

Attenuation of Lead Toxicity by Promotion of Tolerance Mechanism in Wheat Roots by Lipoic Acid

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This study was performed to determine the possible ameliorative effect of alpha-lipoic acid (LA) against oxidative stress evoked by lead (Pb) toxicity on 5-d wheat seedlings and elucidate how this ameliorative process was mediated. Pb toxicity caused a significant reduction in early seedling growth as evidenced by stunted root and coleoptile growth. To cope with the Pb toxicity, the activities of antioxidant enzymes were significantly stimulated compared to the control. However, in spite of high activities of these enzymes, contents of reactive oxygen species (ROS), superoxide anion and hydrogen peroxide and lipid peroxidation level were significantly high compared with the control. Similarly, Pb toxicity caused a marked decrease in the level of reduced forms of ascorbate and glutathione and thus it changed their reduced/oxidized ratio in favor of oxidized forms. On the other hand, LA supplementation further promoted uptake, accumulation, and transportation of Pb by stimulating tolerance mechanism involving ion uptake/accumulation at a high level. Moreover, ROS content and lipid peroxidation level were recorded as lower than that of the stressed-ones alone. In addition, while Pb toxicity markedly reduced amylase activity by decreasing Ca²⁺ content in endosperms, LA supplementation mitigated the reduction in amylase activity by increasing Ca²⁺ content. The changes in amylase activity were supported by isozymes patterns. Taken together, LA carried out its ameliorative effect against Pb toxicity via stimulation of tolerance mechanism, and this mechanism was linked to regeneration of the other main antioxidant compounds due to its own antioxidant property instead of activation of antioxidant enzymes.

Keywords: lipoic acid, Pb, oxidative stress, antioxidant activity

Introduction

Being sessile organisms, plants can be easily affected by biotic and abiotic factors which are ongoing within their surroundings (Perez-Clemente et al. 2013). Unfavourable conditions named as stress have remarkable negative influences on all stages of the plant life cycle from germination to flowering (Ramegowda and Senthil-Kumar 2015). Amongst these stressors, heavy metal pollution is the increasing concern worldwide as it creates hazardous health problems and environmental pollution (Ovecka and Takac 2014).

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Some heavy metals (iron, copper, zinc, manganese etc.) are essential micronutrients for plants and are required in low amounts for a number of important metabolic processes (Turk and Erdal 2015). However, others [lead (Pb), mercury, cadmium etc.] are considered nonessential and are potentially highly toxic for plants (Kumar et al. 2013). Pb is found to be one of the most abundant and dangerous heavy metal contaminants in aquatic and terrestrial ecosystems, responsible for reduced soil fertility and elevated environmental pollution (Genisel et al. 2015). Although it is not an essential element and has no known functions in biological systems, Pb gets easily taken up by plants and accumulated in roots and different plant organs (Pourrut et al. 2013).

Pb toxicity is manifested to have highly deleterious effects on morphology, growth and various physiological, biochemical and molecular processes of plants. Its accumulation may lead to decreases in the percentage and rate of seed germination, as well as seedling growth, disruption of mineral nutrition (Sharma and Dubey 2005), reduction in cell division (Samardakiewicz and Wozny 2005), inhibition of photosynthesis (Wang et al. 2012), DNA damage (Gichner et al. 2008), alterations in membrane permeability (Sharma and Dietz 2009) and enzymatic activities (Chen et al. 2007), chlorosis (Sengar et al. 2008) and decrease in water potential and plant hormones (Israr and Sahi 2008). Similar to other heavy metals, lead (Pb) also has an oxidative stress-inducing effect because it boosts the generation of free radicals and the reactive oxygen species (ROS) (Sharma and Dubey 2005). The overproduction of ROS is the best indicator of secondary stress, which can cause a variety of harmful effects in plant cells (Erdal 2012).

The usage of plant growth regulators is very important in modern agriculture. Their proper applications improve agricultural productivity, and thereby, benefit the environment (Turk et al. 2014). Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid; LA) is a dithiol compound. This compound contains sulfur, which is essential for the function of several mitochondrial enzyme complexes including pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and glycine decarboxylase (Cakatay 2006). In addition to its essential role in energy metabolism, LA is considered to be an “ideal antioxidant” due to its many useful characteristics such as its ability to chelate metal ions, detoxify free radicals, repair oxidatively damaged proteins, inhibit lipid peroxidation and recycle other oxidized antioxidants like ascorbate (AsA), glutathione (GSH), and tocopherol via reduction of their oxidized forms (Gorcek and Erdal 2015; Navari-Izzo et al. 2002; Patrick 2002; Sears 2013). LA is soluble in both lipid and aqueous environments, and can readily convert into its reduced form dihydrolipoic acid (DHLA). Due to the chemical reactivity of its dithiolane ring, it is a unique compound among antioxidants possessing powerful redox properties in both its reduced and oxidized forms (Gorcek and Erdal 2015; Navari-Izzo et al. 2002; Yildiz et al. 2015).

Although there have been a large number of studies about the effects of LA on various metabolic pathways and treatment of diseases in animals, the studies regarding its presence, action, and roles in plants are still in the infancy stages. In the initial studies, the biosynthetic pathway of LA in plastid and mitochondria in *Arabidopsis thaliana* was in-

vestigated (Yasuno and Wada 2002). Similarly, the changes in the endogenous contents of LA and DHLA in copper-stressed wheat seedlings and sodium salts-stressed basil seedlings were studied (Sgherri et al. 2002; Tarchoune et al. 2013). In the following years, investigations regarding the exogenous application of LA have started. One of which, our previous research has illustrated the mitigating effect of exogenous LA application against salt stress in wheat seedlings. The study emphasized that LA significantly strengthened the antioxidant defense system and thereby increased the salinity tolerance of wheat seedlings (Gorcek and Erdal 2015). Another recent study has focused on LA-induced proteomic and biochemical responses of canola under salt stress. The results of this study revealed that LA had an important effect on proteins linked to main metabolic processes like photosynthesis, energy metabolism, signal transduction and stress defense (Yildiz et al. 2015). However, the data are still inadequate to elucidate the effects of LA on morphological characteristics and metabolic processes in plant growth and seed germination under stressed and unstressed circumstances, and little is known about the effects of exogenously-applied LA in plants. And, although it is well documented that LA has the ability to chelate metal ions in addition to its antioxidant property (Sgherri et al. 2002), there is no study on the possible ameliorating effects of LA in plants exposed to heavy metal toxicity.

With this investigation, we aim to elucidate in terms of physiological and biochemical attributes the possible stimulating effect of LA on seed germination and early growth parameters in wheat, and its attenuating effect in the response to oxidative stress generated by excess Pb. To our knowledge, this is the first study examining the stimulating role of LA on early seedling growth and Pb-induced oxidative damage in plants.

Materials and Methods

Experimental Conditions, Seed Treatments, and Growth Analyses

Wheat seeds (*Triticum aestivum* L.) were used in the present study. The seeds were surface sterilized with 5% sodium hypochlorite solution for 10 min and then rinsed with sterile distilled water several times. The sterilised seeds were divided into four different groups for treatments as follows: (1) control group (C): seeds were imbibed with distilled water alone; (2) Pb-exposed seeds (Pb): seeds were imbibed with Pb solution [1.5 mM $\text{Pb}(\text{NO}_3)_2$]; (3) LA-exposed seeds (LA): seeds were imbibed with LA (2 μM solution alone; (4) Pb plus LA-exposed seeds (Pb + LA): seeds were imbibed within Pb solution combined with LA. After swelling period (in dark at 25 °C for 6 h), the 15-swollen seeds were placed on Petri dishes with double-layer of filter paper (Whatman No.1) moistened with its own solutions of each group and germinated at 25 °C for 5 days in dark conditions. The germinating seeds were harvested on day 5, and the root and the coleoptile lengths were measured separately.

Detection of amylase activity and isozymes

Amylase activity was determined by recording absorbance values at 620 nm according to the method described by Juliano and Varner (1969). Amylase in native gel assay was done on 7.5% acrylamide gels.

Analyses of contents of Pb and Ca²⁺

After harvest, tissue samples were oven-dried at 68 °C for 48 h, and then 0.5 g of dried samples were ground with liquid N₂ in a mortar to pass a 1-mm sieve. The contents of Pb and calcium (Ca²⁺) were detected with inductively coupled plasma mass spectrophotometry (ICP/MS).

Determination of changes in oxidative damage indicators

Lipid peroxidation (by estimation of the malondialdehyde (MDA) content) level and hydrogen peroxide (H₂O₂) content was determined in root tissue samples by following the method of Velikova et al. (2000). Estimation of superoxide anion content (O₂⁻) in tissues was carried out according to the method of Elstner and Heupel (1976).

Assays of antioxidant enzyme activities and antioxidant compounds

Superoxide dismutase (SOD) activity was determined according to a method of Agarwal and Pandey (2004). Guaiacol peroxidase (GPX) was estimated according to a method of Ye et al. (2003). The activity of catalase (CAT) was detected by monitoring the disappearance of H₂O₂ at 240 nm using the method described by Du et al. (1995). Ascorbate peroxidase activity (APX) was measured following the decrease in A₂₉₀ nm due to H₂O₂-dependent ascorbate oxidation according to Nakano and Asada (1981). GR activity was assayed following the decrease in 340 nm due to NADPH oxidation, according to Foyer and Halliwell (1976). To calculate the enzyme activities, total soluble protein content was determined according to Smith et al. (1985).

The contents of total glutathione (total GSH), reduced glutathione (GSH), oxidized glutathione (GSSG), total ascorbate (total ASA), reduced ascorbate acid (AsA) and dehydroascorbate (DHA) were determined according to the methods of Hodges et al. (1996).

Statistical analysis

All experiments were performed at least for three times with 6 replicates each time. The mean values are presented with the \pm SE. The significance of the difference between means was computed following the Duncan test ($P \leq 0.05$).

Results

Early seedling growth

As shown in Fig. S1*, LA application had a clear stimulating effect on the root length of wheat seedlings. It enhanced root elongation by 17.3% compared to the control. Similarly, analysis of coleoptile length showed that LA application resulted in a significant increase by 16% compared with the control seedlings. However, Pb toxicity caused markedly inhibitions in root and coleoptile lengths compared to the control. The reductions reached to 47.3 and 22% in root and coleoptiles, respectively. LA was found to decrease the inhibitory effect of Pb on root and coleoptile lengths. The length of coleoptile and roots was enhanced 17% and 26% compared to the stressed-seedling, respectively.

Amylase activity and isozymes

Pb toxicity significantly reduced the amylase activity in the endosperms of wheat seedlings compared to the control (Fig. S2). While the activity was $78.03 \text{ U} \cdot \text{mg}^{-1}$ in the control seedlings, it was only $49.08 \text{ U} \cdot \text{mg}^{-1}$ in the stressed-seedlings. However, LA application with Pb significantly suppressed this reduction by approximately 20%. The highest activity of amylase was recorded at LA-treated seedlings without Pb, where resultant amylase activity was $86.2 \text{ U} \cdot \text{mg}^{-1}$, far above the control seedlings. Similarly, isozymes results were also parallel to the results obtained from enzyme activity. The results of amylase isozyme supported the changes determined in amylase activity. Patterns of bands that suggest the stress caused marked reductions in all isozymes. However, these reductions were, remarkably, sent back by LA application. The intensity analysis of the bands confirmed that Pb and LA treatments, separately and in combination, had a marked effect on amylase activity.

Pb and Ca contents

As shown in Fig. S3, Pb content in roots and coleoptiles showed a dramatic increase in lead-treated seedlings respect to control. LA application in stress-free conditions did not change the lead contents of both tissues; however, a further increase was detected in both root and coleoptiles treated with LA plus Pb as compared to the Pb alone. This data means that LA application stimulated tolerance mechanism and thus increased uptake, accumulation, and transportation of Pb.

Fig. S4 demonstrates that in the endosperms of the stressed-seedlings, while it was determined a significant elevation in Pb content, Ca^{2+} content decreased markedly. Under the stress, LA supplementation decreased the Pb-induced reduction in Ca^{2+} content but it had no statistically significant effect on Pb accumulation.

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Reactive oxygen species and membrane damage

Change of ROS content by LA in roots of wheat seedlings under Pb stress were shown in Table S1. Over a period of 5 days Pb treatment, the contents of O_2^- and H_2O_2 were significantly increased compared with their control groups by 42% and 58%, respectively. However, the addition of LA could effectively inhibit ROS accumulation. The application of LA together with Pb reduced O_2^- and H_2O_2 contents by 19 and 20%, respectively, compared to the stressed-seedlings alone.

In parallel with the changes in ROS content, Pb stress significantly increased MDA content. The MDA level was 37% higher in the stressed seedlings alone than its control seedlings. MDA content of LA-treated seedlings was markedly lower by 14% and 13% than that of non-treated seedlings under stressed and unstressed conditions, respectively (Table S1).

Antioxidant enzyme activities

The changes in antioxidant enzymes' activities are depicted in Table S2. Pb application markedly up-regulated the SOD activities in the roots. It was significantly increased by 25.8% in the stressed-seedlings compared to the control plants. The up-regulation extent was reduced by 10% in the Pb-exposed with LA-treated seedlings. LA treatment alone did not have a significant effect on SOD activity.

Pb toxicity increased the GPX activity of the roots. Compared to seedlings, the GPX activity was 1.43 times greater than that of the control seedlings. While LA application alone had no important effect on GPX activity compared to the control, it markedly reduced GPX activity under the stress. The reduction was 16% compared to the Pb-stressed roots.

Although LA treatment alone did not have a marked effect on CAT activity, Pb toxicity significantly restrained the CAT activity by approximately 30% compared to the control plants. Also, LA application could not up-regulate the CAT activity under Pb toxicity.

In contrast to the CAT activity, Pb stress increased the APX activity in wheat roots. An increase in APX activity is a common response to oxidative stress. When compared to the control seedlings, the increasing extent reached to 20.4% in the stressed seedlings. However, reduced APX activity was recorded at in LA-treated stressed seedlings. Although LA application alone decreased the APX activity compared to the control seedlings, the decreasing extent was not important statistically.

The activity of GR was significantly increased up to 37.4% by Pb toxicity compared to control. LA application did not have a statistically important effect on GR activity under stressed and unstressed conditions.

The compounds of ascorbate-glutathione cycle

The GSH content and GSH/GSSG ratio in wheat roots were significantly decreased (by 36 and 72% under the Pb stress; Figs S5a and S5b), but GSSG content was remarkably

increased (to 136% as compared to the control; Fig. S5b). LA significantly increased the GSH content and GSH/GSSG ratio as compared with the seedlings exposed to the Pb toxicity alone. However, the stress-induced elevation in GSSG level was markedly reduced by the LA treatment. While LA treatment without Pb increased the GSH content and GSH/GSSG ratio (by 19 and 76%) it significantly decreased the GSSG content (to 33% as compared to the control).

In comparison to the control, the Pb toxicity increased the level of DHA by 130% (Fig. S6a), whereas AsA content and AsA/DHA ratio were decreased by 18 and 62%, respectively (Fig. S6b). LA application remarkably decreased the level of DHA, but increased AsA level and AsA/DHA ratio by 19 and 62%, respectively, as compared with the seedlings exposed to the Pb toxicity alone.

Discussion

Pb, even at small amounts, evokes a lot of disorders in metabolic processes in cells and ultimately brings about various morphological symptoms (Sharma and Dubey 2005). Rapid inhibition of root growth and stunted seedling growth are primary visual responses of plants to Pb toxicity (Chen et al. 2007). Pb-induced reductions on germination percentage, germination index and lengths and dry masses of root/shoots have been well-documented in many different plant species (Lamhamdi et al. 2011; Sharma and Dubey 2005). In accordance with these reports, the present investigation confirms the reducing impact of Pb on root and coleoptile lengths of germinating wheat seeds. We found that the roots were more sensitive to toxic Pb ions than the coleoptiles. The inhibition of root growth reached to about 50% of the normal growth, whereas the inhibition recorded at coleoptile length was only 22% as compared to the control. As reported by prior researchers, this severe reduction in root growth might have resulted from Pb-induced retardation of mitotic frequency, differentiation and/or cell elongation within root meristem due to its accumulation in cell wall components in particular pectic substances and hemicelluloses (Jiang and Liu 2010; Pourrut et al. 2013). Similarly, the inhibition of coleoptile growth may be related to the apoplastic or symplastic transportation of the Pb from roots to the coleoptiles. To scrutinize these possibilities, we measured Pb contents in root and coleoptiles. While Pb content in the roots of the stressed-seedlings reached to about 200-fold of the control levels, this ratio in coleoptiles was only 18-fold of the control levels. These data clearly showed that roots were the main place where Pb accumulated, but nevertheless there was a limited movement of Pb from the roots to the coleoptiles. That's why the inhibition in the roots was greater than in the coleoptiles. On the other hand, LA supplementation improved early seedling growth under stressed and stress-free conditions (Fig. S1). We thought that this stimulative effect of LA might have associated with its metal chelation effect as well as its promoting property on different metabolic pathways like mitochondrial respiration and antioxidant defense system. In animals, there are various studies reporting LA is a metal chelating agent (Ou et al. 1995; Patrick 2002; Tarchoune et al. 2013); however, up to now, there have been no studies done to evaluate whether it has a chelating effect on metal ions in plants. The present findings demonstrated that LA

had an important stimulative impact on uptake, transportation, and accumulation of Pb. Compared to the stressed-seedlings alone, LA-treated roots had more Pb content up to 17%. In addition, LA enhanced transportation of Pb from root to coleoptiles. Pb content in LA-treated coleoptiles was 66% more than in the stressed-seedling alone. These data mean the mitigating effect of LA on wheat exposed to Pb toxicity is linked to the activation of tolerance mechanism.

During the germination, various storage materials are reactivated and transformed into new building materials necessary for the initial growth of the embryo and seedling establishment (Koller and Hadas 1982). Alpha-amylase (amylase) is an important component of this process. It initiates hydrolysis of starch into simple sugars, which provide the energy for embryo in the endosperm of cereal grains (Yan et al. 2014). Inhibitory effects of environmental stressors on amylase activity have been well-documented (Genisel et al. 2015; Ye et al. 2015). Similar to prior reports, we found that Pb toxicity significantly reduced amylase activity compared to control. Amylase is a Ca^{2+} -containing metalloenzyme and thus Ca^{2+} ions must be transported into the endosperm to maintain amylase synthesis (Bush et al. 1989). The elemental analysis results demonstrated that Pb toxicity significantly decreased Ca^{2+} accumulation in endosperms compared to the control. Moreover, unlike a reduction in the level of Ca^{2+} ions, there was a big elevation in the level of Pb ions in the endosperm. It is possible to say that the reducing effect of Pb on amylase activity might be attributed to the effect of replacing Pb ions with Ca^{2+} ions due to Pb blockage of the Ca^{2+} -channel and/or competitive transport of Pb through the Ca^{2+} -channel (Sharma and Dubey 2005). On the other hand, under Pb toxicity, LA application prevented the reduction in the levels of Ca^{2+} ions to a degree. That is to say, LA could maintain high amylase activity by holding at certain-concentration Ca^{2+} ions in endosperm. As seen from Fig S2, the changes in amylase isozyme bands and their intensities clearly supported Pb and/or LA-induced changes recorded at enzyme activity.

Overproduction of ROS is a common consequence of environmental stress factors. Plant tolerance to the stressors is associated with a balance between generation and degradation of ROS (Erdal 2012). In this study, to corroborate the enhanced plant tolerance with LA application, we determined the changes in the levels of ROS (O_2^- and H_2O_2) under the stressed and stress-free conditions. There are a number of studies which reported Pb toxicity significantly enhanced O_2^- and H_2O_2 levels (Sharma and Dubey 2005; Verma and Dubey 2003). As expected, the present work also depicted that Pb toxicity caused overproduction of ROS when compared to the control. At the same time, a positive correlation was determined in between ROS content and lipid peroxidation that can be used as a marker of cell membrane injury. In Pb-stressed roots, lipid peroxidation level exhibited a significant elevation compared with the control. However, LA supplementation effectively suppressed the overproduction of both ROS and reduced significantly lipid peroxidation level. These results revealed that Pb-induced oxidative damage was directly linked to the level of ROS, and LA could reduce this damage by detoxifying ROS and/or by inhibiting ROS production.

How does LA confer to tolerance to Pb toxicity: by improving antioxidant system, due to its own antioxidant property, or in combination? To specify whether the mitigating ef-

fect of LA on Pb-induced oxidative damage is linked to activation of the antioxidant system, the changes in activities of enzymatic antioxidants and levels of non-enzymatic antioxidants were detected. Pb toxicity significantly increased the activities of these enzymes (except CAT) in wheat roots. The elevations in ROS and MDA contents despite the high activities of these enzymes in Pb-treated roots clearly indicates that wheat roots tried to resist the Pb toxicity but this effort was not sufficient to fully eliminate or control the excessive production of ROS. On the other hand, although LA application decreased the Pb-induced high activities of SOD, GPX and CAT, the level of ROS and MDA were lower than in the stressed ones alone. These data suggest the mitigating effect of LA on Pb-induced oxidative damage was not associated with the activation of antioxidative enzymes. Until now, there is no study regarding effects of LA against Pb toxicity. Different from the findings obtained in the present study, LA has been reported to protect wheat leaves against salt stress by generally enhancing the activities of antioxidant enzymes (Gorcek and Erdal 2015; Yildiz et al. 2015).

While ROS are scavenged by antioxidant enzymes, their reductive detoxification occurs through the cellular AsA and GSH pools (Erdal 2012). These compounds are major water-soluble antioxidants and redox buffers in plant cells. The coordinating action of these antioxidants on mitigation of metal stress has been demonstrated in many plant species (Mahmood et al. 2010). In this study, we found that Pb toxicity significantly reduced the AsA and the GSH contents compared to the control. Moreover, because of elevation in the contents of DHA and GSSG, AsA/ DHA and GSH/ GSSG ratios lessened markedly compared to the control. These redox couples are indicative of the cellular redox balance and play a crucial role in the maintenance of the cellular homeostasis and signaling system in plants. The regeneration of DHA and GSSG needs high activities of APX and GR, respectively. In the stressed seedlings, despite high activities of these enzymes, determination of high contents of DHA and GSSG indicates that increases in APX and GR activities alone were not sufficient to cope with Pb-induced oxidative damage. As expected, LA supplementation resulted in a marked regeneration in Pb-induced low ratios of AsA/ DHA and GSH/GSSG in favor of AsA and GSH. In spite of the decline in APX activity and insignificant change in GR activity recorded at LA-applied seedlings stressed and unstressed conditions, reduction of AsA-GSH pools indicates that LA itself directly reduced these antioxidants, or protected them from high ROS content due to its unique properties like scavenging of free oxygen radicals and redox interaction with other antioxidants.

In summary, the present study clearly revealed that LA carries out its ameliorative effect against Pb toxicity via tolerance mechanism by increasing uptake, accumulation and transportation of Pb. LA-induced tolerance mechanism is linked to the regeneration of antioxidant compounds due to its own antioxidant property instead of activation of antioxidant enzymes. Further studies, however, are needed to elucidate the changes at the molecular levels of metabolites that are involved in mediating the ameliorating action of LA.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <https://akademai.com/loi/0806>

Electronic Supplementary *Table S1*. Effects of LA supplementation on the contents of hydrogen peroxide, superoxide anion and MDA of 5-day-old wheat roots exposed to Pb toxicity. Different letters in the same column indicate statistically significant differences ($p < 0.05$)

Electronic Supplementary *Table S2*. Effects of LA supplementation on the activities of SOD, GPX, CAT, APX and GR of 5-day-old wheat roots exposed to Pb toxicity. Different letters in the same column indicate statistically significant differences ($p < 0.05$)

Electronic Supplementary *Fig. S1*. Effects of LA supplementation on root and coleoptile lengths of germinating wheat seedlings under Pb toxicity

Electronic Supplementary *Fig. S2*. Effects of LA supplementation on activity, isozyme profiles and intensity of isozymes of amylase in endosperms of germinating wheat seedlings under Pb toxicity

Electronic Supplementary *Fig. S3*. Effects of LA supplementation on Pb content in root and coleoptiles of germinating wheat seedlings under Pb toxicity

Electronic Supplementary *Fig. S4*. Effects of LA supplementation on Ca^{2+} and Pb contents in endosperms of germinating wheat seedlings under Pb toxicity

Electronic Supplementary *Fig. S5*. Effects of LA supplementation on a) the contents of GSH and b) GSSG and GSH/GSSG ratio in roots of germinating wheat seedlings under Pb toxicity

Electronic Supplementary *Fig. S6*. Effects of LA supplementation on a) contents of AsA and DHA and b) AsA/DHA ratio in roots of germinating wheat seedlings under Pb toxicity