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NONOSMOTIC EFFECT OF POLYETHYLENE GLYCOL ON PERCENT SURVIVAL AND GROWTH OF RICE

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ABSTRACT

Salinity is one of the major environmental factors limiting crop productivity. For this reason, two greenhouse experiments were conducted in Faculty of Agriculture, Cairo University, Egypt, during the year 2015 using two rice varieties to evaluate the effects of various levels of osmotic stress caused by polyethylene-glycol 6000 (PEG) and NaCl. Furthermore, it was tested whether the inhibitory effect of salinity on growth, sodium and chloride concentration by two different varieties was greater under NaCl or PEG treatment. The first experiment was undertaken to separate osmotic and ionic aspects of salinity damage to rice (*Oryza sativa L.).*Seedlings of IR28 (salt-sensitive) and Nona Bokra (salt-tolerant) rice varieties were transferred to salinized nutrient solution containing 85 mol m^{-3}NaCl (-3.0 bars) with or without PEG 6000 (-2.0 bars, 45 gL^{-1}). Plants were grown up to 30 days in the salinized solutions. The second experiment was designed to determine the effect of salinity (85 mol m^{-3}NaCl) with or without PEG 6000 (-0.5 bar, 11 g L^{-1})on growth, uptake and transport of sodium and chloride in two rice varieties differing in salt tolerance. The results indicated that survival of salt-tolerant variety (Nona Bokra) was increased significantly by adding PEG (-2.0 bars). The addition of PEG also reduced the rate of death of rice plants compared with NaCl alone. Also, data showed that PEG 6000 (0.5 bar, 11 gL^{-1}) reduce sodium concentration in root of IR28 and Nona Bokra but its effect upon sodium concentration in shoot of the two varieties was more pronounced than the reduction of Na^+ concentration in root. Highly significant differences were obtained between zero and 11 gL^{-1} PEG. The result of this study is strongly indicated that addition of PEG dramatically lessened the toxicity of NaCl to rice seedlings.

INTRODUCTION

Rice is one of the most important cereal crop in the world. Three billion people consider rice as their stable food, yielding one – third of the total carbohydrate source. It considers as a salt – sensitive monocot **(Darwish et al 2009)**. One of the main factors that reduce plant growth in many regions in the world is soil salinity. Excessive concentrations of soluble salts, which adversely affect plant growth in the soils that existed in the arid and semiarid regions. There are many salt excessive, in salt-affected soil, especially NaCl. Also, available water in the salty soil is restricted, resulting osmotic stress **(Pagter et al 2009 and Siringam et al 2011)**.

Plant growth differs in its response to salinity. Most plants severely inhibited growth at low salinity levels **(Moisender et al 2002)**. Excessive salt affects plant physiology through ionic and osmotic stress **(Murphy and Durako, 2003)**. Soil water potential and availability of water decreases by the high concentration of salts in the root zone. This causes dehydration at cellular level and ultimately osmotic stress occurs as a result of the deficiency in available water under saline condition. An ionic imbalance may occurred by reducing the uptake of beneficial ions such as K^+, Ca^{2+} and Mg^{2+} due to the excessive of toxic ions such as Na^+ and Cl^- **(Hasegawa et al 2000)**. High Na^+ concentration adversely affected many enzymatic activities of plants **(Maathuis and Amtmann, 1999)**.

Tolerate salinity is attributed to many mechanisms. These mechanisms include minimizing Na^+ uptake by roots and/or increasing Na^+ efflux back to the soil; potassium retention in the cytosol; tis-

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sue – specific Na^+ sequestration; osmotic adjustment; intercellular Na^+ sequestration; control of xylem ion loading; oxidative stress tolerance and excluding Na^+ from the shoot **(Shabala and Munns, 2012)**. These mechanisms are grouped into three main factors: (1) tissue tolerance mechanisms, (2) osmo-tolerance and (3) sodium exclusion mechanism **(Munns and Tester, 2008)**.

This study was undertaken to examine the nonosmotic effect of PEG on survival and sodium and chloride transport under two different varieties of rice. Also to determine whether the inhibitory effect of salinity on Cl and Na content by rice plant was greater under NaCl or PEG treatment.

MATERIALS AND METHODS

Experiment 1: Nonosmotic effect of polyethylene Glycols on percent survival

In this experiment, polyethylene Glycols (PEG) was initially employed in its conventional role as an inert osmoticum, to separate osmotic and ionic aspects of salinity damage to rice seedlings. Seeds of IR28 (salt- sensitive) and Nona Bokra (salt – tolerant) varieties were obtained from the International Rice Research Institute, Manila, Philippines. 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 hr at temperatures between 25-30^{o}C. 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 the greenhouse, natural light intensity and relative humidity was approximately 60%. 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 2 cm apart. One seedling was transplanted into each hole and held in place by a foam. Thirty seedlings per variety were included in one replication; The treatments were replicated three times.

The amount of PEG needed to bring the osmotic potential of the nutrient solution to a desired level was estimated from data of **Lawlor (1970)**. The actual osmotic potential was checked with Merrill thermocouple psychrometers (No.75-1). Small strips of filter paper were saturated with PEG solution and inserted into psychrometer cups. The readings were obtained after 1.5 hr of thermal and water vapor equilibrium using a Wescor dew point microvoltmeter (HR-33T) operating in the psychrometeric-mode. The output in microvolts was converted to pascals based on calibration of each psychrometer with NaCl solutions of known water potential.

After one day from transplanting, the seedlings were transferred to salinized nutrient solution containing 85 mol m^{-3} NaCl (-3.0 bars) with or without the addition of PEG 6000 (-2.0 bars, 45 gL^{-1}). The containers were then transferred to green house at a constant temperature 29/21^{o}C (day/night). The pH of the solutions were adjusted to 6.0 daily. Solutions were renewed weekly. Plants were grown up to 30 days in the salinized solutions and the survival of the plants was followed.

Table 1. Composition of nutrient solution[a]

Element	Reagent	Concentration of element (mg/l)
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).**

Experiment 2: Nonosmotic effect of polyethylene Glycols (PEG) upon growth, sodium and chloride transport

The present experiment attempt to determine the effect of salinity (85 mol m^{-3}NaCl) with or without PEG 6000 on growth, uptake and transport of sodium and chloride in two rice varieties varying in salt tolerance. Experimental procedure for the study was same as described in experiment 1. The amount of PEG 6000 needed to bring the osmotic potential of the nutrient solution to a desired level was same as described in experiment (1).

After one day from transplanting, the seedlings were transferred to salinized nutrient solution containing 85 mol m^{-3} NaCl (-3.0 bars) with or without the addition of a variable PEG 6000 at (-0.5 bar, 11 g L^{-1}), with an initial pH of 6.0. The containers were then transferred to the green house under natural daylight. The pH of the solutions were adjusted to 6.0 every day. Plants were harvested after 6 days from salinization. After harvest, plant 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 in an oven at 80°C, then subjected to determination of Na and Cl contents, which were extracted in distilled water and analyzed, data were recorded on following aspects: (1) dry weight of root and shoot, (2) sodium and Cl content of root and shoot that were determined using procedures described in **A.O.A.C. (1990)**.

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

RESULTS AND DISCUSSION

Survival of seedlings in salinity

The survival percentage of IR28 and Nona Bokra seedlings are shown in **Fig. (1)**.

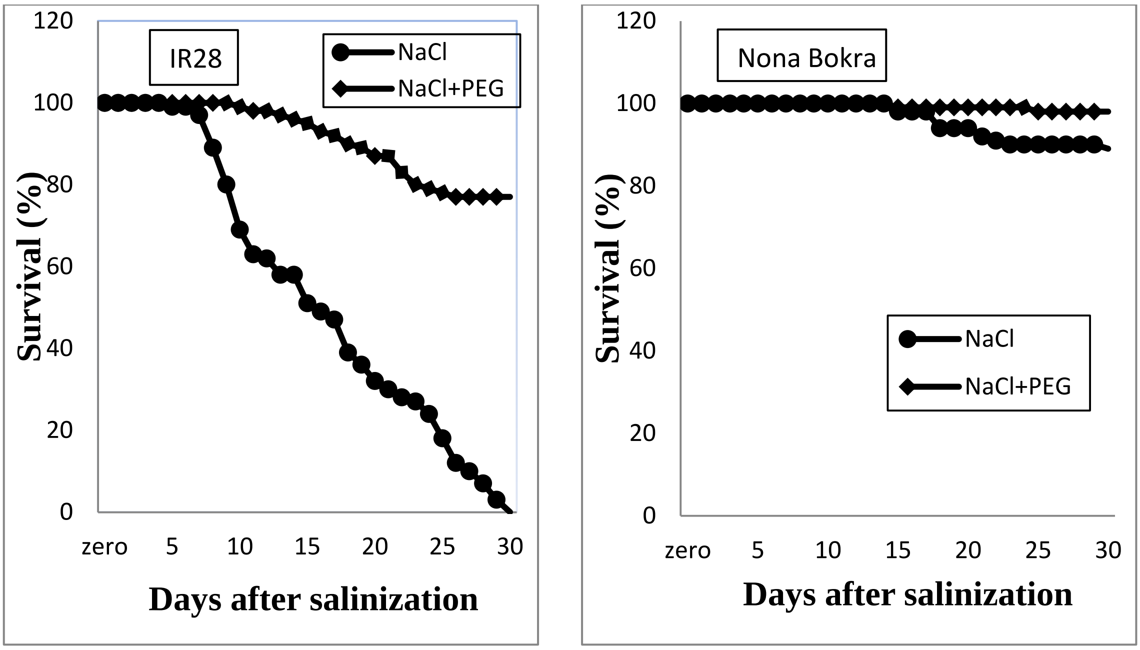

Fig. 1. Time course of survival (% alive) of IR28 and Nona Bokra seedlings salinized with NaCl (85 mol m^{-3}) with or without PEG.

Survival was increased significantly by adding PEG (-2.0 bars) although PEG addition clearly lowers the water potential of the medium, approximately double that of NaCl alone. After 30 days with salt alone, 100% of IR28 plants were recorded dead. The salt tolerant variety (Nona Bokra) survived for more than 30 days at exposure to NaCl with and without PEG. Survival of Nona Bokra was increased significantly by adding PEG (-2.0 bars) to the saline medium (85 mol m^{-3} NaCl). Addition of PEG greatly reduced the rate of death of seedlings compared with NaCl alone. After 30 days with NaCl alone, 11% of Nona Bokra seedlings were reported dead. PEG greatly reduced this value to 2%. The effect of the time of salinization on survival of IR28 and Nona Bokra was highly significant. **Claes et al (1990)** mentioned that PEG is a nonionic (neutral polymer), it has high solubility in water and low toxicity to mammals. Because it cannot penetrate the cell membrane, the composition of the cell is not affected. Polyethylene-glycol causes the mimic specific levels of water stress due to drought **(Attree and Fowke, 1993)**.

The results of this study indicated that, at 85 mol m^{-3} of NaCl, salinity damage was unlikely to be due to water stress since the combined treatment (NaCl +PEG) had the lower water potential but permitted the better survival and seems primarily as Na and Cl ion toxicity. This is a further suggestion that growth reduction of rice in saline conditions was not attributable to water stress per se. This finding is similar to that reported by **Silveria et al (2009)**.

Effects of PEG on growth, Na and Cl concentration

An observation of considerable importance in relation to the mechanism of salt injury was that the NaCl + PEG plants survived so well. They were subjected to approximately twice the osmotic stress experienced by the plants treated with NaCl alone, yet they showed far less injury. It seems, then, most unlikely that salt injury at this concentration (85 mol m^{-3}) was mediated by osmotic factors and much more probable that it was mediated by specific ion toxicity.

Table (2) summarize the data for root and shoot growth, as measured by dry weight, after 6 days of salinity stress with or without PEG. The results show that root and shoot dry weight of the two tested varieties increased significantly with the addition of 11g PEG L^{-1} to the saline medium. However, increasing the rate of dry weight of root and shoot after the addition of 22 and 44 gL^{-1} was very small and insignificant. Measurement of plant growth are important to separate the effects of the osmotic and ionic stress components, which dominate plant growth at different times **(Munns and Tester, 2008)**.

The data of Na and Cl concentration by IR28 and Nona Bokra varieties as affected by adding PEG to the saline medium are presented in **Tables (3 and 4)**. During the 6 days experimental period, PEG 6000 (11 gL^{-1} , -0.5 bar) reduced sodium concentration in root of IR28 and Nona Bokra but its effect on sodium concentration in shoot of the two varieties was more pronounced than the reduction in root sodium concentration. Polyethylene-glycol 6000 (-0.5 bar) reduced shoot sodium concentration from 3.36% to 0.90% in IR28 and from 2.36% to 0.63% in Nona Bokra. The results showed significant differences between zero and the other concentrations of PEG.

The results of this study are strongly indicated that addition of PEG dramatically lessened the toxicity of NaCl to rice seedlings. This was explained by a reduction in the uptake of Na^+ and Cl^- and the greater effect upon shoot Na^+ concentration than root Na^+ concentration. This result is in agreement with that of **Quintero et al (2008)** who reported that many mechanisms of plant tolerance under high soil salinity were related to the maintenance of low Na^+ in shoots. Exclusion of Na^+ ion and its distribution in all leaves were related to salt tolerance **(Haq et al 2009)**. Metabolism of diving and expanding cells was inhibited by high intracellular concentrations of both Na^+ and Cl^- **(Neumann, 1997)**. Osmotic effects solely cause the inhibition of germination in PEG-treated seeds and generally, ionic effects cause the difference in germination of salt-treated relative to PEG-treated seeds **(Dodd and Donovan, 1999)**.

Table 2. Shoot and root dry weight of IR28 and Nona Bokra grown for 6 days in NaCl (85 mol m^{-3}) with and without different concentrations of PEG 6000

PEG treatments (gL^{-1})	Dry weight (g/tray)					
	Root			Shoot		
	IR28	Nona Bokra	PEG-means	IR28	Nona Bokra	PEG-means
0	0.138	0.340	0.239	0.596	1.358	0.977
11	0.239	0.513	0.376	0.831	1.647	1.239
22	0.282	0.504	0.393	0.823	1.666	1.245
44	0.316	0.518	0.417	0.857	1.653	1.255
Variety - means	0.244	0.469	--	0.777	1.581	--
$LSD_{0.05}$ for						
PEG	0.110			0.070		
Variety	0.119			0.046		

Table 3. Sodium ion content (%) in root and shoot of IR28 and Nona Bokra grown for 6 days in NaCl (85 mol m^{-3}) with and without different concentrations of PEG 6000

PEG treatments (gL^{-1})	Sodium ion content (%)					
	Root			Shoot		
	IR28	Nona Bokra	PEG-means	IR28	Nona Bokra.	PEG-means
0	1.60	2.43	2.02	3.36	2.36	
11	1.28	1.45	1.37	0.90	0.63	0.77
22	1.29	1.35	1.32	0.92	0.84	0.87
44	1.13	1.23	1.18	0.71	0.62	0.67
Variety - means	1.33	1.62	-	1.47	1.11	-
$LSD_{0.05}$ for						
PEG	0.044			0.027		
Variety	0.029			0.172		

Table 4. Chloride ion content (%) in root and shoot of IR28 and Nona Bokra grown for 6 days in NaCl (85 mol m^{-3}) with and without different concentrations of PEG 6000

PEG treatments (gL^{-1})	Chloride ion content (%)					
	Root			Shoot		
	IR28	Nona Bokra	PEG-means	IR28	Nona Bokra	PEG-means
0	1.90	2.87		5.94	4.04	
11	1.83	2.46	2.15	2.59	2.46	2.53
22	1.66	2.29	1.98	2.69	2.36	2.53
44	1.52	1.99	1.76	1.99	1.99	1.99
Variety - means	1.73	2.40	--	3.30	2.71	--
$LSD_{0.05}$ for						
PEG	0.017			0.012		
Variety	0.011			0.023		

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