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Proline Reaction, Peroxide Activity and Antioxidant Enzymes in Varieties of Maize (ZEA MAYS L.) Under Different Levels of Salinity

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Abstract: Investigate the effects of salt stress on some physiological and morphological traits in four varieties and four salinity levels, including SC302, SC700, BC662, SC704 and Zero (control), 50, 100 and 150 mM NaCl in three replicates for the factorial experiment in randomized complete block design was carried out. During the experiment, several traits including plant height, chlorophyll a, chlorophyll b, proline and antioxidant enzymes were measured. For the trait days to germination at different levels of salinity significantly different at 5% probability level was seen. Interactions between genotypes and salinity in a significant difference was found. Between different levels of salinity on chlorophyll trait in a significant difference was seen in 1% level. Between different levels of salinity in catalase showed a significant difference. Between genotypes of the enzyme ascorbate peroxidase significant difference was seen, But genotypes showed not significant difference in amount of proline. The most plant height was obtained at zero salinity and with increasing salinity decreased plant height. Maximum chlorophyll a in normal conditions was found in S.C704 with 1.27 mg of chlorophyll per gram fresh weight of leaves. Superoxide dismutase with increasing salinity stress in all genotypes increased. With increasing salinity, the amount of catalase increased and at 100 mM salt stress reached a maximum. Maximum catalase was measured in 100 mM salt in BC666, that with SC302 and SC700 was not significantly different. The least amount of catalase in normal conditions in s.c704 and sc700 respectively 0.3567, 0.3533 units min g fresh weight of leaves was obtained. Of the enzyme ascorbate peroxidase, the enzyme in the greatest amount of salt concentration in 50 mmol in Sc302 with 3.73 units min g fresh weight of leaves was obtained. Lowest of these enzymes in salinity third in s.c704 was obtained. Most of proline, was obtained in 100Mm in variety SC302 with 537 micromole. Between proline and catalase positive and significant correlation was obtained. In other words with increasing salt stress also increases the amount of enzymes. Significant positive correlation was found between the enzyme ascorbate peroxidase and proline.

Key words: Salinity, Proline, antioxidant enzymes , Maize.

INTRODUCTION

The world population is expanding rapidly and is expected to be around 8 billion by the year 2025 (Andersen *et al.,* 1999). This represents an addition of nearly 80 million people to the present population every year. It is forecast that the increase in world population will occur almost exclusively in developing countries, where serious nutritional problems exist at present, and population pressure on agricultural soils is already very high. Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most of the common economic crops, which affect plants through osmotic effects, ion specific effects and oxidative stress (Munns, 2002; Pitman and Lauchli, 2002). Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level (Foyer and Noctor, 2003). If there is a serious imbalance in any cell compartment between the production of reactive oxygen species (ROS) and antioxidant defense, oxidative stress and damage occurs (Mittler, 2002). Even under normal growth conditions, low amounts of ROS such as superoxide radical ($O2^{-}$), hydrogen peroxide (H2O2), hydroxyl radical (OH**.**), and singlet oxygen (O2) are metabolic byproducts of plant cells (Cai-Hong *et al.,* 2005). Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic (Reddy *et al.,* 2004; Demiral and Türkan, 2005). When ROS increases, chain reactions start, in which superoxide dismutase (SOD) catalyzes the dismutation of $O2^{\bullet -}$ radicals to molecular O2 and H2O2 (Meloni *et al.,* 2003). The H2O2 is then detoxified in the ascorbate-glutathione cycle (Willekens *et al.,* 1995; Asada, 1999; Mittler, 2002), which involves the oxidation and re-reduction of ascorbate and glutathione through the ascorbate peroxidase (APX) and glutathione reductase (GR) action (Foyer and Halliwell 1976; Noctor and Foyer, 1998). After wheat and rice, maize (*Zea mays* L.) is the third most important cereal crop grown all over the world in a wide range of climatic condition. Maize, being highly cross pollinated, has become highly polymorphic through the course of natural and domesticated evolution and thus contains enormous variability (Paternian, 1990) in which salinity tolerance may exist. Maize is considered as moderately salt sensitive (Mass and Hofffman, 1977; Katerji *et al.,* 1994; Ouda *et al.,* 2008; Carpici *et al.,* 2009).

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MATERIALS AND METHODS

Investigate the effects of salt stress on some physiological and morphological traits in four varieties and four salinity levels, including S.C302, SC700, BC662, S.C704 and Zero (control), 50, 100 and 150 mM NaCl in three replicates for the factorial experiment in randomized complete block design was carried out. Treatments were planted in pots. During the experiment, several traits including plant height, chlorophyll a, chlorophyll b, proline and antioxidant enzymes were measured. During the experiment, Before dealing amount of proline, chlorophyll a and Chlorophyll b Content were measured in the laboratory. Photosynthetic pigments (chlorophyll a and b) were measured using the method of Arnon (1975) and Ashraf (1994) in fresh leaf samples, a week before the harvest. One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples (0.5 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 5 mL. Then the solution for 10 minutes away in 3000 (rpm) centrifuged. Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula given below :

a) Chlorophyll a (mg/g FW)=[12.7× (A663) _ 2.69× (A645)]×0.5
b) Chlorophyll b (mg/g FW)=[22.9× (A645) _ 4.69× (A663)]×0.5
c) Chlorophyll a+b (mg/g FW)= [20.2× (A645) _ 8.02× (A663)]×0.5

Free proline accumulation was determined using the method of Bates *et al.,* (1975). 0.04 gram dry weight of leafs was homogenized with 3% sulfosalicylic acid and after 72h that proline was released; the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 hour and then absorbance at 520 nm was determined by Uv-visible spectrophotometer. For determination of antioxidant enzyme activities, fresh leaf samples (0.3 g) from control and treated plants were ground with liquid nitrogen, and suspended in specific buffer and pH for each enzyme extraction. The homogenates were centrifuged at 14.000 rpm for 20 min at 4 °C and resulting supernatants were used for enzyme assay. Superoxide dismutase (SOD) activity was determined based on the inhibition of eduction of nitro-blue tetrazolium in the presence of riboflavin in the light at 560 nm as described by Giannopolitis and Ries (1977). A unit of SOD activity is defined as the amount of enzyme, which caused 50% inhibition of the reaction in the absence of enzyme. Catalase activity was measured titrimetrically (Chance and Maehly, 1955), whereas, peroxidase activity was measured on colorimeter, using purpurogallin for standard curve (Chance and Maehly, 1955). APX activity was determined after oxidizing ascorbate to dehydroascorbate, as described by Nakano and Asada (1981). The extraction buffer contained 50 mM phosphate, 0.5 mM ascorbate, 0.1 mM EDTA, and 1% (w/v) polyvinylpolypyrrolidone (PVPP), at pH 7.0, and the assay mixture 50 mM phosphate, 0.5 mM ascorbate, 0.1 mM EDTA and 0.1 mM H2O2, at pH 7.0. Statistical analysis of the data was done on the basis of randomized complete block design. The average of attendances was calculated on the basis of Duncan method at 5% probability level.

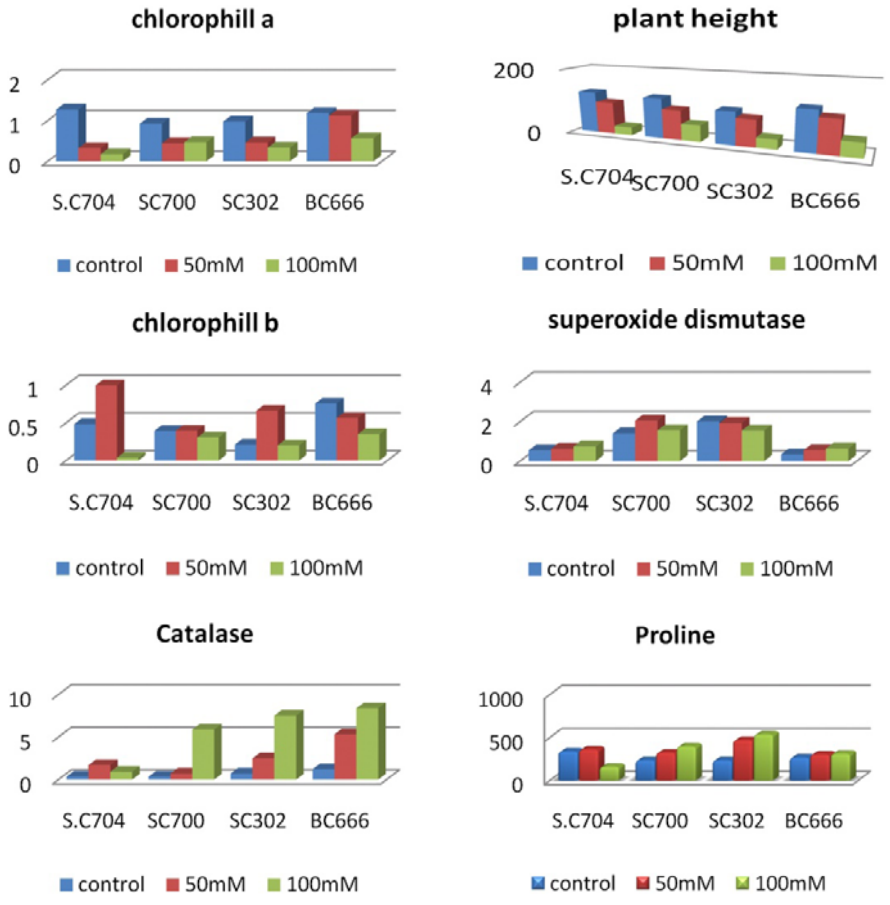
RESULT AND DISCUSSION

Between different levels of salinity on chlorophyll a significant difference was seen at 1% level. Genotypes and salinity levels of superoxide dismutase were not significantly different. Azooz *et al.,* (2009) in the activity of antioxidant enzymes in maize Cultivar showed that in the control (no salt) with salinity levels significant differences was seen, but between salinity 50, 100 150 and 200 differences were not significant. High salt concentrations lead to loss of the plant. Coca *et al.,* 2007; Atari *et al.,* 2008 showed that superoxide dismutase activity in resistant varieties to salinity increases sharply. Enzyme catalase showed a significant difference in different levels of salinity. Between the studied genotypes of the enzyme ascorbate peroxidase significant difference was seen. But in the proline, did not show significant differences between all genotypes. Increasing the salt plant height was significantly reduced. Minimum height of 150mM salt was obtained with the 37.08cm, which was significant with all concentrations. The amount of chlorophyll a decreased significantly with increasing soil salinity than the control. In 100 mM salt was found the lowest amount of chlorophyll a. With increasing salinity to 50 mM increased the amount of chlorophyll b. But decreased with increasing concentration of 100 mM. Tuna *et al.,* (2008) in effect of gibberellic acid and salinity on antioxidants and growth parameters on corn plant showed that with increasing salt concentration, a significant decrease in dry weight, relative amount of chlorophyll and leaf water content was seen. Catalase increased with increasing soil salinity and in salinity of 100 mM highest catalase was measured. The enzyme ascorbate peroxidase increased with increasing salinity to 50 mM, but there was no significant difference between them. Proline accumulation in response to environmental stresses has been considered by a number of authors as an adaptive trait concerned with stress tolerance, and it is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Larher *et al.,* 1993). Its accumulation is caused by both the activation of its biosynthesis and inactivation of its degradation. It is believed that the accumulation of proline, a compatible solute, may help to

maintain the relatively high water content necessary for growth and cellular functions. Furthermore, it was shown that the capability of a number of crop plants to accumulate proline in response to salt or other stresses was highly variable between or within species (Ashraf *et al.*, 2004; Naqvi *et al.*, 1994; Lutts *et al.*, 1996; Aziz *et al.*, 1998; Lutts *et al.*, 1999). Proline levels increased with increasing salinity and in salinity of 50 mM proline was highest (Table 2-3). Mansour *et al.*, (2005) in study two varieties hybrid 321 and Gyza 2,in the salinity level of zero, 75 and 150 mmol showed that, With increasing salinity in both cultivars in the amount of sodium ions in roots and stems and proline increased. Maximum of chlorophyll a in Bc666 with 0.9556 mg chlorophyll per gram fresh weight of leaves was observed. There was no significant difference between genotypes in the chlorophyll b, but the highest and lowest chlorophyll b, respectively, were seen in Bc666 and Sc302. Similar results were also reported by Iqbal *et al.*, (2006), Ashraf *et al.*, (2005), Khan *et al.*, (2009), Oncel and Keles (2002) and Almodares *et al.*, (2008). The highest plant height in normal conditions in S.C704 and BC666 obtained, which together had no significant difference. Superoxide dismutase in all genotypes increased with increasing salinity stress. Most enzymes obtained in the salinity of 50 mmol in SC700. The lowest Enzymes was seen in normal conditions in BC666. Catalase increased with increasing salinity and reached a maximum at 100 mM salt. Catalase increased with increasing salinity and reached a maximum at 100 mM salt. Most of the catalase enzyme was measured at 100 mM salt in the BC666, which with SC302 and SC700 was no significant difference. Lowest amount of catalase in normal conditions were measured in SC704 and SC700 with 0.3567and 0.3533 units respectively. Of ascorbate peroxidase enzymes, most of these enzymes were measured in the salinity concentration of 50 mM in SC302 with 3.73 unit. The lowest of this enzyme obtained in 150 mM salinity in S.C704.

A correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in some plant species (Gossett *et al.*, 1994; Dionisio-Sese and Tobita, 1998; Hernandez *et al.*, 1999). Several studies have pointed out that salt-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to salt treatment, whereas salt-sensitive species failed to do so (Shalata *et al.*, 2001; Demiral and Türkan, 2005).

Between plant height and chlorophyll a (0.594) were positive and significant correlation at the 1% level. In the study by Dlasrda *et al.*, (2003 and 2005) were conducted between chlorophyll a and chlorophyll b, was found significant positive correlation. But Between chlorophyll a and chlorophyll b with proline was obtained significant negative correlation. Between Catalase and proline was found positive and significant correlation. A significant positive correlation was found between enzyme ascorbate peroxidase and proline (0.574).



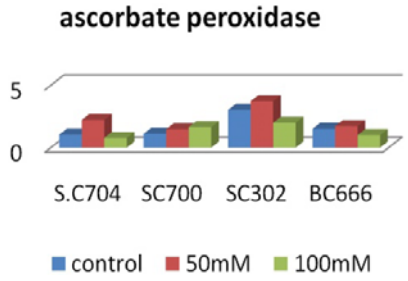


Fig. 1: Diagram of different understudy characteristics in four cultivars of the maize under the normal and salty conditions.

Table 1: Analysis of variance of understudy characteristics in four cultivars of the maize.

Mean Square							df	S.O.V
Superoxide dismutase	proline	Ascorbate peroxidase	Catalase	chlorophyll b	plant height	chlorophyll a		
0.070	15.066	0.0001	0.053	0.007	0.49	0.0001	2	Replication
0.002ns	22.160ns	0.031ns	0.758**	0.007ns	65.089**	0.019**	2	Salinity
0.047ns	16.523ns	0.058**	0.250ns	0.001ns	1.940ns	0.003 ns	3	Genotype
0.002ns	27.959ns	0.008ns	0.095ns	0.002ns	1.096ns	0.002 ns	6	Salt*Genotype
0.029	29.814ns	0.013	0.129	0.003	2.185	0.001	22	Error
3.70	29.26	2.42	7.43	1.26	14.95	0.85	%CV	

* Significant difference in probability level of 5%
** Significant difference in probability level of 1%

Table 2: Comparing the average of understudy characteristics in four cultivars of the maize.

Salinity	variety	plant height(cm)	chlorophyll a mg/gfw	chlorophyll b mg/gfw	Superoxide dismutase Unit/ming fw	proline µmol/gFw	Ascorbate peroxidase Unit/ming fw	Catalase Unit/ming fw
Control	Sc704	126.7a	1.270a	0.4733a	0.5400a	339.9ab	1.041bc	0.2547c
	Sc700	122.7a	0.923ab	0.3933a	1.403a	237.3ab	1.111bc	0.3533c
	Sc302	100.7ab	0.976ab	0.2100a	2.010a	236.4ab	3.037ab	0.7100bc
	Bc666	123.7a	1.183a	0.7567a	0.3333a	268.1ab	1.510bc	1.227bc
50mM	Sc704	98.00ab	0.323c	0.9967a	0.620a	363.8ab	2.227abc	1.707abc
	Sc700	91.33ab	0.443bc	0.3967a	2.057a	325.6ab	1.480bc	0.7100bc
	Sc302	82.33abc	0.460bc	0.6600a	1.930a	471.8ab	3.730a	2.493abc
	Bc666	103.0ab	1.120a	0.5633a	0.550a	304.4ab	1.725abc	5.337abcd
100mM	Sc704	23.67d	0.173c	0.0366a	0.7367a	161.1b	0.779c	0.9267bc
	Sc700	50.33bcd	0.466bc	0.3067a	1.557a	397.3ab	1.647bc	5.907abc
	Sc302	30.00d	0.336c	0.2000a	1.547a	537.0a	1.998abc	7.540ab
	Bc666	44.33cd	0.563bc	0.3500a	0.6367a	312.6ab	1.002bc	8.397a

*Within each column, same letter indicates no significant difference between treatments (p<0.05).

Table 3: Simple Correlation between some growth characteristics in normal and salty conditions.

	SOD	plant height	chlorophyll a	chlorophyll b	Catalase	APX	proline
SOD	1	0.048	-0.069	-0.142	0.076	0.093	0.286
plant height		1	0.594**	0.282	-0.222	0.212	0.150
chlorophyll a			1	0.112	0.033	0.115	0.102
chlorophyll b				1	-0.015	0.265	0.156
Catalase					1	0.293	0.639**
APX						1	0.574**

* Significant difference in probability level of 5%.
** Significant difference in probability level of 1%.

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