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Evaluation of Two Maize Hybrids Under Salinity Stress at Germination Stage

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ABSTRACT

Maize (*Zea mays L.*) is the third most important cereal crop after wheat and rice, besides, it's important and necessary for global food security. Maize is considered a C_4 plant and is sensitive to salinity. Two maize hybrids (SC-168 and TWC-352) were evaluated morphologically and biochemically under salinity stress using levels of NaCl (0, 50, 100, 150, 200, and 250 mM) at the germination stage. The results indicated that the percentage of germination, growth characteristics, pigments in both hybrids significantly decreased with increasing of NaCl concentrations. In addition, proline increased with increasing salinity levels and then decreased at the highest levels of NaCl in both hybrids. Moreover, salinity with high levels of NaCl led to decrease activity in CAT, POX, and PPO enzymes. Finally, under salinity stress, SC-168 had greater values in germination %, growth characteristics, photosynthetic pigments, proline content, antioxidant activity compared to TWC-352. As a result, it's possible that SC-168 has a higher salt tolerance than TWC-352.

Keywords: Salinity stress, *Zea mays* L., photosynthetic pigments, enzyme activity, proline

INTRODUCTION

Maize (*Zea mays* L.) is regarded as one of the most important strategic crops in the world because of its many uses. Maize is used as food for humans and fodder for livestock, and it has many other uses (Alahdadi *et al*., 2011). After wheat and rice, maize is the third most important cereal crop, and it is critical for world food security (Cassman, 1999). Because of the population increase and the high consumption of maize, there is a need to cultivate more newly reclaimed lands, but the cultivation of maize has been faced with many obstacles that impair output, such as salinity stress. Salinity is abiotic stress that affects the productivity of all crops, particularly in arid and semi-arid regions (Khajeh *et al*., 2003). The accumulation of sodium ions in plant tissues causes adverse effects on plant development and metabolism (Abbas *et al*., 1991). Seed germination may be inhibited or delayed due to the toxic effects of Na^+ and Cl^- ions on seed germination (Farsiani and Ghobadi 2009). Maize is a C_4 plant that is salinity sensitive (Mansour *et al*., 2005). The germination period in maize is critical and more vulnerable to salinity stress than the other stages of the plant's life cycle (El-Sayed, 2011). Plant physiological and biochemical activities are disrupted by salinity due to osmotic disruption (Vinocur and Altman, 2005). Salt stress leads to stomata closure and limits the process of CO_2 fixation and thus limits photosynthesis (Murata *et al*., 2007). Under salinity stress, a drop in photosynthetic pigments is linked to a decrease in photosynthesis rate in maize plants (El-Sayed, 2011 and Qu *et al*., 2012). The development of reactive oxygen species, which cause considerable damage to the chloroplasts and mitochondria, is caused by the accumulation of salts in the growth media. Enzymatic antioxidants (such as catalase and peroxidase) and non-enzymatic antioxidants (such as phenolic compound and ascorbic acid) are used by plants to protect cells from harmful free radical (Mittler, 2002). Agami (2013) found that salinity stress by NaCl reduced growth traits, photosynthetic pigment, and activities of catalase, peroxidase in maize plant. El-Katony *et al.* (2019) indicated that salinity stress reduced root and shoot growth, chlorophyll pigments and increased proline in maize leaves. Therefore, the major goal of this study was to evaluate two maize hybrids under salinity stress by NaCl during the germination stage by examining morphological and biochemical characteristics.

MATERIALS AND METHODS

Plant materials and growth conditions

The research was carried out in Biochemistry department labs, faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Two Maize (*Zea mays* L.) hybrids; single cross Giza-168 (SC-168) and three-way cross Giza-352 (TWC-352) were obtained from Field Crops Research Institute (FCRI) of ARC, Giza, Egypt. Seeds of Maize were sterilized by soaking in 1.5% NaOCl solution for 15 minutes and then being washed with distilled water to remove NaOCl residue. After washing, the seeds were left to be air-dried and then they were transferred (20 seeds) into Petri dishes (15 cm) containing one layer of Whatman's filter paper (three replicates). The seeds in the petri dish were moistened with 5 ml daily of distilled water or salty solution of NaCl concentrations (0, 50, 100, 150, 200, and 250 mM), then the dishes were left at a temperature of 25±2 °C for 10 days (Carpici *et al*., 2009). The percentage of seeds germinated (GP%) was calculated according to (Krishnasamy and Seshu 1990). Growth characteristics: shoot length (SL), and root length (RL) as well as seedling

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fresh weight (SFW), and seedling dry weight (SDW) were determined according to (Krishnasamy and Seshu 1990; ISTA, 1999). The calculation of seedling vigor index (SVI) using the following formula: seedling length (SDL) x germination percentage (GP%), according to (ISTA, 1999).

Photosynthetic pigments and proline content

The spectrophotometric method was used to measure chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) in maize leaves, and the results were computed according to (Lichtentaler and Wellburn, 1985). Proline content was determined in maize leaves according to (Bates *et al*., 1973).

Antioxidant enzyme activity

Enzymes extract from maize leaves was prepared according to Esfandiari *et al*. (2007). Catalase activity (CAT) was assayed according to Aebi (1984). Peroxidase activity (POX) was determined using the method by Amako *et al*. (1994). The activity of Polyphenol oxidase (PPO) was measured using the method by Galeazzi *et al*. (1981).

Statistical Analysis

All data was subject to the analysis of variance (ANOVA) of randomized complete block design (RCBD) by SPSS software (version 17.0). LSD test at 5% level of significance was used to compare between means of different variables according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Germination and morphological parameters

Germination percentage and morphological parameters of two maize hybrids under NaCl salt stress are shown in Table (1). The results showed that the germination percentage in both hybrids significantly decreased with increase of NaCl levels. The highest germination percentage was reached 100% under control in SC-168, while the lowest percentage of germination was 43% in TWC-352 at 250 mM NaCl. The highest germination percentage in TWC-352 was 93% under control, then significantly decreased until it reached 88, 76, 70, 62, and 43% at 50, 100, 150, 200, and 250 mM, respectively. Also, the percentage of germination in SC-168 cultivar significantly decreased from 100% under Control to 95, 88, 82, 73, and 67% at the same previous concentrations of NaCl, respectively. The stage of seed germination is a critical and sensitive period in the life of the maize plants, which determines the establishment of seedlings and the success of crop production. Salinity stress due to the presence of NaCl in the growing environment delays initiation and seed germination rate. Accumulation of sodium and chloride ions in the growing environment leads to osmotic stress, which causes difficulty in absorbing water, besides, leads to a decrease in the percentage of germination and all other growth parameters in maize hybrids (Farsiani and Ghobadi, 2009). On the other hand, the presence of 50, 100, 150, 200, and 250 mM NaCl significantly reduced the growth parameters; length of shoot (SL), and root (RL), seedling fresh weight (SFW), and seedling dry weight (SDW) in both hybrids (Table 1). SC-168 had the highest SL (15 cm) and RL (14 cm) at the control, while TWC-352 had the lowest SL (1.5 cm) and RL (2 cm) at 250 mM. Also, SFW and SDW insignificantly decreased in TWC-352 until level 50 mM, but they significantly decreased under other levels until they reached the maximum decrease at 250 mM, which were 0.315 and 0.042 g compared to the control 0.895 and 0.119 g, respectively. In addition, SC-168 had the highest values of SFW and SDW at the control and 250 mM compared to TWC-352 at the same levels. Moreover, seedling vigor index (SVI) more reflects the salinity resistance and germination characteristics of maize hybrids when exposed to NaCl salinity stress. The values of SVI significantly decreased with the increase of NaCl levels in both hybrids. The highest SVI was recorded in SC-168 (2900) under control, while the lowest SVI was noticed in TWC-352 (150) at 250 mM. High salinity causes many disturbances in plants, the most important of which are water stress, ion toxicity, reduction in cell division and expansion, change in metabolic processes, and toxic to the embryo, thus reducing plant growth (Carillo *et al*., 2011 and Filippou *et al*., 2014). In this study, the reduction in germination percentage and all growth parameters in TWC-352 was greater than the reduction in SC-168 under NaCl stress. Therefore, SC-168 is more tolerant of salinity stress compared to TWC-352. Khodarahmpour *et al*. (2012) observed that salinity stress reduces seed germination percentage, mean germination time, germination rate, and seedling vigor index with respect to 8 maize hybrids. Our findings are consistent with those of Dikobe *et al*. (2021). Also, Shtereva *et al*. (2015) and Ali *et al*. (2019) found that a reduction in germination and all growth parameters with increasing of salinity level in three maize genotypes.

Table 1. Germination and morphological parameters of two maize hybrids under NaCl salinity stress

Hybrids	Salinity NaCl (mM)	GP%	SL(cm)	RL(cm)	SDL (cm)	SFW(g)	SDW (g)	SVI
SC-168	0	100	15.0	14.0	29.0	0.916	0.127	2900.0
	50	95	13.5	12.0	25.5	0.805	0.098	2422.0
	100	88	10.0	9.0	19.0	0.784	0.095	1672.0
	150	82	8.5	8.0	16.5	0.586	0.083	1453.0
	200	73	5.5	5.0	10.5	0.529	0.072	766.50
	250	67	2.5	2.5	5.0	0.340	0.047	335.00
TWC-352	0	93	12.5	12.0	24.5	0.895	0.119	2278.5
	50	88	12.0	12.0	24.0	0.894	0.117	2112.0
	100	76	8.5	8.0	16.5	0.683	0.085	1254.0
	150	70	6.0	7.5	13.5	0.528	0.081	945.00
	200	62	4.5	4.0	8.5	0.508	0.062	527.00
	250	43	1.5	2.0	3.5	0.315	0.042	150.00
LSD 5%		1.95	0.4	0.5	0.85	0.01	0.002	80.5

GP%, Germination Percentage; SL, Shoot Length; RL, Root Length; SDL, Shoot length + Root Length; SFW, Seedling Fresh Weight; SDW, Seedling Dry Weight; SVI, Seedling Vigor Index.

Photosynthetic pigments

The results in Table (2) indicated that the maximum contents of chlorophyll a (Chl a) and chlorophyll b (Chl b) were found in SC-168 (1.365 mg/g) and (1.229 mg/g) under control respectively, while the minimum contents of Chl a and Chl b were observed in TWC-352 (0.848 mg/g) and (0.577 mg/g) at 250 mM, respectively. The content of Chl a decreased by 4.33%, 6.88%, 11.36%, 31.22%, and 34.47% at 50, 100, 150, 200, and 250 mM in TWC-352 as compared to control, while percentages of reduction in Chl a in SC-168 were 8.86, 9.67, 21.47, 28.79, and 31.50% at the same levels, respectively. Also, the percentage of reduction in Chl b was 50.13% in TWC-352 and 38.57% in SC-168 at the maximum salinity level by NaCl (250 mM). Total chlorophyll Chl (a+b) had the highest value (2.594 mg/g) in SC-168 under control. On contrast, the lowest value for Chl (a+b) was (1.425 mg/g) in TWC-352 at 250 mM. On the other hand, carotenoids (Car) significantly decreased with increasing salinity levels in both hybrids. SC-168 had the highest content of Car (0.214 mg/g) under the control, while TWC-352 had the lowest content of Car (0.086 mg/g) at 250 mM. NaCl salinity stress also led to an increase in Chl a/b ratio in TWC-352 more than SC-168, reaching the maximum value (1.499 mg/g) at 150 mM in TWC-352, and the minimum value was (1.111 mg/g) under the control in SC-168. A decrease in pigments contents such as chl a, chl b, and carotenoids in seedling maize are also correlated with a decrease in photosynthesis rate under salinity stress (El-Sayed, 2011 and Qu *et al*. 2012). The accumulation of Na^+ and Cl^- ions as a result of salt stress leads to disturbances in photosystems I and II in maize seedlings, which leads to a decrease in photosynthesis (Qu *et al*., 2012). Also, NaCl causes increased activity of the chlorophyllase enzyme, which breaks down chlorophyll by removing the phytol group (Santos, 2004). The rate of decrease in photosynthetic pigments due to the high levels of NaCl was in the salinity-tolerant cultivars less affected than in the salt-sensitive species. Therefore, according to the results, SC-168 was more tolerant of salinity than TWC-352. Similar results were obtained by Zahra *et al*. (2020).

Table 2. Photosynthetic pigments of two maize hybrids seedlings under NaCl salinity stress

Photosynthetic pigments (mg/g fresh weight)						
Hybrids	**Salinity NaCl (mM)**	**Chl a**	**Chl b**	**Chl (a+b)**	**Chl a/b**	**Car**
SC-168	0	1.365	1.229	2.594	1.111	0.214
	50	1.244	1.070	2.314	1.163	0.168
	100	1.233	0.952	2.185	1.295	0.121
	150	1.072	0.926	1.998	1.158	0.115
	200	0.972	0.817	1.789	1.190	0.111
	250	0.935	0.755	1.690	1.238	0.104
TWC-352	0	1.294	1.157	2.451	1.118	0.182
	50	1.238	1.019	2.257	1.215	0.122
	100	1.205	0.847	2.052	1.423	0.119
	150	1.147	0.765	1.912	1.499	0.111
	200	0.890	0.598	1.488	1.488	0.101
	250	0.848	0.577	1.425	1.470	0.086
LSD 5%		0.028	0.02	0.05	0.01	0.006

Proline content

In response to salinity stress by NaCl, proline accumulates as in Fig. (1). The content of accumulated proline differed in both hybrids under NaCl levels. In TWC-352, proline significantly increased with the increase of salinity up to 100 mM (10.91 µmoles/g F.W) and then decreased with increasing salinity levels to (5.97 µmoles/g F.W) at 250 mM. Furthermore, in SC-168, the content of proline significantly increased by increasing the salinity levels up to 200 mM (20.15 µmoles/g F.W) and then decreased at 250 mM (10.74 µmoles/g F.W). Accumulation of proline in many plants has been documented as a defensive response to maintaining osmotic pressure in cells during salt stress (Desingh and Kanagaraj, 2007 and Veeranagamallaiah *et al*., 2007). Accumulation of proline under salinity stress conditions has been linked with stress tolerance in many plant species (Ashraf and Foolad, 2007 and Hasanuzzaman *et al*., 2014). In this study, it was found that SC-168 had the highest value of proline content with a value of (20.15 µmoles/g F.W) at 200 mM, while TWC-352 had the lowest value of proline content with a value of (5.97 µmoles/g F.W) at 250 mM. The higher content of proline in SC-168 may be a reason for its salt tolerance when compared to TWC-352. Similar results in maize have been reported (Agami, 2013 and El-Katony *et al*., 2019).

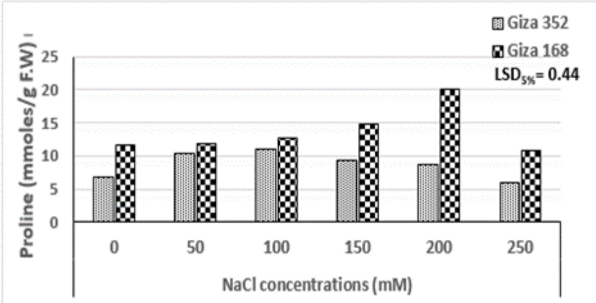

Fig. 1. The effect of salinity stress on proline content in two maize hybrids.

Antioxidant enzyme activity

Catalase activity (CAT) in leaves of two maize hybrids was presented in Fig. (2), CAT activity significantly decreased with increasing levels of NaCl in both hybrids. TWC-352 had the highest and lowest values under the control and 250 mM, which reached 19.45 and 9.42 U/mg F.W respectively. In SC-168, the activity of CAT significantly increased at 50 mM and then decreased under other high levels of NaCl. At the highest levels of NaCl, SC-168 had the highest values of CAT activity with values of 13.15 and 11.23 U/mg F.W at 200 and 250 mM respectively, compared to TWC-352 at the same levels of salinity.

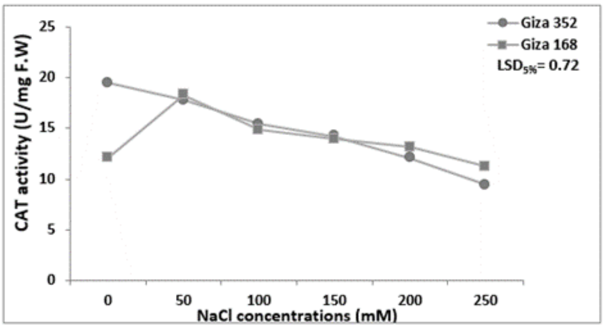

Fig. 2. The effect of salinity stress on CAT activity in two maize hybrids.

Data presented in Fig. (3) showed that a significant increase in peroxidase activity (POX) with an increase in salinity by NaCl up to level of 100 mM and then a gradual decrease at levels of 150, 200, and 250 mM in both hybrids.

The highest value of POX activity (58.40 U/mg F.W) was observed in SC-168 at 100 mM, while the lowest value (26.91 U/mg F.W) was recorded in TWC-352 at 250 mM. It is noticeable in the results that at all levels of NaCl, the values of POX activity in SC-168 were higher than those in TWC-352. Salt stress disrupts metabolic processes and enzymatic activities, causing an increase in the formation and production of ROS, which causes severe damage to cellular structure. Different types of plants have a defense system of antioxidants that provides protection against oxidative damage to get rid of the damages of ROS. The enzymatic defense system consists of enzymatic antioxidants such as CAT, POX, and other enzymes (Heidari and Golpayegani, 2012). CAT work to get rid of hydrogen peroxide and convert it into water and oxygen, thus reducing its danger to cellular components. Peroxidase is a scavenging enzyme and removes toxic oxygen radicles from the cells (Cuypers *et al*., 2011 and Sofo *et al*., 2015).

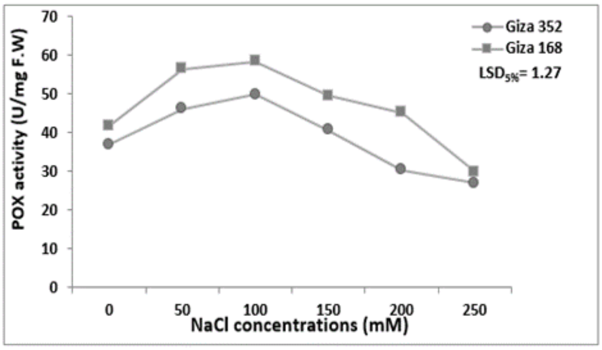

Fig. 3. The effect of salinity stress on POX activity in two maize hybrids.

The results in Fig. (4) showed that activity of polyphenol oxidase (PPO) significantly decreased in TWC-352 with increasing salinity levels, for example, PPO activity decreased from 14.90 U/mg F.W under control to 5.67 U/mg F.W at 250 mM. On the contrary, in SC-168, PPO activity significantly increased from 9.75 U/mg F.W under control to 12.17 and 13.42 U/mg F.W at 50 and 100 mM, respectively, and then followed by a significant decrease to 11.58, 8.88 and 7.42 U/mg F.W at 150, 200 and 250 mM, respectively. At NaCl levels which ranged from 100 to 250 mM, SC-168 had values of PPO activity higher than TWC-352. The activity of the PPO under salt stress indicates its ability to oxidize and break down toxic substances that accumulates during salt stress (Rivero *et al.* 2001).

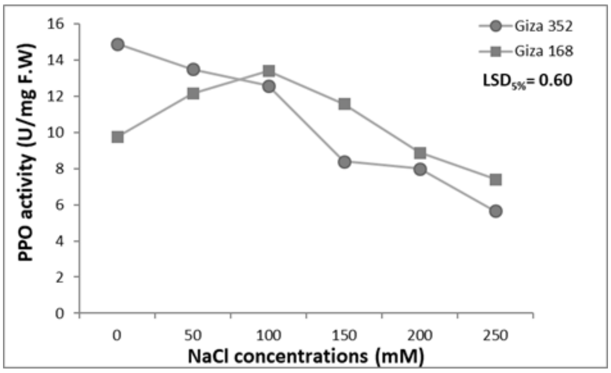

Fig. 4. The effect of salinity stress on PPO activity in two maize hybrids.

In the present study, the decrease in the enzymatic activity of CAT, POX, and PPO in both hybrids of maize under high levels of salinity may be due to the sensitivity of these hybrids to salinity. CAT, POX, and PPO activities were greater in SC-168 than TWC-352 under high levels of NaCl stress (Fig. 2, Fig. 3, and Fig. 4). These results are compatible with (Mickky and Aldesuquy, 2017). Similar results showed a decrease in CAT and POX activities in maize seedlings under NaCl stress (Agami, 2013).

CONCLUSION

Our study evaluated the performance of two maize hybrids using examining morphological and biochemical characteristics under salinity stress by NaCl. The results showed some differences between the two hybrids in response to salinity stress. Salinity stress resulted in a considerable decrease in germination and morphological characteristics as well as photosynthetic pigments. Also, the values of proline content increased with increasing salinity levels and then decreased at the highest levels of NaCl in both hybrids. Moreover, salinity with high NaCl levels reduced enzymatic activity in CAT, POX, and PPO. Finally, SC-168 had higher values compared to TWC-352 and was more tolerant under salinity stress.

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تقييم هجينين من الذرة الشامية تحت الإجهاد الملحي خلال مرحلة الانبات

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تعتبر الذرة الشامية من أهم محاصيل الحبوب بعد القمح والأرز، وهي مهمة وضرورية للأمن الغذائي العالمي. الذرة من النباتات رباعية الكربون وهي حساسة للملوحة. تم تقييم هجينين من الذرة الشامية وهما هجين فردي SC-168 وهجين ثلاثي TWC-352 مورفولوجياً وكيميائيًا تحت الإجهاد الملحي باستخدام مستويات مختلفة من كلوريد الصوديوم (0، 50، 100، 150، 200، 250 ملي مولار) خلال مرحلة الإنبات. أشارت النتائج إلى أن نسبة الإنبات ومقاييس النمو المختلفة وصبغات البناء الضوئي في كلا الهجينين انخفضت معنوياً مع زيادة مستويات كلوريد الصوديوم. بالإضافة إلى ذلك، زاد محتوى البرولين مع زيادة مستويات الملوحة ثم انخفض محتوي البرولين عند أعلى مستويات كلوريد الصوديوم في كلا الهجينين. علاوة على ذلك، أدت المستويات العالية من كلوريد الصوديوم إلى انخفاض النشاط الانزيمي لإنزيمات الكتاليز والبيروكسيديز والبوليفينول اوكسيديز. أخيرًا، تحت الاجهاد الملحي باستخدام كلوريد الصوديوم، كان لـ SC-168 قيمًا أكبر في نسبة الإنبات، ومقاييس النمو المختلفة، وصبغات التمثيل الضوئي، ومحتوى البرولين، والنشاط الانزيمي لإنزيمات مضادات الاكسدة مقارنة بـ TWC-352. من النتائج المتحصل عليها في هذه الدراسة يمكن استنتاج أن الهجين الفردي SC-168 كان اكثر تحملاً للإجهاد الملحي مقارنة بالهجين الثلاثي TWC-352.