Journal of Plant Growth Regulation (2018) 37:1195–1209
https://doi.org/10.1007/s00344-018-9814-y

Jasmonic Acid Improves Growth Performance of Soybean Under Nickel Toxicity By Regulating Nickel Uptake, Redox Balance, and Oxidative Stress Metabolism

Mudaser Ahmad Mir[1] · Geetika Sirhindi[1] · Mohammed Nasser Alyemeni[2] · Pravej Alam[3] · Parvaiz Ahmad[2,4]

Received: 19 February 2018 / Accepted: 2 May 2018 / Published online: 21 May 2018

Abstract
Soil contamination with nickel (Ni) is a persistent threat to crop production worldwide. The present study examined the putative roles of jasmonic acid (JA) in improving Ni tolerance in soybean. Our findings showed that priming of soybean seeds with JA significantly improved the growth performance of soybean when grown under excessive Ni. The enhanced Ni tolerance of soybean prompted by JA could be ascribed to its ability to regulate Ni uptake and accumulation, and to decrease Ni-induced membrane damage as evidenced by reduced levels of reactive oxygen species (ROS), malondialdehyde, lipoxygenase activity, and electrolyte leakage in Ni-stressed plants. JA also boosted redox states and antioxidant capacity in Ni-stressed plants by maintaining increased levels of ascorbate and glutathione, and enhanced activities of ROS-detoxifying enzymes compared with Ni-stressed alone plants. Additionally, methylglyoxal detoxification system was significantly upregulated in JA-primed and "JA-primed + Ni-stressed" plants, indicating an alleviating effect of JA on Ni-induced methylglyoxal toxicity. Our results conclude that JA-mediated regulation of Ni uptake and accumulation, and enhanced ROS metabolism by activating antioxidant defense and glyoxalase systems contributed to improved performance of soybean under excessive Ni, thereby suggesting JA as an effective stress regulator in mitigating Ni toxicity in economically important soybean, and perhaps in other crops.

Keywords Jasmonic acid · Nickel · Soybean · Antioxidants · Lipid peroxidation · Methyl glyoxalase

Introduction

Environmental contaminations by toxic metals have long been recognized in developing and underdeveloped countries; however, the pollution still continues because of rapid urbanization and lack of proper disposal practices of waste materials, especially industrial effluents (Chibuike and Obiora 2014). In this context, nickel (Ni) has arisen as a serious contaminant over the past centuries due to its tremendous applications in agricultural and manufacturing industries (Yusuf et al. 2011). As a consequence, agricultural lands of many countries, including India, are exceedingly polluted with Ni because of unrestrained and regular applications of Ni-containing poultry slurries, pesticides, and untreated industrial wastes to the crop fields (Ayangbenro and Babalola 2017). Soybean (*Glycine max*) is an economically important legume crop grown in many countries in the world, and is considered as one of the major oil-yielding crops in many Asian countries, including India, China, Japan, and Pakistan (Agarwal et al. 2013; FAOSTAT 2015). In Asia, India is the second largest soybean-producing country after China, covering 3.95% of global soybean production (Agarwal et al. 2013; FAOSTAT 2015). Cultivation of legume crops like soybean in Ni-polluted lands results in yield reduction and poor seed quality, as well as causes Ni toxicity to consumers, including animals and humans (Ayangbenro and Babalola 2017).

✉ Parvaiz Ahmad
parvaizbot@yahoo.com

[1] Department of Botany, Punjabi University, Patiala, India

[2] Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

[3] Biology Department, College of Science and Humanities, Prince Sattam bin Abdulaziz University (PSAU), 11942 Alkharj, Kingdom of Saudi Arabia

[4] Department of Botany, S.P. College, Srinagar, Jammu And Kashmir 190001, India

Ni, a cofactor of various metallo-proteins and enzymes, plays pivotal roles in plant development, growth, and defensive mechanisms (Fabiano et al. 2015). However, a marginal accumulation of Ni beyond its beneficial window may cause Ni toxicity, affecting various physio-biochemical processes, including mineral uptake, photosynthesis, membrane permeability, nitrogen metabolism, and senescence in plants (Küpper and Andresen 2016; Sirhindi et al. 2015). Excessive Ni accumulation in plants results in abnormal root growth, and necrotic lesions, chlorosis, and rolling in leaves (Yusuf et al. 2011). Ni can also accelerate the production of reactive oxygen species (ROS) that are responsible for inducing oxidative stress by causing damage to lipids, proteins, and DNA molecules (Küpper and Andresen 2016; Sirhindi et al. 2016). To overcome metal-induced oxidative damage, plant cell organelles are inherently equipped with a vibrant antioxidant defense system that comprises various enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX), glutathione *S*-transferase (GST), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR), as well as non-enzymatic arsenals, such as glutathione (GSH) and ascorbic acid (AsA) (Gill and Tuteja 2010).

Methylglyoxal (MG), a highly cyto-reactive aldehyde is well-known to accumulate at high levels in cellular compartments under various abiotic stresses, including metal toxicity (Hoque et al. 2016; Hossain et al. 2009; Mostofa et al. 2015a). Once overproduced inside cells, MG can exert its cytotoxic effects by (i) directly reacting with biomolecules, (ii) accelerating ROS production, and (iii) overproducing advanced glycation end products (AGEs) (Hoque et al. 2016; Li 2016). As a counter measure, plant cells possess a ubiquitous glyoxalase (Gly) cycle to detoxify MG in a two-step reaction involving conversion of MG to non-toxic D-lactate by using antioxidant GSH, and Gly I and Gly II enzymes. Genetically modified plants harboring *Gly* genes have shown advanced tolerance to abiotic stresses and improved ROS detoxification ability (Álvarez Viveros et al. 2013; Ghosh et al. 2014; Kaur et al. 2014). Effective and coordinated performances of the Gly and antioxidant defense systems have known to dictate improved tolerance to multiple abiotic constraints in various plant species (Mostofa et al. 2015b; Mudalkar et al. 2017; Upadhyaya et al. 2011). Apart from defense mechanisms related to ROS and MG detoxifications, maintenance of Ni homeostasis is critically important in regulating intracellular Ni content to escape Ni toxicity. This could be achieved through numerous mechanisms, including restriction of Ni uptake by forming complexes with root exudates, and intracellular sequestration after binding with phytochelatins or strong ligands, such as cysteine-rich compounds (Yusuf et al. 2011).

Plant responses to environmental stimuli are mostly orchestrated by an array of plant growth regulators, including phytohormones. Jasmonic acid (JA) and methyl jasmonate (MeJA) have been known to activate a number of signaling events during plant responses to abiotic and biotic stresses, thus developing improved safeguard in plants under such stresses (Wasternack 2014). Modification of endogenous JA levels in plants showed promising roles in providing protections against numerous abiotic stresses, including salinity, heat, drought, and metal toxicity (Piotrowska et al. 2009; Sirhindi et al. 2016; Wasternack 2014). However, the interactions of JA with Ni and how JA modulates the physiological and biochemical changes under Ni stress in an economically important legume crop like soybean are still elusive. Moreover, only in few studies JA is known to affect metal tolerance by improving antioxidants (Sirhindi et al. 2015, 2016) but the precise roles of JA in simultaneous regulation of ROS and MG detoxification systems under Ni stress in crop plants are limited, thus warranting in-depth investigations to understand how antioxidant metabolism is changed in crop plants in response to Ni toxicity. The current work is the extension of our previous study (Sirhindi et al. 2015, 2016). Here we aimed to investigate the effects of JA on growth and physio-biochemical processes in soybean by considering the mechanisms related to (i) Ni uptake and accumulation, (ii) JA-induced alterations in growth performance and oxidative parameters, (iii) the effects of JA in modifying non-enzymatic and enzymatic defenses, and (iv) MG detoxification under Ni-stress conditions.

Materials and Methods

Plant Growth and Treatments

Seeds of soybean (*G. max* L. cv.SL-525) were sorted and sterilized with mercuric chloride (0.1%, v/v) for 5 min, and then rinsed five times with ion-free distilled water. Sterilized seeds were divided into two groups and separately soaked in (i) distilled water and (ii) freshly prepared 1 picomolar (pM) JA solution for 8 h. The air-dried seeds of both groups were sown in plastic pots filled with 3 kg of soil that had been composed of peat, perlite, and sand (1:1:1, v/v/v). The pots were arranged in completely randomized block design with three replications in a controlled growth chamber of 26 ± 2 °C temperatures, 14 h/10 h of day/night cycle with 350 µmol photon m^{-2} s^{-1} light intensity, and 65–70% humidity. After 16 days of sowing, soybean seedlings were subjected to Ni treatment by applying nickel chloride (4 mM) solution or water (for control) on the soil surface of pots, and then plants were grown for an additional period of 4 or 8 days in the above-specified conditions. The third trifoliate leaves (from the top) of soybean plants from each treatment

were harvested after 4 and 8 days (20- and 24-day-old plants, respectively) of Ni application for determining various physiological and biochemical parameters.

Estimation of Plant Height and Biomass

The root and shoot lengths of soybean plants were measured using meter scale. For determining plant biomass, DW of third leaves were recorded following the method described in Sirhindi et al. (2016).

Leaf Relative Water Content (RWC), Chl Content, and Proline Content

Leaf RWC was determined according to the method described by Barrs and Weatherley (1962). For determination of total Chl in leaves, 0.5 g of leaf samples was homogenized in 5 mL of 80% aqueous acetone followed by a centrifugation at 10,000×*g* for 8 min. The optical density (OD) of acetone extract was recorded at 645 and 663 nm using spectrophotometer (Beckman 640 D, USA), and total Chl contents were estimated following the formula reported in Arnon (1949). Pro content was estimated using acid ninhydrin solution as previously described by Bates et al. (1973).

Ni Content and Ni Accumulation in Roots and Leaves of Soybean

For estimating Ni content, the separately harvested root and leaf samples were oven-dried at 80 °C for 48 h. The dried samples (0.1 g) were ground and completely digested with HNO_3:$HClO_4$ (5:1 v/v) at 80 °C. The content of Ni in leaves and root samples was analyzed by atomic absorption spectrophotometry (AAS) (Z-5000, Hitachi, Japan), and the Ni concentration and accumulation were calculated using the following formulas:

$$\text{Ni concentration } \left(\text{mg g}^{-1}\ \text{DW}\right) = (\text{Reading of AAS} \times \text{dilution factor})/\text{DW of roots or leaves.}$$

$$\text{Ni accumulation } \left(\mu\text{g seedling}^{-1}\right) = \text{Concentration of Ni} \times \text{DW of roots or leaves per plant.}$$

Lipid Peroxidation, H_2O_2 Content, and Electrolyte Leakage

Lipid peroxidation product malondialdehyde (MDA) in soybean leaves was estimated according to the method of (Heath and Packer 1968) using an extinction co-efficient of 155 mM^{-1} cm^{-1}. H_2O_2 content was determined by conducting a reaction of sample extract and 0.1% $TiCl_4$ prepared in 20% H_2SO_4 following the protocol described in Velikova et al. (2000). Leaf electrolyte leakage was estimated by following the method previously published by Bajji et al. (2002).

Histochemical Detection of $O_2^{\bullet-}$ in Soybean Leaves

$O_2^{\bullet-}$ localization in soybean leaves was detected following the method described by Mostofa and Fujita (2013). In brief, freshly harvested middle leaves of the third trifoliate leaves of 24-day-old plants were immersed in a solution of 0.1% nitroblue tetrazolium (NBT), and incubated at room temperature for 24 h under light. The blue polymerization products in the incubated leaves were detected by decolorizing the leaves in boiling ethanol (90%) for 12 min followed, and photographs were taken using a digital camera (Sony Cyber-shot H300 Point and Shoot Digital camera).

Contents of Non-enzymatic Antioxidants

For determining reduced and oxidized AsA contents, soybean leaves (0.5 g) were ground in 3 mL of 5% metaphosphoric acid containing 1 mM EDTA using ice-cold mortar and pestle followed by a centrifugation at 11,500×*g* for 12 min. Total and reduced AsA contents were spectrophotometrically assayed at 265 nm in potassium phosphate buffer (100 mM, pH 7.0) using 1.0 unit (U) of ascorbate oxidase as described by Dutilleul et al. (2003). Reduced AsA content was subtracted from total AsA content to determine the level of oxidized AsA (DHA). Total GSH [GSH + oxidized GSH (GSSG)] in leaf samples was determined following the method of (Griffith 1980). GSSG content was estimated after eliminating GSH by 2-vinylpyridine derivatization, and the content of reduced GSH was calculated by deducting GSSG content from total GSH content.

Protein Extraction and Determination of Enzyme Activities

Freshly harvested soybean leaves (1.0 g) were homogenized in 1 mL of Tris–HCl (100 mM) containing 5 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM magnesium acetate PVP-40 (1.5%), 1 mM phenylmethanesulfonyl fluoride (PMSF), and 1 µg aprotinin. The homogenates were centrifuged at 11,500×*g* for 20 min at 4 °C, and the supernatants were separated for determining protein content and enzyme activities.

The activity of LOX (EC 1.13.11.12) was determined using linoleic acid as a substrate solution following the method of Doderer et al. (1992). SOD (EC 1.15.1.1) activity was determined using xanthine–xanthine oxidase system according to the method of El-Shabrawi et al. (2010), and expressed in U (one U was the amount of enzyme required to inhibit 50% photoreduction of NBT) min^{-1} mg^{-1} protein. CAT (EC 1.11.1.6) activity was measured according to the method of (Aebi 1984). For measuring APX (EC 1.11.1.11) activity, method of (Nakano and Asada 1981) was followed. MDHAR (EC 1.6.5.4) and DHAR (EC 1.8.5.1) activities were estimated following the methods of (Nakano and Asada 1981) and Hossain et al. (1984), respectively. The activity of GR (EC 1.6.4.2) was measured by observing GSSG-dependent oxidation of NADPH following the protocol developed by (Foyer and Halliwell 1976). GST (EC 2.5.1.18) and GPX (EC: 1.11.1.9) activities were measured according to the method of Hossain et al. (2009) and (Putter and Becker 1984), respectively. The activities of Gly I (EC 4.4.1.5) and Gly II (EC 3.1.2.6) enzymes were determined according to the method of Hossain et al. (2009) using extinction co-efficients of 3.37 and 13.6 mM^{-1} cm^{-1}, respectively.

MG Content Estimation

For extracting MG from soybean leaves, freshly harvested leaves (0.5 g) were homogenized in 0.5 M perchloric acid (2.5 mL), incubated on ice for 15 min, and centrifuged at 11,200×*g* at 4 °C for 10 min. After neutralizing with saturated K_2CO_3, 650 µL of the supernatant was mixed with 330 µL of potassium phosphate buffer (100 mM, pH 7.0) and 20 µL of 0.5 M *N*-acetyl-L-cysteine, and then incubated for 10 min at room temperature. The absorbance was recorded at 288 nm, and the content of MG was estimated using a standard graph developed with known concentrations of MG as described by Wild et al. (2012).

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and the mean differences were compared by Duncan's multiple range test (DMRT) using XLSTAT 2015. Data in tables and figures are represented as means ± standard deviations (SD) of three (3) independent replicates from each treatment. Significant differences between treatments are designated using different alphabetical letters at $P < 0.05$.

Results

JA Improves Soybean Plant Growth Under Ni Stress

Treatment of soybean plants with Ni (4 mM) alone for a period of 8 days resulted in adverse effects on plant performance, such as stunted growth as revealed by shoot height and root length (Fig. 1a). Seedlings treated with only Ni displayed significant reduction in shoot height by 20.34 and 30.31%, and root length by 33.46 and 46.41% at days 20 and 24, respectively, as compared with untreated control plants (Table 1). Priming of seeds with JA relieved the toxic effects of Ni, and enhanced the shoot height by 30.15 and 50.45%, and root length by 60.68 and 88.82% at days 20 and 24, respectively, in comparison with Ni-stressed only seedlings (Table 1). Plant biomass in terms of dry weight (DW) dramatically declined under Ni stress when compared with Ni-free conditions. The decline of DW was 36.65 and 45.93% at days 20 and 24, respectively. However, "JA-primed + Ni-treated" seedlings showed significantly higher DW (by 25.60 and 67.06%, at days 20 and 24, respectively) relative to the Ni-stressed only seedlings, demonstrating the positive impacts of JA on growth performance of soybean under Ni stress.

JA Maintains the Chlorophyll, Relative Water, and Proline Contents

As compared with untreated control, a drastic reduction in total chlorophyll (Chl) content by 34.48% at day 20 and 43.95% at day 24 was noticed in Ni-stressed plants (Table 1). Priming of seeds with JA improved Chl content in Ni-treated plants by 35.08 and 54.90% at days 20 and 24, respectively, in comparison with Ni-stressed only plants. Relative water content (RWC) significantly declined by 20.78 and 28.82% at days 20 and 24, respectively, compared with that of untreated control (Table 1). On the other hand, RWC remained significantly higher in "JA-primed + Ni-treated" seedlings (20.86 and 28.00% at days 20 and 24, respectively), when compared with that of Ni-stressed only seedlings. The content of proline (Pro) significantly increased by 100.73% at day 20 and 128.53% at day 24 in the Ni-stressed plants as compared with that of control (Table 1). Pretreatment of soybean seeds with JA resulted in a lower increase of proline (Pro) content (20.54 and 24.53% at days 20 and 24, respectively) in the Ni-treated seedlings relative to the untreated control seedlings (Table 1).

Exogenous JA Attenuates Ni Uptake and Accumulation in Soybean

Ni uptake and accumulation in roots and leaves were differentially regulated under the condition of seed priming with JA in soybean. Ni content in the roots of the Ni-stressed

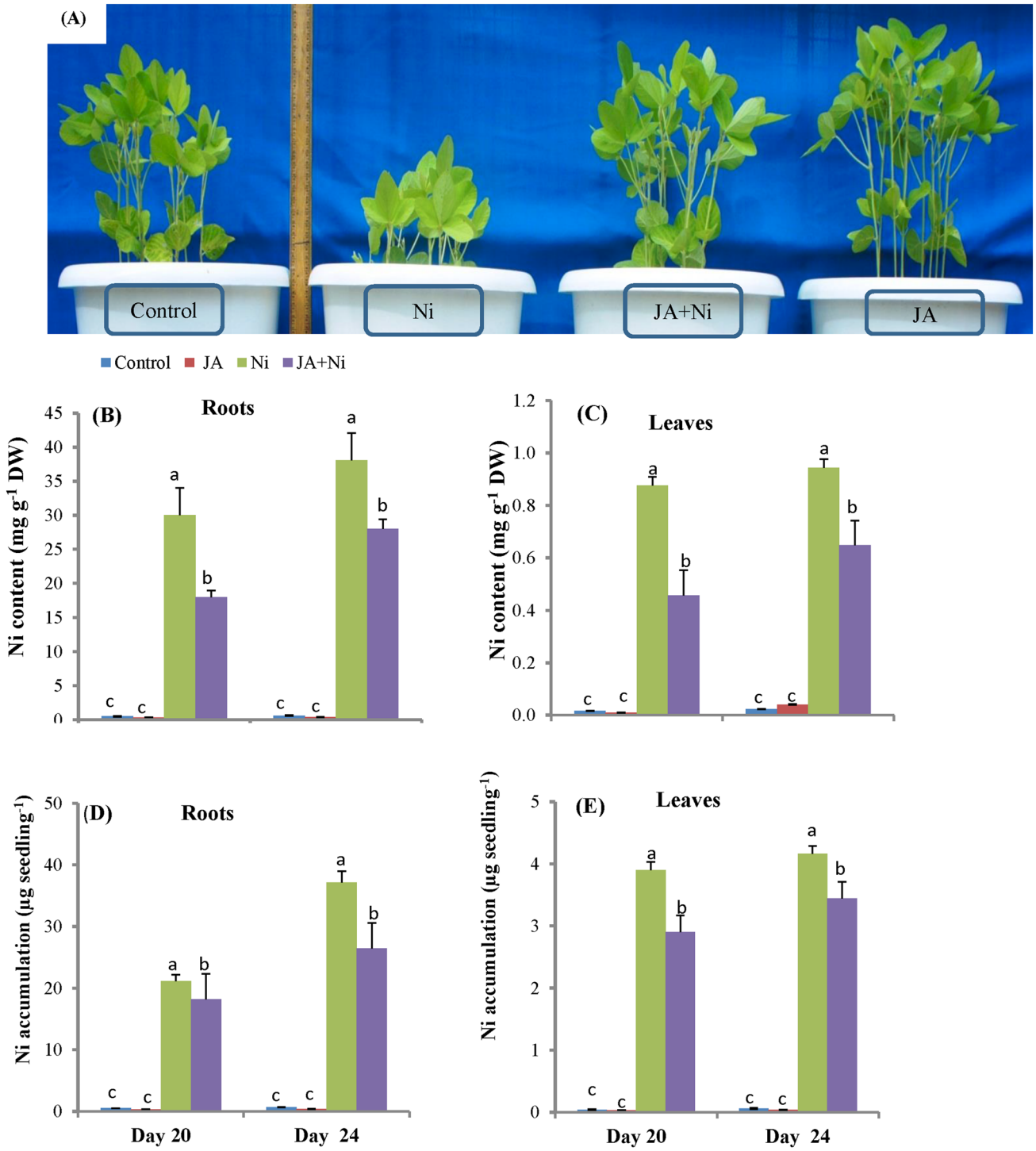


Fig. 1 Effects of seed priming with jasmonic acid (JA) on phenotypes, and Ni uptake and accumulation of soybean seedlings in the presence or absence of Ni stress. **a** Phenotypic appearance of soybean seedlings at day 8th of Ni treatment (day 24), **b** Ni content in roots, **c** Ni content in leaves, **d** Ni accumulation in roots, and **e** Ni accumulation in leaves at days 20 and 24. Control, JA, Ni, and JA + Ni correspond to control, 1 pM jasmonic acid, 4 mM $NiCl_2$, and 1 pM jasmonic acid + 4 mM $NiCl_2$ in nutrient solutions, respectively. Bars represent standard deviation (SD) of the mean ($n = 3$). Different letters (*a*, *b*, *c*) indicate statistically significant differences between treatments, according to Duncan's multiple range test ($P < 0.05$). *DW* dry weight

Table 1 Effects of seed priming with jasmonic acid (JA) on growth (shoot height, root length) and biomass (DW, dry weight), and the levels of total chlorophylls (Chls), relative water content (RWC), and proline (Pro) of soybean plants in the presence or absence of Ni stress at days 20 and 24

Duration	Treatment	Shoot height (cm)	Root length (cm)	DW (mg seedling^{-1})	Total Chls (mg g^{-1} FW)	Leaf RWC (%)	Pro (μmol g^{-1} FW)
Day 20	Control	18.19 ± 1.00^{b}	7.95 ± 0.56^{b}	78.30 ± 1.72^{b}	0.87 ± 0.08^{a}	98.76 ± 0.55^{a}	12.27 ± 1.28^{c}
	JA	24.32 ± 0.93^{a}	12.93 ± 0.86^{a}	88.02 ± 1.14^{a}	0.88 ± 0.07^{a}	98.84 ± 0.93^{a}	13.08 ± 1.58^{c}
	Ni	14.49 ± 1.21^{c}	5.29 ± 0.50^{c}	49.60 ± 1.01^{d}	0.57 ± 0.08^{c}	78.24 ± 0.94^{c}	24.63 ± 0.52^{a}
	JA + Ni	18.86 ± 0.76^{b}	8.50 ± 0.50^{b}	62.30 ± 0.92^{c}	0.77 ± 0.02^{b}	94.56 ± 0.25^{b}	19.57 ± 0.73^{b}
Day 24	Control	20.88 ± 0.39^{b}	10.02 ± 0.99^{b}	87.14 ± 0.89^{b}	0.91 ± 0.06^{a}	98.54 ± 0.77^{b}	14.02 ± 0.66^{c}
	JA	27.13 ± 0.70^{a}	14.01 ± 1.01^{a}	98.34 ± 0.36^{a}	0.88 ± 0.08^{a}	98.91 ± 0.89^{a}	15.12 ± 0.61^{c}
	Ni	14.55 ± 0.39^{c}	5.37 ± 0.29^{c}	47.12 ± 1.00^{d}	0.51 ± 0.06^{c}	70.14 ± 1.05^{d}	32.04 ± 0.77^{a}
	JA + Ni	21.89 ± 0.10^{b}	10.14 ± 0.97^{b}	78.72 ± 1.71^{c}	0.79 ± 0.09^{bc}	89.78 ± 1.04^{c}	24.18 ± 0.63^{b}

Control, JA, Ni, and JA + Ni correspond to the group of plants receiving nutrients only, 1 pM jasmonic acid, 4 mM $NiCl_2$, and 1 pM jasmonic acid + 4 mM $NiCl_2$ in nutrient solutions, respectively. Values are means ± SDs of three independent replications ($n = 3$). Different letters within the column indicate statistically significant differences among the treatments, according to Duncan's multiple range test ($P < 0.05$)

only seedlings increased to 30.02 and 38.05 (mg g^{-1} DW) at days 20 and 24, respectively. However, JA-priming significantly inhibited Ni uptake, and Ni content was reduced to 18.01 (mg g^{-1} DW) at days 20 and 28.00 (mg g^{-1} DW) at days 24 in the roots of JA-primed Ni-treated plants (Fig. 1b). In comparison with control plants, Ni content significantly increased in the leaves of Ni-stressed only plants, whereas JA-priming in Ni-treated plants reduced the content by 0.46 and 0.65 (mg g^{-1} DW) at days 20 and 24, respectively, in Ni-treated plants (Fig. 1c). Consistently, Ni accumulation was significantly higher in Ni-stressed plants than that of control plants; however, JA-priming reduced Ni accumulation in Ni-stressed roots to 18.20 from 21.15 (μg g^{-1} DW) and 26.44 from 37.12 (μg g^{-1} DW) and in Ni-stressed leaves by 2.90 from 3.90 (μg g^{-1} DW) and 3.44 from 4.16 (μg g^{-1} DW) at days 20 and 24, respectively (Fig. 1d, e). These data revealed that JA-priming attenuated Ni toxicity, at least in part, by restricting its uptake and accumulation in soybean seedlings under excessive Ni.

JA Reduces Oxidative Stress, Electrolyte Leakage, and Lipoxygenase Activity

To investigate Ni-induced oxidative stress, we observed ROS generation by estimating superoxide ($O_2^{\bullet-}$) accumulation and hydrogen peroxide (H_2O_2) level in the middle leaves of third trifoliate of soybean plants. Histochemical staining with nitroblue tetrazolium (NBT) showed much higher accumulation of dark-blue polymerization products, indicating overproduction of $O_2^{\bullet-}$ in the Ni-treated plants relative to the Ni-untreated control. However, the accumulation of $O_2^{\bullet-}$ in "JA-primed + Ni-treated" plants markedly reduced in comparison with that of Ni-treated only plants (Fig. 2a). The content of H_2O_2 enormously increased by 45.24% at day 20, which was further, enhanced by 111.43% at day 24 in Ni-stressed plants, as compared with that of untreated control plants. Soaking seeds with JA resulted in reduction of H_2O_2 level by 16.12 and 28.99% at days 20 and 24, respectively, in the leaves of the Ni-stressed plants when compared with that of Ni-treated only plants (Fig. 2b). Moreover, under unstressed conditions exogenous JA showed a reduced tendency (15.87 and 3.61%) in the H_2O_2 content at days 20 and 24, respectively, as compared with JA-unprimed control plants (Fig. 2b).

To observe Ni-mediated membrane damage, we also measured lipid peroxidation in terms of malondialdehyde (MDA) content, electrolyte leakage (EL), and lipoxygenase (LOX) activity in soybean plants. In comparison with untreated control plants, MDA content increased by 157.99 and 179.29% at days 20 and 24, respectively, in Ni-stressed only plants (Fig. 2c). However, "JA-primed + Ni-treated" plants showed a significant reduction in MDA content (25.36 and 23.34% at days 20 and 24, respectively) as compared with Ni-stressed only plants. Ni-treated plants recorded 542.86 and 555.36% increase in EL as compared with control plants at days 20 and 24, respectively; however, priming with JA reduced EL by 39.70 and 22.62% at days 20 and 24, respectively, in Ni-stressed plants when compared with Ni-treated only plants (Fig. 2d). In Ni-stressed soybean plants, LOX activity was amplified by 86.84 and 88.50% at days 20 and 24, respectively, as compared with that of control. However, priming seeds with JA exhibited a decrease in LOX activity by 35.09 and 39.02% at days 20 and 24, respectively, in Ni-treated plants when compared with that of Ni-stressed only plants (Fig. 2e).

JA Maintains Ascorbic Acid and Glutathione Levels, and Redox States Under Ni Stress

In comparison with the control plants, the level of AsA significantly declined at both days 20 and 24 in the Ni-fed

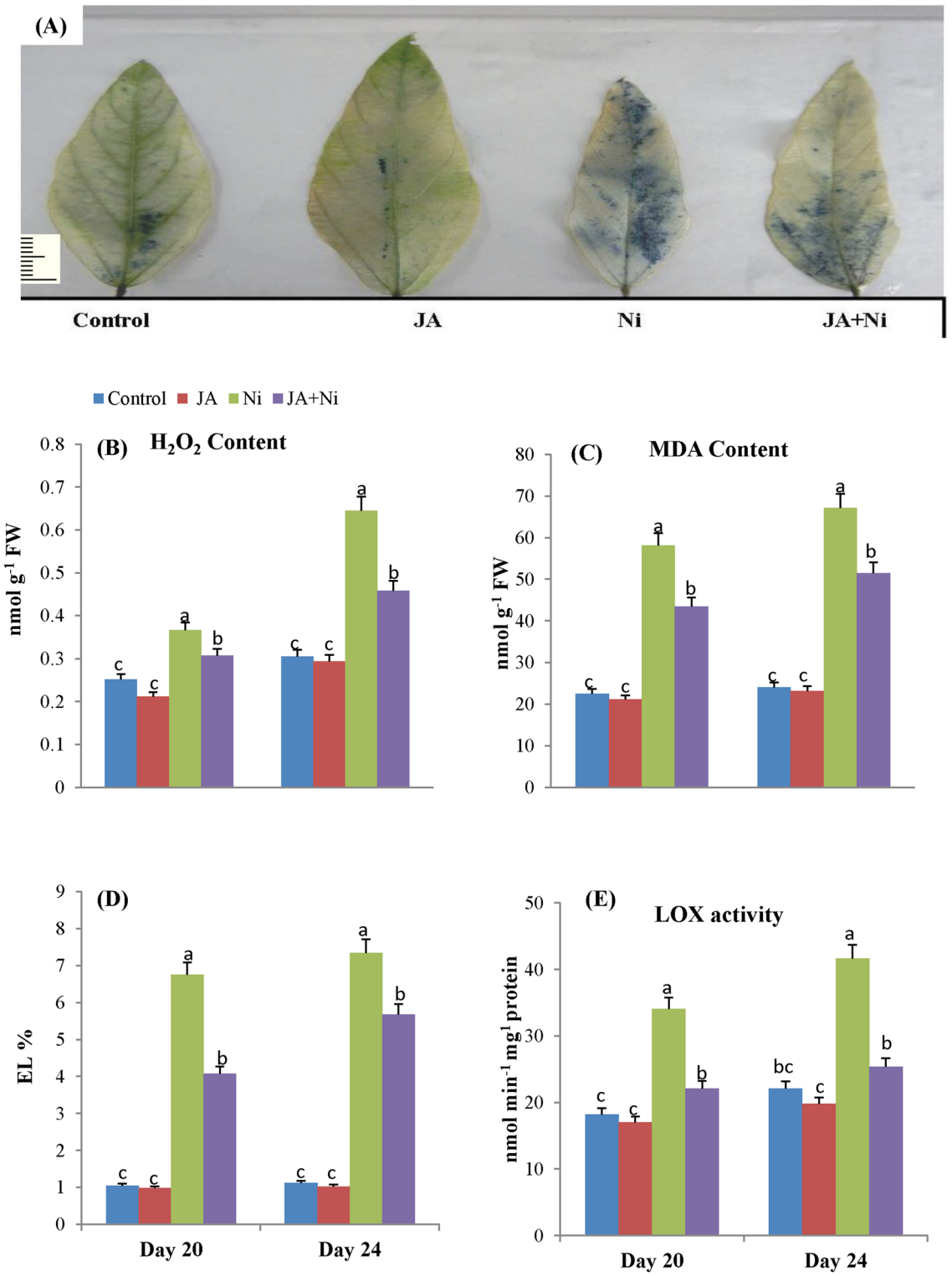


Fig. 2 Effects of seed priming with jasmonic acid (JA) on the accumulation of **a** superoxide ($O_2^{\bullet-}$) in the middle leaf of trifoliate at day 24, and on the levels of **b** hydrogen peroxide (H_2O_2), **c** malondialdehyde (MDA), **d** electrolyte leakage (%EL), and **e** lipoxygenase (LOX) activity in the leaves of soybean seedlings in presence or absence of Ni stress at days 20 and 24. Control, JA, Ni, and JA + Ni correspond to control, 1 pM jasmonic acid, 4 mM $NiCl_2$, and 1 pM jasmonic acid + 4 mM $NiCl_2$ in nutrient solutions, respectively. Bars represent standard deviation (SD) of the mean ($n = 3$). Different letters (*a*, *b*, *c*) indicate statistically significant differences among the treatments, according to Duncan's multiple range test ($P < 0.05$). *FW* fresh weight

seedlings, with the highest decrease (30.42%) recorded at day 24 (Table 2). However, Ni-stressed plants primed with JA showed enhancement in AsA content by 67.48 and 74.82% at days 20 and 24, respectively, in comparison with Ni-stressed only plants. On the other hand, oxidized AsA dehydroascorbic acid (DHA) content increased by 10.91 and 21.24% in Ni-treated plants, and 22.44 and 26.29% in "JA-primed + Ni-stressed" plants at days 20 and 24, respectively, in relation to the levels of control plants. As a result, "JA-primed + Ni-stressed" plants possessed relatively higher DHA level (10.44 and 4.16% at day 20 and 24, respectively) than Ni-stressed plants alone. Because the AsA level was considerably higher in "JA-primed + Ni-treated" plants than in Ni-stressed only plants, the redox state of AsA in terms of AsA/DHA ratio was 47.06 and 61.59% higher in the "JA-primed + Ni-treated" plants at days 20 and 24, respectively, than that of Ni-stressed only plants (Table 2). The content of GSH was found to be varied during the Ni treatment period; that is, it showed a decreased tendency by (11.62%) at day 20, but an increased tendency (by 4.88%) at day 24 in Ni-treated plants as compared with control value. However, JA-priming ensured a significant and consistent rise of GSH content in Ni-treated plants (59.93 and 15.37% at days 20 and 24, respectively) in "JA-primed + Ni-treated" plants when compared with Ni-stressed only plants (Table 2). On the other hand, although the content of oxidized GSH (GSSG) showed insignificant changes in Ni-fed only plants in comparison with control plants, it was significantly increased by 25.04 and 30.20% at days 20 and 24, respectively, in "JA-primed + Ni-stressed" plants relative to control plants. Additionally, "JA-primed + Ni-stressed" plants showed increased GSSG content by 7.56 and 21.82%, respectively, as compared with Ni-stressed only plants (Table 2). Unlike the AsA/DHA ratio, GSH/GSSG ratio did not exhibit significant changes during both days of Ni treatments in either presence or absence of JA-priming. Interestingly, JA significantly boosted the contents of GSH and GSSG in plants generated from JA-primed seeds in comparison with untreated control plants while a significant increase in GSH/GSSG ratio by 43.97% was recorded at day 20 relative to control plants (Table 2).

JA Induces Antioxidant Enzymes in Response to Ni Stress

A slight increase in SOD activity (2.99 and 3.61% at days 20 and 24, respectively) was observed in Ni-stressed plants over untreated control plants. Priming seeds with JA remarkably enhanced the activity of SOD by 59.77 and 62.36% at days 20 and 24, respectively, in Ni-stressed plants as compared with that of Ni-treated plants alone (Fig. 3a). Soybean plants exposed to Ni stress showed a drastic decrease in CAT activity by 24.74 and 40.60% at days 20 and 24, respectively, as compared with control plants (Fig. 3b). In contrast, "JA-primed + Ni-stressed" seedlings displayed significant enhancement in CAT activity (86.50 and 117.43% at days 20 and 24, respectively) as compared with Ni-stressed only seedlings. Moreover, in comparison with control seedlings, CAT activity was found to be increased by 65.63 and 62.61% at days 20 and 24, respectively, in the JA-primed seedlings. The seedlings exposed to excessive Ni showed a reduction in GPX activity by 29.70 and 23.17% at days 20 and 24, respectively, compared with control (Fig. 3c). However, treatment of soybean seeds with exogenous JA resulted in significant increases in GPX activity by 47.11 and 32.45% at days 20 and 24, respectively, in Ni-stressed plants in comparison with the plants treated with Ni alone. Moreover, under

Table 2 Effects of seed priming with jasmonic acid (JA) on the levels of non-enzymatic antioxidants ascorbic acid (AsA) and glutathione (GSH), as well as their redox states (AsA/dehydroascorbic acid, DHA and GSH/oxidized GSH, GSSG ratios) in the leaves of soybean seedlings with or without Ni stress at days 20 and 24

Duration (days)	Treatment	AsA (nmol g^{-1} FW)	DHA (nmol g^{-1} FW)	AsA/DHA ratio	GSH (nmol g^{-1} FW)	GSSG (nmol g^{-1} FW)	GSH/GSSG ratio
20	Control	75.08 ± 1.00^{c}	36.10 ± 1.17^{c}	2.07 ± 0.08^{a}	34.08 ± 1.62^{c}	12.06 ± 0.74^{c}	2.82 ± 0.11^{b}
	JA	100.02 ± 1.98^{b}	38.14 ± 1.11^{c}	2.62 ± 0.14^{a}	57.02 ± 1.14^{a}	21.02 ± 1.72^{a}	4.06 ± 0.14^{a}
	Ni	68.14 ± 1.00^{d}	40.04 ± 0.99^{b}	1.70 ± 0.12^{b}	30.12 ± 1.90^{c}	14.02 ± 0.38^{ab}	2.14 ± 0.08^{c}
	JA + Ni	114.12 ± 1.96^{a}	44.20 ± 0.62^{a}	2.50 ± 0.13^{a}	48.17 ± 1.69^{b}	15.08 ± 0.37^{ab}	3.19 ± 0.10^{b}
24	Control	92.12 ± 1.24^{c}	38.04 ± 0.85^{c}	2.45 ± 0.09^{a}	43.06 ± 1.09^{c}	13.08 ± 0.74^{c}	3.29 ± 0.17^{a}
	JA	120.34 ± 1.15^{a}	42.22 ± 1.96^{b}	2.85 ± 0.08^{a}	68.12 ± 1.42^{a}	27.12 ± 1.73^{a}	2.51 ± 0.6^{b}
	Ni	64.09 ± 0.99^{d}	46.12 ± 1.09^{a}	1.38 ± 0.06^{c}	45.16 ± 0.96^{c}	13.98 ± 1.90^{c}	3.23 ± 0.09^{a}
	JA + Ni	112.04 ± 1.40^{b}	48.04 ± 0.86^{a}	2.23 ± 0.16^{b}	52.10 ± 1.83^{b}	17.03 ± 1.00^{b}	3.05 ± 0.10^{a}

Control, JA, Ni and JA + Ni correspond to the group of plants receiving nutrients only, 1 pM jasmonic acid, 4 mM $NiCl_2$, and 1 pM jasmonic acid + 4 mM $NiCl_2$ in nutrient solutions, respectively. Values are means ± SDs of three independent replications ($n = 3$). Different letters within the column indicate statistically significant differences between treatments, according to Duncan's multiple range test ($P < 0.05$)

FW fresh weight, *DHA* dehydroascorbic acid, *GSSG* oxidized glutathione

non-stressed conditions, application of JA to soybean seeds enhanced GPX activity by 58.84 and 56.94% at days 20 and 24, respectively, compared with JA-untreated control. Soybean seedlings exposed to Ni stress showed a reduction in GST activity by 17.67 and 34.63% at days 20 and 24, respectively, compared with their respective control plant. In contrast, "JA-primed + Ni-stressed" plants exhibited significant enhancement in GST activity by 59.18 and 67.22% at days 20 and 24, respectively, in comparison with the plants treated with Ni alone. Additionally, under normal growing conditions, JA-priming enhanced GST activity by 8.10 and 15.17% at days 20 and 24, respectively, compared with the untreated control (Fig. 3d).

JA Recovers the Activities of the Enzymes Involved in Ascorbate–Glutathione Cycle

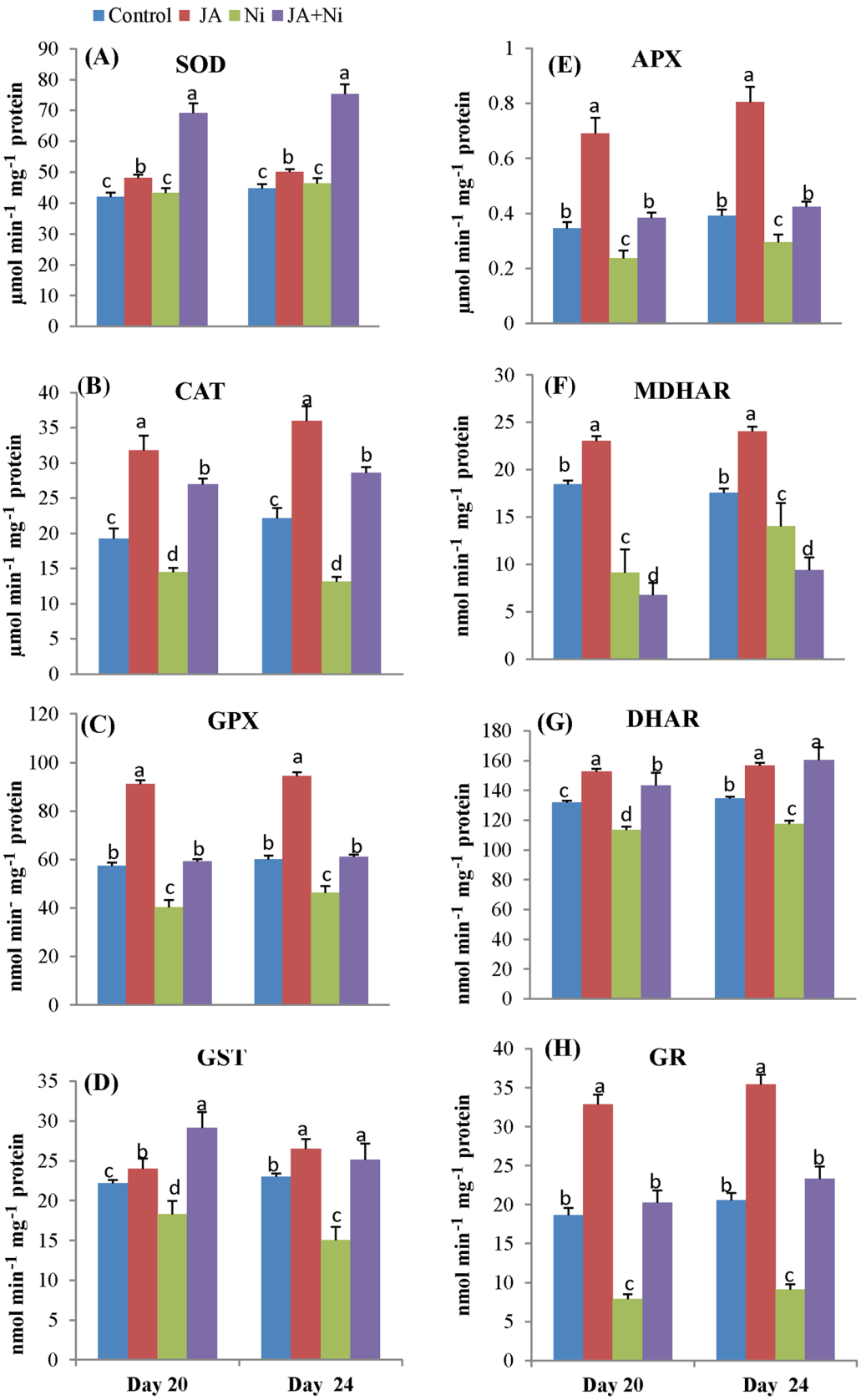
We determined the activities of four enzymes APX, MDHAR, DHAR, and GR to assess the effects of JA on the efficiency of ascorbate–glutathione (AsA–GSH) system in the leaves of soybean plants under Ni stress (Fig. 3e–h). In response to Ni stress, APX activity significantly decreased by 31.70 and 24.74% at days 20 and 24, respectively, relative to that of unstressed control (Fig. 3e). However, seed priming with JA recovered APX activity in excessive Ni-exposed soybean leaves to control level. Additionally, JA-priming enhanced the activity of APX by 98.84 and 105.10% at days 20 and 24, respectively, under unstressed conditions compared with that of control. In comparison with control, MDHAR activity in soybean leaves decreased by 50.46 and 20.03% at days 20 and 24, respectively, under Ni stress (Fig. 3f). JA-priming of soybean seeds did not show recovery effect on MDHAR activity, as the decrease in activity was further pronounced (25.95 and 33.17% at days 20 and 24, respectively) in "JA-primed + Ni-stressed" seedlings when compared with that of Ni-stressed only plants. Ni stress significantly decreased the activity of DHAR by 14.11 and 12.49% at days 20 and 24, respectively, compared with that of control (Fig. 3g). On the contrary, JA-priming significantly increased DHAR activity (26.36 and 33.09% at days 20 and 24, respectively) in Ni-stressed plants than that of the plants subjected to Ni alone. The activity of GR, the last enzyme of the AsA–GSH cycle, was negatively affected by Ni stress, and it was noted to be drastically decreased by 57.62 and 55.55% at days 20 and 24, respectively, in the Ni-stressed seedlings over that of control seedlings (Fig. 3h). However, GR activity noticeably boosted by 156.46 and 155.64% at days 20 and 24, respectively, in "JA-primed + Ni-stressed" plants compared with that of Ni-stressed only plants. Additionally, JA-priming significantly enhanced the activities of DHAR and GR under normal conditions compared with control (Fig. 3g, h).

JA Modulates Gly System to Minimize MG Toxicity

Soybean plants showed a consistent increase in the level of MG (67.17 and 75.50% at days 20 and 24, respectively) under Ni stress in relation to the level in unstressed control plants (Fig. 4a). However, priming of seeds with JA was shown to be effective in decreasing the MG level, and it was significantly lower (14.85 and 14.53% at days of 20 and 24, respectively) in "JA-primed + Ni-stressed" plants than that in the plants exposed to Ni only. The higher level of MG in Ni-stressed only plants was coincided with a significant decrease in the activities of Gly I by 24.05 and 32.53% and Gly II by 28.57 and 19.61% at days 20 and 24, respectively, in comparison with control plants (Fig. 4b, c). In contrast, JA-priming of soybean seeds increased Gly I activity by 25.00 and 28.57% and Gly II activity by 22.85 and 4.88% at days 20 and 24, respectively, in Ni-stressed plants compared with that of plants exposed to Ni alone, suggesting a positive role of JA in relieving MG-induced toxicity under Ni stress.

Discussion

Ni has been added to the list of vital micronutrients because of its requirement as a cofactor for numerous Ni-requiring enzymes essential for optimum growth and development of various species, including plants and endophytes (Fabiano et al. 2015). However, excessive concentrations of Ni are vastly toxic and harmfully affecting growth, metabolism, and life excellence of plants (Fashola et al. 2016; Sirhindi et al. 2016). In the present study, exposure of soybean plants to Ni resulted in reduced root and shoot growth (Table 1), which might be due to inhibition of mitotic activities and destruction of root architecture that limit plants' capacity to absorb water and minerals, leading to reduction of the overall plant growth (Fashola et al. 2016; Yusuf et al. 2011). However, JA-priming of soybean seeds confronted Ni-induced growth inhibition and rescued plant biomass, leading to an improved growth performance of soybean under excessive Ni stress (Table 1). In accordance to our study, JA-mediated improvement of growth-related parameters under metal toxicity was also reported in other plant species, such as *Wolffia arrhiza* and *Vicia faba* (Ahmad et al. 2017; Anjum et al. 2011; Piotrowska et al. 2009). Our data also revealed that Ni exposure of soybean plants caused a significant reduction in total Chl content, whereas JA-priming recovered the loss of Chl to some extent (Table 1), suggesting a shielding effect of JA on Chl pigments to enhance photosynthetic efficiency of soybean under Ni stress. Similar to our results, Ni-induced Chl loss was also recorded in *T. aestivum* and *Pistia*


Control
JA
Ni
JA+Ni
(A) SOD
µmol min-1 mg-1 protein
(B) CAT
µmol min-1 mg-1 protein
(C) GPX
nmol min- mg-1 protein
(D) GST
nmol min-1 mg-1 protein
(E) APX
µmol min-1 mg-1 protein
(F) MDHAR
nmol min-1 mg-1 protein
(G) DHAR
nmol min-1 mg-1 protein
(H) GR
nmol min-1 mg-1 protein
Day 20
Day 24

◂**Fig. 3** Effects of seed priming with jasmonic acid (JA) on the activities of antioxidant enzymes in leaves of soybean seedlings with or without Ni stress at days 20 and 24. **a** Superoxide dismutase (SOD), **b** catalase (CAT), **c** glutathione peroxidase (GPX), **d** glutathione *S*-transferase (GST), **e** ascorbate peroxidase (APX), **f** monodehydroascorbate reductase (MDHAR), **g** dehydroascorbate reductase (DHAR), and **h** glutathione reductase (GR). Control, JA, Ni, and JA + Ni correspond to control, 1 pM jasmonic acid, 4 mM $NiCl_2$, and 1 pM jasmonic acid + 4 mM $NiCl_2$ in nutrient solutions, respectively. Bars represent standard deviation (SD) of the mean ($n = 3$). Different letters (*a*, *b*, *c*, *d*) indicate statistically significant differences among the treatments, according to Duncan's multiple range test ($P < 0.05$)

stratiotes (Siddiqui et al. 2011; Singh and Pandey 2011), and exogenous JA-mediated improvement of Chl content under Cd stress was reported by Keramat et al. (2010) in soybean.

Ni-induced adverse effects also manifested as water shortage in leaf tissues (Table 1), as were also evidenced in *Solanum nigrum* and *Melissa officinalis* under Ni stress (Soares et al. 2016; Soltani Maivan et al. 2017). Moreover, a decrease in leaf RWC was also accompanied with an accumulation of the osmoprotectant Pro, indicating Ni-caused water imbalance and osmotic stress in soybean (Table 1). In support of our findings, a compensating tendency by producing Pro in response to water loss was also observed in wheat under Ni stress (Siddiqui et al. 2011). Pro has been considered as a noble indicator of stress tolerance because of its plausible roles in ROS signaling, osmotic adjustment, and protecting antioxidant enzymes and photosynthetic pigments (Liang et al. 2013). In the current study, JA restored water content in soybean leaves, especially to almost the level of control at day 20, despite allowing Pro accumulation in "JA-primed + Ni-treated" plants as was observed in JA-unprimed Ni-stressed plants, although at lower level (Table 1), suggesting the role of Pro as a stress-responsive metabolite, whose level in cells regulated by the intensity of stress imposed by Ni.

The degree of metal toxicity is associated with the amount of metals accumulated, distributed and partitioned in different organs of the plants. Importantly, roots are the primary sites of plant body that come first to the contact of metals and control their entries into root tissues (Nagajyoti et al. 2010). As expected, soybean roots accumulated much higher amount of Ni, as compared with leaves, under Ni stress either in presence or absence of JA-priming (Fig. 1b–d). However, JA-priming of seeds allowed a lower accumulation of Ni in both roots and shoots (Fig. 1b–d), resulting in smaller amount of Ni in these plant tissues, thereby minimizing detrimental effects of Ni and enhancing the growth performance of soybean plants (Table 1). It is possible that JA stimulated the production of organic acids, such as malate and citrate in root exudates or thiol-containing compounds to sequestrate Ni in the roots, as was observed in studies involving cadmium (Cd) and copper (Cu) (Dresler et al. 2014). Chen et al. (2014) also demonstrated the role of MeJA in reduction of both Cd uptake and Cd translocation to the aerial parts of *Kandelia obovata* seedlings by increasing stomatal closure and decreasing transpiration rate. In addition, being a regulator of various signaling cascades in cells, JA might regulate the genes involved in Ni uptake, transportation, and efflux (Asgher et al. 2015), thus curtailing Ni content to ensure better adaptability of soybean in presence of excessive Ni.

ROS is omnipresent in cellular compartments; however, the production of ROS is drastically amplified and accumulated at enormous quantities in the presence of adverse environmental stresses, including metal toxicity (Gill and Tuteja 2010). Ni, as a non-redox metal, can elicit ROS production by disrupting the balance between detoxification and generation of ROS, particularly through the deactivation of enzymatic and non-enzymatic components of the antioxidant defense system (Sreekanth et al. 2013). In this study, we observed a steady increase in ROS like $O_2^{\bullet -}$ and H_2O_2 contents in soybean leaves over the duration of Ni treatment (Fig. 2a, b), which corroborated the findings in rice (Rajpoot et al. 2016). This ROS accumulation also corresponded with the content of lipid peroxidation product MDA (Fig. 2c), suggesting Ni-mediated oxidative stress in soybean. Moreover, heavy metal-induced LOX activity also contributed to lipid peroxidation and EL (Choudhary et al. 2012; Mostofa et al. 2015a), as also evidenced under Ni stress in this study (Fig. 2d, e). On the other hand, JA-priming of soybean seeds remarkably decreased the production of $O_2^{\bullet -}$ and H_2O_2, and the activity of LOX under Ni stress, both of which positively correlated with the reduced levels of MDA and EL (Fig. 2a–e). These results suggest that JA might restrict ROS accumulation, contributing to the protection of membrane by lowering lipid peroxidation, thereby maintaining a healthy cellular environment to support better growth performance of soybean under Ni stress (Table 1). Apart from the antagonistic effect against Ni stress as observed in this study, JA also played a crucial roles in enhancing tolerance of plants to other heavy metals like Cu and Cd by reducing ROS levels and subsequent membrane damage (Chen et al. 2014; Poonam et al. 2013).

The rate of ROS accumulation and the extent of oxidative stress are controlled by redox status and the whole antioxidant system in plants under environmental stress conditions (Mittler 2017). However, under severe stress, ROS can accumulate at higher pace and overwhelm the antioxidant capacity, resulting in oxidative damage to cellular components (Saleh and Plieth 2010). Non-enzymatic antioxidants AsA and GSH play a pivotal role in scavenging ROS by maintaining cellular redox metabolism (Gill and Tuteja 2010). In this study, decreased AsA and unchanged GSH contents together with decreased AsA/DHA and GSH/GSSG ratios observed under Ni stress represented a compromised redox system (Table 2), which might contribute to an ineffective

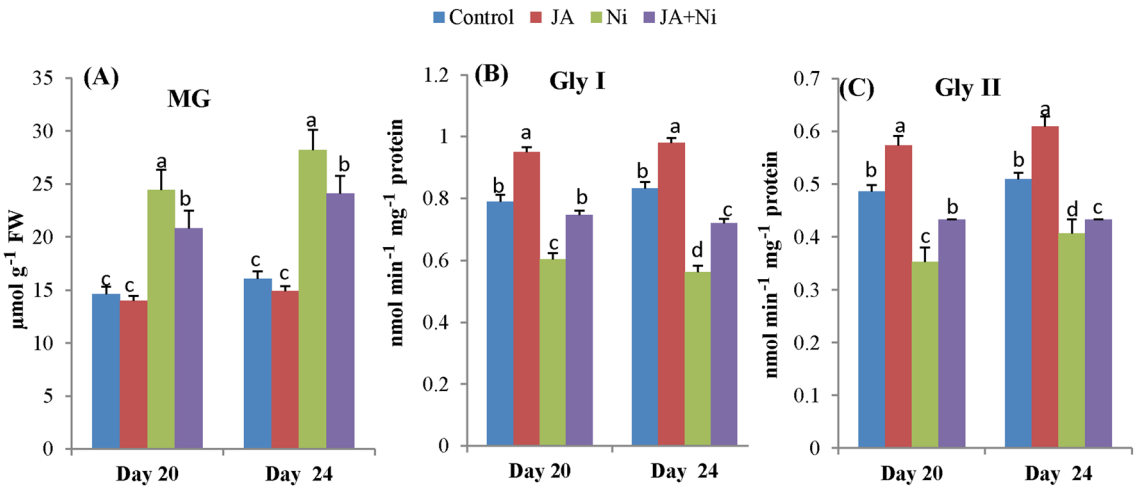


Fig. 4 Effects of seed priming with jasmonic acid (JA) on the glyoxalase system of the leaves of soybean seedlings in presence or absence of Ni stress at days 20 and 24. **a** Methylglyoxal (MG) content, **b** glyoxalase (Gly) I activity, and **c** Gly II activity. Control, JA, Ni, and JA + Ni correspond to control, 1 pM jasmonic acid, 4 mM $NiCl_2$, and 1 pM jasmonic acid + 4 mM $NiCl_2$ in nutrient solutions, respectively. Bars represent standard deviation (SD) of the mean ($n = 3$). Different letters (*a*, *b*, *c*, *d*) indicate statistically significant differences among the treatments, according to Duncan's multiple range test ($P < 0.05$). *FW* fresh weight

ROS detoxification system (Fig. 2a, b). SOD and CAT constitute the frontline of antioxidant batteries by detoxifying $O_2^{\bullet-}$ and H_2O_2, respectively (Gill and Tuteja 2010). In the absence of JA-priming, Ni-stressed plants did not enhance SOD activity, but profoundly declined the activity of CAT (Fig. 3a, b), indicating an imbalanced antioxidant system that led to an overaccumulation of ROS (Fig. 2a, b). Moreover, decline in GST and GPX activities under Ni stress (Fig. 3c, d) indicated inefficient removal of lipid hydroperoxides and reactive aldehydes that are deliberately produced under abiotic stresses (Mano 2012). More importantly, an abrupt decrease in the activities of AsA–GSH cycle enzymes APX, MDHAR, DHAR, and GR further contributed to ROS accumulation (Fig. 2a), and inefficient recycling of AsA and GSH (Fig. 3e–h; Table 2). On the other hand, enhanced SOD and CAT activities, and a well-balanced redox status in terms of AsA/DHA and GSH/GSSG ratios under JA-priming played the crucial roles in executing JA-mediated ROS removal in Ni-stressed plants (Figs. 2a, b, 3a, b; Table 2). Additionally, activation of AsA–GSH cycle enzymes (APX, DHAR, and GR) in addition to upregulated SOD and CAT activities (Fig. 3a, b, e, g, h) has made JA-primed + Ni-stressed plants equipped with a fully functional antioxidant weapon to save cellular constituents from oxidative injuries, as evidenced by lower levels of MDA and EL in JA-primed Ni-stressed seedlings (Fig. 2c, d). It should also be noted that JA-induced upregulation of DHAR and GR activities actively recycled AsA and GSH corresponding to an improved redox state, which eventually helped keep a strict check on the rise of cellular H_2O_2 in the presence of excessive Ni stress (Fig. 2b). A well-controlled redox status further assisted in efficient scavenging of lipid hydroperoxides and reactive carbonyl compounds by ensuring continuous supply of GSH needed for the activities of GPX and GST. Overall, this positive regulatory role of JA on oxidative stress tolerance is in agreement with the previous findings (Farooq et al. 2016; Yan et al. 2015), which demonstrated that MeJA and JA improved growth and better performance of *S. nigrum* (Yan et al. 2015) and *Brassica napus* (Farooq et al. 2016), respectively, by modulating the activities of various antioxidant enzymes, including SOD, APX, CAT, and POD under Cd stress.

In addition, the present study revealed the combined effects of JA and Ni on GSH-dependent Gly system that is particularly involved in MG detoxification and also in redox homeostasis by regenerating GSH (Álvarez Viveros et al. 2013; Hossain et al. 2012). At both durations of Ni treatment, significant increases in the levels of MG coincided with the drastic reductions of the activities of Gly I and Gly II (Fig. 4a–c). These results indicated that Ni aggravated Ni toxicity to soybean plants by disrupting MG detoxification system, allowing accumulation of MG to a toxic concentration (Fig. 4a). Furthermore, a decrease in Gly II activity (Fig. 4c) also indicated a reduced recycling of GSH, which is entrapped by *S*-D-lactoyl GSH (SLG), a highly cytotoxic intermediate in MG metabolism (Álvarez Viveros et al. 2013; Hossain et al. 2012). In contrast, priming of seeds with JA dramatically improved the efficiency of MG detoxification system by maintaining elevated activities of Gly enzymes (Fig. 4b, c), thereby decreasing the accumulation of MG (Fig. 4a). By doing so, JA relieved the load of MG toxicity on cellular components, contributing to the

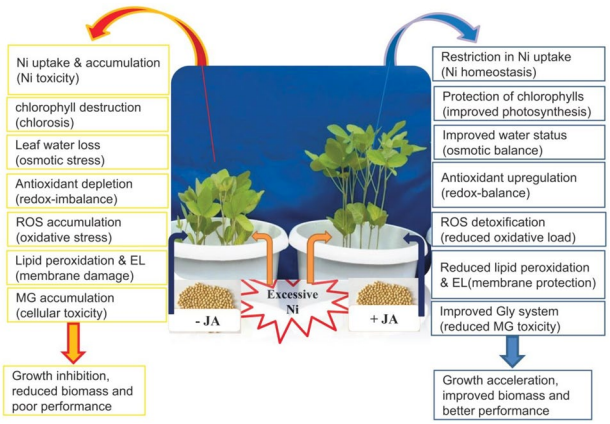


Fig. 5 A schematic representation on the effects of excessive Ni in the presence and absence of jasmonic acid (JA)-priming of soybean seeds. Ni stress resulted in a significant accumulation of Ni, leading to Ni toxicity in association with chlorosis by destroying chlorophyll, osmotic stress by inducing leaf water loss, and redox imbalance by depleting antioxidant levels. Ni toxicity also caused an excessive accumulation of reactive oxygen species (ROS), which was responsible for membrane damage by enhancing lipid peroxidation and electrolyte leakage (EL). All of these negative effects induced by Ni stress led to growth inhibition, reduced biomass production, and poor performance of soybean plants. In contrast, JA-priming of soybean seeds alleviated negative consequences of Ni stress and contributed to Ni homeostasis by restricting Ni uptake and accumulation, improved photosynthesis by protecting photosynthetic pigment chlorophylls, osmotic balance by improving water status, reduced oxidative load and membrane protection by elevating antioxidant system and redox status, and minimized methylglyoxal (MG) toxicity by activating glyoxalase activities. As a result, JA-priming played crucial roles in improving growth, biomass production and performance of soybean under excessive Ni stress

maintenance of redox balance by channeling more GSH into the system (Hossain et al. 2012). Our findings, therefore, suggest that JA enhances soybean tolerance to Ni stress by alleviating Ni-induced MG and ROS toxicity through synchronized biochemical activities of the enzymatic and non-enzymatic antioxidants, and the action of the Gly systems. Barrameda-Medina et al. (2014) also reported a similar cooperation between Gly and antioxidant defense systems in conferring efficient tolerance of *B. oleracea* to Zn stress.

Based on the findings described in the current study, a diagram representing the summary of the potential roles of JA in managing Ni-induced damage in soybean was depicted in (Fig. 5) Our results clearly demonstrated that seed priming with JA is an effective strategy to improve growth performance of soybean under Ni toxicity because of the following important mechanisms revealed from this study, which include: (1) restriction of Ni uptake and accumulation, (2) protection of photosynthetic pigments, (3) maintenance of water status, (4) reduction of oxidative load by detoxifying excessive ROS, and (5) minimization of Ni-induced MG toxicity by accelerating MG detoxification. Moreover, the observation of improved redox balance and simultaneously activated antioxidant defense and Gly systems in the presence of JA illustrates a heightened oxidative stress metabolism in JA-primed soybean plants under Ni stress. However, a detailed study on how seed priming with this phytohormone regulates molecular events in achieving above-listed mechanisms should be considered to facilitate our understandings toward Ni tolerance of soybean, and perhaps other important crops.

Acknowledgements The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding the Research Group No. RG-1438-039. M.A.M appreciates the DST-SERB for the national postdoc fellowship (File No: PDF/2016/002720).

Author Contributions MAM, GS, PA conceived and designed the experiments and MAM carried out the experimental work. MAM, MNA and PA analyzed the data and results. MAM, PA wrote the manuscript. All the authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Agarwal DK, Billore S, Sharma A, Dupare B, Srivastava S (2013) Soybean: introduction, improvement, and utilization in India—problems and prospects. Agric Res 2:293–300

Ahmad P, Alyemeni MN, Wijaya L, Alam P, Ahanger MA, Alamri SA (2017) Jasmonic acid alleviates negative impacts of cadmium stress by modifying osmolytes and antioxidants in faba bean (*Vicia faba* L.) Arch Agro Soil Sci 63:1–11

Álvarez Viveros MF, Inostroza-Blancheteau C, Timmermann T, González M, Arce-Johnson P (2013) Overexpression of GlyI and GlyII genes in transgenic tomato (*Solanum lycopersicum* Mill.) plants confers salt tolerance by decreasing oxidative stress. Mol Biol Rep 40:3281–3290. https://doi.org/10.1007/s11033-012-2403-4

Anjum SA, Xie X-y, Wang L-c, Saleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. Afr J Agric Res 6:2026–2032

Arnon DI (1949) Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1

Asgher M, Khan MIR, Anjum NA, Khan NA (2015) Minimising toxicity of cadmium in plants—role of plant growth regulators. Protoplasma 252:399–413. https://doi.org/10.1007/s00709-014-0710-4

Ayangbenro AS, Babalola OO (2017) A new strategy for heavy metal polluted environments: a review of microbial biosorbents. Int J Environ Res Public Health 14:94

Bajji M, Kinet J-M, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul 36:61–70

Barrameda-Medina Y, Montesinos-Pereira D, Romero L, Blasco B, Ruiz JM (2014) Role of GSH homeostasis under Zn toxicity in plants with different Zn tolerance. Plant Sci 227:110–121

Barrs H, Weatherley P (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15:413–428

Bates L, Waldren R, Teare I (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Chen J, Yan Z, Li X (2014) Effect of methyl jasmonate on cadmium uptake and antioxidative capacity in Kandelia obovata seedlings under cadmium stress. Ecotoxicol Environ Saf 104:349–356

Chibuike G, Obiora S (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. Appl Environ Soil Sci 2014:1–12

Choudhary SP, Oral HV, Bhardwaj R, Yu J-Q, Tran L-SP (2012) Interaction of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus*. J Exp Bot 63:5659–5675

Doderer A, Kokkelink I, van der Veen S, Valk BE, Schram A, Douma AC (1992) Purification and characterization of two lipoxygenase isoenzymes from germinating barley. Biochim Biophys Acta (BBA) 1120:97–104

Dresler S, Hanaka A, Bednarek W, Maksymiec W (2014) Accumulation of low-molecular-weight organic acids in roots and leaf segments of *Zea mays* plants treated with cadmium and copper. Acta Physiol Plant 36:1565–1575. https://doi.org/10.1007/s11738-014-1532-x

Dutilleul C, Driscoll S, Cornic G, De Paepe R, Foyer CH, Noctor G (2003) Functional mitochondrial complex I is required by tobacco leaves for optimal photosynthetic performance in photorespiratory conditions and during transients. Plant Physiol 131:264–275

El-Shabrawi H, Kumar B, Kaul T, Reddy MK, Singla-Pareek SL, Sopory SK (2010) Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. Protoplasma 245:85–96

Fabiano CC, Tezotto T, Favarin JL, Polacco JC, Mazzafera P (2015) Essentiality of nickel in plants: a role in plant stresses. Front Plant Sci 6:754

FAOSTAT (2015) Food and Agriculture Organization of the United Nations Statistics Division. FAO, Rome

Farooq MA et al (2016) Methyl jasmonate regulates antioxidant defense and suppresses arsenic uptake in *Brassica napus* L. Front Plant Sci 7:468. https://doi.org/10.3389/fpls.2016.00468

Fashola MO, Ngole-Jeme VM, Babalola OO (2016) Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. Int J Environ Res Public Health 13:1047

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

Ghosh A, Pareek A, Sopory SK, Singla-Pareek SL (2014) A glutathione responsive rice glyoxalase II, OsGLYII-2, functions in salinity adaptation by maintaining better photosynthesis efficiency and anti-oxidant pool. Plant J 80:93–105. https://doi.org/10.1111/tpj.12621

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 106:207–212

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

Hoque TS, Hossain MA, Mostofa MG, Burritt DJ, Fujita M, Tran L-SP (2016) Methylglyoxal: an emerging signaling molecule in plant abiotic stress responses and tolerance. Front Plant Sci 7:1341

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

Hossain MA, Hossain MZ, Fujita M (2009) Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene. Aust J Crop Sci 3:53–64

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 2012:1–37

Kaur C, Ghosh A, Pareek A, Sopory Sudhir K, Singla-Pareek Sneh L (2014) Glyoxalases and stress tolerance in plants. Biochem Soc Trans 42:485–490. https://doi.org/10.1042/bst20130242

Keramat B, Kalantari KM, Arvin MJ (2010) Effects of methyl jasmonate treatment on alleviation of cadmium damages in soybean. J Plant Nutr 33:1016–1025

Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8:269–285

Li Z-G (2016) Methylglyoxal and glyoxalase system in plants: old players. New Concepts Bot Rev 82:183–203. https://doi.org/10.1007/s12229-016-9167-9

Liang X, Zhang L, Natarajan SK, Becker DF (2013) Proline mechanisms of stress survival. Antioxid Redox Signal 19:998–1011. https://doi.org/10.1089/ars.2012.5074

Mano J (2012) Reactive carbonyl species: their production from lipid peroxides, action in environmental stress, and the detoxification mechanism. Plant Physiol Biochem 59:90–97 https://doi.org/10.1016/j.plaphy.2012.03.010

Mittler R (2017) ROS are good. Trends Plant Sci 22:11–19

Mostofa MG, Fujita M (2013) Salicylic acid alleviates copper toxicity in rice (*Oryza sativa* L.) seedlings by up-regulating antioxidative and glyoxalase systems. Ecotoxicology 22:959–973

Mostofa MG, Hossain MA, Fujita M, Tran L-SP (2015a) Physiological and biochemical mechanisms associated with trehalose-induced copper-stress tolerance in rice. Sci Rep 5:11433

Mostofa MG, Rahman A, Ansary MMU, Watanabe A, Fujita M, Phan Tran L-S (2015b) Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. Sci Rep 5:14078. https://doi.org/10.1038/srep14078

Mudalkar S, Sreeharsha RV, Reddy AR (2017) Involvement of glyoxalases and glutathione reductase in conferring abiotic stress tolerance to *Jatropha curcas* L. Environ Exp Bot 134:141–150. https://doi.org/10.1016/j.envexpbot.2016.11.011

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216. https://doi.org/10.1007/s10311-010-0297-8

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

Piotrowska A, Bajguz A, Godlewska-Żyłkiewicz B, Czerpak R, Kamińska M (2009) Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). Environ Exp Bot 66:507–513

Poonam S, Kaur H, Geetika S (2013) Effect of jasmonic acid on photosynthetic pigments and stress markers in *Cajanus cajan* (L.) Millsp. seedlings under copper stress. Am J Plant Sci 4:817

Putter J, Becker R (1984) Peroxidase. In: BergMeyer HU (ed) Methods of enzymatic analysis, vol 3, 3rd edn. Wiley-VCH, Weinheim, pp 286–293

Rajpoot R, Rani A, Srivastava RK, Pandey P, Dubey R (2016) Terminalia arjuna bark extract alleviates nickel toxicity by suppressing its uptake and modulating antioxidative defence in rice seedlings. Protoplasma 253:1449–1462

Saleh L, Plieth C (2010) Total low-molecular-weight antioxidants as a summary parameter, quantified in biological samples by a chemiluminescence inhibition assay Nat Protoc 5:1627–1634.

http://www.nature.com/nprot/journal/v5/n10/abs/nprot.2010.120.html#supplementary-information

Siddiqui MH, Al-Whaibi MH, Basalah MO (2011) Interactive effect of calcium and gibberellin on nickel tolerance in relation to antioxidant systems in *Triticum aestivum* L. Protoplasma 248:503–511

Singh K, Pandey S (2011) Effect of nickel-stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L. J Environ Biol 32:391

Sirhindi G, Mir MA, Sharma P, Gill SS, Kaur H, Mushtaq R (2015) Modulatory role of jasmonic acid on photosynthetic pigments, antioxidants and stress markers of *Glycine max* L. under nickel stress. Physiol Mol Biol Plants 21:559–565

Sirhindi G, Mir MA, Abd-Allah EF, Ahmad P, Gucel S (2016) Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in *Glycine max* under nickel toxicity. Front Plant Sci 7:591

Soares C, de Sousa A, Pinto A, Azenha M, Teixeira J, Azevedo RA, Fidalgo F (2016) Effect of 24-epibrassinolide on ROS content, antioxidant system, lipid peroxidation and Ni uptake in *Solanum nigrum* L. under Ni stress. Environ Exp Bot 122:115–125

Soltani Maivan E, Radjabian T, Abrishamchi P, Talei D (2017) Physiological and biochemical responses of *Melissa officinalis* L. to nickel stress and the protective role of salicylic acid. Arch Agron Soil Sci 63:330–343

Sreekanth T, Nagajyothi P, Lee K, Prasad T (2013) Occurrence, physiological responses and toxicity of nickel in plants. Int J Environ Sci Technol 10:1129–1140

Upadhyaya CP, Venkatesh J, Gururani MA, Asnin L, Sharma K, Ajappala H, Park SW (2011) Transgenic potato overproducing l-ascorbic acid resisted an increase in methylglyoxal under salinity stress via maintaining higher reduced glutathione level and glyoxalase enzyme activity. Biotechnol Lett 33:2297. https://doi.org/10.1007/s10529-011-0684-7

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151:59–66

Wasternack C (2014) Action of jasmonates in plant stress responses and development—applied aspects. Biotechnol Adv 32:31–39

Wild R, Ooi L, Srikanth V, Münch G (2012) A quick, convenient and economical method for the reliable determination of methylglyoxal in millimolar concentrations: the *N*-acetyl-L-cysteine assay. Anal Bioanal Chem 403:2577–2581

Yan Z, Zhang W, Chen J, Li X (2015) Methyl jasmonate alleviates cadmium toxicity in *Solanum nigrum* by regulating metal uptake and antioxidative capacity. Biol Plant 59:373–381

Yusuf M, Fariduddin Q, Hayat S, Ahmad A (2011) Nickel: an overview of uptake, essentiality and toxicity in plants. Bull Environ Contam Toxicol 86:1–17