

Research Note**Antioxidative enzyme activities in maize genotypes grown under saline water irrigation****B. Nandhini devi^{*1}, T. Chitdeshwari² and R. Ravikesavan³**^{1,2}Department of Soil Science and Agriculture Chemistry, Tamil Nadu Agricultural University, Coimbatore³Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore**E-mail:** nandhu.sac@gmail.com

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Abstract

Plants with high levels of antioxidant enzymes have been reported to have greater tolerance to this oxidative damage caused by salinity. Role of antioxidative enzyme activity in salt stress and screening maize hybrids for salinity tolerance was investigated in this study. A pot culture experiment was conducted to evaluate six maize hybrids (CO 6, CO 7, CO 8, CO 10, NK 6240 and 900M gold) for their tolerance to saline water irrigation. The maize hybrids were irrigated with saline water having a natural EC of 0.06, 3.2, 4.8, 6.2 and 8.9 dSm⁻¹ for 30 days and replicated thrice in a FCRD. The antioxidant activities such as catalase (CAT), peroxidase (POX) and superoxy dismutase (SOD) were measured in maize hybrids grown under saline water irrigation besides recording and dry matter production (DMP) and growth attributes. CO 6 and CO 7 were identified as saline tolerant maize hybrids based on their antioxidant enzyme activities and DMP. However CO 8 has been observed as a salt sensitive maize hybrid as it showed lesser enzyme activities and DMP. The order of tolerance exists among the maize hybrids to irrigation water salinity were: CO 6 > CO 7 > NK6240 > 900M gold > CO 10 > CO8. Antioxidant enzyme activities and dry matter production were found to be useful traits for identifying salinity tolerance in maize hybrids at seedling stage.

Key words

Maize genotypes, saline water, Antioxidant enzymes, saline stress, Plant growth

Water salinity is of increasing importance in agriculture due to their significant role in growth reduction. Hence better understanding of the mechanisms that enable plants to adapt to salinity stress and maintain growth will ultimately help in the selection of stress tolerant cultivars for saline water irrigation (Amirjani, 2010). One of the biochemical changes possibly occurring when plants are subjected to harmful stress conditions is the production of activated oxygen species. The chloroplasts and mitochondria of plant cells are important intracellular generators of activated oxygen species. These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage of lipids, proteins and nucleic acids. Since internal O₂ concentrations are high during photosynthesis, chloroplasts are especially prone to generate activated oxygen species (Dolatabadian *et al.*, 2008). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage. Plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes in order to defend themselves against oxidants. In varying degrees, plants possess a number of antioxidants that protect against the potentially cytotoxic species of activated oxygen (Siringam *et al.*, 2011).

Superoxide dismutase is a major scavenger of O₂⁻ and its enzymatic action results in the formation of H₂O₂ and O₂. Catalase and a variety of peroxidases catalyze the breakdown of H₂O₂. Catalase, which is apparently absent in the chloroplast, dismutates

H₂O₂ into water and molecular oxygen, whereas peroxidase decomposes H₂O₂ by oxidation of co-substrates such as phenolic compounds and/or antioxidants. Dionisio Sese and Tobita (1998) reported that salinity results in higher levels of SOD, CAT, POX activities in rice and cotton respectively. Research on squash and eggplant indicated that superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzyme activities in salt-tolerant genotypes are higher compared to salt-susceptible genotypes in both seedling and callus tissues (Sevengor, 2010).

In India, maize is grown in a wide range of climates, extending from extreme semi-arid to sub-humid to temperate conditions. So, 80 per cent of the maize area at present is grown under water deficit condition and often suffers from abiotic stresses (drought and salinity) which accounted for the low productivity of maize.

Information regarding the relative levels of irrigation water induced salt tolerance among maize genotypes is minimal, therefore in the present study an attempt is made to assess the tolerance potentials of maize cultivars with different sensitivity to salt stress based on antioxidants. Comparison of these responses could be useful in identifying differences related to the relative ability of each cultivar to cope with salinity effect. In this context, the present study was conducted to screen maize hybrids under different levels of irrigation water salinity (EC_{iw}) to test their tolerance and sensitiveness based on the antioxidant enzymes activities.

Plant materials and salinity treatments: Present study was conducted in the Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, TNAU, Coimbatore under *in vitro* condition. Maize cultivars such as CO 6, CO 7 CO 8, CO 10, NK 6240 and 900 M GOLD were chosen and a pot experiment was conducted with five irrigation water salinity (EC_{iw}) levels on a clay loam soil. Water samples were collected from different irrigation sources and analysed for their chemical composition. Based on the similarity in chemical composition, five water samples naturally differing in their electrical conductivity (EC: 0.6, 3.2, 4.8, 6.2 and 8.9 dSm^{-1}) was selected for conducting the experiment. Pots of two kilogram capacity was chosen and filled with one kilogram of processed soil samples. Seeds of a six different Maize genotype were sown in the pots and one plant per pot was maintained. The crop was irrigated with saline water having various EC_{iw} levels at 250 ml/pot/irrigation at an interval of once in 3-4 days. Nutrients were applied basally as per fertiliser recommendation ($250:75:75 \text{ kg NPK ha}^{-1}$), grown upto 30 days and harvested. The plant samples were dried at 70°C in a hot air oven for two days and the dry weight was recorded. Relative chlorophyll content (SPAD index) was also determined on the first fully expanded leaf, by registering three readings per leaf with a portable Minolta chlorophyll meter SPAD-502.

Enzyme assays: Enzyme assays such as SOD, POX and CAT were performed at 30°C under controlled condition and absorbance was recorded using spectrophotometer.

Catalase: Five hundred milligram of leaf sample was weighed and homogenized with phosphate buffer solution at cold condition with PVP (polyvinylpyrrolidone) and centrifuged at $5000 \times g$. Three ml of buffer solution was taken and 50 microliter enzyme extract was added to the cuvette. The absorbance was recorded at 240 nm against standard buffer containing the enzyme. The enzyme activity was calculated and expressed in mg g^{-1} .

Superoxydismutase: Five hundred milligram of leaf sample was weighed and homogenized in 10 ml HEPES-KOH buffer containing 0.1Mm EDTA then centrifuged at 15000 rpm for 15 min. The supernatant was collected and made upto 50 ml volume. One ml of the enzyme extract was mixed with 3 ml of reaction mixture and the absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to result in 50% inhibition of NBT reduction at 560 nm. The result was expressed in units per gram of fresh weight.

Peroxidase: Five hundred milligram of leaf sample was weighed and homogenized with phosphate buffer solution at cold condition and centrifuged at 10000 rpm for 20 minutes. the supernatant was used as enzyme source. One ml of O-dianisidine, 0.5ml of H_2O_2 , 1 ml of phosphate buffer and 2.4ml of distilled water was pipetted into a test tube and incubated at 30°C and the reaction was started by adding 0.2ml of enzyme. After 5min, reaction was stopped by adding 1ml of 2N H_2SO_4 and absorbance at 430nm was recorded using spectrophotometer. The enzyme activity was calculated and expressed as units/min/mg protein or per g weight of sample considering one unit of enzyme as an increase in OD by 1.0 under standard conditions.

Statistical analysis: The experiment was laid out in a completely randomized block design with three replications. Dry matter production and antioxidative enzymes such as Catalase (CAT), superoxydismutase (SOD) and peroxidase (POX) activities were measured and the data was analysed with Agress software.

Saline water irrigation induced salinity significantly reduced the growth of all the maize hybrids through the biochemical changes and the extent of reduction varied with their genotypic differences. Increasing levels of salinity decreased the plant DMP and increases the enzyme activities upto 6.2 dSm^{-1} in all the maize hybrids.

Plant dry matter production: Salinity significantly reduced the plant growth attributes hence results in greater reduction in plant biomass production. There was a marked decrease in dry weights of all the maize hybrids due to the deterrent effect of salinity on plant growth. The plant biomass production by different maize hybrids was reduced from 6.50 to 1.99 grams with increasing irrigation water salinity (Table 1). The highest mean DMP was obtained with CO 6 (5.40 g) and CO 7 (4.92 g) with a per cent reduction of 21.2 to 24.96 per cent respectively. However CO 8 was found highly sensitive by recording lesser DMP (3.73 grams). The mean reduction rate in total plant DMP of genotypes at different EC_{iw} levels was maximum with CO 8 (38.7 %) and lesser reduction in CO 6 (21.2 %) regardless of levels of water salinity. Reduction in plant DMP could be related to the effect of salt-stress which resulted in the limitation of water absorption and biochemical processes (Parida and Das, 2005). The suppression of plant growth under salt-stress may either be due to osmotic reduction in water availability or due to excessive accumulation of ions, known as specific ion effect. There are many reports on osmotic stress and ionic toxicity resulted from salt stress in maize plants (Eker *et al.*, 2006; Siringam *et al.*, 2011). Our results are in accordance with the

findings reported by Akram *et al.*, (2011) who observed that dry weight of the maize hybrids was decreased with increasing water salinity.

Chlorophyll content (SPAD Index): The chlorophyll content in maize hybrids was recorded and presented in the table 2. Increasing salinity decreases the chlorophyll content in the leaves. Among the six maize hybrids CO 6 and CO 7 registered higher mean chlorophyll content (39.7 and 38.4) and the lowest value was recorded with CO 8 (34.7). The order of higher chlorophyll content was: CO 6 > CO 7 > NK 6240 > 900 M Gold > CO 10 > CO 8. Salinity stress also caused similar inhibitory effect on chlorophyll content in blackgram (Ashraf and Basu, 2009) and rice (Peiris and Ranasinghe, 2007). Srivastava *et al.* (2001) reported that tolerant wheat genotypes maintained higher amount of total chlorophyll content at the active growth stage.

Catalase: The catalase activity in the maize hybrids were analyzed and presented in table 3. Increasing level of salinity increases the catalase activity in the plants upto 6.2 dSm⁻¹ after that it gets declined in all the maize genotypes. Among the six genotypes, CO 6 registered the highest activity with the mean value of 7.39 $\mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$ followed by CO 7 (7.35 $\mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$) and the lowest rate was obtained with CO 8 (7.22 $\mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$). Catalase activity in CO 6 at the salinity level of 0.06, 3.2, 4.8, 6.2 and 8.9 dSm⁻¹ were 7.01, 7.32, 7.45, 7.67 and 7.51 $\mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$ respectively. This findings were similar to the findings reported by Sevengor, (2010) in pumpkin under saline condition. Silva *et al.*, (2011) reported that catalase plays an important role in the elimination of H₂O₂ under salinity. Increase in catalase activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue by reducing the toxic levels of hydrogen peroxide produced during cell metabolism. In the present study, the greater increase in catalase activity observed in CO 6 and CO 7 than the other genotypes may suggest its effective scavenging mechanism to detoxify the H₂O₂.

Superoxidismutase (SOD): The SOD activities in maize hybrids under non saline (0.06dSm⁻¹) condition were found to be significantly lower than in saline conditions for all the cultivars (Table 5). Salt stress caused an increase in SOD activity of all the maize genotypes and ranged from 6.20 to 7.59 EU per g and increasing with increasing salinity upto 6.2 dSm⁻¹. Higher SOD activities has noticed in CO 6 (6.95 EU per g) followed by CO 7 (6.60 EU per g) and the lowest rate was observed in CO 8 (6.13 EU per g). The order of higher SOD activity was: CO 6 > CO 7 > NK 6240 > 900 M Gold > CO 10 > CO 8. Increase in SOD activity and differential varietal salt susceptibility have

also been reported in salt-treated wheat (Srivastava *et al.*, 2001), rice (Peiris and Ranasinghe, 2007). Zaefyzadeh *et al.* (2009) reported that higher SOD activity is one of the stress confrontation mechanism under oxidative stress which is activated under salinity and drought conditions.

Peroxidase (POX): Similar to catalase and superoxydismutase, peroxidase activity also increased with increasing salinity upto 6.2 dSm⁻¹ and ranges from 0.42 to 0.61 units min⁻¹ mg⁻¹. The maize hybrids CO 6 and CO 7 registered the highest mean POX activity of 0.54 and 0.53 units min⁻¹ mg⁻¹. The order of decreasing peroxidase activity was CO 8 < CO 10 < 900 M gold < NK 6240 < CO 7 < CO 6. In tolerant plant species, POX activity was found to be higher, which enable the plants to protect themselves against the oxidative stress, where as such higher activity was not observed in sensitive plants (Peter *et al.*, 1999). Tsai *et al.*, (2005) observed higher activity of peroxidase under salinity in rice roots and increase in peroxidase activity was associated with increasing stress condition. The antioxidative capacity conferring salt tolerance in different crops under saline stress has been reported by many scientists (Amirjani 2010; Silva *et al.*, 2011).

Correlation between different enzyme activities and plant DMP was observed and furnished in Table 6. Among the three antioxidant enzymes, superoxy dismutase ($r^2 = 0.98$) and peroxidase ($r^2 = 0.97$) showed higher positive correlation than the catalase ($r^2 = 0.95$) and chlorophyll content ($r^2 = 0.88$) with DMP. The SOD is reported to play an important role in cellular defense against oxidative stress because of its activity directly modulates the amount of O²⁻ and H₂O₂ and the two Haber-Weiss reaction substrates (Bowler *et al.*, 2000). The observed increase in SOD activity could increase the ability of the leaves to scavenge O²⁻ radicals, which could cause membrane damage and hence plant growth may not be reduced much in CO 6 than other genotypes.

It is concluded that evaluation of maize hybrids at early growth stages is a simple and quick method for determining genotypic and physiological differences in response to irrigation water salinity. The present study showed that irrigation water salinity inhibited plant growth and caused considerable decrease in growth and biomass production of all the maize hybrids. The antioxidative enzyme activities such as catalase, peroxidase and superoxydismutase in the plants were increased with increasing salinity upto 6.2 dSm⁻¹ and showed a positive correlation with plant dry matter production. The antioxidative enzyme activities play a protective role against salinity stress and their defense mechanism was effective in providing tolerance to salt stress in maize hybrids grown under saline water irrigation. And

also, it plays important role in the selection of saline tolerant maize hybrids. It was identified that the maize hybrids CO 6 and CO 7 were tolerant to saline water irrigation upto an EC_{iw} of $4.8dSm^{-1}$ based on the better plant growth, DMP and antioxidative enzyme activities. However CO 8 has been observed as a salt sensitive maize hybrid as it showed lesser growth and could perform poor enzyme activities even at low water salinity.

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Table 1. Effect of saline water irrigation on plant dry matter production in maize hybrids

Maize Hybrids/ EC levels (dSm ⁻¹)	Dry matter production (in grams)					Mean
	0.06	3.2	4.8	6.2	8.9	
CO 6	6.50±0.25	6.23±0.22	5.66±0.11	4.75±0.20	3.84±0.13	5.40
CO 7	6.12±0.32	5.67±0.33	5.20±0.25	4.23±0.16	3.36±0.17	4.92
CO 8	5.40±0.27	4.81±0.31	3.80±0.18	2.68±0.27	1.99±0.13	3.74
CO 10	5.55±0.16	4.95±0.35	4.11±0.22	2.97±0.19	2.19±0.11	3.95
NK 6240	5.93±0.37	5.47±0.40	4.89±0.30	3.69±0.16	3.02±0.14	4.60
900 M GOLD	5.74±0.37	5.10±0.41	4.43±0.17	3.39±0.18	2.61±0.13	4.25
MEAN	5.87±0.29	5.37±0.30	4.68±0.20	3.62±0.19	2.83±0.14	4.48
	EC	G	EC x G			
SE(d)	0.06	0.07	0.14			
CD (P=0.05)	0.12**	0.13**	0.29*			

Table 2. Effect of saline water irrigation on leaf chlorophyll content in maize hybrids

Maize Hybrids/ EC levels dSm ⁻¹	Chlorophyll (SPAD Index)					Mean
	<1	2-4	4-6	6-8	>8	
CO 7	42.6±0.21	40.1±0.58	38.4±0.29	36.1±0.63	34.6±0.37	38.4
CO 6	43.8±0.18	42.1±0.87	39.2±0.20	37.3±0.45	35.9±0.50	39.7
CO 8	39.7±0.53	37.4±0.32	34.2±0.69	33.0±0.54	30.3±0.24	34.9
CO 10	40.9±0.43	38.1±0.34	35.9±0.37	33.9±0.72	31.9±0.34	36.1
NK 6240	42.2±0.62	39.8±0.41	37.4±0.64	35.7±0.46	33.5±0.45	37.7
900 M GOLD	41.7±0.30	38.5±0.62	36.1±0.25	35.4±0.59	31.1±0.23	36.6
MEAN	41.8±0.37	39.3±0.52	36.9±0.40	35.2±0.54	32.9±0.36	37.2
	EC	G	EC x G			
SE(d)	0.31	0.34	0.70			
CD (P=0.05)	0.63**	0.69**	1.50*			

Table 3. Effect of saline water irrigation on catalase activity in maize hybrids

Maize Hybrids/ EC levels (dSm ⁻¹)	Catalase activity (µg H ₂ O ₂ min ⁻¹ g ⁻¹)					Mean
	<1	2-4	4-6	6-8	>8	
CO 6	7.01±0.03	7.32±0.14	7.45±0.13	7.67±0.11	7.51±0.09	7.39
CO 7	7.00±0.07	7.25±0.12	7.26±0.12	7.68±0.10	7.57±0.08	7.35
CO 8	7.12±0.04	7.02±0.11	7.34±0.09	7.36±0.06	7.24±0.05	7.22
CO 10	7.00±0.07	7.19±0.12	7.34±0.10	7.48±0.07	7.35±0.13	7.27
NK 6240	7.02±0.16	7.25±0.13	7.36±0.12	7.46±0.09	7.38±0.07	7.29
900 M GOLD	7.11±0.10	7.22±0.08	7.35±0.10	7.45±0.09	7.28±0.06	7.28
MEAN	7.04±0.03	7.21±0.12	7.35±0.11	7.52±0.08	7.39±0.07	7.30
	EC	G	EC x G			
SE(d)	0.10	0.09	0.23			
CD (P=0.05)	0.19*	0.21*	0.46*			

Table 4. Effect of saline water irrigation on peroxidase activity in maize hybrids

Maize Hybrids/ EC levels (dSm ⁻¹)	Peroxidase activity (Units min ⁻¹ mg ⁻¹)					Mean
	0.06	3.2	4.8	6.2	8.9	
CO 6	0.50±0.03	0.52±0.05	0.54±0.04	0.61±0.07	0.55±0.04	0.54
CO 7	0.49±0.02	0.51±0.06	0.53±0.02	0.59±0.09	0.51±0.03	0.53
CO 8	0.42±0.04	0.45±0.02	0.50±0.03	0.53±0.05	0.38±0.02	0.46
CO 10	0.44±0.03	0.47±0.05	0.52±0.04	0.54±0.05	0.41±0.01	0.48
NK 6240	0.49±0.01	0.50±0.03	0.53±0.02	0.55±0.03	0.49±0.03	0.51
900 M GOLD	0.48±0.04	0.49±0.04	0.51±0.06	0.54±0.04	0.46±0.05	0.50
MEAN	0.47±0.03	0.49±0.04	0.52±0.04	0.56±0.05	0.47±0.03	0.50
	EC	G	EC x G			
SE(d)	0.006	0.005	0.016			
CD (P=0.05)	0.013**	0.014**	0.033**			

Table 5. Effect of saline water irrigation on superoxy dismutase activity in maize hybrids

Maize Hybrids/ EC levels (dSm ⁻¹)	Superoxy dismutase (EU per g)					Mean
	0.06	3.2	4.8	6.2	8.9	
CO 6	6.20±0.14	6.48±0.12	7.15±0.11	7.59±0.15	7.32±0.12	6.95
CO 7	6.08±0.13	6.26±0.15	6.58±0.13	7.22±0.11	6.87±0.11	6.60
CO 8	5.78±0.11	5.87±0.13	6.12±0.11	6.50±0.13	6.37±0.14	6.13
CO 10	5.83±0.15	5.99±0.12	6.24±0.12	6.65±0.14	6.47±0.12	6.24
NK 6240	6.05±0.16	6.21±0.14	6.47±0.14	6.87±0.16	6.69±0.13	6.46
900 M GOLD	5.94±0.13	6.10±0.13	6.36±0.15	6.76±0.11	6.58±0.17	6.35
MEAN	5.98±0.14	6.15±0.13	6.49±0.12	6.93±0.13	6.72±0.13	6.45
	EC	G	EC x G			
SE(d)	0.08	0.09	0.20			
CD (P=0.05)	0.17**	0.18**	0.40*			

Table 6. Correlation between plant DMP and antioxidative enzyme activities

	DMP	Chlorophyll	Catalase	Peroxidase	Superoxydismutase
DMP	1.00				
Chlorophyll	0.88*	1.00			
Catalase	0.95**	0.87*	1.00		
Peroxidase	0.97**	0.93**	0.96**	1.00	
Superoxydismutase	0.98**	0.79*	0.97**	0.94**	1.00

*, ** Significant at 5 and 1 per cent level, respectively

Fig 1. Effect of saline water irrigation on antioxidant enzyme activities in maize hybrids A) Plant DMP B) Catalase C) Superoxy dismutase and D) Peroxidase

