EVALUATION OF SOME MAIZE HYBRIDS UNDER DIFFERENT SALINITY CONDITIONS

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ABSTRACTS

Evaluation of maize hybrids to salinity tolerance is an important objective in breeding programs. Laboratory experiments were carried out at the laboratory of Seed Technology Department to study the effects of salinity on seed germination and seedling growth of five hybrids of maize i.e. SC 10, SC 123, TWC 314, TWC 320 and TWC 324. Seeds were treated during germination with either distilled water or one of the four concentrations of either NaCl or CaCl2 salts in addition to a combination of both salts by in a 1:1 ratio. Seed viability and seedling characteristics were measured. Results showed that hybrid SC 10 gave the highest germination percentage, seedling vigor index, first count, cleoptile length, root length and dry weight followed by hybrid SC123. The longest root was recorded for hybrid SC 123, but the shortest one was for hybrid TWC 320.. Results of SDS-PAGE protein electrophoresis indicated that there were wide differences among hybrids in number and position of bands in each treatment. Three markers were shown to be associated with salt treatment CaCl2 with molecular weight of 95 KDa at 4000ppm for all hybrids under study, except for hybrid SC 123, 30 KDa at 8000 ppm for all hybrids and 15 KDa at 6000 and 8000ppm for all hybrids except for SC 10. While one marker was associated with salt treatment NaCl with molecular weight of 85 KDa in SC 10. Both salts decreased values of the studied characters, but NaCl gave higher values than CaCl2. Results indicated that SC 10 is more tolerant to salinity stress than SC 123, TWC 314 and TWC 324, while TWC 320 is considered a sensitive hybrid to salinity stress. It could be concluded that a better understanding of physiological responses to salinity conditions may help in programs, which are involved in the improvement of salt tolerance of crop cultivars.

Key words: Maize, Germination, Seedling characters, Salinity stress, SDS-PAGE.

INTRODUCTION

Maize (Zea mays L.) is one of the important crops, which serves as food and oil for human consumption, feed for livestock and poultry; and raw material for agro-based industries. Salinity tolerance may exist in maize (Paterniani, 1990). Salinity causes not only differences between the mean yield and the potential yield, but also causes yield reduction.

Salinity delays the onset, reduces the rate, and increases the dispersion of germination events, resulting in reducing plant growth and final crop yield (Ashraf and Foolad, 2005). Seeds are particularly vulnerable to stress encountered between sowing and seedling establishment, while plant salt tolerance usually increases with plant ontogeny. Soil salinity may affect the germination of seeds either by creating osmotic potential external

to the seeds preventing water uptake or through the toxic effects of Na and Cl ions on germinating seed (Khajeh-Hosseini et al 2003).

A CALL COMMENTS OF SECURITIONS

Germination and seedling stage of plant life cycle is more sensitive to salinity than the adult stage (Ashraf et al 1986). Effect of salinity at different growth stages in wheat, sorghum and cowpea was investigated and it was found that the early seedling period was the most sensitive one in all the crops and reduction in growth was observed which decreased with increase of salinity (Shalhevet 1995). In maize, Na and Cl accumulation in root and shoot increased as a result of 50 to 200 mM NaCl treatment, while K⁺ content in root and shoot decreased in salinity levels at all the three ages. Beck et al (2004) found that accumulation of Na increased and strong inhibition of K and Ca accumulation in the root, stem and leaves with NaClinduced salinity was observed.

The main objective of the present study was to evaluate the growth pattern of five maize hybrids under two t, pes of salts i.e. sodium chloride (NaCl), calcium chloride (CaCl₂) and a combination of the two salts using four concentrations of the salts.

MATERIALS AND METHODS

Seeds of five maize hybrids, namely TWC 314, TWC 320, TWC 324, SC10, and SC123 were used in this study. Two salts were used: sodium chloride (NaCl), calcium chloride (CaCl₂) and a combination of the two salts. These salts were used in four concentrations i.e. 2000, 4000, 6000, and 8000 ppm.

One hundred seeds of each hybrid were germinated in Petri dishes. Normal seedlings were counted according to the international rules of the International Seed Testing Association (ISTA 1993). Germination percentage was calculated according to Krishnasamy and Seshu (1990).

Germination % = Number of normal seedlings x100 Number of tested seed

First count: It was recorded at the fifth day and the final percentage was recorded at the end of the experiment according to ISTA (1996).

Germination rate: four replicated dishes, each with 25 seeds were used for each treatment and were placed in an incubator. The germination rate was estimated according to Timson (1965) as modified by Khan and Unger (1984) so that seedling shoot and root length were measured after 7 days of germination. Twenty-five seedlings from each petri dish were randomly selected, then shoot and root length were recorded. Seedling fresh weight was determined, then seedling was placed in oven for drying for 24 hrs at 70°C and dry weight of seedling was determined.

Seedling vigor index was calculated according to ISTA (1985) as follows: Seedling vigor index = seedling length x germination percentage

Electrophoresis of total soluble proteins: Electrophoresis methods have been proved to be extremely useful tool in variety identification (Cook 1999). Soluble proteins were extracted from seeds and SDS-PAGE was conducted according to the protocol described by Laemmli (1970).

Statistical analysis: Results were statistically analyzed using the oneway analysis of variance (ANOVA) as described by Snedecor and Cochran (1981). Means were compared by LSD at 5 %.

RESULTS AND DISCUSSIONS

Table (1) revealed that there were significant differences between the five studied genotypes in germination percentage and seedling vigor index. Hybrid SC10 gave the highest germination percentage followed by SC123, while the lowest value was recorded for TWC 320, which was the lowest, also, in seedling vigor index and germination rate. In contrast, SC123 was the highest in these two traits.

Results showed different response of these cultivars to the source of salt and salt concentrations. Sources of salt affected the three characters and were significantly different. Treatment of NaCl had high germination percentage, germination rate, and seedling vigor index. The combination of NaCl and CaCl₂ gave higher effect on these characters than the treatment by each salt alone.

Germination percentage, germination rate, and seedling vigor index were reduced significantly by increasing the concentration of the salt, where 2000 ppm gave the highest values of these traits. While, higher concentration (8000 ppm) was associated with the lowest values. These results were in agreement with Al-Ansari (2003) who found that germination percentage and germination rate were decreased by increasing salt concentration.

The interaction between the source of salt and the salt concentration showed that the highest germination percentage was recorded by the mixture of the two salts at 2000, 4000, and 6000 ppm. While, at 8000 ppm, the lowest germination percentage observed with CaCl₂. Hybrid TWC 314 recorded the highest germination percentage (100%) when treated with 2000 or 4000 ppm of NaCl, while SC 123 recorded the lowest germination percentage when treated with 4000 ppm of NaCl (38%).

Data presented in Table (2) revealed that SC123 recorded the highest values of cleoptile length, radical length and fresh weight. While, TWC320 has the lowest cleaptile and radical length. Hybrid TWC324 had the lowest fresh weight. There were small differences between the five genotypes in

Table1. Effect of cultivar (A) source of salt (B) and salt concentration (C) on germination percentage, germination rate and seed on vigor index.

Treatment	Germination %	Germination rate	Seedling vigor index
Cultivar (A)			
TWC 314	82.80	74.70	1083.0
TWC 320	57.20	43.33	674.7
TWC 324	77.53	71.18	1044.0
SC 10	90.24	74.33	1552.0
SC 123	87 <u>.4</u> 7	77.30	1413.0
LSD (5%)	0.945	1.093	31.68
Sources of Salt (B)			
NaCl	83.27	74.03	1335.0
CaCl ₂	82.08	69.45	1264.0
Na ⁺ + Ca ⁺⁺	71.80	61.02	861.3
LSD (5%)	0.734	0.847	24.54
Salt conc.(ppm) (C)		•	
0.00	97.20	8 5,65	2194.0
2000	84.20	75,25	1327.0
4000	78.87	68.98	1015.0
6000	73.38	59.28	776.3
8000	61.60	51.67	454.0
L.S.D (5%)	0.948	1.093	31.68

Table 2. Effect of cultivar (A) source of salt (B) and salt concentration (C) on cleoptile length, radical length, fresh weigh, and dry weight.

Treatment	Cleoptile length (cm)	Radical length (cm)	Fresh weight (g)	Dry weight (g)
Cultivar (A)				
TWC 314	4.260	7.916	0.294	0.041
TWC 320	4.216	6.910	0.322	0.041
TWC 324	4.834	7.676	0.285	0.041
SC 10	6.437	10.160	0.424	0.056
SC 123	4.956	10.530	0.324	0.051
LSD (5%)	0.159	0.220	0.013	0.0014
Sources of Salt (B)	• * .			
NaCi	5.041	10.06	0.376	0.050
CaCl ₂	5.623	9.003	0.355	0.048
Na ⁺ + Ca ⁺⁺	4.158	6.853	0.259	0.040
LSD (5%)	0.123	0.171	0.010	0.0011
Salt conc.(ppm) (C)				
0.00	8.051	15.85	0.556	0.067
2000	5.771	9.50	0.347	0.053
4000	4.681	7.71	0.300	0.044
6000	3.604	6.24	0.246	0.038
8000	2.596	3.89	0.199	0.027
L.S.D (5%)	0.159	0.220	0.013	0.0014

dry weight. The highest dry weight was recorded for SC10. Source of salt showed significant differences in cleoptile length, radical length, fresh weight and dry weight. The highest value for cleoptile length was associated with CaCl₂ treatments. On the other hand, NaCl treatments recorded the highest radical length, fresh weight, and dry weight. In contrary, the lowest values of these traits were achieved by NaCl and CaCl₂ combination treatments

In response to salt stress, cleoptile length, radical length, fresh weight, and dry weight were significantly decreased with increasing salt concentration. These results are in agreement with those reported by Hussain *et al* (2009).

Table (3) presents the values of the first count, root/shoot ratio, and seedling growth rate. The first count showed significant differences between the studied genotypes. Hybrid SC123 recorded the highest value for the first count (74.53), while, TWC 320 had the lowest value (44.49). For root/shoot ratio, SC10 recorded the highest value (2.016) but TWC 324 recorded the lowest value. Seedling growth rate ranged between 2.423 in SC123 and 1.592 for T.W320.

Type of salt showed the highest value for the first count in NaCl treatment with no significant difference with CaCl₂ treatment. But the combination of the two salts gave the lowest value. Sodium chloride recorded the highest values for root/shoot ratio and seedling growth rate with significant differences with the other treatments. The lowest value of root/shoot ratio was recorded by CaCl₂, while, the lowest seedling growth rate was obtained via the combination of the two salts.

The first count and seedling growth rate values significantly decreased by increasing salt concentration compared with the control. The highest value for root/shoot ratio was recorded when 8000 ppm concentration was used without significant difference with 6000 ppm treatment. But the lowest ratio was obtained with 4000 ppm without significant difference with 2000 ppm.

The SDS-PAGE electrophoretic patterns for water soluble proteins in maize under stresses of NaCl, CaCl₂ and their combination are shown in Figs (1, 2 and 3) and Tables (4, 5, and 6). Maximum number of 16 bands was detected with a molecular weight M.W ranging from 205 to 20 KDa, where 12 of them were monomorphic and the rest were polymorphic. A band of molecular weight 85 KDa was present in SC10 hybrid and was absent in the other hybrids, which could be considered as a marker for this hybrid and band with M.W of 70 KDa which could be considered as a marker for TWC 324.

Table 3. Effect of cultivar (A) source of salt (B) and salt concentration (C) on the first count, root/shoot ratio, and seedling growth rate.

Treatment	First Count	Root/Shoot ratio	Seedling growth rate
Cultivar (A) TWC 314	66.98	1.881	1.741
TWC 320 TWC 324	44.49 61.04	1.881 1.529	1.592 1.790
SC 10 SC 123	74.53 57.60	2.016 1.604	2.423 2.157
LSD (5%)	1.557 ag 1.55.		0.044
CaCl ₂	31 36 <mark>63.64</mark> 8 350 3. 3868 63.63 500 50 3 505 55.52 3 3 83	2.069	2.156 2.095
LSD (5%)	1.206	0.058	0.034
Salt conc.(ppm) (C) 0.00		2.021	3.415
2000 and assessed the 4000 discount gatificated	59.80	1.660	2.186 1.770
6000	<i>-</i> 53.82	1.768	1.403
8000 any gariture tweet	42,67 specification		0.929

Table 4. SDS-PAGE of total proteins extracted from the leaves of five maize hybrids under NaCl and CaCh treatments.

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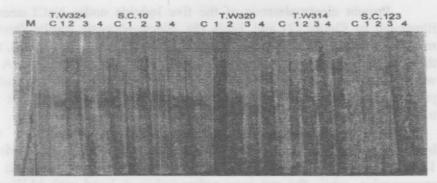


Fig 1. SDS- PAGE protein banding patterns for seed proteins of maize hybrid under salinity with NaCl + CaCl2 salts.

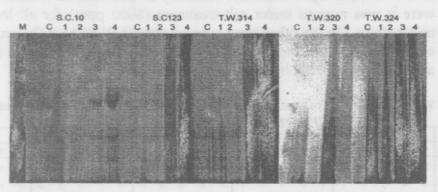


Fig 2. SDS-PAGE protein banding patterns for seed proteins of maize hybrids under salinity with NaCl salt.

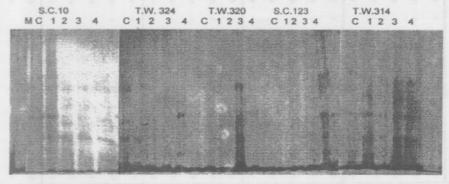


Fig 3. SDS-PAGE protein banding patterns for seed proteins of maize hybrids under salinity with CaCl2 salt.

Protein electrophoresis of the five hybrids under NaCl stress are illustrated in Figure (2) and Table (5). Electrophoresis results showed a maximum number of 16 bands of M.W ranging from 140- to15 KDa, where three of them were monomorphic and the rest were polymorphic. A band with mw of about 20 KDa was absent in the control of four hybrids and present under stress, which may be considered as a marker for NaCl tolerance.

Results of electrophoresis under CaCl₂ stress for the five maize hybrids are shown in Figure (3) and Table (6). Total number of bands were 12 bands of M.W ranging from 105 to 10 KDa. Two bands were monmorphic, while the rest were polymorphic. A band with M.W of 95 KDa could be considered as a positive marker for salt stress tolerance because it was present under salt treatment (4000ppm) for all hybrids under study, except for SC123. Similarly a band with M.W of 30 KDa were it was present under salt treatment (8000 ppm) for all hybrids under study. On the other hand, a band with M.W of 15 KDa could be considered as a positive marker associated with salt tolerance at (6000 and 8000ppm) for all hybrids, except for SC 10.

Table 5. SDS-PAGE of total proteins extracted from the leaves of five maize genotypes under NaCl treatments.

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135	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
115	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
95	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	-	+	
90	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
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Table 6. SDS-PAGE of total proteins extracted from the leaves of five

maize genotypes under CaCl2 treatments.

		SC10 TWC 324 TWC														320 SC123								77710 214							
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In conclusion, three markers were associated with CaCl2 tolerance while one marker associated with NaCl. Tolerance. Similar conclusions were recorded by Zeid (2006) who studied the effect of salinity stress on the metabolic activity of maize seedling and found five new bands having molecular weights of 18,17,16,15, and 14 KDa under salt stress leaves. Garcia et al (2007) noticed that increased soil salinity promoted by irrigation with saline water increased the sodium content and also the relationships Na/Ca, Na/Mg and Na/k, but decreased the contents of Ca, Mg, and K therefore, characterizing the unbalance and the nutritional stress consequent to the progressive saline stress. Zhang et al (2008) indicated that under high salt stress, maize transgenic plants compartmentalized more Na in the roots and kept a relative high K/Na ratio in the leaves compared with wild-type plants. Stepien and Klobus (2005) and Azevedo et al (2006) reported similar results in response to salinity stress. Christian et al (2004) studied the biochemical reation of maize to salt stress at the level of proteins in root and shoot.. They reported that high as well as low NaCl treatment of maize led to an unexpected high number of differentially regulated proteins in root and shoots. Moderate salt stress (25mM NaCl) led to a differential regulation of 31% of shoot proteins and 45% of root proteins, without any effect on the morphology and Na and Cl concentrations of the plant

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تقييم بعض هجن الذرة الشامية تحت ظروف مختلفة من الملوحة مجاهد احمد حلمى مجاهد - رشا يوسف سيد عبد الخالق قسم بحوث تكنولوجيا البذور - معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية - جيزة

إن تقبيم صفات جودة هجن الذرة الشامية لمقاومة الملوحة يعتبر هدف هام في برامج التربية. وقد لجريت هذه الدراسة بقسم بحوث تكنولوجيا البذور لدراسة تأثير الملوحة على البات البذور ونمو البادرات في خمسة هجن هي: (هجين قردي ١٠، هجين فردي ١٢٣، هجين ثلاثي ٢٣٤، هجين ثلاثي ٢٣٠، هجين ثلاثي ٢٣٤). وتم معاملة حبوب الذرة بواسطة أربعة تركيزات من كلوريد الصوديوم ، كلوريد الكالمبيوم ، ومخلوط منهما بنسبة ٢:١ وهي (٢٠٠٠، ٢٠٠٠، ٢٠٠٠، ٨٠٠٠ جزء في المليون) بالاضافة الى معاملة المقارنة.

تم تقدير حبوية الحبوب وصفات البادرة وحبوية البادرة وكذلك التقريد الكهربى للبروتينات باستخدام SDS-PAGE . وقد اشارت التناتج الى ان الهجين الفردى ١٠ اعطى اعلى نسبة انبات ودليل حبوية البادرة والعد الاول وطول الريشة والجنير والوزن الجاف، ينيه الهجين الفردى ١٢٣ . اطول جنير كان في الهجين الفردى ١٢٣ . اطول جنير كان في الهجين الثانثي ٢٣٠.

اشارت نتائج التفريد الكهربى البروتين الى وجود اختلاقات بين هذه الهجن فى عدد ومواقع الحزم البروتينية فى كل معاملة. تم الحصول على ثلاثة معاملت جزيئية بالمعاملة بواسطة كاوريد الكالسيوم عند الاوزان الجزيئية ٥٠ كيلو دالتون (٤٠٠٠ جزء فى المليون) فى كل الهجن ما عدا الهجين الفردى ١٢٣. اما الثانية كانت عند الوزن المجون ٣٠ كيلو دالتون عند اللهجين الفردى ١٠٠ جزء فى المليون) وظهرت فى كل الهجن عند ١٥ كيلو دالتون عند التحريرات ٢٠٠٠،٨٠٠٠ جزء فى المليون ما عدا الهجين الفردى ١٠. بينما لوحظ معلم واحد فقط مرتبط بالمعاملة يكلوريد الصوديوم عند وزن جزيئي ٨٠ كيلو دالتون فى الهجين الفردى ١٠.

من النتائج المتحصل عليها يمكن القول ان كلا الملحين ادى الى نقص فى الصفات المدروسة بزيادة تركيز الملح ولكن كلوريد الصوديوم اعطى أم اعلى من كلوريد الكالسيوم وتشير النتائج الى ان الهجين الفردى ١٠ لكثر تحملاً للملوحة من الهجين الفردى ١٢٣ والهجين الثلاثي ٣٢٤ والهجين الثلاثي ٣٢٠ بينما يعتبر الهجين الثلاثي ٣٢٠ حساس للملوحة.

ئذا فأن التفهم الاقضل للاستجابة الفسولوجية نظروف الاجهاد الملحى ريما تساعد في برامج التربية والانتخاب لتحمل الملوحة.

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