The compensatory effect of glutathione on alleviating salinity – induced modulations in growth and biochemical traits in maize irrigated with diluted seawater

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Abstract: Salinity stress has recently received much attention as an object worthy of research and interest. It is a great challenge for the future global agricultural production that aspires to a large-scale conversion of raw seawater to irrigation use. Our study aims to investigate the antioxidant and free radical scavenging effect of glutathione (GSH) that would enhance maize tolerance to the destructive effect of salinity. A greenhouse trail was conducted in this context during the summer season of 2015 using two salinity (Mediterranean seawater: 3000 and 6000 ppm) and GSH (100 and 200 pm) levels. Tap water was used as a control. Individually, saline water acted in a distinctly different manner than GSH. Irrigation with diluted seawater caused morphological alterations consistent with chemical imbalance. The weight, stem diameter and longitudinal growth of maize were substantially reduced, while enzymatic and non-enzymatic antioxidant components were positively enhanced. Amino acid composition was significantly higher only among plants received low salt concentration (3000 ppm). Glutathione application alone had a strong impact in promoting maize growth. However, lower response was noted at the level of antioxidant-related substances and amino acids content in comparison with salinity treatments. In stressed plants, glutathione mitigated the detrimental effects imposed by salinity, both at the morphological and biochemical levels. Concurrently, the alleviative effect increased as GSH concentration increased. In view of the results obtained irrigation maize with diluted seawater is possible, yet the cumulative adverse effects of salt on land safety should be considered. Our results suggest that using GSH enhances maize tolerance to salinity, and promotes plant recovery from the stress.

Keywords: antioxidants, oxidative stress, reactive oxygen species (ROS), salinity, tolerance, zea mays

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1 Introduction

The serious and wide-ranging implications of the climate and environmental changes appear destined to cast a disastrous shade on plants and crop productivity worldwide, with potentially profound and dangerous consequences for future global food security (Schmidhuber and Tubiello, 2007). Global climate change has already had observable effects on increasing temperatures and subsequently drought and salinity. Extended draught periods lead to increase salinity because less water is available to leach salts (SAIP, 2016). Salinity is one of the major environmental factors that limit plant growth and development. Currently, many of the world's arid and semi-arid regions are suffering hyper salinity. According to the FAO (2008), nearly 20% (45 M ha) of the world's irrigated lands (230 M ha) and 2.1% (32 M ha) of the almost 1500 M ha under dryland agriculture are salt-affected soils.

Increased demand for food worldwide place a greater burden on the agricultural sector as sustained over-exploitation and misuse of the available limited natural resources rise. According to the last estimation, the population of the world is predicted to increase from 6 billion people in 2000 to more than 10 billion in 2050 (Jaggard et al., 2010). This requires the average world

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cereal yield to increase approximately two times its present 3 t/h, with continuing working towards increasing horizontal agricultural expansion (Ahmad et al., 2012). The problem becomes more complex when we considered the escalating challenges related to water deficiency and deterioration in quality that have emerged in the last two decades. Currently, groundwater in many parts around the world, as it is in Egypt, experiences excess salinity due to the exaggerated and unsustainable withdrawal from wells (Ahmed et al., 2013). Today, there is an increasing concern among research community and agricultural planers to use diluted seawater for irrigation in agriculture (Kim et al., 2016).

Existing plants, in their current form, are not fully equipped to withstand the increasing occurrence of extreme events. Plants with greater fitness are required in the foreseeable future. Only stress-acclimated plants can survive in extreme environments or unfavourable growth conditions. Great efforts are being made to develop new resistance plants. Breeding new and higher-performing crops enhances the resistance of plants to a variety of abiotic and biotic stresses (Lane andJarvis, 2007). Diaz-Vivancos et al. (2013) indicated that transformation of plum plants with genes encoding antioxidant enzymes improved the tolerance to salinity. Results from previous research suggested a practical potential of exogenous antioxidants application as an intervention strategy in mitigating imposed adverse effects by low temperature (Ahmad et al., 2014**)**. They have received a great deal of concern for being potentially protective factors (Foyer and Noctor, 2005). One of the earliest responses of plant cells to various biotic and abiotic stresses is the production of the so-called reactive oxygen species [(ROS), Jajic et al., 2015]. Heat, drought, cold, salinity, metal contamination, nutrient deficiency, and ultraviolet-B radiation are the major biotic stresses that enhance generation of ROS in plants (Shukla et al., 2008; Sharma et al., 2012). In a biological context, ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling (*e.g.*, pathogen defense, programmed cell death, stomatal behavior) and homeostasis (Karuppanapandian et al., 2011). They are also produced as secondary messengers in a variety of developmental processes. More thoroughly, ROS influence the expression of a number of genes that are essential to the many of the pivotal physiological responses in plants (Sharma et al., 2012). High concentrations of ROS, however, are extremely harmful to plants; cause cell damage followed by complete growth failure because of the oxidative stress-induced effects. They rapidly degrade macromolecules such as proteins, carbohydrates, lipids and nucleic acids that are important for cell building and plant vitality (Gill and Tuteja, 2010).

Plants innately developed several strategies to adjust ROS level. The intracellular biological system of plants possesses very efficient antioxidative defense system comprising of enzymatic (e.g., superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S-transferase, GST), and non-enzymatic (e.g., ascorbic acid, AsA; GSH; phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidants. This complex network of antioxidant metabolites and enzymes works in a concerted and coordinated manner to contain overproduction of ROS (Asada, 2006). Attenuating the imbalance between generation and scavenging of ROS is fundamental for healthy plants. The equilibrium between both sides is a central element in maintaining steady state conditions [(redox homeostasis), Foyer and Noctor, 2005]. When this neutral equilibrium is disrupted (the accumulation of ROS exceeds the capacity of defense mechanisms) due to multiple abiotic or biotic stress factors, the cell is then called under oxidative stress. ROS production and accumulation of damage is greatly affected by the associated conditions such as light intensity and temperatures (Caverzan et al., 2012). Duration and severity of stress, as wells as the ability of the tissue to withstand or to acclimate to the energy imbalance and restore cellular homeostasis are also closely interlinked to this matter (Miller et al., 2010).

In the current work, we aim to investigate the potential role of GSH in addressing the problems associated with salinity stress on growth and development of maize.

2 Materials and Methods

2.1 Plant materials and growth conditions

In this study, a pot experiment was conducted in a wire-house at the National Research Centre, Dokki, Cairo, Egypt during the summer season of 2015. Maize (*Zea mays* cv. Single Hybrid 10) seeds were purchased from the Agricultural Research Centre, Ministry of Agricultural, Egypt, and subjected to selection for uniformity by choosing those approximately with the same size. An appropriate number of plastic pots (50 cm diameter x 40 cm depth) were filled with clay loam soil. Physical and chemical properties of the soil were as described elsewhere (Orabi and El-Noemani, 2015) and are summarized in Table (1). To improve drainage quality, the soil was mixed partially with sand (3:1; v/v).

Seeds of maize were sown (6 seeds/pot) during the first week of June. The emerged plants were thinned twice (3 and 5 weeks after sowing) to a final number of 4 uniform plants/pot. Fertilizers were added according to the recommendations. Super phosphate (15.5% P_2O_5) and potassium sulfate (K_2O) were added before sowing (during seedbed preparation) at rate of 2.50 and 1.5 g/pot, respectively. The nitrogen fertilizer (ammonium sulfate, 20.5% N) was added (7 g/pot) in two equal doses after four and six weeks from sowing. The soil field capacity was estimated by saturating the pots with water and weighing them after they had drained for 48 h.

Two levels (3000 and 6000 ppm) of diluted saline water (Source: Mediterranean seawater) were used in irrigation starting 35 days after sowing; tap water (250 ppm) was included as a control. Irrigation events occurred alternately with fresh water [2 (salt water) :1 (tap water)], with an equal amount per pot.

Glutathione in the concentration of 100 and 200 ppm was foliar-sprayed [twice at 45 (when the plants have reached 6 to 8 fully developed leaves), and 65 days after sowing (DAS)] with handheld sprayer. Tap water was used as a control. The experiment was set up in a completely randomized block (3×3) factorial design with three replications per treatment.

Plant samples (2 plant/pot) were randomly taken to determine morphological parameters [plant height (cm), dry weight (g), stem diameter (cm), no. of leaves/plant] and biochemical constituents [APX (μ mol/g Fr. Wt.), GR enzyme activities (n mole/g Fr. Wt.), total phenols (mg/g Fr. Wt.), ASA, GSH (μ mol/g Fr. Wt.), amino acids (g/100 g protein)] at 75 DAS.

2.2 Biochemical measurements

The changes in some biochemical parameters due to salinity stress or GSH application including enzymatic and non-enzymatic antioxidants as well as various amino acids were assessed in leaf tissues. All biochemical assessments were performed within 120 h of collecting samples.

2.3 Measurement of enzymatic antioxidants

The analysis included APX and GR enzymes. Activity of the enzymes was determined using 5 g of the frozen leaf tissues. Extraction was done in ice-cold 50 mM potassium phosphate buffer (10 ml; pH 7.0) with 0.1mM ethylene diamine tetra acetic acid (EDTA) and addition of polyvinyl pyrrolidone [PVP; 1% (w:v)]. The extraction step was repeated twice, pooling all the supernatants together. The pooled supernatants, referred as the crude protein extract, were adjusted to a particular volume and stored frozen at –4°C until further analyses. Activity of APX (EC 1.11.1.11) was determined spectrophotometrically according to Nakano and Asada (1981). One unit of APX was defined as the amount of enzyme that degraded 1 μ mole of ascorbate (ASA) per min. Measurement of GR activity (1.6.4.2) was carried out following the method of Zanetti (1979). One unit of GR was defined as the amount of enzyme that decreases 1A340 per min.

2.4 Measurement of non-enzymatic antioxidants

This involved AsA, GSH and total phenols. The content of reduced AsA was assessed as described by Kampfenkel et al. (1995). Total GSH content was measured according to Silber et al. (1992). Total phenols were determined by the Folin–Ciocalteu colorimetric assay according to the method described by Meda et al. (2005). Concentration of the total phenols was plotted from the pyrogallol calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of pyrogallol equivalents/g of fresh sample.

2.5 Determination of amino acids

Samples were assayed for amino acids determination (qualitatively and quantitatively) following the procedures of the standard test methods (AOAC, 1984). This included aspartic, threonine, serine, glutamic, glycine, alanine, valine, methionine, isolaucine, leucine, tyrosine, phenylalanine, histidine, lycine, arginine, proline, and cystin. The crude protein content (in the defatted and dry form) was obtained by the regular micro-kjeldahl method. A reconstituted protein sample of 50 mg protein was hydrolyzed with 5 mL of 5.7 N HCl in sealed ampoules for 24 hr at 110°C. After cooling the contents, the sealed tubes were opened and the hydrolysate was filtered through filter paper Whatman No. 1. The residues with the help of a few milliliters of distilled water were rewashed several times and the final filtrate was completed to 50 mL. Five ml of the filtrate were evaporated to dryness under vacuum at 50°C. The residue was re-extracted with 5 ml of sodium citrate buffer of pH 2.2 and filtered through 0.22 μm membrane. An aliquot of 20 μL was used for the amino acids fraction. Analysis was carried out using an Eppendorf BiotronikLC 3000 Amino Acid Analyzer (Eppendorf-Biotronik, Hamburg, Germany). Operative conditions were: pressure of buffer, 0 to 50 bar; pressure of reagent: 0 to 50 bar; flow rate, 0.2 mL/min; reaction temperature, 123°C. The results were expressed as g/100 g protein.

2.5 Statistical analysis

Data were analyzed for the significant differences between the mean values of the different results. Differences between means were analyzed by two-way analysis of variance using ANOVA table and LSD test at 5% probability (Snedecor and Cochran, 1980).

Table 1 Physico-chemical properties of the soil used in the experiment

Soil texture			Silt, %			Clay, %		Classification	
			36.00			38.00		Clay loam	
Physical characteristics	Soil water capacity/ Others		*F.C., %		W.P. , %	A.W. , %		H.C., cm h^{-1}	B.D., g cm^{-2}
			31.01		16.20	14.81		1.19	1.10
Chemical characteristics	pH	EC, dS m^{-1}	Soluble cations, mole L^{-1}			Soluble anions, mole L^{-1}			
			Ca^{++}	Mg^{++}	Na^{+}	P^{+}	Cl^{--}	CO_3^{-}	HCO_3^{--}	SO_4^{--}
	7.70	0.60	1.11	0.88	2.20	1.48	0.75	2.14	1.14	1.65

Note: * F.C., field capacity; W.P, wilting point; A.W., available water; H.C., hydraulic conductivity; B.D., bulk conductivity; E.C., electrical conductivity.

3 Results

3.1 Vegetative growth

The changes in growth criteria (plant height, number of leaves, stem diameter, and dry matter) of maize due to the different salinity/GSH treatments are illustrated in Tables 2 and 3. Individually, saline water acted in a distinctly different manner than GSH. Irrigation with diluted seawater significantly impaired plant growth at both levels of application (3000 and 6000 ppm). The reduction in plant height, number of leaves, stem diameter and dry weight was estimated at 16.82%-36.76%, 6.84%-23.78%, 9.70%-25.45%, and 25.11%-44.46%, respectively compared to unstressed control plants (Table 3). Dose-related reductions were noted throughout the complete dose range. Glutathione, however, showed pronounced positive effects compared to seawater-treated plants. An estimated 13.25%-21.17%, 26.45%-31.45%, 16.06%-29.40%, and 22.24%-66.17% increase over control were recorded for the different parameters in the same order. Minor insignificant differences were, generally, noted on the no. of leaves/plant. Dose-dependent increases were noted over the two examined concentrations.

On the other hand, glutathione mitigated salinity stress-injury on maize plants, evident in the increased level of plant height, number of leaves, stem diameter, and dry-weight biomass (Table 3). Spraying GSH on plant under irrigation with tap water (250 ppm salts) significantly increased all parameters in a range of 13.25% to 66.14%. The higher the GSH concentration, the greater the impact in avoiding salinity damage. Maximum results were obtained with the 200 ppm concentration, irrespective of the rate of salinity.

Table 2 Growth characteristics of maize as affected by salinity/GSH treatments (75 DAS)

Salinity level, ppm	Glutathione concentration, ppm	Plant height, cm	Stem diameter, cm	No.of leaves	Dr. Wt., g plant^{-1}
250 (tap water)	0	83.00	1.90	10.33	42.15
	100	94.00	2.40	12.00	51.52
	200	101.00	2.50	13.33	69.80
Mean values of salinity		92.66	2.27	11.89	54.49
3000 (Mediterranean seawater)	0	69.00	1.77	9.33	31.53
	100	75.67	1.83	10.00	38.31
	200	80.50	1.90	11.67	44.26
Mean values of salinity		75.06	1.83	10.33	38.03
6000 (Mediterranean seawater)	0	52.50	1.63	7.67	23.26
	100	57.00	1.67	9.33	29.51
	200	61.00	1.77	9.33	32.32
Mean values of salinity		56.83	1.69	8.78	28.36
Mean values of glutathione	0	68.17	1.77	9.11	32.31
	100	75.56	1.97	10.44	39.78
	200	80.83	2.06	11.44	48.79
LSD $_{0.05}$	*S/G	2.09	0.10	0.78	2.20
	S ×G	3.62	0.18	NS	3.82

Note: * S, salinity; G, glutathione; NS, not significant.

Table 3 The inhibitory/stimulatory effect (% of control) of the different treatments on growth and development of maize plants (75 DAS)

Salinity level, ppm	Glutathione conc., ppm	Plant height	Incr.$^{(+)}$/inh.$^{(-)}$ (%) of control	Stem diameter	Incr.$^{(+)}$/inh.$^{(-)}$ (%) of control	No. of leaves	Incr.$^{(+)}$/inh.$^{(-)}$ (%) of control	Dr. Wt.	Incr.$^{(+)}$/inh.$^{(-)}$ (%) of control
250 (tap water)	0	100	--	100	--	100	--	100	--
	100	113.25	$^{+}$13.25	126.45	$^{+}$26.45	116.06	$^{+}$16.06	122.24	$^{+}$22.24
	200	121.71	$^{+}$21.71	131.54	$^{+}$31.54	129.40	$^{+}$29.40	166.14	$^{+}$66.14
Mean values of salinity		111.65	$^{+}$11.65	119.33	$^{+}$19.33	115.15	$^{+}$15.15	129.46	$^{+}$29.46
3000 (Mediterranean seawater)	0	83.18	$^{-}$16.82	93.16	$^{-}$6.84	90.30	$^{-}$9.70	74.89	$^{-}$25.11
	100	91.15	$^{-}$8.85	96.48	$^{-}$3.52	97.27	$^{-}$2.73	91.37	$^{-}$8.63
	200	97.00	$^{-}$3.00	100.00	0.00	113.03	$^{+}$13.03	105.10	$^{+}$5.10
Mean values of salinity		90.45	$^{-}$9.55	96.55	$^{-}$3.45	100.20	$^{+}$0.20	90.45	$^{-}$9.55
6000 (Mediterranean seawater)	0	63.27	$^{-}$36.73	86.22	$^{-}$23.78	74.55	$^{-}$25.45	55.54	$^{-}$44.46
	100	68.69	$^{-}$31.31	87.97	$^{-}$12.03	90.30	$^{-}$9.70	70.36	$^{-}$29.64
	200	73.54	$^{-}$26.46	93.33	$^{-}$6.67	90.61	$^{-}$9.39	76.94	$^{-}$23.06
Mean values of salinity		68.50	$^{-}$31.5	89.17	$^{+}$10.83	85.15	$^{-}$14.85	67.62	$^{-}$32.38
Mean values of glutathione	0	82.15	$^{-}$17.85	93.13	$^{-}$6.87	88.28	$^{-}$11.72	76.81	$^{-}$23.19
	100	91.03	$^{-}$8.97	103.63	$^{+}$3.63	101.21	$^{+}$1.21	94.66	$^{-}$5.34
	200	97.42	$^{-}$2.58	108.29	$^{+}$8.29	111.01	$^{+}$11.01	116.06	$^{+}$16.06
LSD $_{0.05}$	*S/G	2.39	--	5.10	--	6.83	--	5.03	--
	S ×G	4.14	--	8.83	--	NS	--	8.71	--

Note: The data were analyzed as a percentage of control and then the inhibitory or stimulatory effect were deduced from the average obtained.

* Abbreviations are as in Table 2.

3.2 Enzymatic and non-enzymatic antioxidants

Data presented in Tables 4 and 5 describe the effect of the different treatments on enzymatic and non-enzymatic antioxidants in maize-treated plants. Under condition of salinity stress, APX and GR enzyme activities were significantly increased in comparison to the control (unstressed) plants. The results showed higher levels of APX activity (up to 180.67%), but this was associated with relatively less activity in GR (up to 23.62%) enzyme (Table 5). A dose-response relationship was noted between salinity concentration and APX, GR enzyme activities. The higher concentration (6000 ppm) was often associated with more activity. A similar approach but with some noticeable differences was observed with GSH. The activities of APX, and GR enzymes were increased in the plant tissues under GSH treatment (alone) by up to 87.67 and 16.55%, respectively in comparison with the well-watered control plants.

Table 4 Biochemical responses of maize as affected by salinity/GSH treatments (75 DAS)

Salinity level, ppm	Glutathione concentration, pm	Enzymatic antioxidants		Non-enzymatic antioxidant		
		APX, µ mol/g Fr. Wt.	GR, n mol/g Fr. Wt.	GSH, µ mol/g Fr. Wt.	AsA, µ mol/g Fr. Wt.	Phenols, Mg/g Fr. Wt.
250 (tap water)	0	1.21	375.13	5.40	8.12	2.25
	100	1.87	395.22	5.46	8.47	2.47
	200	2.26	435.42	5.81	9.23	2.94
Mean values of salinity		1.78	401.92	5.55	8.61	2.55
3000 (Mediterranean seawater)	0	2.03	406.39	6.24	8.99	2.78
	100	2.28	493.48	7.35	10.19	3.40
	200	3.07	508.77	8.14	10.82	3.52
Mean values of salinity		2.46	469.55	7.24	10.00	3.23
6000 (Mediterranean seawater)	0	3.38	462.21	6.86	10.09	2.44
	100	4.17	544.83	8.36	11.67	3.49
	200	4.85	625.22	9.19	12.31	4.10
Mean values of salinity		4.13	544.09	8.137	11.36	3.35
Mean values of glutathione	0	2.21	414.58	6.16	9.07	2.49
	100	2.77	477.85	7.06	10.11	3.12
	200	3.39	523.14	7.72	10.78	3.52
$LSD_{0.05}$	*S/G	0.11	23.06	0.37	0.44	0.10
	S ×G	0.19	39.94	0.64	NS	0.18

Note: * Abbreviations are as in Table 2.

Table 5 The stimulatory effect (% of control) of the different treatments on the biochemical-related parameters in maize plants (75 DAS)

Salinity level, ppm	Glutathione conc., ppm	Enzymatic antioxidants				Non-enzymatic antioxidant					
		APX	Incr. (%) of control	GR	Incr. (%) of control	GSH	Incr. (%) of control	AsA	Incr. (%) of control	Phenols	Incr. (%) of control
250 (tap water)	0	100	--	100	--	100	--	100	--	100	--
	100	155.82	55.82	105.37	5.37	101.05	1.05	104.54	4.54	109.88	9.88
	200	187.67	87.67	116.55	16.55	107.57	7.57	113.68	13.68	131.08	31.08
Mean values of salinity		147.83	47.83	107.31	7.31	102.87	2.87	106.07	6.07	113.65	13.65
3000 (Mediterranean seawater)	0	169.45	69.45	108.50	8.50	115.82	15.82	110.81	10.81	123.94	23.94
	100	190.19	90.19	131.70	31.70	136.25	36.25	125.91	25.91	151.24	51.24
	200	256.13	156.13	136.15	36.15	151.38	51.38	133.31	33.31	156.60	56.60
Mean values of salinity		205.26	105.26	125.45	25.45	134.48	34.48	123.34	23.34	143.92	43.92
6000 (Mediterranean seawater)	0	280.67	180.67	123.62	23.62	127.03	27.03	124.33	24.33	108.66	8.66
	100	347.40	247.40	145.61	45.61	155.63	55.63	144.27	44.27	155.20	55.20
	200	402.98	302.98	166.88	66.88	170.78	70.78	151.51	51.51	182.66	82.66
Mean values of salinity		343.68	243.68	145.37	45.37	151.15	51.15	140.04	40.04	148.84	48.84
Mean values of glutathione	0	183.37	83.37	110.70	10.70	114.28	14.28	111.71	11.71	110.87	10.87
	100	231.14	131.14	127.56	27.56	130.98	30.98	124.91	24.91	138.77	38.77
	200	282.26	182.26	139.86	39.86	143.24	43.24	132.83	32.83	156.78	56.78
$LSD_{0.05}$	*S/G	11.82	--	6.45	--	8.05	--	5.63	--	4.99	--
	S ×G	20.46	--	11.17	--	13.95	--	NS	--	8.64	--

Note: Explanations/abbreviations are as in Table (2 & 3).

Applying GSH on plants received salt treatments caused relatively higher impact on increasing enzymes activity, particularly with those under high salinity stress (6000 ppm). Highest activity of both enzymes was found in (6000 ppm salinity + 200 ppm GSH), and they were 302.98 and 66.88% over control (Table 5).

Regarding non-enzymatic antioxidant components, almost all comparisons reported a statistically positive significant difference in results. Individually, the effect of salinity was superior to that of GSH in enhancing the different studied components including GSH, AsA, and phenols. Glutathione in interaction with salinity exhibited

higher contents of non-enzymatic antioxidants. As previously reported the effect increased as the concentration increased. The highest values were obtained from GSH plus salinity at 200 ppm and 6000 ppm, respectively (Table 5).

3.3 Amino acids

The data on amino acids concentration appear to display a response resembling, to a large extent, that of the antioxidant components (Tables 6 and 7). We observed more quantitative changes than qualitative changes. The majority of amino acids were largely increased (16.96 to 250.00%) at the 3000 ppm salt level, but a remarkable reduction (up to 100%) was noted at the higher concentration (6000 ppm). An upward trend in results was recorded with GSH level. The increase in amino acids concentration due to the 100 ppm concentration ranged between 4.35 and 165.09%, meanwhile up to more than tenfold was recorded at the 200 ppm concentration in comparison with the unstressed control (Table 7). The presence of the amino acid cystin synchronized with the absence of the amino acid alanine in the control and 100 ppm GSH samples. Cystin was only found in these two treatments.

Table 6 Amino acids composition in response to certain salinity/glutathione treatments (75 DAS)

Amino acid, g/100 g protein	Salinity/GSH concentrations, ppm						
	Control	Salinity		Glutathione		Salinity plus glutathione	
	0.00	3000	6000	100	200	6000+100	600+200
Aspartic	2.30	2.69	0.95	2.40	2.18	2.65	3.01
Threonine	0.28	0.68	0.30	0.47	0.51	0.37	0.41
Serine	0.54	0.91	0.29	0.67	0.61	0.64	0.42
Glutamic	1.21	2.37	0.92	1.40	1.62	1.43	1.74
Glycine	0.24	0.37	0.14	0.27	0.31	0.31	0.43
Alanine	0.00	1.65	0.69	0.00	0.13	0.95	2.20
Valine	0.40	1.06	0.25	0.36	0.55	0.29	0.86
Methionine	1.48	3.02	0.97	1.86	2.26	1.83	3.38
Isolaucine	0.30	0.46	0.17	0.24	0.37	0.30	0.51
Leucine	0.35	0.96	0.23	0.43	0.45	0.54	1.03
Tyrosine	0.20	0.62	0.12	0.59	0.77	0.15	1.11
Phenylalanine	0.17	0.15	0.07	0.10	2.00	0.19	0.16
Histidine	0.22	0.77	0.35	0.54	1.53	0.89	2.15
Lycine	0.34	0.82	0.33	0.56	1.77	0.31	1.25
Arginine	0.85	1.09	0.43	0.77	2.30	0.48	1.72
Proline	1.46	1.71	0.62	3.87	1.60	3.39	3.91
Cystin	3.46	0.00	0.00	4.92	0.00	0.00	0.00
Total	13.80	19.33	6.83	19.45	18.96	14.72	24.29

Table 7 The stimulatory effect of certain selected treatments on amino acids composition in maize plants (75 DAS)

Amino acid	Increasing (%) of control					
	Salinity, ppm		Glutathione, ppm		Salinity plus glutathione, ppm	
	3000	6000	100	200	6000+100	600+200
Aspartic	16.96	*58.70	4.35	*5.21	15.21	30.87
Threonine	142.86	7.14	67.86	82.14	32.14	46.43
Serine	68.52	*46.30	24.07	12.96	18.52	*22.22
Glutamic	95.87	*23.97	15.70	33.88	18.18	43.80
Glycine	54.17	*41.67	12.50	29.17	29.17	79.17
†Alanine	--	--	--	--	--	--
Valine	165.00	*37.50	*10.00	37.50	*27.50	115.00
Methionine	104.05	*34.46	25.68	52.70	23.69	128.38
Isolaucine	53.33	*43.33	*20.00	23.33	0.00	70.00
Leucine	174.29	*34.29	22.86	28.57	54.29	194.28
Tyrosine	210.00	*40.00	195.00	285.00	*25.00	455.00
Phenylalanine	*11.76	*58.82	*41.8	1076.47	11.76	*5.88
Histidine	250.00	59.09	145.46	595.46	304.55	877.27
Lycine	141.18	*2.94	64.70	420.59	*8.82	267.65
Arginine	28.24	*49.41	*9.41	170.59	*43.59	102.35
Proline	17.12	*57.53	165.09	9.59	132.19	167.81
Cystin	*100.00	*100.00	42.20	*100.00	*100.00	*100.00
Total	40.07	*50.51	40.94	37.39	6.67	76.01

Note: * Inhibition; †Alanine was absent in the control sample and found in the others.

Applying GSH over plants irrigated with the higher level of salinity (6000 ppm) amazingly defeated the adverse effect of salinity in reducing amino acids content. The 200 ppm GSH concentration was more effective (30.87 to 877.27% increase) than the 100 ppm concentration (11.76 to 304.55% increase) in this context (Table 7).

3.4 Discussion

The overall objective of this work was to find a clear understanding of the effects of salinity on growth and development of maize, besides exploring the potential role of GSH in mitigating or eliminating such impacts via enhancing antioxidant responses to generated ROS.

Under irrigation by diluted seawater, maize plant growth expressed as plant height, stem diameter, and dry-weight biomass was significantly negatively affected due to the excessive salt uptake. According to our findings the effect increased as the concentration increased. Similar results were obtained on maize by various studies worldwide. In accordance with Hussein et al. (2007), maize plants undergo significant changes from the time salinity stress is imposed. Anatomical alterations

with shift towards disruption in the metabolic processes are among the major impacts of salinity on maize (Farhana et al., 2014). Indeed, others had obtained remarkably similar results on a variety of plant species (Kim et al., 2016).

A causal relationship between salinity or chemically induced oxidative stress and growth damage has been proven by many researchers. Generally, salinity poses adverse health impacts on plants (Yadav et al., 2011). It causes a wide range of morphological, anatomical, metabolic and enzymatic changes that unfavourably affect healthy growth of plants (Ahmad et al., 2012). Regardless of the type of plant, salinity can affect photosynthesis via reducing chlorophyll content, destruction of chloroplast ultrastructure, or damaging many of the related enzymes (Franken et al., 2014; Aldesuquy, 2015). With increasing salinity level and duration of treatment, chloroplasts number and intercellular spaces were found to be dramatically decreased in conjunction with increasing cell-wall thickness and even cracking owing to the increased succulence (Gao et al., 2015). This leads the whole intracellular system to a complete disorganization with a broad failure in the performance of the main tasks.

Individual treatment of GSH, on the other hand, markedly increased plant growth, evident in the increased level of plant height, stem diameter, no. of leaves and dry-weight biomass. These results are in close coordination with those discussed by many researchers who confirmed that GSH is a significant element in improving plant growth, and its level (GSH+GSSG) conflicts the growth-associating conditions (Smirnoff, 2008). It is very essential for healthy growth. Besides functioning as a potent antioxidant in maintaining the intracellular homeostasis, GSH plays a crucial role in numerous biological activities engaged in growth and development during the entire lifespan of the plant (Forman et al., 2009). One of the major themes that has emerged from *in vitro* studies is that GSH promotes cell proliferation, while GSSG promotes organized development (Young et al., 2005).

According to the current findings, GSH efficiently mitigated salinity-induced modulation in growth and biochemical traits, and they were largely consistent with results of reported research in this regard. A primary biological function of GSH is to remove the oxidative stress. A significant number of research studies examined the antioxidant properties of GSH and a great activity was obtained in being capable to alleviate plant resistance to unvafourable growth conditions including salinity (Abogadallah, 2010). Under the no-stress conditions, plants induce antioxidants production to cope with any excess of ROS, which may generate due to the different physiological activities. Under conditions, which promote oxidative stress, endogenous antioxidants are produced in higher concentrations. Glutathione is considered one of the most abundant bioactive substances in this regard (Chakraborty and Chakraborty, 2015). These collective evidences underscore the pivotal role of GSH in detoxifying salinity-induced effects in maize.

Increasing the intracellular activity/content of enzymatic and non-enzymatic antioxidants reflects the positive interactive status of the plants in dealing with the destructive effect of salinity. Ascorbate peroxidase, GR activities, AsA, GSH and total phenol contents concurrently increased with salinity stress. The same occurred with some noticeable differences in response to GSH applied alone or under all treatments of salinity. The results indicated that GSH in interaction with salinity had greater enzymatic and non-enzymatic antioxidant capacity, and therefore much less oxidative damage. It is hypothesized that the availability of antioxidants and antioxidant-related enzymes increases to cope with the situated oxidative stress, which with the help of external dose of GSH may gave maize plants a greater advantage to perform better against even more aggressive conditions. Research has provided a great deal of support for the role of enzymatic and non-enzymatic antioxidants in the protection against ROS-mediated injury (Gill and Tuteja, 2010). The most recent studies on maize have shown that the elevated antioxidant levels can protect the photosynthetic apparatus from oxidative damage (Diao et al., 2014).

In view of the consistent experimental results that have previously been published, these findings seem acceptable for the rational explanation. The correlation

between salt tolerance and antioxidant capacity has been demonstrated in a large number of plants. Salinity stress often causes a series of changes at both the physiological and the molecular level (Cáccoli et al., 2001; Abdul Qados, 2011). The increase in relevant antioxidants including enzymatic and non-enzymatic ones is a normal event of salt-induced action (Hasanuzzaman et al., 2013). Of which the most important that have been reviewed deeply and listed as major contributors to the antioxidant potential in the plants are GSH, AsA, proline, phenolics, GR, SOD, APX, CAT, guaiacol-specific and peroxidase [POX; (Smirnoff, 2008)]. Collectively, Lu et al. (2006) suggested that the oxidative damage occurring under moderate hyposaline and hypersaline conditions is ascribed to the accumulated H_2O_2 and that positively correlates with increasing GR activity, APX activity and GSH content.

Ascorbate peroxidase is a primary enzyme of AsA-GSH cycle. It utilizes AsA as specific electron donor to reduce H_2O_2 to water (Caverzan et al., 2012). Glutathione reductase is responsible for the regeneration of GSH from GSSG using NADPH as a reducing equivalent in the ASA-GSH pathway. The elevated level of GR might act in increasing the ratio of $NADP^+$ to NADPH, and thereby increase the availability of the first to accept electron from the photosynthetic electron transport chain (Orabi and El-Noemani, 2015). Under these conditions, the rate of electron transport to O_2 is reduced, and hence the chances of ROS formation. In parallel, phenolics are highly potent antioxidant compounds. They play a key role in defensive reactions of plants against the adverse effects of environment-inducedabiotic stress factors (Mazid et al., 2011).

Concerning amino acids and their response to the different treatments, there was no conflictions between the results obtained and those discussed by other researchers. Under conditions of salinity stress, amino acids production followed a definite pattern. A remarkable induction was noted at the lower concentration, while the converse occurred with the higher concentration which was bigger than plant tolerance; essentially worked against active production of amino acids. A dose-dependent inhibitory-stimulatory effect was suggested. These results were largely consistent with the past research. Cusido et al. (1987) found that treatment of *Nicotiana rustica* plants with 50 and 100 mM NaCl induced an increase in free amino acids, especially of aspartic acid, glutamic acid and proline. The authors showed that the deficit of K due to the excessive growth of Na created a stat of ionic/chemical dynamic disequilibrium, which in turn leads to increase amino acids composition in plant leaves. Similarly, Abd El-Samad et al. (2010) noted an increase in sodium content in detriment of K^+, Ca^{++}, Mg^{++}, and P in maize and faba bean salt-stressed plants alluding to the viability of proline and amino acids in reshaping the balance between absorption of the different elements in a manner not detrimental to the plant. *Pennisetum glaucum* exposed to a relatively higher concentration (up to 200 mM NaCl) exhibited a similar response (Sneha et al., 2013). The authors suggested that proline and free amino acids act as compatible solutes to protect the cellular macromolecules which are functioned in maintaining the osmotic balance and also scavenge the free radicals. However, increasing salt stress to a level exceeds tolerance threshold of plant can lead to a drop in amino acid pools and subsequently total protein content (Sivasankaramoorthy et al., 2010), which comes in complete agreement with our findings.

Application of GSH on salt-stressed plants was found to be effective in increasing plant composition of the different amino acids. Better results were obtained with the higher concentration. Increasing endogenous amino acids and hence protein composition is one of the characteristic features of GSH-induced impacts in salt stressed plants (Akladious and Abbas, 2013). Glutathione itself is a small protein composed of three amino acids linked together and may have a role in enhancing amino acids pool in plants (Robins and Davies, 1981).

In view of the results obtained irrigation maize with diluted seawater is possible and economically viable, yet accumulating salts and distribution in soil during repeated irrigation should be considered on the long run and in planning future land use. Our results suggest that using GSH enhances maize tolerance to salinity, and promotes

recovery from the stress. Evidence-based efficacy reveals that GSH may provide a new perspective of saline agriculture, which implies the application of brackish and saline waters in irrigation of crops.

References

Abd El-Samad, H. M., M. A. K. Shaddad, and N. Barakat. 2010. The role of amino acids in improvement in salt tolerance of crop plants. *Journal of Stress Physiology & Biochemistry*, 6(3): 25–37.

Abdul Qados, A. M. S. 2011. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, 10(1): 7–15.

Abogadallah, G. M. 2010. Antioxidative defense under salt stress. *Plant Signaling & Behavior*, 5(4): 69–374.

Ahmad, P., M. M. Azooz, and M. N. V. Prasad. 2012. *Ecophysiology and Responses of Plants Under Salt Stress*. New York, USA: Springer.

Ahmad, I., S. M. A. Basra, and A. Wahid. 2014. Exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide improves the productivity of hybrid maize under at low temperature stress. *International Journal of Agriculture & Biology*, 16(4): 1560–8530.

Ahmed, M. A., S. G. Abdel Samie, and H. A. Badawy. 2013. Factors controlling mechanisms of groundwater salinization and hydrogeochemical processes in the Quaternary aquifer of the Eastern Nile Delta, Egypt. *Environmental Earth Sciences*, 68(2): 369–394.

Akladious, S. A., and S. M. Abbas. 2013. Alleviation of sea water stress on tomato plants by foliar application of aspartic acid and glutathione. *Bangladesh Journal of Botany*, 42(1): 31–43.

Aldesuquy, H. S. 2015. Impact of seawater salinity on ultrastructure of chloroplasts and oleosomes in relation to fat metabolism in flag leaf of two wheat cultivars during grain-filling. *Advances in Crop Science and Technology*, 4: 200. Doi: 10.4172/2329-8863.1000200

AOAC. 1984. *Official Methods of Analysis of the Association of Official Analytical Chemists*. 12th ed. Arlington, Virginia, USA: AOAC.

Asada, K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, 141(2): 391–396.

Caverzan, A., G. Passaia, S. B. Rosa, C. W. Ribeiro, F. Lazzarotto, and M. Margis-Pinheiro. 2012. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genetics and Molecular Biology*, 35(4): 1011–1019.

Céccoli, G., J. C. Ramos, L. I. Ortega, J. M. Acosta, and M. G. Perreta. 2011. Salinity induced anatomical and morphological changes in *Chloris gayana* Kunth roots. *Biocell*, 35(1): 9–17.

Chakraborty, B., and U. Chakraborty. 2015. *Abiotic Stresses in Crop Plants*. Wallingford: CABI.

Cusido, R. M., J. Palazon, T. Altabella, C. Morales. 1987. Effect of salinity on soluble protein, free amino acids and nicotine contents in *Nicotiana rustica* L. *Plant and Soil*, 102(1): 55–60.

Diao, M., L. Ma, J. Wang, J. Cui, A. Fu, and H. Liu. 2014. Selenium promotes the growth and photosynthesis of tomato seedlings under salt stress by enhancing chloroplast antioxidant defense system. *Journal of Plant Growth Regulation*, 33(3): 671–682.

Diaz-Vivancos, P., M. Faize, G. Barba-Espin, L. Faize, C. Petri, J. A. Hernandez, and L. Burgos. 2013. Ectopic expression of cytosolic superoxide dismutase and ascorbate peroxidase leads to salt stress tolerance in transgenic plums. *Plant Biotechnology Journal*, 11(8): 976–985.

FAO. 2008. Land and plant nutrition management service. Available at: http://www.fao.org/ag/AGL/public.stm/ (Accessed: 14 November, 2016).

Farhana S., P. Rashid, and J. L. Karmoker. 2014. Salinity-induced anatomical changes in maize (*Zea mays* L. CV. BARI-7). *Dhaka University Journal of Biological Sciences*, 23(1): 93–95.

Forman, H., H. Zhang, and A. Rinna. 2009. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Molecular Aspects of Medicine*, 30(1-2): 1–12.

Foyer, C. H., and G. Noctor. 2005. Oxidant and antioxidant signaling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment*, 28(8): 1056–1071.

Franken, M., F. G. Lima, L. Baracioli, J. Nicolau. 2014. Effect of short term salt stress on chlorophyll content, protein and activities of catalase and ascorbate peroxidase enzymes in pearl millet. *American Journal of Plant Physiology*, 9(1): A1422.

Gao, H., H. Yang, J. Bai, X. Liang, Y. Lou, J. Zhang, D. Wang, J. Zhang, S. Niu, and Y. Chen. 2015. Ultrastructural and physiological responses of potato (*Solanum tuberosum* L.) plantlets to gradient saline stress. *Frontiers in Plant Science*, 5: 787.

Gill, S. S., and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12): 909–930.

Hasanuzzaman, M., K. Nahar, and M. Fujita. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damage. *Ecophysiology and Responses of Plants Under Salt Stress*, 25-87. New York, USA: Springer.

Hussein, M. M., L. K. Balbaa, and M. S. Gaballah. 2007. Salicylic acid and salinity effect on growth of maize plants. *Research Journal of Agriculture and Biological Sciences*, 3: 321–328.

Jaggard, K. W., A. Qi, and E. S. Ober. 2010. Possible changes to arable crop yields by 2050. *Philosophical Transactions of the*

Royal Society B, 365(1554): 2835–2851.

Jajic I., T. Sarna, and K. Strzalkam. 2015. Senescence, stress, and reactive oxygen species. *Plants*, 4: 393–411.

Kampfenkel, K., M. M. Van, and D. Inze. 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Analytical Biochemistry*, 225(1): 165–167.

Karuppanapandian, T., J. C. Moon, C. Kim, K. Manoharan, and W. Kim. 2011. Reactive oxygen species in plants: Their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science*, 5(6): 709–725.

Kim, H., H. Jeong, J. Jeon, and S. Bae. 2016. Effects of irrigation with saline water on crop growth and yield in greenhouse cultivation. *Water*, 8(4): 127. Doi: 10.3390/ w8040127

Lane, A., and A. Jarvis. 2007. Changes in climate will modify the geography of crop suitability: Agricultural biodiversity can help with adaptation. *SAT eJournal*, 4(1): 1–12.

Lu, F., M. S. Sung, and T. M. Lee. 2006. Salinity stress and hydrogen peroxide regulation of antioxidant defense system in *Ulva fasciata*. *Marine Biology*, 150(1): 1–15.

Mazid, M., T. A. Khan, and F. Mohammad. 2011. Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, 3(2): 232–249.

Meda, A., C. Euloge, L. Marco, R. Jeanne, and O. G. Nacoulma. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, 91(3): 571–577.

Miller, G., N. Suzuki, S. Ciftci-Yilmaz, and R. Mittler. 2010. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant, Cell and Environment*, 33(4): 453–467.

Nakano, Y., and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22(5): 867–880.

Orabi, S. A., and A. A. El-Noemani. 2015. Role of proline in improving drought tolerance of faba bean plants via antioxidant responses to enhanced generation of superoxide anion radical and hydrogen peroxide. *American-Eurasian Journal of Sustainable Agriculture*, 9(1): 31–42.

Robins, R. J., and D. D. Davies. 1981. The role of glutathione in amino-acid absorption. Lack of correlation between glutathione turnover and amino-acid absorption by the yeast *Candida utilis*. *Biochemical Journal*, 194(1): 63–70.

SAIP. 2012. Water conservation technical briefs, TB 14 - Technical brief on salinity control. Available at: http://www.saiplatform.org/uploads/Modules/Library/sai-technical-brief-14-salinity-control-2.pdf (Accessed: 26 October, 2012).

Schmidhuber, J., and F. N. Tubiello. 2007. Global food security under climate change. In *Proc. of the National Academy of Sciences of the United States of America*, 104(50): 19703–19708.

Sharma, P., A. P. Jha, R. S. Dubey, and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 2012: 26 p. Doi: 10.1155/2012/217037

Shukla, U. C., R. C. Murthy, and P. Kakkar. 2008. Combined effect of ultraviolet-B radiation and cadmium contamination on nutrient uptake and photosynthetic pigments in *Brassica campestris* L. seedlings. *Environmental Toxicology*, 23(6): 712–719.

Silber, R.,C. M. Farber, E. Papadopoulos, D. Nevrla, L. Liebes, M. Bruck, R. Brown, and Z. N. Canellakis. 1992. Glutathione depletionin chronic lymphocytic leukemia B lymphocytes. *Blood*, 80(8): 2038–2043.

Sivasankaramoorthy, S., T. Balasubramanian, P. Amuthavalli, and P. Sivaramana. 2010. The effect of salinity on organic components of *Excoecaria agallocha* L. *Journal of Experimental Sciences*, 1(12): 7–9.

Smirnoff, N. 2008. *Antioxidants and Reactive Oxygen Species in Plants*. New York, USA: John Wiley & Sons.

Snedecor, G. W., and W. G. Cochran. 1980. *Statistical Methods*, 7th ed. Ames, Iowa, USA: Iowa State University Press.

Sneha, S., A. Rishi, A. Dadhich, and S. Chandra. 2013. Effect of salinity on seed germination, accumulation of proline and free amino acid in *Pennisetum glaucum* (L.) R. Br. *Pakistan Journal of Biological Sciences*, 16(17): 877–881.

Yadav, S., M. Irfan, A. Ahmad, and S. Hayat. 2011. Causes of salinity and plant manifestations to salt stress. *Journal of Environmental Biology*, 32(5): 667–85.

Young, E. C., M. F. Belmonte, L. T. T. Tu, and C. Stasolla. 2005. Glutathione modulation of *in vitro* development. *In Vitro Cellular & Developmental Biology - Plant*, 41(5): 584–590.

Zanetti, G. 1979. Rabbit liver glutathione reductase, purification and properties. *Archives* of *Physiology and Biochemistry*, 198(1): 241–246.