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Promotive effect of exogenously applied thiourea on key physiological parameters and oxidative defense mechanism in salt-stressed *Zea mays* L. plants

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Abstract: A greenhouse experiment was conducted to examine the alleviating role of thiourea (TU) on antioxidants and some vital physiological attributes in salt-stressed plants of two maize cultivars. The maize cv. DK 5783 performed better than cv. Apex 836 in an initial experiment. Of the six TU levels used in the initial experiment, 400 and 500 mg L^{-1} were chosen for subsequent studies. The two cultivars were subjected to saline stress (100 mM NaCl) and two levels of TU were applied presowing or as foliage spray. Salt stress suppressed total biomass, maximum fluorescence yield (F_v/F_m), chlorophyll, and leaf water potential (Ψ_w), but it increased proline, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), leaf osmolality (LO), membrane permeability (MP), and antioxidant enzymes. Exogenous TU application resulted in considerable increases in the dry weight of salt sensitive and tolerant cultivars (38% and 35%, respectively). TU partially improved the salt tolerance of maize plants; it reduced Na^+ but increased N, K^+, Ca^{2+}, and P in the maize plants under saline regimes. TU regulated the growth of maize plants under stress conditions by reducing MP, MDA, and H_2O_2 levels, and altering activities of antioxidant enzymes as well as increasing photosynthetic pigments under a saline regime.

Key words: Maize, thiourea, salt tolerance, oxidative stress, leaf water potential

1. Introduction

Salinization of irrigated land is one of the basic environmental problems for agricultural production (Sreenivasulu et al., 2000). Reduced capacity of crop resistance to salinity is a major obstacle to stabilization of crop performance in stress-prone environments (Chaudhry et al., 2000). Salinity stress causes inhibition of growth and yield production due to high accumulation of hydrogen peroxide, superoxide, and hydroxyl radical (active ROS) (Ashraf, 2009; Miller et al., 2010). Under nonstress conditions, they are produced in nontoxic levels, but their levels are markedly higher in plants grown under stress conditions (Mittler et al., 2010). All these substances, being very reactive, are harmful to vital cellular macromolecules such as proteins and lipids (Ashraf, 2009; Miller et al., 2010; Golldack et al., 2014; 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) (Ashraf, 2009; Sai-Kachout et al., 2013). Consequently, development of stress resistance in crop plants is considered a valid approach by both breeders and molecular biologists (Bartels and Nelson, 1994). Although proper evaluation of genetic modifications for improved stress resistance is attracting considerable attention among plant biologists, there is still a serious lack of concepts, directions, and protocols for accurate measuring and inducing stress resistance in plants (Ashraf and Harris, 2004).

A variety of plant growth regulators are known to regulate growth and development of most plants under stress conditions including salinity stress. Exogenous application of thiourea (TU) is thought to have a significant role in minimizing oxidative damage and processes involved therein. For example, externally applied TU alleviated the injurious effects of salinity in *Brassica juncea* seeds by altering a number of effecter and signaling processes (Srivastava et al., 2011). TU has also been reported to have a key role in plant resistance to a variety of stresses including control of parasitic weeds (Kannan and Zwanenburg, 2014), arsenate toxicity (Srivastava et al., 2014), and heat stress (Asthir et al., 2013).

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However, the effectiveness of TU and related growth regulators has been reported to depend on plant species, environmental conditions, and concentration of soil salt solution. Due to the beneficial role of TU under adverse environmental conditions, it has been assumed that exogenous application of this substance via different modes could be beneficial in ameliorating the adverse effects of high concentration of salt (NaCl) on maize plant growth. Therefore, the current study was aimed to examine the effects of TU applied as seed priming or applied through leaves on plant growth, ROS, enzymatic antioxidants, and mineral nutrition status in maize plants subjected to salt stress.

2. Materials and methods

Based on the results of a pilot experiment, two maize cultivars, namely DK5783 and Apex 836, showed differential salinity tolerance and the two most effective TU doses were selected for the present experiment. The same doses (400 and 500 mg L^{-1}) of TU were applied as seed priming or foliar spray. A glasshouse randomized complete block design replicated three times experiment was arranged at the Research Station of the Agriculture Faculty, Harran University, Turkey, during May to June 2013. Five maize seeds of each cultivar were sown in each pot filled with 10 kg of air-dried soil. The chemical characteristics of the soil used were as follows: pH 7.3, EC 0.45 dS/m, N 1.25 g/kg, and K 1.40 g/kg. Soil was supplemented with NPK at the rates of 100, 50, and 120 mg/kg, respectively. After germination, three seedlings of uniform size were maintained in each pot, and allowed to grow for 35 days at 27 ± 2 °C and RH 60%–70%. Depending on the plant requirement, an aliquot of 50–500 mL of H_2O was applied to each pot. The experiment layout was a randomized complete block design with three replicates and each replicate included three pots (i.e. nine pots per treatment).

Salt stress treatments, control (no NaCl) and 100 mM NaCl, were applied via rooting medium. Salt stress was maintained by adding 5.85 g/kg NaCl to the soil via irrigation water. Addition of 5.85 g kg^{-1} NaCl to the soil brought the salt level to 100 mM. Before germination of seeds, they were disinfected with sodium hypochlorite solution (1% v/v) and then washed with dH_2O. For seed pretreatment with TU, the seeds were soaked for 1 day (24 h) in 400 and 500 mg L^{-1} TU. Plants were sprayed once a week with TU solution (50 mL/pot) prepared in 0.01% T-20, a surfactant. The spray was started 10 days after germination and continued up to day 35. After that, two plants from each replicate were cut at soil level and the whole above-ground plant parts were used to determine fresh and dry weights. After recording fresh weight, the plants were dried to determine dry weights. The remaining plants were used to determine the following attributes.

2.1. Chlorophyll determination

One gram of fully expanded youngest leaf was ground in acetone solution (90%; v/v). The absorbance of the supernatant was measured using a spectrophotometer (Shimadzu UV-1201 V, Japan) and total chlorophyll contents calculated following Strain and Svec (1966).

2.2. Leaf chlorophyll fluorescence

Chlorophyll fluorescence was determined in leaves previously dark- and light-adapted using a fluorometer (PYA Mini-PAM, Walz, Germany). Data for minimum fluorescence (*F*o), maximal fluorescence (*F*m), variable fluorescence (*F*v), and maximum quantum efficiency of PSII (*F*v/*F*m) were recorded.

2.3. Leaf free proline content

The filtrate obtained by grinding a fresh leaf sample (500 mg) in 10 mL of sulfosalicylic acid (3%) was reacted with acid-ninhydrin solution and glacial acetic acid. The mixture was subjected to 100 °C for 60 min and then 4 mL of toluene was added to each sample and OD recorded at 520 nm following Bates et al. (1973).

2.4. Leaf osmolality (LO)

The frozen leaf samples were slightly pressed to extract the sap. The sap so extracted was centrifuged at 5000 × *g* for 5 min. The filtrate was fed to a cryo-osmometer (Osmomat 030, Ganotec) to determine osmomolality.

2.5. Leaf water potential (Ψ_w)

The 3rd leaf from the top was detached from each plant before sunshine and its Ψ_w measured using a pressure chamber (PMS model 600, USA).

2.6. Electrolyte leakage (EL)

Preweighed (0.2 g) fresh leaf (small pieces) was placed in 10 mL of dH_2O, then incubated in a water bath for 2 h at 25 °C, and the electrical conductivity (EC_1) measured. For obtaining released electrolytes, all samples were then subjected to 121 °C in an autoclave for 20 min. Then EC_2 was determined after cooling the mixture to 25 °C. These values were used for the calculations of EL following Dionisio-Sese and Tobita (1998).

2.7. Chemical analysis

Plant dry samples were used for the determination of different ions. Total N was determined using the Kjeldahl method. For the analysis of other nutrients dried and ground samples were ashed in a muffle furnace at 550 °C for 6 h. The white ash was dissolved in 5 mL of 2 M hot HCl, and made up to the final volume to 50 mL with dH_2O. Phosphorus (P) was analyzed by the vanadate–molybdate method and Na, Ca, and K were analyzed using an ICP.

2.8. Antioxidant enzyme assays

Fresh leaf (0.5 g) was triturated in 50 mM Na-phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrrolidine. The mixture was centrifuged at 10,000 × *g* for 15 min at 4 °C and the supernatant collected for the determination

of the following key antioxidant enzymes: following Kraus and Fletcher (1994) for CAT activity, Beauchamp and Fridovich (1971) for SOD activity, and Chance and Maehly (1955) for POD activity determination. The same supernatant was used for the determination of total soluble proteins following Bradford (1976).

2.9. Determination of lipid peroxidation and hydrogen peroxide

Lipid peroxidation in the leaf samples was appraised by measuring malondialdehyde (MDA) content (Weisany et al., 2012). Hydrogen peroxide (H_2O_2) in leaf samples was quantified following Loreto and Velikova (2001). A leaf sample (0.5 g) was ground well in 3 mL of 1% (w/v) TCA and then the extract centrifuged and an aliquot of 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1 M KI. Its OD was read at 390 nm and H_2O_2 contents calculated.

2.10. Statistical analysis

Two parallel experiments were conducted during the same growing period and there were no significant differences between the data of the experiments. However, the data presented here are the means of the data of the two experiments.

Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed using the SAS GLM procedure to examine differences between the two cultivars and treatments at $P \leq 0.05$.

3. Results

3.1. Some key growth parameters

Salt stress significantly suppressed fresh and dry weights of plants of both maize cultivars, but the reduction was higher in the salt sensitive cv. Apex 836 than that in cv. DK 5783 (Table 1). Exogenous application of TU improved fresh and dry weights in both maize cultivars and the mitigating effects of both modes of application were not significantly different on plant dry and fresh biomass.

Salinity stress also reduced both maximum fluorescence yield (*Fv*/*Fm*) and total chlorophyll content, but increased membrane permeability of both cultivars. Externally applied TU improved these key parameters. Overall, there seemed to be no significant differences between the effects of foliar and presowing applications and of TU for either cultivar (Table 2). A considerable difference was observed between the cultivars and treatments for *Fv*/*Fm* and MP, but not for total chlorophyll content (Table 2).

3.2. Water potential and proline

Salinity stress reduced leaf water potential (Ψ_w), but increased leaf osmolality (LO) and proline (Pro) content in both cultivars. Salinity stress was more detrimental on Ψ_w of the salt sensitive cultivar, Apex 836. Salinity stress also resulted in elevated Ψs in the salt sensitive cultivar (Table 3). The results revealed significant differences between the cultivars and treatments for Ψ_w, LO, and Pro ($P \leq 0.05$), as shown in Table 3. Externally applied TU improved Ψ_w

Table 1. Fresh and dry weights of different cultivars of maize grown in salt with or without different levels of thiourea (mg L^{-1}) applied as different modes.

Cultivars	Treatments	FW (g/p)	DW(g/p)
DK 5783	C	16.3 ± 1.4a	1.86 ± 0.16a
	S	9.7 ± 0.8c	1.11 ± 0.09d
	sTU 400	13.2 ± 1.2 b	1.32 ± 0.12c
	sTU 500	13.4 ± 1.3b	1.39 ± 0.13b
	fTU 400	13.5 ± 1.4b	1.40 ± 0.15 b
	fTU 500	13.2 ± 1.2b	1.36 ± 0.14 bc
Apex 836	C	12.3 ± 1.1a	1.29 ± 0.12 a
	S	6.7 ± 0.7e	0.71 ± 0.08 d
	sTU 400	7.5 ± 0.8d	0.86 ± 0.08 c
	sTU 500	8.1 ± 0.9bc	0.91 ± 0.09 b
	fTU 400	8.4 ± 0.7b	0.98 ± 0.09 b
	fTU 500	7.8 ± 0.7c	0.83 ± 0.08 c
Cvs × Treatments		*	*

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with different letters in the same column within the same genotype indicate significant difference between the treatments at $P \leq 0.05$).

Table 2. Maximum fluorescence yield (*Fv*/*F*m), membrane stability (MS), and total chlorophyll (mg/kg FW) of two maize cultivars grown in salt with or without different levels of thiourea (mg L^{-1}) applied through different modes.

Cultivars	Treatments	*Fv*/*F*m	MS (%)	Chl.
DK 5783	C	0.61 ± 0.05a	15 ± 1.2d	1254 ± 109a
	S	0.59 ± 0.05e	25 ± 2.3a	1052 ± 101e
	sTU 400	0.61 ± 0.06cd	20 ± 2.1bc	1148 ± 111d
	sTU 500	0.62 ± 0.06ab	18 ± 1.9cd	1193 ± 116b
	fTU 400	0.61 ± 0.06bc	18 ± 1.7cd	1177 ± 109c
	fTU 500	0.60 ± 0.06d	20 ± 2.1bc	1182 ± 105bc
Apex 836	C	0.61 ± 0.06a	18 ± 1.7c	1196 ± 117 a
	S	0.54 ± 0.05d	29 ± 2.7a	1001 ± 96d
	sTU 400	0.58 ± 0.06c	24 ± 2.2b	1086 ± 106c
	sTU 500	0.58 ± 0.06 c	22 ± 2.1b	1101 ± 103c
	fTU 400	0.59 ± 0.06 b	22 ± 2.0b	1124 ± 104b
	fTU 500	0.58 ± 0.06c	24 ± 2.3b	1103 ± 106c
Cvs × Treatments		*	*	ns

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with different letters in the same column within the same genotype indicate significant difference between the treatments at $P \leq 0.05$). MANOVA: ns: not significant; *: $P \leq 0.05$

Table 3. Leaf water potential (Ψw:MPa), leaf osmolality (LO, Osmol kg^{-1} FW), and proline (Pro, μmol g^{-1} FW) of different cultivars of maize grown in salt with or without different levels of thiourea (mg L^{-1}) applied through different modes.

Cultivars	Treatments	Ψw	LO	Pro
DK 5783	C	–0.34 ± –0.03a	0.046 ± 0.004e	1.04 ± 0.12d
	S	–1.43 ± –0.13e	0.125 ± 0.012a	2.86 ± 0.24a
	sTU 400	–1.34 ± –0.12d	0.102 ± 0.010c	2.34 ±0.23bc
	sTU 500	–1.05 ± –0.10b	0.092 ± 0.009d	2.25 ± 0.21c
	fTU 400	–1.05 ± –0.11b	0.106 ± 0.011c	2.28 ± 0.23c
	fTU 500	–1.14 ± –0.12c	0.112 ± 0.012b	2.41 ± 0.23b
Apex 836	C	–0.31 ± –0.03a	0.041 ± 0.004d	1.11 ± 0.12c
	S	–1.58 ± –0.14e	0.138 ± 0.012a	2.60 ± 0.25a
	sTU 400	–1.35 ± –0.13d	0.125 ± 0.013b	2.39 ± 0.27 b
	sTU 500	–1.15 ± –0.13b	0.109 ± 0.009c	2.12 ± 0.23e
	fTU 400	–1.14 ± –0.13b	0.103 ± 0.011c	2.21 ± 0.24d
	fTU 500	–1.25 ± –0.13c	0.121 ± 0.011b	2.26 ± 0.23d
Cvs × Treatments		*	*	*

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with different letters in the same column within a genotype indicate significant difference between the treatments at $P \leq 0.05$). MANOVA: *: $P \leq 0.05$

and suppressed LO and Pro content in the maize plants. In most cases, seed application of TU (500 mg L^{-1}) and foliar application of TU (400 mg L^{-1}) were more effective.

3.3. Mineral ion contents

Leaf Na^+ concentration accumulated more in cv. Apex 836 as compared to that in cv. DK5783. Both modes of TU application reduced Na^+ content in both cultivars. Moreover, leaf Ca, K, N, and P of both maize cultivars decreased due to salinity, and these reductions were more prominent in maize cv. Apex 836. Significant differences were observed between the cultivars and the treatments for all nutrients tested by MANOVA at $P \leq 0.05$ (Tables 4 and 5).

Both seed and foliar applications of TU reduced leaf Na^+, but increased the levels of other elements analyzed. Seed application of TU at 500 mg L^{-1} and foliar application of TU at 400 mg L^{-1} were more effective in reducing Na^+. The modes of application of TU did not differ in increasing other elements analyzed in the leaves of plants grown in saline conditions.

3.4. Antioxidant enzyme activities

The activities of SOD, POX, and CAT in both maize cultivars increased under salt stress and the salt tolerant cultivar, DK 5783, had higher activities of these enzymes than the salt sensitive cv. Apex 836 did. The activities of all antioxidant enzymes tested decreased with seed and foliar applications of TU, but seed application of TU at 500 mg L^{-1} and foliar application of TU at 400 mg L^{-1} were more effective in reducing the activities of these enzymes. There were significant differences between the cultivars and treatments for SOD and CAT but not for POX according to MANOVA at $P \leq 0.05$ (Table 6).

3.5. Leaf malondialdehyde (MDA) and hydrogen peroxide

In the saline regime, MDA and H_2O_2 contents increased in both maize cultivars (Table 6). Exogenously applied TU via both seed and leaves reduced MDA and H_2O_2 and contents in both maize cultivars. Seed priming with TU at 500 mg L^{-1} and foliar application of TU at 400 mg L^{-1} were also more effective in reducing the reactive oxygen species (ROS). MANOVA showed significant differences between the cultivars and treatments for both H_2O_2 and MDA at $P \leq 0.05$ (Table 7).

4. Discussion

Efforts for improving crop salt tolerance have led researchers to study the effects of various chemicals such as plant growth regulators, mineral nutrients, compatible

Table 4. Sodium and nitrogen concentrations (mmol/kg DW) of two maize cultivars grown in salt with or without varying levels of thiourea (mg L^{-1}) applied through different modes.

Cultivars	Treatments	Na	N
DK 5783	C	33 ± 3.2e	1153 ± 113a
	S	326 ± 31.9a	884 ± 85e
	sTU 400	265 ± 27.3c	1020 ± 99d
	sTU 500	220 ± 21.5d	1100 ± 105b
	fTU 400	225 ± 23.1d	1020 ± 103d
	fTU 500	280 ± 27.9b	1052 ± 103c
Apex 836	C	30 ± 3.2d	1129 ± 111a
	S	398 ± 36.7a	841 ± 82d
	sTU 400	320 ± 31.7b	1005 ±101c
	sTU 500	295 ± 30.6c	1054 ± 102b
	fTU 400	312 ± 31.9b	1067 ± 104b
	fTU 500	321 ± 33.4b	1075 ± 98b
Cvs × Treatments		*	*

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with the different letters in the same column within a genotype indicate significant difference between the treatments at $P \leq 0.05$). MANOVA: *: $P \leq 0.05$

Table 5. Phosphorus, calcium and potassium concentrations (mmol/kg DW) of two maize cultivars grown in salt with or without varying levels of thiourea (mg L^{-1}) applied through different modes.

Cultivars	Treatments	P	Ca	K
DK 5783	C	65 ± 7.1a	174 ± 15a	354 ± 34a
	S	36 ± 3.1d	112 ± 12d	255 ± 26e
	sTU 400	40 ± 3.9c	138 ± 12c	285 ± 27d
	sTU 500	49 ± 4.6b	152 ± 14b	305 ± 31c
	fTU 400	48 ± 4.6b	141 ± 14c	315 ± 32b
	fTU 500	46 ± 4.5b	140 ± 13c	302 ± 29c
Apex 836	C	63 ± 6.4a	164 ± 15a	343 ± 33a
	S	28 ± 2.9e	96 ± 9d	224 ± 23d
	sTU 400	32 ± 3.6d	124 ± 11c	246 ± 25c
	sTU 500	39 ± 3.5c	135 ± 12b	285 ± 29b
	fTU 400	47 ± 4.2b	138 ± 14b	289 ± 29b
	fTU 500	45 ± 4.6b	129 ± 13c	278 ± 28b
Cvs × Treatments		*	*	*

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with different letters in the same column within a cultivar indicate significant difference between the treatments at $P \leq 0.05$). MANOVA: *: $P \leq 0.05$

Table 6. Superoxide dismutase (SOD: Unit/mg protein/min), catalase (CAT: Unit × 100/mg protein), peroxidase (POX: ΔA_{470}/min/mg protein) of two maize cultivars grown in salt with or without varying levels of thiourea (mg L^{-1}) applied through different modes.

Cultivars	Treatments	SOD	CAT	POX
DK 5783	C	47 ± 5e	1.31 ± 014f	8.15 ± 0.83d
	S	172 ± 16a	2.95 ± 0.25a	36.17 ± 3.21a
	sTU 400	109 ± 11b	2.22 ± 0.21b	21.58 ± 2.12c
	sTU 500	90 ± 9d	2.06 ± 0.21c	20.78 ± 1.98c
	fTU 400	86 ± 9d	1.75 ± 0.18e	21.12 ± 2.12c
	fTU 500	98 ± 10c	1.86 ± 0.19d	24.89 ± 2.31b
Apex 836	C	49 ± 5d	1.36 ± 0.14e	8.95 ± 0.81e
	S	154 ± 16a	2.65 ± 0.21a	35.29 ± 3.21a
	sTU 400	96 ± 9b	2.01 ± 0.19b	21.42 ± 2.10d
	sTU 500	67 ± 7c	1.84 ± 0.16c	20.12 ± 1.87c
	fTU 400	78 ± 8c	1.69 ± 0.15d	20.23 ± 1.97c
	fTU 500	95 ± 9b	1.89 ± 0.15c	24.23 ± 2.22b
Cvs × Treatments		*	*	ns

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with different letters in the same column within a cultivar indicate significant difference between the treatments at $P \leq 0.05$). MANOVA: ns: not significant; *: $P \leq 0.05$

Table 7. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations in the leaves of two maize cultivars grown in salt with or without varying levels of thiourea (mg L^{-1}) applied through different modes.

Cultivars	Treatments	H_2O_2 (μmol g^{-1} DW)	MDA (nmol g^{-1} FW)
DK 5783	C	1.14 ± 0.10d	1.39 ± 0.12d
	S	6.53 ± 0.67a	10.21 ± 1.09a
	sTU 400	4.36 ± 0.42b	7.23 ± 0.69b
	sTU 500	3.80 ± 0.36c	6.75 ± 0.66b
	fTU 400	3.75 ± 0.36c	6.84 ± 0.65b
	fTU 500	4.54 ± 0.43b	7.84 ± 0.72b
Apex 836	C	1.22 ± 0.11d	1.52 ± 0.12d
	S	8.69 ± 0.84a	13.29 ± 1.23a
	sTU 400	6.25 ± 0.61b	9.56 ± 0.98b
	sTU 500	5.29 ± 0.51c	8.68 ± 0.82c
	fTU 400	5.46 ± 0.55c	8.29 ± 0.81c
	fTU 500	6.35 ± 0.62b	9.89 ± 1.01b
Cvs × Treatments		*	*

TU: Thiourea; C: control; S: 100 mM NaCl; s: seed application; f: foliar application (mg L^{-1}). Values are the means of two parallel experiments. ±: Standard errors. Means marked with different letters in the same column within a cultivar indicate significant difference between the treatments at $P \leq 0.05$). MANOVA: *: $P \leq 0.05$

solutes, and nonenzymatic antioxidant compounds. Although these chemicals have demonstrated positive effects in inducing salinity tolerance, a number of studies are available showing that application of these chemicals only modulates certain physiological activities that do not translate into salt tolerance (Plaut et al., 2013; Perveen et al., 2014). The success of such chemical applications in inducing salt tolerance depends on absorbance of these chemicals in plant tissues such as leaf and root, with their subsequent translocation to other parts of plants. Absorbance or penetration of these chemicals in plant tissues depends on the type and amount of surfactant used, air temperature and humidity, and mode of application (Athar et al., 2009; Plaut et al., 2013). Due to this reason the efficiency of different modes of application of TU after optimizing surfactant was assessed at the vegetative growth stage in the present study. Seed soaking or foliar application of 400 or 500 mg L^{-1} of TU enhanced the plant growth of both maize cultivars grown under salinity stress. However, seed application of TU at 500 mg L^{-1} and foliar application of TU at 400 mg L^{-1} were most effective in mitigating the deleterious effects of salt stress on both maize cultivars. Some earlier reports reveal that TU application improves stress tolerance and enhances the yield of a broad ranges of crops, e.g., wheat (Sahu et al., 2006), mung bean (Mathur et al., 2006), and potato (Mani et al., 2012). In the present study, although saline stress (100 mM sodium chloride) suppressed growth in terms of plant fresh and dry weights of both maize cultivars, DK 5783 and Apex 836, exogenous application as seed priming or foliar spray improved the growth of maize plants under saline conditions. Similarly, using TU (10 mM) as a foliar spray, Anjum et al. (2011) also observed improved growth of two differentially salt responsive wheat cultivars supplied with salinity (120 mM NaCl) stress. They ascribed this TU-induced increase in growth to triggering of a variety of physio-biochemical processes as recently reported by Pandey et al. (2013). This improvement in plant biomass due to exogenous application of TU could be due to a high endogenous level of TU and its utilization in the leaves, wherein it might have acted as a source of C and N, respectively, as has been earlier reported in different studies (Mitoi et al., 2009; Anjum et al., 2011), because, in the present study, application of TU significantly enhanced the leaf N of maize plants, which was positively associated with enhanced plant biomass production. Furthermore,

this enhancement in biomass production in maize plants due to TU application might have been due to its role in cellular osmotic adjustment (Burman et al., 2004; Seckin et al., 2009).

A number of studies have shown that saline stress can cause alterations in leaf fluorescence of different crops such as sunflower (Akram et al., 2009), okra (Saleem et al., 2011), eggplant (Shaheen et al., 2012), and wheat (Habib et al., 2013; Perveen et al., 2013). In the current study, F_v/F_m of both maize cultivars increased due to exogenously applied TU under saline conditions, which is parallel to the findings of Pandey et al. (2013), who documented improved chlorophyll fluorescence in salt stressed Indian mustard (*Brassica juncea*) plants due to exogenously applied TU and they attributed this growth improvement to TU-induced high efficiency of PSI and PSII. About two decades ago, Sahu et al. (1993) observed that exogenously applied TU enhanced the photosynthetically active leaf area as well as the rate of photosynthesis in maize plants, which was ascribed to TU-induced improvement in the efficiency of photosystems. To date, a number of experiments have been carried out on TU-induced changes/improvement in the rate of photosynthesis under stress in different crops, e.g. clusterbean (Burman et al., 2004), wheat (Nathawat et al., 2007), and maize (Sahu et al., 1993), but the role of TU in efficiency of photosystems has not been well researched. It is well known that the efficiency of photosystems and rate of photosynthesis are closely interlinked (Misra et al., 2006; Geissler et al., 2009; Ashraf and Harris, 2013), but the information on TU-induced changes in the photosynthesis linked to either PSI or PSII still needs to be elucidated.

Generally, high biomass production of a plant is associated with the leaf photosynthetic rate, which ultimately depends upon the stomatal conductance and quantity of leaf photosynthetic pigments such as total chlorophyll. Adverse environmental conditions such as salinity result in the degradation of leaf chlorophyll contents leading to reduced plant photosynthetic rate and thereby reduced biomass production. A number of studies state that exogenous applied organic compounds have been beneficial in ameliorating the deleterious effects of salt stress on leaf photosynthetic pigments coupled with enhanced biomass production (Nawaz and Ashraf, 2010; Ali and Ashraf, 2011). Similarly, in the present study, exogenously applied TU was effective in improving the leaf chlorophyll contents of maize plants, which were positively associated with higher photosynthetic rate and hence higher biomass production.

Ion homeostasis is an important component of the plant salt tolerance mechanism. Accumulation of salt at toxic level in different plant parts disturbs this mechanism (Ali and Ashraf, 2011). In the present study, salt stress decreased the accumulation of mineral nutrients such as N, P, K^+, and Ca^{2+}, whereas it increased accumulation of Na^+ in the leaves of maize plants grown under salt stress. However, both modes of TU at both doses reduced Na^+ contents while increasing N, P, K^+, and Ca^{2+} contents in both maize cultivars under saline conditions. A significant reduction observed in Na^+ uptake shows that TU-induced tolerance is due to salt avoidance strategy (Srivastava et al., 2011). Therefore, the findings of the present study showed that exogenously applied TU has an effective role in cellular ion homeostasis, resulting in increased uptake of N, P, K^+, and Ca^{2+}. These findings can be correlated with some earlier findings indicating that TU has a role in cellular ion transport (Sud and Sharma, 1992).

Activation/upregulation of the antioxidative defense system of plants on exposure to saline conditions is a frequently occurring response (Akram et al., 2012; Perveen et al., 2013). During the present investigation, we also observed increased activities of CAT, SOD, and POX enzymes in both maize cultivars, and they were better in high biomass producing maize cv. DK 5783 as compared to the salt sensitive cv. Apex 836. However, exogenous application of varying concentrations of TU improved the activities of all examined antioxidant enzymes, while reducing the level of H_2O_2 (a strong ROS) significantly under saline conditions (Tables 6 and 7). While reviewing the role of antioxidant potential in stress tolerance, Ashraf and Akram (2009) suggested that, under stress conditions, imbalance between generation of ATP and NADPH through the photosynthetic electron transport chain and their consumption in fixation of CO_2 in sugar causes the generation of ROS via the water–water cycle. Moreover, plants with better antioxidant potential are more tolerant to stress. However, enhancement in the amount and activities of antioxidant enzymes is energetically costly. Thus, the prime objective of application of nonenzymatic antioxidants is to enhance plant antioxidant potential for improved salinity tolerance (Plaut et al., 2013). In view of these findings, it is suggested that exogenous application of TU enhanced the antioxidant potential of maize plants under salt stress while it reduced the metabolic burden on plants. Previously, it was observed that oxidative stress generated by exogenously applied H_2O_2 (10 and 20 mM) in wheat plants was reasonably minimized due to TU-induced increases in the activities of CAT and POD enzymes and levels of total soluble proteins (Hammed et al., 2013). Furthermore, Srivastava et al. (2011), while working with Indian mustard, showed that TU treatment along with NaCl lowered the level of ROS closer to that in nonstressed plants. They attributed the decrease in the level of ROS to the TU-induced upregulation of GSH/GSSG ratio and the activities of DPPH-radical scavenging, and SOD and GR enzymes. Exogenous application of TU

decreased the levels of H_2O_2, MDA, and EL coupled with decreased activities of antioxidant enzymes, which clearly showed the role of TU in minimizing ROS production. These results are in accordance with earlier published studies in which it was reported that application of TU is effective in alleviating oxidative damage to biological membranes (Srivastava et al., 2011).

In conclusion, exogenous use of TU played an active role in maintaining plant water and ion homeostasis, which lowered the generation of ROS. Higher antioxidant potential due to TU application also helped in improving the photosynthetic activity of both maize cultivars. However, the efficiency of TU treatments in improving salinity tolerance in maize plants was greater in the salt tolerant cv. DK 5783 than it was in the salt sensitive cultivar. Thus, exogenous use of TU may be an economically viable strategy for improving crop salt tolerance in plants.

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