinity treatment (SH), the soil salinity in bottom layers was similar with that in the top 0–20 cm layer. During the growing period, cotton may adjust its growth pattern to avoid high salt stresses (Homaee and Schmidhalter 2008). As a result, more roots were distributed in bottom layers under the high soil salinity treatment than under the low soil salinity treatment.

The uptake of nitrate is known to compete with that of Cl, a major ion in saline soil (Xu et al. 2000; Mengel and Kirkby 2001; Abdelgadir et al. 2005). Such an interaction results in diminished N uptake and decreased plant growth at increased Cl concentrations. Our experimental results illustrated that at low soil salinity level, increasing the N application rate could significantly enhance the N uptake. Under
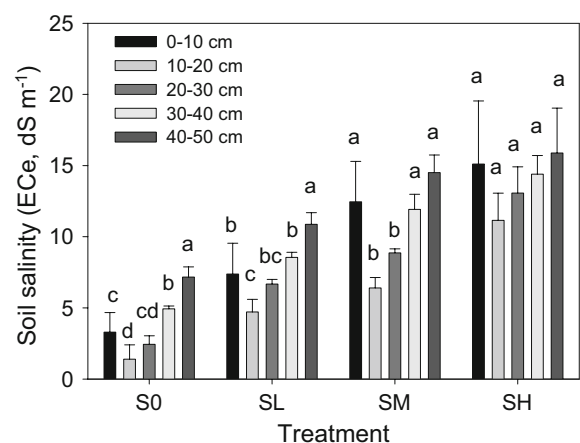

Fig. 6 Soil salinity distribution at different soil depths under salinity treatments. Bars represent mean ± SD. Different letters indicate significant difference under the same soil salinity treatment ($P<0.05$). The symbols are the same as in Fig. 2

moderate soil salinity level, proper use of N fertilizer was necessary. Over fertilization did not benefit the N uptake. Under the high soil salinity, salt was the dominated factor governing the cotton growth and N uptake. The inhibition could not be alleviated by fertilizer application. The findings agree with those observed by Shenker et al. (2003), who demonstrated that increasing the application of N fertilizer could increase N uptake, hence the cotton yield. Our study indicated that N uptake and the cotton yield were not affected by high N application rate, and the N uptake and the cotton yield increased as the soil salinity decreased.

The accumulation of N in different plant parts generally followed a decreasing order of seed > leaf > bur > stem > shed > root, but varied with the soil salinity level (see Table 5). Under the high salinity treatment (SH), the N in the cotton seed accounted for about only 8% of the total N uptake, while under the other three salinity treatments, it accounted for as much as 31% of the total N uptake. Consequently, more N was accumulated in the leaf and stem parts under high salinity level. The results indicated that there was a nutrient imbalance under the high salinity treatment. The nutrient (N) was mainly used for vegetative growth rather than for reproductive growth.

The concentrations of Na, Ca, and Cl in various plant parts of cotton increased as soil salinity increased. The concentrations of these ions among different plant parts followed in a decreasing order: leaf > stem > bur > seed. This kind of distribution pattern of salt ions among different plant tissues was thought to be one of the major salt tolerance mechanisms of cotton (Xin et al. 2005). Leidi and Saiz (1997) demonstrated that the Na concentration in cotton leaves was closely related to plant growth. Fortmeier and Schubert (1995) compared the effect of NaCl and Na_2SO_4 on cotton growth and concluded that the toxicity of salts on cotton was due to Na, not due to C1, or combination of Na and C1. The accumulation of Na may result in decreased uptake of K, and Ca (Rathert 1983; Saqib et al. 2000; Lee et al. 2007). Our experimental results showed that the Na concentration of the cotton roots, leaves and stems increased with the soil salinity, the uptake of K decreased with soil salinity, while the uptake of Ca increased with soil salinity. The high uptake of Ca may be attributed to the soil salinity treatment in which 1:1 of $CaCl_2$ and NaCl was used. Consequently, the Ca

in the soil was significantly increased, thus the uptake of Ca by cotton plant. K and Ca play key roles in several physiological processes, while Na does not function as a macro-nutrient. Thus, the substitution of K/Ca by Na may lead to nutritional imbalances (Gorham and Bridges 1995; Tuna et al. 2007).

Conclusions

Our experimental results illustrated that both the vegetative growth and the reproductive growth were significantly inhibited by the high soil salinity. At low soil salinity level, increasing the N application rate could significantly enhance the N uptake, thus alleviating the adverse effects causing by salinity. Under moderate soil salinity level, proper use of N fertilizer was necessary. Over fertilization did not benefit N uptake. Under the high soil salinity, salt was the dominated factor governing the growth of cotton and N uptake. The inhibition could not be alleviated by fertilizer application.

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