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Authors Contributions

CK, FU, MA, SF and PA designed the experimentation and CK performed the experiments and generated the data. MNA and LW analyzed the data. CK and MA jointly wrote up the manuscript. MA and PA thoroughly edited the entire manuscript

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Combined application of asparagine and thiourea improves tolerance to lead stress in wheat by modulating AsA-GSH cycle, lead detoxification and nitrogen metabolism

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Abstract

Lead (Pb), like other heavy metals, is not essentially required for optimal plant growth; however, plants uptake it from the soil, which poses an adverse effect on growth and yield. Asparagine (Asp) and thiourea (Thi) are known to assuage the negative impacts of heavy metal pollution on plant growth; however, combined application of Asp and Thi has rarely been tested to discern if it could improve wheat yield under Pb stress. Thus, this experimentation tested the role of individual and combined applications of Asp (40 mM) and Thi (400 mg/L) in improving wheat growth under lead (Pb as $PbCl_2$, 0.1 mM) stress. Lead stress significantly reduced plant growth, chlorophyll contents and photosystem system II

(PSII) efficiency, whereas it increased Pb accumulation in the leaves and roots, leaf proline contents, phytochelatins, and oxidative stress related attributes. The sole or combined application of Asp and Thi increased the vital antioxidant biomolecules/enzymes, including reduced glutathione (GSH), ascorbic acid (AsA), ascorbate peroxsidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione *S*-transferase (GST), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Furthermore, the sole or the combined application of Asp and Thi modulated nitrogen metabolism by stimulating the activities of nitrate and nitrite reductase, glutamate synthase (GOGAT) and glutamine synthetase (GS). Asp and Thi together led to improve plant growth and vital physiological processes, but lowered down Pb accumulation compared to those by their sole application. The results suggest that Asp and Thi synergistically can improve wheat growth under Pb-toxicity.

Keywords: Asparagine; Inorganic nutrients; Lead toxicity; osmolytes; phytochelates; oxidative stress; wheat

Introduction

Wheat is one of the most significant cereal crops utilized as a major staple food for the rapidly expanding global population. Each year, millions of tonnes of wheat are harvested worldwide (Grote et al., 2021). However, overall wheat yield is low because of several environmental pressures including heavy metals. (Khan et al. 2006; Rady et al., 2016).

Lad (Pb) is not essentially needed for optimum growth and metabolism of plants; however, a minor rise in its concentration in the growth medium can cause significant damages to several biological events (Sofy et al., 2020), including reduced photosynthetic activity (Fatemi et al., 2020), and leaf water content (Arena et al., 2017). Moreover, Pb-stressed plants can markedly generate reactive oxygen species (ROS) (Huihui et al., 2020). The ROS so generated can effectively injure vital membrane molecules, thereby damaging biological membrane integrity (Khan et al., 2020). However, a sophisticated defense system is

adopted by plants to cope with the adverse effects of stressful environments, including those of Pb-toxicity (Zhang et al., 2019). Modulation of AsA-GSH cycle enzymes is one of the lines of action employed by crops to enable them to thrive under stressful environments (Kaya, 2020). Contrarily, this defense system is not fully able to modulate key functions involved in growth and development in plant species highly susceptible to Pb-toxicity (Ansab et al., 2018).

The growth, productivity, and quality traits of most of plant species are mediated by nitrogen (N) metabolism (Zhong et al., 2017; Ashraf et al., 2018). Nitrate (NO_3^-) is the prevalent form of N taken up plants (Shaikh and Ali, 2021). Nitrate reductase (NR) enzyme reduces NO_3^- to nitrite (NO_2), which is then reduced to NH_4^+ by the action of nitrite reductase (NiR) (Tejada-Jimenez et al., 2019). During the growth and development of plants, the enzymes glutamine synthetase (GS) and glutamate synthetase (GOGAT) are essential for the absorption and reassimilation of ammonia produced from a number of metabolic activities (Yao et al., 2019). Therefore, it is necessary for the metal stressed plants to get upregulated the activities of enzymes involved in N-metabolism required for optimum growth (Ashraf et al., 2018). Numerous reports have shown that Pb stress disrupts N-metabolism in plants (Nas and Ali, 2018; Zanganeh et al., 2018). Hence, an efficient approach is indispensable to diminish the destructive impacts of Pb stress on metabolic processes of plants.

It is well evident that synthetic and natural plant growth regulators can competently control the metabolic events involved in plant growth under both stressful and benign environments (Small and Degenhardt, 2018; Maxiselly et al., 2021). Asparagine (Asp) is one of these intrinsic regulators capable of controlling a range of metabolic events involved in growth (Le Moigne et al., 2018; Han et al., 2021). Asparagine is known to upsurge tolerance to stress in plants (Parida et al., 2018; Ganie, 2021). Various plant species including maize

(Zanganeh et al., 2019) and wheat (Oddy et al., 2020) are known to accumulate Asp in high amount under stressful environments.

Thiourea (Thi) is another bio-regulator which plays a marked role in various biochemical and physiological events in plants under stressful environments including metal toxicity (Patade et al., 2020; Mansoora et al., 2021). Thiourea has a critical function in the modulation of redox status, hormonal regulation and calcium signaling, and can decrease the oxidative stress induced growth impairment by increasing the activities of antioxidant enzymes involved in ROS scavenging (Waqas et al., 2019; Patade et al., 2020; Yadav et al., 2021; Singh et al., 2022). The effects of Asp and Thi on plants subjected to the toxicity of a variety of metals, including Pb, had been studied separately, but the role of the combined application of Asp and Thi in counteracting the injurious influence of high regimes of Pb on plants is not reported in the literature. Thus, it was hypothesized that Pb toxicity would significantly reduce the growth and alter biochemical mechanisms, whereas the combined application of Asp and Thi would reverse these adverse impacts. It was further hypothesized that the combined application of Asp and Thi would result in improved Pb stress tolerance of wheat plants compared to their individual applications. Therefore, the key objective of this research was to examine if the combined supplementation of Asp and Thi could effectively alleviate the adverse impacts of Pb on the wheat plants' growth and key physiological processes.

Materials and Methods

Experimental set-up

The current research was performed in a greenhouse maintained at 20±5 ºC and 10±2 ºC day and night temperatures, 65-70% relative humidity and a 11/13 h light/dark period. Seeds of bread wheat (*Triticum aestivum* L.) cultivar 'Pandas' were decontaminated with 1% NaOCl

solution and sown in 5-L plastic pots containing perlite. Fifty seeds were planted in a separate pot, and after germination the seedlings were uprooted to 35. The Hoagland's nutrient solution (HNS; half strength) was provided to the plants (0.1-1.0 L depending upon the plant size) on alternate days throughout the study. The detailed composition of the HNS is mentioned in Steinberg et al. (2000). The pH of the HNS was adjusted at 5.5. The trial was arranged in a completely randomized design with 3 replicates; each replicate consisted of 3 pots, so there was a total of 9 pots in each treatment.

Before initiating the proper treatments, the germinated seedlings were acclimatized for 10 d. The plants were laid open to Pb-stress (100 µM Pb) using lead chloride ($PbCl_2$) or no-stress (control). The selected concentration was chosen based on our previous work (Kaya, 2020). Lead was supplied through nutrient solution. The treatment solutions of Asp (40 mM) and Thi (400 mg/L) prepared in Tween-20 (0.01%) were sprayed to seedlings on alternate days for 14 days, and then the data for different traits were recorded. The control plants in each pot were foliar-sprayed with 20 mL deionized water. The control pots were placed at a distant place within the greenhouse to avoid spray drift. The source of asparagine is L-asparagine monohydrate (Merck), and thiourea is used as thiourea (Merck). Both chemicals were dissolved in slightly hot water. The concentrations of Asp and Thi chosen were based on our previous works (Kaya et al., 2013; Kaya et al., 2019). The source of asparagine was L-asparagine monohydrate (Merck), and thiourea as thiourea (Merck). Both chemicals were dissolved in slightly hot water. The lead level used in the study was chosen based on our previous work (Kaya, 2020).

After 14 days of imposition of various treatments, the plants were gently removed from the pots to avoid a damage to the roots. The roots and shoots were weighed fresh, and then completely dried in an oven at 75 ˚C. The shoot and root dry weights were then recorded.

Quantification of Pb content, translocation factor (TF), bio-concentration factor (BCF), and biological accumulation coefficient (BAC)

Dried root and shoot samples were digested in $HClO_4$:HNO_3 solution (1:5, v/v) to quantify tissue Pb content. The digested samples were read on an ICP-OES. The protocols listed in Malik et al. (2010) were followed to compute BCF, TF and BAC. The BCF denotes the ration of root Pb growth medium Pb concentration. The TF is the ratio of shoot Pb to root Pb. The BAC indicates the ratio of shoot Pb concentration to growth medium Pb concentration.

Maximal photosystem II quantum yield and key photosynthetic pigments

The procedures described in Arnon (1949) were followed to determine chlorophyll and carotenoid contents. Leaf tissue was homogenized in acetone (5 ml, 80%), and the final volume of each extract was completed to 50 ml with acetone. The optical density was read at 480, 645 and 663 nm for carotenoids, chlorophyll *a* and chlorophyll *b*, respectively.

A portable fluorescence meter (Walz, Germany) was used to determine maximal quantum yield (F_v/F_m) from the leaves previously placed in dark conditions for 30 minutes.

Estimation of RWC, glycine betaine, proline, and soluble sugars

Leaf RWC was appraised employing the procedure illustrated by Barrs and Weatherly (1962). The leaves were separated from the plants and their fresh mass (FM) recorded. The leaf materials were dipped for 3 h in water to record turgid mass (TM). For recording dry mass (DM), the leaves were placed in an oven at 80 ^oC for 12 h. The RWC was computed using Equation 1:

$$RWC\ (\%) = [(FM - DM)/ (TM - DM)] \times 100. \quad \text{-------Eq. 1}$$

Free proline was measured pursuing the protocol illustrated in Bates et al. (1973). An aliquot of 3% sulfosalicylic acid (10 ml) was added to 0.5 g fresh leaf, and centrifuged for 10

min at 3000 RCF. Afterwards, the filtrate (2 ml) was treated sequentially with glacial acetic acid and acid ninhydrin solutions. The resulting mixture was kept at 100 °C for 1 h and then cooled; and toluene (4 ml) was added for separating free proline. The OD was noted at 520 nm.

Glycine betaine (GB) was measured following the protocol outlined in Grieve and Grattan (1983). The anthrone reagent was used to estimate total soluble sugars. The samples (0.1 g) were extracted using 80% ethanol solution. The mixture was centrifuged for 10 minutes at 5000 RCF. To 0.5 ml supernatant, 1 ml HCl (1*N*) was added. The resulting filtrate was subjected to 100 °C maintained in a water bath and then 4.0 ml of 0.2% anthrone were added to it. The ODs of all treated samples were registered at 620 nm.

Quantification of phytochelatins

Phytochelatin (PC) content was computed by deducting glutathione (GSH) contet from that of total non-protein thiols (NPT). Sulfosalicylic acid (3%) was used for macerating fresh leaf tissue. The Ellman's reaction solution consisted of 5 mM EDTA and 0.6 mM DTNB [5,5 o-ithiobis (2-nitrobenzoic acid)]. The NPT was quantified at 412 nm following Ellman (1959).

Determination of ascorbic acid and glutathione

Meta-phosphoric acid buffer (3 mL, 5%) and 1 mM EDTA were used to homogenize 500 mg fresh leaf. The homogenized mixture was subjected to a centrifuge at 11,500 RCF at 4 °C for 12 min. The resulting reaction mixture was used to quantify glutathione and ascorbate.

Potassium-phosphate buffer (pH 7.0; 500 mM) was used to quantify ascorbate following Huang et al. (2005). The assay of reduced ascorbate was conducted in ascorbate oxidase (0.5 units) and potassium-phosphate buffer (pH 7.0; 0.1 M). The treated samples were read at 265 nm.

The samples were extracted with 30 mM dithiothreitol to estimate total AsA. Dehydroascorbate (DHA) was computed by deducting reduced-AsA content from total AsA.

The study of Yu et al. (2003) was pursued for assaying reduced GSH and glutathione disulfide (GSSG). A K-phosphate buffer (0.6 ml, 0.5 M, pH 7.0) was added to 0.4 ml of the sample extract. The GSH was measured by the changes in OD values at 412 nm for NTB (2-nitro-5-thiobenzoic acid) generated by the DTNB reduction. The GSSG level was computed by subtracting the GSH concentration from that of the derivatizing agent, 2-vinylpyridine.

Quantification of oxidative stress related traits

Leaf hydrogen peroxide (H_2O_2) was measured following Loreto and Velikova (2001). Briefly, fresh leaf sample (0.5 g) was extracted in 1% trichloroacetic acid (3 mL). Afterwards, 0.75 ml of the resulting extract was reacted sequentially with 1.0 M KI (1.5 mL) and 10 mM K buffer (0.75 mL). The absorbance was measured at 410 nm.

Leaf MDA was estimated exercising the protocol of Weisany et al. (2012). The leaf samples (each 0.2 g) were extracted in trichloroacetic acid (TCA; 5 mL, 0.1% w/v). The resulting homogenate was subjected for 5 min to a centrifuge adjusted at RCF value of 12,000 at 4 °C. Afterwards, TCA (20%) and 4 ml of 0.5% thiobarbituric acid were added to the homogenate. The optical densities of the treated samples were noted at 532 nm and 600 nm.

The protocol illustrated in Dionisio-Sese and Tobita (1998) was followed for estimating electrolyte leakage (EL). Leaf discs were excised from pre-cleaned leaves. All vials, each containing leaf discs and deionized water (10 mL) vigorously shaken to determine the first electrical conductance (EC1). The resulting materials were incubated at 120 ˚C for 20 min to record the second electrical conductance (EC2). Equation 2 was employed to compute EL.

$$EL\ (\%) = (EC1/EC2) \times 100 \text{ ---------- Eq. 2}$$

Quantification of enzymatic activities

A 500 mg of fresh leaf was macerated in ice-cold K-phosphate buffer (1 ml of 100 mL, pH 7.0) including 1% polyvinylpyrrolidone and then was centrifuged at RCF of 12,000 at 4 ºC for 15 min. The enzyme activities were measured from the extracted mixture.

Van Rossum et al. (1997) were followed to measure SOD activity, and Chance and Maehly (1955) were followed to measure CAT activity. Similarly, Hossain et al. (2010) were followed to appraise the activity of glutathione reductase. The reaction solution consisted of NADPH (0.2 mM), K-phosphate buffer (0.1 M, pH 7.8), EDTA (1.0 mM), GSSG (1.0 mM) and the enzyme extract in a final volume of 1.0 ml. The reaction was started by adding GSSG to the sample mixture to initiate the reaction. The reduction in optical density due to NADPH oxidation was noted for one min at 340 nm.

The activity of monodehydroascorbate reductase was measured according to Hossain et al. (1984). The extract was treated with the chemicals detailed in Hossain et al. (1984) and OD of all treated was read at 340 nm for one min.

The activity of dehydroascorbate reductase was quantified employing the procedure of Nakano and Asada (1981). The samples were treated with all reagents described in the procedure, and their OD was noted at 265 nm for 1 min.

The glutathione-*S*-transferase activity was recorded following Hossain et al. (2006). The reaction mixture comprised Tris–HCl buffer (pH 6.5; 100 mM), GSH (1.5 mM), 1-chloro-2,4-dinitrobenzene (CDNB; 1 mM), and the enzyme extract in a 0.7 ml final volume. The absorbance changes were noted at 340 nm for 1 min. The glutathione-*S*-transferase activity was computed using an extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Axelrod et al. (1981) were followed to record the activity of lipoxygenase (EC: 1.12.11.12).

Measurement of total free amino acids and total soluble proteins

The total amino acids were appraised following the ninhydrin method devised by Rosen (1957). Glycine (µg) present in one g of fresh material was regarded as total free amino acids. Total soluble proteins in the leaves were estimated according to Bradford (1976).

Estimation of N metabolism key enzymes' activities

A proportion of fresh leaves (1:5, w/v) was extracted in 0.1 M K-phosphate buffer (pH 7.5) comprising 2 mM EDTA, 0.5% PVP and 5 mM cysteine in a cold pestle-mortar for estimating the activities of nitrate reductase (NR) and nitrite reductase (NiR). The mixture was centrifuged and used to estimate the activities of NR and NiR.

The activity of NR was recorded following Debouba et al. (2006). An aliquot of 1.4 ml sample mixture containing 0.1 M potassium phosphate buffer (pH 7.5) comprised 7 mM KNO_3, 140 µM NADH, 10 mM $MgCl_2$. To commence the reaction, NADH was added to the sample homogenate and it was kept at 27 °C for 30 min, and then an aliquot of 100 µl of 500 mM zinc acetate was added to it, and centrifuged for 10 min at RCF 3000. Nitrite formation was measured as the formation of diazotation with 0.01% naphthylenediamine dihydrochloride (NEA) and 1% sulfanilamide (SA). The homogenate was cooled and the ODs were noted at 540 nm. The amount of nitrite produced was estimated with a standard curve prepared with a range of $NaNO_2$ solutions

The NiR activity was measured as NO_2^- decrease in the reaction mixture following Debouba et al. (2006).

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247 **Estimation of the activities of glutamine synthetase (GS), glutamate dehydrogenase**
248 **(GDH) and glutamate synthase (GOGAT)**

249 Leaf material (1:5, w/v) was extracted in 50 mM Tris–HCl buffer (pH 7.6) consisting of
250 1 mM EDTA, 1 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1 mM dithiothreitol and 0.5% PVP
251 to determine the activities of GS and GDH. Afterwards, the extract was centrifuged at RCF
252 20,000 for 20 min, and the activities of GS and NADH-GDH were quantified. Agbaria et al.
253 (1998) were followed to quantify the GS.

254 The activity of GDH was recorded at 340 nm at 30 °C by noticing the oxidation of
255 ADH according to Groat and Vance (1981). An aliquot (2 mL) of the sample solution
256 consisting of 100 mM Tris–HCl buffer (pH 8.0), 11 mM 2-oxoglutaric acid, 200 µM NADH
257 and 100 mM NH_4Cl was used to determine the activity of NADH-GDH.

258 **Estimation of total nitrogen, nitrate and ammonium**

259 The leaf samples were dried under 70 °C for 72 h and the Kjeldahl method (Muñoz-Huerta et
260 al. 2013) was used to measure the total nitrogen. Nitrate was quantified as illustrated in
261 Cataldo et al. (1975).

262 Ammonium was measured by the Nessler reagent as detailed in Molins-Legua et al.
263 (2006). The reaction mixture comprised 100 µl of the filtrate, 10 µl of 10% K–Na tartrate,
264 2.4 ml of redistilled water, and100 µl of the Nessler reagent. The OD values were recorded at
265 425 nm.

266

267 **Statistical analysis**

268 The data collected for each attribute were tested for normality on the SAS version 9.1 (SAS
269 Institute Inc. NC, USA). Analysis of variance (ANOVA) was worked out to appraise the
270 variance in the data sets. The data were presented as means and standard errors. The Duncan's

Multiple Range test (at 5% confidence level) was employed to decipher the differences among the mean values where the ANOVA denoted significant differences.

Results

Phenotypic appearance of plants

The leaf size and height of the Pb-stressed plants significantly reduced as well as chlorosis symptoms appeared on their all leaves (Fig. 1). No chlorosis and deformities were noticed in the leaves of plants treated individually with Asp or combined application of Asp and Thi. Lead (Pb) stress significantly increased canopy temperature of the wheat plants compared to that by the control treatment. The canopy temperature increased from 23.4 ºC to 28.1 ºC in the Pb-stressed plants. The sole application of Thi decreased canopy temperature in the Pb-stressed plants to a substantial extent, whereas the combined application of Asp and Thi decreased the canopy temperature close to that of the control treatment (Fig. 1).

Plant growth, photosynthetic pigments, and Pb translocation and accumulation

Lead stress considerably decreased shoot and root biomass compared to that by the controls (Fig. 2A-B). The sole or the application of Asp and Thi together improved dry biomass production under Pb-stress. Shoot and root biomass were found to be reduced by 26% and 42%, respectively, under Pb-stress with respect to that under the normal treatment. The supplementation of Asp and Thi together improved shoot and root biomass by 37% and 78%, respectively, under Pb-stress. These results exhibit that the combined application of Asp and Thi played a critical function in alleviating the detrimental impacts of Pb-stress on biomass of the wheat plants.

Lead toxicity reduced the levels of photosynthetic pigments such as chlorophyll *a*, chlorophyll *b*, and carotenoids as well as the PS II quantum efficiency (*F*v/*F*m) by 48%, 58%,

59% and 36%, respectively (Fig. 2C-F). Exogenous application of Asp or Thi significantly increased these attributes with the highest values recorded due to their combined application. The combined application of Asp + Thi improved Chl *a*, Chl *b*, carotenoids and efficiency of PS II (*F*v/*F*m) by 70%, 112%, 128%, and 51%, respectively, under Pb-toxicity.

High Pb dose in the root zone caused Pb content in the shoots and roots of the wheat plants (Fig. 3A, B). Approximately, 1.6-fold higher Pb was found to be accumulated in the roots over that in the shoots. Foliar applied Asp or Thi reduced Pb accumulation by 5% and 24% in the roots, and 27% and 36% in the shoots, respectively, under Pb stress. Moreover, the Asp and Thi together decreased the root and shoot Pb contents by 44% and 53%, respectively, under Pb toxicity.

Lead toxicity increased BCF, TF and BAC (Fig. 3C-E), whereas they were found to be decreased with the application of Asp or Thi. The Asp and Thi together reduced the BCF, TF and BAC by 44%, 15% and 53%, respectively, under Pb-stress.

Modulation of RWC, soluble sugars, glycine betaine (GB) and proline (Pro) under Pb stress

Lead stress decreased RWC by 27%; however, Asp, Thi and Asp+Thi application improved it by 20%, 21%, and 30%, respectively (Fig. 3F).

Lead stress increased Pro and GB contents by 93% and 194%, respectively, whereas it lowered soluble sugar content by 52% (Fig 3G-I). The sole or the combined application of Asp and Thi led to lower accumulation of Pro, GB, and sugars under Pb-toxicity over the control treatment. The Asp and Thi together increased Pro, GB and soluble sugars by 86%, 34%, and 76%, respectively.

Enhancement in phytochelatin synthesis, GSH and AsA contents

Lead stress increased PC accumulation and GST activity by 5.6- and 2.1-fold, respectively (Fig 4A, B). Moreover, Pb stress raised the GSH and GSSG contents by 29% and 75%, respectively, whereas it decreased the GSH/GSSG rate over that in the control treatment (Fig. 4C-E). Application of Asp or Thi led to a higher rise in GSH and GSSG under Pb-stress. The effect of the Asp and Thi together was more evident than that of their individual application. The Asp and Thi supplementation increased PC and GSH activity, possibly by the detoxification of Pb.

Lead toxicity decreased AsA content by 24%, but increased DHA by 31% compared to that in the normally treated plants (Fig. 5A, B). The AsA/DHA ratio decreased by 42% under the Pb-stress with reference to that in the normally treated plants (Fig. 5C). The combined application of Asp and Thi to the Pb-stressed plants further increased AsA and AsA/DHA ratio. The sole or the combined supplementation of Asp and Thi did not affect these traits in plants subjected to Pb-free environment.

Lead-induced oxidative stress

Lead toxicity significantly increased H_2O_2 (205%), MDA (330%), EL (247%) and LOX activity (122%) over those in the controls (Fig. 5D-G). It was observed that these attributes were reduced due to Asp or Thi treatment. Externally applied Asp + Thi caused 44%, 50%, 53% and 41% reduction in H_2O_2, MDA, EL, and LOX activity, respectively.

Regulation of the antioxidant system

The activities of antioxidant enzymes are shown in Fig. 6A-F. Lead toxicity augmented the activities of SOD (41%), APX (29%) and GR (139%), but it declined CAT (32%), MDHAR (40%) and DHAR (37%) over those in normally treated plants. Foliar supplemented Asp or Thi increased the above-mentioned enzyme activities, whereas the combined application of

Asp + Thi further increased the activities of these enzymes. The Asp or Thi application under Pb-free environment markedly increased the CAT, SOD and APX activities, but the change in the GR, MDHAR and DHAR activities was not significant.

Improvement in nitrogen metabolism under Pb stress

Pb toxicity significantly decreased the activities of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthetase (GOGAT). The activities of these enzymes decreased by 44%, 43%, 43% and 49%, respectively, under Pb stress. Glutamate dehydrogenase (GDH), another enzyme related to nitrogen metabolism, increased by 107% under Pb stress (Fig. 6G-K). Foliar application of Asp or Thi alone increased the NiR, NR, GOGAT and GS activities, but decreased that of GDH. The combined application of Asp and Thi improved the activities of NR, NiR, GS and GOGAT by 56%, 53%, 59%, and 97%, respectively, under Pb toxic regime.

Compared to the control treatment, Pb stress declined total nitrogen (N) by 45% and nitrate (NO_3^-) by 34%, and it enhanced NH_4^+ by 66% (Fig. 7A-C). The wheat plants treated with Asp or Thi showed an increase in total N and NO_3^-, and a reduction in NH_4^+ under Pb toxicity. Foliar application of Asp and Thi jointly to the Pb-stressed plants increased total N by 69% and NO_3^- by 106%, but it decreased NH_4^+ by 58%. A maximal augmentation in N and NO_3^- levels and a drop in NH_4^+ were obtained with Asp + Thi treatment under Pb stress.

Over the control treatment, Pb toxicity upraised the total amino acid level (53%) and decreased total soluble protein level (44%) in plant leaves (Fig 7D, E). Foliar supplementation of Asp or Thi to the Pb-stressed plants decreased total amino acids and upraised total soluble proteins. Compared to the Pb stressed plants, total amino acids decreased by 40% and total proteins by 109% in wheat plants treated with Asp + Thi and Pb stress.

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Discussion

Reduced shoot and root Pb contents

Lead is readily absorbed by plants growing on Pb-rich soils and accumulated mostly in the roots and to a lesser level in the leaves, stems and seeds (Sharma and Dubey, 2005). The cell membrane and cell wall are the main structures that prevent Pb from contacting the cell (Parrotta et al., 2015). Lead influx to the cells is decreased through phytochelatins (Mishra et al., 2006). However, Pb- toxicity led to higher Pb accumulation in plant tissues in the current study, particularly in the roots, which significantly reduced root growth; such reductions have been reported earlier for wheat plants (Kanwal et al., 2020). A variety of methods are being used to mitigate the metal-induced damage to plant growth by preventing metal uptake (Rai et al., 2019). For instance, exogenous application of various endogenously produced substances by plants has been used to alleviate the damaging effects of metals (Bücker-Neto., 2017). The current study investigated the role of sole or joint supplementation of Asp and Thi in assuaging the harmful effects of Pb-toxicity on wheat plants. Both Asp and Thi significantly reduced Pb accumulation in the roots of wheat plants. Furthermore, Asp and Thi reduced the transport of Pb from the roots to above-ground parts. The combined application of Asp and Thi was more effectual in inhibiting Pb uptake from the roots and its transport to the above-ground plant parts. No report could be deciphered from the literature sources on the inhibitory impact of Asp on Pb-stressed plants. Furthermore, decreased root and shoot Pb contents due to Thi application under Pb-toxicity have been reported in *Trigonella foenum graecum* L. (Xalxo and Keshavkant, 2019). Decreased Pb transport in the plant tissues might have been due to formation of a Pb-Thi complex as reported by Patrick (2006), but such occurrence needs a conclusive evidence through future research. In addition, it has been proposed that Thi can protect plants against membrane damage by normally maintaining metabolic

processes, because a small proportion of Pb absorbed by plants is delivered to the shoot cells (Xalxo and Keshavkant, 2019).

Water relations and osmolytes in wheat plants under Pb toxicity

The treatment of Asp and Thi increased the concentrations of osmotic compounds which may enhance tolerance of plants to stress by improving cellular water status (Lea et al., 2006; Ahmad et al., 2021). Proline and GB play a critical role in stress reduction in plants via osmotic adaption (Abbaspour and Ehsanpour, 2020). Moreover, Pb toxicity increases the synthesis of proline (Yang et al., 2011), and GB (Zanganeh et al., 2018) in plants. The supply of Asp and Thi augmented the GB and proline contents in the current study, clearly indicating that both compounds played a significant role in increasing Pb tolerance. Previous studies have also reported that Asp increased proline and GB contents in *Camelina* spp. (Ahmad et al., 2021), whereas Thi increased proline in maize (Kaya et al., 2013). Moreover, our data show that Asp and Thi-induced enhanced proline content could have been due to modulation of proline metabolism as shown by Sofy et al. (2020) under Pb-stress. The wheat plants exposed to Asp or Thi showed a rise in proline, GB and RCW, possibly through improved hydraulic conductivity, as reported by Naz et al. (2021). Furthermore, Pb toxicity is considered to be involved in restricting water uptake (Nas et al., 2018) mediated by diminished root hydraulic conductivity, which can reduce cellular turgor thereby resulting in decreased RWC.

Improvement of Pb detoxification and antioxidant metabolism in wheat plants

Different metabolites including phytochelatins (PCs), glutathione (GSH) and GST are believed to play a significant role in Pb detoxification in plants (Gupta et al., 2010). Lead may

421 bind to GSH through thiol (-SH) group (Vadas and Ahner, 2009), which sequesters it as a
422 precursor of PCs in the vacuole (Malecka et al., 2008). Likewise, PCs are effective chelating
423 substances for binding to Pb (Gul et al., 2021). Lead stress has been stated to promote
424 generation and activation of PCs (Pourrut et al., 2011). This suggests that GSH and PCs
425 jointly detoxify Pb. The wheat plants treated with Pb displayed increased GSH, which was
426 subsequently transformed into GSSG. This might have been one of the causes of higher
427 GSSG in the Pb-stressed wheat plants compared to those in the control. High GSSG and
428 reduced ratio of GSH:GSSG signalize Pb-induced oxidative impairment (Sytar et al., 2013).
429 The treatment of Asp and Thi reversed GSH:GSSG rate and GSH concentration by increasing
430 the GSH level and GSH/GSSG ratio in the current study. Our findings are parallel to those of
431 Srivastava et al. (2014) wherein Thi increased rice GSH and GSH:GSSG ratio under heavy
432 metal stress. Lead stress increased PC synthesis in the wheat plants in the present
433 experimentation, and the plants treated with Asp and Thi had higher PC contents under Pb-
434 stress. This clearly indicates that the sole or combined application of Asp and Thi played a
435 significant role in PC biosynthesis that gave rise to a significant chelation of Pb. Patade et al.
436 (2020) also stated that treatment of Thi enhances chelation of heavy metals in plants. The
437 application of Asp plus Thi was more efficient in promoting PC bio-synthesis. There is no
438 study as yet in the literature reporting the impact of treatment of Asp plus Thi on PC synthesis
439 in plants under Pb toxicity. It has been reported that Asp can bind to lead and make it
440 ineffective for being toxic for plants (Pavlik et al., 2010). Since GSH is a precursor of PC
441 biosynthesis in plants, it is probable that Asp and Thi had a role in the biosynthesis of PC by
442 increasing GSH production, which resulted in higher GSH and PC.

443 Application of Asp or Thi significantly reduced oxidative stress in the wheat plants
444 exposed to Pb toxicity. High H_2O_2 accumulation in stressed plants causes a further damage to
445 proteins and lipids, impacting their ultrastructure and efficacy (Sharma et al., 2019). This was

446 evident in our study with increased MDA and EL. The production of ROS induced by Pb
447 causes formation of LOX (Thakur et al., 2017), which is a symptom of a significant damage
448 to cell lipids. In our experiment, exogenous supplementation of Asp and Thi suppressed the
449 LOX activity in wheat plants under Pb stress. The decreased membrane leakage owing to the
450 externally applied of Asp and Thi might have resulted from the enhanced antioxidant activity,
451 which in turn might have kept the membrane composition and ultrastructure intact.
452
453 Reduced oxidative damage due to Asp and Thi application could be linked with the
454 efficient functioning of antioxidant defense mechanism. The Asp and Thi application under
455 Pb stress noticeably augmented the activities of SOD, APX and CAT as well as those of the
456 AsA-GSH cycle. The increased antioxidant enzyme activities due to Asp application could be
457 attributed to Asp signaling as it interacts with H_2O_2. This has also been reported by Gaufichon
458 et al. (2010). The treatment of Asp and Thi augmented the activity of GR, scavenging H_2O_2
459 via the AsA-GSH cycle, which achieved reduced oxidative stress due to Pb stress. Earlier
460 reports showed that supplementation of Thi augmented the activities of CAT and SOD in salt
461 stressed maize plants (Kaya et al., 2015). Our findings show that Thi upregulated the activities
462 of SOD, and CAT as well as the AsA-GSH cycle, as similarly reported in lentil (Talukdar et
463 al., 2016) and chickpea (Ahmad et al., 2021). There is no report in the literature reporting the
464 joint impact of Asp and Thi on these enzymes' activities. The improved AsA-GSH cycle-
465 connected enzymes' activities with the treatment of Asp and Thi might have imparted higher
466 tolerance to cell organelles against Pb stress.
467 Lead reduced AsA contents and augmented DHA contents in the wheat plants, as
468 earlier reported in *Vallisneria natans* (Wang et al., 2012). The sole or the combined
469 application of Asp and Thi augmented the DHAR and MDHAR activities, which increased
470 AsA/DHA ratio, but reduced DHA content and augmented AsA level in the Pb-stressed wheat

plants. Talukdar et al. (2016) also found that the supply of Thi to lentil plants under arsenic stress increased the DHAR activity.

Enhancement in plant growth, photosynthetic activity and nitrogen metabolism in Pb-stressed wheat plants

Like other stresses, heavy metal pollution negatively affects plant growth (Sabagh et al., 2021). Our study showed that Pb-stress decreased plant growth which is in line with the Pb-induced growth reduction in maize (Rasool et al.,2020) and wheat (Kumar et al.,2018). Decreased mineral and water uptake due to reduced root growth are the possible reasons for poor plant growth under Pb-toxicity (Hussain et al., 2017). Reduced uptake of nutrients and water may decrease chlorophyll synthesis resulting in low photosynthesis, thereby reducing overall growth of plants (Pourrut et al., 2011). The sole or combined application of Asp and Thi reduced Pb-induced adverse effects on the wheat growth in the current study. However, the Asp and Thi together more effectively improved the growth of wheat plants suffering from Pb-toxicity compared to that by their sole application. The earlier studies have reported that Thi promotes growth of plants exposed to Pb-toxicity, e.g. maize (Kaya et al., 2013), and fenugreek (Xalxo and Keshavkant, 2019). The curative impact of Asp or Thi on plant growth under Pb stress can be linked to improved *F*v/*F*m and chlorophyll levels in Pb-stressed plants (Haroun et al., 2010; Ahmad et al., 2021), quite analogous to that found in the wheat plants in the present investigation.

Under Pb stress the canopy temperature increased from 23.4 ºC to 28.1 ºC. Crop development is impeded by high canopy temperatures, which can lower biomass and yield (Rezaei et al., 2015). When Asp and Thi were applied together, the reduced canopy temperature reached the levels similar to those of the control treatment. This demonstrated

unequivocally the relationship between decreased canopy temperature in the Asp+Thi treatments and higher plant growth induced by the Asp+Thi treatment.

The combined supply of Asp and Thi improved Chl and carotenoid contents, and *F*v/*F*m under Pb-free and Pb-stress conditions. Some previous studies have reported that Asp improved photosynthesis in sunflower (Herrera-Rodríguez et al., 2006), and bean (Haroun et al., 2010). In addition, the beneficial impact of Thi has been tested on these traits in wheat (Korat et al., 2020) and chickpea (Vineeth et al., 2016). The favorable impact of Asp and Thi on photosynthesis-associated parameters under Pb-toxicity may relate to decreased production of ROS and chlorophyll damage with increased antioxidant enzymes' activities. Analogous to our data, Patade et al. (2020) stated that Thi increases GSH and chlorophyll synthesis. Diminished chlorophyll disruption, improved GSH contents and increased antioxidant defense system because of Asp or Thi enable plants to growth optimally under Pb stress. In our experiment, lower MDA and H_2O_2 concentrations and higher chlorophyll contents were found in the wheat plants exposed to Asp and Thi compared to those of Pb stressed plants. Previous researchers have observed a remedial role of Thi in fenugreek (Xalxo and Keshavkant, 2019) and that of Asp in maize (Zanganeh et al., 2019). There is no available literature indicating the effect of application Asp+Thi on chlorophyll synthesis.

High Pb can disrupt plant N-metabolism (Singh et al., 2002; Zanganeh et al., 2019). For example, a reduction in total nitrogen (N), nitrate (NO_3) levels nitrate reductase and (NR) activity, and an increase in ammonium (NH4) levels were observed under Pb stress in the current study. Similar findings have been stated earlier exhibiting that Pb suppressed the NR activity and N levels in plants (Gao et al., 2013; Zanganeh et al., 2019). Nitrate (NO_3) is a main N form used by plants (Andrews et al., 2019). NR functions as a key enzyme in the conversion of NO_3 to NO_2 in plant tissues (Imran et al., 2019). Subsequently, NO_2 is reduced

520 to NH_4 via NiR enzyme (Xu et al., 2012). The reduced NO_3 amount due to Pb toxicity in the
521 wheat plants may be related to decreased transpiration, which might have resulted in reducing
522 NO_3 transmission to the shoots of the plant via the xylem system (Xiong et al., 2006). In
523 addition, increased Pb-induced ROS accumulation may cause cell damage, which results in
524 reduced NO_3 absorption by roots (Xiong et al., 2005). Furthermore, the reduction observed in
525 NO_3 uptake and NR activity in the Pb-stressed wheat plants in the current study can be related
526 to what Xiong et al., (2006) observed in Pb-stressed Chinese cabbage. Furthermore, increase
527 in NH_4 content of plants exposed to Pb stress may have been due to inhibition of ammonia
528 assimilation (Xiao et al., 2008). Surplus ammonium accumulation is injurious for the plant
529 cells (Wang et al., 2020). Fortunately, plants have effective strategies such as the GS/GOGAT
530 cycle or the GDH pathways to mitigate harmful ammonia accumulation (Gao et al. 2013).
531 Ammonium is quickly converted to organic compounds via GS/GOGAT pathway (Liu et al.,
532 2021). Our results exhibited that decreased GS and GOGAT activity in the Pb-stressed plants
533 may be associated with impaired NH_4 assimilation, as observed by decreased N and protein
534 concentrations and augmented NH_4 levels in the current study. In addition, increased GDH
535 activity due to Pb toxicity could be the reason for decreased activities of GS and GOGAT.
536 Increased GDH activity is insufficient to get continued NH_4^+ assimilation: This was obvious
537 in terms of reduced growth and increased NH_4 concentration in the wheat plants suffering
538 from Pb toxicity. Furthermore, improved GDH is considered effective in reducing NH_4^+
539 content and producing glutamate molecule for the synthesis of defensive agents (Gangwar et
540 al., 2011).

541 Foliar application of Asp and Thi increased NR activity, total N, NO_3 and NO_2 levels,
542 and decreased NH_4^+ level as GS and GOGAT utilize ammonium for amino acid synthesis.
543 This led to more N usage in the chlorophyll synthesis, and improved growth of Pb-stressed
544 plants. The NR enzyme adjusts the rate of limiting reactions in N-metabolism, thereby

involving in important metabolic events such as synthesis of secondary compounds containing N and amino acids (Mokhele et al., 2012; Teixeira et al., 2018). It has been stated by other researchers that Thi increases the N concentration and NR activity in plants (Garg et al., 2006; Mani et al., 2014). Increased absorption and assimilation of NO_3 converts stored nitrogen into amino acids (Miller et al., 2008). Furthermore, raised NR activity results in increased N assimilation (Nazar et al., 2011), which may enhance stress tolerance by increasing protein synthesis. Moreover, Asp and Thi application decreased the activity of GDH enzyme in the wheat plants exposed to Pb toxicity, showing that GDH can improve the assimilation of NH_4^+ through adjusting the GS/GOGAT cycle under Pb toxicity. Furthermore, the amelioration of Pb stress by Asp and Thi is probably due to increased protein content.

Conclusion

Generally, lead toxicity severely inhibited the growth of wheat plants and impaired water relations, N metabolism and the AsA-GSH pathway. The supplementation of Asp plus Thi reduced the damage caused due to oxidative stress by increasing antioxidant enzyme activities. Furthermore, Asp + Thi promoted N absorption, metabolism, and assimilation by regulating the NR and NiR activities in the wheat plants under Pb toxicity. The findings show that the supplementation of Asp plus Thi is effective in establishing a stress response in plants exposed to lead toxicity; however, a large-scale field research is needed in future to strengthen the claim.

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570

571 **Authors Contributions**

572 CK, FU, MA, SF and PA designed the experimentation and CK performed the experiments
573 and generated the data. MNA and LW analyzed the data. CK and MA jointly wrote up the
574 manuscript. MA and PA thoroughly edited the entire manuscript

575 **Compliance with Ethical Standards**

576 All research ethical standards where obligatory were truly practiced

577 **Conflict of interest**

578 The authors declare no conflict of interest for the publication of this paper.

579

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986 **Figures Legends**

987 **Fig. 1.** Effects of foliar-applied (singly or jointly) asparagine (Asp; 40 mM) or thiourea (Thi;
988 400 mg/L) on the growth and canopy temperature of wheat seedlings exposed to lead (Pb)
989 toxicity (100 µM Pb). Thermal and digital images were obtained at the extremity the
990 experiment.

991

992 **Fig. 2.** Dry weights of shoot (A) and root (B), leaf chlorophyll a (C), chlorophyll b (D),
993 carotenoids (E), and Photosystem II quantum efficiency [*F*v/*F*m (F)] in normally grown
994 wheat plants (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40
995 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Different alphabets on bars
996 within each variable exhibit significant differences (at $P \leq 0.05$) among average values

997

998 **Fig. 3.**Leaf lead (A), root Cd (B) on dry weight (DW) basis, biological concentration factor
999 [BCF (C)], translocation factor [TF (D)] and biological accumulation factor [BAC (E)] of Pb,
1000 leaf relative water content [RWC; F)], proline (G), and glycine betaine [GB (H)] content on
1001 fresh weight (FW) basis and sugar content (I) in wheat plants grown under control (C) and Pb
1002 stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L
1003 thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ
1004 significantly ($P \leq 0.05$) based on Duncan's multiple range test.

1005

1006 **Fig. 4.** Phytochelatins [PC (A)], glutathione-*S*-transferase [GST (B)], reduced glutathione
1007 [GSH (C)], oxidized glutathione [GSSG (D)] on fresh weight (FW) basis, and GSH/GSSG in
1008 the leaves of normally grown wheat plants (C) and Pb stress (100 µM Pb) and sprayed singly

or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Different alphabets on bars within each variable exhibit significant differences (at $P \leq 0.05$) among average values.

Fig. 5. Ascorbate [AsA (A)], and dehydroascorbate [DHA (B)] on fresh weight (FW) basis, and AsA/DHA ratio (C), hydrogen peroxide [H_2O_2; D)], and malondialdehyde [MDA; E)] on fresh weight basis, and electrolyte leakage [EL (F)], and Lipoxygenase [LOX (G)] in the leaves of normally grown wheat plants (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Different alphabets on bars within each variable exhibit significant differences (at $P \leq 0.05$) among average values.

Fig. 6. Activities of superoxide dismutase [SOD (A)], catalase [CAT (B)], ascorbate peroxidase [APX (C)], glutathione reductase [GR (D)], monodehydroascorbate reductase [MDHAR (E)], and dehydroascorbate reductase [DHAR (F)] in the leaves, activities of nitrate reductase [NR (G)], nitrite reductase [NiR (H)], glutamine synthatase [GS (I)], glutamate synthase [GOGAT (J)] and glutamate dehydrogenase [GDH (K)] on fresh weight (FW) basis of wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

Fig. 7. Leaf total nitrogen [N (A)] on dry weight (DW) basis, leaf nitrate [NO_3^- (B)], leaf ammonium [NH_4^+ (C)], total amino acid (D) and total soluble protein contents on fresh weight (FW) basis in normally grown wheat plants (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Different

1034 alphabets on bars within each variable exhibit significant differences (at $P \leq 0.05$) among
1035 average values.

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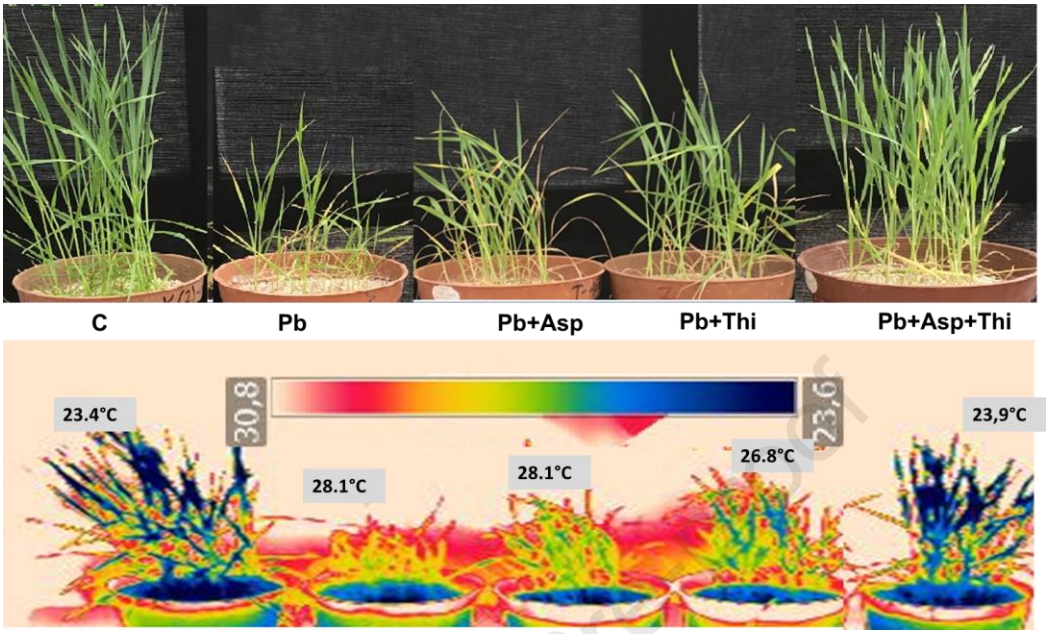

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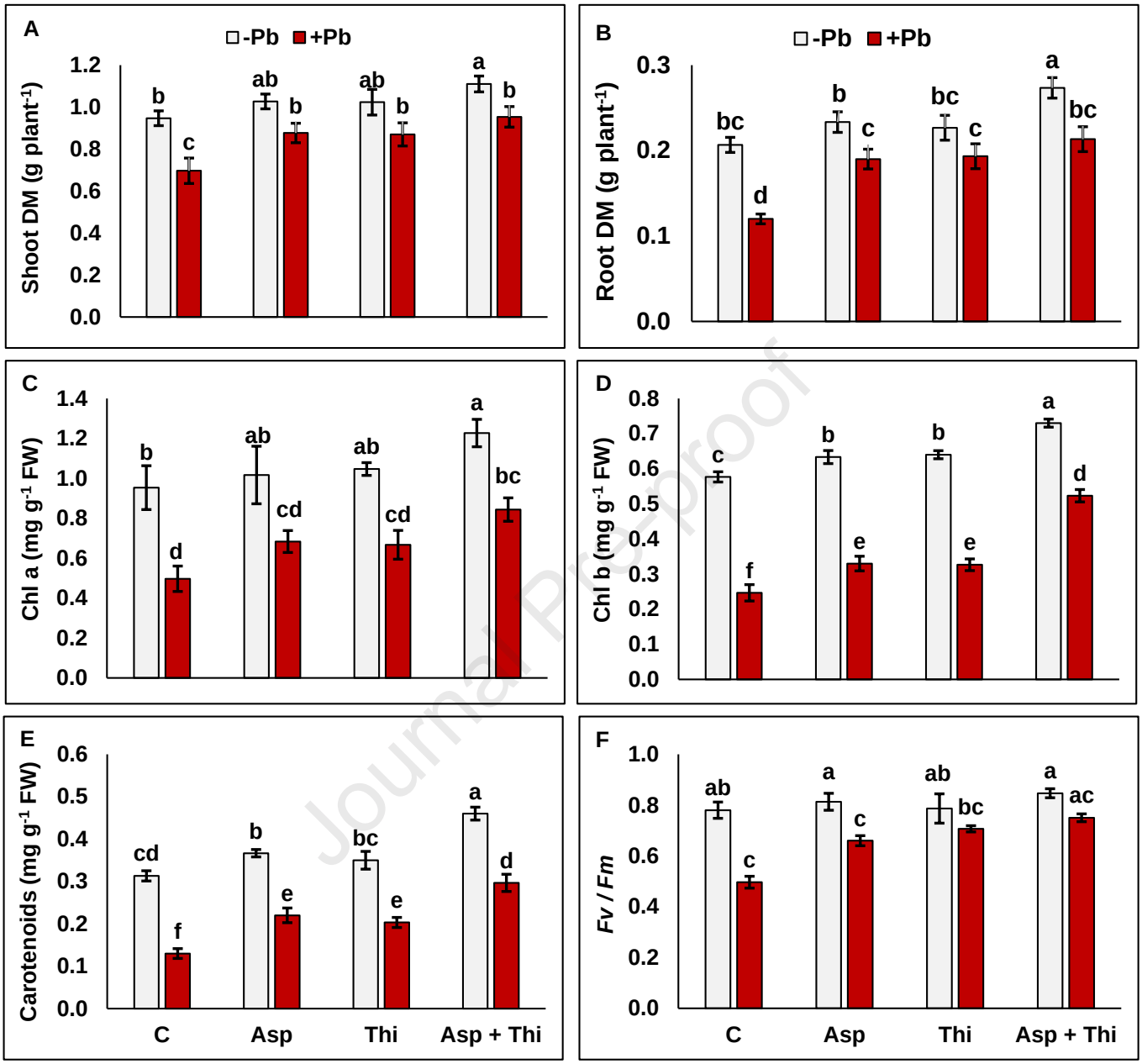

Fig. 2. Shoot (A) and root (B) dry weight (DW), leaf chlorophyll a (C), chlorophyll b (D), carotenoids (E) on fresh weight (FW) basis, and Photosystem II quantum efficiency [*Fv*/*Fm* (F)] in wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

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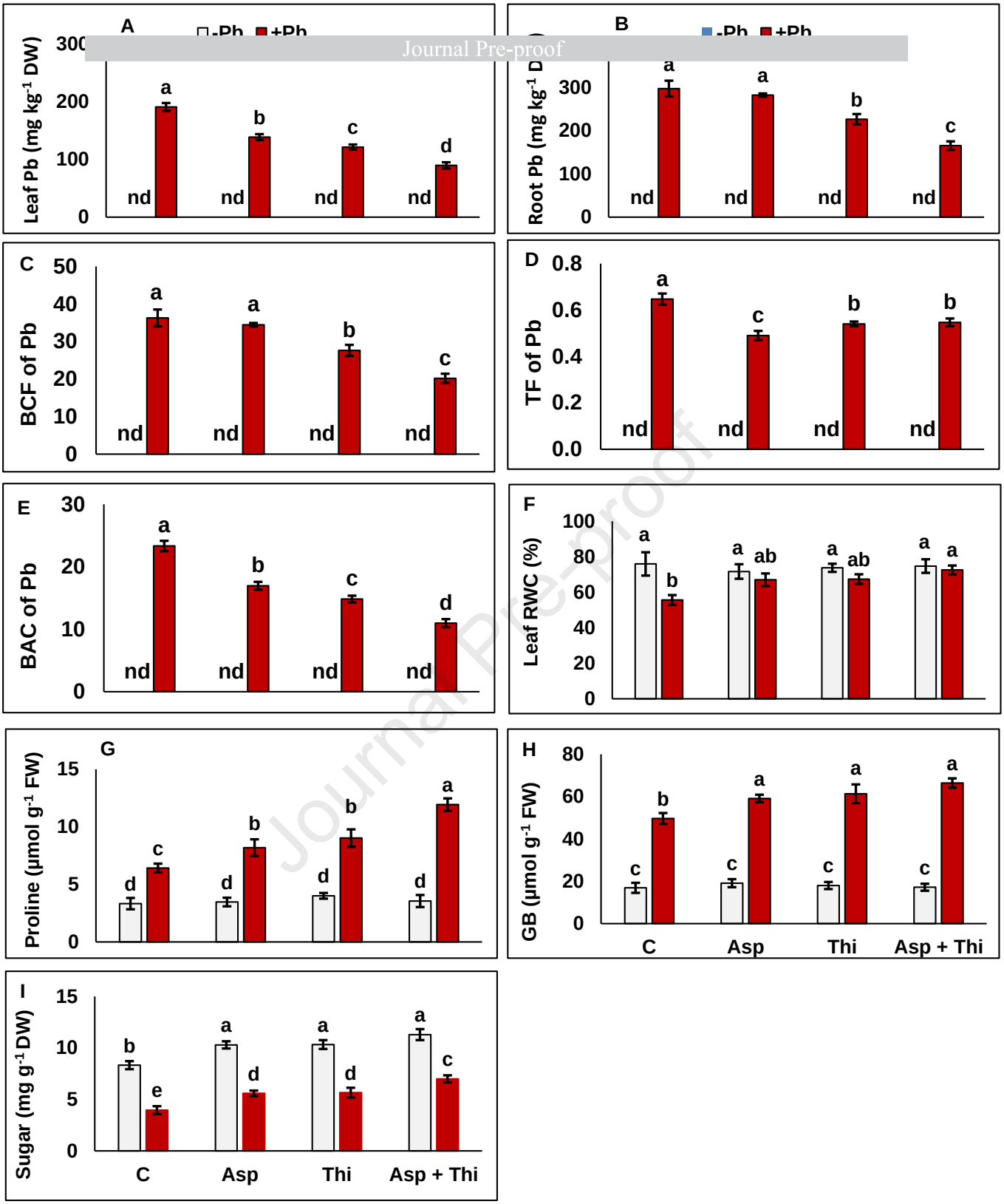

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Fig. 3. Leaf lead (A), root Cd (B) on dry weight (DW) basis, biological concentration factor [BCF (C)], translocation factor [TF (D)] and biological accumulation factor [BAC (E)] of Pb, leaf relative water content [RWC; F)], proline (G), and glycine betaine [GB (H)] content on fresh weight (FW) basis and sugar content (I) in wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

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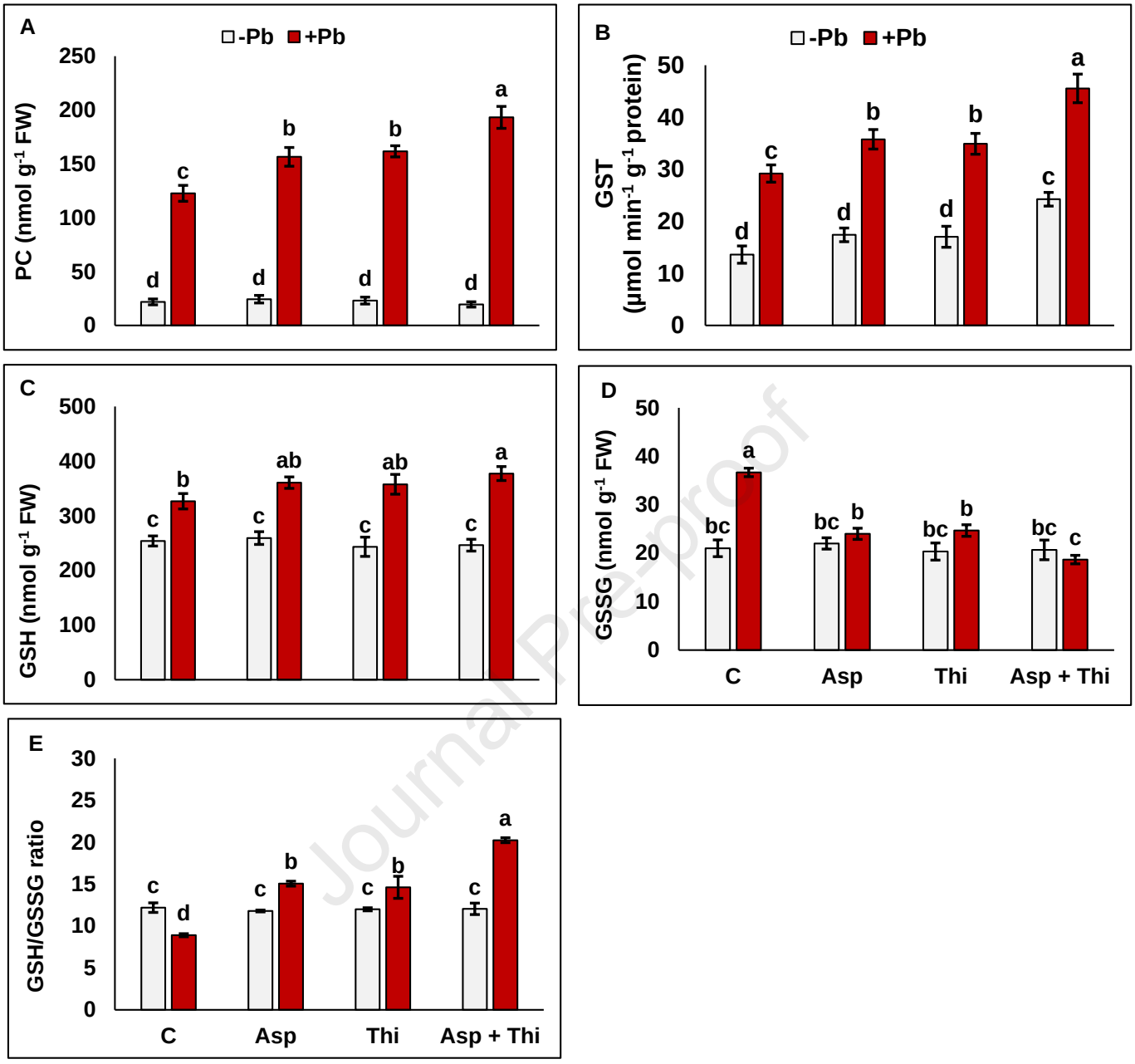

Fig. 4. Phytochelatins [PC (A)], glutathione-*S*-transferase [GST (B)], reduced glutathione [GSH (C)], oxidized glutathione [GSSG (D)] on fresh weight (FW) basis, and GSH/GSSG in the leaves of wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

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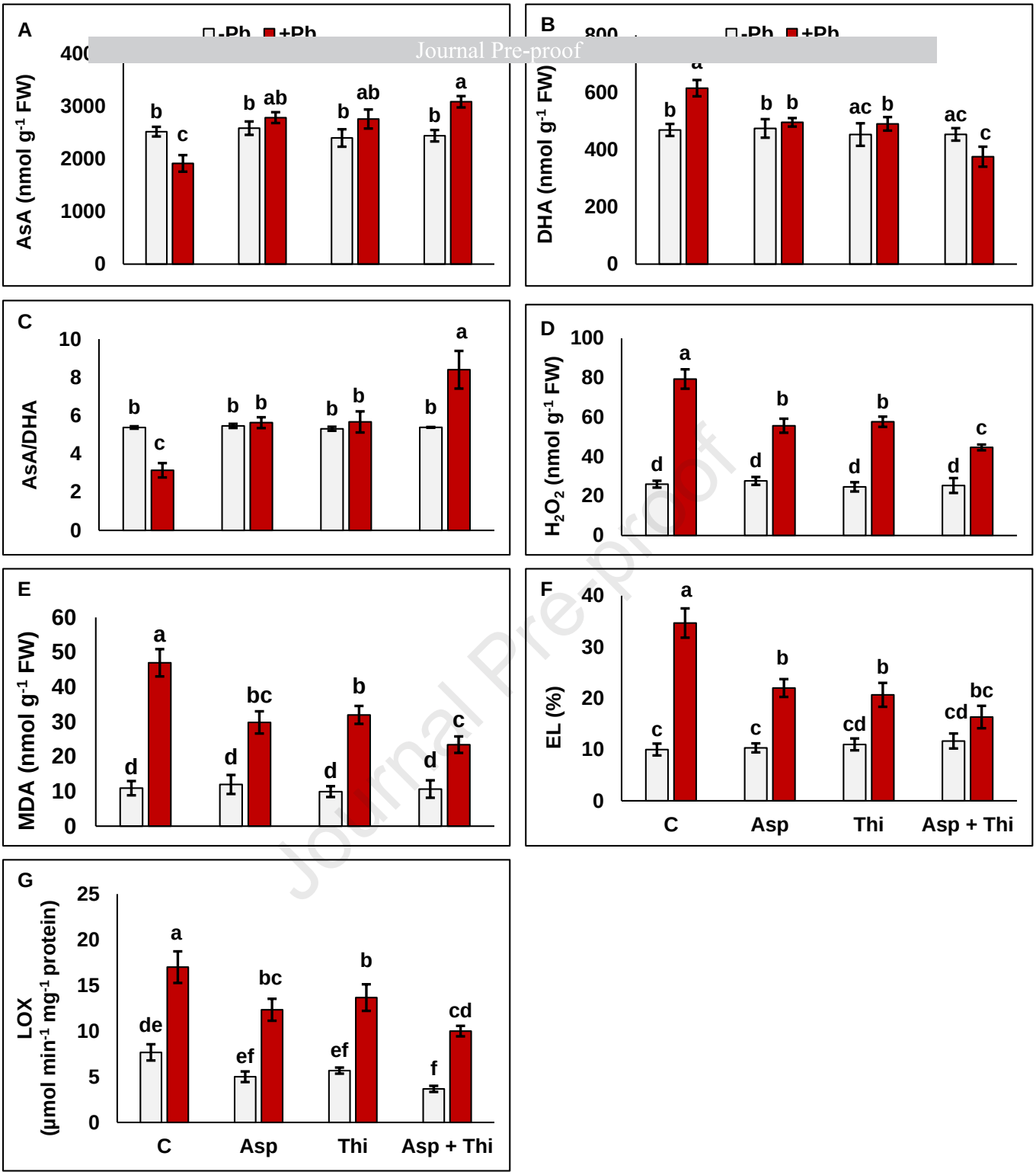

Fig. 5. Ascorbate [AsA (A)], and dehydroascorbate [DHA (B)] on fresh weight (FW) basis, and AsA/DHA ratio (C), hydrogen peroxide [H_2O_2; D)], and malondialdehyde [MDA; E)] on fresh weight basis, and electrolyte leakage [EL (F)], and Lipoxygenase [LOX (G)] in the leaves of wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

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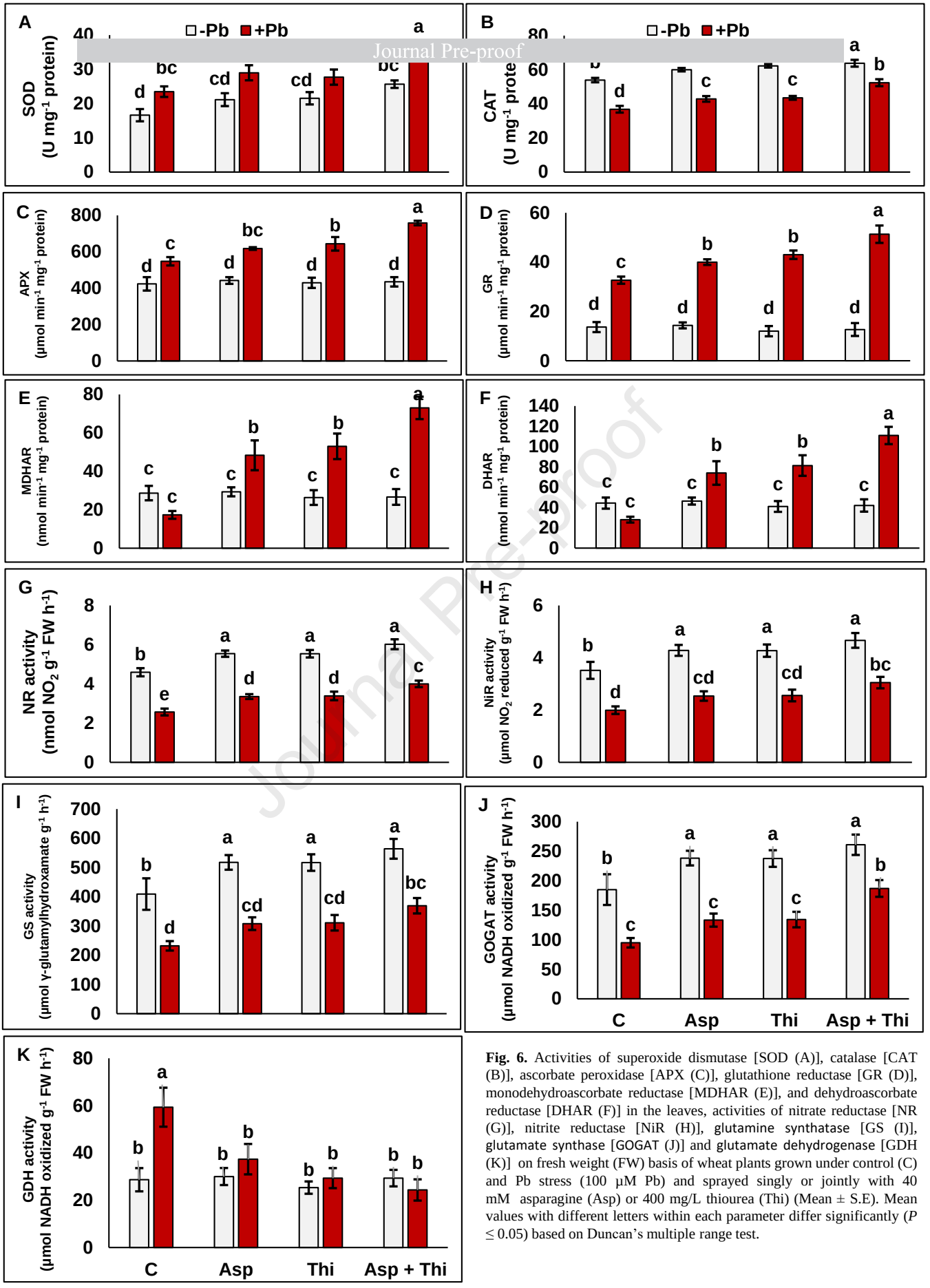

Fig. 6. Activities of superoxide dismutase [SOD (A)], catalase [CAT (B)], ascorbate peroxidase [APX (C)], glutathione reductase [GR (D)], monodehydroascorbate reductase [MDHAR (E)], and dehydroascorbate reductase [DHAR (F)] in the leaves, activities of nitrate reductase [NR (G)], nitrite reductase [NiR (H)], glutamine synthatase [GS (I)], glutamate synthase [GOGAT (J)] and glutamate dehydrogenase [GDH (K)] on fresh weight (FW) basis of wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

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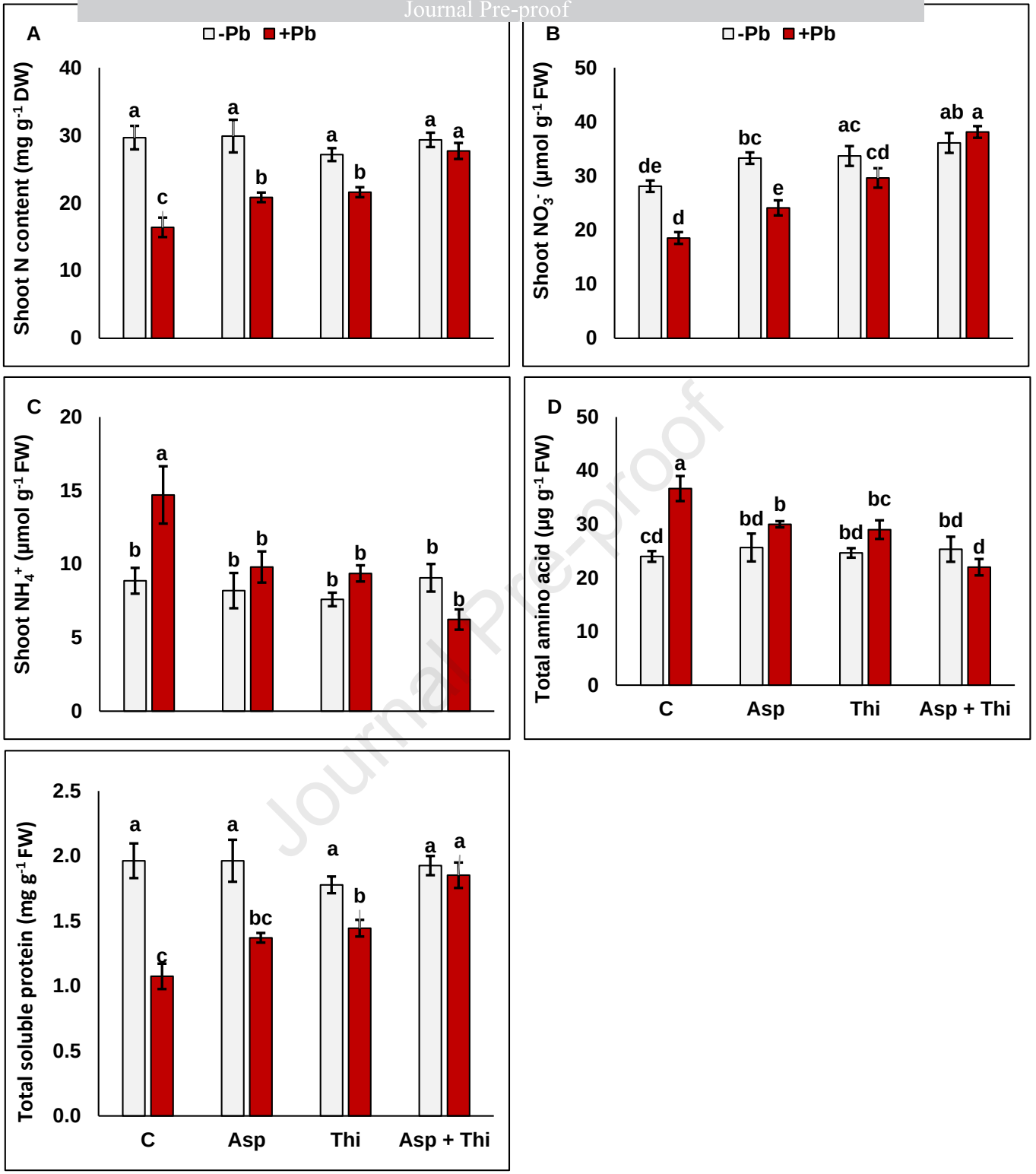

Fig. 7. Leaf total nitrogen [N (A)] on dry weight (DW) basis, leaf nitrate [NO_3^- (B)], leaf ammonium [NH_4^+ (C)], total amino acid (D) and total soluble protein contents on fresh weight (FW) basis in wheat plants grown under control (C) and Pb stress (100 µM Pb) and sprayed singly or jointly with 40 mM asparagine (Asp) or 400 mg/L thiourea (Thi) (Mean ± S.E). Mean values with different letters within each parameter differ significantly ($P \leq 0.05$) based on Duncan's multiple range test.

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Highlights

- Lead (Pb) reduced biomass and pigment content, and increased oxidative stress.
- The application of Asp and Thi together was more effective in enhancing Pb tolerance in the wheat
- Asp and Thi supplied improved ascorbate-glutathione related enzymes.
- Asp and Thi supplied enhanced key nitrogen metabolism related enzymes.

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Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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