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SCREENING FOR SALT TOLERANCE IN MAIZE (*ZEA MAYS* L.) HYBRIDS AT AN EARLY SEEDLING STAGE

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Abstract

An efficient and simple mass screening technique for selection of maize hybrids for salt tolerance has been developed. Genetic variation for salt tolerance was assessed in hybrid maize (*Zea mays* L.) using solution-culture technique. The study was conducted in solution culture exposed to four salinity levels (control, 40, 80 and 120 m*M* NaCl). Seven days old maize seedlings were transplanted in themopol sheet in iron tubs containing ½ strength Hoagland nutrient solutions and salinized with common salt (NaCl). The experiment was conducted in the rain protected wire house of Stress Physiology Laboratory of NIAB, Faisalabad, Pakistan. Ten maize hybrids were used for screening against four salinity levels. Seedling of each hybrid was compared for their growth under saline conditions as a percentage of the control values. Considerable variations were observed in the root, shoot length and biomass of different hybrids at different salinity levels. The leaf sample analysed for inorganic osmolytes (sodium, potassium and calcium) showed that hybrid Pioneer32B33 and Pioneer30Y87 have high biomass, root shoot fresh weight and high K^+/Na^+ ratio and showed best salt tolerance performance at all salinity levels on overall basis.

Introduction

Salinization is the accumulation of water soluble salts in the soil column or regolith to a level that has a drastic impact on agricultural production, environmental health and economic welfare of the country (Rengasamy, 2006). Soil salinity is one of the most serious problems for irrigated agriculture, which drastically affect crop productivity throughout the world. This is mainly due to low precipitation and high transpiration causing disturbance in salt balance in the soil; this also renders ground water brackish and affects plant growth adversely (Rhoades & Loveday, 1990; Evans, 1998).

The impact and severity of salinity has been exacerbated by the activities of man. With the steady increase in population, especially in the under-developing countries of the world and the concomitant decline in new agriculture lands, the need to tackle these stresses is urgent (Ali *et al.*, 2002). According to Wild (2003) about 15% of the total land area of the world has been degraded by soil erosion and physical and chemical degradation including soil salinization and that global food production should increase by at least 38% by the year 2025 and 50% by the year 2050 if food supply to the growing world population is to be maintained at current levels. It is also worth mentioning here that most of suitable lands all over the world have been extensively cultivated and expansion into new areas to increase food production is rarely possible or desirable. Therefore, more efforts are needed to improve the productivity per unit area.

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High concentration of complex inorganic salts present in the growing medium, retard the growth in most of the crop plants depending on the nature of salt present, the growth stages and the salt tolerance or avoidable mechanism of the plant tissue (Ashraf *et al.,* 2002). High salinity causes both hyper osmotic and ionic stress, which results in alteration in plant metabolism including reduced water potentials, ionic imbalances and specific ion toxicity (Cramer *et al.,* 1990; Tester & Devenport, 2003).

Salinity not only affects the morphology, but also modifies the metabolisms of plants. Extent of modification depends upon cultivars, duration and intensity of stress (Khan *et al.,* 2003b; Munns & James, 2003).

Most of the crops tolerate salinity to a threshold level and above which yield decreases as the salinity increases (Khan *et al.,* 2006). Plant scientists have adopted various strategies to overcome the salinity. One of the important of them is to exploit genetic variability of the available germplasm to identify a tolerant genotype that may sustain a reasonable yield on salt affected soil (Ashraf *et al.,* 2006).

Screening of large number of genotypes of a crop is necessary to identify the salt tolerant germplasm for breeding programs to evolve the salt tolerant and high yielding crop varieties. This approach involves understanding the response of plants at different growth stages under saline conditions as reported in different crops such as maize (Khan *et al.,* 2003 a), wheat (Ali *et al.,* 2002; Khan *et al.,* 2003 b), soybean (Kamal *et al.,* 2003), sorghum (Azhar & Khan, 1997), rice (Shannon, 1998), cotton (Azhar & Ahmad, 2000). These also provide clues to breeders looking for plants of economic importance with improved salt tolerance. According to Flowers & Yeo (1995) one of the important ways to develop salt tolerant crop in shorter time is to use the variation already present in the existing crops. Screening for salinity tolerance in the field is difficult, due to spatial heterogeneity of soil physico-chemical properties and seasonal fluctuations in rainfall.

After wheat and rice, maize (Z*ea mays* L.) is the third most important cereal crop and it is grown all over the world under a wide range of environmental condition. It has become highly polymorphic and is perhaps the cultivated species that contains the greatest amount of genetic variability. Being cross pollinated, salinity tolerance may exist in maize (Paterniani, 1990).

Keeping in view this idea, present investigation was planned for screening 10 maize hybrids in a solution culture experiment at 40 to 120 m*M* NaCl. This research describes the effect of soil salinity on the germination and seedling growth of these maize hybrids. The screened material can be recommended for cultivation on salinity-hit areas.

Materials and Methods

The solution culture studies were conducted in the rain protected wire house of Plant Stress Physiology and Biochemistry Laboratory, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. Seeds of 10 different maize (*Zea mays* L.) hybrids i.e., Pioneer 3062, Pioneer 32B33, Pioneer 30Y87, Pioneer 31R88, Dekalb 919, Dekalb 979, Dekalb 922, Hycorn 984, Hycorn 11 plus and Hycorn 993 were collected from three different seed companies i.e., Pioneer, Monsanto and ICI. Healthy seed of these hybrids were sown in trays containing pre-washed sand. Separate trays were used for the germination of each hybrid. Water was sprinkled daily over these trays to maintain optimum moisture contents for seed germination.

Seven days after sowing uniform sized seedlings were transplanted in a thermopol sheet containing ½ strength Hoagland's solution (Hoagland & Arnon, 1950) and salinized with control (S_0), 40 (S_1), 80 (S_2) and 120 (S_3) m*M* NaCl. The salinity was applied in splits i.e., 40 m*M* five days after transplantation and remaining salinity was applied seven days after transplantation. The experiment was laid out in factorial CRD and each treatment was replicated three times. The culture solution was changed twice a week. The pH of nutrient solution was monitored daily. The EC of treatment solutions was maintained at desired level by topping up with culture-solution during the entire periods of study. Plants were harvested 17 days after transplantation. Harvested plants were washed with tap water once and twice with distilled water and plants were blotted dry using blotting paper and their roots and shoots were separated. Data on root and shoot length and plant fresh weight was determined. All the plant samples were dried at 65 ±2°C for 2 days in a forced air-driven oven to a constant weight and dry weight (g plant^{-1}) was determined. After drying shoot was ground, Na^+, K^+ and K^+/Na^+ were recorded. Na^+ and K^+ contents (mg g^{-1} dry weight) of shoot was determined from a 0.5g dried digested sample using a flame photometer (Jenway PFP-7).

Statistical analysis: Data were subjected to statistical analysis according to standard procedures (Steel *et al*., 1997) using 'MSTAT-C' (Russell & Eisensmith, 1983), and the methods described by Gomez & Gomez (1984). Completely randomized factorial design (factorial CRD) was employed for analysis of variance (ANOVA).

Results

Data regarding mean root length of various maize hybrids at different levels of salinity are presented in Table 1. Statistical analysis of data showed that both source of variation (maize hybrids and salinity) were highly significant for root length. The maximum mean root length was recorded in hybrid Pioneer30Y87, Pioneer32B33 and Hycorn 11 plus while minimum was noted in Dekalb922, Hycorn984 and Dekalb979. At highest salinity level maximum reduction in root length was recorded in Dekalb979 (47%) at 200 m*M* NaCl and minimum reduction was observed in Pioneer30Y87 (29%). Salinity levels means indicated that with the increase in the salinity level the root length decreased significantly. The interaction between different salinity levels and maize hybrids were found non-significant.

Shoot length was affected significantly with the increase in the salinity (Table 2). Maize hybrids mean indicated that maximum shoot length was observed in Pioneer32B33 while minimum was recorded in Dekalb979. All the other were in between these two hybrids.

Due to increase in salinity, biomass accumulation was severely affected consequently; fresh and dry weights were reduced. The data delineates the means of plant fresh weight of maize hybrids under control and different levels of salinity is presented in Table 3. Under controlled condition maximum plant fresh weight (4.54 g) was exhibited by Pioneer32B33. However, at 40, 80 and 120 m*M* salt stress it was reduced up to 4.31, 4.02 and 3.55g plant^{-1} respectively. Hybrid Pioneer30Y87, Pioneer31R88 and Pioneer32B33 successfully tolerated 120 m*M* NaCl salinity and exhibited less than 50% reduction in shoot fresh weight of seedling. At maximum level of salinization (120m*M*) Dekalb979 proved to be sensitive and shoot fresh weight was severely reduced up to 63%. Although hybrid mean differences were statistically significant but hybrid Hycorn993, Pioneer30Y87 and Pioneer31R88 were statistically non-significant with each other.

Table 1. Effect of salinity on root length of different maize hybrids at seedling stage.

Maize hybrids	Root length (cm)							
	Treatments NaCl (m*M*)							Mean
	Control (S_0) = 4	S_1= 40	% Reduc.	S_2= 80	% Reduc.	S_3= 120	% Reduc.	
Hycorn 984	18.00	15.31	15	12.70	29	9.96	45	14.00 c
Dekalb 979	16.73	15.99	4	11.33	32	8.80	47	13.22 c
Dekalb 922	16.93	16.55	2	12.53	26	10.06	41	14.02 c
Pioneer 32B33	19.76	19.40	2	17.43	12	10.97	44	16.89 a
Pioneer 3062	15.63	14.41	8	12.63	19	9.10	42	12.95 c
Pioneer 31R88	18.20	17.18	6	14.36	21	11.53	37	15.32 b
Pioneer 30Y87	19.93	19.91	0.1	16.56	17	14.06	29	17.62 a
Hycorn 993	18.06	17.68	2	15.20	16	11.00	39	15.49 b
Hycorn 11 plus	21.03	17.58	16	15.59	26	12.63	40	16.71 a
Dekalb 919	19.85	16.85	15	14.50	27	11.06	44	15.57 b
Mean	**18.51 a**	**17.16 b**		**14.29 c**		**10.92 d**		

LSD at $p \leq 0.05$
Maize hybrids (H) = 1.07
Salinity levels (S) = 0.68
Interaction (H x S) = Non-significant
Means sharing same letters do not differ significantly at the 5% level of significance
% Reduc. = % reduction over control

Table 2. Effect of salinity on shoot length of different maize hybrids at seedling stage.

Maize hybrids	Shoot length (cm)							
	Treatments NaCl (m*M*)							Mean
	Control (S_0) = 4	S_1= 40	% Reduc.	S_2= 80	% Reduc.	S_3= 120	% Reduc.	
Hycorn 984	23.33	22.66	3	19.80	15	18.53	21	21.08 de
Dekalb 979	23.35	21.23	9	16.80	28	14.13	39	18.88 f
Dekalb 922	27.60	25.63	7	22.13	20	20.43	26	23.95 c
Pioneer 32B33	31.13	30.76	1	29.00	7	27.40	12	29.58 a
Pioneer 3062	28.93	27.13	6	24.86	14	22.13	24	25.77 b
Pioneer 31R88	28.20	26.10	7	23.50	17	22.63	20	25.11 b
Pioneer 30Y87	28.09	26.93	4	24.33	13	21.93	22	25.32 b
Hycorn 993	23.70	23.20	2	19.33	18	17.53	26	20.94 de
Hycorn 11 plus	25.83	22.13	14	20.02	22	18.83	27	21.71 d
Dekalb 919	23.80	23.03	3	19.00	20	16.30	32	20.53 e
Mean	**26.39 a**	**24.88 b**		**21.87 c**		**19.98 d**		

LSD at $p \leq 0.05$
Maize hybrids (H) = 0.75
Salinity levels (S) = 0.47
Interaction (H x S) = Non-significant
Means sharing same letters do not differ significantly at the 5% level of significance
% Reduc. = % reduction over control

Table 3. Effect of salinity on plant fresh weight of different maize hybrids at seedling stage.

Maize hybrids	Plant fresh weight (g $plant^{-1}$)							
	Treatments NaCl (m*M*)							Mean
	Control (S_0) = 4	S_1= 40	% Reduc.	S_2= 80	% Reduc.	S_3= 120	% Reduc.	
Hycorn 984	3.08 ghi	2.88 i	6	2.21 lm	29	1.33 pqr	57	2.37 e
Dekalb 979	2.98 hi	2.52 jk	16	1.78 n	40	1.11 r	63	2.10 f
Dekalb 922	3.76 cd	3.39 efg	10	2.33 klm	38	1.65 no	56	2.78 c
Pioneer 32B33	4.54 a	4.31 ab	5	4.02 bc	12	3.55 def	22	4.11 a
Pioneer 3062	3.38 efg	3.21 gh	5	2.11 m	38	1.31 qr	61	2.50 de
Pioneer 31R88	4.13 b	3.37 efg	18	2.55 jk	38	2.10 m	49	3.04 b
Pioneer 30Y87	3.69 de	3.25 fgh	12	2.88 i	22	2.55 jk	31	3.09 b
Hycorn 993	4.03 bc	3.69 de	8	2.86 i	29	1.56 nopq	61	3.03 b
Hycorn 11 plus	3.40 efg	2.81 ij	17	2.45 kl	28	1.45 opq	58	2.52 d
Dekalb 919	3.37 efg	2.99 hi	11	2.37 klm	30	1.62 nop	52	2.58 d
Mean	**3.64 a**	**3.24 b**		**2.55 c**		**1.82 d**		

LSD at $p \leq 0.05$
Maize hybrids (H) = 0.14
Salinity levels (S) = 0.09
Interaction (H x S) = Significant
Means sharing same letters do not differ significantly at the 5% level of significance
% Reduc. = % reduction over control

Data regarding mean plant dry weight of various maize hybrids under control, 40, 80 and 120 m*M* of NaCl salinity is presented in Table 4. Plant dry weight showed the same pattern as in fresh weight. Dry weight of plant when compared on the basis of percent reduction under different levels of salinity in comparisons of their respective control, it was observed that dry weights reduced to varying degree among these hybrids. The highest reduction was observed in hybrid Dekalb979 where degree of reduction was increased from 51 to 62% at 80 to 120m*M* of NaCl. At 120m*M* NaCl, 4 maize hybrids (Dekalb979, Hycorn984, Pioneer3062 and Hycorn993) exhibited more than 50% reduction in their dry weight while the remaining 6 hybrids maintained less than 50% reduction. The maximum decrease in shoot dry weight was observed in Dekalb979 and minimum in Pioneer32B33.

The results showed that there was substantial increase in sodium content in all the hybrids with increase in the salinity levels (Fig. 1a). At highest level of salinity maximum Na^+ uptake was recorded in Dekalb979 which is closely followed by Pioneer3062 and Pioneer31R88 while minimum was recorded in Pioneer32B33. The trend in case of potassium was almost reverse, showing decreased K^+ content in all the hybrids (Fig. 1b). Pioneer32B33 was successful in maintaining high level of K^+ at all the salinity levels.

Calcium uptake decreased with increase in the salinity levels (Fig. 2a). Non-significant difference was found in hybrids mean for Ca^{2+} content. The interaction between maize hybrids and different salinity levels was also found to be non-significant. The increasing uptake of Na^+ with increase in the salinity levels resulted in a decrease of K^+/Na^+ and Ca^{2+}/Na^+ ratios (Fig. 2b, 3). The highest potassium contents at higher salinity level had resulted in maintaining higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in hybrids Pioneer32B33 and Pioneer30Y87, showed better performance under saline conditions. The interaction between maize hybrids and salinity levels were non-significant. From the data it is clear that salt tolerant hybrids maintained higher level of K^+ as compared to Na^+ while accumulation of Na^+ was more in the shoot of salt sensitive hybrids. In general, hybrids with higher plant fresh and dry weights, and content of K^+ in their shoots had shown more salt tolerance.

Discussion

This study showed the existence of an impressive variation in tolerance to increasing NaCl treatments during the early growth stages. The maize hybrids responded varyingly under saline conditions. The growth performance of two maize hybrids (Pioneer32B33 and Pioneer30Y87) was comparatively better as compared to others.

Salinity tolerance is obviously necessary at whole plant level through the whole life cycle to seed production in grain producing species. It has been shown in several crops that tolerance at the seedling stage also reflects the enhanced salinity tolerance at the adult plant level. This has been exploited with success, as a means for selecting enhanced salinity tolerance in maize (Ashraf & McNeilly, 1990), pearl millet (Kebebew & McNeilly, 1994), in several forage grass species (Ashraf *et al.*, 1986), and in lucerne (Al-Khatib *et al.*, 1993). Maiti *et al*., (1996) reported that variation in maize at early seedling stage in response to salinity reflects potential grain yield at maturity. This implies that for preliminary selection for salinity tolerance in these species, screening of seedling is a productive method, assuming that variability at the seedling stage is genetically based.

Table 4. Effect of salinity on plant dry weight of different maize hybrids at seedling stage.

Maize hybrids	Plant dry weight (g $plant^{-1}$)							
	Treatments NaCl (m*M*)							Mean
	Control (S_0) = 4	S_1= 40	% Reduc.	S_2= 80	% Reduc.	S_3= 120	% Reduc.	
Hycorn 984	0.44 cd	0.40 def	9	0.33 ghi	24	0.21 lmn	51	0.34 cd
Dekalb 979	0.43 cd	0.30 ijk	31	0.21 lmn	51	0.16 n	62	0.28 e
Dekalb 922	0.43 cde	0.36 fgh	15	0.27 jkl	38	0.23 lm	47	0.32 cd
Pioneer 32B33	0.54 a	0.52 ab	4	0.47 bc	13	0.40 def	26	0.48 a
Pioneer 3062	0.50 ab	0.32 ghij	36	0.26 kl	49	0.18 mn	64	0.32 d
Pioneer 31R88	0.45 cd	0.32 ghij	28	0.36 fghi	20	0.26 kl	42	0.34 c
Pioneer 30Y87	0.44 cd	0.43 cde	2	0.36 fgh	18	0.30 hijk	31	0.38 b
Hycorn 993	0.51 ab	0.42 cde	18	0.35 fghi	32	0.21 lmn	60	0.37 b
Hycorn 11 plus	0.43 cd	0.30 hijk	30	0.32 ghij	25	0.23 lm	46	0.32 cd
Dekalb 919	0.44 cd	0.37 efg	14	0.22 lm	49	0.24 lm	46	0.32 cd
Mean	**0.46 a**	**0.37 b**		**0.32 c**		**0.24 d**		

LSD at $p \leq 0.05$
Maize hybrids (H) = 0.03
Salinity levels (S) = 0.02
Interaction (H x S) = Significant
Means sharing same letters do not differ significantly at the 5% level of significance
% Reduc. = % reduction over control

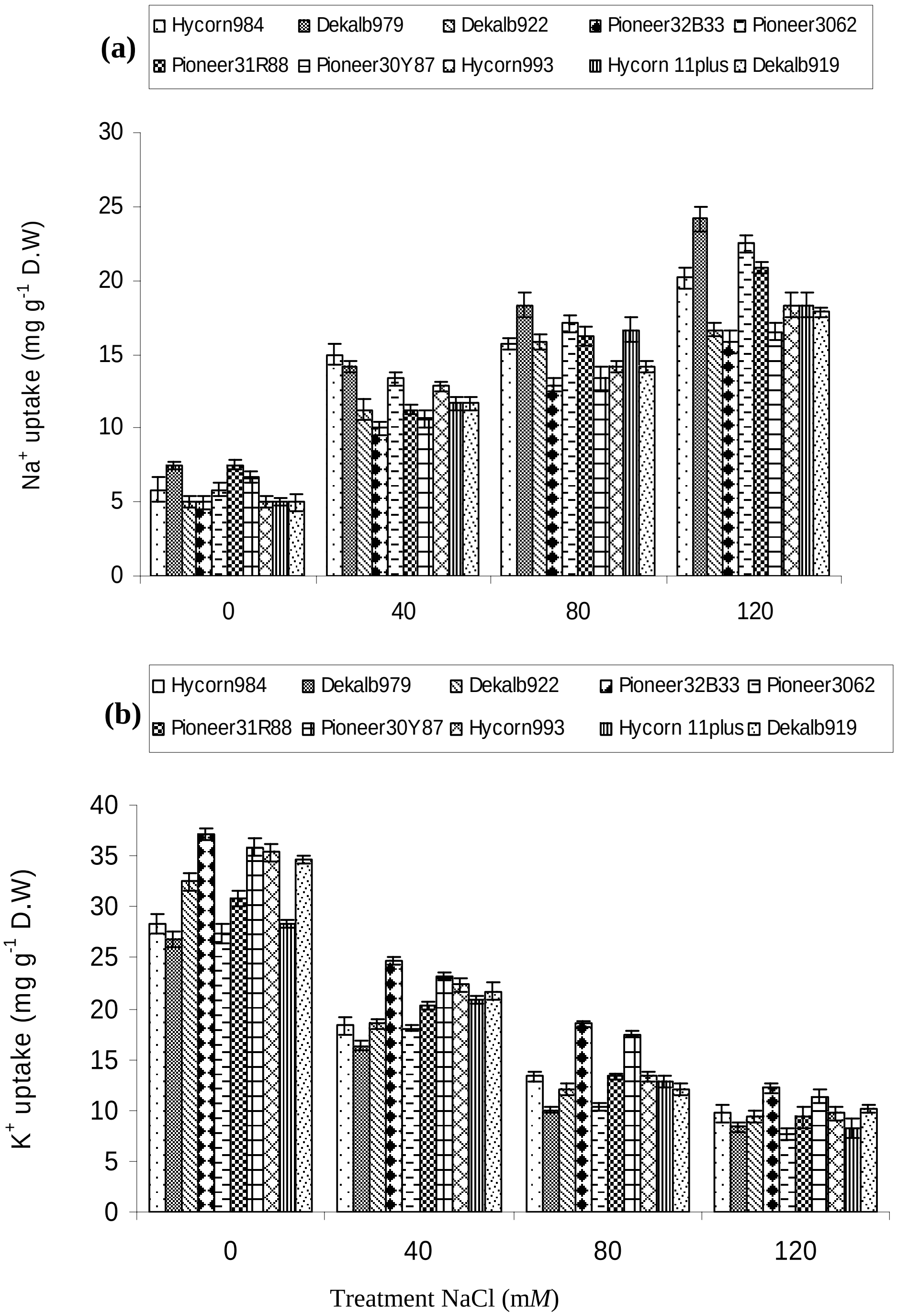

Fig. 1. Effect of different salinity levels on Na^+ (a) and K^+ (b) uptakes in maize hybrids.

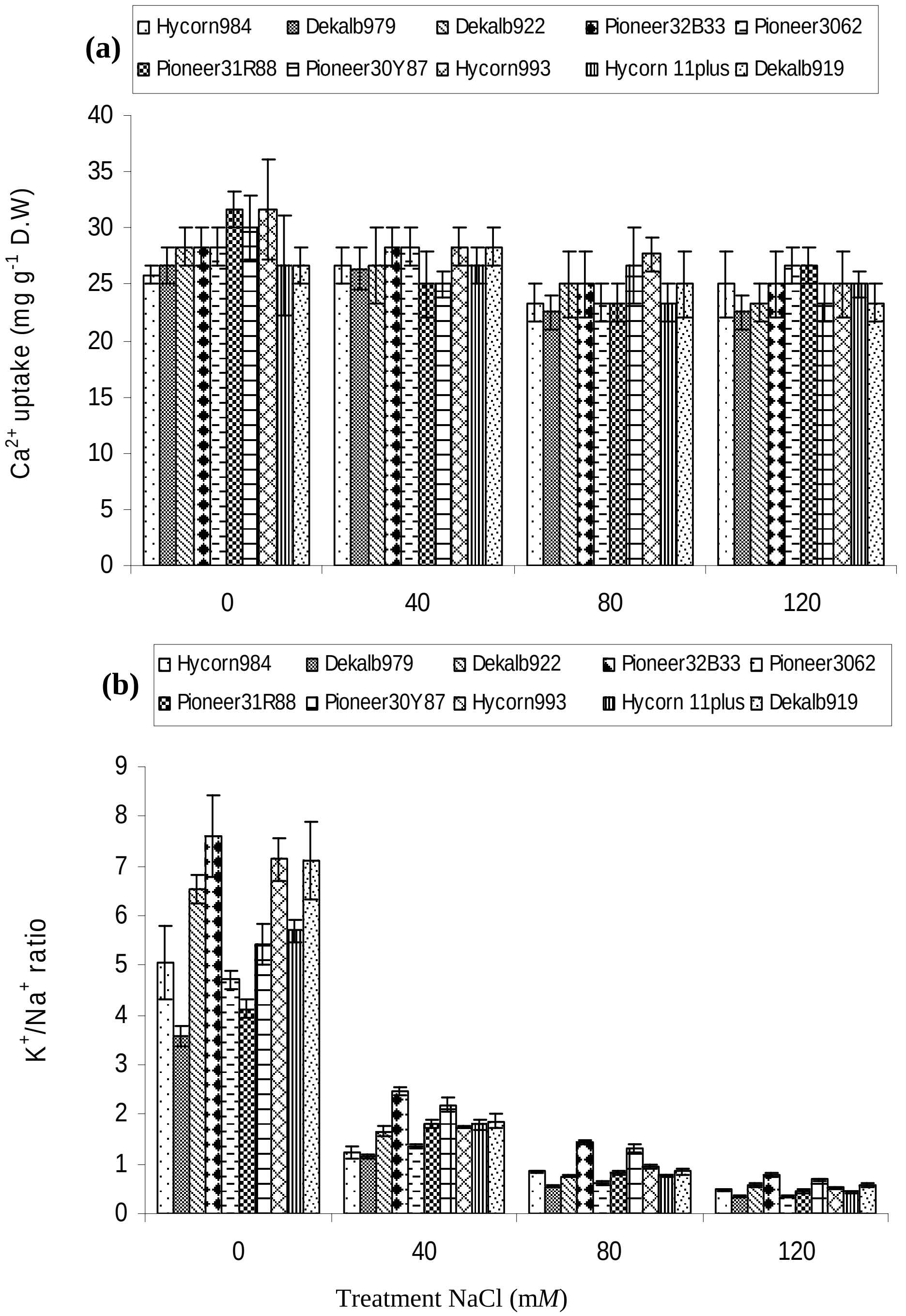

Fig. 2. Effect of different salinity levels on Ca^{2+} (a) contents and K^+/Na^+ ratio (b) in maize hybrids.

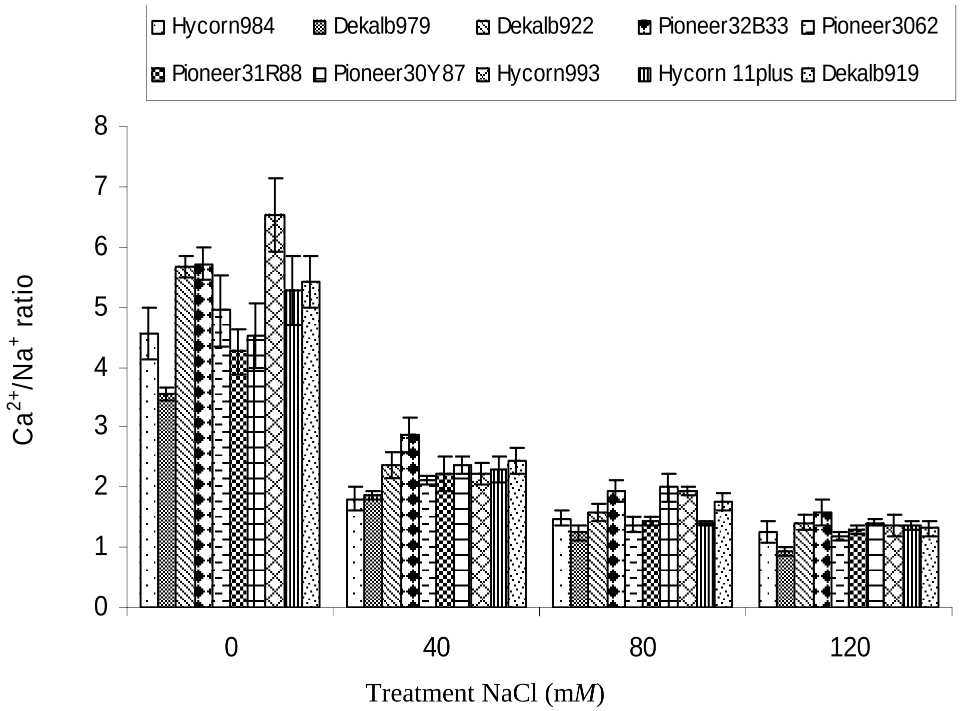

Fig. 3. Effect of different salinity levels on Ca^{2+}/Na^{+} ratio in maize hybrids.

Root is the plant organ which supplies all the nutrients from growth medium to growing plant and has direct contact with the medium so rooting behavior provides the useful information regarding the salt tolerant potential of the plants. In the present investigation, root growth was severely affected due to salinity (Table 1). It is reported that root growth is sensitive to high salt concentrations in the medium. That is why roots are rapidly reduced or prevented by salinity (Cramer *et al*., 1988; Ashraf *et al.,* 2005).

Increasing salinity is accompanied by a significant reduction in shoot length (Table 2), plant fresh and dry biomass (Table 3, 4). Similar results were reported by Mohammad *et al.* (1998) in tomato. In the present study, almost all the maize hybrids responded varyingly to imposition of different salinity levels. Maize hybrids Pioneer32B33, Pioner30Y87 and Hycorn993 showed better performance in term of plant fresh and dry weights and proved to be salt tolerant to lower and higher levels of salinity. Similar results were reported by Meloni *et al*., (2001) for cotton and Sarwar & Ashraf (2003) for wheat.

Potassium is essential for plant survival in saline habitats. It contributes in osmotic adjustment and overall water balance of plants. Sodium transport from the environment to cytoplasm of plant cell is a passive process. It depends on electrochemical potential gradient of Na^{+} and presence of Na^{+} permeable channels in the plasma membranes, which allow Na^{+} permeation. Regulation and selectivity of such channel seems to be responsible for Na^{+} exclusion in many salt tolerant plants (Jacoby, 1999). This differential selectivity of plasma membrane may be contributing factor in sensitivity/tolerance of these hybrids.

High salt (NaCl) uptake competes with the uptake of other nutrient ions, especially K^+, leading to K^+ deficiency. High NaCl induces increase in the Na^+ and decrease in Ca^{2+} and K^+ in plants (Khan *et al.*, 2000 a; Khan, 2001). In the present study Na^+ concentration (Fig. 1a) increased in all the maize hybrids whereas with the increase in the salinity and was higher in Dekalb979 and Pioneer3062 (salt sensitive), while presence of comparatively less amount of Na^+ in Pioneer32B33 and Pioneer30Y87 (salt tolerant hybrids) could be due to its less uptake in root and genetic variability. This could be due to their adaptive character towards saline environments. Similar observations were reported by Khan *et al*., (1992) in case of sorghum, Ashraf *et al*., (1991, 1998) for wheat and rice. In all the hybrids K^+ and Ca^{2+} contents (Fig. 1b, 2a) decreased with increasing salinity levels, the effect was higher in Dekalb979 and Pioneer3062. Salt tolerance has been associated with higher K^+ and Ca^{2+} contents because of their involvement in osmotic regulation and competitive effect with Na^+ (Ashraf *et al.*, 2006). However, salt tolerance is not simply a matter of ion avoidance or accumulation rather a regulation of ions induces osmotic adjustment to avoid nutrient imbalance in tissue leading to further disturbances in plant metabolisms.

Calcium is an essential element in all the plants (Marschner, 1995). The ability of calcium to form intercellular linkages gives it an important role in maintaining the integrity and structure of membrane and cell wall (Hanson, 1984). Ca^{2+} is also used as a second messenger in many signal transduction pathways with in the cell (Bush, 1995).

In the present study, salinity decreased the Ca^{2+} (Fig. 2a) uptake of plant. There is abundant evidence that salinity alters the ion transport and contents of plants. It reported that uptake of Ca^{2+} from soil solution may decrease because of ion interaction, precipitation and increase in ionic strength that reduce the activity of Ca^{2+} (Garg & Gupta, 1997). The ability of plant genotypes to maintain higher level of K^+ and Ca^{2+} and low levels of Na^+ with in the tissue is one of the key mechanisms contributing to expression of high salt tolerance. In most cases salt tolerant genotypes are capable of maintaining higher K^+/Na^+ ratios in tissues (Mansour, 2003; Zeng *et al.*, 2003).

The results of the present study indicate that increasing attention should be paid to high uptake of K^+ by root when a maize hybrid is tested for salt stress tolerance. Among the measurement needed for reliable ranking of hybrids for salt stress tolerance, an important emphasis should be given to the ability of biomass production and ion contents. The genotype Pioneer32B33 and Pioneer30Y87 with the highest tolerance Dekalb979 and Pioneer3062 with the largest susceptibility to salt stress are currently being investigated for characterization of molecular mechanisms explaining the differential expression of salt stress tolerance within the maize hybrids.

Conclusion

From the results it can be concluded that screening can be done through laboratory experiments using accumulation of plant biomass and K^+/Na^+ ratio under saline conditions. Our results provide guidelines for the selection of salt tolerant maize hybrids and this information is relevant and very important to breeders and plant physiologists interested in improving salt tolerance of maize. A refinement of current screening tool could be desirable to facilitate germplasm evaluation. The screened material can be used to evolve high yielding salt tolerant maize hybrids or can directly be introduced for cultivation on saline areas.

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