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Interactive effects of pH, EC and nitrogen on yields and nutrient absorption of rice (*Oryza sativa* L.)

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ABSTRACT

Soil salinity and sodicity can not only directly restrain crop growth by osmotic and specific ion stresses, it also may reduce grain yield indirectly by impacting plant absorption of essential nutrients. Ensuring adequate nitrogen is an important management aspect of rice production in saline-sodic soils. The objective of this study was to investigate the interaction of soil pH, salinity and nitrogen application on rice yield and nutrient absorption. We conducted a rice experiment in containers in a greenhouse. The soils were first leached with 9 target salt solutions of pH 7, 8 and 9 and electrical conductivity (EC) of 2, 6 and 10 dS m^{-1}. Nitrogen application rates were 100, 200 and 300 kg N ha^{-1}. Rice grain yield and shoot weight significantly decreased with increasing soil pH and increasing soil EC, and significantly increased with increasing nitrogen application ($P < 0.001$). However, at high EC and/or high pH yield was not significantly increased by increased N. High pH and high EC in soil significantly influenced the mineral nutrient content of rice shoots ($P < 0.05$). High soil pH and soil EC stresses were superimposed on each other, the negative effects on rice were compounded. The results by stepwise regression analysis showed that soil pH very significantly and adversely impacts grain yield and was the major factor impacting rice grain yield ($R^2 = 0.565$, $P < 0.001$). Nitrogen application provided a positive response under control and pH 7 at all salinity values, and at pH 8 under low salinity only. There was no significant response to additional N under pH 8 and elevated EC and no significant response at pH 9 for all EC values. Thus, adequate nitrogen application is an important technical measure for improving rice yield and promoting nutrient absorption in rice of high EC soils but not of high pH soils where pH is the major limiting factor for rice production in saline-sodic soils.

1. Introduction

Soil salinity and sodicity are major environmental factors limiting plant growth and productivity throughout the world. It is estimated that the annual rate of loss of agricultural lands by salinization is about 1.5 million hectares; so far about 77 million hectares of agricultural lands have been degraded by soil salinity and sodicity (Eynard et al., 2006). Salinity and sodicity can not only inhibit plant growth by ion toxicity (mainly of Na^+ and Cl^-) and osmotic stress (Borsani et al., 2001; Eraslan et al., 2007; Tarakcioglu and Inal, 2002), but also may reduce productivity by altering ionic relations and in combination with pH changes (sodic soils are generally elevated in pH) lead to nutritional imbalance (Caines and Shennan, 1999).

The relations between soil salinity/sodicity and mineral nutrition of plants are extremely complex. Grattan and Grieve (1999a,b) reviewed mineral nutrient response by plants grown in saline environments. They concluded that in the presence of salinity, low nutrient ion activities and extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}, and Cl^-/NO_3^-, can cause reduction of nutrient uptake, alteration of nutrient partition, nutritional disorders; and reduction of crop growth. Hu and Schmidhalter (2005) compared the effects of drought and salinity on mineral nutrition of plants, and clarified that drought could mainly affect nutrient uptake and impair acropetal translocation of some nutrients. On the other hand, salin-

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ity might cause nutrient deficiencies or imbalances due to the competition during plant uptake of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+}, and NO_3^-.

A large number of studies that examined salinity utilized neutral salts, namely those containing chloride or sulfate anions. However, there are many soils in arid and semiarid regions that contain bicarbonates and carbonates in solution. Due to the high alkalinity (where alkalinity is defined as the sum of the titratable bases, primarily bicarbonate and carbonate) and resultant elevated pH, these soils are low in dissolved calcium, and with elevated sodium, become soils with high levels of exchangeable sodium (sodic soils). According to the FAO/UNESCO Soil Map of the world (Martinez-Beltran and Manzur, 2005), the total global area of salt-affected soils is 831 million ha, which includes 397 million ha of saline soils and 434 million ha of sodic soils (Rengasamy, 2006). The Western Songnen Plain of China is one of the three larger saline-sodic soil regions in the world, with a total area of 3.42 million hectares (Song et al., 2003; Wang et al., 2003), with soil pH ranging from 7.02 to 10.16 (Chi et al., 2012). High pH and high sodicity are adverse to soil physical properties including hydraulic conductivity (Suarez, 2012; Suarez et al., 1984). Huang et al. (2010, 2015) indicated that soil nitrogen deficiency had seriously impacted crops growth and the yield in saline-sodic soils in Western Songnen Plain of Northeast China.

Rice (*Oryza sativa* L.), one of the main cereal crops in the world, is considered as not salt tolerant (Maas and Hoffman, 1977; Shannon et al., 1998; Zeng and Shannon, 2000) but is moderately tolerant to sodicity (Pearson, 1960; Ayers and Westcott, 1989). Rice cropping could potentially reduce the negative effects of degraded soil structure by irrigation, progressively removing salinity and sodicity from the surface soil (Obrejanu and Sandu, 1971). Therefore, rice cropping has been recommended as the preferred method of biological improvement of saline-sodic soils (Maianu, 1984; Chi et al., 2012; Huang et al., 2016). Numerous experimental studies had reported on the physiological response of rice under saline or sodic stress (Ali et al., 2004; Qi et al., 2009a,b; Aref and Rad, 2012; Zhang et al., 2015). Recently, the Chinese government launched a project for developing approximately 0.3 million hectares of moderate or severe saline-sodic soils (soil pH is generally above 8.5) for rice production. It has become a top priority to clarify the interaction of pH, salinity and major soil nutrients to rapidly increase rice yields.

Compared to the extensive studies of rice salt tolerance, the study of rice tolerance to sodicity is still very limited, and especially with respect to its' relationship with high pH, salinity and the major plant nutrients. The present study focused on the interaction of pH, salinity and nitrogen on rice yield and nutrient absorption under soil sodicity stress. The objectives of this study were (1) to determine the separate effects of pH and salinity on rice growth and nutrient absorption, (2) to determine the interactive relationship among soil pH, EC, nitrogen and rice yield, and (3) to determine the effects of pH, EC and nitrogen fertilizer on rice nutrient absorption under different soil pH and salinity stresses.

2. Materials and methods

A rice experiment in containers was conducted in a greenhouse at the USDA-ARS, U.S. Salinity Laboratory (33°58′24″ N, 117°19′12″ W). The height of the containers was 36 cm, and the radius was 15 cm (volume was about 25 Liters). Each container was filled with 18 kg of sandy soil (Arlington sandy loam). There was a 0.5 cm (radius) opening and rubber stopper near the bottom of each container for draining water. We placed gravel and plastic gauze around the opening thus the rubber stopper can be removed for draining water or closed to retain water, as desired.

Table 1
Composition of target solutions used to equilibrate soils, the amount of various salts in the target solutions were firstly calculated and obtained by the Extract Chem software, and then prepared with deionized water.

Target solutions	Solution composition ($mmol\,L^{-1}$)					
	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-
pH_7EC_2	16.0	1.00	1.00	9.91	4.96	0.18
pH_8EC_2	16.5	1.00	1.00	9.25	4.63	2.00
pH_9EC_2	20.0	0.05	2.00	1.10	0.55	21.89
pH_7EC_6	49.0	3.00	3.00	30.40	15.20	0.20
pH_8EC_6	49.5	3.00	3.00	29.50	14.75	2.50
pH_9EC_6	59.4	0.05	3.50	18.00	9.00	30.48
pH_7EC_{10}	85.0	5.25	5.25	53.00	26.38	0.25
pH_8EC_{10}	85.0	5.18	5.50	52.00	26.00	2.36
pH_9EC_{10}	107.8	0.04	2.59	29.10	14.53	54.89

Arlington soil was collected at site (33°58′11″ N, 117°20′27″ W). The pH of the saturation extract was 6.8 and electrical conductivity (EC) was approximately 14.0 $dS\,m^{-1}$. We washed the soil repeatedly with tap water (EC 0.63 $dS\,m^{-1}$), leaching the original soil salinity and soluble nutrients, and then immersed and leached the soils with target salt solutions. The 9 target salt solutions were pH 7 EC 2 $dS\,m^{-1}$, pH 7 EC 6 $dS\,m^{-1}$, pH 7 EC 10 $dS\,m^{-1}$, pH 8 EC 2 $dS\,m^{-1}$, pH 8 EC 6 $dS\,m^{-1}$, pH 8 EC 10 $dS\,m^{-1}$, pH 9 EC 2 $dS\,m^{-1}$, pH 9 EC 6 $dS\,m^{-1}$ and pH 9 EC 10 $dS\,m^{-1}$. The target solutions given in Table 1 were prepared with different salts and deionized water; the salt composition was calculated by the Extract Chem software (Suarez and Taber, 2012, http://www.ars.usda.gov/services/software/software.htm?modecode=20-36-05-00). We analyzed the pH and EC of the solutions that drained from the individual holes. Once the drainage solutions achieved the target pH and EC, the soil treatment was completed and the containers were ready for planting.

We utilized the rice cultivar FL478, a California cultivar very sensitive to salinity, with early maturity, high straw content and a medium-size grain. Seeds were sterilized in $HgCl_2$ (0.5 $g\,L^{-1}$) for 2 min, rinsed with formaldehyde (16 $mL\,L^{-1}$) and methanol (4 $mL\,L^{-1}$) for 15 min, and then rinsed and soaked in deionized water at 37 °C for 24–48 h. Seeds were planted in a rectangular nursery seedling tray filled with wet vermiculite, and then covered with plastic film for germination.

The experiment was conducted in the greenhouse between January and June 2013. The rice nutrient requirements were provided using Yoshida Solution (Zeng and Shannon, 2000). In addition to nitrogen, the nutrient requirements were calculated with consideration of soil mass and then the same amount of nutrient solution was applied to the soil in each container. At each pH and EC we had containers with different nitrogen fertilizer application levels, 100, 200 and 300 $kg\,N\,ha^{-1}$ as urea. Half of the nitrogen fertilizer was applied before planting rice, and the other half was divided into two applications, one during rice tillering and the other at rice heading. A randomized block design was used with three replicates (3 containers) for 27 treatments, thus a total of 81 containers. The design details of the experimental treatments are provided in Table 2.

When rice seedlings had four leaves, they were transplanted into the containers (January 7, 2013). There were three hills in each container, and 4–5 seedlings in each hill. Irrigation was with Riverside tap water (EC = 0.63 $dS\,m^{-1}$) twice a week. Water depth in the containers was controlled at 3–5 cm during the first week and at 6–8 cm thereafter, with no drainage to maintain pH and salinity concentrations. Hourly mean temperatures were integrated across each day and summed to obtain cumulative thermal time. Air temperature ranged from 25 to 33 °C during the day and 18–23 °C during the night. Humidity ranged from 40 to 85%. Light averaged 1050 $\mu mol\,m^{-2}\,s^{-1}$ with a minimum of 200 and a maximum

Table 2
Design of the experiment treatments, N means nitrogen, 100, 200 and 300 kg N ha^{-1}; pH and EC (electric conductivity) were determined in drainage solutions after soil leaching, EC unit value was dS m^{-1}.

	EC_1	EC_2	EC_3	
N_1	pH_1	$N_{100}pH_7EC_2$	$N_{100}pH_7EC_6$	$N_{100}pH_7EC_{10}$
	pH_2	$N_{100}pH_8EC_2$	$N_{100}pH_8EC_6$	$N_{100}pH_8EC_{10}$
	pH_3	$N_{100}pH_9EC_2$	$N_{100}pH_9EC_6$	$N_{100}pH_9EC_{10}$
N_2	pH_1	$N_{200}pH_7EC_2$	$N_{200}pH_7EC_6$	$N_{200}pH_7EC_{10}$
	pH_2	$N_{200}pH_8EC_2$	$N_{200}pH_8EC_6$	$N_{200}pH_8EC_{10}$
	pH_3	$N_{200}pH_9EC_2$	$N_{200}pH_9EC_6$	$N_{200}pH_9EC_{10}$
N_3	pH_1	$N_{300}pH_7EC_2$	$N_{300}pH_7EC_6$	$N_{300}pH_7EC_{10}$
	pH_2	$N_{300}pH_8EC_2$	$N_{300}pH_8EC_6$	$N_{300}pH_8EC_{10}$
	pH_3	$N_{300}pH_9EC_2$	$N_{300}pH_9EC_6$	$N_{300}pH_9EC_{10}$

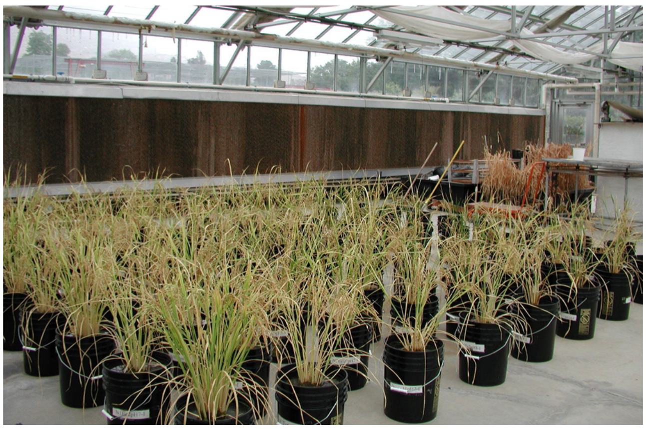

Fig. 1. The conditions of potted rice growth in greenhouse, cultivar: FL478 grown under three nitrogen supply levels, three pH and three EC stresses, U.S. Salinity Lab., ARS-USDA.

1400 μmol m^{-2} s^{-1} at noon. The container grown rice plants of the various treatments are shown in Fig. 1.

We ceased irrigation on June 4, 2013 to enable drying of the soils and plants. The rice plants were harvested on June 19, 2013. Yield-related parameters such as plant height and number of productive tillers were recorded for each plant. The rice plants were cut 5 cm above the soil surface, washed with tap water, and rinsed with deionized water. Next, the plants were blotted dry using paper towels. The samples were transferred into large paper bags, one container of plants per bag. The bags were left in the greenhouse, to sun-dry for three weeks. Next, the samples were processed to obtain yield, straw weight, panicle length, number of grains/panicle, grain yield and 100 grain weight. Shoot nutrient concentrations were analyzed. Total nitrogen content in shoots was measured by a N combustion system (Elementar Pyrocube Germany) and P, K, Ca, Mg and Na were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, 138 Perkin Elmer Ultrace, Edison, USA).

We utilized the GLM procedures of SAS (version 9.3, SAS Institute, Cary, N.C.) applying a factorial ANOVA to determine significances at $P \le 0.05$ of individual nitrogen level, pH level and salinity effects and their interactions on growth parameters, shoot macro and micro nutrient and salt accumulation after testing for normal distributions of the data for each dependent variable. Data was transformed for some dependent variables in order to obtain a normal distribution. Specific significant differences of each dependent variable among the pH values, salinities and nitrogen levels, respectively, were determined at $P \le 0.05$ using the Bonferroni multi-comparison method in the SAS GLM procedure. Stepwise regression analysis was used to examine the contribution of soil pH, EC and nitrogen application to grain yield and shoot weight of rice.

3. Results

3.1. Rice growth and yield characteristic as related to pH, salinity (EC) and nitrogen

3.1.1. Grain yield

Soil pH, salinity (EC) and N all significantly influenced rice grain yield. Rice grain yield significantly decreased with increasing pH value under the same N application levels and the same salinity (EC) (Fig. 2A & B). If the influence of soil salinity (EC) was not considered, rice grain yield significantly increased with increased N application rates at pH 7 (Fig. 2A), but there was no significant yield response to increased N when soil pH was greater than 8. Increased N application improved rice grain yield at low EC and low pH only.

Grain yield also decreased with increasing EC compared at the same pH value without regard to N application levels. When soil pH was 7, there were no significant differences in grain yield among the three different EC treatments ($P < 0.05$). However, the data done show a significant decrease in yield with increasing EC at both pH 8 and pH 9 (Fig. 2B). At pH 8 rice grain yields at EC_6 and EC_{10} were significant lower than those at comparable N at EC 2. Similarly, when the soil pH was 9, and N application rate was 100 kg ha^{-1}, rice grain yields at EC 6 and EC 10 were significantly reduced as compared to that at EC 2, and less than 26% of the $N_{100}pH_9EC_2$ treatment.

3.1.2. Shoot weight

Similar to the changes in grain yield, soil pH and EC decreased rice shoot weight under the same N application levels, and N application significantly increased rice shoot weight when soil pH was 7 or 8 without regard to EC (Fig. 2C). However differences between N_2 and N_3 treatments were generally not significant (Fig. 2C). When soil pH was 7 and soil EC was 2 dS m^{-1}, N application rates increased from 100 kg ha^{-1} to 200 kg ha^{-1}, and rice shoot weight increased more than 50%. At soil pH 9, and soil EC 10 dS m^{-1}, an increase in N application from 100 kg ha^{-1} to 300 kg ha^{-1}, increased rice shoot weights more than doubled. However, there were significant differences of shoot weight among 3 different N levels when soil pH reached 9.

Rice shoot weight also significantly decreased with increasing soil pH under the same soil EC, if the effect of N application on shoot weight was not temporarily considered. When soil pH was 7 and 8, rice shoot weight decrease with increasing EC was not significant ($P > 0.05$). When soil pH reached 9, soil EC reached 6 dS m^{-1} and 10 dS m^{-1}, rice shoot weight significantly declined with increasing soil EC ($P < 0.05$) (Fig. 2D). Soil pH significantly decreased rice shoot weight under high EC especially, and soil salinity also significantly decreased rice shoot weight under high pH ($P < 0.05$).

3.1.3. 100-grain weight

Rice 100-grain weight was small generally non-significant declines with increasing EC when soil pH was 7, and there was a relatively small but statistically significant decline in 100-grain weight with increasing EC when soil pH was 8 and 9 (Fig. 3A). Nitrogen application had little effect on rice 100-grain weight under various soil pH and EC treatments. When soil pH was 9 and nitrogen application rates were 100 kg ha^{-1} and 200 kg ha^{-1}, 100-grain weight significantly decreased with increasing EC ($P < 0.05$). Fig. 3A shows that rice 100-grain weight significantly decreased with increasing pH only when soil EC was 6 and 10 dS m^{-1}($P < 0.05$).

3.1.4. Number of panicle

Soil pH and EC reduced rice panicle number at each N level, although not all were statistically significant (Fig. 3B). Increased N application increased rice panicle number in almost all instances, however differences were significant only at pH 7 ($P < 0.05$). At pH 8

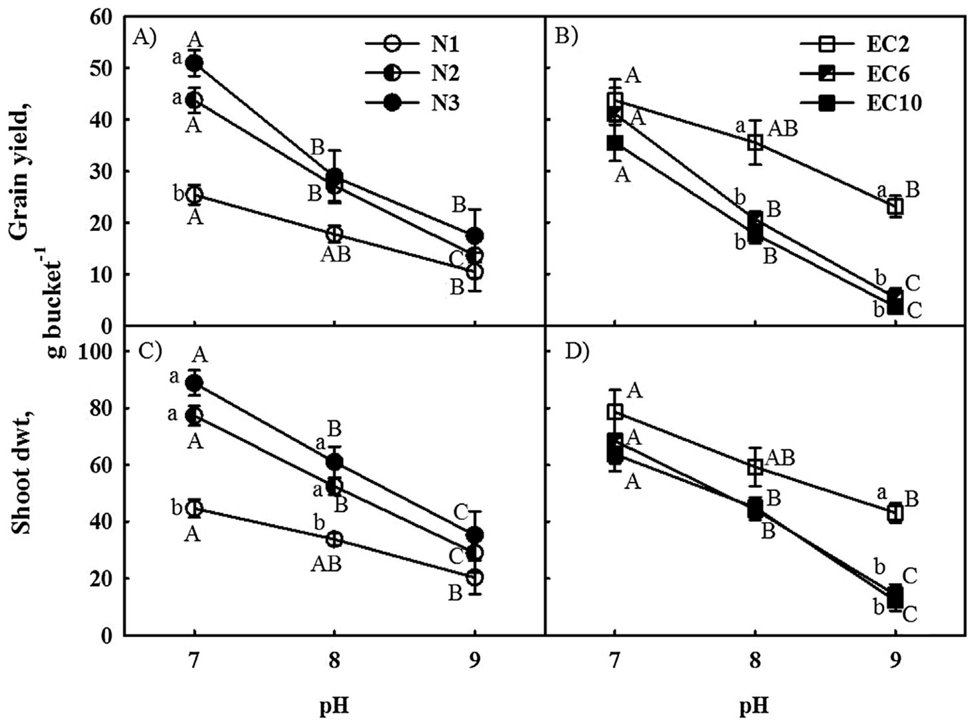

Fig. 2. Effects of soil pH and salinity on grain yields (A & B), and shoot wt (weight) (C & D) of rice (*Oryza sativa* L.), cultivar: FL478 grown under three nitrogen supply levels (N_1, N_2 and N_3).

Chart line with stick bar are Means with 1SE (standard error), n = 9 (buckets). At the same pH value, different lower case letters are given to those with significant difference at $P \le 0.05$ among the three nitrogen application levels without regard to salinities (A & C), or among the three salinities (EC) without regard to nitrogen application levels (B & D). At the same nitrogen level (A & C) or salinity (EC) (B & D), different capital letters are given to those with significant difference at $P \le 0.05$ among the three pH values. Within each comparison group, same letter means no significant difference at $P > 0.05$. No letter was marked within any comparison group if there is no any significant difference at $P > 0.05$ among any of the corresponding three treatment levels.

Table 3
Summary of statistical test significances of the effects of soil nitrogen level (Nitrogen), salinity (EC) and pH on grain wt (weight), grain wt per 100 grains (100 grain wt), shoot wt and number (No.) of panicle of rice (*Oryza sativa* L.), cultivar: FL478, respectively, and their interactions (marked with * between the effects) on the effects.

Items	Grain wt	100 grain wt	Shoot wt	No. of panicle
Nitrogen	***	n.s.	***	***
EC	***	***	***	n.s.
pH	***	***	***	***
Nitrogen*EC	n.s.	n.s.	n.s.	n.s.
Nitrogen*pH	*	n.s.	**	n.s.
EC*pH	***	***	*	n.s.
Nitrogen*EC*pH	n.s.	n.s.	n.s.	n.s.

Significance: ***, at $P < 0.001$, **, at $0.001 < P < 0.01$ and *: $0.01 < P \le 0.05$. n.s. means no significance at $P > 0.05$.

and 9 increased N increased the number of panicles but the results were not significant (Fig. 3B). Rice panicle number was significantly reduced with increasing soil pH when nitrogen application rates were 100 and 200 kg ha^{-1} ($P < 0.05$). When N application rates were increased from 100 kg ha^{-1} to 300 kg ha^{-1}, rice panicle number increased 25%–60% at pH 7.

3.1.5. Interaction of pH, EC and nitrogen on rice growth and yield

The statistical results of combined effects of N, pH and EC on rice growth and yield are shown in Table 3. Nitrogen application significantly increased rice grain weight, shoot weight and panicle number ($P < 0.001$), but there was no significant influence of N on rice 100-grain weight. Soil EC significantly decreased rice grain weight, shoot weight and 100-grain weight ($P < 0.001$), and soil pH significantly decreased all four growth parameters ($P < 0.001$). However, the interactive effects of N, EC and pH on rice were not significant ($P < 0.05$). Similarly, the interactive effect of N and EC on grain weight (yield) was not significant, meaning that the response to N is similar at different EC values. The interactive effect of N and pH (Table 3) was significant ($P < 0.05$), in this instance increased N provided a positive yield response at low pH but no response at high pH (as seen in Fig. 2A). The interaction of EC and pH significantly impacted rice grain weight, 100-grain weight and shoot weight ($P < 0.001$ or $P < 0.05$). Stepwise regression analysis showed that soil pH was the major factor affecting grain yield and shoot weight of rice ($R^2 = 0.565$, $R^2 = 0.504$, $P < 0.001$, respectively).

3.2. Rice nutrient absorption as related to pH, salinity (EC) and nitrogen

3.2.1. Nitrogen, phosphorus and potassium

Without regard to N application rates, the N concentration in the rice shoots increased as soil EC or pH increased (Fig. 4A). When soil pH was 7 or 9, shoot N concentrations significantly increased as soil EC increased from 2 dS m^{-1} to 10 dS m^{-1} ($P < 0.05$). When soil EC was 6 dS m^{-1}, N concentration in shoots was significantly increased with an increase in soil pH was from 7 to 9 ($P < 0.05$). For example, shoot N concentration at soil pH 9 increased 62.2% relative to that at pH 7 when N application rate was 100 kg ha^{-1} and soil EC was 6 dS m^{-1}. However, there were no significant changes in shoot N concentration with increasing N application rates under the same soil pH or the same soil EC. Decreased growth due to pH or EC stress resulted in decreased total N uptake and thus increased N in soils solution and increased concentration of plant N.

There were no significant changes in shoot phosphorus (P) concentration as N application rate increased but similar to N, shoot P

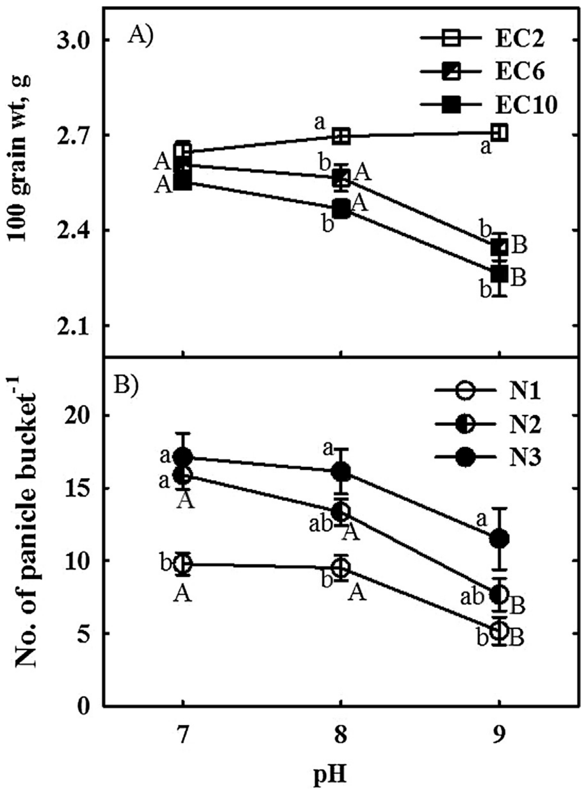

Fig. 3. Effects of soil pH and salinity on 100 grain wt (grain weight per 100 grains) (A), and No. (number) of panicle (B) of rice (*Oryza sativa* L.), cultivar: FL478 grown under three nitrogen supply levels (N_1, N_2 and N_3).
Chart line with stick bar are Means with 1SE (standard error), n = 9 (buckets). At the same pH value, different lower case letters are given to those with significant difference at $P \leq 0.05$ among the three salinities (EC) without regard to nitrogen application levels (A), or among the three nitrogen application levels without regard to salinities (B). At the same salinity (A) or nitrogen level (B), different capital letters are given to those with significant difference at $P \leq 0.05$ among the three pH values. Within each comparison group, same letter means no significant difference at $P > 0.05$. No letter was marked within any comparison group if there is no any significant difference at $P > 0.05$ among any of the corresponding three treatment levels.

concentration increased as soil pH and EC increased (Fig. 4B). When soil EC was 6 dS m^{-1}, P concentration in rice shoots significantly increased as soil pH increased ($P < 0.05$). Shoot K concentrations, significantly decreased with increasing soil pH when soil EC was 6 dS m^{-1} and 10 dS m^{-1} ($P < 0.05$) (Fig. 4C). Under the same soil pH, shoot K concentrations also significantly decreased with increasing soil EC ($P < 0.05$). At a N application rate of 100 kg ha^{-1} and soil pH of 9, K concentration in shoots decreased 79.2% as soil EC increased from 2 dS m^{-1} to 10 dS m^{-1}. Shoot K concentration decreased 67.3% as soil pH increased from 7 to 9 when the N application rate was 200 kg ha^{-1} and soil EC was 6 dS m^{-1}, An increase in N application did not significantly increase shoot K concentration ($P < 0.05$) at each soil pH and soil EC (Fig. 4D).

3.2.2. Calcium, magnesium and sulfur

Nitrogen application did not significantly affect shoot Ca and Mg concentrations at the same soil pH ($P < 0.05$) (Fig. 5A & C). Under low N (100 kg N ha^{-1}) or low salinity (EC was 2 and 6 dS m^{-1}), shoot Ca concentrations significantly decreased with increasing soil pH ($P < 0.05$) (Fig. 5A & B). Shoot Ca concentrations were significantly decreased with increasing EC only when soil pH was 7 and 8 ($P < 0.05$) (Fig. 5B). Magnesium concentration in shoots was significantly increased with increasing pH under the same N application levels or the same soil EC (Fig. 5C & D). When soil pH was 7, shoot Mg concentrations significantly increased with increasing N application levels ($P < 0.05$), When soil pH was more than 8, shoot Mg concentrations were also significantly increased with increasing soil EC ($P < 0.05$).

Sulfur concentrations in shoots significantly increased ($P < 0.05$) with increasing EC at the same soil pH. Similarly, in some treatments, shoot S concentrations significantly increased as soil pH increased at the same soil EC (Fig. 6A). Shoot S concentration significantly increased with N application rate ($P < 0.05$) when soil pH was 8 and soil EC was 6 dS m^{-1}.

3.2.3. Sodium and chlorine

Sodium concentrations in shoots increased significantly ($P < 0.05$) with increasing pH at N application level 100 kg ha^{-1} or at the same soil EC (Fig. 5E & F) as would be expected. For example, when the N application rate was 100 kg ha^{-1}, the shoot Na concentrations increased 3.0 times as pH increased from 7 to 9. Shoot Na concentration also significantly increased as EC increased, at the same soil pH (Fig. 5F), likely due to increased solution Na at elevated pH. Furthermore, when N application rates were 100 kg ha^{-1}, 300 kg ha^{-1} and soil EC was 6 dS m^{-1}, shoot Cl concentrations also significantly increased with increased pH ($P < 0.05$) (Fig. 6B). Under the same soil pH, shoot Cl concentrations significantly increased with increasing EC ($P < 0.05$). There were no significant effects of N application on Na and Cl at the same soil pH and the same soil EC.

3.2.4. Iron, copper, manganese and zinc

The effects of different soil pH, EC and N application rate on four micronutrients concentration in rice are shown in Fig. 7. At the same N application rates, soil pH or soil EC had no effect on shoot Fe, Cu, Mn and Zn concentrations. However, N had a positive effect, increasing micronutrients, in some treatments. When soil EC was 6 dS m^{-1}, shoot Fe concentrations significantly increased as soil pH increased ($P < 0.05$) (Fig. 7A). Conversely, when soil EC was 6 and 10 dS m^{-1}, shoot Cu concentrations significantly decreased as soil pH increased ($P < 0.05$) (Fig. 7B). Shoot Mn concentrations significantly decreased with increasing pH at all EC levels. ($P < 0.05$), and also with increasing EC at all pH levels (Fig. 7C). Shoot Zn concentration significantly decreased with increasing soil pH ($P < 0.05$), or with increasing soil EC only when soil pH was 7 and 8 (Fig. 7D).

3.2.5. Interaction of pH, EC and nitrogen on rice nutrients absorption

The results of the interactive effect of N, pH and EC on various nutrients absorption in rice are shown in Table 4. Nitrogen application significantly influenced shoot K, Na, Ca and Mg concentrations but there was no significant influence of N on other nutrient concentrations in rice ($0.01 < P \leq 0.05$). Soil EC significantly influenced most nutrient concentrations (except P), and soil pH significantly influenced all nutrient concentrations (Table 4). The interactive effects of soil pH and EC significantly impacted most nutrient concentrations (except P and Cu). However, the interactive effects of N and soil EC were significant only for Zn concentrations. Similarly, the interaction of N and pH on shoot nutrients was not significant. The interaction of N, EC and pH significantly affected only shoot Na concentration ($P < 0.05$).

4. Discussion

4.1. Impact of pH, EC and nitrogen on rice yield and yield components

Many studies have demonstrated that rice yield and shoot weight significantly decrease with an increase in salinity (Murtaza et al., 2000; Abdelgadir et al., 2005; Hakim et al., 2014). Our results demonstrate the impact of salinity and alkalinity superimposed stress resulting in a greater decrease in plant growth and decreased

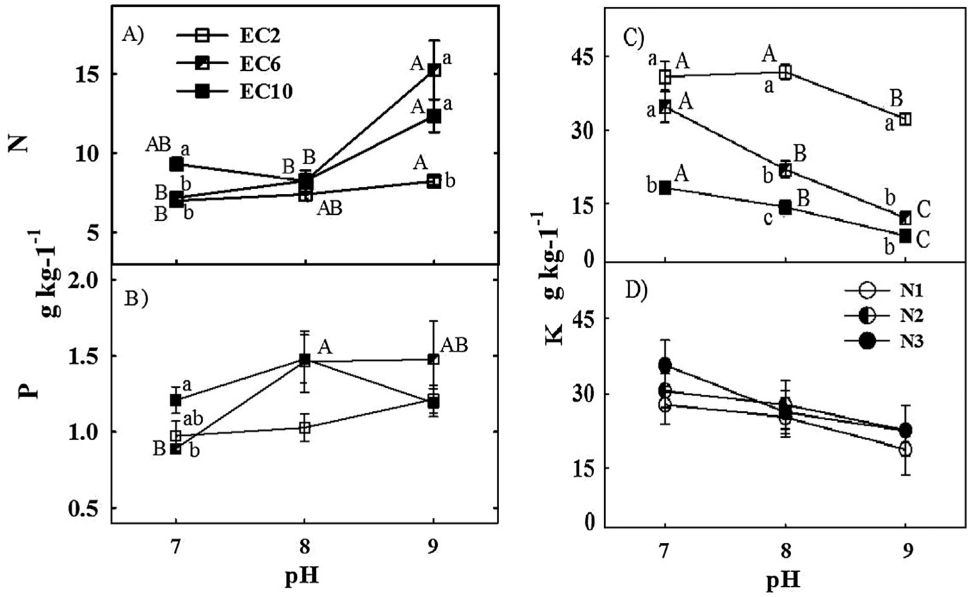

Fig. 4. Effects of soil pH and salinity on Nitrogen (A), Phosphorus (B) and Potassium (C & D) concentrations in shoot of rice (*Oryza sativa* L.), cultivar: FL478 grown under three nitrogen supply levels (N_1, N_2 and N_3).
Chart line with stick bar are Means with 1SE (standard error), n = 9 (buckets). At the same pH value, different lower case letters are given to those with significant difference at $P \leq 0.05$ among the three salinities (EC) without regard to nitrogen application levels (A, B & C), or among the three nitrogen application levels without regard to salinities (D). At the same salinity or nitrogen levels, different capital letters are given to those with significant difference at $P \leq 0.05$ among the three pH values. Within each comparison group, same letter means no significant difference at $P > 0.05$. No letter was marked within any comparison group if there is no any significant difference at $P > 0.05$ among any of the corresponding three treatment levels.

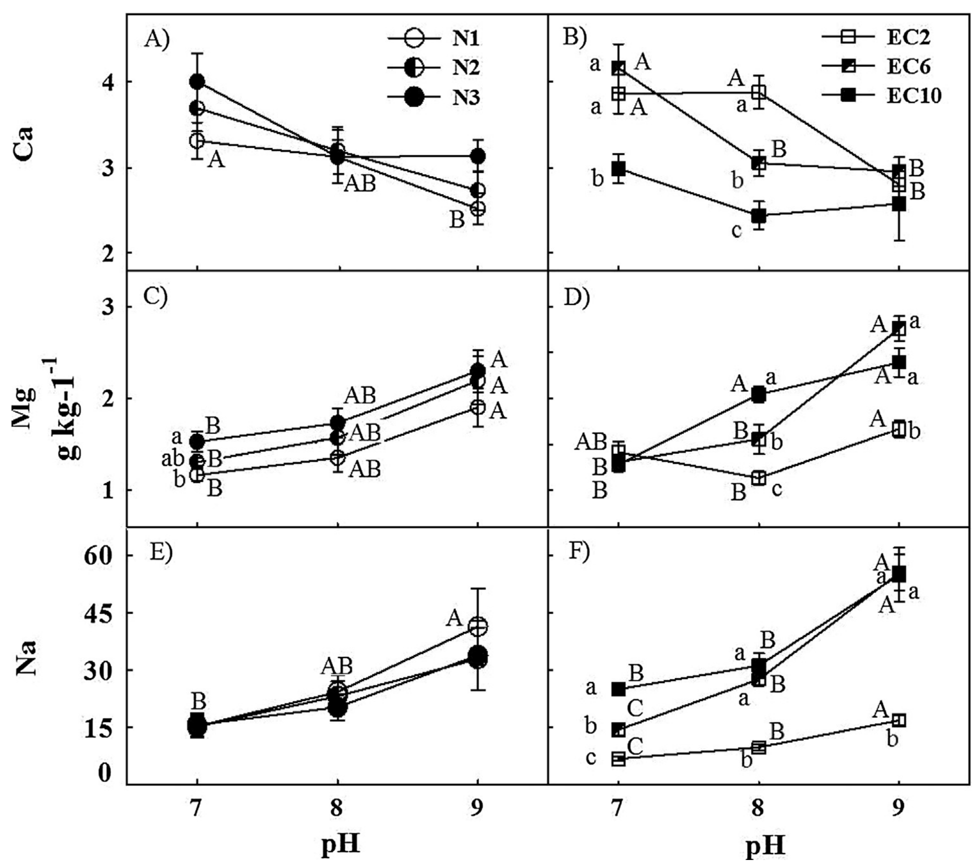

Fig. 5. Effects of soil pH and salinity on Calcium (A & B), Magnesium (C & D) and Sodium (E & F) concentrations in shoot of rice (*Oryza sativa* L.), cultivar: FL478 grown under three nitrogen supply levels (N_1, N_2 and N_3).
Chart line with stick bar are Means with 1SE (standard error), n = 9 (buckets). At the same pH value, different lower case letters are given to those with significant difference at $P \leq 0.05$ among the three nitrogen application levels without regard to salinities (A, C & E), or among the three salinities (EC) without regard to nitrogen application levels (B, D & F). At the same nitrogen levels or salinity, different capital letters are given to those with significant difference at $P \leq 0.05$ among the three pH values. Within each comparison group, same letter means no significant difference at $P > 0.05$. No letter was marked within any comparison group if there is no any significant difference at $P > 0.05$ among any of the corresponding three treatment levels.

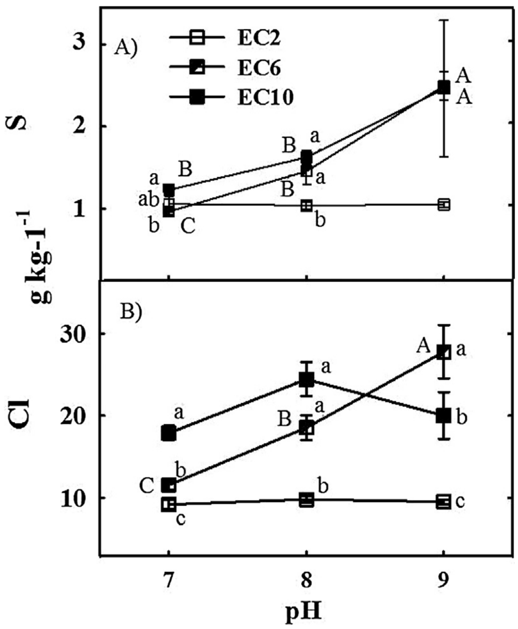

Fig. 6. Effects of soil pH and salinity on Sulfur (A) and Chlorine (B) concentrations in shoot of rice (*Oryza sativa* L.), cultivar: FL478 grown under three nitrogen supply levels (N_1, N_2 and N_3).
Chart line with stick bar are Means with 1SE (standard error), n = 9 (buckets). At the same pH value, different lower case letters are given to those with significant difference at $P \leq 0.05$ among the three salinities (EC) without regard to nitrogen application levels. At the same salinity, different capital letters are given to those with significant difference at $P \leq 0.05$ among the three pH values. Within each comparison group, same letter means no significant difference at $P > 0.05$. No letter was marked within any comparison group if there is no any significant difference at $P > 0.05$ among any of the corresponding three treatment levels.

grain yield, shoot weight, 100-grain weight and panicle number of rice (Figs. 2 and 3). Increased N application was beneficial under non-saline and saline conditions at pH 7 and non-saline conditions at pH 8, There was no benefit to adding higher levels of N beyond 100 kg ha^{-1} under saline conditions at pH 8 or under any conditions at pH 9 (Fig. 2A & C).

High pH and salinity are usually regarded as the major obstacles for crop/plant production in saline-sodic soil. Some previous studies have reported that rice was sensitive to salinity (Maas and Hoffman, 1977; Shannon et al., 1998), rice seedling survival and growth were significantly reduced under salinity stress, tillering and panicle initiation were more sensitive to salinity than other growth stages, salinity affected other yield components and eventually decreased yield (Zeng and Shannon, 2000; Shereen et al., 2005; Rad et al., 2012). However, these negative effects were often greater under alkaline salt stress due to increased soil pH. Our experimental results showed by stepwise regression analysis that soil pH very significantly and adversely impacts grain yield and was the major factor impacting yield in our experiment ($R^2 = 0.565$, $P < 0.001$). Also 100-grain weight, shoot weight and panicle number of rice were adversely impacted by pH ($P < 0.001$). Soil EC also significantly influence grain yield, 100-grain weight and shoot weight ($P < 0.001$) (Table 3). When high pH and high EC stresses were superimposed on each other, the negative effects were compounded (Figs. 2 B, D & 3 A).

Nitrogen is as one of the most important plant nutrients in arable agriculture. Background values of N were lower than optimal in saline-sodic soils in Western Songnen Plain of Northeast China, and N was the critical limiting nutrient for rice growth in that saline-sodic region (Huang et al., 2016). In our study N application above 100 kg ha^{-1} significantly improved grain yield, shoot weight and

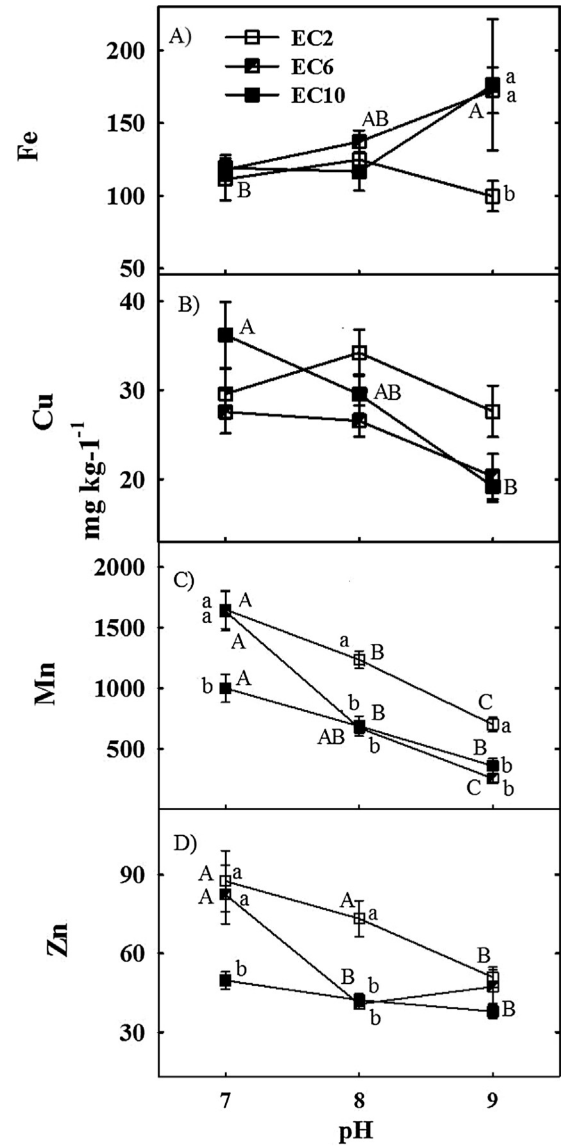

Fig. 7. Effects of soil pH and salinity on Iron (A), Copper (B), Manganese(C) and Zinc (D) concentrations in shoot of rice (*Oryza sativa* L.), cultivar: FL478 grown under three nitrogen supply levels (N_1, N_2 and N_3).
Chart line with stick bar are Means with 1SE (standard error), n = 9 (buckets). At the same pH value, different lower case letters are given to those with significant difference at $P \leq 0.05$ among the three salinities (EC) without regard to nitrogen application levels. At the same salinity, different capital letters are given to those with significant difference at $P \leq 0.05$ among the three pH values. Within each comparison group, same letter means no significant difference at $P > 0.05$. No letter was marked within any comparison group if there is no any significant difference at $P > 0.05$ among any of the corresponding three treatment levels.

the number of panicle ($P < 0.05$) under non saline and saline conditions at pH 7 (Figs. 2 and 3). These results are consistent with earlier reports that N application was beneficial even at higher levels of soil salinity/sodicity (Murtaza et al., 2000). At pH 7 in our study the relative response to N under saline conditions was comparable to that under non-saline conditions. In this instance additional N is beneficial but the response is comparable to that under non-saline conditions. The recommendation for rice at soil pH of 7 is thus to use the same N levels under saline conditions (below soil water EC of 10 dS m^{-1}) as needed under non-saline conditions. It has been documented that a positive response to N exists with other crops, however in general less N is required under saline as compared to non-saline conditions, as found for pepper (Semiz et al., 2014) and corn (Lacerda et al., 2016) due to reduced plant growth. In our

Table 4
Summary of statistical test significances of the effects of nitrogen level (N), salinity (EC) and pH on shoot nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), chloride (Cl), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) of rice (*Oryza sativa* L.), cultivar: FL478, respectively, and their interactions (marked with * between the effects) on the effects.

	N	P	K	Ca	Mg	S	Na	Cl	Fe	Cu	Mn	Zn
Nitrogen	n.s.	n.s.	*	*	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
EC	***	n.s.	***	***	***	***	***	***	**	*	***	***
pH	***	*	***	***	***	***	***	***	*	**	***	***
Nitrogen*EC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Nitrogen*pH	n.s.	n.s	n.s	n.s.	n.s.	n.s.	n.s	n.s	n.s.	n.s.	n.s.	n.s.
EC*pH	**	n.s.	**	**	***	***	***	**	*	n.s.	**	*
Nitrogen*EC*pH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.

Significance: ***, at $P < 0.001$, **, at $0.001 < P < 0.01$ and *: $0.01 < P \leq 0.05$. n.s. means no significance at $P > 0.05$.

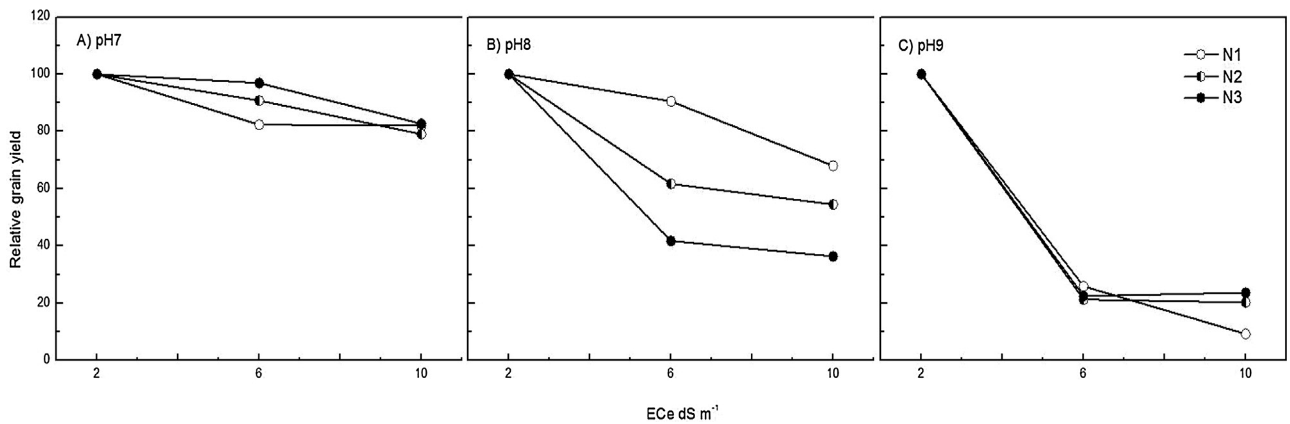

Fig. 8. The changes of relative rice grain yield with ECe under different soil pH.

experiment at pH 8 there was a positive response to increased N only under non-saline conditions. At EC 6 and 10 dS m^{-1} at pH 8 there was no significant response to added N. Nitrogen requirements are thus lower under high pH at all salinity levels and at pH 8 under saline conditions. Thus the recommendation at pH 8 and 9 under saline conditions is to apply much less N (100 kg ha^{-1}) than would be optimal under control conditions (300 kg ha^{-1}). We did not evaluate N application below 100 kg ha^{-1}.

The relative salt tolerance of rice grain yield using the model of Maas and Hoffman (1977) is shown in Fig. 8. In this instance the control was calculated as the non-saline treatment for each N and pH. For example in Fig. 8A we displayed 3 different salt tolerance curves based on 3 different N application levels. At soil pH 7, the yields increased with N application rate but the relative yield data was relatively insensitive to N application (Fig. 8A). Rice appears moderately tolerant as there is limited yield loss at EC 2 and 6, especially at high N application. At soil pH 8, the relative yields decreased faster with EC increase when compared to pH 7, meaning that rice appears more sensitive to EC (Fig. 8B). Also with increased N application rice appeared to be more sensitive to salinity (as observed by Semiz et al., 2014 for pepper). At pH 9 (Fig. 8C) rice is much more sensitive to salinity as compared to pH 7. Also N levels did not affect relative yield at pH 9. These data show that not only is elevated pH the most detrimental factor in our study but it also increased sensitivity to EC effects. These data indicate that the data from salt tolerance tables are not good predictors of plant response to EC when other stresses are operating. Other stresses (such as a moderate increase in pH from 7 to 8) are not always known and may serve to at least partially explain discrepancies in crop salt tolerance in different studies. These discrepancies may be better understood if the absolute yields in the controls of salt tolerance studies are compared to optimal yields for that crop.

4.2. Impact of pH, EC and nitrogen on nutrients absorption of rice

A comprehensive understanding of the interaction between salinity and plant nutrient is of great importance to improve crop productivity and nutrient use efficiency in saline-sodic soil. Under saline/sodic conditions, salinity could not only reduce crop growth but also might lead to nutritional disorders, and a nutrient imbalance could also result in an increase in the plant's internal requirement for the essential element (Grattan and Grieve, 1993). In most soils (saline or non-saline), N accounts for about 80% of the total mineral nutrients absorbed by plants (Marschner, 1995), and it is usually the most growth-limiting plant nutrient. Some studies have noted that N application improved plant growth and yield at a given salinity level (Papadopoulos and Rendig, 1983; Soliman et al., 1994), but the N application rate determined as best under optimal conditions was not necessary for crops under saline conditions because decreased biomass reduced total N requirement under higher saline condition (Semiz et al., 2014).

Our results showed that soil pH and soil EC significantly influenced mineral nutrients accumulated in rice shoots (Figs. 4–7). According to Table 4 and Figs. 4–7, the effects of soil pH and EC on mineral nutrients absorption by rice could be summarized as the following five types: 1) High pH or high EC reduced rice growth and biomass which lead to excessive accumulation of some mineral nutrients, such as nitrogen and iron (Fe); 2) The presence of high pH and EC caused some salt ions such as Na, Cl and S to accumulate in rice shoots; 3) The presence of high pH and EC caused nutrient concentrations of K, Ca and Mg to decrease in rice shoots; 4) Due to high pH and to a less or extent high EC in the soil, availability of Mn and Zn were reduced (likely due to decreased ion activity in the soil solution as a result of both increased soil adsorption and mineral precipitation) and this caused their concentrations to decrease in rice shoots; 5) The concentrations of some nutrients were little influenced by soil pH and soil EC, such as P and Cu. The experimental results (Table 4) further showed that the above nutrient concentrations in rice were significantly influenced by pH, EC (except P) and their interaction (except P and Cu) ($P < 0.001$, $P < 0.01$ or $P < 0.05$).

Plant response to N application rates was different than the response to pH and EC. There were no significant effects of N application on other mineral nutrient concentrations in rice under the same soil pH or soil EC. The interaction of N and EC also had no significant effects on absorption of other mineral nutrients (Table 4). Nitrogen application promoted the absorption of K in rice only when soil pH was 9 and soil EC was 10 dS m^{-1}(Fig. 4C). In many field studies, researchers set out to test the hypothesis that N applications can improve yields under saline conditions (Grattan and Grieve, 1993, 1999). Our results have confirmed that increased N under saline and non-saline conditions increased rice yield. Therefore, nitrogen application is an important measure to improve agricultural productivity in saline soil but the need under high pH soil is greatly reduced.

In saline-sodic soils, high soil EC imposed osmotic and ionic stresses on rice, and high soil pH also influenced the uptake and transport of essential nutrients such as K^+ and Ca^{2+}. The relationship between nutrients absorption and rice grain yield became more complex under high soil pH and high soil EC. Therefore, further comprehensive studies are merited on the interaction between plant essential nutrients and both soil pH and soil EC.

5. Conclusions

In summary, rice grain yield and shoot weight significantly decreased with increasing soil pH and soil EC, and under low pH and EC significantly increased with increasing N application rates. High pH and high EC in soil significantly influenced mineral nutrient absorption in rice shoots. High soil pH and soil EC stresses were superimposed on each other, the negative effects on rice were compounded. The results by stepwise regression analysis showed that soil pH very significantly and adversely impacts grain yield and was the major factor impacting rice grain yield ($R^2 = 0.565$, $P < 0.001$). Nitrogen application did not alleviate the negative effects of soil pH and soil EC on rice growth, grain yield and nutrients absorption. Nitrogen application of 100 kg ha^{-1} appears sufficient for rice production under elevated pH, but higher N levels would be required as pH and EC decrease with reclamation.

Acknowledgements

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