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## Research Paper

# Brassinolide ameliorates the detrimental effects of arsenic in tomato: Insights into iron and arsenic absorption, antioxidant capacity, nitrogen, and sulfur assimilation

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A B S T R A C T

The role of brassinosteroids (BRs) in enabling plants to respond effectively to adverse conditions is well known, though the precise mechanism of action that helps plants cope with arsenic (As) toxicity is still difficult to interpret. Therefore we tested the effect of brassinolide (BL) spray (0, 0.5, and 1 mg $\cdot$ L$^{-1}$) on As (0, and 10 mg $\cdot$ L$^{-1}$) stressed tomato defense responses As stress led to the induction of oxidative stress, impaired chlorophyll and nitrogen metabolism, and Fe uptake, in conjunction with a reduction in plant growth and biomass. BL spray, on the contrary, protected the photosynthetic system and helped plants grow better under As stress. This was achieved by controlling the metabolism of chlorophyll and proline and lowering the amounts of methylglyoxal and H$_2$O$_2$ through glyoxalase I and II and antioxidant enzymes. BL decreased arsenic accumulation by directing As sequestration towards vacuoles and increased Fe amount in the leaves and roots by regulating the expression of As (Lsi1 and Lsi2) and Fe (IRT1, IRT2, NRAMP1, and NRAMP3) transporters in As-stressed tomatoes. Furthermore, BL boosted adaptability against As phytotoxicity, while reducing the damaging impacts on photosynthesis, nitrogen metabolism, sulfur asimilation, and Fe absorption. These results offer a solid framework for the development of exogenous BRs-based breeding strategies for safer agricultural development.

*Keywords:* Arsenic toxicity; Brassinosteroid; Fe transporters; Nitrogen metabolism; Sulfur assimilation; Oxidative stress

## 1. Introduction

Anthropogenic activities, such as manufacturing arsenic (As)-containing pesticides, using fossil fuels, and extracting minerals, lead to the transfer of arsenic into water and soil, resulting in environmental damage (Ghorbani et al., 2024a). Contaminated soil or water with As has toxic effects on plant biomass and productivity, posing a severe threat to global food security. The effects of As contamination on food production can vary significantly depending on the crop type and cultivar, as well as the level of As found in the irrigation water and soil. For example, rice is particularly susceptible to As contamination, and even low levels can have a detrimental effect on yields. On the other hand, in tomatoes, an essential component of the

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human diet across the globe, (global consumption of tomatoes is extensive, yielding approximately 183 million tons of fruit at harvest) high levels of As contamination can lead to complete crop failure (Ghorbani et al., 2019, 2022). Because, specific tomato cultivars concentrate As in the fruit to a level of 20 ppm dry weight (Marmiroli et al., 2014). This crop is vulnerable to As-induced stress, negatively affecting plant metabolism (Singh and Upadhyay, 2014; Ghorbani et al., 2024b). The concentration of As in the soil and water irrigation directly affects the As level in both the roots and surface of the tomato plants. Therefore, As accumulation in the fruit is possibly risky for human health (Ghorbani et al., 2024a).

The accumulation of As causes an increase in reactive oxygen species (ROS), which plants counteract by activating their glyoxalase (Gly) and antioxidant systems (Finnegan and Chen, 2012). Ghorbani et al. (2021) conducted a study that demonstrated the disruption of chlorophyll metabolism in plants due to As accumulation. This disruption resulted in detrimental effects on the photosynthesis process, ultimately reducing plant growth and biomass. Another study demonstrated that the presence of toxicity triggered oxidative stress, impeded nutrient absorption, and negatively impacted several cellular components such as lipid membranes and enzyme proteins. The toxicity also caused disturbances in ion equilibrium (Bidi et al., 2021). Therefore, critical steps should be taken to reduce As levels in soil and water. These include engineering solutions such as removing contaminated soil and replacing it with an uncontaminated medium, or using specialized filters and treatments to remove As from water sources. Strategies applying external phytohormones to plants might also be useful. One of the foremost approaches to deal with toxic metals is the detoxification of HMs using compounds containing sulfur, such as glutathione (GSH) and phytochelatins (PCs), which highlights the significance of sulfur assimilation in enhancing plant adaptation to toxic metals (Gill et al., 2012). Metal(oid)s elevate the production of PCs and GSH via sulfur metabolism stimulation, demonstrating the activation of an adaptive system to counteract the phytotoxicity (Emamverdian et al., 2023b). The Arabidopsis seedlings with PC synthesis abnormalities exhibited increased sensitivity to As, Zn, and Cd, highlighting PCs' importance in reducing HM phytotoxicity (Tennstedt et al., 2009). Ghorbani et al. (2021) and Bidi et al. (2021) also linked the increase in PC and GSH contents to a rise in plant adaptability to As and lead stresses. Accordingly, more intrinsic PCs and GSH (sulfur-rich compounds) produced by the sulfur absorption process may substantially improve plant adaptability to As stress. Plant growth and development are also significantly impacted by nitrogen (N) metabolism under HM stress. Once the plant absorbs nitrate ($NO_3^-$), nitrate reductase (NR) and nitrite reductase (NiR) change it into $NO_2^-$ and then $NH_4^+$. This is the first step in N assimilation. Then, the glutamate synthase (GOGAT) converts $NH_4^+$ into glutamate, while the glutamine synthetase (GS) converts it into glutamine (Lea and Miflin 2011). Previous research (Gill et al., 2012) showed that toxic metals make plants absorb nitrogen less effectively, which leads to a loss of biomass. This shows the importance of keeping the best N metabolism (GOGAT, GS, NR, and NiR enzyme activities) when plants are under As stress. Two central As transporters, enabling As uptake into the plant have been discovered to date (Lsi1 and Lsi2). The

Lsi1 protein is located on the outside surface of both exodermis and endodermis cells, At the same time, Lsi2 plays a crucial role in facilitating the translocation of As to the upper parts (Ghorbani et al., 2021).

On the other hand, researchers have identified a family of metal transporters (NRAMPs) as membrane-spanning proteins with approximately 12 transmembrane domains that exhibit high hydrophobicity. This family of metal transporters has gained recognition due to its extensive distribution across many organisms, including plants, mammals, fungi, and bacteria, with several homologous variants (López-Lorca et al., 2022). Some NRAMPs, such as NRAMP1 and NRAMP3, serve as high-affinity Fe transporters (Bozzi and Gaudet, 2021). NRAMP1 and NRAMP3, which exhibit comparable functions in Fe absorption, have also demonstrated the transport capacities of Mn in Arabidopsis (Castaings et al., 2016; Kozak et al., 2022). Additionally, several studies have reported that NRAMPs can transport Ni and Cd as well (Bozzi and Gaudet, 2021; Haider et al., 2024).

IRT1, the first member of the ZIP family, was identified using an Arabidopsis cDNA, which was shown to restore the function of the S. cerevisiae fet3fet4 mutant successfully and had deficiencies in both low- and high-affinity Fe absorption (Lo et al., 2016). The incorporation of IRT1 into S. cerevisiae resulted in the development of a novel, high-affinity pathway for ferrous iron absorption and also led to increased uptake activities for Mn (II) and Zn (II) (Quintana et al., 2022). Furthermore, the IRT2 gene, which belongs to the ZIP family and closely resembles IRT1, encodes a high-affinity Fe transporter (Vert et al., 2001). When Arabidopsis roots lack Fe, they induce the expression of both IRT1 and IRT2. Consequently, IRT1 and IRT2 likely play a pivotal role in the Fe-deficiency response of roots, making them strong candidates for facilitating Fe uptake from the soil.

As steroid hormones, brassinosteroid (BR)s play a fundamental role in controlling key signaling networks in plants, including root, shoot, seedling, fruit growth, senescence, and flowering at low concentrations (Bajguz et al., 2020). By modulating the antioxidant gene transcription and the phytochelatin synthesis (Surgun-Acar and Zemheri-Navruz, 2019), the external application of active analogs of BRs effectively provides adaptation to plant abiotic stresses such as NaCl (Kolomeichuk et al., 2020), HMs (Singh and Prasad, 2017), or drought (Kaya et al., 2019), which indicates possible involvement of the hormone in plant defense against As toxicity. Although the role of BRs in increasing plant tolerance to adverse conditions is widely acknowledged, it is unclear how exactly they help tomato plants withstand As toxicity. Therefore, this study aimed to investigate the potential role of BRs in the mechanisms that enable plants to tolerate the adverse effects of As toxicity. These mechanisms include As absorption and transfer to leaves and reduced uptake of essential elements such as Fe. The expression of transporter genes for As and Fe, and the genes involved in the sequestration of As were analyzed as new targets here. The in vitro effects of brassinolide (BL), a synthetic analog with potent BR activity, were therefore investigated in tomato plants subjected to As stress. The specific molecular and biochemical processes related to the absorption and movement of Fe and As, N and sulfur assimilation, antioxidant and Gly defense systems, as well as proline and chlorophyll metabolisms were examined.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Tomato (*Lycopersicon esculentum* L.) seeds were germinated in sterilized moss peat after surface sterilization. Ten days after germination, seedlings were transplanted into a Hoagland nutrient solution (1/2-strength). Seven days after transplanting the seedlings, different concentrations of As (NaAsO$_2$, 0 and 10 mg · L$^{-1}$) were added to the nutrient solutions. Brassinolide (Solarbio, Beijing, China) (0, 0.5, and 1 mg · L$^{-1}$) was applied via spraying twice during the experimental period (the first application was conducted three days prior to the start of As treatment while the second was administered five days following the initiation of As stress). The concentrations of As and BL were selected based on the results of our previous research (Ghorbani et al., 2021) and other literature (Su et al., 2020; Xu et al., 2023). The treatments used in the study were as follows: T1. control (without As and BL); T2. BL 0.5 mg · L$^{-1}$; T3. BL1 mg · L$^{-1}$; T4. As; T5. As + BL 0.5 mg · L$^{-1}$; T6. As + BL1 mg · L$^{-1}$. The pH of the nutrient solution was set to 6.0 and replaced every four days. The growth conditions of tomato plants were set at 16 h of light, 25 °C/18 °C, and a humidity level of (65 ± 3)%. After 14 days following the As treatment, the samples were harvested. The harvested samples were maintained at 68 °C (48 h) to estimate the total dry weight (Fig. S1).

### 2.2. Photosynthesis parameters

By extracting fresh leaves using 80% acetone and recording the absorbance of the extracts at 645, 663, and 450 nm, carotenoids, chlorophyll *a*, and chlorophyll *b* were obtained, as per Arnon et al. (1949). The $F_v/F_m$ index was determined by a portable LI-6400 fluorometer (LI-COR, USA) in tomato leaves that had been pre-darkened for 20 min. Several photosynthesis-related metrics, including stomatal conductance (Gs), internal CO$_2$ concentration (Ci), net photosynthetic rate (Pn), and transpiration rate (Tr), were measured using the Li-6400 portable photosynthetic system.

### 2.3. Fe, Si, and As contents

The dried leaf and root tissues were incubated in an acidic mixture of HNO$_3$:HClO$_4$ (5:1, V:V). Afterwards, the Fe, Si, and As concentrations of the leaves and roots were measured using an ICP-OES. All experiments were repeated two times.

### 2.4. Proline content

To measure proline contents, fresh leaves were homogenized in 3% sulphosalicylic acid. The resulting extract, after centrifugation, was subjected to a 1-h reaction with glacial acetic acid and acid ninhydrin at 100 °C, followed by termination in an ice bath. The reaction mixture was then extracted with toluene. The toluene containing the chromophore was brought to room temperature, and the optical density was assessed at 520 nm (Bates et al., 1973).

### 2.5. Sulfur-contained defense compounds

Non-protein thiols (NPTs) were quantified using the Ellman reagent (Howe and Merchant, 1992). The cysteine and GSH contents of tomato leaves were measured according to Gaitonde (1967) and Anderson (1985). The leaf content of PCs was computed using the formula PCs = NPTs − GSH.

### 2.6. Oxidative stress parameters and electrolyte leakage (EL)

To quantify H$_2$O$_2$, fresh leaves were homogenized with tri-chloroacetic acid (0.1%, w/v), and after centrifugation, the resulting supernatant was mixed with potassium phosphate buffer (10 mmol · L$^{-1}$, pH 7.0) and KI (1 mol·L$^{-1}$) prior to spectrophotometric analysis at 390 nm (Loreto and Velikova, 2001). The protocol described by Mostofa et al. (2015) was employed to detect methylglyoxal (MG) levels in the leaves based on the N-Acetyl-L-cysteine assay and read at 288 nm. The supernatant of the homogenized solution of fresh leaves with a 1% trichloro-acetic acid extraction was used for measuring malondialdehyde (MDA). Subsequently, the supernatant was mixed with a 5% thiobarbituric acid solution diluted in 20% trichloroacetic acid, and the mixture was placed in a 95 °C water bath for 25 min. After cooling, calculations were performed as per Heath and Packer (1968) based on the 532 nm and 600 nm readings. The electrical conductivity (EC) of the leaf discs was recorded by setting samples in deionized water at 10 °C for 24 h (S1) and in a water bath at 95 °C for 15 min (S2). The following formula was used to calculate EL: EL = (EC$_{S1}$/EC$_{S2}$) × 100 (%).

### 2.7. Antioxidant and Gly defense systems

A mixture including 0.5% TX-100, 100 mmol · L$^{-1}$ K–P phosphate buffer (pH 7.0), and 1% polyvinylpyrrolidone was used for enzyme extraction.

The reaction solution containing enzymatic extract along with potassium phosphate buffer (50 mmol · L$^{-1}$, pH 7.6), NBT (75 μmol · L$^{-1}$), methionine (13 mmol · L$^{-1}$), and EDTA (0.1 mmol · L$^{-1}$) was used to measure the activity of the enzyme superoxide dismutase (SOD). After adding lactochrome and light exposure at 25 °C for 30 min, the solution was read at 560 nm (Dhindsa and Matowe, 1981). Catalase (CAT) activity was determined by monitoring the absorbance changes at 240 nm resulting from the decomposition of H$_2$O$_2$ in a reaction mixture consisting of an enzymatic extract, potassium phosphate buffer (50 mmol · L$^{-1}$, pH 7.0), and H$_2$O$_2$ (Aebi, 1984). Ascorbate peroxidase (APX) activity in the leaves was determined by measuring the drop in absorbance at 290 nm resulting from ascorbate oxidation (Nakano and Asada, 1981). The activity of glutathione reductase (GR) was quantified with the method by monitoring the oxidation level of NADPH through changes in absorption at 340 nm over 1 min (Foyer and Halliwell, 1976).

The method of Hossain et al. (1984) was used to measure the activity of monodehydroascorbate reductase (MDHAR). The oxidation of NADH at 340 nm was monitored by measuring a decline in absorbance. Dehydroascorbate reductase (DHAR) activity was assessed under ambient conditions by measuring absorbance at 265 nm, in accordance with the method described by Nakano and Asada (1981). The spectrophotometric measurement of the enhanced absorbance resulting from the GSH-dependent generation of AsA was performed. By recording the rise in absorbance of the reaction mixture enzyme extract, potassium phosphate buffer (15 mg · L$^{-1}$, pH 7), MG (3.5 mg · L$^{-1}$), and GSH (1.7 mg ·

$L^{-1}$) at 240 nm over 1 min, the activity of Gly I was determined, as outlined by Hossain et al. (2010). The activity of Gly II was also defined by quantifying GSH formation through changes in absorbance at 412 nm (Principato et al., 1987).

### 2.8. Enzyme activities in proline and chlorophyll metabolisms and sulfur assimilation pathway

A potassium phosphate buffer (50 mg · $L^{-1}$, pH 7.0) including 1 mg · $L^{-1}$ ascorbic acid, 100 mg · $L^{-1}$ KCl, 10% glycerol (V: W), and 5 mg · $L^{-1}$ 2-mercaptoethanol was combined to extract leaf chlorophyll metabolism, proline metabolism, and the sulfur assimilation pathway enzymes.

The protocols previously described by Rena and Splittstoesser (1975) and Costa et al. (2005) were employed to detect proline dehydrogenase and chlorophyllase activities of the leaves, with readings at 550 and 663 nm, respectively. Pyrroline-5-carboxylate synthetase (P5CS) activity was quantified by measuring the amount of gamma-glutamyl formation at 535 nm (Garcia-Rios et al., 1997). Jain and Gadre's (2004) method was employed to quantify aminolevulinic acid dehydratase activity.

**Table 1  The effects of brassinolide on morphological traits, the photosynthetic apparatus-related traits, and the leaf proline content of hydroponically grown tomato plants**

| Treatments | Height (cm) | Total dry weight (g) | Chlorophyll $a$ (mg · $g^{-1}$ FW) | Chlorophyll $b$ (mg · $g^{-1}$ FW) | Carotenoids (mg · $g^{-1}$ FW) | $F_v/F_m$ | Proline |
|---|---|---|---|---|---|---|---|
| Control | 20.82 ± 0.56a | 4.23 ± 0.19a | 2.13 ± 0.17a | 1.18 ± 0.12a | 0.47 ± 0.01a | 0.84 ± 0.03a | 2.51 ± 0.14d |
| 0.5 BL | 20.92 ± 0.52a | 4.32 ± 0.21a | 2.17 ± 0.18a | 1.15 ± 0.14a | 0.47 ± 0.02a | 0.84 ± 0.03a | 2.53 ± 0.14d |
| 1 BL | 20.89 ± 0.61a | 4.33 ± 0.24a | 2.26 ± 0.22a | 1.22 ± 0.12a | 0.47 ± 0.02a | 0.84 ± 0.03a | 2.47 ± 0.17d |
| 10 As | 15.08 ± 0.32c | 2.26 ± 0.13d | 1.02 ± 0.11c | 0.52 ± 0.07c | 0.23 ± 0.02c | 0.63 ± 0.03d | 7.30 ± 0.34c |
| 10 As + 0.5 BL | 18.16 ± 0.29b | 3.32 ± 0.19c | 1.66 ± 0.09b | 0.75 ± 0.07b | 0.34 ± 0.02b | 0.71 ± 0.03c | 10.62 ± 0.37b |
| 10 As + 1 BL | 18.77 ± 0.46b | 3.69 ± 0.18b | 1.81 ± 0.13b | 0.92 ± 0.13b | 0.37 ± 0.02b | 0.75 ± 0.02b | 12.79 ± 0.44a |

Note: Values (Means ± SD, $n = 5$) followed by the same letters are not significantly different ($P < 0.05$; Duncan's test).
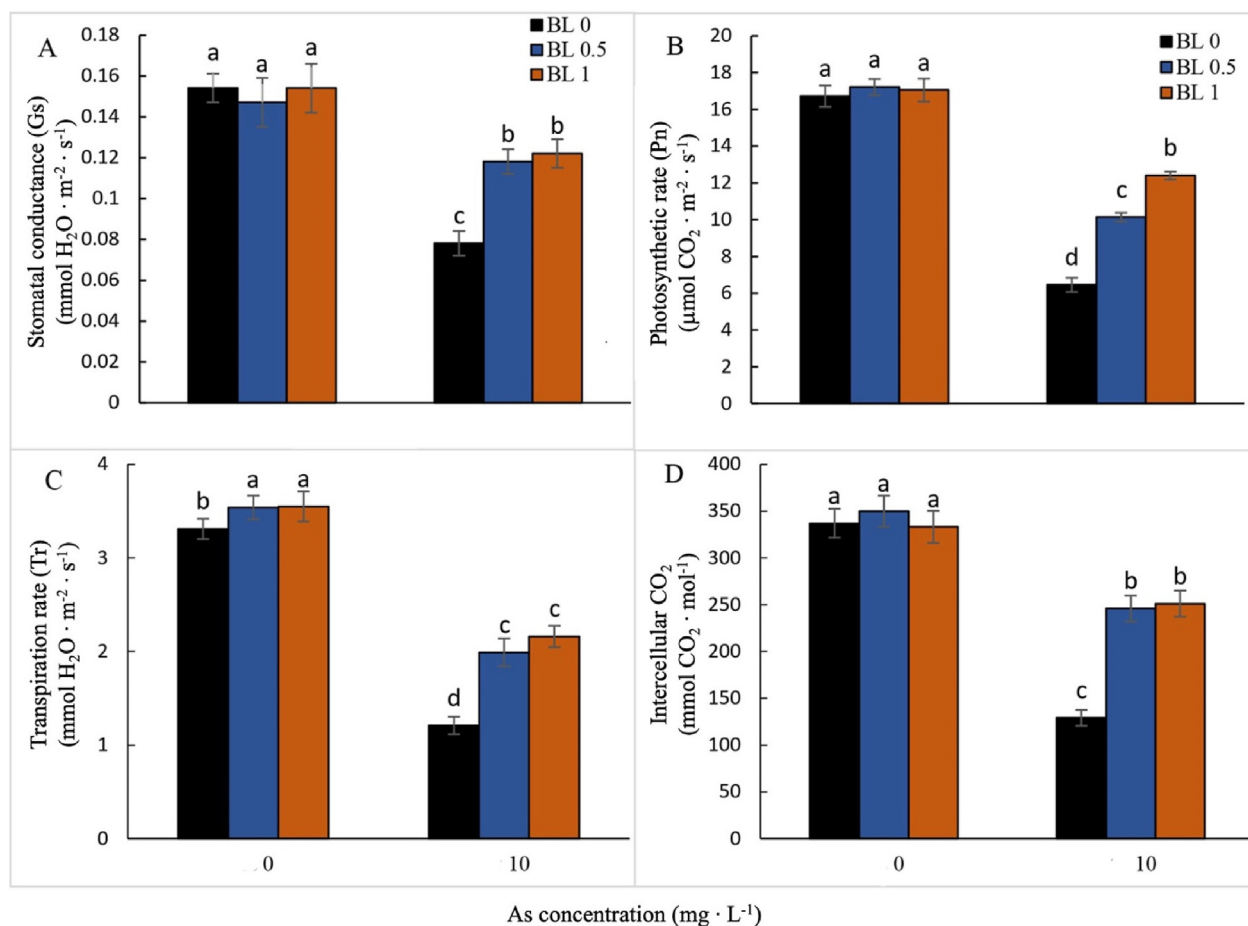


**Fig. 1  The effects of foliar brassinolide spray on physiological indicators of hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on stomatal conductance (A), photosynthetic rate (B), transpiration (C), and intercellular $CO_2$ concentration (D) of hydroponically grown tomato seedlings under arsenic stress. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).
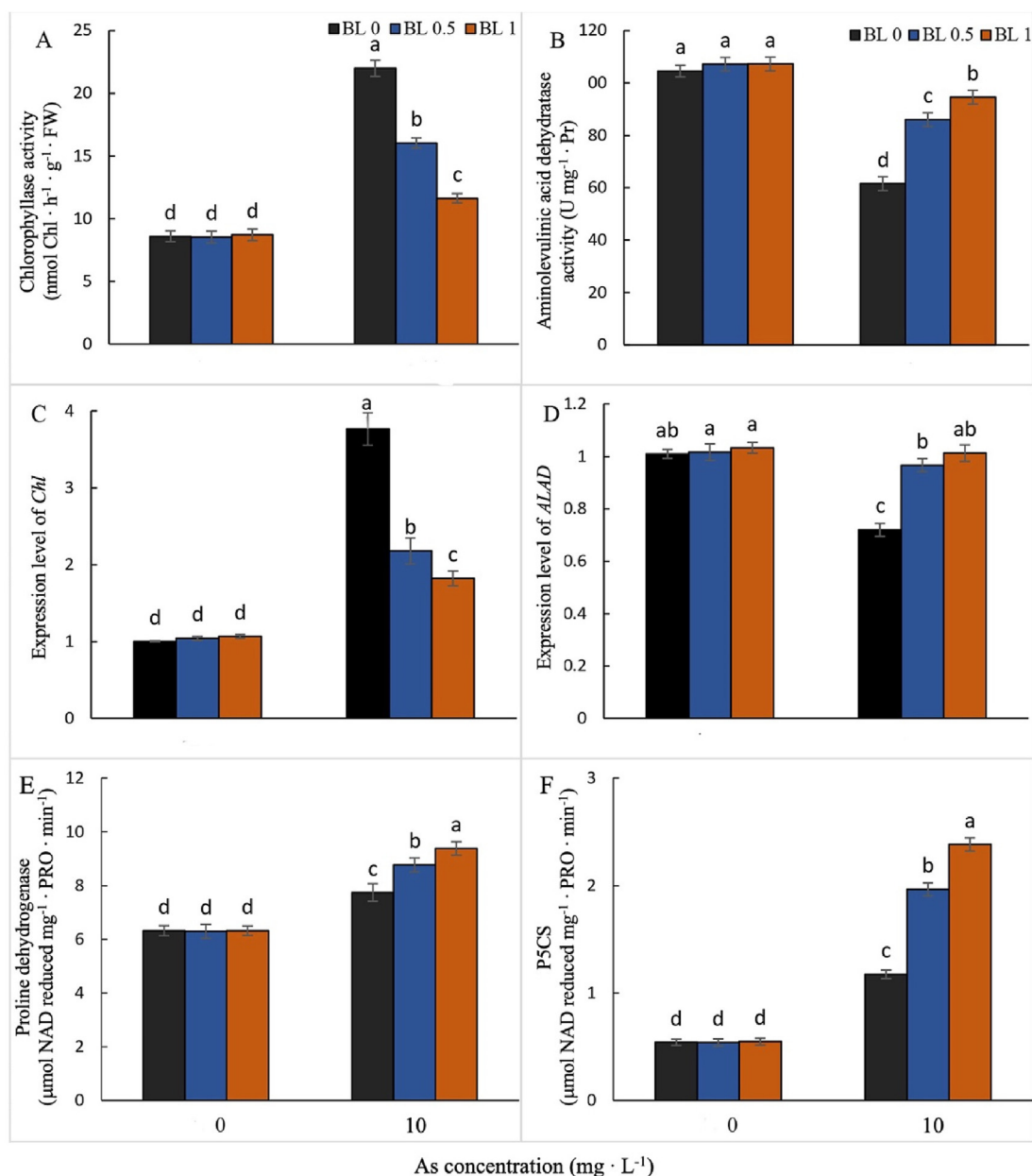
**Fig. 2 The effects of foliar brassinolide spray on enzymes of chlorophyll metabolism, and proline metabolism of hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on the activity and expression of chlorophyll metabolism enzymes (A, B, C, D) and activity of proline metabolism enzymes (E, F) of hydroponically grown tomato seedlings under arsenic stress. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

The methods described by Jez et al. (2004) and Orlowski and Meister (1973) were used to measure the activities of $\gamma$-glutamylcysteine synthetase (GCS) and $\gamma$-glutamyl transpeptidase (GT) at 340 and 530 nm, respectively. The activities of 5′-adenylylsulfate reductase (ASR), glutathione S-transferase (GST), serine acetyltransferase (SAT), and cysteine synthase (CS) were obtained from the leaves using the procedures (Peck et al., 1965; Gaitonde, 1967; Habig and Jakoby 1981; Błaszczyk et al., 2002), respectively.

### 2.9. Nitrogen metabolism

The protocol of Muñoz-Huerta et al. (2013) was employed to quantify the N content through the Kjeldahl method using dried leaf tissues. After homogenizing fresh leaves with distilled water and heating, the resulting extract was utilized to determine $NO_3^-$ and $NH_4^+$ contents. The leaf $NH_4^+$ and $NO_3^-$ concentrations were obtained, respectively (Cataldo et al., 1975; Molins-Legua et al., 2006).

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6      Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1−21.

**Table 2** The effects of brassinolide spray (BL) on arsenic (As), iron (Fe), nitrogen (N), nitrate ($NO_3$), and ammonium ($NH_4$) concentrations of hydroponically grown tomato plants under arsenic exposure (As)

| Treatments (mg · L⁻¹) | As (µg · g⁻¹) DW | | Fe (µg · g⁻¹) DW | | Si (µg · g⁻¹) DW | | N (µg · g⁻¹) DW | $NO_3^-$ (µg · g⁻¹) DW | $NH_4^+$ (µg · g⁻¹) DW |
|---|---|---|---|---|---|---|---|---|---|
| | in root | in leaf | in root | in leaf | in root | in leaf | in leaf | in leaf | in leaf |
| Control | n.d. | n.d. | 300 ± 19a | 71.1 ± 4.1a | 2.34 ± 0.12a | 1.95 ± 0.14a | 33.69 ± 0.71a | 25.98 ± 0.89a | 7.17 ± 0.39d |
| 0.5 BL | n.d. | n.d. | 304 ± 23a | 70.2 ± 4.4a | 2.23 ± 0.14a | 1.93 ± 0.17a | 33.49 ± 0.89a | 25.98 ± 0.86a | 6.95 ± 0.41d |
| 1 BL | n.d. | n.d. | 303 ± 22a | 72.0 ± 5.1a | 2.29 ± 0.13a | 1.92 ± 0.17a | 33.68 ± 0.75a | 26.06 ± 0.96a | 6.92 ± 0.47d |
| 10 As | 539 ± 29a | 104 ± 11a | 160 ± 13c | 35.6 ± 4.1c | 1.90 ± 0.16b | 1.05 ± 0.16c | 18.99 ± 0.76d | 14.72 ± 0.50d | 13.93 ± 0.46a |
| 10 As + 0.5 BL | 463 ± 11b | 56 ± 7b | 247 ± 17b | 54.5 ± 4.4b | 2.18 ± 0.11a | 1.46 ± 0.06b | 25.22 ± 0.78c | 21.60 ± 0.58c | 9.66 ± 0.54b |
| 10 As + 1 BL | 426 ± 13c | 37 ± 6c | 257 ± 17b | 65.3 ± 4.1a | 2.19 ± 0.18a | 1.65 ± 0.10b | 28.36 ± 1.15b | 24.57 ± 0.43b | 8.30 ± 0.42c |

Note: Values (Means ± SD, n = 5) followed by the different letters are not significantly different (P < 0.05; Duncan's test).

Following the extraction of fresh leaves in an extraction solution comprising 100 mg · L⁻¹ K−P buffer (pH 7.5), 2 mg · L⁻¹ EDTA, 0.5% polyvidone, and 5 mg · L⁻¹ cysteine, the enzyme extract was obtained. The activities of NR and NiR were measured by recording changes in absorbance at 540 nm, resulting from the increase or decrease in $NO_2^-$ levels (Debouba et al., 2006).

To specify the activities of GS, GOGAT, and glutamate dehydrogenase (GDH), fresh leaves were extracted in a mixture of 50 mg · L⁻¹ Tris−HCl buffer (pH 7.5), 1 mg · L⁻¹ magnesium chloride, 1 mg · L⁻¹ EDTA, Cleland's reagent (1 mg · L⁻¹), 0.5% polyvinylpyrrolidone, and 2-mercaptoethanol (10 mg · L⁻¹). The supernatants were read at 540 and 340 nm for GS and GOGAT activities, respectively, in accordance with the methods described by Agbaria et al. (1998) and Groat and Vance (1981), respectively.

The activity of GDH was obtained, by reading the reaction solution (0.1 mmol · L⁻¹ Tris−HCl buffer (pH 8.0), $\alpha$-ketoglutaric acid (11 mmol · L⁻¹), ammonium chloride (0.1 mol · L⁻¹), NADH (0.2 mmol · L⁻¹), and enzyme extracts) at 340 nm by Groat and Vance (1981).

### 2.10. Gene expression

The total RNA of root and leaf was extracted by Plant RNA Miniprep Kit (Zymo, USA). The Revert Aid Reverse Transcriptase (Thermo, Germany) was used to synthesize cDNA. The SYBR Premix (without ROX) was used to perform qPCR in triplicates on the C1000TM Thermal Cycler(BioRad). The oligonucleotide sequences for the internal control gene and target genes (Table S1) were designated using Oligo 7.0. The relative transcription levels of genes were calculated based on Livak and Schmittgen (2001).

### 2.11. Statistics

Statistical analyses were conducted by SPSS 20, and the Duncan's test ($P \leq 0.05$) was used to detect the differences among groups. The data were expressed as the (Mean ± Standard Deviation) of at least five replicates.

## 3. Results

### 3.1. BL reduces the effects of As toxicity on growth and photosynthetic apparatus

As treatment decreased the Height (27.6%) and total dry weight (46.6%) over untreated plants. However, 0.5 and 1 mg · L⁻¹ BL increased length (20.4% and 24.5%, respectively) and total dry weight (46.6% and 63.3%, respectively) in As-treated tomato plants (Table 1). Applying As stress recorded 52.1%, 55.9%, and 51.6% decreases in chlorophyll *a*, *b*, and carotenoids compared to the control treatment. Treatments of 0.5 and 1 mg · L⁻¹ BL improved the photosynthetic pigment concentrations in As-treated plants. As stress lead to a 33.5% decrease in the $F_v/F_m$ index compared to untreated plants. However, 19.5% and 29.2% improvements in the $F_v/F_m$ were found with treatments of 0.5 and 1 mg · L⁻¹ BL in As-subjected plants, respectively (Table 1).

Applying As stress significantly reduced Gs (49.4%), Pn (61.4%), Tr (63.4%), and Ci (61.6%) compared to the control plants. However, applying both BL concentrations significantly increased all
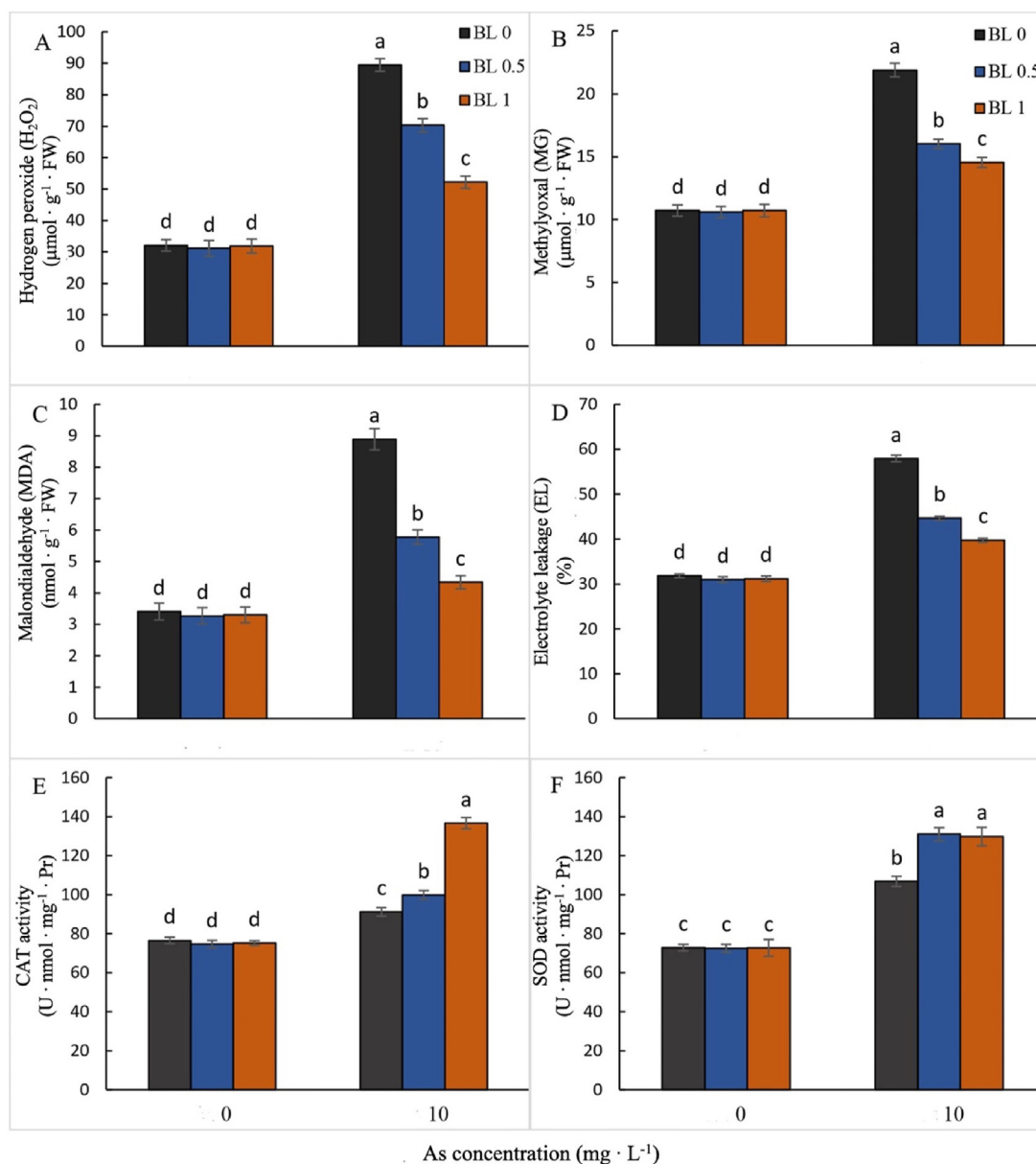
**Fig. 3  The effects of foliar brassinolide spray on oxidative stress markers and antioxidant enzymes activity of hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on oxidative stress markers (A, B, C, and D) along with catalase (E) and superoxide dismutase (F) activities in hydroponically grown tomato seedlings under arsenic exposure The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test (P < 0.05).

parameters related to photosynthesis in As-stressed plants. Associated with Pn, the most significant increase was obtained under high concentrations of BL. Still, in other characteristics, no significant difference was observed between the responses caused by two BL concentrations (Fig. 1, A, B, C, D).

### 3.2. BL regulates the activity and expression of chlorophyll metabolism enzymes under As stress

As stress increased chlorophyllase activity by 156% while decreasing aminolevulinic acid dehydratase activity by 41.1%. However, the sprayed treatments of 0.5 and 1 mg · $L^{-1}$ BL downregulated chlorophyllase activity by 27% and 47.1% and upregulated aminolevulinic acid dehydratase activity by 39.8% and 53.7%, respectively, over As-subjected plants alone (Fig. 2, A and B).

Applying As substantially raised the mRNA transcript level of the *CHL* in the leaves of tomato plants compared to controls, while the application of foliar BL spray declined the transcriptional level of this gene in the leaves of As-subjected plants (Fig. 2, C). The transcriptional level of *ALAD* in tomato leaves was significantly decreased by As treatment over control plants.

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8                                    Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1—21.
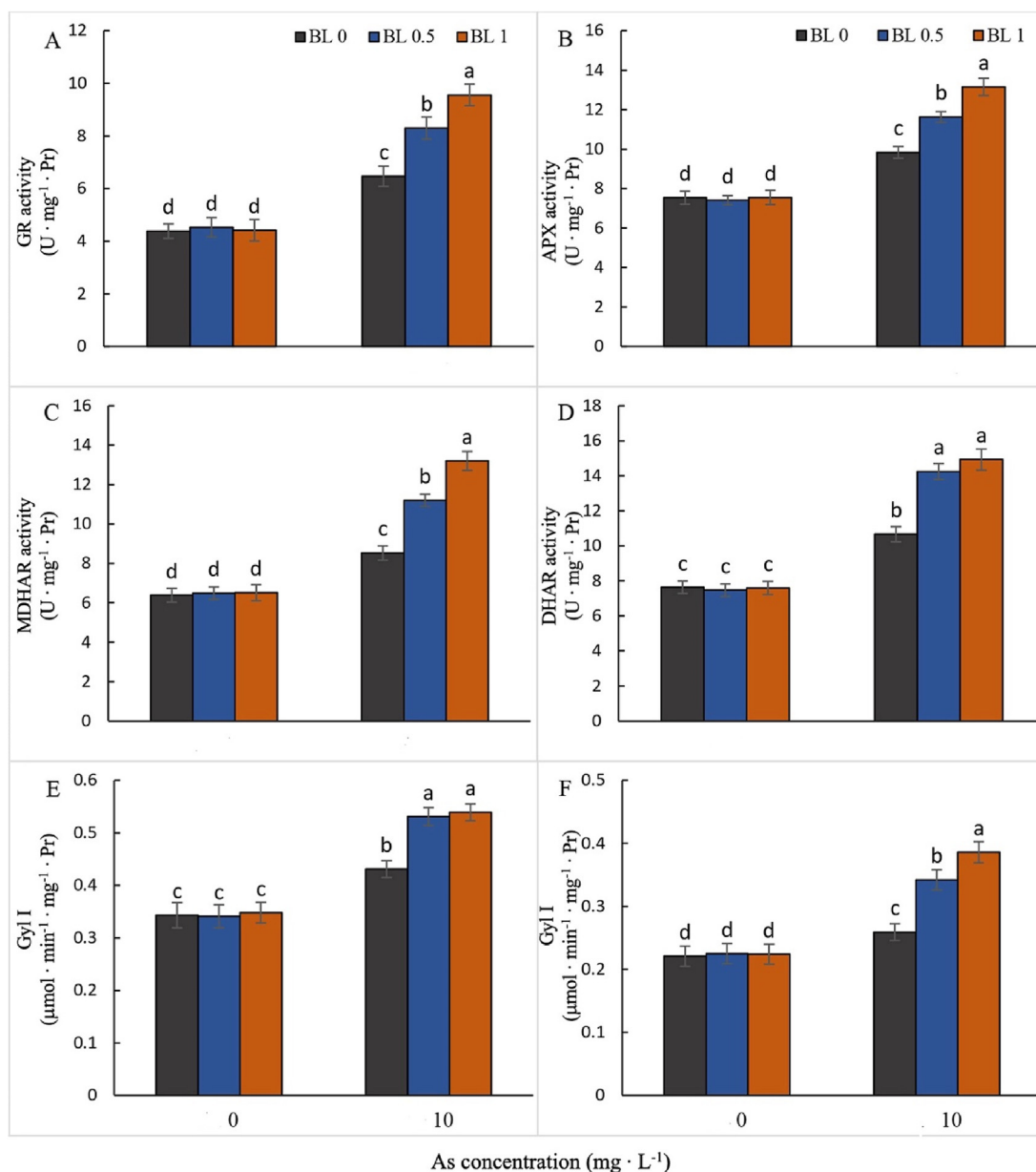


**Fig. 4  The effects of foliar brassinolide spray on glutathione reductase (GR), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glyoxalase (Gly) I, and Gly II of hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on the activity of glutathione reductase (GR) (A), ascorbate peroxidase (APX) (B), monodehydroascorbate reductase (MDHAR) (C), dehydroascorbate reductase (DHAR) (D), glyoxalase (Gly) I (E), and Gly II (F) in hydroponically grown tomato seedlings under arsenic exposure. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

However, BL spraying significantly raised the gene expression of the *ALAD* in the leaves (Fig. 2, D).

The effects of foliar brassinolide spray on activity and expression of chlorophyll metabolism enzymes and proline metabolism enzymes of hydroponically grown tomato seedlings under arsenic stress. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

### 3.3.  BL regulates the concentration of As, Fe, and Si in roots and leaves under As toxicity

As (10 mg · $L^{-1}$) in the Hoagland medium caused the accumulation of As in the roots (539 µg $g^{-1}$ DW) and leaves (104 µg $g^{-1}$ DW). Nevertheless, As accumulation in leaves and roots was decreased in As-stressed plants by 46.2% and 64.4% and 14.1% and 21%, respectively, following foliar applications of 0.5 and
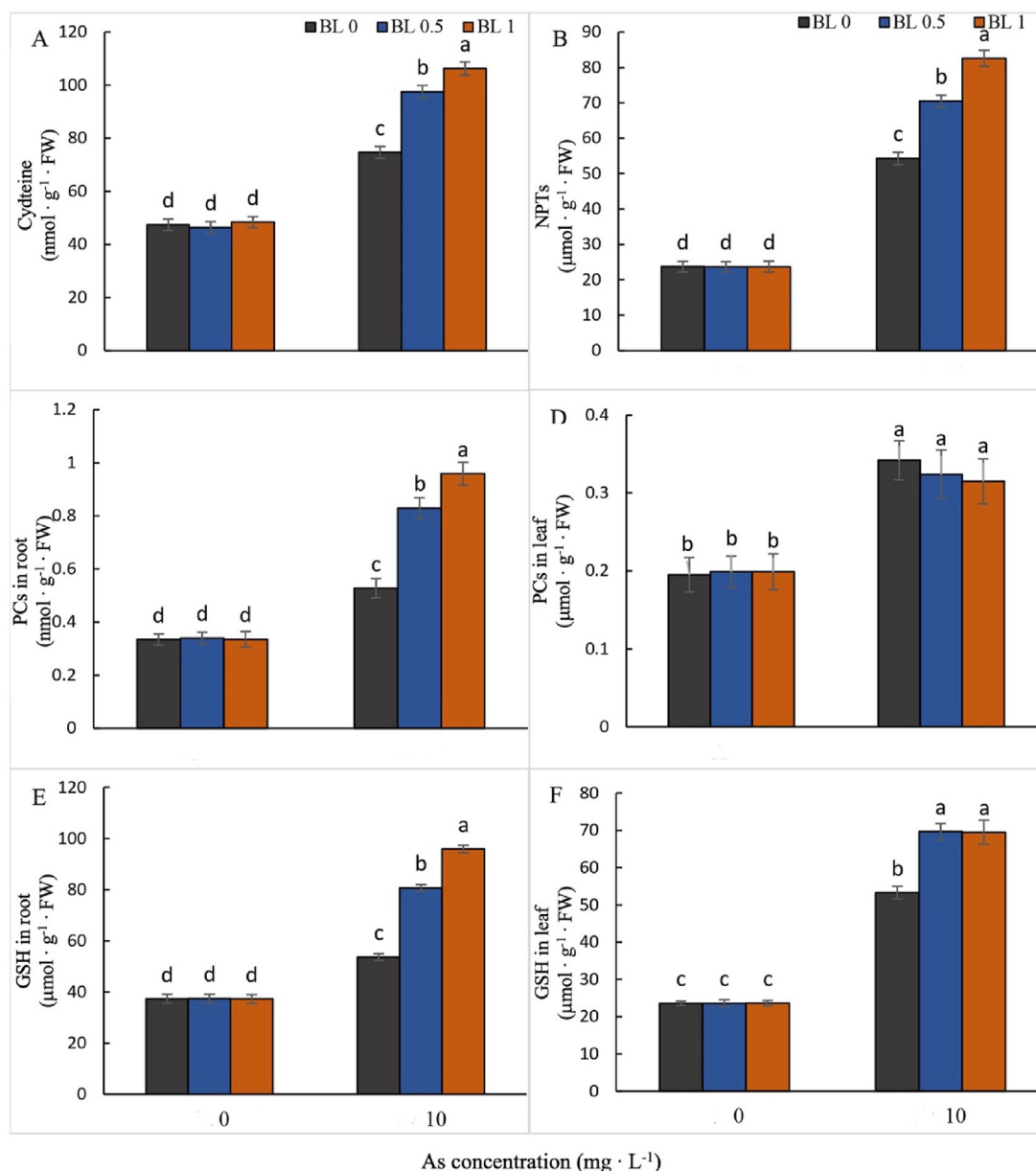
**Fig. 5  The effects of foliar brassinolide spray on content of cysteine, non-protein thiols (NPTs), phytochelatins (PCs) and glutathione (GSH) of hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on the content of cysteine (A), non-protein thiols (NPTs) (B), phytochelatins (PCs) (C, D) and glutathione (GSH) (E, F) of hydroponically grown tomato seedlings under arsenic exposure. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

1 mg · $L^{-1}$ BL (Table 2). A 49.9% and 43.6% reduction in Fe concentration was found in the leaf and root of As-stressed tomatoes, respectively, compared to untreated plants. While BL treatments caused restoration of Fe concentrations in both tissues, the highest increase was found in 1 mg · $L^{-1}$ BL As-subjected plants (Table 2). The application of stress reduced Si levels in both roots and leaves by 18.8% and 46.2%, respectively, compared to plants that were not subjected to As treatment. Nevertheless, applying BL at concentrations of 0.5 and 1 mg · $L^{-1}$ resulted in a significant enhancement of Si levels in the roots, exhibiting an increase of 14.7% and 15.3%,

respectively. Similarly, in the leaves, the Si levels resulted in a substantial rise of 39.1% and 57.1% in plants subjected to As stress (Table 2).

### 3.4. BL modulates proline level and the activity of proline metabolism enzymes in roots and leaves under As stress

As treatment significantly raised leaf proline content by 191% over untreated controls. The foliar spray of 0.5 and 1 mg · $L^{-1}$ BL increased proline content by 45.5% and 75.2%, in As-exposed tomatoes, respectively, compared to plants subjected to As alone (Table 1).

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10                          Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1—21.
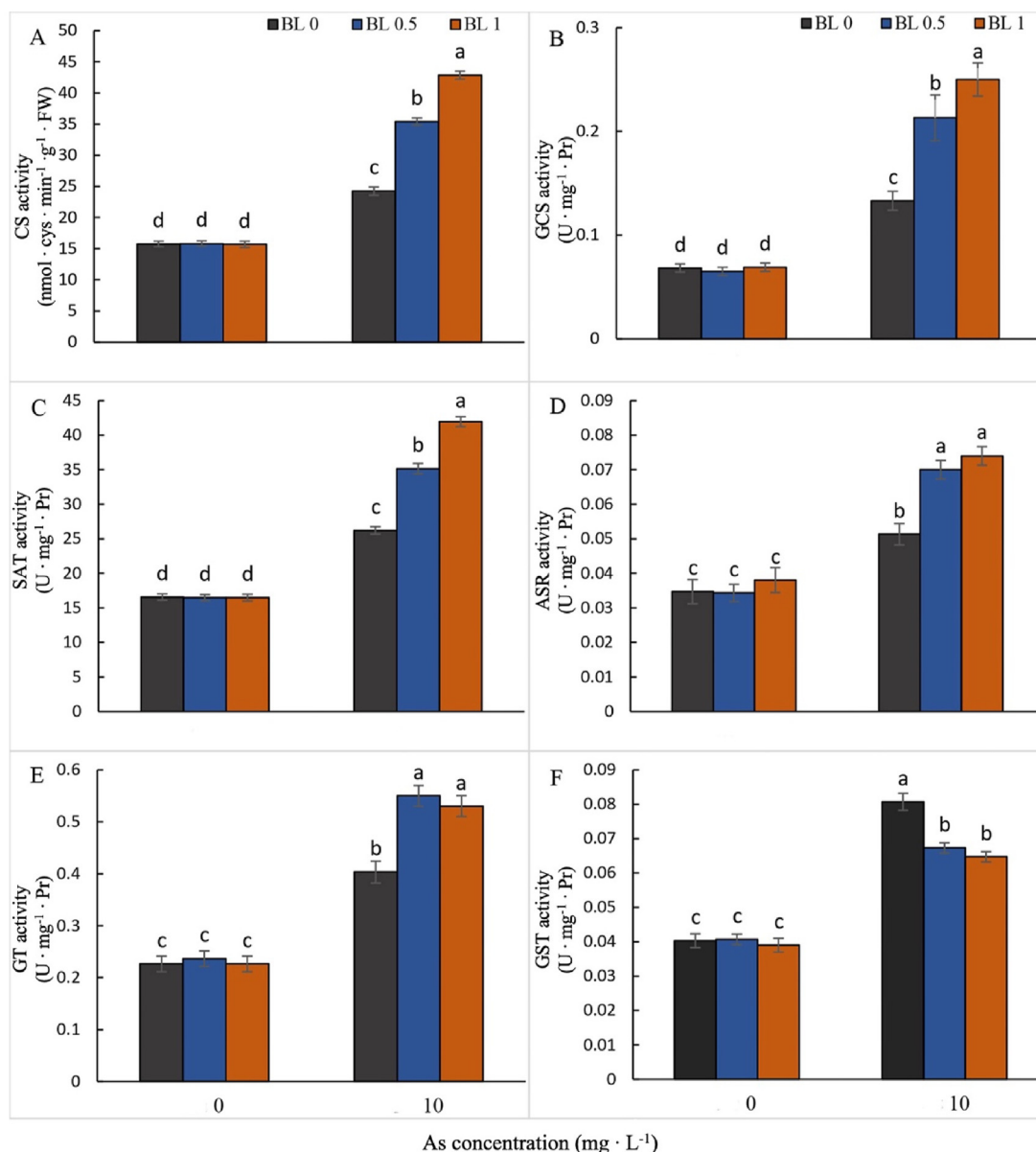


**Fig. 6 The effects of foliar brassinolide spray on the activity of enzymes involved in sulfur assimilation of hydroponically grown tomato seedlings under arsenic stress**
CS: Cysteine synthase (A); GCS: γ-glutamylcysteine synthetase (B); SAT: serine acetyltransferase (C); ASR: 5′-adenylylsulfate reductase (D); GT: γ-glutamyl transpeptidase (E); GST: glutathione S-transferase (F). The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

The activities of P5CS and proline dehydrogenase were markedly raised by As stress, by 116.8% and 22.5%, respectively. Spraying BL on the leaves of As-subjected tomatoes provoked a further peak in both enzymes, with the highest activity documented under $1 mg \cdot L^{-1}$ BL treatment (Fig. 2, E and F).

### 3.5. BL relieves levels of oxidative stress markers in leaves under As stress

As treatment increased $H_2O_2$ (179%), MG (104%), MDA (161%), and EL (82%), in leaves, compared to untreated plants. In tomatoes exposed to As, spraying BL on the leaves lowered oxidative stress indicators, with a $1 mg \cdot L^{-1}$ BL treatment showing the most drastic decrease (Fig. 3, A, B, C, D).

### 3.6. BL enhances the activity of antioxidant enzymes in tomato leaves under As stress

The activities of CAT and SOD increased to 19.2% and 46.6%, respectively, in tomato plants exposed to As treatment in comparison to untreated tomatoes. Applying 0.5 and $1 mg \cdot L^{-1}$ BL sprays increased CAT by 9.5% and 49.9%, and SOD enzyme by 23% and 35%, respectively, in As-stressed plants (Fig. 3, E and F). As stress provoked an upregulation in the
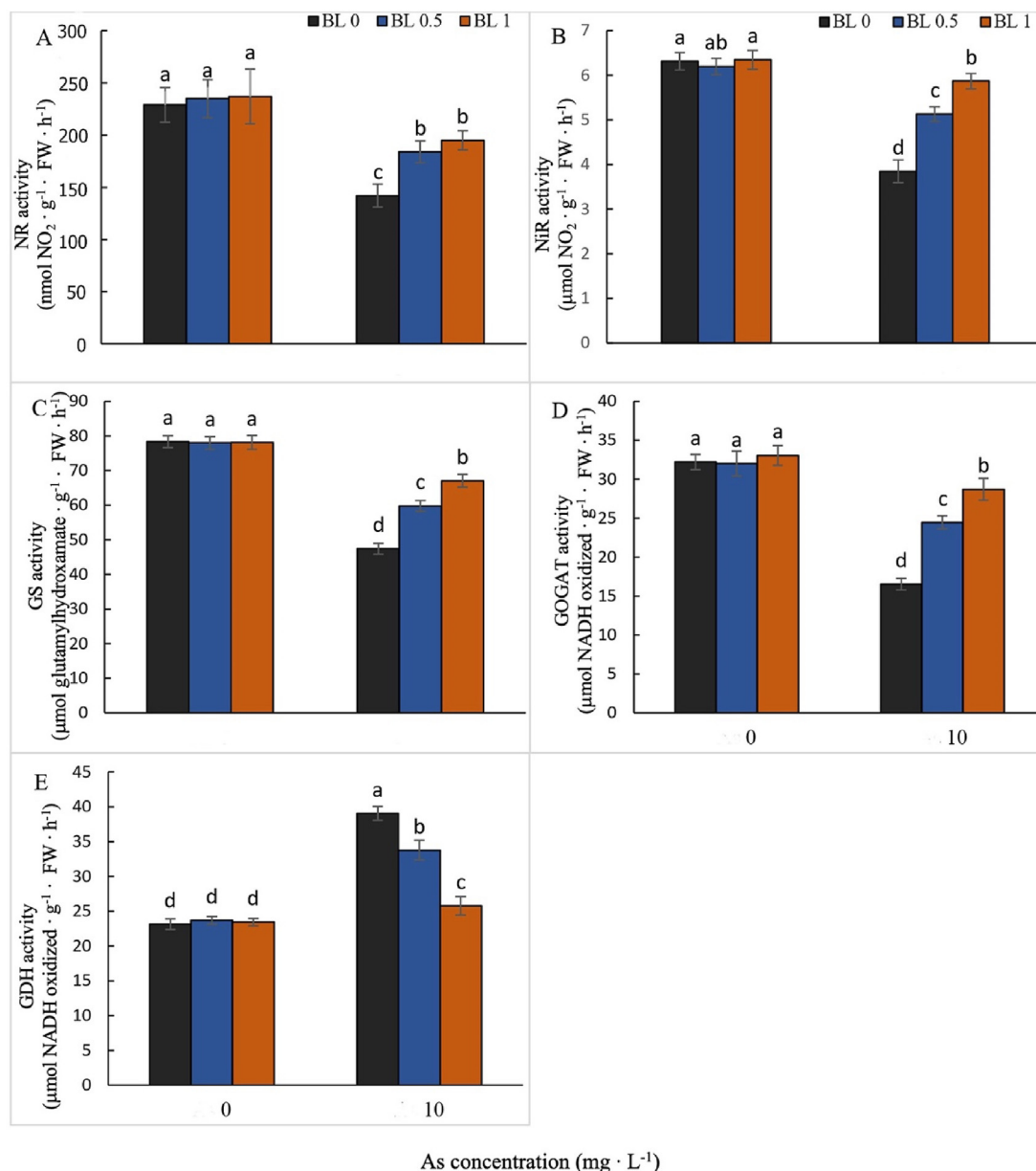
**Fig. 7  The effects of foliar brassinolide spray on the activity of enzymes involved in nitrogen metabolism of hydroponically grown tomato seedlings under arsenic stress**

NR: Nitrate reductase (A); NiR: nitrite reductase (B); GS: glutamine synthetase (C); GOGAT: glutamate synthase (D); GDH: glutamate dehydrogenase (E). The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

activities of GR (47.4%), APX (30.5%), MDHAR (33.7%), and DHAR (39.8%) over control samples. These enzymes were, however, elevated by BL foliar spray in As-stressed plants, with the maximum upregulation recorded under 1 mg · L$^{-1}$ BL conditions (Fig. 4, A, B, C, D).

### 3.7.  BL upregulates the activity of Gly enzymes in tomato leaves under As toxicity

Gly I and II exhibited a rise of 25.7% and 17.2%, respectively, under As treatment over control plants. However, 0.5 and 1 mg · L$^{-1}$ BL foliar spray upregulated the activity of Gly I by 23.2% and

25.1%, and Gly II by 32.1% and 49%, respectively, in As-stressed plants (Fig. 4, E and F).

### 3.8.  BL induces thiol compound contents and the activity of sulfur metabolism enzymes in the leaves during As treatment

As stress substantially rose the leaf accumulation of cysteine (57.8%) and NTP (129%) compared to untreated groups. Foliar spraying of BL enhanced the leaf cysteine levels, and NTPs in As-stressed tomatoes, and the highest accumulation in leaves was recorded in plants sprayed with 1 mg · L$^{-1}$ BL (Fig. 5, A and B). As stress also increased PCs levels in root and leaf by 57.8% and 75.4%, respectively, over control groups. However, 0.5 and 1 mg · L$^{-1}$ BL in

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12           Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1–21.
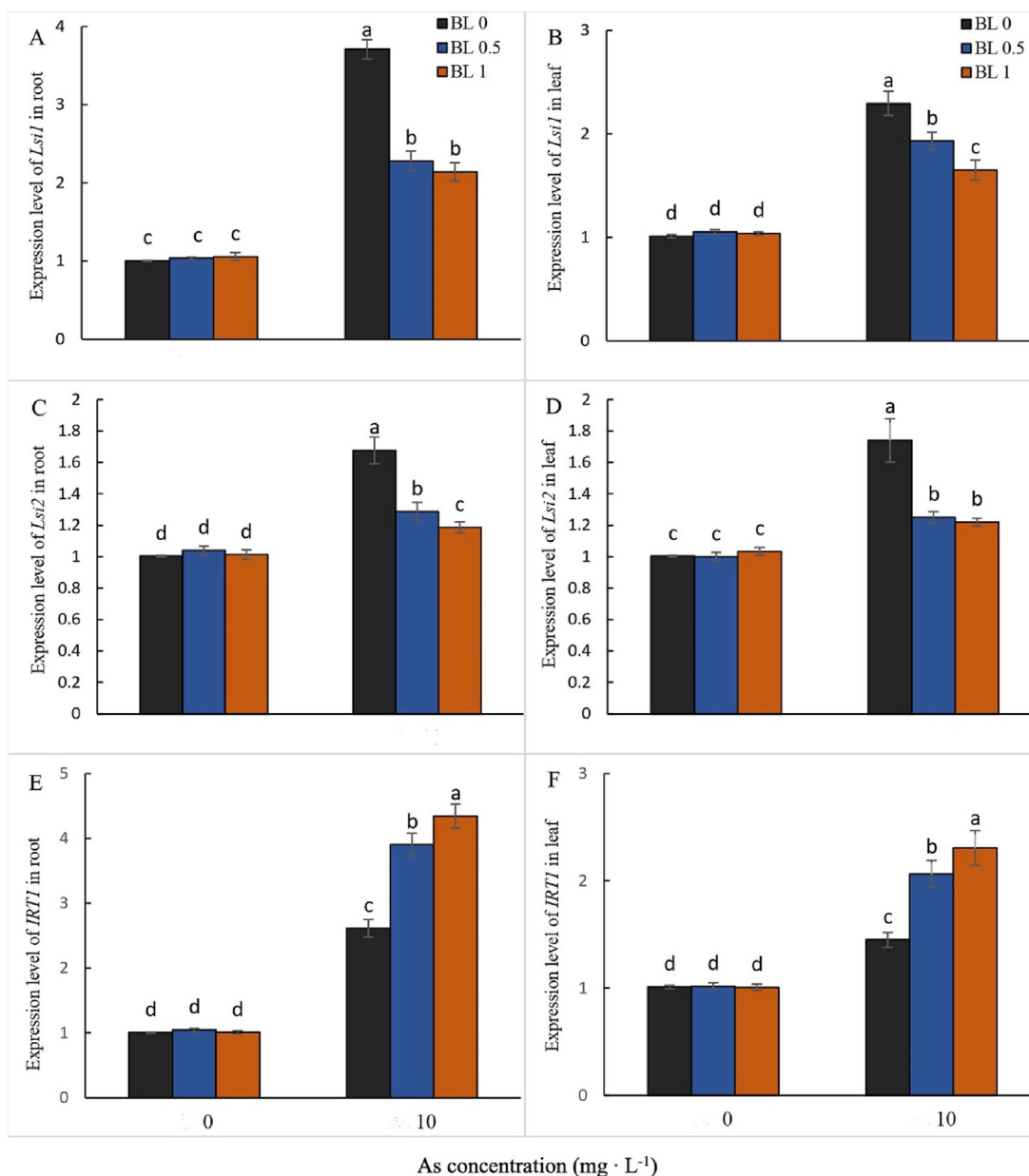


**Fig. 8**   **The effects of foliar brassinolide spray on transcript abundance of As transporter genes Lsi1 in root and leaf, Lsi2 in root, in leaf, and iron (Fe) transporter IRT1 in root and leaf of hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on the transcript abundance of As transporter genes Lsi1 in root (A) and leaf (B), Lsi2 in root (C), in leaf (D), and iron (Fe) transporter IRT1 in root (E) and leaf (F) in hydroponically grown tomato seedlings under arsenic exposure. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

As-exposed tomatoes caused an increase of PCs in roots by 57.3% and 82% and a decrease in leaves by 5.3% and 7.9%, respectively (Fig. 5, C and D). An increase of 43.8% and 125.8% in GSH levels in the leaves and roots was recorded upon As stress application over untreated controls. However, BL provoked an additional accumulation in the natural GSH in root and leaf tissues in As-exposed tomatoes (Fig. 5, E and F).

Adding As to the hydroponic culture medium enhanced the leaf sulfur metabolism enzymes, CS(54.1%), GCS(95.6%), SAT(58.2%), ASR(47.8%), GT(77.9%), and GST(100%) compared to control plants. However, BL treatments raised the activities of CS, GCS, SAT, ASR, and GT and diminished the activity of GST in the leaves of As-subjected tomatoes (Fig. 6, A, B, C, D, E, F).

### 3.9. BL modulates the leaf levels of N, $NO_3^-$, and $NH_4^+$ and the activity of leaf N metabolism enzymes in As-stressed tomato plants

As toxicity declined, the concentrations of N (43.6%) and $NO_3^-$ (43.3%) raised the $NH_4^+$ content (94.3%) in tomato plants compared to control samples. Spraying plants with 0.5 and 1 mg · L$^{-1}$ BL increased N and $NO_3^-$ by 32.8% and 49.3%, 46.7% and 66.9%, respectively, while a 30.7% and 40.4% respective decrease in $NH_4^+$ concentrations were calculated in the As-subjected tomato leaves (Table 2).
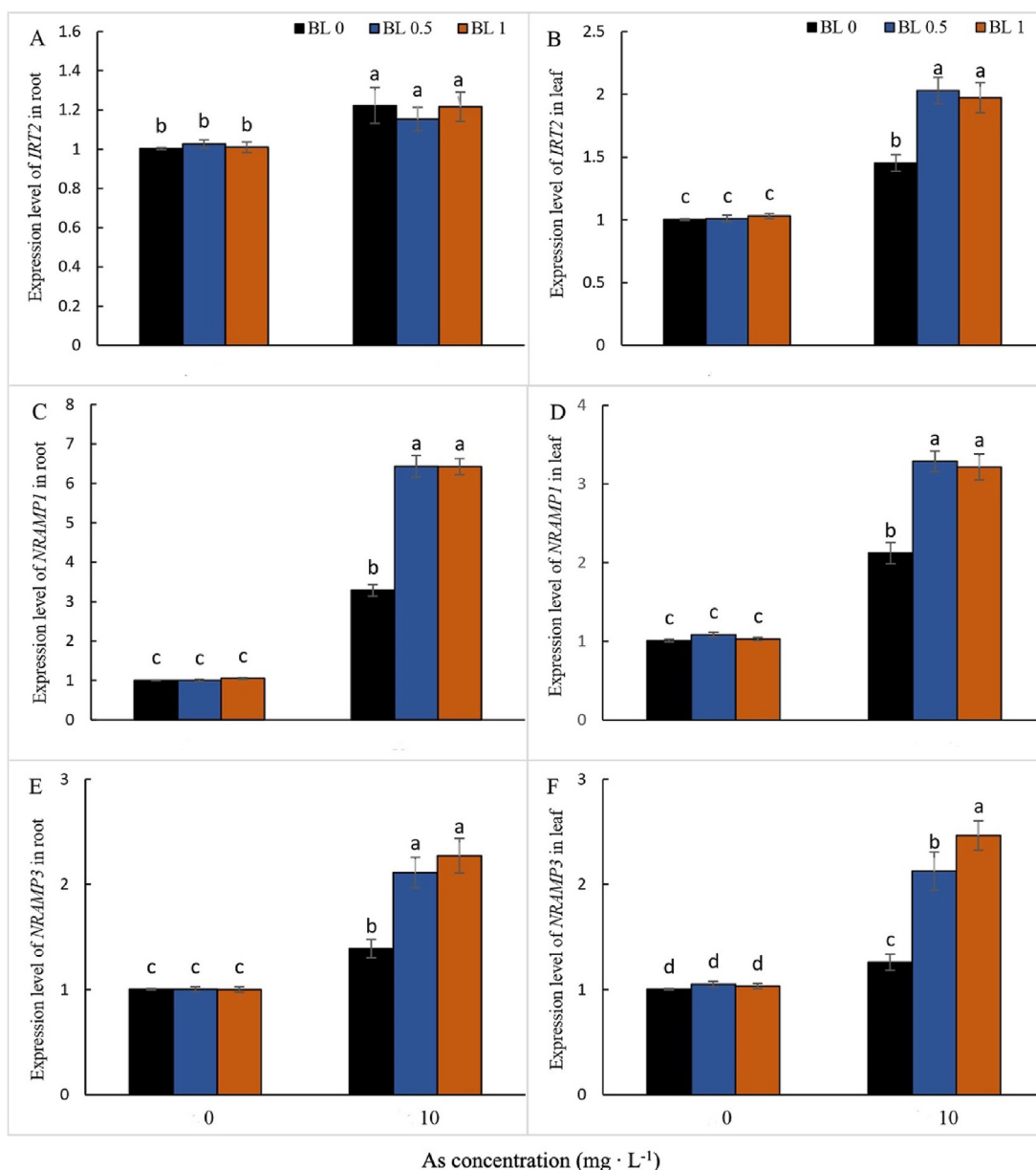
**Fig. 9  The effects of foliar brassinolide spray on the transcript abundance of iron (Fe) transporter *IRT2* in root and in leaf, metal transporter genes *NRAMP1* in root and in leaf, and *NRAMP3* in root and in leaf in hydroponically grown tomato seedlings under arsenic stress**

The effects of foliar brassinolide spray on the transcript abundance of iron (Fe) transporter IRT2 in root (A) and in leaf (B), metal transporter genes NRAMP1 in root (C) and in leaf (D), and NRAMP3 in root (E) and in leaf (F) in hydroponically grown tomato seedlings under arsenic exposure. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

The results revealed that As treatment declined the activities of NR, NiR, GS, and GOGAT by 38%, 39.1%, 39.5%, and 48.7%, respectively, and enhanced the activity of GDH by 68.9% compared with non-stressed plants. Applying foliar BL caused NiR, NR, GOGAT, and GS restoration and reduced GDH enzyme activity in the leaves of As-subjected tomatoes (Fig. 7, A, B, C, D, E).

### 3.10. BL regulates the relative expression of As and Fe transporters in roots and leaves during As stress

Applying As treatment substantially raised the mRNA transcript levels of the *Lsi1* and *Lsi2* in the roots and leaves of tomato compared to controls, while the application of foliar BL spray
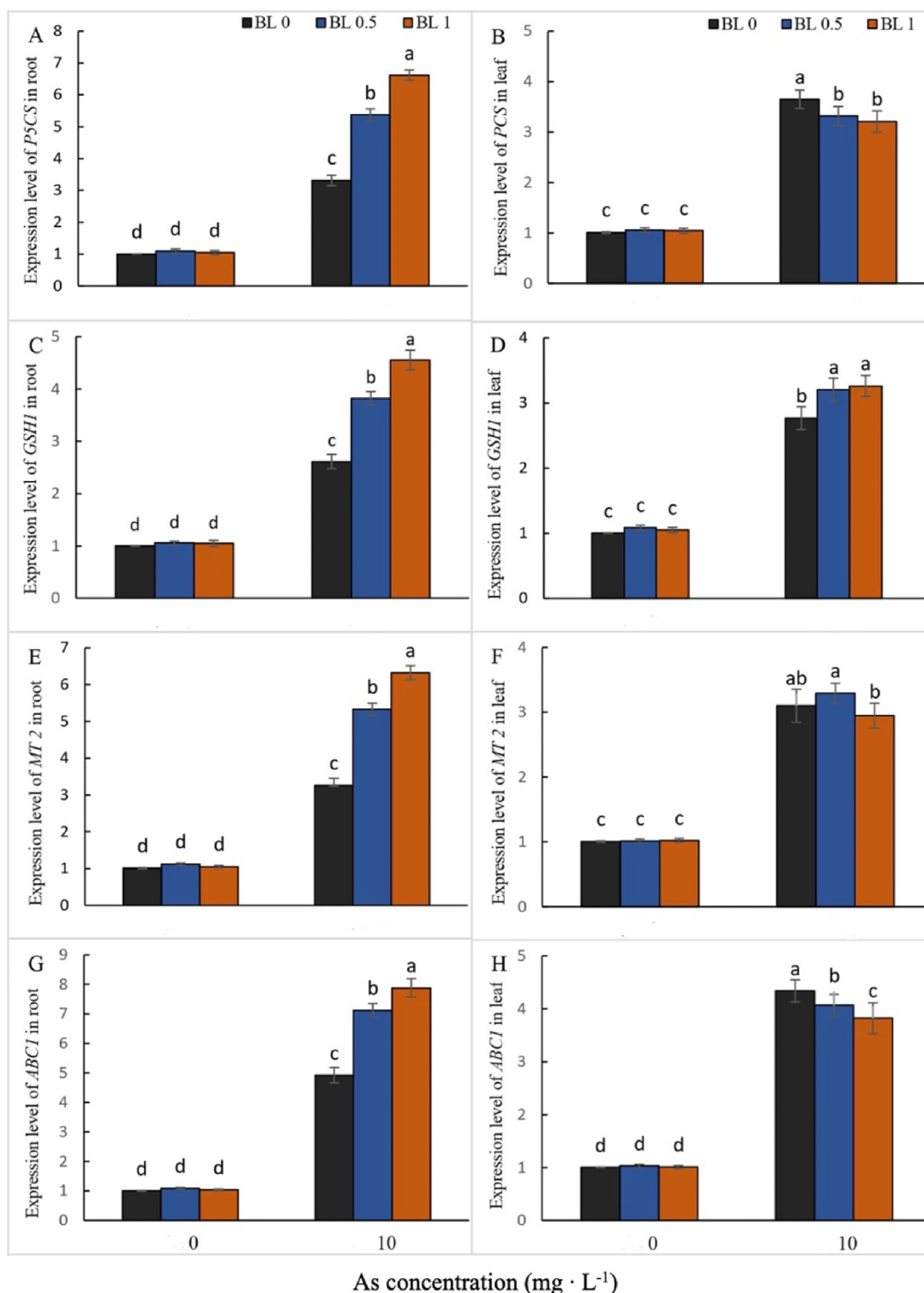
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14                    Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1—21.

**Fig. 10  The effects of foliar brassinolide spray on the transcript abundance of Pyrroline-5-carboxylate synthetase (P5CS) in root and in leaf, γ-glutamylcysteine synthetase gene (GSH1) in root and leaf, metallothionein (MT) genes (MT2) in root and in leaf, and ATP-binding cassette transporter genes (ABC1) in root and in leaf genes in hydroponically grown tomato seedlings under arsenic stress**
The effects of foliar brassinolide spray on the transcript abundance of Pyrroline-5-carboxylate synthetase (P5CS) in root (A) and in leaf (B), γ-glutamylcysteine synthetase gene (GSH1) in root (C) and leaf (D), metallothionein (MT) genes (MT2) in root (E) and in leaf (F), and ATP-binding cassette transporter genes (ABC1) in root (G) and in leaf (H) genes in hydroponically grown tomato seedlings under arsenic exposure. The lowercase letters (a, b, c, d) indicated significant differences among treatments based on Duncan's test ($P < 0.05$).

decreased the transcriptional level of these genes in the root and leaves of As-subjected plants (Fig. 8, A, B, C, D).

The transcriptional levels of IRT1 and IRT2 in the root and leaves of tomatoes were significantly improved by As treatment over control plants. However, BL spraying significantly raised the gene expression of the IRT1 in the roots and leaves. The IRT2 gene in As-stressed plants was also upregulated in the leaves (Fig. 8, E and F; Fig. 9, A and B).

As stress caused an increase in transcriptional levels of NRAMP1 and NRAMP3 in root and leaf compared to non-treated tomato plants. However, BL treatment further raised the expression of these genes in root and leaf tissues. Nevertheless, no significant difference was observed between the responses provoked by the two doses of BL (except for NRAMP3 expression in leaf tissues, which was most enhanced under 1 mg · L$^{-1}$ BL administration) (Fig. 9, C, D, E, F).

### 3.11. BL upregulates the relative expression of As sequestering genes in roots and leaves under As treatment

In roots and leaves, a significant upregulation in the PCS and GSH1 transcription was found by As treatment. However, applying BL raised the PCS expression in roots and GSH1 both in roots and leaves, while decreasing the transcription of the PCS in leaves (Fig. 10, A, B, C, D). In addition, the expression level of MT2 was upregulated by approximately 3.3- and 3.1-fold in the roots and leaves of tomato plants, respectively, over untreated plants. Spraying BL in roots increased the MT2 expression (Fig. 10, E, F). Also, As in the hydroponic medium, significantly increased ABC1 expression in roots and leaves. However, BL treatments increased ABC1 expression in roots while decreasing it in leaves in As-stressed plants (Fig. 10, G and H).

## 4. Discussion

In this work, As toxicity damaged the photosynthetic machinery by upregulating the activity of chlorophyllase and decreasing the activity of aminolevulinic acid dehydratase, resulting in an overall reduction in chlorophyll content, which was associated with a limitation in the efficiency of photosynthetic machinery leading to stunted growth. The toxic impacts of As on the photosynthetic system and the reduction of plant growth were also previously reported by Emamverdian et al. (2023a, 2023c). On the other hand, Ghorbani et al. (2021) indicated that As toxicity caused damage to thylakoid membranes and disruption of chlorophyll metabolism, which was associated with a decrease in Gs, Pn, and CO$_2$ fixation, supporting the findings of current work. Damage to thylakoid lipids induced by oxidative stress is another negative effect caused by As stress on the photosynthetic machinery and gas exchange parameters, as previously reported by Zulfiqar and Ashraf (2022) and Bidi et al. (2021). Here, BL foliar application restored chlorophyll pigments and improved photosynthetic apparatus functionality by modifying chlorophyll metabolism activity, which was related to increased elongation and biomass of tomato plants under As stress. Under fluctuating conditions during environmental stress, BL protects the reaction centers of the photosystem and preserves chlorophyll content (Jan et al., 2018), suggesting that this phytohormone has a defense-modulating effect on the photosynthetic machinery.

The BL hormone has been shown to enhance photosynthetic competence and chlorophyll content under stress by upregulating the transcription level of genes involved in photosynthesis and chlorophyll formation through stimulating the activities of Rubisco and chlorophyllase (Zhao et al., 2017). In addition, Shu et al. (2016) indicated that BL maintains the thylakoid membrane's resilience and improves the photosynthetic apparatus's capacity by facilitating the attachment of chlorophylls to the proteins in the photosynthetic reaction center and improving gas exchange parameters. Furthermore, chlorophyllase catalyzes the hydrolysis reaction of chlorophyll to phytol and chlorophyllide, and it is the first enzyme of chlorophyll catabolism shown to be upregulated under stress (Szafrańska et al., 2017). Therefore, maintaining chlorophyll metabolism through regulating chlorophyllase and aminolevulinic acid dehydratase activities may also play a crucial role in maintaining the functionality of the photosynthetic apparatus. Therefore, the decreased activity of aminolevulinic acid dehydratase by As may involve its interaction with thiol groups since aminolevulinic acid dehydratase is a metal-sensitive enzyme that necessitates the presence of thiols (Jain and Gadre, 2004).

Under severe stress, free proline is also an essential defensive component that works as an osmolyte, signal and antioxidative defense molecule, and a metal chelator (Pehlivan et al., 2023). Increasing free proline levels under As toxicity, through osmotic balance and reduction of internal ROS levels, alleviates damage to membrane lipids and EL. As a result, membrane function is preserved. Here, As treatment was associated with the enhancement in P5CS activity and free proline levels, corroborating with the findings of Surgun-Acar and Zemheri-Navruz (2019) and Ghorbani et al. (2021). The P5CS enzyme is one of the critical plant enzymes in proline biosynthesis derived from glutamate (Guan et al., 2020). Proline dehydrogenase, on the other hand, a FAD-dependent enzyme, initiates and controls the initial step of proline oxidation, resulting in the formation of Δ$^1$-pyrroline-5-carboxylate (Servet, 2012). According to Wang et al. (2022), HM-induced water stress can be managed by elevation in proline levels during detrimental HM stress. Here, in our work, BL enhanced the proline level in As-stressed tomato plants by also modifying proline metabolism. In soybean, Chandrakar et al. (2017) observed similar results of superior leaf proline accumulation driven by BL treatments during As treatment, showing BL's regulatory impact on proline metabolism. Surgun-Acar and Zemheri-Navruz (2019) also indicated that the exogenous BL enhanced the P5CS gene transcript levels, raised the endogenous proline, and enabled the adaptation of Arabidopsis to As phytotoxicity. In BR mutants (bin2-1 and det2-1), which exhibited a decrease in the transcription of enzymes involved in proline biosynthesis, the proline accumulation decreased under NaCl treatment (Zeng et al., 2010). This indicates the regulatory effect of the BL hormone on proline metabolism and plant tolerance under As stress. Thus, our results suggest a regulatory role of BL in proline metabolism, which may be crucial for enhancing the response of tomato to As toxicity.

One of the detrimental effects of toxic metal stress is also the excessive generation of ROS and conditions brought by oxidative stress, which profoundly compromise plants' metabolic and physiological activities by attacking cellular components. Our results showed a substantial increase in H$_2$O$_2$, MG, and MDA and an elevated EL during As stress, suggesting the induction of oxidative stress provoked by As. Indeed, the production of toxic free radicals

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16                     Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1—21.

and the detrimental impact of As toxicity on the electron transport chain in chloroplasts and mitochondrial organelles can trigger oxidative stress (Nahar et al., 2022), which alters the stability and permeability of membranes by deteriorating lipids. Similar results of excessive $H_2O_2$ and MG levels accompanying membrane lipid impairment under As in tomato (Ghorbani et al., 2021) and potato plants (Shahid et al., 2019) have previously been reported. In addition, Nahar et al. (2022) observed that plants increase the activity of antioxidant machinery enzymes in response to elevated levels of ROS and oxidative stress caused by As toxicity. This adaptive response is aimed at restoring ROS levels to a homeostatic range. Also, several studies have shown that plants employ a highly effective strategy to mitigate the toxicity of HMs by upregulating the expression of genes associated with the antioxidant enzyme system (Pehlivan et al., 2021; Emamverdian et al., 2023a) and the Gly cycle, thereby enhancing their antioxidant capacity (Navabpour et al., 2020). In times of stress, SOD facilitates the elimination of superoxide anion by converting it into $H_2O_2$ and $O_2$ through dismutation, while CAT converts the generated $H_2O_2$ to $O_2$ and water. GR facilitates the conversion of oxidized GSH into reduced GSH, while APX utilizes AsA as a specific electron donor to neutralize $H_2O_2$, converting it to water. MDHAR utilizes either NADPH or NADH for the reduction of monodehydroascorbate to AsA, while DHAR catalyzes the reduction of dehydroascorbate using GSH, resulting in the production of AsA. In the Gly system, MG is detoxified into the non-toxic compound d-Lactate through two-step reactions catalyzed by Gly I and Gly II (Rajput et al., 2021). Here in this work, applying BL foliar spray decreased $H_2O_2$ and MG accumulation and preserved bio-membrane integrity under As toxicity. By raising the cellular concentrations of growth hormones, particularly salicylic acid and ethylene, the BR hormone promotes the antioxidant mechanisms of defense and optimizes plant tolerance (Fariduddin et al., 2013). Additionally, it has been shown that reinforcing the Gly barrier and antioxidant system efficiently lowers the ROS level and minimizes the oxidative damage brought by As overdose (Bidi et al., 2021; Ghorbani et al., 2022). Although CAT, DHAR, MDHAR, GR, SOD, APX, Gly I, and Gly II activities were increased in As-stressed plants in our work, these were inadequate in effectively reducing oxidative stress or the negative impacts of toxic substances, especially $H_2O_2$ and MG. However, spraying BL on the As-subjected tomato significantly augmented antioxidant capacity and Gly systems, which was supported by the oxidation mitigation provided by the external BL in HM-stressed *Arabidopsis* (Surgun-Acar and Zemheri-Navruz, 2019). In addition, the BRs trigger salicylic acid accumulation under stress by regulating the BR signaling kinase (BSK 1) (Jan et al., 2018), which is associated with the strengthening of the antioxidant machinery. Therefore, the link between redox status and BR signaling is fundamentally critical for improving plant response and adaptation during stress. In this context, the different mechanisms by which BR signaling pathways optimize the defense system include 1) regulating the transcription of genes involved in adaptation to environmental stresses; 2) turning on the antioxidant machinery and Gly cycle; and 3) increasing the synthesis and accumulation of defense system enhancing substances called osmoprotectants (Fabregas et al., 2018). Lv et al. (2018) showed that the mutants defective in BR biosynthesis (*det2-9*) increased the quantity of superoxide anion radicals inside cells, indicating an association between BRs signaling and the ROS pathway. Surgun-

Acar and Zemheri-Navruz (2019) also showed that BRs strengthened the antioxidant defense machinery and improved overall plant tolerance to As phytotoxicity by upregulating the target antioxidant gene expressions. Jan et al. (2018) demonstrated that applying BL diminished the cellular level of MG and enhanced the plant's capacity to respond to metal phytotoxicity by promoting the activities of Gly I and II. Corroborating with these data, our findings have confirmed that BL, a vital plant defense hormone, can reduce the $H_2O_2$ and MG accumulation, while simultaneously reinforcing the antioxidant system and the Gly cycle, ultimately leading to increased stability of membranes in the face of As toxicity.

Two transporters, low silicon rice 1 (*Lsi1*) and (*Lsi2*) play a vital role in the absorption and transfer of silicon in plants. These transporters have been found to be involved in the absorption of As at the root surface and As transfer to the aerial parts (Ma et al., 2011). *Lsi1* and *Lsi2* transporters have also been implicated in the influx and efflux of Si/As, respectively, in tomato plants (Sun et al., 2020). In the present work, we investigated the interaction between BL and As transporters for the first time. The accumulation of As in the roots and leaves of tomato plants following As treatment was shown to be compatible with the increased transcription levels of *Lsi1* and *Lsi2*. Since SlLsi1 and SlLsi2 are homologs of *Lsi1* and *Lsi2* transporters in rice (Sun et al., 2020), according to the confirmed function of *OsLsi1* and *OsLsi2* in As uptake and transport (Ma et al., 2008), the increased concentration of As with upregulated expression of *SlLsi1* and *SlLsi2* transporters can indicate the functionality of these genes in As transport. However, more molecular-level research is needed to verify the notion. Thus, the increase in the transcription levels of *Lsi1* and *Lsi2* may compensate for the decrease in Si absorption and translocation triggered by As stress. Because any rise in As concentration in the rhizosphere is inhibited by the transfer of Si through the same transporter in plants (Kiany et al., 2022). Although As treatment increased the expression level of *Lsi1* and *Lsi2* transporter genes, it was accompanied by decreased Si uptake and accumulation. The observed phenomenon may be attributed to the competing influence of Si and As on the functioning of *Lsi1* and *Lsi2* transporters, as previously documented by Kiany et al. (2022) and Khan and Gupta (2018). However, here, BL decreased the mRNA levels of *Lsi1* and *Lsi2* in roots and leaves, which was associated with the decrease in As concentration. We observed that, BL could lessen the absorption of As and its subsequent transfer to the leaves, by reducing the transcriptional levels of *Lsi1* and *Lsi2*, thus protecting the photosynthetic organs of the leaf from As toxicity.

On the other hand, As treatment enhanced the activity of sulfur metabolism-related enzymes (GCS, CS, ASR, SAT, GST, and GT), which led to an upward trend in cysteine, NTPs, GSH, and PC concentrations, demonstrating the induction of defense response to counteract As phytotoxicity. Therefore, sulfur absorption process can also contribute significantly to the enhancement of tomato adaptation under As exposure by supplying protective molecules that promote plant adaptation, such as GSH and PCs. GSH and PCs alleviate toxic metal stress by interacting with free cytosolic toxic metals and transporting ions into the vacuole (Metwally et al., 2005). As a rate-limiting substrate in the assembly of critical defensive molecules in the antioxidant system, and an HM chelator, cysteine here performs as a structural component in synthesizing GSH and PCs (Fujii et al., 2023).

Accordingly, the modulation of sulfur-containing metabolites in response to HM stress has been documented in several plant species. For instance, Bashir et al. (2015) reported induction of the sulfur metabolism and endogenous levels of defense compounds containing sulfur in mustard and *Arabidopsis* (Khare et al., 2017). Moreover, Sharma et al. (2017) highlighted the role of sulfur-containing compounds in protecting wheat from HM toxicity. These findings demonstrate the significance of sulfur metabolism in response to HM stress.

In addition to raising the levels of PCs and GSH, As exposure also increased relative transcription of the *GSH1, PCS, MT2,* and *ABC1* genes, which are involved in the course of As sequestration in the vacuole. The expression levels of As-sequestering genes and the accumulation of GSH and PCs increased with BL treatment. Upregulated sequestering genes may be involved in the biosynthesis of GSH which is a derivative of PCs and have a role in As detoxification or/and sequestration in tomato plants (Basit et al., 2022). Therefore, our data demonstrated that BL enhances the detoxification mechanism of As by increasing sulfur assimilation, providing protective agents such as GSH and PCs, and raising the mRNA levels of genes associated with As sequestration.

Insufficient Fe has been shown to increase the mRNA level of Fe transporters in tomato seedlings (Eckhardt et al., 2001). Bidi et al. (2021) and Panthri and Gupta (2022) reported that a high concentration of As around the rhizosphere reduces Fe absorption and, as a result, Fe deficiency in the plant through physical competition. Therefore, As phytotoxicity generally decreases Fe accumulation in leaves and roots (Bidi et al., 2021), showing detrimental effects on the function of the photosynthetic system and growth. In this context, increasing Fe transporter expression, is generally aimed at increasing Fe absorption to compensate for the Fe loss caused by As stress. Therefore, the interaction between BL and the mechanism of Fe absorption under As toxicity was also investigated here. Adverse effects were accompanied by upregulation in gene transcript levels of target transporters involved in Fe absorption and distribution (*IRT1, IRT2, NRAMP1,* and *NRAMP3*). Indeed, Eckhardt et al. (2001) discovered that *IRT1* and *IRT2* transporters transport certain HMs. Thus, As may have caused Fe deficiency by competing with and physically inhibiting the absorption and transport of Fe by *IRT1* and *IRT2* transporters. BL, on the other hand, increased the expression of *IRT1, IRT2, NRAMP1,* and *NRAMP3* in As-subjected tomato plants, which was corroborated by an increase in Fe content in tomato roots and leaves. Similarly, Ijaz et al. (2022) proved that the increase in the expression levels of *NRAMP1* and *NRAMP3* were associated with increased plant adaptation to HM toxicity. Indeed, our results revealed that BL improved Fe absorption and distribution by triggering the transcription of transporters involved in Fe absorption and distribution.

Given the significance and relevance of N as a component of crucial molecules such as nucleotides, amino acids, proteins, and enzymes, sustaining N metabolism has also a substantial effect on managing plant development and response to environmental challenges (Zayed et al., 2023). Here, As stress lowered the activities of NiR, NR, GS, and GOGAT and the concentrations of N and $NO_3^-$, while increased the GDH activity and leaf $NH_4^+$ content, which indicates a disturbance in N metabolism ($NO_3^-$ is converted into $NO_2^-$ and $NH_4^+$ by NR and NiR enzymes, respectively). The drop in leaf $NO_3^-$ levels might be related to a decrease in $NO_3^-$ transport from the roots to the leaves due to As damage to gas exchanges

and a slowing in the transpiration rate (Singh and Prasad, 2017). In addition, oxidative stress and the augmentation of toxic radicals, which are the result of As toxicity, could be another reason leading to damage of plasma membranes' permeability and a decrease in $NO_3^-$ absorption by root cells, potentially causing a decrease in leaf $NO_3^-$ levels in As-stressed tomato plants (Kaya et al., 2021). On the other hand, the increase in $NH_4^+$ accumulation might be due to a disruption in $NH_4^+$ assimilation caused by As exposure. Plants have an alternative mechanism for reducing excessive $NH_4^+$ formation under stress, mainly when the activities of GOGAT or GS are blocked (Yang et al., 2013). As a result, a rise of the GDH activity under As implies an inhibition in the GS and GOGAT activities and, consequently of, N metabolism. However, the augmentation of GDH activity in As-stressed tomato plants was not sufficient to reduce $NH_4^+$ quantity and sustain its assimilation, as evidenced by the decline in growth and the increase in leaf $NH_4^+$ concentration. Kaya et al. (2021) demonstrated that Cd also provoked a decline in wheat growth and biomass by disrupting N metabolism. However, in our work, BL spraying caused restoration of N metabolism in As-stressed plants by increasing the activities of NiR, NR, GS, and GOGAT and improving the N and $NO_3^-$ levels in the leaves, accompanied by a decline in GDH activity and a reduction in leaf $NH_4^+$ content. Xing et al. (2022) demonstrated that BRs could raise the expression level of $NO_3^-$ transporters under N-deficient conditions, suggesting that this hormone has regulatory effects on N absorption. BRs have also previously been shown to induce N metabolism and elevate N and $NO_3^-$ levels under Cd exposure (Singh and Prasad, 2017) and low temperature (Shu et al., 2016). Therefore, the reduction of oxidative stress resulting from BL and the protection provided by GS and GOGAT proteins in As-stressed tomato plants can be credited with BL's protective effects on N metabolism (Bajguz and Hayat, 2009). The study conducted by Devi et al. (2022) demonstrated the capacity of BR's to enhance root cell elongation and promote root development through their interaction with auxin. Hence, it is likely that one of the potential mechanisms triggered by BL is the enhancement of root development and absorption capacity, consequently facilitating the adaptation of tomato plants to As toxicity. This could be achieved through the promotion of $NO_3^-$ uptake and the stimulation of N assimilation processes under stress.

## 5. Conclusion

Arsenic stress was found to interfere with N and chlorophyll metabolism, leading to a decrease in the concentrations of N, $NO_3^-$, and chlorophylls and, consequently, a reduction in the growth of tomato plants. Furthermore, exposure was observed to increase As accumulation and raise the endogenous $H_2O_2$ and MG contents, resulting in oxidative stress and damage to the permeability of membranes, which was linked to a reduction in the Fe absorption and distribution in tomatoes. BL foliar spray modified the proline and chlorophyll metabolisms, and photosynthetic machinery. Spraying BL also lessened the amounts of $H_2O_2$ and MG and upregulated the activity of Gly and antioxidant enzymes, maintaining the integrity and permeability of biomembranes. By augmenting sulfur assimilation enzymes' activity and upregulating the mRNA levels of As sequestering genes (*GSH1, MT2, PCS,* and *ABC1*), BL increased sulfur-based defensive

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18                    Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1−21.

compounds` contents (PCs and GSH) and declined As toxicity. Regulating the transcription level of transporters involved in the absorption and transfer of Fe (IRT1, IRT2, NRAMP1, and NRAMP3) and As (Lsi1 and Lsi2) in roots and leaves, BL also enhanced Fe accumulation and lessened the As uptake in tomato plants. Furthermore BL treatment improved $NH_4^+$ assimilation and increased leaf N and $NO_3^-$ content under As stress via modulating the enzyme activities of N metabolism. Thus, the capacity of BRs and related gene transcription alterations to produce pleiotropic positive responses connected to several agronomic variables raises the possibility that BRs might be a significant target for concurrently optimizing plant production and performance to combat As toxicity. In this context, the molecular and biochemical responses to the interaction of BL and As will assist in a better comprehension of the anti-stress responses of the BR hormone, thereby improving appropriate procedures to protect plants against HM toxicity.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.hpj.2024.05.010.

R E F E R E N C E S

Aebi, H., 1984. Catalase in vitro. In: Colowick, S., Kaplan, N. (Eds.), Methods in Enzymology. Elsevier, Florida, pp. 121−126.

Agbaria, H., Heuer, B., Zieslin, N., 1998. Rootstock-imposed alterations in nitrate reductase and glutamine synthetase activities in leaves of rose plants. Biol Plant (Prague), 41: 85−91.

Anderson, M.E., 1985. Determination of glutathione and glutathione disulfides in biological samples. Meth Enzymol, 113: 548−570.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts Polyphenoloxidase in Beta vulgaris. Plant Physiol, 24: 1−15.

Bajguz, A., Hayat, S., 2009. Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Biochem, 47: 1−8.

Bajguz, A., Chmur, M., Gruszka, D., 2020. Comprehensive overview of the brassinosteroid biosynthesis pathways: substrates, products, inhibitors, and connections. Front Plant Sci, 11: 1034.

Bashir, H., Ibrahim, M.M., Bagheri, R., Ahmad, J., Arif, I.A., Baig, M.A., Qureshi, M.I., 2015. Influence of sulfur and cadmium on antioxidants, phytochelatins and growth in Indian mustard. AoB Plants, 7: plv001.

Basit, F., Bhat, J.A., Hu, J., Kaushik, P., Ahmad, A., Guan, Y., Ahmad, P., 2022. Brassinosteroid supplementation alleviates chromium toxicity in soybean (Glycine max L.) via reducing its translocation. Plants, 11: 2292.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studied. Plant Soil, 39: 205−207.

Bidi, H., Fallah, H., Niknejad, Y., Barari Tari, D., 2021. Iron oxide nanoparticles alleviate arsenic phytotoxicity in rice by improving iron uptake, oxidative stress tolerance and diminishing arsenic accumulation. Plant Physiol Biochem, 163: 348−357.

Błaszczyk, A., Sirko, L., Hawkesford, M.J., Sirko, A., 2002. Biochemical analysis of transgenic tobacco lines producing bacterial serine acetyltransferase. Plant Sci, 162: 589−597.

Bozzi, A.T., Gaudet, R., 2021. Molecular mechanism of nramp-family transition metal transport. J Mol Biol, 433: 166991.

Castaings, L., Caquot, A., Loubet, S., Curie, C., 2016. The high-affinity metal transporters NRAMP1 and IRT1 team up to take up iron under sufficient metal provision. Sci Rep, 16: 37222.

Cataldo, D.A., Maroon, M., Schrader, L.E., Youngs, V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal, 6: 71−80.

Chandrakar, V., Yadu, B., Meena, R.K., Dubey, A., Keshavkant, S., 2017. Arsenic-induced genotoxic responses and their amelioration by diphenyleneiodonium, 24-epibrassinolide and proline in Glycine max L. Plant Physiol Biochem, 112: 74−86.

Costa, M.L., Civello, P.M., Chaves, A.R., Martínez, G.A., 2005. Effect of ethephon and 6−benzylaminopurine on chlorophyll degrading enzymes and a peroxidase−linked chlorophyll bleaching during post−harvest senescence of broccoli (Brassica oleracea L.) at 20 °C. Postharvest Biol Technol, 35: 191−199.

Debouba, M., Gouia, H., Valadier, M.H., Ghorbel, M.H., Suzuki, A., 2006. Salinity-induced tissue-specific diurnal changes in nitrogen assimilatory enzymes in tomato seedlings grown under high or low nitrate medium. Plant Physiol Biochem, 44: 409−419.

Devi, L.L., Pandey, A., Gupta, S., Singh, A.P., 2022. The interplay of auxin and brassinosteroid signaling tunes root growth under low and different nitrogen forms. Plant Physiol, 189: 1757−1773.

Dhindsa, R.S., Matowe, W., 1981. Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. J Exp Bot, 32: 79−91.

Eckhardt, U., Mas Marques, A., Buckhout, T.J., 2001. Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants. Plant Mol Biol, 45: 437−448.

Emamverdian, A, Ding, Y, Hasanuzzaman, M, Barker, J, Liu, G, Li, Y, Mokhberdoran, F, 2023c. Insight into the biochemical and physiological mechanisms of nanoparticles-induced arsenic tolerance in bamboo. Front. Plant Sci., 14: 1121886. https://doi.org/10.3389/fpls.2023.1121886.

Emamverdian, A., Ghorbani, A., Li, Y., Pehlivan, N., Barker, J., Ding, Y., Liu, G., Zargar, M., 2023a. Responsible mechanisms for the restriction of heavy metal toxicity in plants via the co-foliar spraying of nanoparticles. Agronomy, 13: 1748.

Emamverdian, A., Ghorbani, A., Pehlivan, N., Alwahibi, M.S., Elshikh, M.S., Liu, G., Li, Y., Barker, J., Zargar, M., Chen, M., 2023b. Co-application of melatonin and zeolite boost bamboo tolerance under cadmium by enhancing antioxidant capacity, osmolyte accumulation, plant nutrient availability, and decreasing cadmium absorption. Sci Hortic, 322: 112433.

Fabregas, N., Lozano-Elena, F., Blasco-Escámez, D., Tohge, T., Martinez-Andujar, C., Albacete, A., Osorio, S., Bustamante, M., Riechmann, J.L., Nomura, T., Yokota, T., Conesa, A., Alfocea, F.P., Fernie, A.R., Cano-Delgado, A.I., 2018. Overexpression of the vascular brassinosteroid receptor BRL3

confers drought resistance without penalizing plant growth. Nat Commun, 9: 4680.

Fariduddin, Q., Yusuf, M., Ahmad, I., Ahmad, A., 2013. Brassinosteroids and their role in response of plants to abiotic stresses. Biol Plant (Prague), 58: 9–17.

Finnegan, P.M., Chen, W., 2012. Arsenic toxicity: the effects on plant metabolism. Front Physiol, 3: 182.

Foyer, C.H., Halliwell, B., 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 133: 21–25.

Fujii, J., Osaki, T., Soma, Y., Matsuda, Y., 2023. Critical roles of the cysteine-glutathione Axis in the production of γ-glutamyl peptides in the nervous system. Int J Mol Sci, 24: 8044.

Gaitonde, M.K., 1967. A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J, 104: 627–633.

Garcia-Rios, M., Fujita, T., LaRosa, P.C., Locy, R.D., Clithero, J.M., Bressan, R.A., Csonka, L.N., 1997. Cloning of a polycistronic cDNA from tomato encoding-glutamyl kinase and -glutamyl phosphate reductase. Proc Natl Acad Sci USA, 94: 8249–8254.

Ghorbani, A., Ghasemi Omran, V.O., Razavi, S.M., Pirdashti, H., Ranjbar, M., 2019. Piriformospora indica confers salinity tolerance on tomato (Lycopersicon esculentum Mill.) through amelioration of nutrient accumulation, K$^+$/Na$^+$ homeostasis and water status. Plant Cell Rep, 38: 1151–1163.

Ghorbani, A., Pishkar, L., Roudbari, N., Pehlivan, N., Wu, C., 2021. Nitric oxide could allay arsenic phytotoxicity in tomato (Solanum lycopersicum L.) by modulating photosynthetic pigments, phytochelatin metabolism, molecular redox status and arsenic sequestration. Plant Physiol Biochem, 167: 337–348.

Ghorbani, A., Pishkar, L., Roodbari, N., Ali Tavakoli, S., Moein Jahromi, E., Chu, W., 2022. Nitrate reductase is needed for methyl jasmonate-mediated arsenic toxicity tolerance of rice by modulating the antioxidant defense system, glyoxalase system and arsenic sequestration mechanism. J Plant Growth Regul, 42: 1107–1119.

Ghorbani, A., Emamverdian, A., Pehlivan, N., Zargar, M., Razavi, S.M., Chen, M.X., 2024a. Nano-enabled agrochemicals: mitigating heavy metal toxicity and enhancing crop adaptability for sustainable crop production. J Nanobiotechnol, 22: 91.

Ghorbani, A., Pehlivan, N., Zargar, M., Chen, M., 2024b. Synergistic role of melatonin and hydrogen sulfide in modulating secondary metabolites and metal uptake/sequestration in arsenic-stressed tomato plants. Sci Hortic, 331: 113159.

Gill, S.S., Khan, N.A., Tuteja, N., 2012. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (Lepidium sativum L.). Plant Sci, 182: 112–120.

Groat, R.G., Vance, C.P., 1981. Root nodule enzymes of ammonia assimilation in alfalfa (Medicago sativa L.): developmental patterns and response to applied nitrogen. Plant Physiol, 67: 1198–1203.

Guan, C., Cui, X., Liu, H.Y., Li, X., Li, M.Q., Zhang, Y.W., 2020. Proline biosynthesis enzyme genes confer salt tolerance to switchgrass (Panicum virgatum L.) in cooperation with polyamines metabolism. Front Plant Sci, 11: 46.

Habig, W.H., Jakoby, W.B., 1981. Assays for differentiation of glutathione S-Transferases. Meth Enzymol, 77: 398–405.

Haider, F.U., Zulfiqar, U., Mehmood, T., Shahzad, B., Liqun, C., Yong, J.W.H., Binobead, M.A., 2024. Effects of titanium oxide nanoparticles and 24-epibrassinosteroid to mitigate the toxic effect of cadmium (Cd) and improve physio-morphological traits of soybean (Glycine max L.) cultivated under Cd-contaminated soil. Environ Technol Innov, 103811: 1–16.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stochiometry of fatty acid peroxidation. Arch Biochem Biophys, 125: 189–198.

Hossain, M.A., Nakano, Y., Asada, K., 1984. Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiol, 25: 385–395.

Hossain, M.A., Hasanuzzaman, M., Fujita, M., 2010. Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiol Mol Biol Plant, 26: 259–272.

Howe, G., Merchant, S., 1992. Heavy metal activated synthesis of peptides in Chlymodomonas reinhartii. Plant Physiol, 98: 127–136.

Ijaz, M., Ansari, M., Alafari, H.A., Iqbal, M., Alshaya, D.S., Fiaz, S., Ahmad, H.M., Zubair, M., Ramzani, P.M., Iqbal, J., Abushady, A.M., Attia, K., 2022. Citric acid assisted phytoextraction of nickle from soil helps to tolerate oxidative stress and expression profile of NRAMP genes in sunflower at different growth stages. Front Plant Sci, 13: 1072671.

Jain, M., Gadre, R., 2004. Inhibition of 5-amino levulinic acid dehydratase activity by arsenic in excised etiolated maize leaf segments during greening. J Plant Physiol, 161: 251–255.

Jan, S., Alyemeni, M.N., Wijaya, L., Alam, P., Siddique, K.H., Ahmad, P., 2018. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in Pisum sativum L. seedlings. BMC Plant Biol, 18: 146.

Jez, J.M., Cahoon, R.E., Chen, S., 2004. Arabidopsis thaliana glutamate-cysteine ligase. J Biol Chem, 279: 33463–33470.

Kaya, C., Ashraf, M., Wijaya, L., Ahmad, P., 2019. The putative role of endogenous nitric oxide in brassinosteroid-induced antioxidant defence system in pepper (Capsicum annuum L.) plants under water stress. Plant Physiol Biochem, 143: 119–128.

Kaya, C., Ugurlar, F., Ashraf, M., Noureldeen, A., Darwish, H., Ahmad, P., 2021. Methyl jasmonate and sodium nitroprusside jointly alleviate cadmium toxicity in wheat (Triticum aestivum L.) plants by modifying nitrogen metabolism, cadmium detoxification, and AsA–GSH cycle. Front Plant Sci, 12: 654780.

Khan, E., Gupta, M., 2018. Arsenic–silicon priming of rice (Oryza sativa L.) seeds influence mineral nutrient uptake and biochemical responses through modulation of Lsi-1, Lsi-2, Lsi-6 and nutrient transporter genes. Sci Rep, 8: 10301.

Khare, R., Kumar, S., Shukla, T., Ranjan, A., Trivedi, P.K., 2017. Differential sulphur assimilation mechanism regulates response of Arabidopsis thaliana natural variation towards arsenic stress under limiting sulphur condition. J Hazard Mater, 337: 198–207.

Kiany, T., Pishkar, L., Sartipnia, N., Iranbakhsh, A., Barzin, G., 2022. Effects of silicon and titanium dioxide nanoparticles on arsenic accumulation, phytochelatin metabolism, and antioxidant system by rice under arsenic toxicity. Environ Sci Pollut Res Int, 29: 34725–34737.

Kolomeichuk, L.V., Efimova, M.V., Zlobin, I.E., Kreslavski, V.D., Murgan, O.K., Kovtun, I.S., Khripach, V.A., Kuznetsov, V.V., Allakhverdiev, S.I., 2020. 24-Epibrassinolide alleviates the toxic effects of NaCl on photosynthetic processes in potato plants. Photosynth Res, 146: 151–163.

Kozak, K., Papierniak-Wygladala, A., Palusińska, M., Barabasz, A., Antosiewicz, D.M., 2022. Regulation and function of metal uptake transporter NtNRAMP3 in tobacco. Front Plant Sci, 13: 867967.

Lea, P.J., Miflin, B.J., 2011. Nitrogen assimilation and its relevance to crop improvement. Annu Plant Rev, 42: 1–40.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods, 25: 402–408.

Lo, J.C., Tsednee, M., Lo, Y.C., Yang, S.C., Hu, J.M., Ishizaki, K., Kohchi, T., Lee, D.C., Yeh, K.C., 2016. Evolutionary analysis of iron (Fe) acquisition system in Marchantia polymorpha. New Phytol, 211: 569–583.

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20                        Abolghassem Emamverdian et al. 2024. Horticultural Plant Journal, xxx (xxxx): 1−21.

López-Lorca, V.M., Molina-Luzón, M.J., Ferrol, N., 2022. Characterization of the NRAMP gene family in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. J Fungi (Basel), 8: 592.

Loreto, F., Velikova, V., 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol, 127: 1781−1787.

Lv, B., Tian, H., Zhang, F., Liu, J., Lu, S., Bai, M., Li, C., Ding, Z., 2018. Brassinosteroids regulate root growth by controlling reactive oxygen species homeostasis and dual effect on ethylene synthesis in *Arabidopsis*. PLoS Genet, 14: e1007144.

Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P., Zhao, F.J., 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc Natl Acad Sci USA, 105: 9931−9935.

Ma, J.F., Yamaji, N., Mitani-Ueno, N., 2011. Transport of silicon from roots to panicles in plants. Proc Jpn Acad Ser B Phys Biol Sci, 87: 377−385.

Marmiroli, M., Pigoni, V., Savo-Sardaro, M.L., Marmiroli, N., 2014. The effect of silicon on the uptake and translocation of arsenic in tomato (*Solanum lycopersicum* L.). Environ Exp Bot, 99: 9−17.

Metwally, A., Safronova, V.I., Belimov, A.A., Dietz, K.J., 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exper Bot, 56: 167−178.

Molins-Legua, C., Meseguer-Lloret, S., Moliner-Martinez, Y., Campíns-Falcó, P., 2006. A guide for selecting the most appropriate method for ammonium determination in water analysis. TrAC Trends Anal Chem, 25: 282−290.

Mostofa, M.G., Rahman, A., Ansary, M.M.U., Watanabe, A., Fujita, M., Tran, L.P., 2015. Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. Sci Rep, 5: 14078.

Muñoz-Huerta, R.F., Guevara-Gonzalez, R.G., Contreras-Medina, L.M., Torres-Pacheco, I., Prado-Olivarez, J., Ocampo-Velazquez, R.V., 2013. A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. Sensors, 13: 10823−10843.

Nahar, K., Rhaman, M.S., Parvin, K., Bardhan, K., Marques, D.N., García-Caparrós, P., Hasanuzzaman, M., 2022. Arsenic-induced oxidative stress and antioxidant defense in plants. Stresses, 2: 179−209.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol, 22: 867−880.

Navabpour, S., Yamchi, A., Bagherikia, S., Kafi, H., 2020. Lead-induced oxidative stress and role of antioxidant defense in wheat (*Triticum aestivum* L.). Physiol Mol Biol Plants, 26: 793−802.

Orlowski, M., Meister, A., 1973. γ-Glutamyl cyclotransferase distribution, isozymic forms, and specificity. J Biol Chem, 248: 2836−2844.

Panthri, M., Gupta, M., 2022. An insight into the act of iron to impede arsenic toxicity in paddy agro-system. J Environ Manage, 316: 115289.

Peck Jr, H.D., Deacon, T.E., Davidson, J.T., 1965. Studies on adenosine 5′-phosphosulfate reductase from *Desulfovibrio desulfuricans* and *Thiobacillus thioparus* I. The assay and purification. Biochem Biophys Acta, 96: 429−446.

Pehlivan, N., Gedik, K., Eltem, R., Terzi, E., 2021. Dynamic interactions of *Trichoderma harzianum* TS 143 from an old mining site in Turkey for potent metal (oid)s phytoextraction and bioenergy crop farming. J Hazard Mater, 403: 123609.

Pehlivan, N., Gedik, K., Wang, J.J., 2023. Tea-based biochar-mediated changes in cation diffusion homeostasis in rice grown in heavy metal (loid) contaminated mining soil. Plant Physiol Biochem, 201: 107889.

Principato, G.B., Rosi, G., Talesa, V., Govannini, E., Uolila, L., 1987. Purification and characterization of two forms of glyoxalase II

from rat liver and brain of Wistar rats. Biochem Biophys Acta, 911: 349−355.

Quintana, J., Bernal, M., Scholle, M., Holländer-Czytko, H., Nguyen, N.T., Piotrowski, M., Mendoza-Cózatl, D.G., Haydon, M.J., Krämer, U., 2022. Root-to-shoot iron partitioning in Arabidopsis requires IRON-REGULATED TRANSPORTER1 (IRT1) protein but not its iron (II) transport function. Plant J, 109: 992−1013.

Rajput, V.D., Singh, R.K., Verma, K.K., Sharma, L., Quiroz-Figueroa, F.R., Meena, M., Gour, V.S., Minkina, T., Sushkova, S., Mandzhieva, S., 2021. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. Biology, 10: 267.

Rena, A.B., Splittstoesser, W.E., 1975. Proline dehydrogenase and pyrroline-5-carboxylate reductase from pumpkin cotyledons. Phytochemistry, 14: 657−661.

Servet, C., 2012. Proline dehydrogenase: a key enzyme in controlling cellular homeostasis. Front Biosci, 17: 607−620.

Shahid, M.A., Balal, R.M., Khan, N., Zotarelli, L., Liu, G.D., Sarkhosh, A., Fernández-Zapata, J.C., Martínez Nicolás, J.J., Garcia-Sanchez, F., 2019. Selenium impedes cadmium and arsenic toxicity in potato by modulating carbohydrate and nitrogen metabolism. Ecotoxicol Environ Saf, 180: 588−599.

Sharma, S., Anand, G., Singh, N., Kapoor, R., 2017. Arbuscular mycorrhiza augments arsenic tolerance in wheat (*Triticum aestivum* L.) by strengthening antioxidant defense system and thiol metabolism. Front Plant Sci, 8: 906.

Shu, S., Tang, Y., Yuan, Y., Sun, J., Zhong, M., Guo, S., 2016. The role of 24-epibrassinolide in the regulation of photosynthetic characteristics and nitrogen metabolism of tomato seedlings under a combined low temperature and weak light stress. Plant Physiol Biochem, 107: 344−353.

Singh, S., Prasad, S.M., 2017. Effects of 28-homobrassinoloid on key physiological attributes of *Solanum lycopersicum* seedlings under cadmium stress: photosynthesis and nitrogen metabolism. Plant Growth Regul, 82: 161−173.

Singh, V.K., Upadhyay, R.S., 2014. Effects of arsenic on reactive oxygen species and antioxidant defense system in tomato plants. Toxicol Environ Chem, 96: 1374−1383.

Su, Q., Zheng, X., Tian, Y., Wang, C., 2020. Exogenous brassinolide alleviates salt stress in *Malus hupehensis* Rehd. by regulating the transcription of NHX-Type Na$^+$(K$^+$)/H$^+$ antiporters. Front Plant Sci, 11: 479411.

Sun, H., Duan, Y., Mitani-Ueno, N., Che, J., Jia, J., Liu, J., Guo, J., Ma, J.F., Gong, H., 2020. Tomato roots have a functional silicon influx transporter but not a functional silicon efflux transporter. Plant Cell Environ, 43: 732−744.

Surgun-Acar, Y., Zemheri-Navruz, F., 2019. 24-Epibrassinolide promotes arsenic tolerance in *Arabidopsis thaliana* L. by altering stress responses at biochemical and molecular level. J Plant Physiol, 238: 12−19.

Szafrańska, K., Reiter, R.J., Posmyk, M.M., 2017. Melatonin improves the photosynthetic apparatus in pea leaves stressed by Paraquat via chlorophyll breakdown regulation and its accelerated de novo synthesis. Front Plant Sci, 8: 878.

Tennstedt, P., Peisker, D., Bottcher, C., Trampczynska, A., Clemens, S., 2009. Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. Plant Physiol, 149: 938−948.

Vert, G., Briat, J.F., Curie, C., 2001. *Arabidopsis IRT2* gene encodes a root-periphery iron transporter. Plant J, 26: 181−189.

Wang, Z., Yang, Y., Yadav, V., Zhao, W., He, Y., Zhang, X., Wei, C., 2022. Drought-induced proline is mainly synthesized in leaves and transported to roots in watermelon under water deficit. Hortic Plant J, 8 (5): 615−626.

Xing, J., Wang, Y., Yao, Q., Zhang, Y., Zhang, M., Li, Z., 2022. Brassinosteroids modulate nitrogen physiological response and promote nitrogen uptake in maize (*Zea mays* L.). Crop J, 10: 166—176.

Xu, Z., Huang, S., Xie, Y., Wang, S., Jin, N., Jin, L., Tie, J., Meng, X., Li, Z., Lyu, J., Yu, J., 2023. Physiological responses of coriander (*Coriandrum sativum* L.) to exogenous 2,4-epibrassinolide at different concentrations. BMC Plant Biol, 23: 649.

Yang, Y.J., Lu, X.M., Yan, B., Li, B., Sun, J., Guo, S.R., Takafumi, T., 2013. Bottle gourd rootstock-grafting affects nitrogen metabolism in NaCl-stressed watermelon leaves and enhances short-term salt tolerance. J Plant Physiol, 170: 653e661.

Zayed, O, Hewedy, OA, Abdelmoteleb, A, Ali, M, Youssef, MS, Roumia, AF, Seymour, D, Yuan, ZC, 2023 Sep 25. Nitrogen journey in plants: from uptake to metabolism, stress response, and microbe interaction. Biomolecules, 13 (10): 1443. https://doi.org/10.3390/biom13101443. PMID: 37892125; PMCID: PMC10605003.

Zeng, H., Tang, Q., Hua, X., 2010. *Arabidopsis* brassinosteroid muatants *det2*-1 and *bin2*-1 display altered salt tolerance. J Plant Growth Regul, 29: 44—52.

Zhao, G., Xu, H., Zhang, P., Su, X., Zhao, H., 2017. Effects of 24-epibrassinolide on photosynthesis and Rubisco activase gene expression in *Triticum aestivum* L. seedlings under a combination of drought and heat stress. Plant Growth Regul, 81: 377—384.

Zulfiqar, F., Ashraf, M., 2022. Antioxidants as modulators of arsenic-induced oxidative stress tolerance in plants: an overview. J Hazard Mater, 427: 127891.