



## Combination of nitric Oxide and thiamin regulates oxidative defense machinery and key physiological parameters in salt-stressed plants of two maize cultivars differing in salinity tolerance

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### ABSTRACT

A glasshouse experiment was conducted to appraise the influence of combined nitric oxide (NO) and thiamin (TA) on oxidative defense system and some key physiological attributes in two maize cultivars (DK 5783 and Apex 836) stressed with 0 (control) or 100 mM NaCl. Of six NO and TA levels used in the initial germination experiment, 2 levels of combined NO and TA (3 +100 or 6+ 125 mg l<sup>-1</sup> respectively) were chosen for subsequent studies as seed soaking or as a spray to seedlings. Salinity resulted in rising leaf free proline content and osmolality, but in a decrease in plant dry biomass and maximum fluorescence yield ( $F_v/F_m$ ) in cultivars. Both modes of applied NO and TA were found to be effective in alleviating the adverse effects of NaCl on shoot growth. Salt stress resulted in enhancing leaf Na<sup>+</sup>, but reducing leaf K<sup>+</sup> and Ca<sup>2+</sup> in plants. Both modes of application of NO and TA resulted in increased Ca<sup>2+</sup> and K<sup>+</sup> contents, but decreased those of Na<sup>+</sup> in salt stressed maize plants. Salt stress caused the enhanced accumulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA). Salinity promoted the activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in maize. The growth improvement in maize plants due to exogenously-applied NO and TA in combination was found to be due to decreased leaf Na<sup>+</sup>, H<sub>2</sub>O<sub>2</sub> and MDA levels, and altered activities of SOD, CAT, and POD as well as improved maximum fluorescence yield under saline stress.

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### Introduction

Salt stress leads to huge losses to crop production worldwide. The research related to the effects of salt stress on plants has been under intensive investigation since a long span of time. These salt-induced effects have been explored using a variety of physiological parameters such as rate of photosynthesis, water and ion uptake, osmotic

adjustment, and oxidative metabolism, etc. (Liu *et al.*, 2014). Several studies have shown that accumulations of reactive oxygen species (ROS) are linked primarily to the antioxidant enzyme system, which is one of the premier adaptive responses of plants to saline stress (Dong *et al.*, 2014), although plants generate a variety of antioxidant compounds both enzymatic and non-enzymatic antioxidants to

nullify the ROS. The key enzymatic antioxidants include catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) which can effectively scavenge ROS before they cause injury to cells (Noctor and Foyer, 1998; Perveen *et al.*, 2013). The modulation of the activities of these enzymes could be important in improving tolerance of plants to environmental stresses (Dong *et al.*, 2014).

During the last few decades, researchers have used a variety of growth substances as a shot-gun approach to overcome the adverse effects of environmental stresses on plants (Ashraf and Foolad, 2007; Nawaz and Ashraf, 2010). Thiamin is one of such plant growth substances required for growth and differentiation of some plant species (Proebsting *et al.*, 1990; Rapala-Kozik *et al.*, 2012). Some *in vitro* studies have shown that thiamin is one of the potential antioxidants known so far (Tunc-Ozdemir *et al.*, 2009), so by virtue of being an antioxidant it can play a key role in plant stress tolerance.

Nitric oxide (NO), being ubiquitous signal in plants, is believed to play a vital role in plant responses to environmental stresses (Freschi 2013; Liu *et al.*, 2014). NO has also been reported to mitigate the stress-induced oxidative stress in plants (Chen *et al.*, 2010; Mihailovic and Drazic, 2011).

The present study was conducted to examine to what extent salinity stress has triggered oxidative stress in maize plants and whether an extra supply of combined nitric oxide and thiamin could mitigate the adverse effects of salt stress on antioxidative defense system.

## Materials and methods

### *Plant growth conditions*

The study was carried out during May and June 2013 at the Research Station of the Agriculture Faculty, University of Harran (Turkey) under glasshouse conditions maize (*Zea mays* L. cvs. DK5783 and Apex 836). Seeds of each of two maize cultivars DK

5783 and Apex 836 were planted at a rate of five seeds per pot. Each pot contained 10 kg air-dried loamy clay soil. The chemical characteristics of the soil used were as follows. pH (1.2.5 water, v.v) 7.3, electrical conductivity 0.45 dS/m, N and K 1.25 and 1.40 g/kg, respectively. Nitrogen, phosphorus, and potassium were added to the soil at the rates of 100, 50 (P<sub>2</sub>O<sub>5</sub>) and 120 (K<sub>2</sub>O) mg/kg, respectively. Salt treatment in the soil was developed before planting the seeds by adding appropriate amount of NaCl to irrigation water to keep salt level to 100 mM. The electrical conductivity of the soil was checked weekly during the whole experimentation.

After germination, thinning of the seedlings was done to maintain three seedlings in each pot, and then they were further allowed to grow for 35 days under 27±2 °C and mean daytime RH 60-70%. The experiment was arranged in the glasshouse in a randomized complete block design replicated thrice. Every day an aliquot of 50 mL to 500 mL of water depending on plant size and time from planting was applied to each pot.

Two levels (100 and 125 mg/l) of thiamin and two (3 and 6 mg/l) of nitric oxide in combination were applied as presowing seed treatment or through leaves to 10 days old seedlings. The salt used for NO source was sodium nitroprusside (SNP). All seed samples were sterilized with NaOCl solution (1% v/v) before use. For presowing seed treatment, the seed samples were soaked for 24 h in 3 mg/l NO +100mg/l TA or 6 mg/l NO +125 mg l<sup>-1</sup> TA solution. However, for foliar spray, the plants were applied 50 ml per pot of NO and TA solution prepared in 0.01% tween-20. The foliar spray was initiated when the seedlings were 10 days old and continued up to day 35. Data for different growth and physio-biochemical attributes were measured before terminating the experiment.

### *Chlorophyll fluorescence measurements*

Maximal quantum yield (Fv/Fm) was measured using a portable chlorophyll fluorometer

(Photosynthesis Yield Analyzer Mini-PAM, Walz, Germany). The data were recorded using previously dark-adapted leaves for 30 min.

#### ***Leaf free proline content***

The protocol described by Bates et al. (1973) was employed to determine leaf free proline content. The filtrate obtained by grinding 0.5 g fresh leaf sample in 10 ml of 3% aqueous sulfosalicylic acid, was reacted appropriately with acid-ninhydrin solution and glacial acetic acid. The mixture was subjected to 100 °C for one hour. Thereafter the mixture was treated with 4 ml of toluene and its absorbance read at 520 nm.

#### ***Leaf osmolality***

The leaf samples were placed in liquid nitrogen for 72 h and thereafter they were slightly pressed to extract the sap. The sap so extracted was centrifuged at 5,000g for 5 minutes. The filtrate was fed to a Cryo-osmometer (Osmomat 030, Ganotec) to determine osmolarity.

#### ***Soluble protein content***

The protocol described by Bradford (1976) was used to determine soluble protein content of fresh leaf samples.

#### ***Antioxidant enzyme assays***

For determining the activities of different enzymatic antioxidants 0.5 g of fresh leaf material was triturated in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrrolidone. The mixture was centrifuged at 20,000 g for 15 minutes at 4° C and then used to determine the activities of the key antioxidant enzymes such as POD, CAT and SOD.

The method described by Kraus and Fletcher (1994) was used to determine CAT activity, whereas that of Beauchamp and Fridovich (1971) was used to determine the SOD activity. The POD activity was determined following the method described by Chance and Maehly (1955).

#### ***Inorganic nutrient analysis and dry weight measurement***

Plant samples were dried well in an oven at 65 °C and their dry weights measured. For determining inorganic nutrients, the well ground plant samples were placed in a muffle furnace at 550 °C for 6 h. The ash was dissolved in 5 mL of 2 M hot HCl and the final volume was raised to 50 mL by adding distilled water (Chapman and Pratt 1982). An ICP was used to quantify sodium (Na<sup>+</sup>), Ca<sup>2+</sup>, and K<sup>+</sup>.

#### ***Determination of lipid peroxidation and hydrogen peroxide***

The leaf malondialdehyde (MDA) content, a product of lipid peroxidation, was determined using the method described by Cakmak and Horst (1991) with some modifications as suggested by Weisany et al. (2012).

The quantification of H<sub>2</sub>O<sub>2</sub> in leaves was carried out following Loreto and Velikova (2001). Half gram of fresh leaves was triturated in 3 mL of 1% (w/v) trichloro-acetic acid (TCA). The mixture was centrifuged at 10,000 rpm for 10 minutes. 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1M KI were added to an aliquot of 0.75 ml of the supernatant and then its OD read at 390 nm.

#### ***Statistical analysis***

A statistical package SAS version 9.1 was employed to work out analysis of variance of data for each attribute. The Least Significance Difference (LSD) test at P = 0.05 was employed to determine the differences among data means within each parameter.

## Results

While screening nine maize cultivars at the germination stage (unpublished data) cv. DK 5783 was found to be salt tolerant and cv. Apex 836 as salt sensitive. Also while optimizing the levels of NO and TA, out of six two levels (3 and 6 mg l<sup>-1</sup>) of NO and two (100 and 125 mg l<sup>-1</sup>) TA in combination were found more effective in promoting seed germination of the two maize cultivars under saline conditions and thus they were used for further glasshouse studies.

### *Some key growth parameters*

Salt stress caused a significant reduction in plant dry biomass and maximum fluorescence yield (*F<sub>v</sub>/F<sub>m</sub>*) in both maize cultivars (Tables 1 and 2). However, these salt-induced reductions were less in cv. DK 5783 than those in cv. Apex 836. Combined application of varying levels of NO and TA as presowing seed treatment or foliar spray improved both attributes in the salt stressed plants of both maize cultivars, however, the effect of the former mode of application was slightly better than that of the latter in improving plant dry biomass in the salt-stressed maize plants.

Leaf osmolality (LO) and free proline content increased in both maize cultivars subjected to saline stress. Cv. Apex 836 had slightly greater leaf osmolality than that in DK 5783, whereas the reverse was true for proline content (Table 2). Both modes of application of NO and TA considerably decreased proline content and leaf osmolality in salt stressed plants of both maize cultivars exposed to saline regimes.

### *Inorganic elements*

Saline stress caused a marked increase in leaf Na<sup>+</sup> in the plants of both maize cultivars, however, it was significantly higher in the salt sensitive cultivar Apex 836 than that in the salt tolerant cultivar DK5783 (Table 3). Both presowing seed treatment

and foliar spray of both growth substances in combination significantly decreased leaf Na<sup>+</sup>. Salinity stress also lowered down leaf Ca<sup>2+</sup> and K<sup>+</sup> content in both maize cultivars, but these reductions being greater in the salt sensitive cultivar Apex 836. Overall, pre-sowing seed treatment with combined NO and TA was more efficient in decreasing leaf Na<sup>+</sup>, and foliar application in enhancing leaf Ca<sup>2+</sup> and K<sup>+</sup> contents.

### *Antioxidant enzymes and ROS*

Salt stress caused a significant improvement in the activities of SOD, CAT and POX in both maize cultivars and these enhancement being greater in cv. DK 5783 as compared to those in cv. Apex 836. Both modes of combined NO and TA applications decreased the activities of all antioxidant enzymes examined (Table 4).

Leaf H<sub>2</sub>O<sub>2</sub> and MDA contents were found to increase in both cultivars exposed to salt and they being higher in the salt sensitive cultivar (Table 5). Both modes of applications of the two growth regulators, i.e. NO and TA in combination, caused a marked reduction in the levels of leaf H<sub>2</sub>O<sub>2</sub> and MDA contents in both maize cultivars. However, presowing seed treatment of the two growth regulators was found to be more efficient in decreasing the ROS than foliar application.

## Discussion

Enhanced salinity tolerance in different plants has been reported to be associated with enhanced growth and yield as well as a variety of physio-biochemical adaptations/mechanisms (Batool *et al.*, 2014; Gupta and Huang, 2014) including low uptake of toxic ions (Kader and Lindberg, 2005; Sabir and Ashraf 2007), low accumulation of ROS (Fayez and Bazaid 2014), upregulation of oxidative defense system (Ashraf 2009; Akram *et al.*, 2012), high accumulation of key osmoprotectants (Ashraf and Foolad, 2007, Kaya *et al.*, 2013a), efficient photo-respiratory machinery

**Table 1:** Dry weights of two cultivars of maize grown in salt with or without different levels of combined nitric oxide (NO) and thiamin (TA) applied as pre-sowing seed treatment or foliar spray

<b>Cultivars</b>			
	<b>DK 5783</b>	<b>Apex 836</b>	
<b>Treatments</b>	<b>DW(g/p)</b>	<b>DW(g/p)</b>	<b>Treatments vs Cultivars</b>
<b>C</b>	1.86 a	1.29 a	*
<b>S</b>	1.11d	0.71c	*
<b>sNO+TA3+100</b>	1.29 b	0.85b	*
<b>sNO+TA6+125</b>	1.32 b	0.87 b	*
<b>fNO+TA3+100</b>	1.21 c	0.78 bc	*
<b>fNO+TA6+125</b>	1.12 d	0.86 b	*

*C: control; S: 100 mM NaCl; s:seed application; f: foliar application (mg/l). Within each column, means with different letters are significantly different ( $P \leq 0.05$ ). \*LSD test: shows significant differences between treatments and cultivars ( $P < 0.005$ ).*

**Table 2:** Maximum fluorescence yield ( $F_v/F_m$ ), leaf osmolality (LO, Osmol/kg) and free proline (pro,  $\mu\text{mol/g}$ ) of two cultivars of maize grown in salt with or without varying levels of combined nitric oxide (NO) and thiamin (TA) applied as pre-sowing seed treatment or foliar spray

<b>Cultivars</b>		<b>DK 5783</b>			<b>Apex 836</b>	
<b>Treatments</b>	<b><math>F_v/F_m</math></b>	<b>LO</b>	<b>Pro</b>	<b><math>F_v/F_m</math></b>	<b>LO</b>	<b>Pro</b>
<b>C</b>	0.62 a*	0.045 d	1.09 c	0.59a	0.042 c	1.12 c
<b>S</b>	0.58 c	0.126 a	2.89 a	0.54 c	0.136 a	2.62 a
<b>sNO+TA3+100</b>	0.60 b	0.105 c	2.39 b	0.56 b	0.128 ab	2.43 b
<b>sNO+TA6+125</b>	0.59 bc	0.103 c	2.32 b	0.56 b	0.126 b	2.39 b
<b>fNO+TA3+100</b>	0.59 bc	0.115 b	2.45 b	0.56 b	0.126 b	2.36b
<b>fNO+TA6+125</b>	0.60 b	0.112 b	2.41 b	0.56 b	0.129 ab	2.39 b

*C: control; S: 100mM NaCl; s:seed application; f: foliar application (mg/l). \*Within each column, means with different letters are significantly different ( $P \leq 0.05$ ).*

**Table 3:** Sodium, calcium and potassium concentrations (mmol/kg) of two cultivars of maize grown in salt with or without varying levels of combined nitric oxide (NO) and thiamin (TA) (mg/l) applied as pre-sowing seed treatment or foliar spray

Cultivars	DK 5783			Apex 836		
	Na <sup>+</sup>	Ca <sup>2+</sup>	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	K <sup>+</sup>
Treatments						
C	34 d*	172 a	355 a	31d	165 a	341 a
S	325a	110 c	254 d	395 a	95 c	221 d
sNO+TA3+100	274c	134 b	276 c	331 c	130 b	272 c
sNO+TA6+125	278bc	129 b	284 c	334 c	124 b	280 bc
fNO+TA3+100	284 b	134 b	304 b	347 b	122 b	284 bc
fNO+TA6+125	285 b	131 b	302 b	342 b	124 b	289 b

C: control; S: 100 mM NaCl; s:seed application; f: foliar application (mg/l). \*Within each column, means with different letters are significantly different ( $P \leq 0.05$ ).

**Table 4:** Superoxide dismutase (SOD: Unit/mg protein/min), catalase (CAT: Unit x100/mg protein), peroxidase (POX:  $\Delta A_{470}$ /min/mg protein) of two cultivars of maize grown in salt with or without varying levels of combined nitric oxide (NO) and thiamin (TA) (mg/l) applied as pre-sowing seed treatment or foliar spray

Cultivars	DK 5783			Apex 836		
	SOD	CAT	POX	SOD	CAT	POX
Treatments						
C	45 c*	1.33 d	8.24 c	48 d	1.39 e	8.93 c
S	173 a	2.95 a	36.25 a	155 a	2.74 a	35.20 a
sNO+TA3+100	126 b	2,36 b	24,63 b	105 c	2,14 c	24,63 b
sNO+TA6+125	127 b	2,45 b	25,68 b	104 c	2,36 b	25,36 b
fNO+TA3+100	135 b	2,12 c	26,45 b	112 b	1,98 d	24,23 b
fNO+TA6+125	127 b	2,10 c	25,65 b	114 b	1,96 d	24,25 b

C: control; S: 100mM NaCl; s:seed application; f: foliar application (mg/l). \*Within each column, means with different letters are significantly different ( $P \leq 0.05$ )

**Table 5:** Hydrogen peroxide ( $H_2O_2$   $\mu\text{mol g}^{-1}$  DW) and malondialdehyde (MDA  $\text{nmol g}^{-1}$  FW) concentrations in the leaves of two cultivars of maize grown in salt with or without varying levels of combined nitric oxide (NO) and thiamin (TA) (mg/l) applied as pre-sowing seed treatment or foliar spray

Cultivars	DK 5783		Apex 836	
	$H_2O_2$	MDA	$H_2O_2$	MDA
Treatments				
C	1.12 d*	1.30 d	1.28 d	1.58 d
S	6.54 a	10.22 a	8.67 a	13.29 a
sNO+TA3+100	3.73 c	5.28 c	5.36 c	7.32 c
sNO+TA6+125	3.75 c	5.32 c	5.34 c	7.46 c
fNO+TA3+100	4.70 b	6.25 b	6.37 b	8.28 b
fNO+TA6+125	4.80 b	6.38 b	6.31 b	8.69 b

C: control; S: 100mM NaCl; s:seed application; f: foliar application (mg/l). \*Within each column, means with different letters are significantly different ( $P \leq 0.05$ )

(Muranaka *et al.*, 2002), etc. However, different plant species/cultivars respond differently with regard to these processes. In the present study, although plant dry weight of both maize cultivars reduced significantly under saline conditions, more reduction was observed in the salt sensitive cv. Apex 836 as compared to that in the salt tolerant cv. DK 5783. Exogenous combined application of nitric oxide (NO) and thiamin (TA) improved dry weights of both maize cultivars under saline conditions. Although the growth regulatory effects of NO and TA have been already assessed individually and both have been shown effective in approving growth and yield under saline conditions, little information exists in the literature on the effects of these two bioregulators applied in combination. For example, NO-induced growth improvement has already been observed in different plant species such as rice (Habib and Ashraf, 2014), tomato (Wu *et al.*, 2011), wheat (Xie *et al.*, 2008), *Kandelia obovata* (Chen *et al.*, 2014), and cucumber (Fan *et al.*, 2012). Exogenously applied thiamin has also been reported to be effective in regulating plant growth and some key processes such as decreased membrane damage, low uptake of  $\text{Na}^+$ , enhanced uptake of  $\text{K}^+$ , and increased leaf relative water contents and total amino acids in sunflower plants under saline stress (Sayed and Gadallah, 2002).

In the present study, maximum fluorescence yield ( $F_v/F_m$ ) of both maize cultivars decreased significantly, and the maximum reduction was observed in the salt sensitive maize cv. Apex 836. Exogenously-applied varying levels of TA and NO significantly improved  $F_v/F_m$  in both cultivars under saline conditions. Salinity-induced reduction in  $F_v/F_m$  could be due to the inactivation and destruction of PSII reaction center (Santos *et al.*, 2001; Yan *et al.*, 2012; Ashraf and Harris, 2013; Dong *et al.*, 2014). However, combined TA and NO application ameliorated the adverse effects of salt on this fluorescence parameter. Analogous to this in another study on cotton plants, Dong *et al.* (2014) inferred that exogenously applied NO improved the activity of PSII under saline conditions.

Furthermore, they observed that the salt-induced reduction in  $F_v/F_m$  was due to the inhibitory effects of salinity on photosynthetic machinery of cotton plants. It has also been reported that thiamin plays an important role in protecting PSI by minimizing over-accumulation of  $\text{H}_2\text{O}_2$  contents as well as up-regulation of oxidative pentose phosphate pathway in plants (Goyer, 2010). Thus, the TA-induced increase in  $F_v/F_m$  in both maize cultivars under saline conditions may have been due to the protective effect of TA on D1 protein of PSII (Ashraf and Harris, 2013). In another study, grain soaking in thiamin at the rate of 0.3 mM alleviated the adverse effects of salt stress by reducing NaCl-induced accumulation of proline in wheat seedlings (Al-Hakimi and Hamada, 2001).

Salinity stress increased proline content in both maize cultivars which is parallel to some earlier reports in which salt-induced increase in proline contents has been observed in different plants under saline conditions, e.g. sunflower (Akram *et al.*, 2012), maize (Cha-um and Kirdmanee, 2009), *Jerusalem artichoke* (Huang *et al.*, 2013), okra (Saleem *et al.*, 2011), cauliflower (Batool *et al.*, 2013), eggplant (Shaheen *et al.*, 2013), etc. Proline is one of the essential osmoprotectants that play a critical role in osmoregulation in different plants under saline stress (Ashraf and Foolad, 2007; Sabir *et al.*, 2011; Akram *et al.*, 2012). High accumulation of proline in plant tissues is generally associated with enhanced salt tolerance (Ashraf and Foolad 2007; Banu *et al.*, 2012). In the present study, proline contents were higher in the relatively salt tolerant maize cv. DK 5783 and both seed and foliar applications of NO + TA reduced proline contents in both maize cultivars. A number of reports available in the literature show that both NO and TA have multifunctional roles when applied individually as an external application. However, not a single report could be deciphered from the literature on the effects of combined application of NO and TA on proline accumulation, therefore, there is a need to explore the effects of NO and TA alone or in combination in different plant species.

Recently, Fan *et al.* (2013) suggested that exogenously applied NO alleviated the salt-induced damages to cucumber seedlings and they attributed this alleviation due to the regulation of the content and proportions of different polyamines (Fan *et al.* 2013).

In the current study, the salt sensitive cv. Apex 836 had higher tissue  $\text{Na}^+$  concentration than that in the salt tolerant cv. DK5783. Both modes of combined application of NO and TA in different doses reduced tissue  $\text{Na}^+$  content. Furthermore,  $\text{Ca}^{2+}$  and  $\text{K}^+$  concentrations in the leaves of both maize cultivars decreased under saline conditions. Seed application of NO and TA in combination was more effective than foliar application in reducing  $\text{Na}^+$  contents. While working with wheat plants, Kausar *et al.* (2013) observed that varying levels of NO caused a slight reduction in  $\text{Na}^+$  accumulation in root and shoot tissues of wheat plants, however, exogenously applied NO enhanced the activities of SOD, POD and CAT enzymes, and soluble protein and proline contents in wheat plants under saline and non-saline conditions which were pointed out to be major factors of improving growth in wheat plants under saline stress. Similarly, Dong *et al.* (2014) have observed NO-induced improvement in  $\text{K}^+$  content, antioxidant enzyme activities, while decrease in  $\text{Na}^+/\text{K}^+$  ratio, TBARS and MDA contents under saline regimes in cotton plants.

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and malondialdehyde (MDA) contents increased in both maize cultivars subjected to saline conditions. Of both maize cultivars, salt sensitive cv. Apex 836 accumulated more  $\text{H}_2\text{O}_2$  and MDA contents than did the salt tolerant maize cultivar under saline conditions. Foliar and seed combined applications of NO + TA reduced  $\text{H}_2\text{O}_2$  and MDA contents in the leaves of both maize cultivars. It has been speculated that efficient scavenging of ROS produced under saline conditions requires the action of several enzymatic and non-enzymatic antioxidants (Ashraf 2009; Akram *et al.*, 2012; Sharma *et al.*, et al. 2012; Kaya *et al.*,. 2013b). In the present study, seed application of combined NO and TA at varying doses was more

effective in reducing the  $\text{H}_2\text{O}_2$  and MDA contents than the foliar supplementation. During a study with *Arabidopsis thaliana*, it has been observed that under adverse environmental conditions, accumulation of thiamin increased which induced tolerance against salinity-induced oxidative stress by decreasing the over-accumulation of ROS particularly that of  $\text{H}_2\text{O}_2$  (Tunc-Ozdemir *et al.*, 2009). In the present study, activities of CAT, SOD and POX were found to be accelerated in both maize cultivars on their exposure to saline stress. However, seed and foliar applications of combined NO and TA suppressed the activities of all these antioxidant enzymes. In contrast, the supplementation of SNP, the source of NO (1.0 mM) suppressed the superoxide anions synthesis but enhanced the activities of antioxidants such as CAT and SOD which suggested that the application of SNP at moderate concentration protected plants from oxidative damage under salt stress (Dong *et al.*, 2014).

## Conclusions

Overall, salinity stress caused high reductions in plant dry matter and  $F_v/F_m$ , but increased proline content and leaf osmolality in both maize cultivars. These suppressions were greater in the salt sensitive Apex 836 than those in the salt tolerant DK 5783. Application of NO and TA in combination as presowing seed treatment or foliar application were effective in mitigating salinity-induced suppression in shoot growth with the exception of foliar application of NO and TA (6+125 mg/l) being not effective for cv. DK 5783. Seed treatment with both levels of combined NO and TA was more effective in improving dry weights than that with foliar treatments. Salt stress increased leaf  $\text{Na}^+$  contents, but reduced those of  $\text{K}^+$  and  $\text{Ca}^{2+}$  in both cultivars. Both modes and combined application of NO and TA increased tissue  $\text{Ca}^{2+}$  and  $\text{K}^+$  contents, whereas reduced  $\text{Na}^+$  in the salt stressed maize plants. Salt stress caused oxidative stress in plants by generating the accumulation of ROS such as MDA and  $\text{H}_2\text{O}_2$ .



In both cultivars increased activities of POD, CAT and SOD were observed under saline regime. Externally-applied NO and TA in combination induced growth improvement in maize was ascribed to reduced leaf  $\text{Na}^+$ , osmolality, MDA and  $\text{H}_2\text{O}_2$  levels, and modulated activities of CAT, SOD and POD as well as increased  $F_v/F_m$  under saline conditions.

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