Salicylic Acid Improves Boron Toxicity Tolerance by Modulating the Physio-Biochemical Characteristics of Maize (Zea mays L.) at an Early Growth Stage

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Abstract: The boron (B) concentration surpasses the plant need in arid and semi-arid regions of the world, resulting in phytotoxicity. Salicylic acid (SA) is an endogenous signaling molecule responsible for stress tolerance in plants and is a potential candidate for ameliorating B toxicity. In this study, the effects of seed priming with SA (0, 50, 100 and 150 µM for 12 h) on the growth, pigmentation and mineral concentrations of maize (Zea mays L.) grown under B toxicity were investigated. One-week old seedlings were subjected to soil spiked with B (0, 15 and 30 mg kg⁻¹ soil) as boric acid. Elevating concentrations of B reduced the root and shoot length, but these losses were significantly restored in plants raised from seeds primed with 100 µM of SA. The B application decreased the root and shoot fresh/dry biomasses significantly at 30 mg kg⁻¹ soil. The chlorophyll and carotenoid contents decreased with increasing levels of B, while the contents of anthocyanin, H₂O₂, ascorbic acid (ASA) and glycinebetaine (GB) were enhanced. The root K and Ca contents were significantly increased, while a reduction in the shoot K contents was recorded. The nitrate concentration was significantly higher in the shoot as compared to the root under applied B toxic regimes. However, all of these B toxicity effects were diminished with 100 µM SA applications. The current study outcomes suggested that the exogenously applied SA modulates the response of plants grown under B toxic conditions, and hence could be used as a plant growth regulator to stimulate plant growth and enhance mineral nutrient uptake under B-stressed conditions.

Keywords: biomass reduction; cereal crops; growth regulators; metal stress

1. Introduction

Abrupt changes in climate along with the potential abiotic and biotic stresses are serious challenges for plant growth and production worldwide [1]. Environmental stresses negatively influence the
germination, growth and yield of the crop plants. The continuous yield losses caused by abiotic stresses are one of the important reasons for socioeconomic imbalance [2]. Drought reduces the yield of staple food crops throughout the world up to 70% [3], and the effects of drought and salt stress on plant growth mechanisms and patterns have been discussed [3,4]. In the last few decades, soil and water resources are being contaminated with toxic elements due to industrial revolution and urbanization together with the use of artificial fertilizers [5,6]. Increasing levels of these toxic elements are imposing harmful effects on plants, plant-dependent animals and ultimately human health [7].

Boron is an important micronutrient in many plants for their normal functioning [8]. It is also considered to be an essential element for vascular plants according to the defined criteria for essentiality. The indirect association of B with photosynthesis has been reported in crop plants—e.g., soybean [9]. However, the rate of emergence and productivity is also decreased in many plants, including tomato, maize, wheat, alfalfa and carrot under B toxicity [10]. The B toxicity significantly reduces the yield of crop plants in relatively dry areas of the world [11]. Some of the factors contributing to the elevating levels of B are the use of fertilizers, mining and irrigation [12,13]. The B-induced toxicity occurs more commonly in saline soil in semi-arid geographical zones [14]. The interplay between salt stress and B nutrition in plants has been described, with contrasting results showing antagonistic and synergistic relations even within the same plant species [15]. It has been observed that salinity increases B toxicity [16], but the interaction of salinity and B is not fully understood [17], making it an important area of research in plant physiology and ecotoxicology.

Oxidative stress may result from a deficiency or excess of B, which triggers the over-production of reactive oxygen species (ROS). The ROS and their derivatives are highly toxic agents and damage cellular membranes due to lipid peroxidation, causing protein denaturation and mutations in DNA [18]. Different nutrients such as silicon (Si) [19], zinc (Zn) [20,21], potassium (K) [22] and calcium (Ca) [23] can ameliorate B toxicity in different crop plants. The SA signal molecule [24] plays an important role in reducing the hazardous effects posed by biotic and abiotic stresses. Thus, SA has been used by many researchers to reduce the hazardous effects of different stresses such as osmotic stress [25], heat, saline and B toxicity in wheat [26].

Among the most important staple foods, maize holds an important position after wheat and rice [27]. Maize is well known for its high potential of extracting heavy metals from soil [28]. Despite this phytoextraction ability, maize is affected by various environmental stresses along with the high metal concentrations. The abiotic stress effects on maize growth and yield have been studied [29,30]. In the current study, the main objective was to assess the effects of high B toxicity under the remodeling effects of SA in terms of physio-biochemical improvements in the maize cultivar Gohar-19.

2. Results

For assessing the effects of SA on mitigating the effects of B toxicity, plants were supplied with 0, 50, 100 and 150 µM of SA. The B toxicity levels were 0, 15 and 30 mg kg$^{-1}$ soil. Roots transport B via passive diffusion or facilitate transport [30] in the plant body through transpiration streams and it is accumulated in older shoots without being translocated [31], therefore the study parameters include both the root and shoot data of maize cv. Gohar-19.

2.1. Root and Shoot Length

The B toxicity significantly reduced the root and shoot length of maize seedlings. High B concentrations in soil inhibit the root and shoot growth due to the decreased photosynthetic activity and net plant productivity. Elevating the B concentration in soil decreased the root and shoot length up to 21.77% and 25.25%, respectively, which are significant reductions (Table 1, Figure 1). The priming of seeds with SA reduced the B toxic effects and retained the root and shoot lengths. Plant seeds that were primed with various concentrations (0, 50, 100 and 150 µM) of SA improved the root and shoot lengths. Significant increases in the root and shoot lengths were observed at 100 µM SA (Figure 2, Table 1). A 23.8% increase in root length was observed with the application of 100 µM of SA in 30 mg kg$^{-1}$ of
B-treated plants, while a 26.7% decrease was observed in the shoot length of 30 mg kg\(^{-1}\) B-treated plants as compared with the control. The SA application at 100 µM was found to be the best treatment and caused increases in the shoot length in 30 mg kg\(^{-1}\) B-treated plants up to 31.8%.

**Table 1.** Effects of SA (0, 50, 100 and 150 µM) on the plant root and shoot length of maize cultivar Gohar-19 under different B toxicity levels (0, 15 and 30 mg kg\(^{-1}\)).

|          | Root Length (cm) | Shoot Length (cm) |
|----------|------------------|--------------------|
|          | 0 mg kg\(^{-1}\) B | 15 mg kg\(^{-1}\) B | 30 mg kg\(^{-1}\) B | 0 mg kg\(^{-1}\) B | 15 mg kg\(^{-1}\) B | 30 mg kg\(^{-1}\) B |
| SA       |                  |                    |                   |
| 0 µM     | 27.1 ± 0.89 c    | 24.3 ± 1.05 b      | 21.2 ± 0.88 c     |
| 50 µM    | 27.8 ± 1.01 b    | 24.2 ± 0.97 b      | 21.34 ± 1.03 c    |
| 100 µM   | 29 ± 0.98 a      | 28.2 ± 0.87 a      | 26.4 ± 0.77 a     |
| 150 µM   | 26.4 ± 1.12 d    | 23 ± 1.24 c        | 21.8 ± 1.02 b     |

LSD 5% = 0.44. Values in the same column with different letters in superscript differ significantly.

**Figure 1.** Score (a) and loading plot (b) of principal component analysis (PCA) on different attributes of maize cultivar Gohar-19 plants supplemented with and without SA while grown under B stress. Score plot represents the separation of treatments as T1: 0 mg B without SA; T2: 0 mg B with 50 µM SA; T3: 0 mg B with 100 µM SA; T4: 0 mg B with 150 µM SA; T5: 15 mg/kg B without SA; T6: 15 mg/kg B with 50 µM SA; T7: 15 mg/kg B with 100 µM SA; T8: 15 mg/kg B with 150 µM SA; T9: 30 mg/kg B without SA; T10: 30 mg/kg B with 50 µM SA; T11: 30 mg/kg B with 100 µM SA; T12: 30 mg/kg B with 150 µM SA. Attributes evaluated include R L = root length; Car = carotenoids; R Nit = root nitrate; R K = root potassium; R Ca = root calcium, Antho = anthocyanin; L ASA = leaf ascorbic acid.
2.2. Plant Biomass

Plant fresh and dry biomass were also reduced in response to increasing the B treatment levels. The results obtained exhibited a positive correlation with the root and shoot lengths. Reducing and increasing patterns in plant fresh and dry biomass were observed in the root and shoot lengths. We observed 30% and 32.89% decreases in the root and shoot fresh biomass, respectively. The maximum increase in plant biomass was noted in plants raised from seeds primed with 100 µM of SA, as shown in Table 2. This gain in the plant growth biomarkers was due to the enhanced photosynthetic activity and improved antioxidant status of the plant body (Figure 2).
The carotenoid contents were reduced due to B toxicity. An increase of 30.4% in the chl \(a\) contents was recorded through priming seeds with SA. Seeds primed with 100 \(\mu\)M of SA expressed the maximum chl \(a\) content, which suggests reduced toxicity effects. The SA priming reduced the anthocyanin contents overall, but only 100 \(\mu\)M of SA caused a 47.5% reduction in the anthocyanin contents (Figures 2 and 3c). The toxic effects of B increased the ASA contents of maize seedlings. The B treatment of 30 mg kg\(^{-1}\) B. However, under a high boron toxicity, the ASA contents increased with increasing the levels of B toxicity. Significant increases were also reduced under B toxicity as compared to the control. An increase in the chl \(b\) contents was observed with respect to the control in the plants emerging from primed seeds. An increase of 30.4% in the chl \(b\) contents was observed at 100 \(\mu\)M SA treatment, while a non-significant increase was observed at 150 \(\mu\)M SA treatment as compared to the control (Figure 3a). An improvement in the chl \(a\) concentration was recorded through priming seeds with SA. Seeds primed with 100 \(\mu\)M of SA expressed the maximum chl \(a\) content, which suggests reduced toxicity effects.

Figure 3. Effect of SA on chlorophyll \(a\) (A), chlorophyll \(b\) (B), carotenoids (C) and anthocyanin (D) contents of maize cultivar Gohar-19 under varying B toxicity levels.

The chl \(b\) contents were also reduced under B toxicity as compared to the control. An increase in the chl \(b\) contents was observed with respect to the control in the plants emerging from primed seeds. An increase of 30.4% in the chl \(b\) contents was observed at 100 \(\mu\)M SA treatment, while a non-significant increase was observed at 150 \(\mu\)M SA treatment as compared to the control (Figure 3b). The carotenoid contents were reduced effectively under 30 mg kg\(^{-1}\) B. The B application at 30 mg Kg\(^{-1}\) caused reductions of 52.6%, 31.3% and 45% in the chl \(a\), chl \(b\) and carotenoids, respectively. The SA

### Table 2. Effect of SA (0, 50, 100 and 150 \(\mu\)M) on the plant fresh and dry weight of maize cultivar Gohar-19 under varying B toxicity levels (0, 15 and 30 mg kg\(^{-1}\)).

| Treatment | Root Fresh Weight (g) | Root Dry Weight (g) | Shoot Fresh Weight (g) | Shoot Dry Weight (g) |
|-----------|-----------------------|---------------------|------------------------|----------------------|
| 00 \(\mu\)M SA + 00 mg kg\(^{-1}\) B | 1.00 ± 0.89 \(^{abc}\) | 0.75 ± 0.21 \(^{bc}\) | 3.80 ± 0.33 \(^{abc}\) | 2.53 ± 1.01 \(^{abcd}\) |
| 00 \(\mu\)M SA + 15 mg kg\(^{-1}\) B | 0.89 ± 0.95 \(^{bc}\) | 0.65 ± 0.34 \(^{d}\) | 3.10 ± 0.41 \(^{bc}\) | 2.07 ± 0.85 \(^{abcd}\) |
| 00 \(\mu\)M SA + 30 mg kg\(^{-1}\) B | 0.70 ± 0.55 \(^{c}\) | 0.5 ± 0.21 \(^{d}\) | 2.55 ± 0.53 \(^{c}\) | 1.70 ± 0.33 \(^{d}\) |
| 50 \(\mu\)M SA + 00 mg kg\(^{-1}\) B | 1.25 ± 0.45 \(^{ab}\) | 1.02 ± 0.35 \(^{ab}\) | 3.85 ± 1.01 \(^{ab}\) | 2.57 ± 0.65 \(^{abcd}\) |
| 50 \(\mu\)M SA + 15 mg kg\(^{-1}\) B | 1.15 ± 0.75 \(^{abc}\) | 0.95 ± 0.34 \(^{ab}\) | 3.00 ± 0.95 \(^{abc}\) | 2.00 ± 0.35 \(^{abcd}\) |
| 50 \(\mu\)M SA + 30 mg kg\(^{-1}\) B | 0.85 ± 0.65 \(^{bc}\) | 0.62 ± 0.32 \(^{d}\) | 2.70 ± 0.55 \(^{bc}\) | 1.80 ± 0.45 \(^{cd}\) |
| 100 \(\mu\)M SA + 00 mg kg\(^{-1}\) B | 1.50 ± 0.76 \(^{ab}\) | 1.26 ± 0.22 \(^{a}\) | 4.50 ± 0.25 \(^{a}\) | 2.87 ± 0.27 \(^{a}\) |
| 100 \(\mu\)M SA + 15 mg kg\(^{-1}\) B | 1.35 ± 0.55 \(^{ab}\) | 1.09 ± 0.36 \(^{abc}\) | 3.60 ± 0.97 \(^{abc}\) | 2.40 ± 0.85 \(^{abc}\) |
| 100 \(\mu\)M SA + 30 mg kg\(^{-1}\) B | 1.15 ± 0.75 \(^{abc}\) | 0.9 ± 0.23 \(^{abc}\) | 3.00 ± 0.85 \(^{abc}\) | 2.00 ± 0.33 \(^{abcd}\) |
| 150 \(\mu\)M SA + 00 mg kg\(^{-1}\) B | 1.25 ± 0.82 \(^{ab}\) | 0.99 ± 0.45 \(^{abc}\) | 3.90 ± 0.21 \(^{abc}\) | 2.60 ± 0.43 \(^{abc}\) |
| 150 \(\mu\)M SA + 15 mg kg\(^{-1}\) B | 1.00 ± 0.71 \(^{abc}\) | 0.75 ± 0.35 \(^{bc}\) | 3.25 ± 0.85 \(^{abc}\) | 2.17 ± 0.55 \(^{abcd}\) |
| 150 \(\mu\)M SA + 30 mg kg\(^{-1}\) B | 0.95 ± 0.66 \(^{bc}\) | 0.71 ± 0.32 \(^{c}\) | 2.85 ± 0.79 \(^{abc}\) | 1.90 ± 0.65 \(^{bcd}\) |
| LSD 5% | 0.51 | 0.49 | 1.46 | 0.98 |

Values in the same column with different letters in superscript differ significantly.

### 2.3. Photosynthetic Pigments

Elevated B levels significantly reduced the photosynthetic pigment contents of maize seedlings. It was observed that the chl \(a\) contents were reduced with increasing B treatment levels. The 30 mg kg\(^{-1}\) B imposed deteriorative effects and reduced the chl \(a\) contents effectively (Figure 3a). An improvement in the chl \(a\) concentration was recorded through priming seeds with SA. Seeds primed with 100 \(\mu\)M of SA expressed the maximum chl \(a\) content, which suggests reduced toxicity effects.
priming improved the carotenoid contents by reducing the drastic effects of B toxicity. A non-significant change in the carotenoid contents was observed in 50 and 150 µM SA primed seeds, while 100 µM SA significantly enhanced the carotenoid contents as compared to the control (Figures 2 and 3c).

2.4. Anthocyanin

The anthocyanin contents increased with increasing the levels of B toxicity. Significant increases in the anthocyanin contents were observed in plants treated with 15 and 30 mg kg⁻¹ B. There was a 33.33% increase in anthocyanin contents when 30 mg kg⁻¹ soil B was applied, as compared to the control. The SA priming reduced the anthocyanin contents overall, but only 100 µM of SA caused a 47.5% reduction in the anthocyanin contents (Figure 2 and Figure 3c).

2.5. Ascorbic Acid

The toxic effects of B increased the ASA contents of maize seedlings. The B treatment of 30 mg kg⁻¹ significantly increased the ASA content up to 44% as compared with the control (Figure 4). Priming with SA reduced the B toxic effects. Only 100 and 150 µM of SA effectively mitigated the toxic effects on plants grown in pots containing 15 mg kg⁻¹ B. However, under a high boron toxicity, only 100 µM of SA significantly reduced the ASA content up to 36% as compared to the control, as shown in Table 3, Figure 2.

Figure 4. Effect of SA on the K (A,B), Ca (C,D) and nitrate (E,F) contents in the roots and leaves of maize cultivar Gohar-19 under varying B toxicity levels.
Table 3. Effect of SA (0, 50, 100 and 150 µM) on the leaf ascorbic acid, \( \text{H}_2\text{O}_2 \), proline and glycine betaine contents of maize cultivar Gohar-19 under varying B toxicity levels (0, 15 and 30 mg kg\(^{-1} \)).

| Treatment | Leaf ASA (µmoles/g FW) | Leaf \( \text{H}_2\text{O}_2 \) (mg/g FW) | Leaf Proline (µMole/g FW) | Leaf GB (µg/g FW) |
|-----------|------------------------|------------------------------------------|--------------------------|-------------------|
| 00 µM SA + 00 mg kg\(^{-1} \) B | 210 ± 1.53 | 0.80 ± 0.15 | 32.00 ± 0.5 | 1.60 ± 0.05 |
| 00 µM SA + 15 mg kg\(^{-1} \) B | 320 ± 1.15 | 1.80 ± 0.06 | 36.50 ± 0.5 | 1.80 ± 0.03 |
| 00 µM SA + 30 mg kg\(^{-1} \) B | 375 ± 0.58 | 2.50 ± 0.10 | 46.00 ± 0.5 | 1.90 ± 0.03 |
| 50 µM SA + 00 mg kg\(^{-1} \) B | 209 ± 1.00 | 0.70 ± 0.05 | 33.00 ± 0.5 | 1.70 ± 0.05 |
| 50 µM SA + 15 mg kg\(^{-1} \) B | 300 ± 0.58 | 1.34 ± 0.03 | 37.00 ± 0.58 | 2.50 ± 0.06 |
| 50 µM SA + 30 mg kg\(^{-1} \) B | 360 ± 0.58 | 2.40 ± 0.03 | 47.00 ± 0.50 | 2.50 ± 0.08 |
| 100 µM SA + 00 mg kg\(^{-1} \) B | 200 ± 1.00 | 0.64 ± 0.02 | 33.00 ± 0.29 | 1.80 ± 0.05 |
| 100 µM SA + 15 mg kg\(^{-1} \) B | 260 ± 0.58 | 1.00 ± 0.03 | 46.00 ± 0.76 | 2.00 ± 0.05 |
| 100 µM SA + 30 mg kg\(^{-1} \) B | 240 ± 0.58 | 1.90 ± 0.03 | 58.00 ± 0.29 | 2.80 ± 0.05 |
| 150 µM SA + 00 mg kg\(^{-1} \) B | 211 ± 1.15 | 0.78 ± 0.02 | 37.00 ± 0.29 | 1.90 ± 0.05 |
| 150 µM SA + 15 mg kg\(^{-1} \) B | 276 ± 0.76 | 1.45 ± 0.05 | 37.00 ± 0.58 | 2.50 ± 0.09 |
| 150 µM SA + 30 mg kg\(^{-1} \) B | 335 ± 0.29 | 2.20 ± 0.20 | 48.00 ± 0.29 | 2.40 ± 0.08 |
| LSD 5% | 0.51 | 0.49 | 1.46 | 0.98 |

2.6. \( \text{H}_2\text{O}_2 \) Concentration

Increasing the B toxicity enhanced the \( \text{H}_2\text{O}_2 \) content effectively. The \( \text{H}_2\text{O}_2 \) content was highly affected by 30 mg kg\(^{-1} \) B in soil. The effective treatment in term of reducing the \( \text{H}_2\text{O}_2 \) content was 100 µM SA, which decreased the \( \text{H}_2\text{O}_2 \) content up to 84% (Table 3, Figure 2).

2.7. Proline Content

The toxic effects of B significantly increased the proline contents. The B treatment of 30 mg kg\(^{-1} \) increased the proline contents up to 43.75% as compared to the control. Priming with SA remained productive in reducing the B toxic effects. Only 100 and 150 µM SA effectively mitigated the toxic effects in plants grown in pots containing 15 mg kg\(^{-1} \) B. However, under a high boron toxicity, only 150 µM SA significantly reduced the toxic effects by increasing the proline content in comparison with the control, as shown in Table 3, Figure 2.

2.8. Glycine Betaine

An outstanding improvement was noted in the leaf GB contents of plants grown under B stress. Exogenous applications of SA further increased the GB content in the leaves of plants experiencing B toxicity stress. All the treatments of SA affected the GB level, however 100 µM SA increased the GB contents up to 100% under 30 mg kg\(^{-1} \) of B treatment as compared to the control, as indicated in Table 3, Figure 2.

2.9. Potassium Content

An increase in the K contents was observed in response to the B toxicity. Applications of SA reduced the K contents and a maximum reduction of up to 27.8% was noted in the plants primed with 100 µM of SA. The K uptake and accumulation exhibited quite similar patterns in the plant root and shoot (Figures 2 and 4a,b).

2.10. Calcium Content

Boron toxicity significantly influenced the Ca accumulation in the root of the maize cultivar Gohar-19. The Ca content was reduced at a lower SA treatment level as compared to the control. With increasing the SA concentration up to 100 and 150 µM, higher increments in the Ca contents relative to the control were recorded. A total of 100 µM SA treatment was found to be effective in reducing toxic effects by lowering the Ca content up to 28.7% in the 30 mg kg\(^{-1} \) B group. The Ca
accumulation in the plant leaves was reduced due to the B toxicity even at high concentrations of SA applications. No significant effects of SA on Ca contents were observed (Figures 2 and 4a,b).

2.11. Nitrate Concentration

An increase in the nitrate contents was observed with an increasing B level. Various SA concentrations (0, 50, 100 and 150 µM) reduced the nitrate content. The application of 100 µM of SA reduced the nitrate content up to 20% in the most destructive B treatment of 30 mg kg\(^{-1}\) (Figures 2 and 4a,b).

The relationship between B toxicity and the morpho-physiological attributes of maize under SA application is illustrated in Figure 2.

A Pearson correlation analysis was conducted to quantify the interactive effects of B toxicity and SA application on plant growth and biomass, chlorophyll contents, lipid peroxidation and the antioxidant and nutrient uptake of maize (Figure 2). B toxicity was negatively correlated with plant growth and biomass, photosynthetic pigments, oxidative stress and antioxidative response. Chlorophyll contents were positively correlated with plant biomass accumulation. Positive correlations were also identified among growth attributes and K, Ca and nitrate contents.

2.12. Principal Component Analysis

The combinatorial effect of B toxicity and SA application was evaluated on important attributes of maize plants by the synthesis of the score and loading plots of PCA, as presented in Figure 4. All the three applied B treatments with and without SA were successfully dispersed by the first two principal components (Figure 1a). The maximum variance among all the components was based on extracted components—i.e., PC1 (Dim1) and PC2 (Dim2), where component Dim1 contributed 69.9% while the contribution of Dim2 was 18.9% (Figure 1b).

3. Discussion

The only non-metallic element of group 13 of the periodic Table is B, which exhibits a trivalent oxidation state. Naturally, B is present in the form of borate, boric acid and borosilicate mineral. In the Earth crust, the B level varies between 1–500 mg kg\(^{-1}\) and 2–100 mg kg\(^{-1}\) as per the geographical region and soil composition status [32].

B has a considerable importance due to its supportive role in plant development and growth. It helps in the processes of cell division, the formation of cell wall and the elongation of cells [33]. However, B causes toxic effects at very high or very low levels. B toxicity mostly co-exists together with some other abiotic stresses—e.g., salt and drought stress [34]. A high B toxicity reduces the plant growth and other attributes.

El-Shazoly [25] conducted a study to describe the SA effects on B toxicity stress in wheat. The results of such a study were in agreement with those of the present study. The SA application also enhanced the root and shoot length, supporting the findings of the previous works [35]. It has been reported that a high level of B causes abnormal cell division in the root meristematic zone [35], hypoderms formation and suberin deposition [36], thus limiting plant growth and development. The excess of B also causes cytotoxic effects during mitosis, which in turn reduces the root and shoot biomass [37,38]. In the present study, 100 µM of SA significantly enhanced the plant biomass by mitigating the B toxicity. Sarafi et al. [39] reported that the B toxicity reduces the plant dry weight up to 48%, the number of leaves and the root dry weight in the pepper plant (Capsicum annuum). In this study, the applications of melatonin (MEL) and resveratrol (RES) were studied, where a treatment of 100 µM of RES and 1 µM of MEL effectively reversed the reductions in fresh and dry weights under B toxic effects, respectively. Eser and Aydemir [22] reported that kinetin application prevented the B-induced reductions in the plant fresh and dry weight of wheat plants under B stress. Moreover, the high B content (50 mg kg\(^{-1}\)) in soil reduced the shoot fresh and dry weight of tomato plants [40]. It has been particularized that B toxicity causes the down-regulation of the photosystem biochemical
components and the inhibition of the electron transport rate [41], thus lowering the activity of carbon fixation enzymes [41,42]. High levels of B can also cause the root growth inhibition, accompanied by a decrease in plant dry weight [43]. The reduction in root growth may be due to the intense lignification of cell wall [44]. However, it has been reported that lignification is not mainly responsible for root growth inhibition, but is rather a defensive attribute for reducing B uptake [36].

The high B level (30 mg kg$^{-1}$) reduced the photosynthetic pigments biosynthesis. However, SA application reversed these negative effects, and the most effective treatment was 100 µM of SA. The findings of present study are in line with those of El-Shazoly [25]. Plant growth and development are considerably dependent on photosynthetic pigments. It has been reported that the inhibition of plant growth by B stress is associated with reduced photosynthetic pigments. Indeed, the present study indicated that the biosynthesis of chlorophyll and carotenoid was negatively affected by B toxicity stress. Our results depicted a negative relationship between the biosynthesis of photosynthetic pigments and the increasing applied B stress regimes. This decline in photosynthetic pigments might be owing to H$_2$O$_2$ accumulation, which damages the photosynthetic reaction centers. Papadakis et al. [45] reported that one of the possible reasons for the reduction in photosynthetic activity in plants grown under excess of B was the structural damage of thylakoids. In general, SA, being a versatile molecule, interacts with other hormones to promote the induction of enzymes and antioxidants to alleviate the toxic effects of stress [46].

Regarding the mineral contents, Kaya and Ashraf [46] described that B toxicity significantly reduces the N, K and Ca contents in tomato. However, nitric oxide application induced the level of minerals and minimized the B toxicity effects. El-Shazoly [25] described that a low level of boron (3 mg kg$^{-1}$ soil) does not affect the K content in wheat plants, however a high level could decrease it. Moreover, the Ca level was reduced due to the B toxicity (3 mg kg$^{-1}$ soil), but increased upon SA and thiamin application.

High levels of B increased the anthocyanin contents in sweet basil (Ocimum basilicum L.) plants, indicating possible stress responses or poor nutrient mobilization from the plant root [47]. We also found that B stress elevated the anthocyanin levels in the root and shoot of maize cultivar Gohar-19. The application of SA at higher levels reduced the stress level. Additionally, the reduction in anthocyanin content in plants treated with SA predicts a reduction in stress severity.

Ascorbic acid is an important antioxidant and scavenger of ROS [48–50]. The ASA content was significantly affected by B stress in the present study. Abiotic stresses result in a higher accumulation of ASA than that of other stress. Increased ROS scavenging enzymatic and nonenzymatic activity by excessive B concentrations has already been reported in barley, chickpea, tomato and grapes [51–54].

In general, plants up-regulate the synthesis of different osmolytes in cytosol and other organelles to cope with the deleterious effects of environmental stresses. Proline and GB are considered to be key osmolytes for the osmotic adjustment. Proline, being a secondary metabolite, plays a key role in stress tolerance as an antioxidant and osmoprotectant [55]. Stress-related genes are activated by GB to detoxify ROS and protect photosynthetic machinery under stressed conditions [56]. In the current study, SA applications triggered the accumulation of proline and GB to cope with the B toxicity effects through scavenging ROS and the activation of the antioxidant defense systems.

The PCA results depicted that the application of salicylic acid had a significant ameliorative effect for B toxicity on the studied parameters of maize plants. The same effects of SA have been reported in salt-stressed sunflower plants [57]. Overall, the applied B stress exerted hazardous effects on the growth and ecophysiological attributes of maize. These results were in accordance with the findings of previous reports which have reported decreases in the growth traits of various plant species grown under environmental stress conditions [58–66]. Based on the findings of the current study, we conclude that SA applications improved the growth of B-stressed maize plants at the seedling stage through increasing the biosynthesis of photosynthetic pigments, osmolytes and antioxidants. The high level of B deteriorates photosystem II centers, as the low levels of chlorophyll and carotenoids are linked with biomass reduction caused by B toxicity. High levels of osmoprotectants such as proline may
act as signaling molecules for scavenging ROS, thus stabilizing the membrane structures as well as 
cascading the stress-tolerant gene expression. Further studies at the molecular level may elaborate 
the comprehensive understanding lying behind these modulations of SA against B toxicity in maize. 
The induction of B toxicity tolerance in maize plants after SA application is also associated with 
antioxidant defense system improvement.

4. Materials and Methods

4.1. Plant Material and Experimental Design

Seeds of maize (cultivar Gohar-19) were obtained from the Maize & Millets Research Institute, 
Yusafwala, Sahiwal, Pakistan, and the experiments were conducted at the Department of Botany, 
Government College University Faisalabad, Pakistan. The seeds were surface-sterilized using a 1% 
sodium hypochloride solution for 5 min. Surface-sterilized seeds were thoroughly washed with 
distilled water and air-dried for 12 h. These seeds were soaked in 0, 50, 100 and 150 
µM of SA solution 
for 12 h. Plastic pots were filled with 1 kg of washed and air-dried sand at the botanical garden, 
Government College University Faisalabad, and a 100% field capacity was maintained in pots by 
adding boron-free water. The experiment was carried out in a completely randomized design (CRD) 
with three replicates. Ten seeds were sown in each pot. After 5 days of germination, 5 seedlings 
were selected based on their similarity in size and vigor. B stress was applied using Nable’s solution 
containing boric acid (H\textsubscript{3}BO\textsubscript{3}) by maintaining pH at 5.7. The final B concentrations of 0, 15 and 
30 mg Kg\textsuperscript{-1} soil were maintained in each pot for one week. After one week of B stress application, 
the plants were harvested and stored in a freezer for further analysis.

4.2. Morphological Parameters and Plant Biomass

The root and shoot lengths were measured for individual plants using a meter scale. The root and 
shoot fresh and dry weight, after drying at 70 °C for 72 h, was calculated using the same weight balance.

4.3. Physiological and Biochemical Analysis

Different physio-biochemical analyses were carried out as described below.

4.3.1. Photosynthetic Pigments

Contents of chlorophyll a, b and carotenoids were determined using a 0.5 g fresh leaf sample. The Arnon [67] method with minor modifications was used for the determination of photosynthetic pigments. The collected sample was ground in 15 mL of 85% acetone and centrifuged at 10,000× g for 15 min. The absorbance of the supernatant was measured at 480, 645 and 663 nm using a spectrophotometer (Hitachi U-2001, Tokyo, Japan).

4.3.2. Anthocyanin Content

The anthocyanin content was measured as reported previously [68]. Fresh root and shoot samples (0.1 g each) were ground separately in 2 mL of 1% acidified methanol (1 mL HCl and 99 mL methanol), then the extract was heated up to 50 °C for one in a water bath. The anthocyanin content was then quantified using a spectrophotometer (Hitachi U-2001, Tokyo, Japan) at 535 nm.

4.3.3. Ascorbic Acid Content

The protocol of Mukherjee and Choudhuri [68] was followed to determine the ascorbic acid content. Root and shoot fresh samples (0.1 g) were taken and ground in 5 mL of trichloroacetic acid (TCA) using a pestle and mortar. The extract was filtered, and 4 mL of the homogenate sample was allowed to react with 2 mL of 2% dinitrophenyl hydrazine in an acidified medium. One drop of 10% thiourea (prepared in 70% ethanol) was added and the mixture was then allowed to boil at 100 °C
for 15 min. The absorbance at 530 nm was recorded through UV-spectrophotometer (Hitachi U-2001, Tokyo, Japan) for calculating the ascorbic acid content.

4.3.4. \( \text{H}_2\text{O}_2 \) Content Determination

Shoot samples were extracted in a cold acetone for \( \text{H}_2\text{O}_2 \) content determination. One milliliter of extract was mixed with 1 mL of 0.1% titanium dioxide in 20% sulfuric acid and centrifuged at 8000 rpm for 15 min. The supernatant was then used to measure the absorbance at 415 nm. \( \text{H}_2\text{O}_2 \) content was calculated using a standard curve plotted in a range of 0.5–5 mM \( \text{H}_2\text{O}_2 \) and was expressed as mg g\(^{-1}\) FW.

4.3.5. Potassium Content

Dry samples of plant root and shoot (1 mg) were dissolved in 9 mL of distilled water. Flame photometer was used for the determination of potassium content, as reported previously [69].

4.3.6. Calcium Content

Dry samples of plant root and shoot (1 mg) were added to 9 mL of distilled water. Flame photometer method was used for the determination of the calcium content [69].

4.3.7. Nitrate Content

Dry root and shoot samples were dissolved in 1 mL of TCA (1.24 g TCA + 500 mL \( \text{H}_2\text{SO}_4 \)) and 1 mL of distilled water. For determining the nitrate oxide, the absorbance was recorded at 530 nm using a UV vis spectrophotometer (Hitachi U-2001, Tokyo, Japan).

5. Statistical Analysis

Collected data were subjected to an analysis of variance (ANOVA) using the statistical software Co-Stat version 6.2, Cohorts Software, 2003 (Monterey, CA, USA). The treatment means were equated by the least significant difference method (Fisher’s LSD) at \( p \) value of \( \leq 0.05 \) level. Before applying ANOVA, the data were standardized using means of inverse or logarithmic transformations wherever necessary. The correlations and PCA of the mean values of all variables were found using XL-STAT 2010.

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References

1. Jaradat, A.; Simulated, A. Climate change deferentially impacts phenotypic plasticity and stoichiometric homeostasis in major food crops. *Emir. J. Food Agr.* 2018, 30, 429–442.
2. Khan, N.; Ali, S.; Shahid, M.A.; Kharabian-Masouleh, A. Advances in detection of stress tolerance in plants through metabolomics approaches. *Plant Omics* 2017, 10, 153. [CrossRef]
3. Mantri, N.; Patade, V.; Penna, S.; Ford, R.; Pang, E. Abiotic stress responses in plants: Present and future. In *Abiotic Stress Responses in Plants*; Springer: New York, NY, USA, 2012; pp. 1–19.
4. Bakhsh, A. Engineering crop plants against abiotic stress: Current achievements and prospects. *Emir. J. Food Agr.* 2015, 27, 24–39. [CrossRef]

5. Cheng, S. Heavy metal pollution in China: Origin, pattern and control. *Environ. Sci. Pollut. Res.* 2003, 10, 192–198. [CrossRef]

6. Kellertzer, E. Accumulation of heavy metals in agricultural soils of Mediterranean: Insights from Argolida basin, Peloponnesse, Greece. *Geoderma* 2014, 221, 82–90. [CrossRef]

7. Zhao, F.; Ma, Y.; Zhu, Y.G.; Tang, Z.; McGrath, S.P. Soil contamination in China: Current status and mitigation strategies. *Environ. Sci. Technol.* 2014, 49, 750–759. [CrossRef]

8. Hussain, S.; Zhang, J.H.; Zhong, C.; Zhu, L.F.; Cao, X.C.; Yu, S.M.; Jin, Q.Y. Effects of salt stress on rice growth, development characteristics and the regulating ways. *J. Integr. Agr.* 2017, 16, 2357–2374. [CrossRef]

9. Liu, P.; Yang, Y.S.; Xu, G.D.; Fang, Y.H.; Yang, Y.A.; Kalin, R.M. The effect of molybdenum and boron in soil on the growth and photosynthesis of three soybean varieties. *Plant Soil Environ.* 2005, 51, 197–205. [CrossRef]

10. Khan, N.; Bano, A.; Babar, M.A. The stimulatory effects of plant growth promoting rhizobacteria and plant growth regulators on wheat physiology grown in sandy soil. *Arch. Microbiol.* 2019, 201, 769–785. [CrossRef]

11. Ayvaz, M.; Avci, M.K.; Yamaner, C.; Koyuncu, M.; Guven, A.; Fagerstedt, K. Does excess boron affect the malondialdehyde levels of potato cultivars? *Eurasia J. Biol. Sci.* 2013, 7, 47–53. [CrossRef]

12. Stiles, A.R.; Liu, C.; Kayama, Y.; Wong, J.; Doner, H.; Funston, R.; Terry, N. Evaluation of the boron tolerant grass, Puccinellia distans, as an initial vegetative cover for the phytoremediation of a boron-contaminated mining site in southern California. *Environ. Sci. Technol.* 2011, 45, 8922–8927. [CrossRef] [PubMed]

13. Princi, M.P.; Lupini, A.; Araniti, F.; Longo, C.; Mauceri, A.; Sunseri, F.; Abenavoli, M.R. Boron toxicity and tolerance in plants: Recent advances and future perspectives. In *Plant Metal Interaction*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 115–147.

14. Kayihan, C.; Oz, M.T.; Eyidogan, F.; Yucel, M.; Oktem, H.A. Physiological, biochemical and transcriptomic responses to boron toxicity in leaf and root tissues of contrasting wheat cultivars. *Plant Mol. Biol. Rep.* 2017, 35, 97–109. [CrossRef]

15. Yermiyahu, U.; Ben-Gal, A.; Keren, R.; Reid, R.J. Combined effect of salinity and excess boron on plant growth and yield. *Plant Soil* 2008, 304, 73–87. [CrossRef]

16. Diaz, F.J.; Grattan, S.R. Performance of tall wheatgrass (*Thinopyrum ponticum* cv. Jose) irrigated with saline-high boron drainage water: Implications on ruminant mineral nutrition. *Agric. Ecosyst. Environ.* 2009, 131, 128–136. [CrossRef]

17. Zhang, B.; Chu, G.; Wei, C.; Ye, J.; Li, Z.; Liang, Y. The growth and antioxidant defense responses of wheat seedlings to omethoate stress. *Pestic. Biochem. Phys.* 2011, 100, 273–279. [CrossRef]

18. Khan, N.; Zandi, P.; Ali, S.; Mehmood, A.; Adnan Shahid, M.; Yang, J. Impact of salicylic acid and PGPR on the drought tolerance and phytoremediation potential of Helianthus annus. *Front. Microbiol.* 2018, 9, 2507. [CrossRef]

19. Tavallali, V. Interactive effects of zinc and boron on growth, photosynthesis and water relations in pistachio. *J. Plant Nutr.* 2017, 40, 1588–1603. [CrossRef]

20. Nasim, M.; Rengel, Z.; Aziz, T.; Regmi, B.D.; Saqib, M. Boron toxicity alleviation by zinc application in two barley cultivars differing in tolerance to boron toxicity. *Pak. J. Agric. Sci.* 2015, 52, 151–158.

21. Samet, H.; Cikili, Y.; Dursun, S. The role of potassium in alleviating boron toxicity and combined effects on nutrient contents in pepper (*Capsicum annuum* L.). *Bulg. J. Agric. Sci.* 2015, 21, 64–70.

22. Siddiqui, M.H.; Al-Whaibi, M.H.; Sakran, A.M.; Ali, H.M.; Basalah, M.O.; Faisal, M.; Al-Amri, A.A. Calcium-induced amelioration of boron toxicity in radish. *J. Plant Growth Regul.* 2013, 32, 61–71. [CrossRef]

23. Moustafa-Farag, M.; Mohamed, H.I.; Mahmoud, A.; Elkelish, A.; Misra, A.N.; Gyu, K.M.; Kamran, M.; Ai, S.; Zhang, M. Salicylic Acid Stimulates Antioxidant Defense and Osmolyte Metabolism to Alleviate Oxidative Stress in Watermelons under Excess Boron. *Plants 2020*, 9, 724. [CrossRef] [PubMed]

24. Maghsoudia, K.; Arvinb, M.J. Salicylic acid and osmotic stress effects on seed germination and seedling growth of wheat (*Triticum aestivum* L.) cultivars. *Plant Ecophysiol.* 2010, 2, 7–11.

25. El-Shazoly, R.M.; Metwally, A.A.; Hamada, A.H. Salicylic acid or thiamin increases tolerance to boron toxicity stress in wheat. *J. Plant Nutr.* 2019, 42, 702–722. [CrossRef]

26. Hussain, H.A.; Men, S.; Hussain, S.; Zhang, Q.; Ashraf, U.; Anjum, S.A.; Ali, I.; Wang, L. Maize Tolerance against Drought and Chilling Stresses Varied with Root Morphology and Antioxidative Defense System. *Plants 2020*, 9, 720. [CrossRef] [PubMed]
51. Gunes, A.; Soylemezoglu, G.; Inal, A.; Bagci, E.G.; Coban, S.; Sahin, O. Antioxidant and stomatal responses of grapevine (Vitis vinifera L.) to boron toxicity. *Sci. Hortic.* 2006, 110, 279–284. [CrossRef]

52. Cervilla, L.M.; Blasco, B.; Rios, R.; Romero, L.; Ruiz, J. Oxidative stress and antioxidants in tomato (Solanum lycopersicum) plants subjected to boron toxicity. *Ann. Bot.* 2007, 100, 747–756. [CrossRef]

53. Ardic, M.; Sekmen, A.H.; Tokur, S.; Ozdemir, F.; Turkan, I. Antioxidant responses of chickpea plants subjected to boron toxicity. *Plant Biol.* 2009, 11, 328–338. [CrossRef] [PubMed]

54. Khan, N.; Bano, A.; Curá, J.A. Role of Beneficial Microorganisms and Salicylic Acid in Improving Rainfed Agriculture and Future Food Safety. *Microorganisms* 2020, 8, 1018. [CrossRef] [PubMed]

55. Shaki, F.; Maboud, H.E.; Niknam, V. Effects of salicylic acid on hormonal cross talk, fatty acids profile, and ions homeostasis from salt-stressed safflower. *J. Plant Interact.* 2019, 14, 340–346. [CrossRef]

56. Ahmad, P.; Abdel Latef, A.A.; Hashem, A.; Abd-Allah, E.F.; Guzel, S.; Tran, L.S.P. Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Front. Plant Sci.* 2016, 7, 347. [CrossRef]

57. Hossain, M.A.; Hoque, M.A.; Burritt, D.J.; Fujita, M. Proline Protects Plants against Abiotic Oxidative Stress: Biochemical and Molecular Mechanisms. In *Oxidative Damage to Plants*; Academic Press: Cambridge, MA, USA, 2014; pp. 477–522.

58. El-Esawi, M.A.; Elkelish, A.; Soliman, M.; Elansary, H.O.; Zaid, A.; Wani, S.H. *Serratia marcescens* KM4 Improves Cadmium Stress Tolerance and Phyto remediation Potential of Soybean Through Modulation of Osmolytes, Leaf Gas Exchange, Antioxidant Machinery, and Stress-Responsive Genes Expression. *Antioxidants* 2020, 9, 43. [CrossRef]

59. Abdelaal, K.A.; El-Maghraby, L.M.; Elansary, H.; Hafez, Y.M.; Ibrahim, E.I.; El-Banna, M.; El-Esawi, M. Treatment of Sweet Pepper with Stress Tolerance-Inducing Compounds Alleviates Salinity Stress Oxidative Damage by Mediating the Physio-Biochemical Activities and Antioxidant Systems. *Agronomy* 2020, 10, 26. [CrossRef]

60. Alhaithloul, H.A.; Soliman, M.H.; Ameta, K.L.; El-Esawi, M.A.; Elkelish, A. Changes in Ecophysiology, Osmolytes, and Secondary Metabolites of the Medicinal Plants of *Mentha piperita* and *Catharanthus roseus* Subjected to Drought and Heat Stress. *Biomolecules* 2020, 10, 43. [CrossRef]

61. El-Esawi, M.A.; Alaraidh, I.A.; Alsaahi, A.A.; Alzahrani, S.M.; Ali, H.M.; Alayafi, A.A.; Ahmad, M. *Serratia liquefaciens* KM4 Improves Salt Stress Tolerance in Maize by Regulating Redox Potential, Ion Homeostasis, Leaf Gas Exchange and Stress-Related Gene Expression. *Int. J. Mol. Sci.* 2018, 19, 3310. [CrossRef]

62. Zafar-ul-Hye, M.; Naem, M.; Danish, S.; Khan, M.J.; Fahad, S.; Datta, R.; Brtnicky, M.; Kintl, A.; Hussain, G.S.; El-Esawi, M.A. Effect of Cadmium-Tolerant Rhizobacteria on Growth Attributes and Chlorophyll Contents of Bitter Gourd under Cadmium Toxicity. *Plants* 2020, 9, 1386. [CrossRef]

63. Naveed, M.; Bukhari, S.S.; Mustafa, A.; Ditta, A.; Alamri, S.; El-Esawi, M.A.; Rafique, M.; Ashraf, S.; Siddiqui, M.H. Mitigation of Nickel Toxicity and Growth Promotion in Sesame through the Application of a Bacterial Endophyte and Zeolite in Nickel Contaminated Soil. *Int. J. Environ. Res. Public Health* 2020, 17, 8859. [CrossRef]

64. Imran, M.; Hussain, S.; El-Esawi, M.A.; Rana, M.S.; Saleem, M.H.; Riaz, M.; Ashraf, U.; Potcho, M.P.; Duan, M.; Raiput, I.A.; et al. Molybdenum Supply Alleviates the Cadmium Toxicity in Fragrant Rice by Modulating Oxidative Stress and Antioxidant Gene Expression. *Biomolecules* 2020, 10, 1582. [CrossRef] [PubMed]

65. Ali, Q.; Shahid, S.; Ali, S.; El-Esawi, M.A.; Hussain, A.I.; Perveen, R.; Iqbal, N.; Rizwan, M.; Nasser Alyemeni, M.; El-Serehy, H.A.; et al. Fertigation of Ajwain (*Trachyspermum ammi*) with Fe-Glutamate Confers Better Plant Performance and Drought Tolerance in Comparison with FeSO₄. *Sustainability* 2020, 12, 7119. [CrossRef]

66. Soliman, M.; Alhaithloul, H.A.; Hakeem, K.R.; Alharbi, B.M.; El-Esawi, M.; Elkelish, A. Exogenous Nitric Oxide Mitigates Nickel-Induced Oxidative Damage in Eggplant by Upregulating Antioxidants, Osmolyte Metabolism, and Glyoxalase Systems. *Plants* 2019, 8, 562. [CrossRef] [PubMed]

67. Arnon, D.I. Copper enzymes in isolated chloroplasts. Polypehenoloxidase in *Beta vulgaris*. *Plant Physiol.* 1949, 24, 1–15. [CrossRef] [PubMed]

68. Mukherjee, S.P.; Choudhuri, M.A. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. *Physiol. Plant* 1983, 58, 166–170. [CrossRef]
69. Stark, D.; Wray, V. Anthocyanins. In Methods in Plant Biology; Volume 1: Plant Phenolics; Harborne, J.B., Ed.; Academic Press: London, UK; Harcourt Brace Jovanovich: London, UK, 1989; pp. 325–356.

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