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Glutathione and Proline Attenuates Injury Induced by Boron Toxicity in Wheat

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ABSTRACT

Given the increasing importance of boron (B) toxicity, the present study investigates the roles of glutathione (20 mM, GSH) and proline (20 mM) in the improvement of wheat (*Triticum aestivum* cv. Altındane) resistance to B toxicity (10 mM B). The plants were raised in hydroponic culture with control, B toxicity, B+glutathione, B+proline, glutathione and proline. B+glutathione and B+proline resisted the detrimental influences of B toxicity on the root and shoot lengths, the total chlorophyll, and phenolic contents. B toxicity increased superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), and proline contents while B+glutathione and B+proline applications diminished the mentioned parameters with the exception of the proline content. Individual B toxicity and combined B+glutathione and B+proline applications increased generally total ascorbic acid and glutathione levels in the wheat while the B+proline application decreased GSH content. The B toxicity decreased superoxide dismutase, catalase and guaiacol peroxidase activities in compared with control with the exception of the ascorbate peroxidase activity. Exogenous glutathione and proline augmented all enzyme activities in the wheat exposed to B toxicity. As a result, it can be suggested that glutathione and proline mitigates B toxicity; by preventing oxidative damage in the membrane, by increasing enzymatic and non-enzymatic antioxidant and by decreasing $O_2^{\cdot-}$, H_2O_2, and MDA contents. Glutathione is generally more effective than proline in mitigating the above detrimental effects of B toxicity. The datum submitted in the current work are significant and the first to indicate that effects of exogenous glutathione and proline in improving a culture plant strength to B toxicity.

Keywords: Antioxidants, B toxicity, Glutathione, Proline, Wheat

1. Introduction

Boron (B) toxicity is a noticeable agricultural problem that limits crop productivity in different regions of the world. It can occur in B-rich soils or in soils exposed to B-rich irrigation waters, fertilizers, sewage sludge, or fly ash (Cervilla et al. 2012; Çapar et al. 2016; Nable et al. 1997). B is a unique essential micronutrient (Marschner 1995) that has a narrow concentration between its deficiency and toxicity. B toxicity causes phytotoxicity in plants and considerably decreases crop productivity and quality worldwide; Peru, Chile, Iraq, California, India, Israel, South Australia, West Asia, Morocco, Egypt, Malaysia Jordon, North Africa, Libya, Syria and Turkey (Yau & Ryan 2008).

A thiol tripeptide glutathione (with the formula γ- L-glu- L-cys-gly; a crucial multifunctional metabolite in plants) has been localized and measured in mitochondria, chloroplasts, peroxisomes, the apoplast, and vacuoles of different plant species (Zechmann 2014). Glutathione is a precursor of phytochelatins and is involved in several physiological processes; the arrangement of growth, development and cell cycle regulation, enzymatic regulation and pathogen resistance, abiotic stress tolerance, detoxification of xeneobiotics and heavy metals, protection of thiol groups, regulating the expression of stress defence genes and signaling for sulfur metabolism, regulation of sulfate transport, signal transduction and conjugation of metabolites (Jozefczak et al. 2012; Hasanuzzaman et al. 2019).

Under stress conditions, proline as an excellent osmolyte accumulates in the cytosol. Proline has acts as an antioxidative defense molecule, a metal chelator and a signaling molecule (Xiong & Zhu 2002).

There are some applications, such as phytoremediation and the effective management of water and vegetation that can prevent the harmful effects of B toxicity (Balal et al. 2017; El-Shazoly et al. 2019). As an alternative approach, the application of stimulants such as glutathione and proline enhances oxidative defenses and increase B toxicity-tolerance in plants. For this purpose, this research sought to understand the effects of exogenous glutathione and proline applications on the growth (root-shoot), proline, total chlorophyll and phenolic contents, and oxidant ($O_2^{\cdot -}$, H_2O_2, MDA) accumulation, non-enzymatic [ascorbic acid (AsA) and GSH] and enzymatic antioxidative system [superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX)] in the wheat grown under the oxidative stress related with B toxicity. Additionally, this study provides a strategy to maintain sustainable and friendly production and to provide tolerance to B toxicity-exposed plants. As far as we know, this is the first study demonstrating the effects of exogenous glutathione and proline in the improvement of wheat resistance to B toxicity. Silva et al. (2016) (Proline but not glutathione actively participates in the tolerance mechanism of young *Schizolobium parahyba* var. amazonicum plants exposed to B toxicity) don't investigates effects of exogenous glutathione and proline in their study.

2. Material and Methods

Before germination, the wheat seeds (*Triticum aestivum* cv. Altındane) were surface sterilized in ethyl alcohol (95%) for 2 min and then transferred to sodium hypochlorite activated with 1% Cl for 10 min and washed with sterile dH_2O. The seeds were grown in control conditions for 11 days in a hydroponic culture system in which each container was filled with 2.8 L of Hoagland and Arnon nutrient solution. The hydroponic systems were continuously aerated with an air pump. The pH of hydroponic growth mediums was adjusted to 6.0. On the 11th day, the entire foliar region of the plants was foliar sprayed with glutathione (20 mM) and proline (20 mM) (an approximate volume of 2 mL) or simply with distilled water as a control (repeated three times at 2-h intervals). All the experiments were kept in a growth room at 22±2 ^{o}C under fluorescent white light (100 µmol m^{-2} s^{-1} at leaf level) with a 14-h light/10-h dark photoperiod. Afterward the plants were exposed to B toxicity stress [10 mM, boric acid (H_3BO_3)] for 3 days (preliminary studies showed that 20 mM glutathione and proline solutions were optimum to increase B tolerance in the wheat seeds). On the 14th day after the treatment began, all the plants were harvested (the tissues were rinsed three times in distilled water after harvesting) and analysed.

The total chlorophyll content was assayed according to Witham et al. (1971). The total soluble phenolic contents were determined according to Dewanto et al. (2002). Proline was determined according to the method described by Bates et al. (1973). Each experiment was repeated at least three times.

The superoxide level was measured according to Elstner & Heupel (1976). Sodium nitrite was used as a standard solution to calculate the production rate of superoxide anion. The hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents were measured according to Loreto & Velikova (2001). The MDA level was calculated using an extinction coefficient of (ε=155 mM^{-1} cm^{-1}) and was expressed as µmol g^{-1} fresh mass.

For the enzyme assays (SOD, CAT, GPX and APX), the leaf tissues (0.5 g) were homogenized in liquid nitrogen, and 5 mL 10 mmol L^{-1} K–P buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone and 1 mmol L^{-1} disodium ethylenediaminetetraacetic acid was added. The homogenates were centrifuged at 12.000×g and 4 ^{o}C for 15 min, and the supernatant was used to determine enzymes activities. The activity of SOD was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the methods of Agarwal & Pandey (2004). One unit of SOD (unit mg^{-1} protein min^{-1}) was defined as the amount of enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50%. CAT activity was measured at A_{240} for H_2O_2 decomposition rate using the extinction coefficient of (ε=40 mM^{-1} cm^{-1}) according to the method of Gong et al. (2001). One unit of CAT activity (µmol H_2O_2 mg^{-1} protein min^{-1}) was assumed to be the amount of enzyme that decomposed 1 nmol of H_2O_2 per mg of soluble protein per minute. GPX activity was determined in the homogenates by measuring the increase in absorption, and colour development at 470 nm due to the guaiacol (hydrogen donor) oxidation was recorded for 5 min, as described by Yee et al. (2002). The GPX activity (µmol g'col mg^{-1} protein min^{-1}) was estimated by the increase in absorbance of oxiguaiacol at 470 nm (extinction coefficient of ε = 26.6 mM^{-1} cm^{-1}) and was expressed as nmol of guaiacol consumed per mg of soluble protein per minute. APX activity was determined by monitoring the decrease in absorbance at 290 nm as reduced AsA was oxidized (extinction coefficient of ε = 2.8 mM^{-1} cm^{-1}) according to the method described by Nakano & Asada (1981). The APX activity (µmol AsA mg^{-1} protein min^{-1}) was calculated as the amount of enzyme that oxidizes 1 nmol of ascorbate consumed per mg of soluble protein per minute (ε=2.8 mM^{-1} cm^{-1}).

Tissue samples (0.2 g) were powdered in liquid nitrogen and then 2 mL of 5% (w/v) trichloroacetic acid was added and homogenized. After centrifugation at 12.000×g for 10 min at 4 ^{o}C, the supernatant was collected to determine the total AsA and GSH contents. The total AsA content (AsA+DHA) was estimated as described by Mukherjee & Choudhuri (1983). The reduced total GSH (µmol g^{-1})

content was determined according to Griffith (1980). The levels of GSH were estimated as the difference between total GSH and oxidised glutathione (GSSG).

The experiment was organized as a completely random design with three replications. All data obtained were subjected to a two-way analysis of variance (ANOVA) and the significant differences between treatment means were determined by the Duncan multiple range test using the SPSS 20.0 to separate the means. Data are shown as means with three replicates and the significance was determined at the 95% confidence (α=0.05) limits.

3. Results

As shown in Figure 1 and Table 1, B toxicity significantly inhibited the root and shoot lengths of the wheat seedlings by approximately 13.00% when compared to the control. Glutathione and proline applications reverted these B toxicity-based inhibitions to a degree of 8.36% in glutathione and 7.86% in proline. Individual glutathione and proline showed an important increase in the growth parameters compared to their control by 9.05% in glutathione and 2.73% in proline.

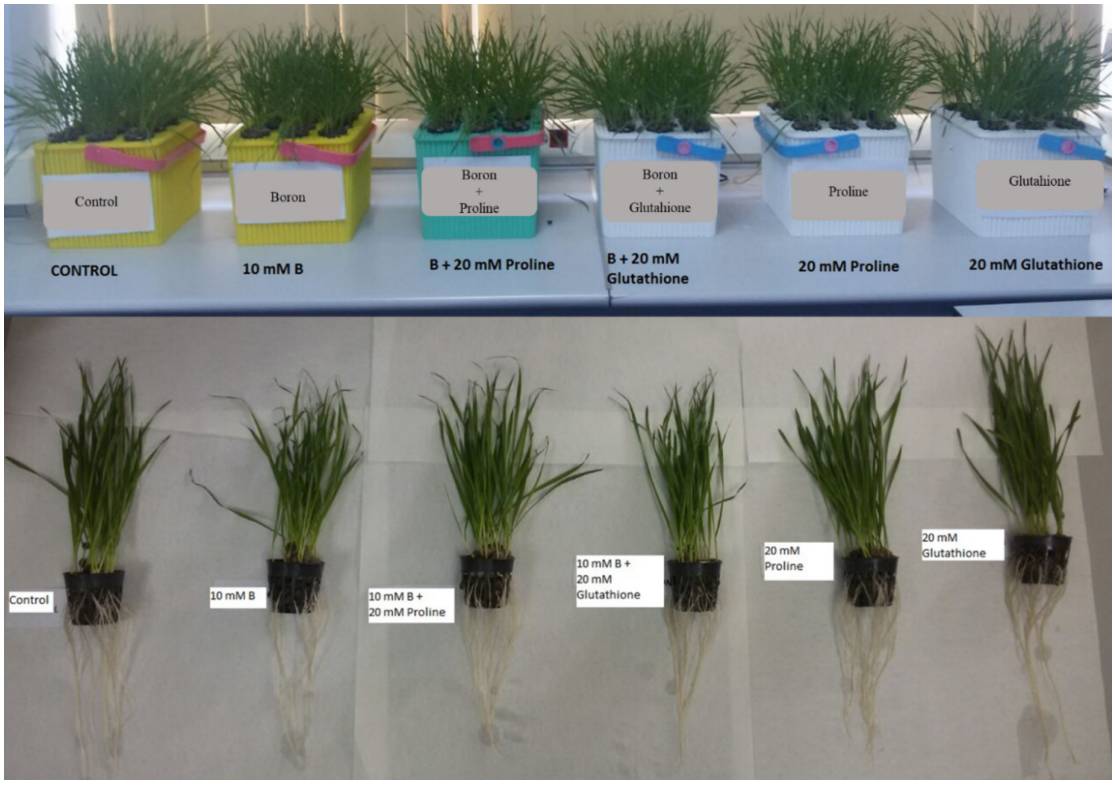

Figure 1- Effects of glutathione and proline on root-shoot lengths on the 14th day the wheat had been exposed to B toxicity in hydroponic culture

The B toxicity markedly reduced the total chlorophyll content by 26.65% compared to the control (Table 1). Glutathione and proline applications remarkably inversed the B toxicity-based inhibition in total chlorophyll content by 22.69% in glutathione and 14.15% in proline. Similarly, individual glutathione and proline applications resulted in significant a rise in total chlorophyll content by 6.88% in glutathione and 2.11% in proline when compared with the control.

The B toxicity markedly reduced total the phenolic substance by 5.61% compared to the control (Table 1). Glutathione and proline applications remarkably inversed the B toxicity-based inhibition in total phenolic substance by 19.80% in glutathione and 26.73% in proline. Similarly, individual glutathione and proline applications resulted in a noticeable elevation in total phenolic substance by 2.80% in glutathione and 11.2% in proline when compared to the control.

The B toxicity remarkably boosted proline content by 40.79% compared to the control (Table 1). B+glutathione and B+proline applications significantly more increased the proline content by 19.63% in glutathione and 36.45% in proline. Similarly, individual

glutathione and proline applications caused an increase in proline content by 3.95% in glutathione and 28.95% in proline when compared to the control.

Table 1- Effects of glutathione and proline on root-shoot lengths, total chlorophyll, total phenolics and proline contents on the 14th day of the wheat exposed to B toxicity in hydroponic culture

Treatments	*Root lengths (cm)*	*Shoot lengths (cm)*	*Total chlorophyll (mg.g^{-1} FW)*	*Total phenolics (µg.g^{-1} FW)*	*Proline (µg.g^{-1})*
Control	20.87±0.55^{c}	22.11±0.34^{c}	7.48±0.25^{c}	10.7±0.34^{e}	76±70.0^{f}
B toxicity	18.06±0.32^{e}	19.24±0.55^{f}	5.48±0.40^{e}	10.1±0.43^{f}	107±5.4^{c}
B+Proline	19.48±0.18^{d}	20.56±0.23^{e}	6.26±0.15^{d}	12.8±0.28^{a}	146±6.6^{d}
B+Glutathione	19.57±0.34^{d}	21.44 ±0.30^{d}	6.73±0.26^{d}	12.1±0.14^{b}	128±5.8^{b}
Proline	21.44±0.47^{b}	23.06±0.36^{b}	7.63±0.40^{b}	11.9±0.10^{c}	98±3.9^{a}
Glutathione	22.76±0.66^{a}	24.46±0.66^{a}	7.99±0.33^{a}	11.0±0.18^{d}	79±4.6^{e}

Data are the means ± standard deviation of three independent replicates. The different small letters indicate significant differences at $p<0.05$ according to Duncan's multiple range test at $p<0.05$

As determined in Table 2, $O_2^{\cdot-}$ production and H_2O_2 level were significantly increased by B toxicity respectively 35.50% and 63.76% compared to control in wheat while glutathione and proline applications significantly reduced B toxicity-induced increases in these parameters (12.75% and 28.63% in glutathione and 8.42% and 21.40% in proline, respectively). Similarly, individual glutathione and proline applications caused decreases in $O_2^{\cdot-}$ production and H_2O_2 levels by 1.27% and 12.27% in glutathione and 5.55% and 5.32% in proline, respectively when compared to the control.

When compared with the control, the MDA content aggressively increased up to 27.44% under the B toxicity. However, glutathione and proline applications reduced MDA content by 8.08% and 5.92% ratio, respectively when compared to B toxicity (Table 2). Similarly, individual glutathione and proline applications caused decreases in MDA content by 7.55% in glutathione and 5.49% in proline when compared to the control.

As indicated in Table 2, B toxicity significantly augmented the total AsA and GSH amounts by 2.21-fold, and 46.07%, respectively. B+glutathione and B+proline applications significantly more increased total AsA and GSH contents by 26.42% and 17.10% in glutathione and 14.40% and 7.88% in proline, respectively when compared to the control. Similarly, individual glutathione and proline applications significantly increased the total AsA and GSH contents by 52.45% and 22.73% in glutathione, and 83.04% and 9.37% in proline, respectively.

Table 2- Effects of glutathione and proline on superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), total AsA and GSH contents on the 14th day the wheat exposed to B toxicity in hydroponic culture

Treatments	*$O_2^{\cdot-}$ (nmol.min g^{-1})*	*H_2O_2 (µmol g^{-1})*	*MDA (nmol ml^{-1})*	*Total AsA (nmol g^{-1})*	*Total GSH (nmol g^{-1})*
Control	6.31±0.24^{d}	23.48±1.00^{d}	5.83±0.28^{c}	572±46^{f}	1782±89^{f}
B toxicity	8.55±0.28^{a}	38.45±1.20^{a}	7.43±0.22^{a}	1264±68^{c}	2603±111^{b}
B+Proline	7.83±0.21^{b}	30.22±0.80^{b}	6.99±0.15^{b}	1446±54^{b}	2398±73^{c}
B+Glutathione	7.46±0.24^{c}	27.44±0.60^{c}	6.83±0.19^{b}	1598±77^{a}	3048±146^{a}
Proline	5.96±0.44^{e}	22.25±1.20^{e}	5.51±0.36^{d}	1047±39^{d}	1949±51^{e}
Glutathione	6.23±0.56^{d}	20.60±0.94^{f}	5.39±0.24^{e}	872±74^{e}	2187±99^{d}

GSH: Glutathione, AsA: Ascorbic acid. Data are the means ± standard deviation of three independent replicates. The different small letters indicate significant differences at $p<0.05$ according to Duncan's multiple range test at $p<0.05$

As shown in Table 3, B toxicity remarkably reduced SOD, CAT and GPX activities by 29.4%, 18.2% and 36.2% respectively while increasing APX activity by 52.2%. B+glutathione and B+proline applications remarkably augmented SOD, CAT, GPX and APX activities by 18.1%, 5.6%, 27.4% and 41.3% in glutathione, and 26.2%, 8.8%, 14.1% and 23.0% in proline, respectively when compared

to the control. Similarly, individual glutathione application increased SOD and APX activities by 8.3% and 24.8% and decreased CAT and GPX activities by 8.2% and 21.1%, respectively. Individual proline applications increased SOD and APX activities by 5.6% and 10.1% and decreased CAT and GPX activities by 3.4% and 14.7%, respectively.

Table 3- Effects of glutathione and proline on the enzymatic antioxidative system (SOD, CAT, GPX and APX enzyme activities) on the 14th day the wheat exposed to B toxicity in hydroponic culture

Treatments	*SOD (unit mg^{-1} protein min^{-1})*	*CAT (µmol H_2O_2 mg^{-1} protein min^{-1})*	*GPX (µmol g'col mg^{-1} protein min^{-1})*	*APX (µmol AsA mg^{-1} protein min^{-1})*
Control	27.46±1.00^{c}	39.46±1.20^{a}	1452±79^{a}	14.09±2.30^{f}
B toxicity	19.38±0.90^{f}	32.28±0.78^{f}	926±42^{f}	21.44±1.40^{e}
B+Proline	24.46±1.60^{d}	35.11±1.28^{d}	1057±54^{e}	26.37±1.10^{b}
B+Glutathione	22.89±1.40^{e}	34.07±1.14^{e}	1180±66^{c}	30.30±3.50^{a}
Proline	28.99±2.10^{b}	38.12±2.00^{b}	1239±75^{b}	15.52±1.90^{e}
Glutathione	29.75±1.30^{a}	36.22±1.60^{c}	1145±38^{d}	17.58±1.00^{d}

SOD: Superoxide dismutase, CAT: Catalase, GPX: Guaiacol peroxidase, APX: Ascorbate peroxidase. Data are the means ± standard deviation of three independent replicates. The different small letters indicate significant differences at $p<0.05$ according to Duncan's multiple range test at $p<0.05$

4. Discussion

In this study, the effect of B toxicity on physiological and biochemical parameters in wheat was investigated in the presence and absence of glutathione and proline supplementation. The B toxicity significantly reduced the root and shoot growth of wheat compared to the control (Figure 1 and Table 1). The reduction in the growth of root and shoot in many plants is the typical syndrome of plants exposed to B toxicity (Landi et al. 2013; Seth & Aery 2017; El-Shazoly et al. 2019). The results are coherent with previous studies demonstrating that B toxicity reversely affects plant fruitfulness by disrupting membrane stability, photosynthetic pathways, photosynthetic pigments and the generation of reactive oxygen species (ROS) (Seth & Aery 2017; El-Shazoly et al. 2019). B toxicity can delay elongation and cell division by binding to ATP and NADPH thereby disturbing their proper working in plant metabolism (Cervilla et al. 2012). B can cause metabolic disruption, also an inhibition and/or dysfunction of the enzyme, and disruption of cell division, and elongation. Exogenous glutathione and proline notably decreased B toxicity-induced inhibitions in the root-stem lengths. The positive effects of glutathione and proline applications on wheat growth occurred not only in the existence of B toxicity but also in non-stressed wheat. The highest valuations in root-shoot lengths were registered at individual glutathione and proline applications (Figure 1 and Table 1). Exogenous GSH application improved the germination and growth of Arabidopsis, tobacco, and pepper under mercury (Hg) stress. Exogenous GSH also conferred Cd, Cu and Zn stress tolerance (Hasanuzzaman et al. 2019). Anjum et al. (2015) reported that plants tailor to excessive situations of abiotic stresses either by synthesizing S-rich complexes, such as reduced glutathione or by osmotic arrangement owing to proline accumulation (proline acts as a nitrogen welding in the course of the plant growth).

B toxicity decreased the total chlorophyll content in the wheat leaves (Table 1). Other studies have also recorded that B toxicity reduces carotenoid, chlorophyll, biomass contents, and the internal carbon dioxide (CO_2) concentration in some plants (El-Shazoly et al. 2019; Silva et al. 2016). B toxicity can cause oxidative stress by producing excess ROS or diminishing pigment biosynthesis through different mechanisms, including altering the enzyme activities and limiting the uptake of elements, thereby decreasing the total chlorophyll content (Catav et al. 2022). The above-mentioned effects are not only related to a specific target of B toxicity at the cellular level but also are the monitored replies of the capability of B to compose complexes to molecules that are involved in different cellular processes. Glutathione and proline applications reversed considerably the B toxicity-based decreases in total chlorophyll content in the presence and absence of B toxicity. Glutathione and proline can contribute to increased stability of the thylakoid membranes and plastid biogenesis. Glutathione and proline can preserve membranes from the destructive effect of B toxicity by increasing the enzyme activities or through overexpression of some responsible genes in photosynthesis, or by maintaining the photosynthetic device (Xia et al. 2009).

Phenolics have multiple biological effects such as antioxidant activity and the markers of stress in plants. B toxicity decreased the total phenolic content, but exogenous glutathione and proline applications increased the total phenolic content in all applications (Table 1). Phenolics can behave as a direct antioxidant, an absorption strainer for radiation, and can restrict the stimulation of chlorophyll under

stress terms for the photosynthetic device (Cervilla et al. 2012). GPX activity and phenolic content decreased in the B toxicity although glutathione and proline applications increased both of them (Tables 1, 3). GPX activity is one of the most "noticeable signs" of the actuation of phenolic metabolism under B toxicity. Exogenous glutathione and proline can augment the pentose phosphate pathway, which protects a high percentage of reduced antioxidants like glutathione for the scavenging of ROS and supply erythrose-4-phosphate for the biosynthesis of phenolic compounds (Lu et al. 2014; Mishra & Heckathorn 2016).

Proline maintains cellular homeostasis and rehabilitates plant toleration under abiotic stresses by scavenging ROS and stabilizing protein structure (Seth & Aery 2017; Catav et al. 2022;). To analyze the relationship between exogenous glutathione and proline applications and B toxicity tolerance, we examined the stress-stimulated proline backlog and the impacts of exogenous glutathione and proline. In this study, B toxicity enhanced the quantity of proline in the wheat (Table 1), This increase may be due to augmented proline biosynthesis and protein degradation and/or the decreased degradation of proline, or by inducing osmotic stress (Seth & Aery 2017; Catav et al. 2022;). Exogenously proline application has been used as a joint way to provide more proline welds and enhancement proline accumulation. That proline increase under B toxicity is significant since a decline in proline can lead to greater lipid peroxidation (MDA) (hence, membrane damage) (Molassiotis et al. 2006). Proline is constantly associated as a ROS antagonist that decreases oxidative stress. Proline also inhibits apoptosis-like cell death. Proline protects the protein structure against denaturation and strengthens the cell membranes during interaction with phospholipids (Silva et al. 2016). Similar results have also been noted in how toxic B concentrations enhanced proline content in peppers and tomatoes (Eraslan et al. 2007). Our study suggests that proline and photosynthetic pigments are both synthesized from a similar substrate. In this way, the decrease in chlorophyll content under the B toxicity could be due to an increase in proline accumulation (Balal et al. 2017). Exogenous glutathione and proline applications also further increased the proline content in all applications (Table 1). In our study, the improved toleration to B toxicity was correlated with an increase in the activities of antioxidant enzymes and in the proline accumulation in wheat like other abiotic stresses, particularly drought and salt. Previous studies also reported that proline accumulation is related to stress toleration under abiotic stresses (Balal et al. 2017; Khan et al. 2015). Exogenous glutathione and proline applications can increase abiotic stress tolerance by mitigating the adverse effects of ROS, preventing MDA in the membranes, and by reducing MDA content in wheat. The effects of glutathione and proline on the reduction of B stress were also partially due to the stimulatory impact on the proline accumulation. Proline can be used by plants as a source of endogenous nitrogen to strengthen the structure of the protein, enzymes, and photosynthetic apparatus and maintain cellular homeostasis under conditions of abiotic stress (Seth & Aery 2017). Thus, glutathione and proline applications may be a possible cause of increased proline accumulation in response to glutathione and proline in B toxicity. It was concluded that the role of proline as a free radical scavenger is more important than simply as an osmolyte in stress reduction (Hong et al. 2000).

To monitor the oxidative damage in wheat under B toxicity, MDA content as used as an indicator of oxidative stress in the different abiotic stresses was calculated (Aghaleh et al. 2011). Our study has shown that MDA content was increased significantly by B toxicity (Table 2). With respect to the existing outcomes, some researchers declared that B toxicity increased MDA content (Balal et al. 2017; El-Shazoly et al. 2019). An increase in MDA content in exposed and non-exposed to B toxicity was alleviated by exogenous glutathione and proline applications decreasing the production of extremely disruptive free radicals (that is by decreasing the $O_2.^-$ and H_2O_2 contents).

The phytotoxicity induced by B toxicity causes the generation of ROS that is required for different biological processes in plants, including cellular proliferation, stress acclimation, and signal transduction (Catav et al. 2022). B toxicity significantly increased $O_2.^-$ production together with enhanced H_2O_2 content in wheat (Table 2). Some researchers also found identified increases in the MDA and H_2O_2 contents and electrolyte leakage in reply to B toxicity (Balal et al. 2017; El-Shazoly et al. 2019). The increased $O_2.^-$, H_2O_2 and MDA levels by B toxicity showed antioxidative systems could not bring concentrations of ROS within normal ranges (at a steady and secure grade for plant oxidative stress). For this reason, glutathione and proline applications may be appropriate for the plants to scavenge extreme ROS and to hinder MDA. Glutathione and proline applications in exposed and non-exposed to stress B toxicity have reduced the $O_2.^-$, H_2O_2, and MDA quantities while augmenting the activities of antioxidant enzymes, suggesting that these two compounds may play a role in the ROS quenching or stopper of ROS production. Our results support those of previous studies (Hasanuzzaman et al. 2019).

The total AsA and GSH amounts as non-enzymatic antioxidants found in chloroplast and cellular compartments significantly increased in B toxicity when compared to the control (Table 2). Cervilla et al. (2007) recorded AsA and GSH intensified with an increment in the B concentration in the culture ambiance, and Mittler (2002) recorded that its concentrations differ in the many abiotic stress conditions. Our results support previous studies that also indicated an increment in total GSH level in pear leaves under B-toxicity (Wang et al. 2011). Some researchers declared that the upregulation or overexpression of AsA-GSH pathway enzymes

and the enhancement of the AsA and GSH levels conferred plants better tolerance to abiotic stresses by reducing the ROS ($O_2^{\cdot-}$ and $OH^{\cdot}$) (Hasanuzzaman et al. 2019). Although defensive measures against stress occasionally occur regardless of glutathione, an increase in the GSH content is interrelated with the capability of plants to stand against B stress-induced oxidative stress (Foyer & Noctor 2011). B+glutathione and B+proline applications significantly increased the total AsA and GSH contents with respect to B toxicity alone (Table 2). The exogenous application of proline upregulates the enzyme activities in the AsA-GSH cycle (such as APX in this study). A multifunctional metabolite GSH is a crucial transport and repository form of non-protein reduced sulphur and has a protective role against the protein degradation sourced by the oxidation of protein thiol groups. GSH also has a key role in intracellular antioxidative defense and protection mechanisms by regenerating ascorbic acid via the AsA-GSH cycle during stress in plants (Hasanuzzaman et al. 2019). In addition, some researchers reported that increasing GSH biosynthesis augmented cadmium and nickel stress toleration in the various plants, and the Arabidopsis mutant that produces less GSH was hypersensitive in both cadmium and copper stresses (Yadav 2010). Increased antioxidant activity resulting from GSH accumulation may preserve many photosynthetic enzyme activities in B toxicity. In this study, less oxidative damage was reported in glutathione and proline applicated plants by increasing non-enzymatic compounds (total AsA and GSH). Finally, the stimulation of GSH synthesis like AsA as the strongest ROS scavenger could prevent B toxicity. These results are partially in accord with Ruiz et al. (2003) that B stress decreased glutathione accumulation in the sunflower leaves but an external application of GSH diminished the harmful effects stimulated by B toxicity.

B toxicity decreased SOD, CAT and GPX activities but increased APX activity in wheat plants (Table 3). The response of enzymatic antioxidants against to B toxicity significantly relies on the species and stresses. A decrease in SOD activity in the B toxicity shows that detoxification of $O_2^{\cdot-}$ radicals by SOD under B toxicity is not enough. In addition, the decreases in CAT and GPX activities suggest that these enzymes are unable to completely detoxify H_2O_2 generated by B toxicity. Our results don't support the previous results that found increases in SOD, CAT and GPX activities in the different plants (Molassiotis et al. 2006; Balal et al. 2017; Sent & Aery 2017). Similarly, an increment of APX activity was observed in citrus while a decline in CAT activity was registered after B toxicity in diverse plants (Oluk et al. 2012). In this study, elevated APX activity (has a higher affinity for H_2O_2) likely aided in maintaining the H_2O_2 amount at normal levels. However, exogenous glutathione and proline applications and individual glutathione and proline treatments enhanced SOD, CAT, GPX and APX activities (Table 3). Exogenous glutathione and proline provide protection by boosting the SOD, CAT and GPX activities against to B toxicity. The increased SOD activity with B+glutathione and B+proline applications may enhance the ability to scavenge $O_2^{\cdot-}$ reducing membrane damage in the wheat. Exogenous glutathione and proline applications suppress H_2O_2 accumulation accompanied by a rise in the CAT and GPX activities. An increase in GPX activity demonstrates its role as a defensive measure to counteract B toxicity-induced oxidative damage in wheat. Thus, we observed that CAT, GPX and APX enzymes in the wheat with B+glutathione and B+proline were successfully detoxified H_2O_2 induced by B toxicity. Other studies have also found that exogenous proline applications significantly enhanced these enzyme activities in some stress conditions (salinity and Cd stresses) (Hayat et al. 2012). Proline is known to act as an enzyme protector under abiotic stress conditions. The roles of glutathione and proline in preventing the deleterious effects of B toxicity can be the result of the activation of key antioxidant enzymes mediated owing to the arrangement at the levels of transcriptional, translational and/or enzyme activities. The results of this and previous studies demonstrate that plants are needed an efficient antioxidative system to protect from oxidative damage and to increase resistance to environmental stresses (Cervilla et al. 2012; Hasanuzzaman et al. 2019).

5. Conclusions

This study suggests that glutathione and proline confer toleration to B toxicity in the wheat by boosting the SOD, CAT, GPX and APX activities and decreasing the $O_2^{\cdot-}$, H_2O_2, and MDA amounts. Therefore, this work provides an efficient eco-friendly route for farmers to minimize the B-toxicity worldwide.

Data availability: Data are available on request due to privacy or other restrictions.

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