

1-1-2020

Thiamine-induced nitric oxide improves tolerance to boron toxicity in pepper plants by enhancing antioxidants

CENGİZ KAYA

MUSTAFA ASLAN

FERHAT UĞURLAR

MUHAMMAD ASHRAF

Follow this and additional works at: <https://journals.tubitak.gov.tr/agriculture>



Part of the [Agriculture Commons](#), and the [Forest Sciences Commons](#)

Recommended Citation

KAYA, CENGİZ; ASLAN, MUSTAFA; UĞURLAR, FERHAT; and ASHRAF, MUHAMMAD (2020) "Thiamine-induced nitric oxide improves tolerance to boron toxicity in pepper plants by enhancing antioxidants," *Turkish Journal of Agriculture and Forestry*. Vol. 44: No. 4, Article 6. <https://doi.org/10.3906/tar-1909-40>
Available at: <https://journals.tubitak.gov.tr/agriculture/vol44/iss4/6>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Agriculture and Forestry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Thiamine-induced nitric oxide improves tolerance to boron toxicity in pepper plants by enhancing antioxidants

Cengiz KAYA^{1*}, Mustafa ASLAN², Ferhat UĞURLAR¹, Muhammad ASHRAF³

¹Department of Soil Science and Plant Nutrition, Agriculture Faculty, Harran University, Şanlıurfa, Turkey

²Department of Biology, Education Faculty, Harran University, Şanlıurfa, Turkey

³University of Agriculture Faisalabad, Pakistan

Received: 10.09.2019 • Accepted/Published Online: 17.02.2020 • Final Version: 02.08.2020

Abstract: The role of thiamine (THI)-induced nitric oxide (NO) synthesis in the improvement of tolerance to boron (B) toxicity in pepper (*Capsicum annuum* L.) was studied. A solution of THI (50 or 100 mg L⁻¹) was sprayed to the foliage of pepper plants exposed to high B regime (2.0 mM H₃BO₃) once a week for 4 weeks. A scavenger of NO (0.1 mM), cPTIO, along with THI was sprayed once a week to ascertain whether or not endogenous NO played a role in the alleviation of B stress in pepper plants by THI. There were significant reductions in plant dry weight, PSII quantum efficiency (F_v/F_m), total chlorophyll, leaf calcium (Ca²⁺) and potassium (K⁺) contents as well as leaf water potential by 47.57%, 28.78%, 34.42%, 45.45%, 55.53%, and 471.4%, respectively, but there was a significant increase in the leaf proline, ascorbate (AsA), glutathione (GSH), hydrogen peroxide, malondialdehyde, electron leakage, B levels and NO by 2.6-, 2.3-, 1.9, 3.7-, 5.6-, 2.2-, 3.6-, and 3.0-fold, respectively in B-stressed plants. Both treatments of THI mitigated B-induced oxidative damage and led to a further increase in NO synthesis. The positive effect of THI on plants grown under B stress was fully eliminated by the cPTIO application by lowering leaf NO content. These findings exhibit that NO may function as a downstream signal in THI-induced tolerance to B toxicity in pepper plants by reversing oxidative stress, enhancing the antioxidant defense mechanism and sustaining mineral nutrient homeostasis. Thus, NO and THI both contributed to improved B toxicity tolerance in pepper plants.

Key words: Antioxidant system, boron toxicity, nitric oxide, pepper, thiamine

1. Introduction

Sweet pepper is commonly grown in dry areas where the soils are enriched with boron (B), so it was chosen as a test crop for the present investigation. It is moderately sensitive to B toxicity (Ayers and Westcot, 1985), so B toxicity can damage it when the concentration of B in soil solution exceeds 1–2 mg L⁻¹ (Yermiyahu et al., 2008). Since B is needed at very low quantities for plants, it is regarded as a micronutrient (Fang et al., 2016). Conversely, its levels are reasonably high in arid and semiarid areas wherein precipitation and irrigation practices are not adequate (Pardossi et al., 2015). Furthermore, use of water containing high B for irrigation purpose is another possible cause of B toxicity in plants (Wakeel et al., 2018). Boron deficiency is often easy to counteract by using fertilizers enriched with B, but boron toxicity seems to be a relatively more difficult issue because the harmful effects of high B levels on plants, especially in areas where annual rainfall is less than 550 mm, cannot be reversed (McDonald et al., 2010; Wang et al., 2014). Boron toxicity causes some of the characteristic symptoms on plants, such as restriction of

plant growth and induction of chlorosis due to inhibition of chlorophyll synthesis (Nali et al., 2015). It also leads to deprivation of lipids, increased membrane leakage, reduced photosynthesis, and impaired regulation of oxidative defense mechanisms (Mesquita et al., 2016; Shah et al., 2017; Kaya et al., 2018a).

Boron toxicity could be relieved by using bio-promoters such as thiamine (THI) (Yusof et al., 2015). Thiamine is involved in all living beings since it participates in a number of vital events including the synthesis of carbohydrates, ATP, NADPH, and nucleic acids (Nosaka 2006). Thiamine is believed to activate defense systems in plants (Bettendorf and Wins, 2013; Yusof et al., 2015) and it improves tolerance to DNA damage induced by stress factors (Tunc-Ozdemir et al., 2009). Furthermore, thiamine was reported to efficiently allay the harmful effects of water stress and saline stress in maize (Rapala-Kozik et al., 2008), reduce oxidative stress induced by low temperature, high light intensity, and osmotic and salt stresses in *Arabidopsis* (Tunc-Ozdemir et al., 2009). In the former investigations, thiamine has been proved to be a

* Correspondence: c_kaya70@yahoo.com

potential biochemical for improving tolerance to stress in plants, so it is proposed that the continuous synthesis of thiamine may improve severe stress tolerance in plants (Ribeiro et al., 2005; Abidin et al., 2016). Although thiamine has a crucial function in alleviating deleterious effects on plants under a stress as mentioned earlier, there appears to be a rare investigation concerning its effects against boron toxicity, so it logically justifies that thiamine should be examined in boron-stressed plants.

Nitric oxide (NO) is reported to control different metabolic processes such as stomatal regulation, photomorphogenesis, leaf senescence, plant resistance, pollination, blossoming, etc. (Misra et al., 2011; Simontacchi et al., 2015; Fancy et al., 2017). NO also contributes to tolerance mechanisms in plants exposed to exceptionally unfavorable environments (Ahmad et al., 2016; Tripathi et al., 2017). As an example, it maintains reactive oxygen species (ROS) at low levels through changing the activities of key antioxidant enzymes (Niu and Guo, 2012; Groß et al., 2013; Ahmad et al., 2016). Various investigations have shown that NO is implicated in several hormonal and biological responses in plants (Hasanuzzaman et al., 2012; Corpas and Palma, 2018), but the likely contribution of NO to thiamine-induced antioxidant defense needs to be elucidated. Thus, this study aimed to assess if thiamine-induced synthesis of NO was implicated in alleviation of B stress in pepper plants.

2. Material and methods

2.1. Plant growth and treatments

Greenhouse experimentation was set up with pepper (*Capsicum annuum* L.) cv. "Semerkand". Before designing the study, the seeds of pepper were sterilized with NaOCl (1%) solution. For germination purpose, 5 seeds were sown in a pot consisting of perlite (5 L in each pot), and then three plants were retained to grow in each pot alone for assessing various traits mentioned below. The plants were irrigated using a nutrient solution (0.5 strength). The composition of NS was outlined in detail by Kaya and Ashraf (2015) and the pH of NS was kept at 5.5. The plants were grown at 11 h light period per day during the entire experimentation period.

The experiment comprised the following treatments: control treatment of nutrient solution only with 0.05 mM B or 2.0 mM (toxic) B. One week after germination, the seedlings were subjected to two B levels. There were two levels of thiamine (THI) (50 or 100 mg L⁻¹) in 0.01% Tween-20; the treatment solutions were sprayed to plant leaves once a week for 4 weeks. To gain insight into if NO participated in mitigation of B stress by THI, an NO scavenger (0.1 mM), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide potassium salt (cPTIO) was sprayed once a week for 4 weeks along with

THI application to plants subjected to nonstressful and stressful regimes. The control plants were supplied with an equal volume of solution with no THI. Depending on plant size, 100–1000 mL of 1/2-strength NS was supplied every other day to each pot. The trial consisted of 3 replications, each having 9 plants.

Four weeks after the start of B supplementation, three plants from each replication were divided into shoot and roots and their fresh weights were noted. The leaves (fully expanded) of the remaining six plants of each replicate (e.g., 18 plants per treatment) were used to appraise different traits mentioned below. For appraising dry weights of the plant samples, they were subjected to 72 °C for 3 days.

After quantifying the fresh mass, the roots and shoots of plants were divided and dried at 75 °C for 24 h to quantify dry mass. The plant growth and physio-biochemical traits described below were determined.

2.2. Chlorophyll determination

A leaf sample (1.0 g) was homogenized in a 90% acetone solution. The mixture was then filtered and absorbance was measured using a spectrophotometer to quantify chlorophyll levels following the method of Strain and Svec (1966).

2.3. Chlorophyll fluorescence measurements

Prior to determining this parameter, the leaf samples were adapted in dark for 30 min and then used to measure the PSII photochemical efficiency (F_v/F_m) using Mini-PAM Photosynthesis Yield Analyzer (Walz, Germany).

2.4. Leaf water potential

Leaf water potential was quantified by using a recently extended leaf of plant by subjecting it to a water potential measurement apparatus (PMS model 600, USA).

2.5. Free proline

Free proline was quantified by employing the ninhydrin protocol outlined by Bates et al. (1973). A fresh leaf tissue (each 500 mg) was macerated in aqueous sulfosalicylic acid (10 mL, 3%) and then the macerated solution was filtrated. An aliquot (2 mL) was treated with 2 mL each of glacial acetic acid and acid ninhydrin. All samples were kept at 80 °C in a water bath for 1 h. Next, to last the reaction, the samples were kept in an ice bath. By adding toluene (4 mL) to the mixture, a mixer was used to mix the solution for 15–20 s. The absorbance readings of all the treated samples were noted at 520 nm.

2.6. Determination of ascorbate (AsA)

The method outlined by Mukherjee and Choudhuri (1983) was employed to appraise AsA. Leaves were homogenized in liquid nitrogen followed by in a cocktail containing dinitrophenyl-hydrazine (2%) and trichloroacetic acid (6%) prepared in ½-strength H₂SO₄ and 10% thiourea dissolved in 70% ethanol. The sample solution was boiled in a water bath for 15 min and then cooled down to 25

°C. After centrifugation at $1000 \times g$ for 10 min at 4 °C, the resultant pellet was liquefied in H_2SO_4 (80%). The absorbance values were noted at 530 nm.

2.7. Glutathione (GSH) assay

The total GSH was quantified employing the method of Ellman (1959). A supernatant of sulfosalicylic acid (3 mL of 4%) was added to 0.5 mL of the extract in phosphate buffer. Following centrifugation of the mixture at $3000 \times g$, a 0.5 mL of sample supernatant was reacted with Ellman's reagent. The absorbance values were recorded at 412 nm after an interval of 10 min.

2.8. Chemical analysis

Determination of the leaf B was performed following the Azomethine-H method outlined by Wolf (1971). Further details are given in Kaya et al. (2019). For the quantification of leaf Ca^{2+} and K^+ , ground leaf samples were heated in a muffle furnace at 500 °C for 6 h. A 5 mL of hot HCl (2 M) were added to the resultant ash, and then the mixture volume was completed to 50 mL by adding deionized water. An ICP was used to read the samples for the quantification of Ca^{2+} and K^+ (Chapman and Pratt, 1982).

2.9. Analysis of nitric oxide (NO)

Leaf NO content was quantified following the protocol outlined by Zhou et al. (2005) with slightly modified. Following homogenising fresh leaf material (0.6 g) in 50 mM cold acetic acid buffer (3 mL) at pH 3.6 containing 4% zinc diacetate, the mixture was centrifuged at $10,000 \times g$ at 4 °C for 15 min. The collected aliquots were mixed followed by adding 100 mg of charcoal to each sample. After the samples were filtered and vortexed, the mixture (1 mL) and the Greiss reagent (1 mL) at room temperature were added. The absorbance readings were noted at 540 nm.

2.10. Hydrogen peroxide (H_2O_2)

The method outlined by Velikova et al. (2000) was followed to appraise leaf H_2O_2 content. A fresh leaf material (each 500 mg) was extracted in 3 mL of TCA (1% w/v). After centrifuging each sample, an aliquot of 0.75 mL of the sample was added to 0.75 mL of K buffer (10 mM) and 1.5 mL of KI (1 M). The absorbance readings were recorded at 390 nm.

2.11. Leaf malondialdehyde (MDA)

The method depicted by Weisany et al. (2012) was employed to quantify leaf MDA, a lipid peroxidation product.

2.12. Electrolyte leakage (EL)

The EL was appraised by employing the detailed procedure outlined by Dionisio-Sese and Tobita (1998). Detailed information on the procedure used can be found in Kaya and Ashraf (2015).

2.13. Leaf soluble proteins

Leaf soluble protein was appraised adopting the procedure reported by Bradford (1976). Fresh leaf material (each 500 mg) was homogenised in the solution of phosphate buffer (0.2 mM) at pH 6.2. The homogenized samples were then centrifugated at $2000 \times g$ for 10 min. The volume of the aliquot was brought up to 10 mL by adding the phosphate buffer solution. A supernatant of the Coomassie Brilliant blue reagent (5 mL) was added to the solution of sample (1 mL). It was then shaken in a vortex mixer for 30 s. The absorbance values were noted at 595 nm.

2.14. Activities of antioxidant enzymes

A proportion of fresh leaf (500 mg) was homogenized in Na-P buffer (50 mM) containing soluble polyvinyl pyrrolidone (1%). The solution so extracted was centrifuged at 4 °C at $20,000 \times g$ for 15 min. The CAT activity was appraised following Kraus and Fletcher (1994), the SOD activity by Van Rossum et al. (1997), and that of POD by Chance and Maehly (1955).

2.15. Statistical analysis

The data for various traits were subjected to a two-way analysis of variance using the statistical package, CoStat program (Version v6.303). The Duncan's Multiple Range test at 5% probability was adopted to assess if the means varied significantly from one another.

3. Results

When pepper plants were exposed to B toxicity (BT), substantial decreases were found in dry mass, chlorophyll contents, Fv/Fm and Ψl of the plants ($P \leq 0.05$) by 47.6%, 34.4%, 28.8%, and 471.4%, respectively in comparison with those in the nonstressed plants (Table; Figures 1A–1D). Treatments of THI, 50 and 100 mg L^{-1} , considerably ($P \leq 0.05$) improved total dry weight by 50.1% and 57.0%, chlorophyll content by 52.5% and 56.3%, Fv/Fm by 21.4% and 33.9%, Ψl by 21.7% and 33.0%, respectively in comparison with those in BT plants.

To understand the possible involvement of endogenous NO in THI-improved B toxicity stress tolerance in pepper plants, cPTIO, a scavenger of NO, was sprayed together with THI to pepper plants under BT stress. The use of cPTIO plus THI completely overturned the benefit effects of THI on the traits mentioned above. These findings obviously provide a strong evidence that NO was participated in THI-improved BT tolerance in pepper plants.

Leaf potassium (K^+) and calcium (Ca^{2+}) levels considerably ($P \leq 0.05$) decreased (Figures 2A and 2B), but boron (B) content (Table; Figure 2C) increased in the boron-stressed plants. However, THI supply significantly ($P \leq 0.05$) increased leaf K^+ and Ca^{2+} contents, but reduced leaf B in the boron-stressed plants. When cPTIO along with THI was applied to the BT-stressed plants, the positive effects of THI applications on these parameters were reversed, and this suggests that lowering NO in the

Table. Two-way completely randomized analyses of variance (ANOVA) of the data (mean squares) for growth and different biochemical attributes of pepper plants.

Source of variations	df	Plant dry weight	Total Chl.	F_v/F_m	Leaf water potential	Leaf K^+	Leaf Ca
BS	1	221***	2.54***	0.203***	9.52***	750***	295***
T	4	7.23ns	0.57ns	0.013ns	0.106**	48.4ns	9.12**
BS x T	4	6.63ns	0.98*	0.012ns	0.090*	3.07*	9.31**
Error	20	2.708	0.28	0.007	0.014	7.947	1.89
		Leaf B	Proline	AsA	GSH	H₂O₂	MDA
BS	1	47600***	395***	64.9***	4.3***	6077***	3141***
T	4	818***	7.0ns	9.7**	0.28ns	198.2**	172***
BS x T	4	973***	6.8ns	14.3***	0.30ns	165.4**	110**
Error	20	99.0	4.2	1.57	0.12	34.0	20.0

BS: Boron stress; T: Treatments; ns = nonsignificant; *, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively.

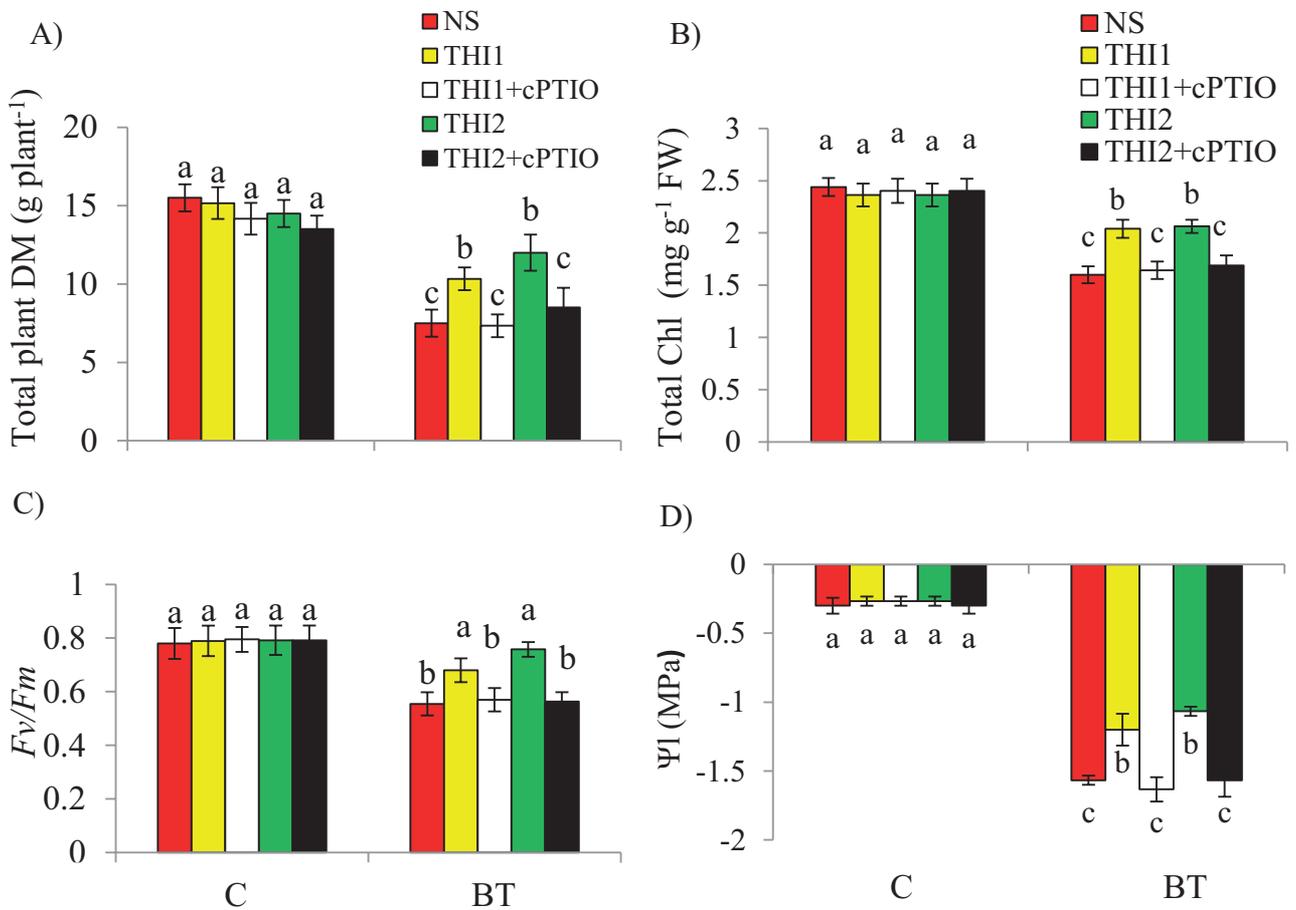


Figure 1. Total plant dry matter [DM; A)], total chlorophyll on fresh weigh (FW) basis (B), maximum fluorescence yield [F_v/F_m ; C)] and leaf water potential [Ψ_l ; D)] in the leaves of pepper plants grown under control (C) and boron toxicity (BT) sprayed with thiamine (THI1: 50 and THI2: 100 mg L⁻¹) combined with 0.1 mM scavenger of NO, cPTIO, or nonsprayed (NS). (Mean \pm SE). Mean values carrying different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

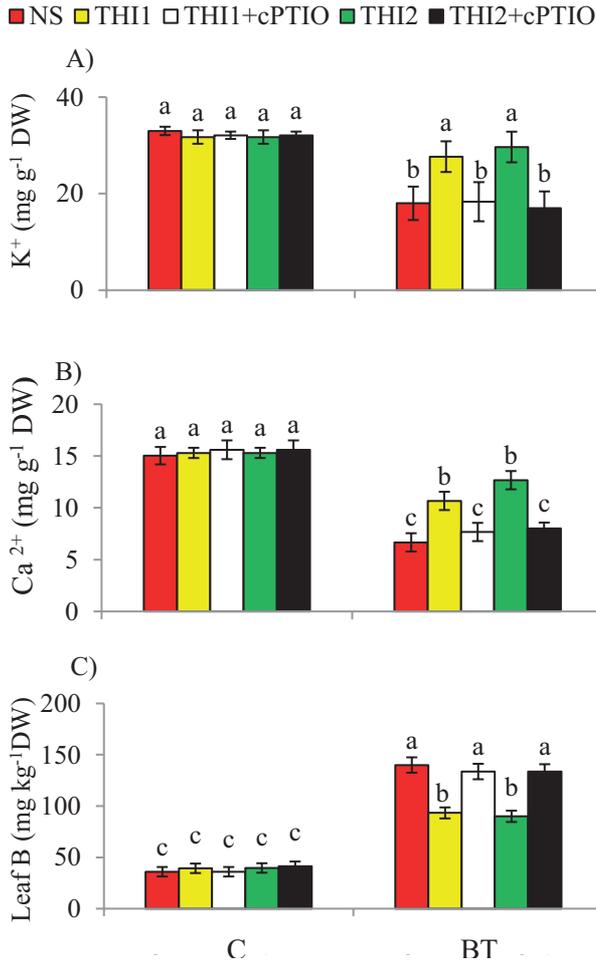


Figure 2. Leaf potassium [K⁺; A), calcium [Ca²⁺; B) and boron [B; C) contents on dry weight (DW) basis in the leaves of pepper plants grown under control (C) and boron toxicity (BT) sprayed with thiamine (THI1: 50 and THI2: 100 mg L⁻¹) combined with 0.1 mM scavenger of NO, cPTIO, or nonsprayed (NS). (Mean ± SE). Mean values carrying different letters within each parameter differ significantly (P ≤ 0.05) based on Duncan's multiple range test.

leaves of plants possibly caused THI applications being not effective in increasing leaf K⁺ and Ca²⁺ contents and decreasing leaf B in the plants subjected BT stress.

Leaf proline content, ascorbate (AsA), and glutathione (GSH) increased significantly (P ≤ 0.05) under BT stress, but they all were reduced due to THI supply (Table; Figures 3A–3C). When BT-stressed plants were sprayed along with cPTIO, the effects of THI applications on these traits were reversed, suggesting that when NO was possibly blocked, THI applications were not effective in reducing these traits in the plants under BT stress.

The overgeneration of hydrogen peroxide and malondialdehyde as well as electrolyte leakage were

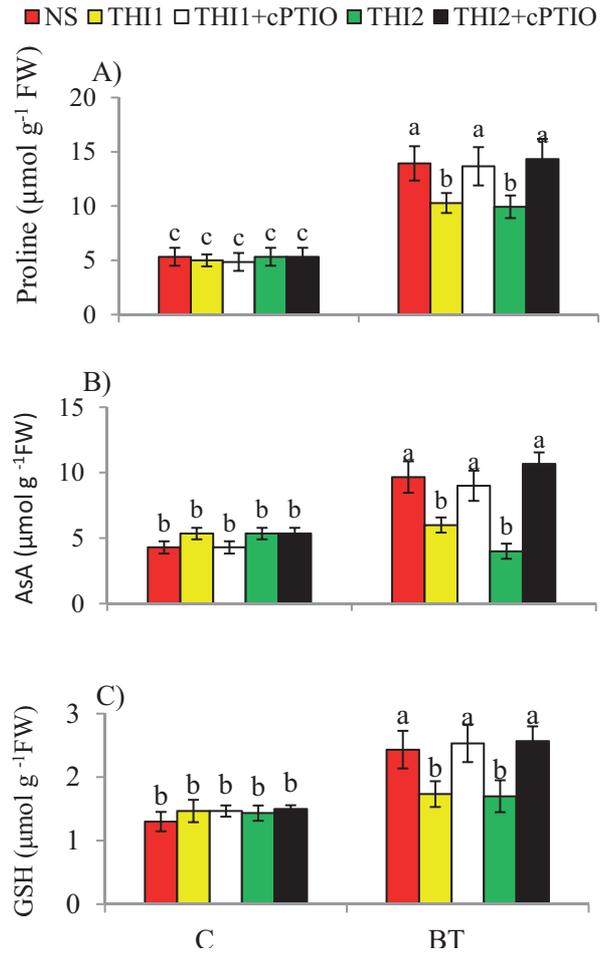


Figure 3. Leaf proline (A), ascorbate [AsA; (B) and glutathione content [GSH; (C) on fresh weight (FW) basis in the leaves of pepper plants grown under control (C) and boron toxicity (BT) sprayed with thiamine (THI1: 50 and THI2: 100 mg L⁻¹) combined with 0.1 mM scavenger of NO, cPTIO, or nonsprayed (NS). (Mean ± SE). Mean values carrying different letters within each parameter differ significantly (P ≤ 0.05) based on Duncan's multiple range test.

significantly augmented in pepper plants under BT stress (Figures 4A–4C). Both THI treatments relieved BT-induced the oxidative stress by significantly reducing oxidative stress parameters, but supplementation of cPTIO completely reversed the alleviation effects of the THI treatments. When endogenous NO was blocked by using cPTIO, THI alone was not effective in reducing the oxidative stress. Thus, these findings further reveal that NO and THI might jointly play a vital role in improving BT stress tolerance of pepper plants due to THI application.

Boron stress considerably (P ≤ 0.05) elevated the endogenous NO in the leaves of pepper plants (Figure 4D). The treatments of THI, 50 and 100 mg L⁻¹, led to further

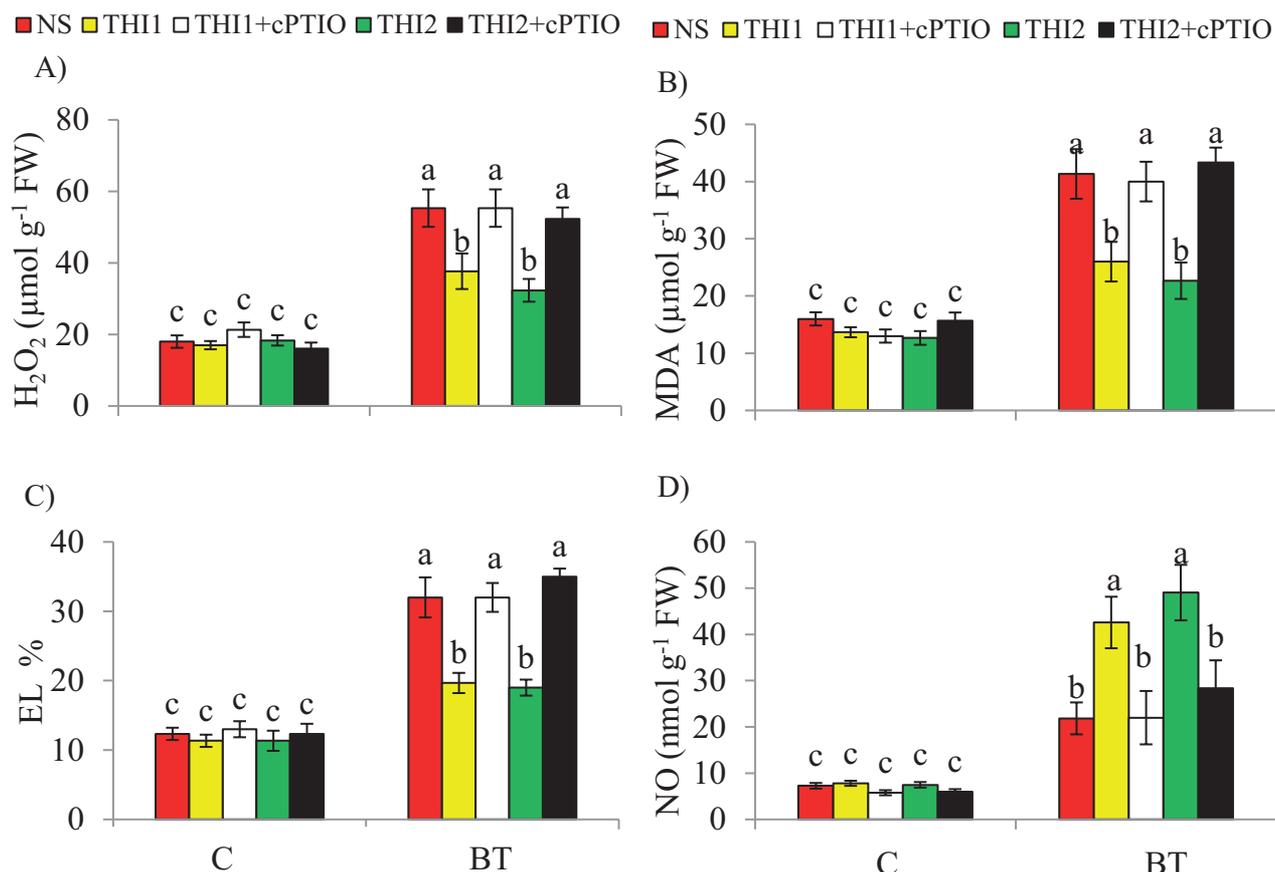


Figure 4. Hydrogen peroxide [H₂O₂; A)], malondialdehyde [MDA; B)], electrolyte leakage [EL; C)] and nitric oxide [NO; D)] on fresh weight (FW) basis in the leaves of pepper grown under control (C) and boron toxicity (BT) sprayed with thiamine (THI1: 50 and THI2: 100 mg L⁻¹) combined with 0.1 mM scavenger of NO, cPTIO, or nonsprayed (NS). (Mean ± SE). Mean values carrying different letters within each parameter differ significantly (P ≤ 0.05) based on Duncan's multiple range test.

increases significantly (P ≤ 0.05) in this metabolite in the leaves of pepper plants under BT condition relative to those in BT-stressed plants alone. The significant increases in endogenous NO induced by THI treatments were reversed totally with the supply of cPTIO. These results indicate that THI triggers NO in the leaves and so NO may be a downstream signal produced by THI.

Antioxidant defense system which might be induced by THI in the BT-stressed plants was investigated and it was also proposed to get an insight into whether THI was involved in endogenous NO-triggered antioxidant system under BT stress. The results clearly indicate that BT stress considerably (P ≤ 0.05) augmented the activities of antioxidant enzyme (SOD, POD, and CAT) (Figures 5A–5C). The treatments of THI led to additional elevations in the activities of all these enzymes. However, combining cPTIO with THI completely reduced these enzyme activities. These results reveal that both endogenous NO and THI are jointly responsible in regulating the antioxidant defense system in pepper plants under BT stress.

4. Discussion

The results of many investigations show that although B is needed in a small amount for higher plants, it can be toxic at high concentration in a growing medium (Kot 2008; Kayihan et al., 2017). An obvious decline in plant biomass under BT could be due to the harmful effects of B toxicity on physiological processes and mineral acquisition (Metwally et al., 2018). The possible reason of increased plant growth by the THI in the present experimentation might have been that THI is needed for division of meristematic stem cells and organ primary cells (Martinis et al., 2016). Furthermore, the accumulation of some osmo-regulators, e.g., free soluble sugars and amino acids in the THI-treated plants can enhance the water status of plants thereby raising the turgor pressure, required for cell expansion and plant growth (Sayed and Gadallah, 2002). Thiamine has been tested in various stress situations, e.g., under salinity and water stress in *Arabidopsis* (Rapala-Kozik et al., 2012), under salt stress in maize (Kaya et al., 2015), under drought stress in white clover (Ghaffar et al.,

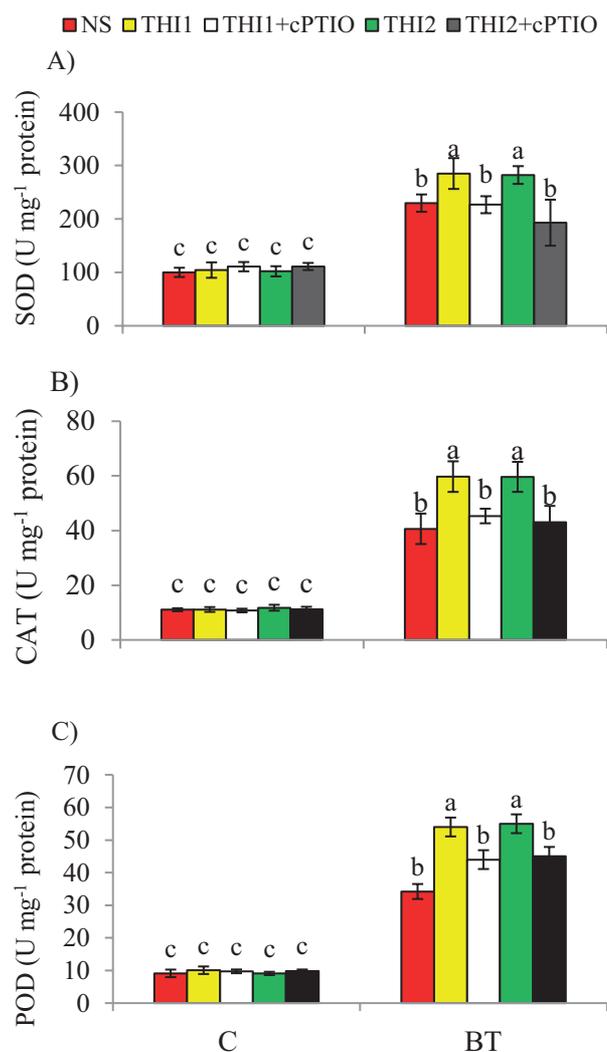


Figure 5. Activities of superoxide dismutase [SOD; A)], catalase [CAT; B)], and peroxidase [POD; C)] in the leaves of pepper plants grown under control (C) and boron toxicity (BT) sprayed with thiamine (THI1: 50 and THI2: 100 mg L⁻¹) combined with 0.1 mM scavenger of NO, cPTIO, or nonsprayed (NS). (Mean ± SE). Mean values carrying different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

2019), and under osmotic, oxidative and salt stresses in oil palm (Abidin et al., 2016). However, a rare report seems to be available in the literature on the effect of thiamine tested on the B-stressed plants. Thus, one of the priorities of our investigation was to assess its possible positive effects on plants under B toxicity stress. The levels of THI (50 or 100 mg L⁻¹) chosen for foliar spray to maize plants were already used for the same crop in a previous study (Kaya et al., 2015). The allaying effect of THI on BT-induced suppression in plant growth is likely due to reduced leaf B content, and enhanced K⁺ and Ca²⁺ contents, chlorophyll

content and *Fv/Fm*, which were otherwise suppressed by BT stress. In the present investigation, plant dry matter was nonlinearly correlated ($P \leq 0.001$) with leaf B content ($r = -0.701$) (Figure 6A), but linearly correlated ($P \leq 0.001$) with leaf K⁺ and Ca²⁺ content, and chlorophyll content ($r = 0.850$, $r = 0.868$, and $r = 0.744$, respectively) (Figures 6B–6D). These results showed that THI might have participated in the response of the pepper plants to BT, as already proposed by Kaya et al. (2015) in salinity-stressed maize plants.

Although NO acts as a signaling molecule in the response of plants to a variety of stressful environments (Singh et al., 2008; Panda et al., 2011), there is no sufficient information in the literature on the interaction between NO metabolism and BT-induced stress. An elevated NO content in pepper plants under BT stress was recorded, as previously shown for other plants subjected to growth conditions supplemented with various heavy metals (Yuan and Huang, 2016; Farag et al., 2017). Therefore, these findings propose that NO could effectively participate in some key metabolic phenomena in plants subjected to boron toxicity. Moreover, THI treatments caused a further elevation in endogenous NO synthesis in the plants under BT stress. Thus, it is soundly promising that the treatment of THI may induce endogenous NO synthesis, which may be involved in improving stress tolerance of plants as an antioxidant. However, it is believed that oversynthesis of NO can cause damaging effects in plants (da-Silva et al., 2018). Therefore, an optimum level of cellular NO is needed for mitigating the deleterious effects of a stress. In the present experiment, the levels of endogenous NO induced by THI treatments were not higher than the threshold level for pepper plants; thus, its destructive effects on physiological processes could be expected. There seems to be no report relating to the interaction of THI with NO in the response of plants to a stress in the available literature. Thus, this study provides a novel insight into our understanding in this respect. Nitric oxide could be a downstream signal molecule induced by THI in enhancing tolerance to boron toxicity in pepper plants. A similar suggestion was put forth by Liu et al. (2015) who proposed that NO might be a downstream signal of melatonin in the improvement of tomato tolerance to alkaline conditions. On the other hand, when NO synthesis was scavenged by treating plants with cPTIO, the alleviation effects of THI were reverted thereby resulting in reduced leaf NO content.

Environmental stress is widely believed to deleteriously affect chlorophyll synthesis (Kalaji et al. 2016), but during the mechanism of photosynthesis, the chlorophylls are required in a large amount to absorb a considerable amount of light to expedite the shifting of electrons to the reaction center. Similarly, *Fv/Fm* is known as an important indicator of assessing photo-oxidative outcome

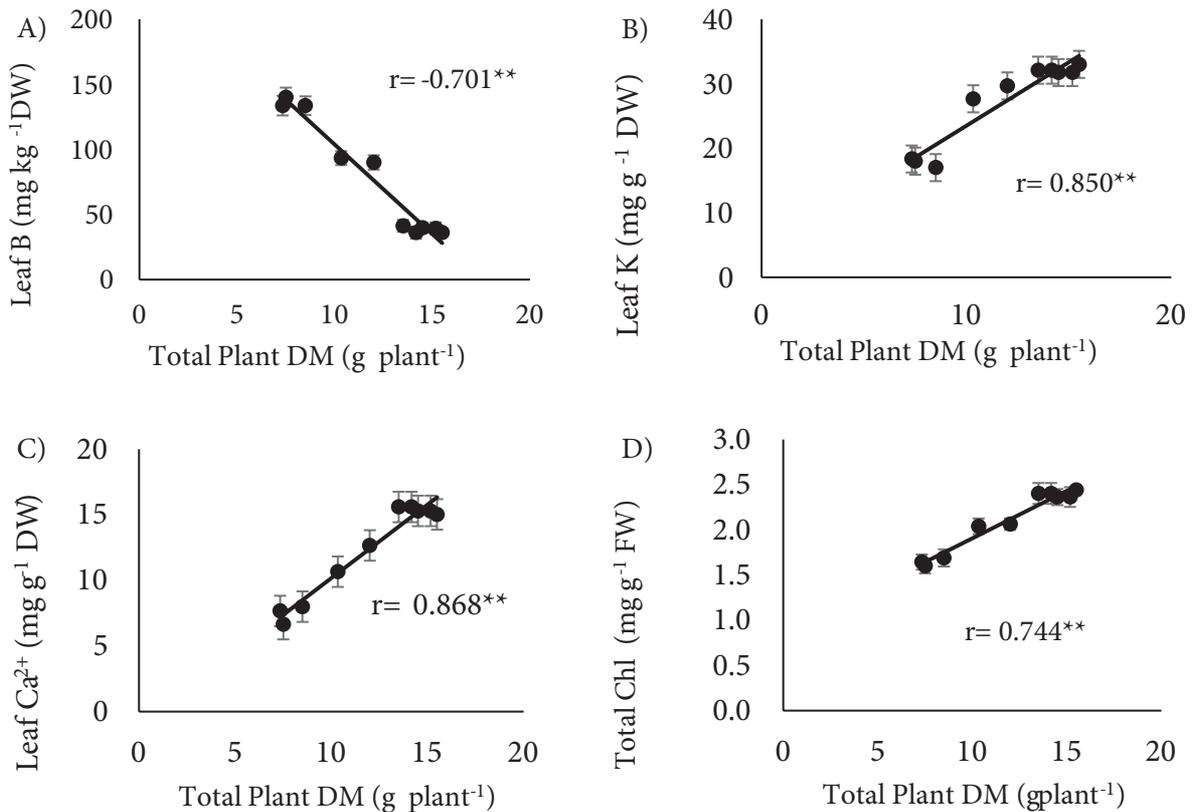


Figure 6. Correlation of total plant dry mater (DM) with leaf B (A), potassium [K⁺; B]) and calcium [Ca²⁺; C]) contents on dry weight (DW) basis, and total chlorophyll (D) content on fresh weight (FW) basis in the leaves of pepper plants under boron toxicity, supplied with thiamine and sprayed with or without 0.1 mM scavenger of NO, cPTIO, **: Correlation is significant (P ≤ 0.001)

of photosystem II (Wang et al., 2013). In our study, the treatments of THI enhanced chlorophyll levels and *Fv/Fm* of pepper plants under BT stress (Figures 1B and 1C). In an earlier study, Soltani et al. (2014) noted that exogenously supplied THI improved the chlorophyll contents in marigold plant. Thiamine is known to scavenge reactive oxygen species, thereby improving the chlorophyll content (Mady, 2009; Kaya et al., 2018b). The present results additionally show that the overproduction of H₂O₂ within the leaves of pepper plants subjected to BT could be connected to decreased chlorophyll level. Thus, it can be inferred that THI is efficiently involved in alleviating the injurious effect of BT on chlorophyll synthesis, likely by reducing the production of H₂O₂. An alternative reason for improvement of tolerance of pepper plants to BT could be because of the role of THI in improving the antioxidant defense mechanism to eliminate H₂O₂, thereby elevating the levels of chlorophyll. This statement was similarly verified in our study where THI applications further improved SOD, CAT, and POD, and chlorophyll levels. In view of the available literature, it is likely that thiamine has a defensive effect on the D1 protein of PSII; therefore, it

might have resulted in increased PSII activity in pepper plants under BT as previously reported in wheat treated with ascorbic acid (Athar et al., 2008).

It has been reported that BT can disturb water uptake by affecting the water flow (Wimmer and Eichert, 2013). Severe deterioration of the xylem vessels can decrease the flow of water to plant shoots (Wimmer and Eichert, 2013). An explanation of greater leaf water potential (Ψ) by THI treatment might have been that application of thiamine lowered the osmotic stress by accumulating proline, thereby enhancing leaf water potential of the pepper plants under BT stress, as shown in the present experiment. Similar ameliorating effects of THI treatment on leaf water and proline contents have already been reported in sunflower (Sayed and Gadallah, 2002), and maize (Tuna et al., 2013) under saline stress.

It has been well documented that ascorbate (AsA) and glutathione (GSH) are the nonenzymatic substances which scavenge the ROS including H₂O₂ to mitigate oxidative stress induced by boron stress (Metwally et al., 2018). Similarly, the results of the present study clearly show that the leaf AsA and GSH levels were increased in the plants

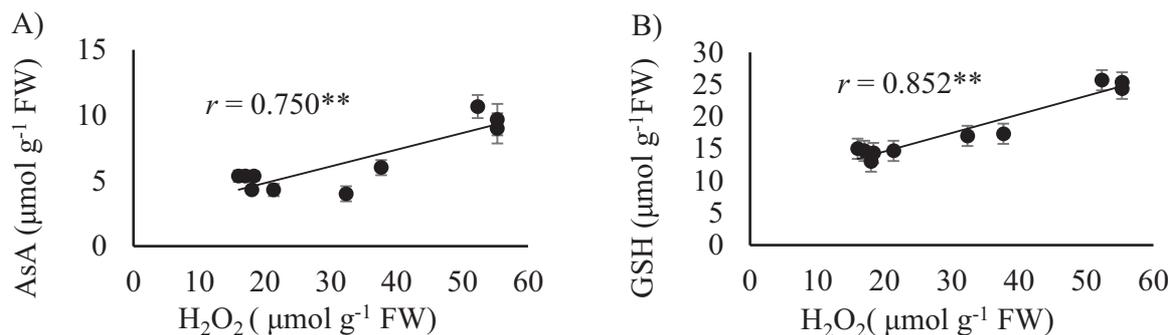


Figure 7. Correlation of hydrogen peroxide (H₂O₂) with ascorbate [AsA; (A)] and glutathione [GSH; (B)] on fresh weight (FW) basis of pepper plants under boron toxicity supplied with thiamine and sprayed with or without 0.1 mM scavenger of NO, cPTIO, ** and *: Correlation is significant at ($P \leq 0.001$) and ($P \leq 0.05$), respectively.

under BT stress, wherein H₂O₂ content was also increased. The correlation coefficients indicate that leaf H₂O₂ content is significantly and linearly correlated with ASA and GSH (Figures 7A and 7B). When pepper plants were exposed to BT stress, they also produced ASA and GSH to ameliorate B-induced oxidative stress.

Boron stress has been reported to decrease Ca²⁺ contents in apple (Mouhtaridou et al., 2004) and K⁺ and Ca²⁺ contents in sweet pepper (Piñero et al., 2017) as similarly shown in our experiment. This could have been due to the decrease in the uptake of nutrients mediated by reduced transpiration (Piñero et al., 2017). THI-induced improvement in tolerance to B toxicity of the pepper plants could be related to decreased B levels and increased leaf K⁺ and Ca²⁺. In the present study, it could be inferred that THI treatments enhanced the nutrient concentrations by improving plant growth, thereby improving nutrients' uptake from the growing medium.

Boron toxicity caused significant increases in H₂O₂, MDA, and EL in the pepper plants. The present investigation proposes that B toxicity led to an injurious effect on membrane integrity; thus, membrane damage was increased. Oxidative impairment and membrane peroxidation were the most usual symptoms appearing in plants under B stress (Wang et al., 2010; Pandey, 2013). Earlier studies have shown that B stress triggers H₂O₂ and superoxide radicals in tomato plants (Cervilla et al., 2009). These alterations might disturb consistent ion exchange capability of plasma membrane and the whole metabolic events linked with cell membrane functions (Wang et al., 2016; Moussa and Algamal, 2017). The redox potential of the cell can be impaired by over-accumulation of H₂O₂ and this leads to the elevations of antioxidants and the reinforcement of antioxidant defense system (Hassanein et al., 2015). However, THI treatments significantly mitigated BT-induced oxidative impairments by lowering H₂O₂ accumulation, MDA levels, and EL.

Plants evolve a strategy to improve their ability to protect plant cells against the damage and dysfunctions caused by heavy metal contaminated soil by triggering antioxidant enzyme activities including SOD, CAT, and POD to scavenge H₂O₂ (Sharma and Dubey, 2005). Furthermore, in the present experiment, THI enhanced the antioxidant defense system in the pepper plants under B toxicity which was evident by lowered H₂O₂ generation and lipid peroxidation, and improved chlorophyll content and antioxidant defense system. Blocking the NO synthesis induced by THI caused THI to be ineffective in plants grown under B toxicity. This further proves that the alleviating effect of THI on oxidative damage caused by BT stress is NO-dependent.

5. Conclusion

Overall, thiamine-triggered nitric oxide was found to participate in improving tolerance to B toxicity in pepper plants. The results suggest that NO could be a downstream signaling molecule induced by THI in pepper plants under B toxicity. When NO was scavenged using cPTIO, THI alone was not effective in improving tolerance to B toxicity. Thus, externally applied THI and endogenous NO are both jointly responsible for improved tolerance to B toxicity in pepper plants. Furthermore, endogenous NO induced by THI might be a key metabolite which improves antioxidant defense system to reduce oxidative damage in pepper plants under B toxicity. On the other hand, we need to further investigate how far the molecular network works in the case of THI, which increases tolerance of plants to B toxicity. Additional studies are still needed to investigate the role of other signaling molecules in response of plants to BT stress induced by THI.

Acknowledgment

The support of Harran University (HUBAK-19011) to this study is thankfully acknowledged.

References

- Abidin AAZ, Wong S, Rahman NSA, Idris ZHC, Balia Yusof ZN (2016). Osmotic, oxidative and salinity stresses upregulate the expressions of thiamine (Vitamin B1) biosynthesis genes (THIC and THI1/THI4) in oil palm (*Elaeis guineensis*). Journal of Oil Palm Research 28: 308-319.
- Ahmad P, Abdel Latef AA, Hashem A, Abd-Allah EF, Gucl S et al. (2016). Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. Frontiers in Plant Science 7: 347.
- Athar HR, Khan A, Ashraf M (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environmental and Experimental Botany 63: 224-231.
- Ayers R, Westcot W (1985). Water quality for agriculture. Irrigation and Drainage Paper No. 29. FAO, Rome.
- Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207
- Bettendorff L, Wins P (2013). Thiamine triphosphatase and the CYTH superfamily of proteins. FEBS Journal 280: 6443-6455.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of micro gram quantities of protein utilizing the principle of protein-dye binding Analytical Biochemistry 72: 248-254.
- Cervilla LM, Rosales MA, Rubio-Wilhelmi MM, Sánchez-Rodríguez E, Blasco B et al. (2009). Involvement of lignification and membrane permeability in the tomato root response to boron toxicity. Plant Science 176: 545-552
- Chance B, Maehly C (1955) Assay of catalase and peroxidases Methods in Enzymology 2: 764-775.
- Chapman HD, Pratt PF (1982). Methods of Plant Analysis I Methods of Analysis for Soils, Plants and Water Chapman Publishers, Riverside, California
- Corpas FJ, Palma JM (2018). Assessing nitric oxide (NO) in higher plants: an outline. *Nitrogen 1*: 12-20.
- da-Silva CJ, Canatto RA, Cardoso AA, Ribeiro C, de Oliveira JA (2018). Oxidative stress triggered by arsenic in a tropical macrophyte is alleviated by endogenous and exogenous nitric oxide. Brazilian Journal of Botany 41: 21-28.
- Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress Plant Science 135: 1-9.
- Ellman GL (1959). Tissue sulfhydryl groups. Archives Biochemistry and Biophysics 82: 70-77.
- Fancy NN, Bahlmann AK, Loake GJ (2017). Nitric oxide function in plant abiotic stress. Plant Cell Environment 40: 462-472.
- Fang K, Zhang W, Xing Y, Zhang Q, Yang L et al. (2016). Boron toxicity causes multiple effects on *Malus domestica* pollen tube growth. Frontiers in Plant Science 7: 208.
- Farag M, Najeeb U, Yang J, Hu Z, Fang ZM (2017). Nitric oxide protects carbon assimilation process of watermelon from boron-induced oxidative injury. Plant Physiology and Biochemistry 111: 166-173.
- Ghaffar A, Akram NA, Ashraf M, Ashraf Y, Sadiq M (2019). Thiamin-induced variations in oxidative defense processes in white clover (*Trifolium repens* L.) under water deficit stress. Turkish Journal of Botany 43: 58-66.
- Groß F, Durner J, Gaupels F (2013). Nitric oxide, antioxidants and prooxidants in plant defence responses. Frontiers in Plant Science 4: 419
- Hassanein RA, Amin AAE, Rashad EM, Ali H (2015). Effect of thiourea and salicylic acid on antioxidant defense of wheat plants under drought stress. International Journal of Chemistry Technical Research 7: 346-354.
- Hasanuzzaman M, Nahar K, Alam MM, Fujita M (2012). Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. Australian Journal Crop Science 6: 1314-1323.
- Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M et al. (2016). Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiologia Plantarum 38: 102
- Kaya C, Ashraf M (2015) Exogenous application of nitric oxide promotes growth and oxidative defense system in highly boron stressed tomato plants bearing fruit. Sci Hortic 185: 43-47
- Kaya C, Ashraf M, Sonmez O, Tuna AL, Polat T et al. (2015). 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. Acta Physiologia Plantarum 37: 1741.
- Kaya C, Akram NA, Ashraf M (2018a). Kinetin and Indole Acetic Acid Promote Antioxidant Defense System and Reduce Oxidative Stress in Maize (*Zea mays* L.) Plants Grown at Boron Toxicity. Journal of Plant Growth Regulation 37 (4): 1258-1266.
- Kaya C, Ashraf M, Sonmez O (2018b). Combination of nitric Oxide and thiamine regulates oxidative defense machinery and key physiological parameters in salt-stressed plants of two maize cultivars differing in salinity tolerance. Advances in Agricultural Science 6: 34-44.
- Kaya C, Sarioğlu A, Akram NA, Ashraf M (2019). Thiourea-mediated nitric oxide production enhances tolerance to boron toxicity by reducing oxidative stress in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) plants. Journal of Plant Growth Regulation 1-16. doi: 10.1007/s00344-019-09916-x
- Kayihan C, Öz MT, Eyidoğan F, Yücel M, Öktem HA (2017). Physiological, biochemical, and transcriptomic responses to boron toxicity in leaf and root tissues of contrasting wheat cultivars. Plant Molecular Biology Reports 35: 97-109.
- Kot SF (2008). Boron sources, speciation and its potential impact on health. Reviews in Environmental Science and Bio/Technology 8: 3-28.
- Kraus TE, Fletcher RA (1994). Paclobutrazol protects wheat seedlings from heat and paraquat injury Is detoxification of active oxygen involved? Plant Cell Physiology 35: 45-52

- Liu N, Gong B, Jin Z, Wang X, Wei M et al. (2015). Sodic alkaline stress mitigation by exogenous melatonin in tomato needs nitric oxide as a downstream signal. *Journal of Plant Physiology* 186: 68-77
- Mady MA (2009). Effect of foliar application with salicylic acid and vitamin E on growth and productivity of tomato (*Lycopersicon esculentum*, Mill.) plant. *Journal of Agricultural Sciences, Mansoura University* 34 (6): 6735-6746.
- Martinis J, Gas-Pascual E, Szydłowski N, Crèvecoeur M, Gisler A et al. (2016) Long-distance transport of thiamine (vitamin B1) is concomitant with that of polyamines. *Plant Physiology* 171: 542-553.
- McDonald GK, Eglinton JK, Barr AR (2010). Assessment of the agronomic value of QTL on chromosomes 2H and 4H linked to tolerance to boron toxicity in barley (*Hordeum vulgare* L.). *Plant and Soil* 326: 275-290.
- Mesquita GL, Zambrosi FC, Tanaka FA, Boaretto RM, Quaggio JA et al. (2016). Anatomical and physiological responses of citrus trees to varying boron availability are dependent on rootstock. *Frontiers in Plant Science* 7: 1-12.
- Metwally AM, Radi AA, El-Shazoly RM, Hamada AM (2018). The role of calcium, silicon and salicylic acid treatment in protection of canola plants against boron toxicity stress. *Journal of Plant Research* 1-14
- Misra AN, Misra M, Singh R (2011). Nitric oxide ameliorates stress responses in plants. *Plant Soil and Environment* 57: 95-100
- Mouhtaridou GN, Sotiropoulos TE, Dimassi KN, Therios IN (2004). Effects of boron on growth, and chlorophyll and mineral contents of shoots of the apple rootstock MM 106 cultured in vitro. *Biologia Plantarum* 48: 617-619.
- Moussa HR, Algama SMA (2017). Does Exogenous Application of Melatonin Ameliorate Boron Toxicity in Spinach Plants? *International Journal of Vegetable Science* 23: 233-245.
- Mukherjee SP, Choudhuri MA (1983). Implications of water stress-induced changes in the leaves of indigenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiologia Plantarum* 58: 166-170.
- Nali C, Francini A, Pellegrini E, Loppi S, Lorenzini G (2015). Visible injury, CO₂ assimilation and PSII photochemistry of eucalyptus plants in response to boron stress. In: Öztürk M, Ashraf M, Aksoy A, Ahmad MSA, Hakeem KR (editors) *Plants, pollutants and remediation*. Dordrecht, the Netherlands: Springer, pp. 1-11
- Niu YH, Guo FQ (2012). Nitric oxide regulates dark-induced leaf senescence through EIN2 in Arabidopsis. *Journal of Integrative Plant Biology* 54: 516-525
- Nosaka K (2006) Recent progress in understanding thiamin biosynthesis and its genetic regulation in *Saccharomyces cerevisiae*. *Applied Microbiology Biotechnology* 72: 30-40.
- Panda P, Nath S, Chanu TT, Sharma GD, Panda SK (2011). Cadmium stress induced oxidative stress and role of nitric oxide in rice (*Oryza sativa* L.). *Acta Physiologia Plantarum* 33: 1737-1747.
- Pandey N (2013). Antioxidant responses and water status in *Brassica* seedlings subjected to boron stress. *Acta Physiologia Plantarum* 35: 697-706.
- Pardossi A, Romani M, Carmassi G, Guidi L, Landi M et al. (2015). Boron accumulation and tolerance in sweet basil (*Ocimum basilicum* L.) with green or purple leaves. *Plant and Soil* 395: 375-389.
- Piñero MC, Pérez-Jiménez M, López-Marín J, Del Amor FM (2017). Amelioration of boron toxicity in sweet pepper as affected by calcium management under an elevated CO₂ concentration. *Environmental Science Pollution Research* 24: 10893-10899.
- Rapala-Kozik M, Kowalska E, Ostrowska K (2008). Modulation of thiamine metabolism in *Zea mays* seedlings under conditions of abiotic stress. *Journal of Experimental Botany* 59: 4133-4143.
- Rapala-Kozik M, Wolak N, Kujda M, Banas AK (2012). The upregulation of thiamine (vitamin B 1) biosynthesis in *Arabidopsis thaliana* seedlings under salt and osmotic stress conditions is mediated by abscisic acid at the early stages of this stress response. *BMC Plant Biology* 12: 2.
- Ribeiro DT, Farias LP, De Almeida JD, Kashiwabara PM, Ribeiro AF et al. (2005). Functional characterization of the th1 promoter region from *Arabidopsis thaliana*. *Journal of Experimental Botany* 56: 1797-1804.
- Sayed SA, Gadallah MAA (2002). Effects of shoot and root application of thiamin on salt-stressed sunflower plants. *Plant Growth Regulation* 36: 71-80.
- Shah A, Wu X, Ullah A, Fahad S, Muhammad R et al. (2017). Deficiency and toxicity of boron: Alterations in growth, oxidative damage and uptake by citrange orange plants. *Ecotoxicology and Environmental Safety* 145: 575-582
- Sharma P, Dubey RS (2005) Pb toxicity in plants. *Brazilian Journal of Plant Physiology* 17: 35-52.
- Simontacchi M, Galatro A, Ramos-Artuso F, Santa-Maria GE (2015). Plant survival in a changing environment: the role of nitric oxide in plant responses to abiotic stress. *Frontiers in Plant Science* 6: 977.
- Singh HP, Batish DR, Kaur G, Arora K, Kohli RK (2008). Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. *Environmental and Experimental Botany* 63: 158-167.
- Soltani Y, Saffari VR, Maghsoudi Moud AA (2014). Response of growth, flowering and some biochemical constituents of *Calendula officinalis* L. to foliar application of salicylic acid, ascorbic acid and thiamine. *Ethno- Pharmaceutical Products* 1: 37-44.
- Strain HH, Svec WA (1966). Extraction, separation, estimation and isolation of the Chlorophylls. In: Vernon LP, Seely, GR (editors). *The Chlorophylls*. New York, NY, USA: Academic Press.
- Tripathi DK, Singh S, Singh S, Srivastava PK, Singh VP et al. (2017). Nitric oxide alleviates silver nanoparticles (AgNps)-induced phytotoxicity in *Pisum sativum* seedlings. *Plant Physiology and Biochemistry* 110: 167-177.
- Tuna AL, Kaya C, Altunlu H, Ashraf M (2013). Mitigation effects of non-enzymatic antioxidants in maize (*Zea mays* L.) plants under salinity stress. *Australian Journal of Crop Science* 7: 1181.

- Tunc-Ozdemir M, Miller G, Song L, Kim J, Sodek A et al. (2009). Thiamine confers enhanced tolerance to oxidative stress in *Arabidopsis*. *Plant Physiology* 151: 421-432.
- Van Rossum MWPC, Alberda M, Van Der Plas LHW (1997). Role of oxidative damage in tulip bulb scale micropropagation. *Plant Science* 130: 207-216.
- Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant system in acid rain treated bean plants: protective role of exogenous polyamines. *Plant Science* 151: 59-66.
- Wakeel EUH, Aftab M, Rasheed F, Muhmood A, Naz A et al. (2018). Boron concentration in irrigation water used for wheat-cotton cropping system in alkaline calcareous soils of Southern Punjab. *Science Letters* 6: 6-12.
- Wang BL, Shi L, Li YX, Zhang WH (2010). Boron toxicity is alleviated by hydrogen sulfide in cucumber (*Cucumis sativus* L.) seedlings. *Planta* 231: 1301-1309.
- Wang P, Sun X, Chang C, Feng F, Liang D et al. (2013). Delay in leaf senescence of *Malus hupehensis* by long-term melatonin application is associated with its regulation of metabolic status and protein degradation. *Journal of Pineal Research* 55: 424-434.
- Wang R, Liu G, Liu L, Jiang C (2014). Relationship between leaf chlorosis and different boron forms in *Trifoliate* orange seedlings under excessive boron supply. *Soil Science and Plant Nutrition* 60: 325 -332.
- Wang Y, Duan X, Xu S, Wang R, Ouyang Z et al. (2016). Linking hydrogen-mediated boron toxicity tolerance with improvement of root elongation, water status, and reactive oxygen species balance: a case study for rice. *Annals of Botany* 118: 1279-1291.
- Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Ghassemi-Golezani K (2012). Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L). *Plant Omics Journal* 5: 60-67.
- Wimmer MA, Eichert T (2013). Mechanisms for boron deficiency-mediated changes in plant water relations. *Plant Science* 203: 25-32.
- Wolf B (1971). The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. *Communication in Soil Science and Plant Analysis* 2: 363-374.
- Yermiyahu U, Ben-Gal A, Keren R, Reid RJ (2008). Combined effect of salinity and excess boron on plant growth and yield. *Plant and Soil* 304: 73-87.
- Yuan HM, Huang X (2016). Inhibition of root meristem growth by cadmium involves nitric oxide-mediated repression of auxin accumulation and signalling in *Arabidopsis*. *Plant, Cell and Environment* 39: 120-135.
- Yusof ZB, Borhan FP, Mohamad FA, Rusli MH (2015). The effect of *Ganoderma boninense* infection on the expressions of thiamine (vitamin B1) biosynthesis genes in oil palm. *Journal of Oil Palm Research* 27: 12-18.
- Zhou B, Guo1 Z, Xing J, Huang B (2005). Nitric oxide is involved in abscisic acid-induced antioxidant activities in *Stylosanthes guianensis* *Journal of Experimental Botany* 56: 3223-3228.