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EFFECT OF DIFFERENT CALCIUM CONCENTRATIONS IN SOIL ON SURVIVAL PERCENT AND UPTAKE OF Na^+ AND Cl^- IONS BY RICE PLANT

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ABSTRACT

Salinity is a stress factor affecting the production of crop in many regions. Calcium can reduce Na^+ transport to shoots in rice. Two greenhouse experiments were conducted in Faculty of Agriculture, Cairo University, Egypt, during 2015 growing season of rice to evaluate the effect of different calcium concentrations on survival percent along with uptake of Na^+ and Cl^- ions by two varieties of rice (*Oryza sativa* L.) differing in salt-tolerance. The first experiment was undertaken to study the effect of different calcium concentrations on survival percent of IR28 (salt-sensitive) and Nona Bokra (Salt-tolerant) seedlings which were transferred to salinized nutrient solution containing 0.5% NaCl and a variable calcium concentrations at 4, 40, 100 and 200 ppm; plants were grown up to 40 days. The second experiment investigated the effect of different calcium concentrations on growth, uptake and transport of Na^+ and Cl^- ions in the two rice varieties differing in salt-tolerance. The seedlings were transferred to salinized nutrient solution containing 0.5% NaCl and calcium ion concentrations at two levels, 4 and 40 ppm. Plants were harvested at 0, 1, 3, 5 and 7 days from salinization. The results indicated that the salt-tolerant variety (Nona Bokra) survived for more than 40 days under exposure to 0.5% NaCl when calcium concentration of the culture solution ranged from 40 to 200 ppm Ca^{++}. The low calcium ion concentration (4 ppm) depressed the growth of plants at 5 and 7 days after salinization. In Nona Bokra, the shoot had less sodium and Cl than the root. This implies that the salt tolerance of Nona Bokra may be attributed to the restricted translocation of Na^+ and Cl^- from the root to the shoot. Sodium as well as cloride content in the shoot of IR28 was more than twice that of Nona Bokra. An adequate amount of Ca^{+2} tended to lower the salt injury caused by high levels of salinity in rice plants. The effect of calcium ion on salt tolerance varied greatly between Nona Bokra and IR28 varieties.

INTRODUCTION

Salinity is a stress factor affecting the production of crop in many regions, especially in arid and semi-arid regions. Approximately, over 800 million hectares in the world are affected by salinity and sodicity **(Munns, 2005)**. Salinity leads to > 35% decrease in crop productivity in the world **(Tanji, 2002)**. More than 2 billion people consider rice (*Oryza sativa*) is a staple food in Asia, Africa and latin America **(Khush, 2005)**. Young seedlings of rice are sensitive to salinity in the most rice cultivars **(Lutts et al 1995)**. Cultivars have mechanisms to protect themselves from salinity. Calcium is considered one of these mechanisms which regulate physiological processes to adjust to stresses **(Kader and Lindberg, 2008; Kader and Lindberg, 2010)**.

Hussein et al (2010) reported that Ca is important to restrict the entry of Na into the cells of plant. In rice root, high levels of Ca^{++} are important for keeping high root uptake and shoot of Ca^{++} and K^+ in saline soils as to decrease the damage of salinity in rice, **(Song et al 2006)**. Calcium is essential to maintain the integrity and selectivity of cell membrane **(Aslam et al 2000)**. Also, Ca^{++} can minimize the leakage of cytosolic potassium **(Maathuis and Amtamann, 1999)**. In addition, Ca^{++} can decrease the uptake and transport of Na.

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Damage of salinity occurs due to extra transport of Na^+ and Cl^- to the shoots **(Yeo et al 1999)**. It was reported that Ca^{++} can reduce Na^+ transport to shoots in rice **(Gong et al 2006)**. The objective of this study was to determine the influence of increasing Ca^{++} concentration in the root medium on survival, uptake and transport of Na^+ and Cl^- in two rice varieties differing in salt tolerance.

MATERIAL AND METHODS

Experiment (I): Effect of different calcium concentrations on survival percent

The experiment was conducted to elucidate the influence of increasing Ca^{+2} concentration in the root medium on salt injury, and adverse effects of low Ca^{+2} concentration on survival of salt-susceptible (IR28) and salt-tolerant (Nona Bokra) varieties in saline culture medium in the green house.

IR28 (salt-sensitive) and Nona Bokra (salt-tolerant) seeds were obtained from the Plant Breeding Department of the International Rice Research Institute. Seeds were surface-sterilized with 0.1% $HgCl_2$ for 5 minutes and washed twice with distilled water and then soaked for 24h in distilled water. The seeds were germinated in petri dishes over three layers of cotton saturated with distilled water. The dishes were then covered and left in the incubator for 48 h at temperature between 28-30^oC. The germinated seeds were raised on a nylon net seedbed floating in 7 liter plastic tray containing nutrient solution **(Table 1)**. The experiment was conducted in a glasshouse. After 7 days, the seedlings which had grown to three-leaf stage were selected and transplanted into a transferable styrofoam board with 60 holes spaced 2cm apart. One seedling was transplanted into each hole and held in place by a foam. Thirty seedlings per variety were included in each replication; The treatments were replicated three times.

Table 1. Composition of nutrient solution [a]

Element	Reagent	Concentration of element (ppm)
N	NH_4NO_3	40
P	$NaH_2PO_4 . 2H_2O$	10
K	K_2SO_4	40
Ca	$CaCl_2$	40
Mg	$MgSO_4. 7 H_2O$	40
Mn	$MnCl_2. 4 H_2O$	0.5
Mo	$(NH_4)_6. MO_7 O_{24}. 4 H_2O$	0.05
B	$H_3 BO_3$	0.2
Zn	$ZnSO_4. 7H_2O$	0.01
Cu	$CuSO_4. 5H_2O$	0.01
Fe	$FeCl_3. 6H_2O$	2.0

[a] **Yoshida et al (1976).**

After growing in the nutrient solution for one day, the plants were transferred to the nutrient solution containing 0.5% NaCl and different calcium concentration at 4, 40, 100 and 200 ppm, with an initial pH of 6.0 and an electric conductivity of about 10.5 dS/m. The salinized solution were renewed once a week and the pH adjusted to 6.0 every day using 1N NaOH or 1N H_2SO_4. Also, the electric conductivity was monitored every day. Plants were grown up to 40 days in the salinized solution and the number of surviving plants recorded every day. A plant was judged dead when more than 90% of the area of the last surviving leaf blade had become necrotic and dry.

Experiment (II): Effect of different calcium ion concentrations on uptake of Na^+ and Cl^- ions

The objectives of this experiment was to investigate the role of calcium ion on uptake and transport of Na^+ and Cl^- in two rice varieties differing in salt tolerance.

The experiment was conducted, in a similar approach to the first experiment, in the greenhouse, one day after transplanting, the seedlings were transferred to salinized nutrient solution containing 0.5% NaCl and calcium ion concentrations at two levels, 4 and 40 ppm, with an initial pH of 6.0 and an electric conductivity of about 10.2 dS/m. The pH of the salinized nutrient solutions was adjusted to 6.0 every day. Electric conductivity was likewise monitored every day and the salinized nutrient solutions were renewed once a week. Plants were harvested at 0, 1, 3, 5 and 7 days after salinization. After harvest, plants were first washed with tap water several times and then rinsed in deionized distilled water three times and blotted dry. Each plant was divided into shoot and root. Fractions were dried and the dry weights recorded. Then Na and Cl contents of root and shoot were determined using procedures described in **AOAC (1990)**.

The data were statistically analyzed according to the technique of analysis of variance (ANOVA) of randomized complete block design by **Sendecor and Cochran (1990)**.

RESULTS AND DISCUSSION

Survival of seedlings

The results **(Fig. 1)** showed that the effect of salinity on rice seedlings were dependent on the concentration of calcium in the nutrient solution and the concerned variety. The medium survival percent of the salt-sensitive variety (IR28) was obtained at around 9 days but it was earlier in case of solution of 200 ppm Ca^{+2} concentration.

Considerable variabilities in the survival of individual plant seedlings were recorded within the variety. Some individual seedlings (less than 10% of the population) survived in excess of 17 days and less than 3% survived in excess of 23 days. The effect of calcium on success of salt-sensitive variety (IR28) was not great.

The salt-tolerant variety (Nona Bokra) survived for more than 40 days under exposure to 0.5% NaCl when the calcium concentration of the culture solution ranged from 40 to 200 ppm Ca^{+2} **(Fig. 1)**. The median survival for this variety at higher concentrations of calcium (40 to 200 ppm) was more than 40 days especially at 40 ppm calcium. However plants exposed to 0.5% NaCl at low calcium concentration (4 ppm) suffered greater damage and the median survival for these plants was 25 days.

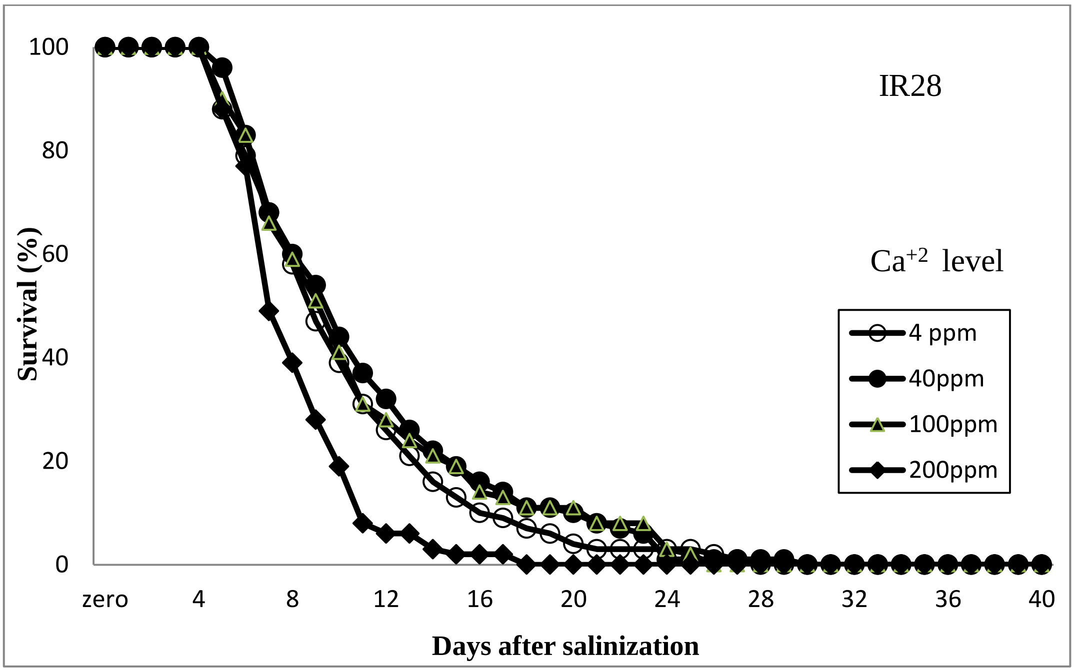
Among the seedlings there was considerable variability in the survival of individual plants within Nona Bokra variety. After salinization at calcium concentration ranged from 40 to 100 ppm Ca^{+2}, less than 10% individually died in 23 days and less than 25% at 200 ppm Ca^{+2}, while at low concentration (4 ppm) less than 10% survived in excess of 35 days.

The appropriate concentrations of calcium increased the ability of Nona Bokra variety to withstand the effects of high concentration of sodium chloride. In the presence of adequate concentration of calcium, the plants were able to survive more than 40 days with strong root systems and tiller number. On the other hand, it was observed that the plants grown at low concentration showed necrotic root tips, poor root systems and lower tiller number. However, low calcium concentration had no effect on the survival of IR28, but very high calcium concentration (200 ppm) depressed the survival of both varieties more with IR28 variety than with Nona Bokra.

In accordance with these findings, **Song et al (2006)** demonstrated that an adequate amount of Ca^{+2} tended to lower the salt injury caused by high levels of salinity in rice plants. **Cha-um et al (2012)** added that the increase in Ca^{+2} in the external solution seemed to alter greatly the salt and Na^+ tolerance of rice plants and Ca^{+2} acts as salt defense mechanisms due to Ca^{+2} sensing stomatal closure when plant subjected to salt stress.

Root and shoot dry weights

Dry weight of roots and shoots influenced by varying concentration of calcium in the external medium is presented in **Tables (2 and 3)**. The root and shoot dry weights of IR28 plants did not vary significantly at 4 and 40 ppm calcium concentrations. Although there was an increase in dry weight of shoot and little increase in the dry weight of root. In Nona Bokra variety, root and shoot growth was slow and showed increase in the weight. The low calcium ion concentration (4 ppm), compared to 40 ppm calcium, depressed the growth of plants at 5 and 7 days after salinization. The finding was similar to that reported by **Aslam et al (2001), Kader and Lindberg (2010)**.



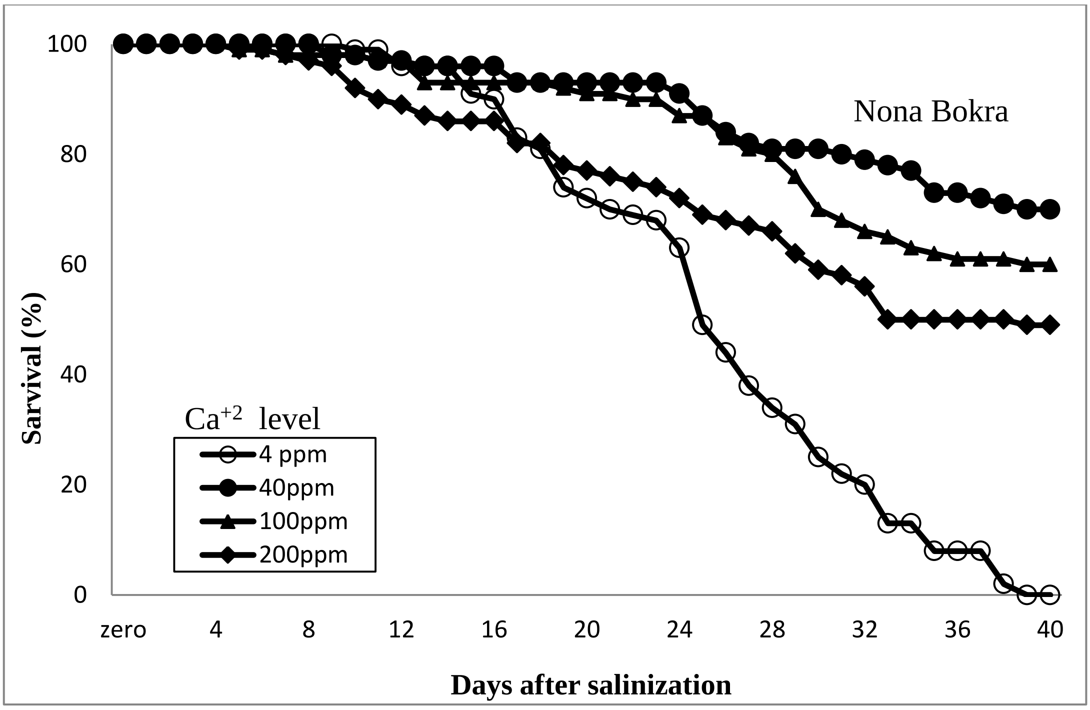


Fig. 1. Effect of calcium ion on survival percent of IR28 and Nona Bokra

Table 2. Variability in root dry weight of seedlings of IR28 and Nona Bokra under salinity and different calcium concentration

Days after Stalinization	Dry weight (g/tray)						
	IR28			Nona Bokra			Time means
	4 ppm	40 ppm	Mean variety	4 ppm	40 ppm	Mean variety	
0	0.117	0.120	0.119	0.193	0.187	0.190	0.154
1	0.164	0.168	0.166	0.232	0.253	0.243	0.204
3	0.172	0.174	0.173	0.332	0.419	0.376	0.274
5	0.169	0.170	0.170	0.360	0.420	0.390	0.280
7	0.169	0.179	0.174	0.446	0.570	0.508	0.341
Calcium mean	0.158	0.160	0.160	0.313	0.370	0.341	0.251
$LSD_{0.05}$							
Calcium	0.11						
Variety	0.16						
time	0.16						

Table 3. Variability in shoot dry weight of seedlings of IR28 and Nona Bokra under salinity and different calcium concentration

Days after Stalinization	Dry weight (g/tray)						
	IR28			Nona Bokra			Time means
	4 ppm	40 ppm	Mean variety	4 ppm	40 ppm	Mean variety	
0	0.481	0.452	0.467	0.747	0.699	0.723	0.595
1	0.638	0.605	0.622	0.930	0.955	0.943	0.782
3	0.709	0.669	0.689	1.353	1.358	1.356	1.022
5	0.793	0.738	0.765	1.493	1.696	1.595	1.180
7	0.827	0.833	0.830	2.096	2.342	2.219	1.523
Calcium mean	0.690	0.659	0.675	1.324	1.410	1.367	1.020
$LSD_{0.05}$							
Calcium	0.079						
Variety	0.045						
Time	0.046						

Sodium and chloride uptake and transport

The amount of Na^+ ions absorbed by the plants in the shoot was higher in IR28 than in Nona Bokra, which explains the susceptibility of IR28 to salinity **Tables (4 and 5)**. Additional calcium ion at 40 ppm did not decrease the translocation of Na ion from the root to the shoot in IR28 variety. However, in Nona Bokra variety absorption of Na ion was reduced both in the root and shoot; chloride ion absorption decreased significantly in both varieties **Tables (6 and 7)**.

These studies indicate generally higher uptake and translocation of Na and Cl in the root and shoot of susceptible variety IR28. Nona Bokra (Tolerant variety) appeared to tolerate high salinity by regulating salt uptake and low Na and Cl accumulation in the shoot. In the presence of inadequate concentrations of calcium, Nona Bokra plants was not greatly effective in excluding sodium and chloride ions. Plants with poor root systems, as a result of inadequate calcium, transfer relatively more quantities of Na^+ and Cl^- ions into the shoot.

Table 4. Sodium ion content (%) in root of IR28 and Nona Bokra as a subjected to NaCl (0.5%) and different Ca^{+2} concentration

Days after Stalinization	Sodium ion concentration (%)						
	IR28			Nona Bokra			Time means
	4 ppm	40 ppm	Mean variety	4 ppm	40 ppm	Mean variety	
0	0.30	0.30	0.30	0.28	0.29	0.29	0.29
1	1.56	1.56	1.56	1.75	1.69	1.72	1.64
3	1.98	1.94	1.96	2.11	2.01	2.06	2.01
5	2.35	2.39	2.37	2.29	2.15	2.22	2.30
7	2.52	2.52	2.52	2.55	2.32	2.44	2.48
Calcium mean	1.74	1.74	1.74	1.80	1.69	1.74	1.74
$LSD_{0.05}$							
Calcium	0.141						
Variety	0.045						
Time	0.046						

Table 5. Sodium ion content (%) in shoot of IR28 and Nona Bokra as a subjected to NaCl (0.5%) and different Ca^{+2} concentration

Days after Stalinization	Sodium ion concentration (%)						
	IR28			Nona Bokra			Time means
	4 ppm	40 ppm	Mean variety	4 ppm	40 ppm	Mean variety	
0	0.11	0.10	0.11	0.09	0.08	0.08	0.10
1	0.79	0.83	0.81	0.62	0.60	0.61	0.71
3	2.02	2.04	2.03	1.26	1.09	1.18	1.60
5	3.12	3.15	3.14	1.78	1.41	1.60	2.37
7	4.45	4.49	4.47	2.12	1.67	1.90	3.18
Calcium mean	2.10	2.12	2.11	1.17	0.97	1.07	1.59
$LSD_{0.05}$							
Calcium	0.050						
Variety	0.060						
time	0.083						

Table 6. Chloride ion content (%) in root of IR28 and Nona Bokra as a subjected to NaCl (0.5%) and different Ca^{+2} concentration

Days after Stalinization	Chloride ion concentration (%)						
	IR28			Nona Bokra			Time means
	4 ppm	40 ppm	Mean variety	4 ppm	40 ppm	Mean variety	
0	0.77	0.75	0.76	0.85	0.81	0.83	0.80
1	1.27	1.17	1.22	1.75	1.92	1.84	1.53
3	2.83	2.76	2.80	2.48	2.42	2.45	2.62
5	2.43	2.36	2.40	2.88	2.68	2.78	2.59
7	2.79	2.66	2.73	3.22	2.68	2.95	2.84
Calcium mean	2.02	1.94	1.98	2.24	2.10	2.17	2.076
$LSD_{0.05}$							
Calcium	0.050						
Variety	0.128						
Time	0.166						

Table 7. Chloride ion content (%) in shoot of IR28 and Nona Bokra as a subjected to NaCl (0.5%) and different Ca^{+2} concentration

Days after Stalinization	Sodium ion concentration (%)						
	IR28			Nona Bokra			Time means
	4 ppm	40 ppm	Mean variety	4 ppm	40 ppm	Mean variety	
0	1.11	1.08	1.10	0.87	0.88	0.88	0.99
1	1.46	1.59	1.53	1.26	1.30	1.28	1.40
3	3.36	3.39	3.38	1.86	1.89	1.88	2.63
5	4.59	4.69	4.64	2.89	2.63	2.76	3.70
7	6.49	6.62	6.56	3.03	2.69	2.86	4.71
Calcium mean	3.40	3.47	3.44	1.98	1.89	1.93	2.69
$LSD_{0.05}$							
Calcium	0.050						
Variety	0.056						
Time	0.074						

Although uptake of sodium as well as chloride in the roots of IR28 and Nona Bokra were comparable, the sodium as well as the chloride content in the shoot of IR28 was more than twice that of Nona Bokra. In Nona Bokra, the shoot has less sodium than the root. This implies that the salt tolerance of Nona Bokra may be attributed to the restricted translocation of sodium and chloride from the root to the shoot.

Compared to 4 ppm calcium, 40 ppm calcium has a slight depressing effect on the uptake and translocation of sodium and chloride in both the root and shoot of Nona Bokra as well as a slight improvement in the root and shoot growth. Calcium has no great effect on the growth and uptake of sodium and chloride in IR28.

These results partially agree with those of **Wu and Wang (2012)** who showed that at a high concentration of calcium chloride, rice plants absorbed and translocated relatively more potassium and less sodium than at low concentration of $CaCl_2$.

Results of this study also indicated that the effect of calcium ion on salt tolerance varied greatly between Nona Bokra and IR28.

It cannot be excluded the suggestion that the responses of salt tolerant genotype related to decrease high Na^+ accumulation in the Young leaves **(Nemati et al 2011)**. Also, **Tester and Davenport (2003)** reported that the main mechanism of salt tolerant include reducing uptake into the cytosol of Na^+ and Cl^-, and its sequestration into the apoplast or into the vacuole.

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