



Response of grain sorghum to alpha-tocopherol and thiamine under salinity

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IN ORDER to evaluate the oxidative defense and growth of grain sorghum [*Sorghum bicolor* (L.) Moench] grown under salt stress (2500 and 5000 ppm than the tap water as a control) to application of alpha-tocopherol and thiamine (as antioxidants) a pot experiment was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt. The obtained results showed that salinity decreased the dry weight of root, stem and whole plant and root more affected than stem and whole plant but leaves seemed to be without effect. On reverse, T/R ratio (top to root ratio) increased as the salinity level increased. This means that salinity affect the root more than any part of grain sorghum plants. It is clearly shown that different grain sorghum plants parts dry weight and their whole weight increased by tocopherol or tocopherol+thiamine (spraying on vegetative parts) in comparable with the control plants. On the opposite side, T/R decreased with tocopherol+thiamine only. This means that this treatment increased dry weight of root than the other part of sorghum plant. It is clearly shown from data that different grain sorghum plants parts dry weight increased by tocopherol or tocopherol+thiamine spraying in comparable with the control plants. On the opposite side, T/R decreased with tocopherol+thiamine only. This means that this treatment increased dry weight of root than the other part of sorghum plant. O₂- and MDA (malondialdehyde) its concentrations negatively responded to alpha-tocopherol or alpha-tocopherol+thiamine. The depression in these parameters by alpha-tocopherol were: 52.07 and 30.08 and by alpha-tocopherol+thiamine were 59.50, and 38.52%, respectively compared to that of the control. On the other side, APX (ascorbate peroxidases), GR (glutathione reductase), PPO (polyphenole oxidases) and CAT (catalase) level increased by: 83.81, 53.50, 61.88 and 19.64 % as the grain sorghum plants sprayed by alpha-tocopherol and thiamine, respectively it is compare to the plants received distilled water. These Data lead to conclusion that application of alpha-tocopherol or thiamine increased tolerance of grain sorghum plants.

Keywords: Grain sorghum (*Sorghum bicolor* L.), Moench, Alpha, tocopherol, Thiamine, Salinity, Dry matter, Antioxidant enzymes.

Introduction

Salinity of soil is a global increasing problem and main obstacle to agricultural productivity especially Environmental stresses are considered the main factor affect adversely the world food production. There are over 800 million hectares of land worldwide are affected by salinity (Munns, 2005). Salt stress is a major abiotic stress and induce pig problem in arid and semi-arid regions and irrigation lands. It represents approximately 7% of the world's land area, 20% of the world's cultivated land, it affected nearly half of the irrigation and with high salt contents (Rhoades and Loveday 1990 and Szabolcs 1994). Salinity adversely affected growth and yield of different crops cultivated in these areas or irrigated by saline water especially in areas suffer from water shortage and the only available the saline water (Hassanain and Azab, 1990; Hussein, et al 2008; Sairam and Srivastava, 2002;

Hussein, et al 2012; Gondim, et al 2013 and Hussein, et al 2017) in regions where irrigation is necessary (Sagar, et al 2019). Salinity is a major threat to plant growth, development, and overall plant productivity (Hussein and Abu Bakr, 2018). Additions of toxic salts in the water lower the osmotic potential of water that leads to water shortage and less absorption of water by roots and ultimately causes the development of osmotic stress. Osmotic stress results in the disturbance of physiological (Khalil, et al 2012) and biochemical response (Hussein and Orabi, 2008) of plants and adversely affect the plant development, yield, and production (Huang, et al 2006; Hussein, et al 2008; Ashraf, et al 2013 and Kausar and Gull, 2018). Different researches were done and revealed that salinity affected most the physiological and metabolic processes such as photosynthesis, water and mineral

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uptake, anatomical structure and oxidative defense (Redondo-Gomez, et al 2007; Hussein, et al 2008; Hussein, et al 2011 and Orabi, et al 2018). A number of defense compounds accumulated in plants such as ascorbate (vitamin C), glutathione, N-acetyl Cys, tocopherols (vitamin E), carotenoids, phenyl propanoids, polyamines, and indoles in response to abiotic stresses (Iriti and Faoro, 2007). In Photosynthetic organisms, tocopherols are lipophilic antioxidants that are synthesized exclusively, while α -tocopherol accumulates predominantly in photosynthetic tissue, seeds are rich in γ -tocopherol. Recently, little is known about the specific roles of α - and γ -tocopherol in different plant tissues (Abbasi, et al 2007). The α -Tocopherol is the major vitamin E compound found in leaf chloroplasts, where it is located in the chloroplast envelope, thylakoid membranes and plastoglobul (Munné-Bosch, 2005).

Tocopherols deactivates photosynthesis-derived reactive oxygen species (mainly $1O_2$ and OH), and prevents the propagation of lipid peroxidation by scavenging lipid peroxy radicals in thylakoid membranes. The level of α -Tocopherol, differentially change in response to environmental constraints, and this process depending on the magnitude of the stress and species-sensitivity to stresses (Munné-Bosch, 2005). Thiamine pyrophosphate (TPP) involved in a number of important metabolic processes and is an essential cofactor required by enzyme. This metabolic processes such as the production of acetyl-CoA, the tricarboxylic acid cycle, the pentose phosphate pathway/Calvin cycle, branched chain amino acid biosynthesis, and isoprenoid biosynthesis (Hohmann and Meacock, 1998). While in vitro studies suggest that thiamine can directly act as an antioxidant, the association between oxidative stress and thiamine-dependent enzymes may also indicate a vital cofactor role for thiamine under stress conditions (Tunc-Dzdemir, et al 2009).

Wanti, et al (2013) reported that oxidative stress and eventual cell death in plants can be caused by excess H_2O_2 accumulation. Since stress factors provoke enhanced production of H_2O_2 in plants, severe damage to biomolecules can be possible due to elevated and non-metabolized cellular H_2O_2 . H_2O_2 -metabolizing enzymes endowed plants such as catalases (CAT), ascorbate peroxidases (APX), some peroxiredoxins, glutathione/thioredoxin peroxidases, and glutathione sulfo-transferases. However, CAT and APX are the most notably distinguished enzymes since the former mainly occurs in peroxisomes and does not require a reductant for catalyzing a dismutation reaction. In particular, APX has a higher affinity for H_2O_2 and reduces it to H_2O in chloroplasts, cytosol, mitochondria and peroxisomes, as well as in the apoplasmic space, utilizing ascorbate as specific electron donor. On the other hand, APX, GR and PPO concentration increased by: 83.81, 53.50 and 81.88

% as the grain sorghum plants sprayed by alpha-tocopherol (plants received distilled water).

Sorghum (*Sorghum bicolor* L. Moench) is a moderately saline tolerant grain and forage crop, (Tabatabaei and Anaghali, 2012), can tolerate up to 8.6 dS m⁻¹ soil salinity (Maas, et al 1986). It is one of the five major cultivated species in the world having several economically important uses such as 55% as food, 33% as feed and others are as fuel, fibre, fermentation and fertilizer.

Moreover, the antioxidant and vitamins and its effect of amelioration of salt stress in plants were reported in several investigations (Hussein and El-Greatly, 2007; Hussein, et al 2008; Ashraf, et al 2009; Hussein, et al 2011 and Bekheta and Hussein, 2014).

Therefore, this work aimed to investigate the effect of alpha-tocopherol and thiamine on growth and antioxidant enzymes of grain sorghum plants grown under salinity condition.

Materials and Methods

A pot experiment was carried out at the greenhouse of the National Research Center, Dokki, Egypt during the summer season of 2017. Seeds were provided from the Agricultural Research Center, Giza, Egypt and directly sown on the 1st of April in earthenware pots of 40 cm diameter filled with 15 kg of clay loam soil. Plants were thinned to two plants/pot at 10 days after sowing. The treatments were as follows:

Salinity treatments: irrigation by diluted sea water 2500 and 5000 ppm more than tap water as a control.

Antioxidants: Alpha-tocopherol (100 ppm) and tocopherol (100 ppm)+Thiamine (100 ppm) more than distilled water (as a control) were sprayed twice, the 1st was 21 days and the 2nd was applied two weeks later.

All pots received a recommended doses of N, P and K fertilizers, namely 6 g calcium super phosphate (15.5% P₂O₅), 1.5 g potassium sulphate (48.5% K₂O) and 1.5 g ammonium nitrate (33.5% N), which was added immediately before sowing. Seeds of grain sorghum (*Sorghum bicolor* (L.) Moench) were regularly irrigated with tap water and saline water starting 21 days. The general principal stated by (Boutraa and Sanders, 2001) was used for the water treatments application. The three exogenous of antioxidants treatments and all possible combinations between them were tested. This experiment included 9 treatments which included all combinations between three salinity treatments and three exogenous antioxidants application. Treatments were arranged in a split plot design with six replicates each.

At 50 days from sowing the plants were sampled at random to estimate the following characters: fresh

and dry weights (g) of root, stem, leaves and whole plant and for Biochemical measurements in leaves.

Enzymatic and non-enzymatic antioxidants measurements: Extraction of the antioxidant enzymes Ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) were determined as 5g of frozen leaves tissues were homogenized in prechilled mortar in presence of 10 ml of 50 mM potassium phosphate buffer (pH7) with 1% (W/V) insoluble polyvinyl pyrrolidone (PVP) and 0.1 mM EDTA. The extraction procedures were repeated twice and supernatants were pooled, raised to a certain volume, referred as crude enzyme extract, all operations were carried out at -4°C for further analysis. The activity of APX (EC1.11.1.11) was determined according to (Nakano and Asada, 1981). One unit of APX was defined as the amount of enzyme that breaks down 1 μ mol of ascorbate per min. and GR activity (EC 1.6.4.2) was determined according to (Zanetti, 1979). One unit of GR was defined as the amount of enzyme that decreases 1A340 per min. The activity of catalase (EC1.11.1.6) was determined according to the method of Aebi (1983) by the decrease of absorbance at 240 nm for 1 min as a consequence of H₂O₂ consumption. Poly phenole oxidase (PPO)(EC 1.10.3.1) was determined using the method of Oktay et al. (1995). Total phenolic compound contents were determined by the colourimetric method of folin-Denis as described by (Daniel and George, 1972). A calibration curve of pyrogallol was prepared, and the results were expressed as mg Pyr. (Pyrogallol).

Oxidative damage measurements and scavenging: Determination of the generated superoxide radical (O₂^{·-}) was base on the reduction of nitro blue tetrazolium (NBT) according to the method described by (Doke, N.1983). Lipid peroxidation was determine by measuring Malondialdehyde (MDA) content as described by (Dhindsa, et al 1982) Superoxide radical (O₂^{·-}) scavenging activity was determined as described by (Beauchamp and Fridovich, 1971) and modified by (Ibrahim, et al 2013). Ascorbic acid was use as a positive control compound.

The collected data were subject to the statistical analysis of variance using the normal (F) test and means were evaluated by using Least Significant Difference (LSD) test at the 5% level according to (Snedecor and Cochran, 1980).

Results and discussion

Fresh and dry matter

Salinity

Data in Table (1) showed that salinity decreased the dry weight of root, stem and whole plant and root more affected than stem and whole plant but leaves seemed to be without effect. On reverse, T/R ratio

increased as the salinity level increased. This means that salinity affect the root more than any part of grain sorghum plants. Different researches has been done reported the adverse effect of salinity on different crops: **Hussein and Mandour (1979); Abd El-Rasoul, et al 1980); Nour, et al (1982); Ashoub, et al (1984); Mousa, et al(1987); Netonodo, et al (2004) Nimir, et al (2017) and Machad and Serralheiro (2017).**The study of **Sagar, et al (2019)** showed that salinity opposite to the regular growth of sorghum seedlings, as indicated by shoot and root length stress tolerance index. Consequently, the root-shoot ratio and total dry matter decreased with increasing salinity. These results are consistent (**Qu, et al (2012)**) They found NaCl salinity led to be short of growth characters and the shoot was more affected than the root, while leaf indices was declined significantly measured up to the control plants. Reduction in growth may be caused by a reduced number of elongated cells and the reduced rate of cell elongation (**Sagar, 2017**) and these happen due to lower transport rate of essential ions like due to salinity that reduce the N compounds and increased Na n plant under high salinity (**Hamid, et al 2008**).

Concerning this phenomenon, salinity from the accumulation of salts in soil or irrigation by saline water and both affected the soil properties and plant metabolism. **Hassanain and Azab (1990)** noticed that the increasing of soil salinity levels significantly decreased dry weight of shoots and roots as well as root volume of the tested genotypes at seedling stage. **Hussein, et al (2017)** used diluted sea water on irrigation of jojoba and concluded that this depressed the in vegetative growth and dry matter of plants. The adverse effect of salinity may be attributed to the inhibition effect on photosynthetic apparatus and carbohydrates decreased which reflected on the dry matter accumulation (**Sudhir and. Murthy, 2004; Koyro, 2006 and Hussein et al 2015**), protein building depression (**Zagorchev, et al 2016**), enzymes activity (**Sozharajan and Natarajan, 2013**), disturbance in nutrients uptake, and distribution (**Munns and Taker, 2002; Hussein, et al 2008; Abd El-Halin, et al 2019 and Hussein, et al 2019**), effects on osmotic adjustment (**Alarcon, et al 1994 and Neto, et al 2004**) and as in our study the effect on oxidative defense (**Orabi, et a l 2018; Hussein and Orabi, 2008 and Abd Elgawad, et al 2016**). Salt stress causes decrease in plant growth and productivity by disrupting physiological processes, especially photosynthesis. The accumulation of sodium ions intracellular at salt condition changes the ratio of K :Na, which seems to affect the bioenergetic processes of photosynthesis (**Sudhar and Murthy, 2004**).

Table 1. Response of grain sorghum growth to salt stress.

Sal	Fresh weight g				Dry weight g				T/R
	Root	Stem	Leaves.	Whole	Root	Stem	Leaves.	Whole	
T.W	7.58	32.96	22.87	63.41	3.98	8.01	6.94	18.93	3.76
S1	4.60	26.69	19.77	51.06	2.29	5.38	7.11	14.78	5.45
S2	3.61	28.51	17.23	49.35	1.81	5.24	6.21	13.26	6.33
LSD	1.11	4.49	N.S	N.S	N.S	2.94	N.S	N.S

T.W=Tap water S1=2500 ppm S2=5000 ppm T/R= Top/ root ratio.

Oxidative stress and eventual cell death in plants can be caused by excess H_2O_2 accumulation. Since stress factors provoke enhanced production of H_2O_2 in plants, biomolecules severe damage can be possible due to elevated and non-metabolized cellular H_2O_2 . Plant tissues are endowed with H_2O_2 -metabolizing enzymes such as catalases (CAT), ascorbate peroxidases (APX), some peroxidoxins, glutathione/thioredoxin peroxidases, and glutathione sulfo-transferases. **Netonodo, et al (2004)** mentioned that roots and stems accumulated substantial amounts of sodium, saturated at 150 mM external NaCL. K^+ , and Ca^{++} accumulation in the root stem and leaves was strongly inhibited and was minimally impaired but that of the stem and leaves was affected strongly. Sodium continuously accumulated in leaves which was preferentially deposited in the sheaths. Mature leaves contain more K^+ and Mg^+ ions than the young leaves.

Additionally, salinity injury in plant growth may be due to the disturbance in mineral uptake and contents. **Anubha Sinha and Rana (1986)** noticed that there occurred an increase in the concentration of certain nutrients such as N, Ca, Mg, TNC in the plants in response to salinity, which along with increased root: shoot ratios was inferred as an adaptive feature of the plant for persistence under saline conditions. K concentration, K/Na and Ca/Na ratios decreased significantly, while Na concentration increased in root, shoot, and seed as the increase in soil salinity. In shoots, P concentration significantly decreased under salinity stress. Moreover, **Weisany, et al (2014)** stated that calcium significantly decreased in root, but increased in seed with increased salinization. **Munnus and Tester (2008)** concluded that salinity affects plants through osmotic stress and ion imbalance and toxicity. Salt-induced decrease in the Alpha-tocopherol and Thiamin soil water potential through

osmotic effects. High salts inside the plant take time to accumulate before they affect plant function. A wide range of mechanisms have developed in plants to sustain productivity under salt stress environment. These mechanisms are osmotic adjustment, Na^+ and/or Cl^- exclusion, and tissue tolerance of high concentrations of Na^+ and/or. Moreover, many researches on salt tolerance of various crops has indicated that salt tolerance largely depends on genera and species and even on cultivars within certain species (**Niu, et al 2012**). **Nimir, et al (2017)** indicated that Na^+ content was gradually increased with salt increase. Stomatal conductance, photosynthetic rate, transpiration rate, and K^+ content were significantly decreased due to high salt concentration. The medium salinity level of 2 g NaCl per kg dry soil decreased Ca^{2+} , P and Mg^{2+} content. **Hussein, et al (2015) on onion, Hussein, et al (2017) on jojoba and Hussein, et al (2008)** on grain sorghum, related the adverse effect of salinity to the disturbance on mineral absorption and distribution and also to the changes in anatomical structure of plant. They related this effects to the decrease in stomatal closer, CO_2 exchange and photosynthesis activity. This bade effect may be due to the unbalance in growth regulators. While **Hussein and Oraby (2008) and Abd El-Baky, et al 2014** attributed this negative effects on growth and yield of plants to the depression antioxidant activity in to the depression on antioxidant defense. **Sairam, et al (2005)** indicated that salinity stress decreased relative water content (RWC) and membrane stability index (MSI) that was confirmed by **Semida, et al (2015)**. The application of α TOC, particularly at 0.50 mM significantly increased RWC and MSI, maintaining cells turgid for healthy metabolic processes and membrane integrity.

Table 2. Response of grain sorghum plants growth to alpha-tocopherol and thiamine.

Antiox.	Freh weight g				Dry weight g				T/R
	Root	Stem	Leaves.	Whole	Root	Stem	Leaves.	Whole	
Dw	3.55	20.35	14.61	38.51	1.91	4.66	5.62	12.19	5.38
Toc	5.11	29.33	18.10	52.45	2.47	6.80	6.50	15.77	5.39
To.+Th.	6.23	39.40	27.18	72.81	3.71	7.87	7.47	19.07	4.14
LSD	1.05	4.99	5.52	3.88	1.81	N.S	4.19	5.44

Dw= Distilled water Toc=alpha tochopherol Thi=Thiamine T/R= Top/ root ratio.

It is clearly shown from Data in Table (2) that different grain sorghum plants parts dry weight and their whole weight increased by tocopherol or tocopherol + thiamine spraying in comparable with the control plants. On the opposite side, T/R decreased with tocopherol+ thiamine only. This means that this treatment increased dry weight of root than the other part of sorghum plant.

Tocopherol enhance growth and yield through its effect on growth regulators as found by **El-Hariri, et al (2012)** who noticed an increase in IAA content in flax plants by the foliar application of α TOC, and attributed this increased IAA to the role of α TOC in activating the biosynthesis of endogenous hormone, reflecting in stimulating cell division and/or the cell enlargement. **Barkat, et al 2003, Hussein and Orabi (2008) and Abdel-Baky, et al (2008). Hussein, et al 2007 and Simadi, et al (2014)** reported that all enhanced parameters (i.e., growth traits, plant water relations, contents of nutrients and their relations, and final yields) by the foliar application of α TOC which were accompanied with the improved stem and leaf anatomy. This gave an opportunity to a good translocation of the absorbed nutrients into healthy cells to be used in different metabolic processes positively reflecting in vigorous growth and satisfactory yield under the adverse conditions of the tested newly-reclaimed saline soil. **Sayed and Gaballah (2002)** found that root or shoot application of thiamine reduced membrane injury

by either heat or dehydration stress, lowered leaf ψ_w , improved uptake of K^+ and increased leaf RWC, Chl, SS, TAA contents and dry mass production. However, in vitro studies of **Hu, et al (1995) Jung and Kim (2003)** showed that thiamine is a potent scavenger of hydroxyl radicals and superoxide and could protect membranes from lipid peroxidation. **Lukienko et al (2000)** and our results in this work confirming these findings. **Gadallah (2002)** found that thiamine-induced reduced membrane injury and increased leaf RWC. Excess lipid oxidation will eventually result in membrane damage. **Kaya, et al (2014)** reported that exogenously applied thiamine induced growth improvement in maize plants was found to be associated with reduced membrane permeability, MDA and H_2O_2 levels, and altered activities of some key antioxidant enzymes such as CAT, SOD and POD as well as increased photosynthetic pigment concentration under saline regime.

Interaction

Tocopherol deactivates photosynthesis-derived reactive oxygen species (mainly O_2 and OH), and prevents the propagation of lipid peroxidation by scavenging lipid peroxy radicals in thylakoid membranes. α -Tocopherol levels change differentially in response to environmental constraints, depending on the magnitude of the stress and species-sensitivity to stress (**Munné-Bosch, 2005**).

Table 3. Response of grain sorghum plants growth to alpha-tocopherol and thiamin and salt stress.

Sal	Antiox.	Fresh weight g				Dry weight g				T/R
		Root	Stem	Leaves.	Whole	Root	Stem	Leaves.	Whole	
T.W	Dw	5.88	23.43	16.41	40.42	2.62	5.64	5.18	12.94	7.41
	Toc	7.01	35.72	20.30	36.66	2.84	7.73	6.40	17.27	6.08
	Toc.+Thi	9.85	39.73	31.91	81.49	6.27	10.67	9.23	26.13	8.71
S1	Dw	4.43	16.92	13.77	35.02	1.63	3.25	6.80	11.68	6.18
	Toc	4.87	28.11	17.62	50.60	2.52	5.21	7.50	15.23	6.05
	Toc.+Thi	4.50	38.05	27.93	66.43	2.73	7.69	7.04	17.46	5.40
S2	Dw	3.03	20.71	13.65	37.93	1.29	5.09	4.88	9.36	5.48
	Toc	3.44	24.15	16.36	43.95	2.04	7.45	5.61	12.74	5.25
	Toc.+Thi	4.35	40.67	21.69	55.71	2.11	5.24	8.13	17.69	7.38
LSD		1.81	4.49	N.S	6.71	1.51	N.S	N.S	N.S

T.W=Tap water S1=2500 ppm S2=5000 ppm Dw= Distilled water Toc=alpha tocopherol Thi=Thiamine T/R= Top/ root ratio.

Examination of Data in Table (2) showed that generally antioxidant substances improved the fresh and dry matter of grain sorghum plants grown under salinity or fresh water irrigation. Moreover, application of tocopherol plus thiamine gave the highest values of root as well as whole plant dry matter. This was true under both fresh water or salinity treatments. In addition, it was observed that the combined of thiamine+tocopherole more effective under fresh water than salinity treatments. **Semida, et al (2014)** found that α TOC-treated plants, grown under the abovementioned adverse conditions, had enhanced all growth parameters (i.e., shoot length and numbers). In

addition, performance index, relative water content, membrane stability index, nutrients and their relations, and anatomy of stem and leaf were significantly improved in α TOC-treated plants compared to control plants of leaves and branches, leaf area, and shoot fresh and dry weights) and yield and its components (i.e., number of dry pod per plant, average 100-seed weight, and dry seed yield per plant and per hectare) of both varieties compared to control plants. **Hariri, et al. (2010)** found an increase in IAA content in flax plants by the foliar application of α TOC, and attributed this increased IAA to the role of α TOC in activating the biosynthesis of endogenous hormone, reflecting in

stimulating cell division and/or the cell enlargement that lead to the improvement in plant growth under stress. The same authors proved an increase in total phenols by α TOC application, which play a mechanism in regulation of plant metabolic processes, act as a substrate for many antioxidant enzymes. **Sayed and Gadallah (2000)** mentioned that the effects of salinity (ψ_s), thiamin (Thi.) and their interaction ($\psi_s \times \text{Thi}$) on the parameters tested were significant. Salinity was dominant (as indicated by η^2 values) in affecting the contents of Ca^{2+} , Cl^- , TAA and membrane stability to heat and leaf ψ_w . The role of thiamin was dominant for Na^+ , K^+ and SS contents and the contribution of interaction was dominant for growth parameters, Chl. and root ψ_w .

Salt stress markedly suppressed shoot and root dry mass, total chlorophylls ($-\text{al} + -\text{bl}$), leaf water potential and maximum fluorescence yield (Fv/Fm) in the plants of both maize cultivars, but it increased proline accumulation, leaf osmotic pressure, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations, electrolyte leakage (EL) as well as activities of some key antioxidant enzymes, superoxide dismutase (SOD; EC. 1.15.1.1), peroxidase (POD; EC. 1.11.1.7) and catalase (CAT; EC. 1.11.1.6). Salt-induced reduction in plant growth parameters was higher in the salt-sensitive cultivar, Apex 836, which was found to be associated with relatively increased EL, and MDA and H_2O_2 levels, and decreased activities of the key antioxidant enzymes (**Kaya, et al. 2015**).

affects both plant growth and productivity by causing high production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide and hydroxyl radical (**Mittler, 2002**). All these substances being very reactive are harmful to vital cellular macromolecules such as proteins and lipids (**Noctor et al 2014**). However, to counteract ROS, plants can upregulate their antioxidative defense mechanism by

stimulating the activities of key antioxidative enzymes including superoxide dismutases (SOD), catalases (CAT) and peroxidases (POX) (**Sai-Kachout et al 2013**). The salt stress is known to cause reduction in cell division and elongation (**Pitann et al 2009**) which is mainly due to salt induced alterations in the nutrient uptake, induced formation of reactive oxygen species (Ashraf, 2009), inhibition of cytoplasmic enzymes, turgor loss (**Pitann et al 2009**) and hormonal imbalance (**Ashraf et al 2010**) which will naturally impair plant growth and finally the yield.

found that he single TOC foliar application recorded the positive results significantly improved soil physical and chemical properties, which positively reflected on plant growth and productivity, physio-biochemical attributes, mineral nutrients (N, P, K and Ca), osmo-protectants (soluble sugars and proline), non-enzymatic (ascorbic acid, glutathione and TOC) and enzymatic (superoxide dismutase, catalase and guaiacol peroxidase (GPOX)) antioxidants compared to untreated controls. Integrated KH + TOC treatment was most effective compared to the single treatments. The above results recommended benefits of this integrated KH + TOC for the possibility of sustainable agronomic performance of common beans grown on saline soils.

Anti-Oxidative system

Salinity

As the Data in Table (4) showed, salinity induced increases in O_2^- and MDA in leaves tissues of grain sorghum plants as compared to the control **Kaya, et al (2015)** noticed that salinity increased malondialdehyde (MDA) i.e. lipid peroxidation and H_2O_2 in maize cultivars. On the opposite side, APX, GR, CAT and PPO activity increased parallel to the increase in salinity level up to the highest level used in comparable with plants irrigated by fresh water.

Table 4. Anti-Oxidative system as affected under salinity condition.

Sal	O_2^- A680/ g FW	MDA $\mu\text{mole/g}$ FW	Phenols mg/g FW	O_2^- -scavenging activity %	APX $\mu\text{mole/g}$ g FW	GR $\mu\text{mole/g}$ FW	PPO $\mu\text{mole/g}$ FW	CAT $\mu\text{mole/g}$ FW
Tw	0.32	7.71	2.51	76.29	2.76	3.9374	21.12	88.37
S1	0.84	9.64	3.86	84.52	4.25	4.9571	23.47	98.75
S2	1.18	12.50	4.84	83.96	5.02	5.9770	26.36	109.58
LSD	0.03	0.73	0.72	0.80	0.68	1.102	1.678	1.52

T.W=Tap water S1=2500 ppm S2=5000 ppm Apx= Ascorbate peroxidase GR= Glutathione reductase PPO=Poly phenol oxidase CAT= Catalase

Soil salinity adversely affects both plant growth and productivity by causing high production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide and hydroxyl radical (**Mittler 2002**). All these substances being very reactive are harmful to vital cellular macromolecules such as proteins and lipids (**Noctor et al 2014**). However, to counteract ROS, plants can upregulate their

antioxidative defense mechanism by stimulating the activities of key antioxidative enzymes including superoxide dismutases (SOD), catalases (CAT) and peroxidases (POX) (**Sai-Kachout et al 2013**). **Kaya, et al (2015)** reported that salinity increased the activity of key antioxidant enzymes, SOD, POD and CAT in plants of maize cultivars. **Wani, et al (2013)** mentioned that salinity induced modulation in antioxidant system.

Oxidative stress and eventual cell death in plants can be caused by excess H_2O_2 accumulation. Since stress factors provoke enhanced production of H_2O_2 in plants, severe damage to biomolecules can be possible due to elevated and non-metabolized cellular H_2O_2 . Plants are endowed with H_2O_2 -metabolizing enzymes such as catalases (CAT), ascorbate peroxidases (APX), some peroxiredoxins, glutathione/thioredoxin peroxidases, and glutathione sulfo-transferases. However, the most notably distinguished enzymes are CAT and APX since the former mainly occurs in peroxisomes and does not require a reductant for catalyzing a dismutation reaction. In particular, APX has a higher affinity for H_2O_2 and reduces it to H_2O in chloroplasts, cytosol, mitochondria and peroxisomes, as well as in the apoplasmic space, utilizing ascorbate as specific electron donor. Based on recent reports, thiamine biosynthetic enzymes (Sofa, et al 2015). Based on recent reports, the data revealed that the antioxidant enzyme activity [catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) increased in response to the concentrations of NaCl in the soil in both the varieties

[catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD)].

Alpha-tocopherols and thiamine

Data presented in Table (5) showed that O_2^- and MDA its concentrations negatively responded to alpha-tocopherol or alpha-tocopherol+thiamine. The depression in these parameters by alpha-tocopherol were: 52.07 and 30.08 and by alpha-tocopherol+thiamine were 59.50, and 38.52%, respectively compared to that of the control. The effects of this compound in intracellular signaling may be either direct, by interacting with key components of the signaling cascade, or indirect, through the prevention of lipid peroxidation or the scavenging of singlet oxygen. In the latter case, α -tocopherol may regulate the concentration of reactive oxygen species and plant hormones, such as jasmonic acid, within the cell, which control both the growth and development of plants, and also plant response to stress (Munné-Bosch, and Alegre, 2002).

Table 5. Anti-Oxidative system as affected with alphatocopherol and thiamine spraying.

Sal	O_2^- -A680/g FW	MDA μ mole/g FW	Phenols mg/g FW	O_2^- -scavin -ging activity %	APX μ mole/g FW	GR μ mole/g FW	PPO μ mole/g FW	CAT μ mole/g FW
Dw	1.21	12.90	2.79	78.52	2.78	3.8549	17.60	88.45
Toc	0.58	9.02	3.81	81.57	4.15	5.0986	24.86	101.81
Toc.+Thi	0.49	7.93	4.61	84.67	5.11	5.9173	28.49	105.82
LSD	0.05	0.66	0.98	0.52	0.181	0.261	N.S.	2.48

Dw= Distilled water Toc=alpha tocopherol Thi=Thiamine Apx= Ascorbate peroxidase GR= Glutathione reductase PPO=Poly phenol oxidase CAT= Catalase

On the other side, APX, GR, PPO and CAT level increased by: 83.81, 53.50, 61.88 and 19.64 % as the grain sorghum plants sprayed by alpha-tocopherol and thiamine, respectively if it is compared to the plants received distilled water. Hemada, et al (2017) found that the single TOC foliar application recorded effects on enzymatic (superoxide dismutase, catalase and guaiacol peroxidase (GPOX)) and antioxidants compared to untreated controls.

Tunc-Dzdemir, et al (2009) pointed out that the accumulation of these compounds (thiamin or thiamin pyrophosphate) in plants subjected to oxidative stress was accompanied by enhanced expression of transcripts encoding t). Oxidative stress and eventual cell death in plants can be caused by excess H_2O_2 accumulation. Since stress factors provoke enhanced production of H_2O_2 in plants, severe damage to biomolecules can be possible due to elevated and non-metabolized cellular H_2O_2 . Plants are endowed with H_2O_2 -metabolizing enzymes such as catalases (CAT), ascorbate peroxidases (APX), some peroxiredoxins, glutathione/thioredoxin peroxidases, and glutathione sulfo-transferases. However, the most notably distinguished enzymes are CAT and APX since the former mainly occurs in peroxisomes and does not require a reductant for catalyzing a dismutation

reaction. In particular, APX has a higher affinity for H_2O_2 and reduces it to H_2O in chloroplasts, cytosol, mitochondria and peroxisomes, as well as in the apoplasmic space, utilizing ascorbate as specific electron donor. Based on recent reports, thiamine biosynthetic enzymes (Sofa, et al 2015). The effects of this compound in intracellular signaling may be either direct, by interacting with key components of the signaling cascade, or indirect, through the prevention of lipid peroxidation or the scavenging of singlet oxygen. In the latter case, α -tocopherol may regulate the concentration of reactive oxygen species and plant hormones, such as jasmonic acid, within the cell, which control both the growth and development of plants, and also plant response to stress (Munné-Bosch, and Alegre, 2002).

Interaction

The interaction between salinity and vitamins on APX, GR, PPO and CAT were illustrated in Table (6). Alpha-tocopherol alone decreased MDA as well as O_2^- by both salinity treatments but under the second level of salinity was more than that under the first treatment of salts. Nevertheless, the increment % in total phenols was more with the 1st level of salt stress than that resulted under the second level of salt stress. Recent studies indicate that compensatory mechanisms exist to afford adequate protection to the photosynthetic

apparatus in the absence of alpha-tocopherol, and provide further evidence that it is the whole set of antioxidant defenses (ascorbate, glutathione, carotenoids, tocopherols and other isoprenoids, flavonoids and enzymatic antioxidants) rather than a single antioxidant, which helps plants to withstand environmental stress (Munne-Bosch, 2005). Regarding the effect of this interaction on enzymes activity, tocopherol solitary increased pronouncedly GR activity in green leaves of grain sorghum, however PPO activity did not affect by the moderate salt stress and markedly increased by the highest salt stress used more than that in plants received of water in irrigation contains 275 ppm (Tap water) salts only (Table 6). Under normal conditions, ROS are generated at very low levels and a homeostasis is maintained between production and quenching of these molecules. This balance could be disturbed by the environmental stress, giving rise to a rapid increase in intercellular ROS levels which induce oxidative damage to lipids, proteins, and/or nucleic acids. In order to cope with the oxidative damages under stress, plants raise the level of endogenous enzymes (CAT, POX and SOD) and the non-enzymatic component such as proline (Sharma, et al 2010).

This Data showed that all the O₂⁻ as well as MDA decreased with mixed alpha-tocopherol and thiamine under both salinity levels, but the decrement in the 1st level of salinity is more than that caused by the second level, these decrement percentages in the case of the two damage criteria be less than the control while the reverse was true for phenol which still more than the control. Concerning the interaction effect of tocopherol and thiamine and salt stress are presented in the same Table. This Data also showed that both vitamins decreased the level of the O₂⁻ as well as MDA. On

reverse total phenols markedly increased continuously which its percentage of increment under the second level of salinity approach to be the 2 folds of those induced under fresh water of irrigation.

High salinity causes ion toxicity and osmotic stress, leading to the excessive production of reactive oxygen species (ROS) in plant cells, including superoxide radicals, hydrogen peroxide, hydroxyl anions, and singlet oxygen. These ROS cause damage to lipids, proteins and DNA (Yasar, et al 2006). Also, the authors suggested that a-tocopherol application could effectively plants from salt stress damage presumably by quenching the excessive reactive oxygen species to protect the photosynthetic pigments and by enhancing osmotic adjustment (Ye, et al 2017).

Application of thiamine as seed soaking or foliar spray partly mitigated the deleterious effects of salinity on plants of both maize cultivars. The most promising effect of Thiamin on alleviation of adverse effects of salt stress on maize plants was found when it was applied as foliar spray at 100 mg l⁻¹. Thiamin application considerably reduced tissue Na⁺ concentration, but improved those of N, P, Ca²⁺ and K⁺ in the salt-stressed maize plants. Exogenously applied thiamin-induced growth improvement in maize plants was found to be associated with reduced membrane permeability, MDA and H₂O₂ levels, and altered activities of some key antioxidant enzymes such as CAT, SOD and POD as well as increased photosynthetic pigment concentration under saline regime. Exogenous application of thiamin promotes growth and antioxidative defense system at initial phases of development in salt-stressed plants of two maize cultivars differing in salinity tolerance (Kaya, et al. 2015).

Table 6. Anti-Oxidative system as affected with alpha-tocopherol and thiamin spraying and salt stress.

Sal	Antiox.	O ₂ ⁻ A680/gFW	MDA μmole/g FW	Phenols mg/g FW	O ₂ ⁻ scavenging activity%	APX μmol e/g FW	GR μmole/g FW	PPO μmole/g FW	CAT μmole/g FW
Tw	Dw	0.44	8.17	2.06	73.82	2.05	3.2154	16.00	83.14
	Toc	0.28	7.87	2.46	76.30	2.73	3.95.23	17.92	87.95
	Toc.+Thi	0.23	7.10	3.02	78.76	3.51	4.6445	18.88	90.97
S1	Dw	1.32	12.39	2.83	80.42	2.63	3.8630	21.44	88.88
	Toc	0.69	8.78	4.09	85.43	4.19	4.7785	24.96	102.26
	Toc.+Thi	0.52	7.74	4.66	87.70	5.92	6.2298	28.16	105.12
S2	Dw	1.87	18.15	3.48	81.33	3.65	4.4882	25.92	93.33
	Toc	0.94	10.41	4.89	82.99	5.52	6.5651	27.52	114.04
	Toc.+Thi	0.73	8.95	6.15	87.56	5.89	6.8777	32.04	121.37
LSD		0.08	1.15	1.30	0.90	0.314	0.454	N.S.	N.S.

T.W.=Tap water S1=2500 ppm S2=5000 ppm Dw= Distilled water Toc=alpha tocopherol Thi=Thiamine Apx= Ascorbate peroxidase GR= Glutathione reductase PPO=Poly phenol oxidase CAT= Catalase.

In spite of the increases in APX, PPO, GR and CAT values (as a self produced plant defense against stress), the application of tocopherol or tocopherol combined with thiamine increased the activity of these enzymes. The addition of thiamine with tocopherol increased the effect of tocopherol on enzymes activity under moderate salinity or control treatment but under high

saline treatment used the difference caused by tocopherol or addition of thiamine in these activities (except for PPO or CAT) seemed to be equal. However, under moderate salinity treatment CAT activity approximately equal either by tocopherol solitary or combined with thiamine. It is clear from Data in Table (6) that salinity increased O₂⁻ and on reverse the

treatments with tocopherol or tocopherol+ thiamine decreased it. Addition of thiamin with tocopherol enhancing the role of tocopherol in this phenomenon. Also, Data showed that, in spite of the increases in O₂-scavenging, the application of vitamin and nitrogen basic increased the scavenging activity.

When supplemented with exogenous thiamin, wild-type plants displayed enhanced tolerance to oxidative stress induced by paraquat. Thiamin application was also found to protect the reactive oxygen species-sensitive ascorbate peroxidase1 mutant from oxidative stress. Thiamin-induced tolerance to oxidative stress was accompanied by decreased production of reactive oxygen species in plants, as evidenced from decreased protein carbonylation and hydrogen peroxide accumulation. Because thiamin could protect the salicylic acid induction-deficient1 mutant against oxidative stress, thiamin-induced oxidative protection is likely independent of salicylic acid signaling or accumulation (**Tunc-Dzdemir, et al 2002**).

Application of antioxidants including vitamins has gained considerable attention for alleviating the adverse impact of moisture and salinity stress on plants. This can show in terms of plant growth and yield quantity and quality. They concluded that Vitamins could be regarded as natural and safety bio-regulators; low concentrations can exert considerable effects on different chemical activities (**Sadak and Dawood, 2014**). **Falk and Munne- Bosch (2010)** stated that tocopherols have been found to have a role in protection of thylakoids or other purified membranes from oxidative stress. The discovery of the function, for instance, of alpha- tocopherols in protecting photosystem II from singlet oxygen using this approach (**Kruk, et al 2005**). In the recent studies (**Hincha, 2008**) about the protective role of tocopherols in photosynthetic membranes are remarkable.

Our obtainable results with respect to antioxidant enzymes activity were in line with many scientists, antioxidant enzymes (CAT, SOD, and POD) are considered the most effective biomolecules in preventing cellular damage Ashraf, 2009. In his study, the activities of CAT, SOD, and POD were enhanced reasonably in the green pods of both mung bean cultivars under water stress. Likewise, in an earlier study with oilseed rape (*Brassica napus* L.), **Abedi and Pakniyat (2010)** reported a significant stimulation in the activities of SOD and POD enzymes, and a decrease in the activity of CAT enzyme under water-deficit stress. Similarly, an increase in the activities of CAT, GPX, and SOD enzymes was observed in chickpea plants grown under dryland conditions (**Mohammadi, et al 2011**). However, exogenously applied α -Toc significantly improved the activities of CAT, SOD and POD in the green pods of both mung bean cultivars. In a comprehensive review by **Szarka et al (2012)**, it has been reported that stress-induced ROS accumulation is controlled by antioxidants (enzymatic and non

enzymatic) including α -Toc. They are usually capable of chelating metal ions, eliminating their catalytic activity to form excessive ROS. No reports on the role of exogenous application of α -Toc on the antioxidative defense system of plants grown under stress conditions are available in the literature. However, it could be concluded that α -Toc supplementation could compensate for the harmful effects of stress-induced ROS through its powerful antioxidant properties (**Bughdadi, 2013**) but recent reports were mentioned by **Orabi et al, (2014, 2017, 2018)** and **Orabi and Abdelhamid, (2016)** using α -Toc on thyme, cucumber, mango and faba baen plants respectively in respect to tolerance enhancement via elevation of the antioxidative defense system.

Increase in tocopherol content around the second day under both stresses did not correlate with the chlorophyll degradation while such correlation was observed from the fifth day of severe stress. The activities of GSH-Px and GST as well as TBARS content showed NaCl-induced enhancement which was dose- and time-dependent. However, chloroplastic SOD was rather not involved in the response of tomato plants to NaCl stress. under the moderate stress similarly as in the early phase of severe stress tocopherol functions as a typical antioxidant, while in the late phase of the latter it may be involved in senescence signaling pathway and enables the recovery and recycling of the compounds significant for a plant organism (**Skłodowska, et al 2009**).

Biochemical and molecular features of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) play crucial roles in scavenging ROS in the different cell compartments and in response to stress conditions (**Racchi, 2013, Mansour, et al 2021 and Zhung, et al 2021**). Among the non enzymatic defenses, particular attention is paid to ascorbic acid, glutathione, flavonoids, carotenoids, and tocopherols. The operation of ROS scavenging systems during the seasonal cycle and specific developmental events, such as fruit ripening and senescence, are discussed in relation to the intense ROS formation during these processes that impact fruit quality. Particular attention is paid to *Prunus* (**Racchi, 2013**) and *Citrus* species because of the nutritional and antioxidant properties contained in these commonly consumed fruits.

Tocopherols and tocotrienols scavenge lipid peroxy radicals, thereby preventing the propagation of lipid peroxidation in membranes, and the ensuing products tocopheroxyl and tocotrienoxyl radicals, respectively, are recycled back to tocopherols and tocotrienols by the concerted action of other antioxidants. Furthermore, tocopherols and tocotrienols protect lipids and other membrane components by physically quenching and reacting chemically with singlet oxygen. The scavenging of singlet oxygen by α -tocopherol in chloroplasts results in the formation of, among other products, α - tocopherol quinone, a known contributor to cyclic electron transport in thylakoid membranes, therefore providing photo

protection for chloroplasts. Moreover, given that α -tocopherol increases membrane rigidity, its concentration, together with that of the other membrane components, might be regulated to afford adequate fluidity for membrane function. Furthermore, α -tocopherol may affect intracellular signaling in plant cells. The effects of this compound in intracellular signaling may be either direct, by interacting with key components of the signaling cascade, or indirect, through the prevention of lipid peroxidation or the scavenging of singlet oxygen. In the latter case, α -tocopherol may regulate the concentration of reactive oxygen species and plant hormones, such as jasmonic acid, within the cell, which control both the growth and development of plants, and also plant response to stress (Munné-Bosch and Alegre, 2002).

Conclusion

Salinity is one of the main challenges face the productivity of agriculture and intern food through its bad effects on soil and plants. Our work designed to investigate the effect of some chemicals on growth and antioxidant activity of grain sorghum plants. Results showed that salinity adverse affected growth and increased free radicals and decreased the anti-oxidative system. Generally, the reverse was true for antioxidants application. This led us to conclude that both alpha tocopherols and thiamine enhanced oxidative defense and helped grain sorghum plants to tolerate salinity stress.

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