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PHENOLIC ACIDS MEDIATE BORON EXCESS TOLERANCE IN TOMATO CALLUS TISSUES BY REGULATING ANTIOXIDANT ENZYMES AND BORON ACCUMULATION

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The participation of salicylate (SalA), gallate (GalA), and benzoate (BenA), in various physiological and biochemical processes in the plant under conditions of boron excess (BE), is largely unknown. The relationship between phenolic acids (PhAs) and regulation of antioxidant enzymes and B forms has been studied in the alleviation of oxidative stress caused by BE within the tomato callus. Tomato calli were subjected to BE (2 mM) in the absence or presence of three levels of BenA, GalA and SalA. The results demonstrated that different levels of PhAs attenuated the oxidative stress of BE by reducing hydrogen peroxide, B accumulation, and lipoxygenase activity (LOX) activity, and the moderate level was the most effective. Phenolic acid treatments reduced the stimulatory effects of BE on catalase (CAT) and superoxide dismutase (SOD) activity. Similarly, BenA and GalA increased the effect of BE stimulation on the activities of ascorbate peroxidase (APX) and peroxidase (POD), while SalA decreased these impacts on both enzymes. The results highlight that PhAs perform an important function in alleviating BE stress in tomato calli by regulating antioxidant enzymes and forms of B accumulation. This research supplies new viewpoints for strategies associated with BE tolerance in tomato plants and therefore can be employed as plant growth stimulators.

Keywords: Benzoic acid, Boron excess, Gallic acid, Salicylic acid, Tomato callus

INTRODUCTION

Boron is a micro-nutrient and is fundamental for plants that have significant physiological functions and therefore regulate the productivity of crops (Landi *et al*., 2019). Climate change is raising sea levels and reducing rainfall in many regions of the world, leading to increased levels of B in irrigation water affecting plant growth (Princi *et al*., 2016). Boron is necessary for normal plant growth and development in small quantities such as 1µg g^{-1} : 1 mg g^{-1} and is toxic to plants when present in excessive quantities. After exposure to biotic/abiotic stresses such as EB, some kinase cascades, and ion channels are stimulated (Fraire-Velázquez *et al*., 2011), reactive oxygen species (ROS) are stimulated (Laloi *et al*., 2004), genes are reprogrammed, which play a role in defensive actions and stimulating plant resistance to reduce stress damage (Fujita *et al*., 2006). Among the components associated with adaptive actions, important functions are performed by compounds of the LOX pathway, which is characterized by the major signaling system (Rejeb *et al*., 2014). Recently, in tomato plants, accumulation of H_2O_2, B, and lipid peroxidation in tissues was reported to result from BE (Kaya *et al*., 2020; Farghaly *et al*., 2021).

Plants have complex defensive action strategies that can emerge primarily or after stress challenges. After a plant recognizes a stress, its constitutive defense mechanisms release chains of complex defenses, which vary from stress to stress (Rejeb *et al*., 2014). Plants mainly deal with oxidative stress through an intrinsic defense strategy consisting of non-enzymatic and enzymatic antioxidants such as SOD, CAT, APX, and POD (Hasanuzzaman *et al*., 2020). Under B toxicity, Kaya *et al*. (2020) revealed an increase in some antioxidant enzymes (SOD, POD, and CAT) within tomato seedlings. Nonetheless, other researchers have shown that CAT in citrus plants (Han *et al*., 2009) and APX in lettuce (Eraslan *et al*., 2007) were low or insensitive in tomato plants to some doses of B toxicity (Cervilla *et al*., 2007). Also, phenolic compounds, secondary metabolites, are important and act as potent antioxidants (Hossain *et al*., 2009).

Phenolic compounds are used in many applications such as antioxidant, growth promotion, bioremediation, and allelochemicals (Bujor *et al*., 2015). Benzoic acid, GalA, and SalA are PhAs associated with the regulation of plant growth and development, and their response to biotic/abiotic stresses (Farghaly *et al*., 2021). Senaratna *et al*. (2003) concluded that BenA is known to provide tolerance to abiotic stress similar to that shown for SalA. Benzoic Acid is proven to be resistant to temperature extremes (Senaratna *et al*., 2003), water stress (Anjum *et al*., 2013), heavy metals (Pan-pan *et al*., 2020), and B toxicity (Farghaly *et al*., 2021). In plants, GalA is not yet known to be involved in defense against abiotic stress to a large extent; it is only known to reduce the effect of UV stress (Rudolphi-Skórska and Sieprawska, 2016), relieve cold stress (Ozfidan-Konakci *et al*., 2019), increase tolerance against Cu and Cd stress (Ozfidan-Konakci and Kabakci, 2020), and relieve B toxicity stress (Farghaly *et al*., 2021). Salicylic acid performs a critical function in controlling many physiological activities and tolerance to different environmental stresses (Khan *et al*., 2019). In addition, SalA regulates the synthesis of ROS by attenuating the antioxidant system (Farghaly *et al*., 2021).

Tomato plants are among the most abundant vegetable crops worldwide (Srividya *et al*., 2014) and a rich source of minerals, vitamins (USDA, 2016), and antioxidant systems (Di Masico *et al*., 1989). It is considered one of the most prominent vegetable crops in Egypt and is grown throughout the year. Boron toxicity has been reported in tomato-growing fields in many countries of arid and semi-arid regions, such as Egypt (Landy *et al*., 2012).

Effects of BenA and GalA applications on antioxidant enzymes and forms of B accumulation have not been demonstrated in tomato plants under BE conditions, whereas, SalA is an active hormone to alleviate B toxicity on antioxidant enzymes (Radi *et al*., 2014). This study provides new perspectives on the regulatory effect of antioxidant enzymes of BenA, GalA or SalA and B accumulation to stimulate tolerance against BE stress in tomato callus tissues.

MATERIALS AND METHODS

Callus generation

Solanum lycopersicum L. (Castle Rock cultivar) seeds were sterilized with 5% sodium hypochlorite prior to germination at half strength of Murashige and Skoog medium (Murashige and Skoog, 1962). The seedlings were grown for 10 days under growth room conditions (16 h light and 8 h dark, temperature 25 ± 1 °C). Explants (~1.0 cm hypocotyl) were cultivated in a MS medium and incubated in a growth chamber for 30 days (Farghaly *et al*., 2021). The medium included 4.4 g/L MS, 30 g/L sucrose, 0.1 mg/L alpha-naphthalene-acetic acid, and 0.1 mg/L 6-benzyl-amino-purine, 4 different treatments were as follows:

1-	Control	MS-nutrient medium alone
2-	BE	2 mM boric acid at the MS-nutrient medium
3-	BenA group	0.1 µM BA + 0 mM B, 1 µM BA + 0 mM B, 10 µM BA + 0 mM B, 0.1 µM BA + 2 mM B, 1 µM BA + 2 mM B, 10 µM BA + 2 mM B
4-	GalA group	1 µM GA + 0 mM B, 10 µM GA + 0 mM B, 20 µM GA + 0 mM B, 1 µM GA + 2 mM B, 10 µM GA + 2 mM B, 20 µM GA + 2 mM B
5-	SalA group	5 µM GA + 0 mM B, 50 µM GA + 0 mM B, 100 µM GA + 0 mM B, 5 µM GA + 2 mM B, 50 µM GA + 2 mM B, 100 µM GA + 2 mM B

Calli were harvested after 30 days, rinsed with sterile distilled water, and fresh weight (FW) was estimated; oven-dried calli (DW), and other plants were frozen at −80 °C.

Analytical methods

Hydrogen peroxide: The method of Velikova *et al.* (2000) was used to estimate the H_2O_2 content in the calli. The absorbance was estimated at 390 nm. The value was expressed as mg/g FW.

Enzyme extraction: Frozen samples (0.5 g) were ground in PPB (100 mM and pH 7.8) including ethylenediaminetetraacetic acid (EDTA; 0.1 mM) and polyvinylpyrrolidone (0.1 g) and centrifuged. In filtrates, the method of Lowry *et al.* (1951) was applied to estimate soluble proteins. The activity of the investigated enzymes was estimated based on the difference in absorption wavelength (nm)/mg protein/minute.

Lipoxygenase (EC 1.13.11.12): The Minguez-Mosquera *et al.* (1993) method was applied to estimate LOX activity. The changes in absorbance were estimated at 234 nm.

Superoxide dismutase (EC 1.15.1.1): The method of Misra and Fridovich (1972) was used to estimate SOD activity. The changes in absorbance were estimated at 480 nm.

Catalase (EC 1.11.1.6): The method of Aebi (1984) was applied to estimate CAT activity. The changes in absorbance were estimated at 240 nm.

Peroxidase (EC 1.11.1.7): The method of Zaharieva *et al.* (1999) was applied to estimate POD activity. The changes in absorbance were estimated at 470 nm.

Ascorbate peroxidase (EC 1.11.1.11): The method of Nakano and Asada (1981) was applied to estimate APX activity. The changes in absorbance were estimated at 290 nm.

Boron forms: Various forms of B were measured as described by Du *et al.* (2002) and Li *et al.* (2017). The dry sample of calli powder was mixed with distilled water, placed on a shaker for 24 h, and then the free B was measured in the filter. The pellets were mixed with 1 M NaCl, placed on a shaker for 24 h, and then the semi-bound B was measured in the filter. The pellets were mixed with 1 M HCl, placed on a shaker for 24 h, and then the bound B was measured in the filter. The method of curcumin-acetate was used to estimate the concentration of B forms (Mohan and Jones, 2018).

Statistical analysis: The data obtained were averages (± standard deviation) of four replicates, and statistical tests were estimated by SPSS software. One-way analysis of variance (ANOVA) was used and followed by Tukey's multiple range test for all PhA treatments with or without BE. Pearson's correlation test was used to measure the relationship between the averages of different parameters of tomato calli under BenA, GalA, SalA with or without BE and asterisks showed a significant correlation (* and ** at 5 and 1%, respectively). The t-test was applied in Excel to test the significant difference between treatments of PhA with or without BE.

RESULTS

Hydrogen peroxide

The contents of H_2O_2, the product of oxidized metabolites, were measured in tomato callus tissues, which were subjected to various treatments to evaluate the efficacy of BenA, GalA, and SalA in reducing oxidative damage caused by BE stress (Fig. 1A). As demonstrated in Fig. 1A, BE stress increased the H_2O_2 content in calli by 60.22% compared to control plants. Applications of PhA to B-stressed calli reduced the impact of stimulating BE stress on the H_2O_2 content. Compared with B-stressed calli, the callus treated with moderate levels of BenA, GalA, and SalA demonstrated a decrease in the H_2O_2 content by 23.0%, 47.9%, and 25.8%, respectively. The high level of BenA and SalA led to slight decreases in the H_2O_2 content, while GalA significantly reduced this content. Under normal B conditions, treatments of BenA and GalA at low and medium concentrations did not change the content of H_2O_2, however, this content was catalyzed by higher concentrations. Furthermore, the content of H_2O_2 was increased with increasing concentrations of SalA in the medium. The application of BenA (0.85**, 0.61*, 0.77**), GalA (0.90**, 0.93**, 0.97**), and SalA (0.55, 0.92**, 0.89**) to B-stressed calli demonstrated a strong positive correlation between the H_2O_2, free, semi-bound and bound B content.

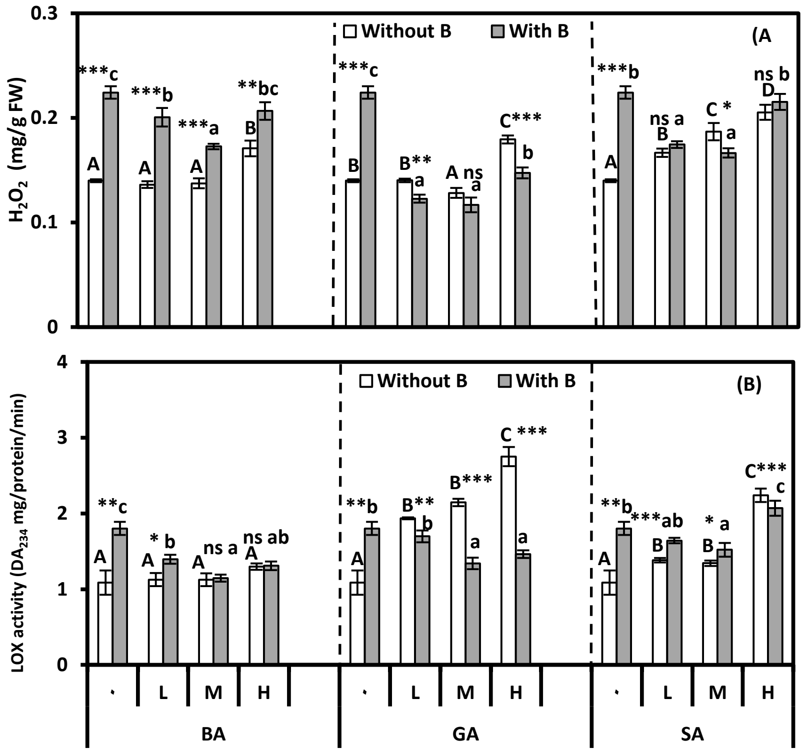

Fig. 1. The H_2O_2 content (A) and lipoxygenase (LOX; B) activity of *Solanum lycopersicum* calli under the influence of different concentrations (Low, L; moderate, M; high, H) of benzoic acid (BA), gallic acid (GA), or salicylic acid (SA) and excess boron for 30 days. The different letters, capital for phenolic acid without boron treatments and small for phenolic acid with boron treatments, indicate significance, and asterisks indicate significant differences between treatments with or without boron.

Lipoxygenase activity

To examine whether the beneficial impacts of PhA treatments on the B-stressed callus were associated with their capability to decrease membrane damage, the activity of LOX was evaluated in calli subjected to BE stress with or without PhA applications (Fig. 1B). LOX activity was increased in the callus with more B in the medium (66%) compared with the control callus. Treatment of the B-stressed callus with PhA reduced the negative effects of BE stress on the LOX activity. In most cases, LOX activity was significantly decreased and the highest decrease was recorded in moderate concentrations of BenA, GalA, and SalA, while the decrease was 36.4%, 25.6%, and 15.7%, respectively, compared with B-stressed calli. Under normal B conditions, significant increases in LOX activity were observed with PhA applications, and BenA induced only minor changes. Further, applications of BenA, GalA, and SalA caused a strong positive correlation between the H_2O_2 content and LOX activity in the callus treated with or without BE stress.

Superoxide dismutase activity

SOD activity was evaluated as it eliminates ROS (Fig. 2A). SOD activity in tomato calli was considerably increased under BE stress, and the increase was 23.6%, compared with the control callus. The application of GalA and SalA did not significantly alter the SOD activity in the B-stressed callus; however, BenA significantly reduced this activity. Under normal B conditions, BenA and GalA treatments did not change the activity of SOD, whereas the activity increased with increasing concentration of SalA in MS medium. Moreover, the relationship between the H_2O_2 content and SOD activity was negligible in the callus treated with PhAs and not stressed or stressed with BE; only the correlation in calli treated with SalA (0.89**) without BE and BenA (0.58*) with BE was significant.

Catalase activity

CAT activity was evaluated in tomato callus that was exposed to different concentrations of PAs with or without EB as it helps in the degrading of H_2O_2 to water and oxygen (Fig. 2B). As shown in Figure 2B, BE stress significantly stimulated CAT activity in calli (about twice that of the control). Nonetheless, applications of different concentrations of BenA, GalA, or SalA to the B-stressed callus reduced the stimulatory effects of BE on the CAT activity. Treatment of the B-stressed calli with moderate concentrations of BenA or SalA showed lower decreases in the CAT activity (39.5% and 34.4%, respectively) than the moderate concentration of GalA that recorded a 60.5% reduction in the B-stressed callus. Under normal B conditions, the application of PhA, in most cases, considerably stimulated the CAT activity in calli compared to control calli. Interestingly, PhA applications induced a strong positive relationship between the H_2O_2 content and CAT activity and in the unstressed or stressed callus with BE, only GalA without BE stress induced an insignificant correlation.

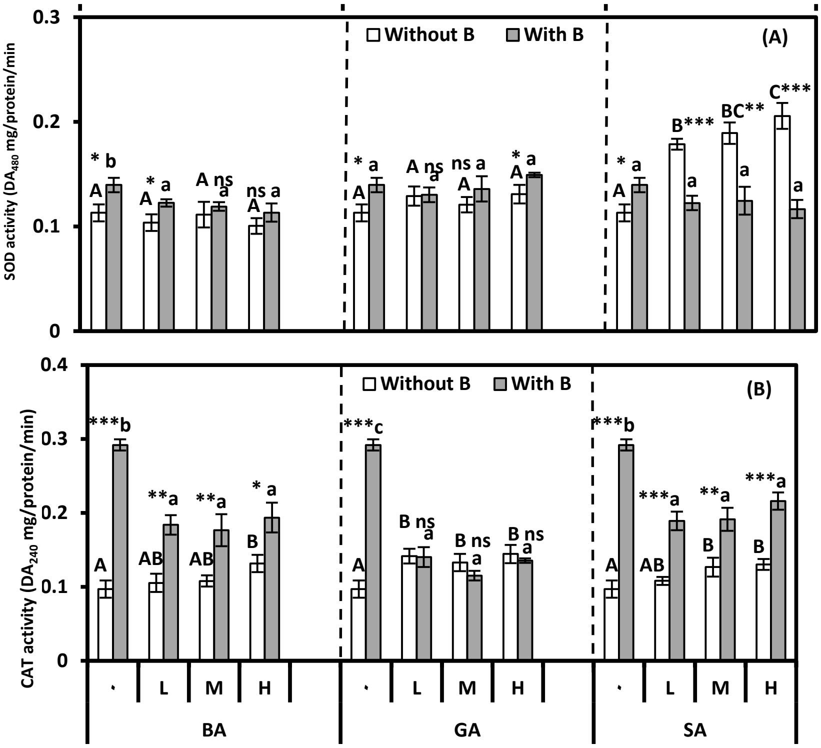

Fig. 2. The superoxide dismutase (SOD; A) and catalase (CAT; B) activity of *Solanum lycopersicum* calli under the influence of different concentrations (Low, L; moderate, M; high, H) of benzoic acid (BA), gallic acid (GA), or salicylic acid (SA) and excess boron for 30 days. The different letters, capital for phenolic acid without boron treatments and small for phenolic acid with boron treatments, indicate significance, and asterisks

indicate significant differences between treatments with or without boron.

Peroxidase activity

POD activity was evaluated as it catalyzes the oxidation by H_2O_2 for wide ranges of organic materials (Fig. 3A). The activity of POD was increased with the increase of B in the medium, which demonstrated a 35.1% increase in the POD activity compared to the control callus. The application of BenA or GalA at different levels with BE stimulated the POD activity, whereas, SalA treatment significantly reduced this activity. Compared with the B-stressed callus, moderate concentrations of BenA or GalA increased the POD activity by 26.2% and 83.5%, respectively, whereas, SalA at a moderate level reduced this activity (43.6%).Under normal B conditions, BenA or GalA treatments gradually improved the POD activity compared to the absolute control. In contrast, the POD activity was considerably reduced using concentrations of SalA in the medium. Furthermore, the calli treated with BE, BenA, GalA, or SalA the correlations between the H_2O_2 content and POD activity were significant (-0.60*, -0.79**, and +0.87**, respectively), while these correlations were not significant in calli treated with PhAs only.

Ascorbate peroxidase activity

The activity of APX was evaluated as it catalyzes the H_2O_2-dependent oxidation of ascorbate (Fig. 3B). Treatment of tomato calli with BE positively affected the APX activity. Under BE stress, the increase in the APX activity was 35.0% compared to the control callus. Different concentrations of BenA or GalA with BE stimulated the APX activity (10.5%, 22.3%, 16.2% for BenA and 10.3%, 9.2%, 20.3% for GalA, respectively, above the B-stressed callus), whereas, SalA treatment did not considerably change this activity. Under normal B conditions, BenA treatments decreased the APX activity in the callus, only the highest concentration did not alter this activity. In most cases, GalA treatments stimulated the APX activity under normal B conditions. However, under normal B conditions, the use of SalA did not alter the activity of APX, only the highest concentration stimulated this activity. In addition, our findings revealed that the activity of APX in calli treated with SalA (0.66*) or BenA with BE (-0.74**) showed a strong relationship with H_2O_2 content, while treatments of GalA with or without BE showed a non-significant correlation.

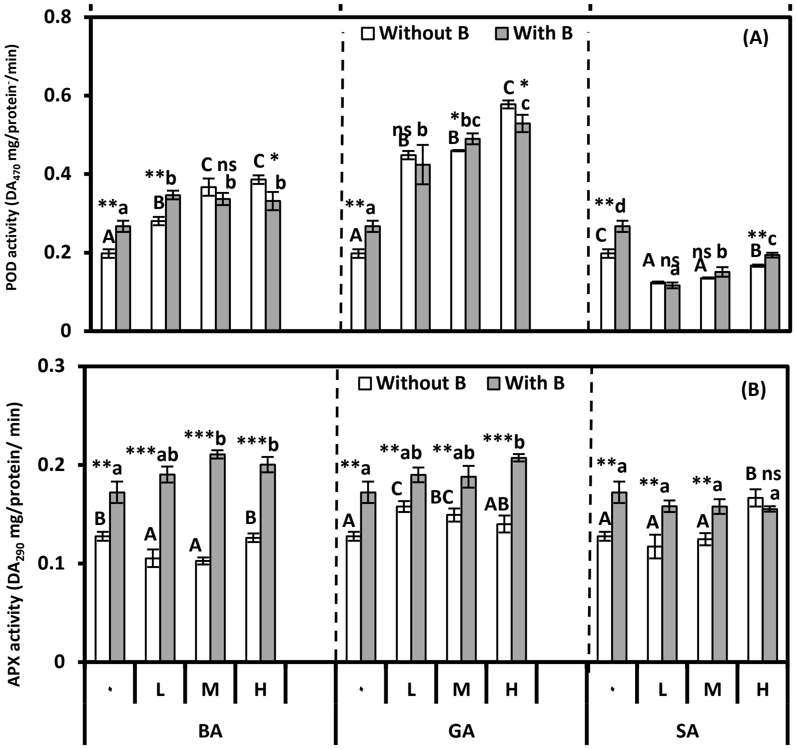

Fig. 3. The peroxidase (POD; A) and ascorbate peroxidase (APX; B) activity of *Solanum lycopersicum* calli under the influence of different concentrations (Low, L; moderate, M; high, H) of benzoic acid (BA), gallic acid (GA), or salicylic acid (SA) and excess boron for 30 days. The different letters, capital for phenolic acid without boron treatments and small for phenolic acid with boron treatments, indicate significance, and asterisks indicate significant differences between treatments with or without boron.

Boron forms

In normal B cases, free B was the highest amount of B (68.40%), followed by semi-bound B (23.40%), followed by bound B (8.20%) of B present in the callus (Fig. 4A-C). In the presence of BE, bound B was increased by 11.3%, while free and semi-bound B were decreased by 6.9% and 4.4%, respectively. Also, it can be noticed that BE induced a considerable increase in the content of free, semi-bound, and bound B in the stressed callus higher than in the control callus. Boron excess stress stimulated the content of free, semi-bound, and bound B in the callus by 74.6%, 57.7%, and 362.6%, respectively compared to the control calli.

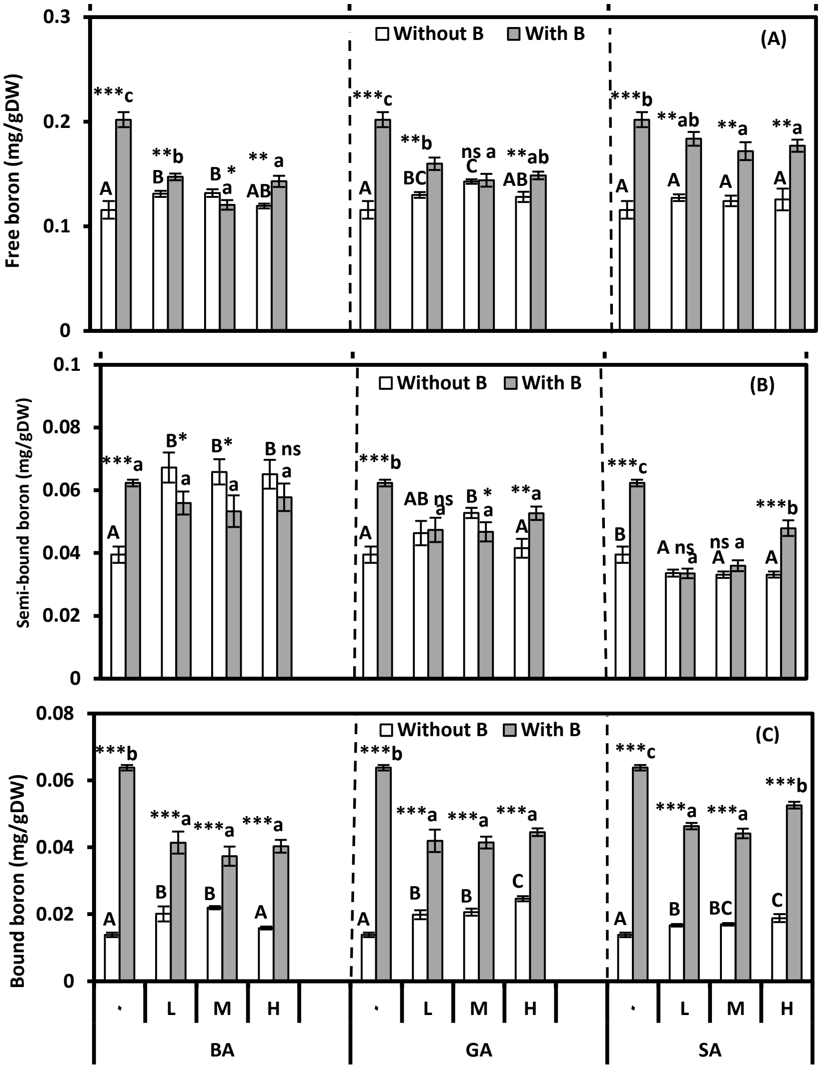

Fig. 4. Free boron (A), semi-bound boron (B), and bound boron (C) of *Solanum lycopersicum* calli under the influence of different concentrations (Low, L; moderate, M; high, H) of benzoic acid (BA), gallic acid (GA), or salicylic acid (SA) and excess boron for 30 days. The different letters, capital for phenolic acid without boron treatments and small for phenolic acid with boron treatments, indicate significance, and asterisks indicate significant differences between treatments with or without boron.

The application of a moderate concentration of BenA, GalA, or SalA reduced the accumulation of free, semi-bound, and bound B in the B-stressed callus by 40.4%, 28.7%, 14.9% for free B, 14.4%, 25.0%, 42.3% for semi-bound B, 41.5%, 35.1%, and 30.8% for bound B, respectively, compared with the B-stressed callus. Under normal B conditions, BenA or GalA treatments led to a significant or insignificant increase in the different concentrations of B form in the callus compared to the absolute control. However, the different concentrations of SalA did not alter the accumulation of B forms in the callus.

DISCUSSION

Boron excess stress like other ionic stresses leads to the formation of ROS in plant cells (Kaya *et al*., 2020). Hence, BE may facilitate the transport of boric acid into plant cells, which can be moderately converted to borate as a result of endogenous cellular pH (Wimmer *et al*., 2002), consequently releasing ROS (Dat *et al*., 2000). In this work, the content of H_2O_2 in tomato calli was evaluated to show the oxidative damage caused by BE stress. The results showed that the presence of BE in the nutrient medium stimulated the production of H_2O_2 in the callus tissues. These results indicate that BE in callus tissues, mostly, stimulates NADPH oxidase compounds (Agrawal *et al*., 2003) that reduce oxygen to the $^{\bullet}O_2^{-}$, which decomposes to H_2O_2. Furthermore, we speculate that BE stimulates H_2O_2 accumulation by affecting electron transport in mitochondria and plastids. Shah *et al*. (2017) also declared that BE leads to oxidative damage mostly through electron transport leakage during photosynthesis. These results affirmed our previous findings with wheat cultivars (El-Shazoly *et al*., 2019).

However, the application of most concentrations of PhAs considerably inhibited H_2O_2 production in B-stressed callus tissues. These results appeared that applications of PhA contributed to the inhibition of ROS release under BE stress through regulating B uptake (Farghaly *et al*., 2021). Chen *et al*. (2019) also concluded that phenolic compounds are efficient in scavenging or neutralizing ROS that reduce the oxidative damage to lipids, proteins, and DNA and thus reduce cellular oxidative stress. Among the PhAs tested, GalA was the most effective in inhibiting H_2O_2 accumulation in the B-stressed callus, and this suggests that this phA contains the largest number of OH groups, donating H atoms to produce antioxidant properties. Accordingly, Chen *et al*. (2020) explained that the existence and site of the hydroxyl group in PhAs perform important functions in antioxidant activity. Furthermore, Sekher Pannala *et al*. (2001) mentioned that more hydroxyl groups of phenolic acid, antioxidant molecules, have a greater ability to remove hydroxyl radicals. These reports affirmed the minimal effect of BenA in removing the accumulation of H_2O_2 under BE stress. The high content of H_2O_2 in the callus that treated with all concentrations of SalA and high concentrations of BenA or GalA without BE stress revealed that H_2O_2 resulted in the onset of SalA creating systemic acquired resistance (Rao *et al*., 1997).

Under conditions of different stresses, lipolysis and oxidation of membrane-bound fatty acids are associated with increased LOX activity (Babenko *et al*., 2017). Our results demonstrated that the activity of LOX was significantly increased in the B-stressed callus, while applications of PhAs, in most cases, significantly reduced this activity under B-stress. These indicate that PhAs increased the elimination of ROS in the tomato callus under the stress of BE. Therefore, PhAs can reduce oxidative stress in tomato calli caused by BE stress. The strong positive relationships between the LOX activity and H_2O_2 content in the callus affirmed these results suggesting that PhAs without or with BE reduced oxidative damage, resulting in increased H_2O_2 and LOX activity. Generally, applications of moderate concentrations of BenA, GalA, or SalA can reduce the LOX activity and H_2O_2 content, thereby increasing callus resistance to BE stress. In this consistency, the release of ROS species, factors assessing BE stress, and ion imbalance, have been demonstrated in tomato plantations (Cervilla *et al*., 2012). Siquet *et al*. (2006) also clarified that the antioxidant effect of PhAs should be considered as a useful means to retard membrane peroxidation. Similarly, the functions of BenA, GalA, or SalA in enhancing the effects of stress on free radical scavenging activities have been examined by other authors that could support our findings (Shaki *et al*., 2019). On the contrary, the significant increase in the LOX activity in GalA or SalA treated callus tissues without BE indicated that the fatty acid oxidative metabolite resulting from the LOX activity was primarily involved in growth rather than senesce. In agreement with our findings, Siedow (1991) declared that the oxygenated fatty acid products of the LOX activity have a clear function in growth, aging, and interaction with external stress.

Almost all antioxidant enzymes were influenced under BE stress without or with PhAs, indicating that these enzymes qualify the tomato callus to withstand BE stress. The enzyme SOD constitutes the main frontier of defense against ROS, and it is metallic in nature (Jackson *et al*., 1978). Our results showed that the SOD activity was significantly stimulated in the B-stressed callus, indicating that BE stress stimulated a key defense enzyme that assisted the callus to resist this stress. Likewise, Kaya *et al*. (2020) demonstrated increased SOD activity in tomatoes under excessive B stress. Statistics demonstrated that GalA or SalA did not change the SOD activity in B-stressed calli, indicating that the callus contained suitable interior antioxidants to eliminate low ROS content conforming to low LOX activity and low H_2O_2 content, compared to B-stressed calli alone. These findings confirmed the insignificant relationships between H_2O_2 content and SOD activity in calli tread with GalA or SalA alone or B-stressed calli. However, the strong relationship between the H_2O_2 content and SOD activity in the callus treated with BenA and BE also affirmed the reduced effect of BenA in reducing the accumulation of H_2O_2 under BE stress. In harmony with our findings, Chandrakar *et al*. (2016) reported that application of the PhA hormone, SalA, did not change the SOD activity in arsenic-stressed soybean plants. In contrast, the increase in the SOD activity with SalA treatment in normal B cases was associated with an increase in the content of H_2O_2, and this result is consistent with previous reports indicated that SalA stimulates H_2O_2 production by inactivating H_2O_2 removal enzymes and increasing H_2O_2 generation of enzymes like SOD (Rao *et al*., 1997). In addition, this result confirmed the strong relationship between H_2O_2 content and SOD activity within the callus treated with SalA without increasing B.

The important role of the CAT enzyme in plant resistance, and its increased activity under stresses are signals of tolerance to oxidative stress, as it breaks down the H_2O_2 product of SOD enzymes (Mittler, 2002). Based on our results, CAT activity was considerably increased in callus tissues exposed to BE, suggesting that excess exposure to B stress stimulated ROS production in calli, while also stimulating the defense system. Also, Kaya *et* *al*. (2020) found an increase in CAT activity under B toxicity in tomato. However, supplementation of PhAs to B-stressed calli significantly reduced CAT activity, confirming their function in decreasing oxidative stress caused by BE. Additionally, these results indicated that other enzymes could be enough to suppress low H_2O_2 levels. The significant positive relationship between H_2O_2 content and CAT activity in the callus treated with BE and PhAs confirmed the low content of H_2O_2 in callus cells as CAT activity has a low affinity for H_2O_2. These indicate that the H_2O_2 content was not stimulated and therefore, the CAT activity was also not stimulated in the plant treated with an excess of B and PhAs. Foyer *et al*. (2009) reported that CAT has a remarkably low affinity for H_2O_2, and therefore only eliminates a high amount of H_2O_2. Consistent with our findings, Ozfidan-Konakci and Kabakci (2020) recognized that GalA alone or GalA with cadmium did not stimulate the CAT activity within wheat plants. Also, Amist and Singh (2018) noticed a reduction in the CAT activity of wheat plants treated with water stress and BenA. In addition, a decrease in CAT activity was observed during the application of SalA to B-stressed canola plants (Radi *et al*., 2014).

Both POD and CAT enzymes, in concert with the SOD enzyme, perform an important defensive function in eliminating ROS (Jaleel *et al*., 2009). This study manifested that the POD activity in tomato tissues was increased due to BE stress, suggesting that the callus was adapted by an effective defense system to withstand BE stress. Furthermore, an increase in POD activity was associated with a high content of phenolic compounds content, as noticed in our previous study (Farghaly *et al*., 2021). Similarly, Kaya *et al*. (2020) showed that B toxicity stimulated POD activity in tomato cultivar SC 2121. In normal or BE conditions, the application of BenA or GalA further stimulated POD activity in the callus, while the application of SalA reduced its activity. Additionally, the results showed that the relationships between H_2O_2 content and POD activity were negatively strong in the callus treated with BenA and BE or GalA with BE, whereas they were positively strong in the callus treated with SalA with BE. However, the same relationships were insignificant in calli treated with PhAs without BE stress. These findings indicated that in BE, the POD eliminated H_2O_2 in calli treated with BenA or GalA, while POD did not eliminate H_2O_2 in the callus treated with SalA.

Accordingly, Yadav and Singh (2013) found that increasing POD activity by adding BenA to cadmium-stressed wheat plants was dose-dependent. Yetişsin and Kurt (2020) reported that GalA increased POD activity in copper-stressed maize plants. In contrast, Radi *et al*. (2014) demonstrated that SalA reduced the POD activity in B-stressed canola plants.

APX is the main antioxidant enzyme to reduce the toxic level of H_2O_2 (Apel and Hirt, 2004) and has a pivotal function in eliminating ROS since very low level is enough to break down H_2O_2 (Anjum *et al*., 2014). The current data manifested that BE increased the APX activity in calli, suggesting that BE stress stimulated antioxidant enzymes, which help tomatoes resist this stress. Furthermore, these increases in APX activity could be sustained by high ascorbic acid contents, as observed in the previous study in B-stressed tomatoes (Farghaly *et al*., 2021). Likewise, the increase in APX activity was previously detected in tomatoes under B toxicity by Cervilla *et al*. (2007). The results also revealed that APX activity was further increased in B-stressed tissues by the use of BenA or GalA as a result of their effective association in the detoxification of ROS, as well as the management of oxidative stress. The significant negative relationships between the H_2O_2 content and APX activity in the BenA-treated callus with BE stress confirmed reports of Amist and Singh (2018) concluding that APX and POD were common peroxidase enzymes, which converted H_2O_2 to water using ascorbic acid and guaiacol, and increased in wheat plants treated with BenA and drought. In addition, our findings are agreement with the results of Ozfidan-Konakci and Kabakci (2020) who concluded that GalA stimulated APX activity in wheat subjected to cadmium stress. However, SalA applications without or with BE stress did not change APX activity, in most cases, indicating that the APX enzyme was not responsible for H_2O_2 removal in this plant. Similarly, the APX activity was not considerably changed by SalA with or without B toxicity in canola plants (Radi *et al*., 2014).

Normally, B is present within the cell in free, semi-bound, and bound forms, the soluble form is free and directly available for physiological function, the semi-bound form is involved in the biosynthesis of the cell wall, and the bound form is involved in the cell wall components (Du *et al*., 2002). The results demonstrated that the BE in the nutrient medium increased the uptake of different forms of B by the tomato callus. The results showed that the free forms of B (61.5%) were the most prevalent in callus tissues that could be linked to the symptoms of BE. The B-bound forms of about 11% were induced by BE stress compared to the control callus, and this might be due to the increase of binding sites on the cell wall by rhamnogalacturonan RG-II. In agreement with our findings, Reid (2007) illustrated that BE may bind to the cell wall, disrupting cell expansion and growth. However, co-applications of PhAs with BE resulted in a significant reduction in the forms of B accumulated in callus tissues, resulting in significant increases in callus growth. Free forms of B were probably related to chlorosis in plant leaves (Wang *et al*., 2014), and the decrease in the accumulation of these forms could be linked to tolerance to BE stress (Landi *et al*., 2015). Our findings are in agreement with the previous results of Farghaly *et al*. (2021) who illustrated that PhAs decreased B accumulation in tomato tissues under BE stress.

CONCLUSION

The involvement of BenA, GalA, or SalA in various physiological and biochemical processes under BE is not yet known in plants. This study illustrated that PhAs (BenA, GalA, or SalA) treatments of B-stressed tomato calli reduced the accumulation of H_2O_2 and B (free, semi-bound, and bound), as well as altered the activity of antioxidant enzymes to withstand the stress of BE. The strategies of PhAs applied to boost vallus growth under BE were to reduce H_2O_2 synthesis through activation of CAT activity and to reduce the accumulation of various B forms, specially bound forms. The most efficient PhA order in this study was BenA > GalA > SalA. In addition, a moderate concentration (1 µM) of BenA was the most effective concentration, which was the lowest concentration of all the PhAs used. These results could contribute to elucidating strategies that mitigate BE stress in tomato plants using PhAs, and further studies are still needed.

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الأحماض الفينولية تتوسط تحمل البورون الزائد في أنسجة كالوس الطماطم عن طريق تنظيم الإنزيمات المضادة للأكسدة وتراكم البورون

إن مشاركة حمض الساليسيلك، وحمض الجاليك، وحمض البنزويك في مختلف العمليات الفسيولوجية والكيميائية في النبات في ظل ظروف سمية فائض البورون غير معروفة إلى حد كبير. في هذه الدراسة تمت دراسة العلاقة بين الأحماض الفينولية وتنظيم إنزيمات مضادات الأكسدة وتراكم أشكال البورون المختلفة في التخفيف من الإجهاد التأكسدي الذي يسببه فائض البورون داخل كالوس الطماطم. تم تعرض كالوس الطماطم لتركيز ٢ مل مول حمض البوريك في وجود أو غياب ثلاثة مستويات من حمض الساليسيلك، وحمض الجاليك، أو حمض البنزويك. وأظهرت النتائج أن المستويات المختلفة من الأحماض الفينولية الثلاثة، في معظم الحالات خففت من الإجهاد التأكسدي لفائض البورون عن طريق تقليل تراكم فوق أكسيد الهيدروجين وكذلك خفض نشاط انزيم ليبوكسجينيز (LOX)، وكان المستوى المتوسط هو الأكثر فاعلية. وأدت الأحماض الفينولية الثلاثة الي خفض تحفيز فائض البورون لنشاط انزيم الكتاليز (CAT) وسوبر أكسيد الديسميوتيز (SOD). وزاد كل من حمض البنزويك وحمض الجاليك من التأثير التحفيزي لفائض البورون على أنشطة انزيمي أسكوربك بيروكسيديز (APX) وبيروكسيديز(POD)، في حين خفض حمض الساليسيلك من هذه التأثيرات على كلا من الإنزيمين. وسلطت النتائج الضوء على أن الأحماض الفينولية تؤدي وظيفة مهمة في التخفيف من سمية إجهاد فائض البورون علي كالوس الطماطم من خلال تنظيم أنزيمات مضادات الأكسدة وخفض تركم الأشكال المختلفة من البورون. وقدم هذا البحث وجهات نظر جديدة للاستراتيجيات المرتبطة بتسامح سمية فائض البورون علي نباتات الطماطم وبالتالي يمكن استخدامها كمحفزات لنمو النبات.