Lead exposure dose-dependently affects oxidative stress, AsA-GSH system, photosynthesis, and mineral element content in pakchoi (Brassica Chinensis L.)

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Abstract

Lead (Pb) stress can cause oxidative stress and abnormal plant growth. The ascorbic acid-glutathione (AsA-GSH) cycle mainly exists in the chloroplast and is involved in resisting oxidative stress, scavenging reactive oxygen radicals in the chloroplast, and maintaining normal photosynthesis. However, whether Pb can affect the photosynthesis of pakchoi in a dose-dependent manner through the oxidative stress and AsA-GSH system is unclear. In this study, the low-dose (300 mg/kg), medium-dose (600 mg/kg) and high-dose (900 mg/kg) Pb stress models were established. In this experiment, methods such as ICP-MS, detection of photosynthetic characteristics and fluorescence characteristics, qRT-PCR, principal component analysis and correlation analysis were used. The results showed that Pb stress induced a dose-dependent increase in Pb content in pakchoi leaves. Principal component analysis discovered that Se, B and Pb were significantly negatively correlated. Pb stress caused an increase in MDA content and the decrease in SOD, GSH-Px and T-AOC activities. We also found that the Vc content and the GSH/GSSG ratio decreased. In addition, Pb stress resulted in the decreases of Pn, Tr, Gs, Ci, and VPD, and attenuated Fv/Fm and Fv/Fo. In the high-dose group, the contents of chlorophyll a, chlorophyll b and carotenoids were significantly decreased, and the expression of chloroplast development genes (GLK, GLN2) were abnormal. Taken together, our data suggests that Pb stress leads to aberrant photosynthesis in a dose-dependent manner by inhibiting the AsA-GSH system in pakchoi. The study expands the field of Pb toxicology research and provides indications for screening antagonists.

1. Introduction

Lead (Pb) is a non-essential heavy metal pollutant for plants, mainly from waste gas, batteries, canned products, etc. (Ye, Wang et al. 2018, Dong, Liu et al. 2021). The Pb content in the Yangtze River and the estuary in winter was 11.3 to 669.4 μg/g in China. From the 1980s to 2016, the Pb content increased by 77–78% due to pollution (Yu, Junchuan et al. 2021). The Pb in household ash mainly comes from coal burning and solid waste incineration and is consistent with the Pb in urban air and soil surface. Therefore, household dust is considered the main route for kids' environmental Pb treatment (Dong, Liu et al. 2021). Besides, in the soil within 20 kilometers of the La Oroya metallurgical complex in Peru, Pb content was 217.81 ± 39.48 mg/kg, of which 9.5% was transferred to the grass (Doris, Jorge et al. 2021). In urban garden soil planting leafy vegetables, the Pb content in lettuce leaves and Chinese cabbage reached 0.05 mg/kg FW and found that the metal concentration in vegetables was positively correlated with the metal consistence in the soil (Gao, Zhang et al. 2021). The over-standard rate of Pb in the 673 plant samples provided by the typical intensive production system in Hainan Province is 2.67%, and leafy vegetables have a higher degree of pollution than non-leaf vegetables (Yang, Wang et al. 2021). Crop mainly absorbs Pb by absorbing Pb^{2+} in the soil solution. Hence, when the soil is acidic, the insoluble $PbCO_3$ in the soil is easily released and absorbed by plants. Most of the Pb absorbed by plants accumulates in the roots and then migrates to the stems and leaves (Dalyan, OLu et al. 2018). Pb can hinder the formation of plant roots, resulting in a decrease in plant seed germination rate, plant height, leaf number, biomass and yield (Ye, Wang et al. 2018, Kanwal, Farhan et al. 2020). Thus, excessive Pb

content in the soil will threaten the growth and development of plants and even cause plant death. Studies have found that Pb stress can reduce chlorophyll pigment and gas exchange characteristics, leading to plant oxidative damage (Bamagoos, Mallhi et al. 2021). After Pb exposure (3000 mg/kg), chlorophyll production, the efficiency of photosynthesis and PSII (the reaction center of photosystem II) decreases (Xie, Pu et al. 2021). Besides, it has been reported that Pb exposure affected ascorbic acid metabolism, resulting in oxidative damage and chloroplast damage (Zhang, Yang et al. 2020).

In the study of plant stress physiology, the ascorbic acid-glutathione (AsA-GSH) circulatory system participates in resisting oxidative stress and scavenging reactive oxygen free radicals in chloroplasts (Ahmad, Jaleel et al. 2010, Ahmad, Tripathi et al. 2019, Kohli, Khanna et al. 2019). Studies have confirmed that AsA and reduced GSH are important non-enzymatic antioxidants whose content is closely related to the stress resistance of plants. It has been reported that in the AsA-GSH cycle, the ratio of AsA/DHA and the ratio of GSH/GSSG can be used to measure the response of plants to environmental stress (Li, Huang et al. 2022). Dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), ascorbate peroxidase (APX) and glutathione reductase (GR), are important enzyme components in the AsA-GSH cycle of plants, which play the important roles in the regeneration of reduced AsA and GSH (Gao and Chen 2005). Studies have confirmed that drought stress could affect the antioxidant capacity of cotton leaves. The content of AsA and GSH increase under drought stress, and the ability of AsA-GSH cycle to eliminate ROS is weakened (Raja, Wani et al. 2021). In salt-treated soybeans, the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), MDHAR and DHAR will increase with the increase of salt concentration (Rahman, Rahman et al. 2021). Therefore, the AsA-GSH cycle is critical to prevent plant oxidative damage caused by stress.

Many studies have proved that Pb stress has an impact on plant growth and photosynthesis (Ye, Wang et al. 2018, Kanwal, Farhan et al. 2020, Xie, Pu et al. 2021). Pakchoi *(Brassica Chinensis L.)* is a kind of miniature Chinese cabbage, which belongs to a subspecies of Cruciferae Brassicae. And it is loved by the Chinese because of its small size, high nutritional value, and easy planting. However, it is still unclear whether Pb stress will have a dose-dependent effect on the mineral element content, AsA-GSH cycle, photosynthesis, and chloroplast development in pakchoi. Therefore, we took pakchoi as the research object in this experiment and established the low, medium and high dose exposure models of Pb stress. And we also measured the whole element content, AsA-GSH cycle level, antioxidant enzyme activity, photosynthesis, chlorophyll content and chloroplast development-related genes through ICP-MS, kits, handheld photosynthesis measurement instrument, portable chlorophyll fluorometer and qPT-PCR methods. This experiment aims to elucidate the dose-dependent influences of Pb stress on AsA-GSH cycle and photosynthesis in pakchoi. Also, this paper will provide a reference for Pb toxicology and an indication for the culture and management of pakchoi.

2. Methods And Materials

2.1 The planting and processing of pakchoi

The varieties of pakchoi used are conventional field seeds (Wanlida, China). The purity of the variety is ≥ 92.0%, and the clarity is over 98.0%. There were 4 experimental treatments in this experiment: control group (C), 300 mg/kg Pb group (L), 600 mg/kg group (M) and 900 mg/kg group (H). Three more repetitions were performed for each group. In the experiment, 300 mm × 200 mm (upper diameter x height) ceramic pots were used, and 3.0 kg of soil and Pb mixture were filled in the pots. We added deionized water along the pot wall to make the soil moisture reach the maximum capillary water holding capacity. After standing for 24 h, we sown the pakchoi seeds. One week after the emergence of the seedlings, the seedlings were thinned, and 6 sturdy seedlings were retained in each pot. We arranged the potted plants randomly and changed the position of the potted plants every day to ensure that each pot got even light. In addition, during the growth period, the pakchoi was regularly and quantitatively supplemented with water. The experiment was carried out in the greenhouse of Tarim University. It was planted on March 4, 2021 and harvested in 45 days.

The potted test soil is sandy loam, and the basic characters of the tested soil was shown in Table 1. Apply basal fertilizer to the soil: urea 0.33 g/kg, potassium dihydrogen phosphate 0.10 g/kg, potassium chloride 0.09 g/kg, and no top dressing during the growth period of pakchoi.

Table 1
Physical and chemical properties of potting soil

Soil properties	Potting soil
pH	7.05 ± 0.04
Organic matter (g/kg)	23.5 ± 0.20
Total nitrogen (g/kg)	1.35 ± 0.02
Available phosphorus (mg/kg)	9.05 ± 0.1
Available potassium (mg/kg)	81.51 ± 1.04
Pb (mg/kg)	0.22 ± 0.05

2.2 Inductively coupled plasma mass spectrometry (ICP-MS) analysis, principal component analysis (PCA) and correlation analysis

When harvesting, cut off the leaves of pakchoi, washed them and put them in clean envelopes and placed them in an oven. After placing them at 105 ℃ for 30 min, they will be dried at 60 ℃. For specific steps, please refer to the experiment conducted by Shi et al. (Xu, Xiaojing et al. 2021). After acid digestion of pakchoi, all the content of elements (Lithium (Li), Beryllium (Be), Boron (B), Sodium (Na), Magnesium (Mg), Aluminum (Al), Phosphorus (P), Potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Gallium (Ge), Arsenic (As), Selenium (Se), Rubidium (Rb), Strontium (Sr), Molybdenum (Mo), Silver (Ag), Cadmium (Cd),

Tin (Sn), Antimony (Sb), Barium (Ba), Mercury (Hg), Thallium (Tl), Lead (Pb) and Bismuth (Bi)) were detected using ICP-MS technology (iCAP Q, Thermo).

In the Pb-dose-stressed pakchoi leaves, we took the value of the total element content as the logarithm based on 10. Then, we used SPSS (version 25.0) software to perform PCA through dimensionality reduction. Then we made use of the Origin (version 2021) software for correlation analysis.

2.3 The determination of photosynthetic characteristics

From 9:00–11:00 on April 17, 2021, the experiment was carried out under clear weather and occasional cloud conditions. The light intensity was 800 μmol m^{-2} s^{-1}, the CO_2 consistence was 500 μmol mol^{-1} and the humidity was 62%. We selected the same leaf position and good growth of pakchoi leaves for photosynthetic index determination, and randomly selected 3 leaves from each group for determination. Under the condition that the water pressure difference between the pakchoi leaves and the air was 1.0-1.2 kPa, we used the handheld photosynthesis measurement system (LI-6400XT, Lincoln) to gauge the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and atmospheric CO_2 concentration (Ca) and other indicators of pakchoi (Lei, Zhu et al.).

2.4 The determination of fluorescence characteristic parameters of pakchoi

According to the report by Li et al (Li, Yang et al. 2018), we used a portable chlorophyll fluorometer (FMS-2, UK) to measure the fluorescence parameters of the same position and well-growing pakchoi leaves under the set light intensity. Before the measurement, the pakchoi leaves were dark treatment for 15 min, and low light intensity (1 μmol m^{-2} s^{-1}) was applied to gauge the initial fluorescence (Fo). After that, we used saturated pulsed light intensity (3000 μmol m^{-2} s^{-1}) to gauage the maximum fluorescence (Fm), the variable fluorescence (Fv = Fm-Fo), the maximum photochemical efficiency of PS (Fv/Fm), the potential of PS Photochemical activity (Fv/Fo), 100 μs photoreaction center closed purification rate (dVG/dto) and 300 μs photoreaction center closed purification rate (dV/dto). Three leaves were chosen for each group.

2.5 The determination of photosynthetic pigment content in leaves of pakchoi

We put 0.5 g fresh pakchoi leaves in 95% ethanol and protected from light for 24 h to extract photosynthetic pigments. A spectrophotometer (Hitachi UV-3100 UV/VIS; TECHCOMP, China) was used to measure the absorbance of the extract at 665, 649 and 470 nm. We referred to Wu's article for the calculation formulas of chlorophyll a, chlorophyll b and carotenoids (Wu, Tian et al. 2021). Each group selected the leaves of different pakchoi plants to repeat the experiment three times.

2.6 The contents of AsA and DHA in pakchoi

We used colorimetric method and phenanthroline colorimetric method to detect the AsA and DHA contents of the pakchoi leaves in the four treatment groups. According to the instructions, we used the Vitamin C (VC) content test kit (A009-1-1, Jiancheng Nanjing) and the DHA test kit (TC2041, Leagene Beijing).

2.7 The detection of oxidative stress level in pakchoi

We use phosphate buffer (pH 7.4) to grind a weighed 0.1 g of pakchoi. After centrifugation at 3500 r/min for 15 min, the supernatant was collected. Then, we detected according to the kit instructions. Six indicators of oxidative stress were all determined with kits, which were purchased from Nanjing Jiancheng Institute of Biology in China. Additionally, the detailed product numbers were as follows: MDA Determination Kit (Item No. A003-1-2), Glutathione Peroxidase (GSH-Px) Determination Kit (Item No. A005-1-2), Superoxide Dismutase (SOD) Test Kit (Item No. A001-1-2), Total Antioxidant Capacity (T-AOC) Test Kit (Item No. A015-1-2) and total glutathione (GSH)/oxidized glutathione (GSSG) determination kit (Item No. A061-2-1).

2.8 The analysis of mRNA levels of pakchoi

According to the method recorded in Shi's article (Xu, Xiaojing et al. 2021), TRIzol™ reagent (Item No. 12183555, Invitrogen) was used to collect total RNA from pakchoi leaves. Then we used a cDNA synthesis kit (BioFlux, China) to reverse transcribed total RNA into cDNA for reverse transcription. In this experiment, the primers of genes detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR) were shown in Table S1. β-actin was used as an endogenous control to standardize other target genes. According to the manufacturer's manuals, the qRT-PCR reaction program was executed using SYBR Green fluorescent dye (BioFlux, China). Relative mRNA levels genes were calculated using $2^{-\Delta\Delta Ct}$ method (Ali Shah, Zhang et al. 2021).

2.9 Statistical analysis

In this experiment, we used GraphPad Prism (version 8.0) and SPSS (version 25.0) to perform one-way analysis of variance on all data. The relevant experimental data of Pb-stressed pakchoi were all normally distributed and passed the equal variance test. The relevant results of this study were expressed in terms of mean ± standard deviation (M ± SD). Groups with the same letter represent insignificant differences; groups with different letters represent significant differences.

3. Results

3.1 Pb content and total element content of leaves of pakchoi

We replicated successfully pakchoi Pb stress model, and we found the Pb content to be 0.48518 mg/kg (C group), 3.108726 mg/kg (L group), 6.696257 mg/kg (M group) and 12.96486 mg/kg (H group) by ICP-MS (Fig. 1a). It can be seen that the Pb contents of pakchoi leaves among the Pb stress groups increased

exponentially. Given that IRT (iron-regulated transporter 1), as a member of the Zip family (ZRT IRT-like protein), participates in the transport of heavy metal ions in plants. The results of this experiment showed that the levels of IRT1 and IRT2 mRNA in group M increased by 147.3% and 75.0%, respectively, and the levels of IRT1 and IRT2 mRNA in group H increased by 296.3% and 159.1% ($P < 0.05$), while the increase in group L was not statistically significant ($P > 0.05$) (Fig. 1c, 1d). The above results indicated that Pb applied in the soil is transported to the leaves of pakchoi and presents a dose-dependent effect. The heat map of the total element content showed that the content of Rb in the H group decreased, and the contents of V, Fe, Cu, Co, Cd, Zn, As, and Mo increased. It is worth noting that the content of Mn in the H group increased by nearly twenty times (Fig. 1b).

The results of all-element principal component analysis found that Pb, Cd, As had the negative correlation with Se, Hg, B, Rb on the first and second components. Pb, Cd, As and Tl, Ba, Ca, Be, Sr, Sn, Bi, Ti, Ag, Cr, P, Sb, Mg, Ni, Mo, Li, K, Na, V, Co, Al were negatively correlated with component one and positively correlated with component two (Fig. 2a). Correlation analysis results found that Pb and B, Rb, Se, Ba, Hg, Tl presented a strong negative correlation. Pb had a strong positive correlation with Cd, As, Ge, Zn, Cu, Co, Fe, Mn, V and Al (Fig. 2b). The above results indicate that the increase of Pb content reduces the contents of B, Rb, Se, Hg and Tl, and increases the contents of Cd, As, Ge, Zn, Cu, Co, Fe, Mn, V and Al.

3.2 The effect of Pb stress on the AsA-GSH cycle of pakchoi

We detected the content and activity of oxidative stress-related indicators (GSH-Px, T-AOC, SOD, GSH, MDA and GSSG) (Fig. 3a), and found that the content of MDA increased ($P < 0.05$), the contents of T-AOC, GSH-Px and SOD were obviously reduced with dosage dependent manner ($P < 0.05$). This shows that with the increase of Pb dose, the oxidative stress level of pakchoi gradually increases. In addition, the GSH contents of the M and H group decreased, compared with the control group, and the GSSG content was significantly increased to 134.05% and 151.95% of the control group, which appeared dosage dependent ($P < 0.05$) (Fig. 3a). And GSH/GSSG, as a measure of plant response to ecological environmental stress, showed a dose-dependent decrease ($P < 0.05$) (Fig. 3b). Subsequently, the contents of AsA and DHA were significantly decreased in the M and H groups (Fig. 3c, 3d). Glutamate dehydrogenase (GLDH) is the key rate-limiting enzyme for AsA synthesis. APX and DHAR are genes related to AsA-GSH system. Thus, we detected their mRNA levels by qRT-PCR (Fig. 3e-g). In the M and H groups, the transcription level of APX was up-regulated ($P < 0.05$), with the increase of Pb dose, while the transcription levels of DHAR and GLDH were down-regulated. These results confirm that the AsA-DHA system of pakchoi is in a state of disorder under the Pb dose stress.

3.3 Influence of Pb stress on the dose-dependent effect of photosynthesis of pakchoi

Photosynthesis uses inorganic matter to produce organic matter and store energy, which is the basis for the survival of plants. In order to gauge the influence of Pb stress on photosynthesis of pakchoi, we used a handheld photosynthesis measuring instrument to detect Pn, Tr, Gs, Ci, VPD and Ca. Compared with the control group, under low-dose Pb treatment, Pn, Tr and Gs did not change obviously. The values of Pn (Fig. 4a), Tr (Fig. 4b) and Gs (Fig. 4c) were significantly reduced ($P < 0.05$) in the M group and H group, but the value of Gs was no difference between the M and H dose group ($P > 0.05$). Besides, the Ci values of the L and M groups did not change significantly (Fig. 4d), compared with the control group, while the H group showed a significant downward trend ($P < 0.05$). Pb treatment decreased the value of VPD slightly (Fig. 4e), but the dose dependence was not significant. The atmospheric CO_2 concentration did not change significantly among the four groups (Fig. 4f). Finally, we tracked the changes in the fluorescence intensity of pakchoi leaves over time (Fig. 4g). It was found that Pb treatment decreased the fluorescence intensity. Although no dose-dependent effect was found, the fluorescence intensity of the low-dose Pb treatment decreased most significantly over time. The above results indicate that Pb stress weakens the photosynthesis of pakchoi, which appeared dosage dependent.

3.4 Pb stress has a dose-dependent effect on the PS response system of pakchoi

In order to gauge the influence of Pb stress on the PS reaction system in pakchoi, we used a portable chlorophyll fluorometer to determine the Fo, Fm, Fv/Fm, Fv/Fo, dVG/dto and dV/dto. The results showed that Fo showed an upward trend after Pb treatment (Fig. 5a), and significant difference only appeared in the M group ($P < 0.05$), and the dose effect was not obvious. In addition, we found that with the increase of Pb dose, Fm showed an upward trend (Fig. 5b), and also the middle dose treatment began to show significant differences ($P < 0.05$). PSII reaction is the photosynthesis unit in the light-reactive thylakoid membrane. Fv/Fm can be used to measure the original light energy conversion efficiency of PSII. The results showed that with the increase of Pb dose, Fv/Fm showed a significant downward trend ($P < 0.05$) (Fig. 5c), which indicated that Pb stress inhibited the PS response system of pakchoi. In addition, compared with the other three groups, high-dose Pb exposure decreased significantly Fv/Fo value (Fig. 5d), which meant that the maximum light energy conversion potential of PS reaction was reduced. Then, we selected the purification rate of extensive initiation center closure at two time points of 100 μs and 300 μs (Fig. 5e, 5f). The results showed that with the increase of Pb dose, both dVG/dto and dV/dto showed an upward trend, and the dose dependence was not obvious. The above results indicated that Pb stress had a dose-dependent inhibitory effect on the PS response system of pakchoi.

3.5 The influence of Pb treatment on the chlorophyll content and chloroplast formation of pakchoi

In order to gauge the influence of Pb stress on the chloroplast of pakchoi, we explored from the perspective of chlorophyll content and chloroplast formation. The results displayed that no significant changes were observed in chlorophyll a and chlorophyll b between the control group and the L group

(Fig. 6a, 6b). But in the M group, we observed that the chlorophyll a and chlorophyll b contents decreased by 22.7% and 38.1%, respectively; the H group decreased by 17.4% and 37.4%, respectively ($P < 0.05$). After that, the carotenoid content changed slightly, and only slightly decreased in the H group (Fig. 6c). In addition, the vesicle-inducing protein plastid 1 (VIPP1) is located in the thylakoid, and it was found that the transcription level of VIPP1 did not change significantly (Fig. 6d). Subsequently, we detected the transcription levels of chloroplast development-related genes Golden 2-like (GLK), Glutamine synthetase 2 (GLN2), ethylene-dependent geotropism and yellow-green 1 (EGY1). The results showed that EGY1 was significantly reduced only when high-dose Pb treatment ($P < 0.05$) (Fig. 6e). Compared with the control and L groups, the transcription levels of GLK and GLN2 in the M and H groups decreased, which appeared dosage dependent (Fig. 6f, 6g). THF1 is a protein encoded by a nuclear gene located in the chloroplast. This experiment found that middle-dose and high-dose Pb treatments reduced the mRNA level of THF1 with dosage dependent manner ($P < 0.05$) (Fig. 6h). Additionally, phytochrome interacting factor (PIF) and high chlorophyll fluorescence (HCF) are involved in chloroplast development and chlorophyll biosynthesis. This study found that the middle and high dose of Pb treatments reduced the transcription levels of PIF and HCF with dosage dependent manner ($P < 0.05$) (Fig. 6i, 6j). The mRNA expression of the light-harvesting chlorophyll a/b protein complex (LHC) decreased, which appeared dosage dependent (Fig. 6k). The above results manifested that the influence of Pb stress on the chlorophyll content and chloroplast formation of pakchoi was a dose-dependent effect. As the Pb dose increases, the chlorophyll content and chloroplast formation decreased.

4. Discussion

Pb residues were found in both industrial and human living soils, which will be further transferred to plants to accumulate (Doris, Jorge et al., Gao, Zhang et al. 2021). A study reported that Pb can cause chlorosis, oxidative stress and growth and development disorders in plant leaves (Kanwal, Farhan et al. 2020). This study evaluated the effects of different doses (300 mg/kg, 600 mg/kg, 900 mg/kg) of Pb stress on the leaf chlorosis, oxidative stress and growth and development of pakchoi. The results showed that Pb stress in pakchoi caused oxidative stress, abnormal mineral content, inhibition of AsA-GSH system and photosynthesis, abnormal chlorophyll content, and abnormal expression of chloroplast development genes in a dose-dependent manner.

Heavy metal stress can cause mineral imbalance in humans, animals and plants (Xu, Xiaojing et al. 2021). In addition, Cd and As are proven toxic mineral elements, which are easy to accumulate in plants and affect growth and development (Irshad, Noman et al. 2021). The ICP-MS method can gauge the content of all elements in plant and animal tissues, and principal component analysis and correlation analysis can simplify the complex relationship between the elements (Xu, Xiaojing et al. 2021), so that we can observe that Pb is exposed to small changes of element content in pakchoi leaves. This experiment found that as the dose of Pb in the soil increased, the content of growth important elements B and Se decreased, while the content of toxic mineral elements such as Cd, As and Cu increased. These results indicate that Pb stress decreases the absorption of beneficial elements in pakchoi leaves in a dose-dependent manner, while the deposition of other toxic metal elements increases. Boron is an vital element

for plant reproduction and growth, which plays an important role in the physiological processes of crop plant leaf expansion and meristem development (Pinho, Monnerat et al. 2015). In PCA, Se, B, Hg, Tl and Ba belong to the first component. Li, K, Na, Mo, V, Co, Al, Mn, Cu, Zn and Fe belong to the second component. Se and B were positively correlated with component one; Pb was negatively correlated with component one. Therefore, Se and B are negatively correlated with Pb, which is similar to that obtained by correlation analysis. So, this also suggests to us that adding B or Se to Pb-stressed pakchoi may be used as an antagonist of Pb stress. Pb treatment can reduce Se content and Se supplementation also can reduce Pb content. There was a negative correlation between Pb and Se (Huang, Chen et al. 2021).

The AsA-GSH system is composed of the AsA (that is, vitamin C)-DHA and GSH-GSSG processes and the enzymes involved in these two processes and resists environmental stresses such as low light (Hu, Li et al. 2019). According to reports, Cd and Cu will accumulate in plants, causing the activities of MDHAR, APX and DHAR to decrease, the abnormal levels of AsA and DHA, and the decreases of GSH and GSSG content, leading to the oxidative stress and the imbalance of AsA-GSH cycle (Zhou, Huo et al. 2018, Jung, Lee et al. 2021). Salt-alkali mixed stress reduces the key enzymes of AsA synthesis pathway L-galactose dehydrogenase (GDH) and L-galactose-1,4-lactone dehydrogenase (GLDH) activities, and weakens the AsA-GSH cycle efficiency, thereby causing oxidative damage to naked oats (Liu, Liu et al. 2021). Additionally, ammonia gas stress decreased the activities of antioxidant systems (SOD, T-AOC, and GSH-Px), whereas increased the concentration of MDA in chickens (Han, Zhang et al. 2020). Boron (B) and chromium (Cr) stress increased MDA and caused oxidative stress in wheats (Ashraf, Rasheed et al. 2022). The results of this study are similar to the above-mentioned literature. It was found that under medium and high doses of Pb treatment, the contents of AsA and vitamin C synthesis key enzyme (GLDH) continued to decrease, indicating that Pb has a dose-dependent inhibitory effect on the vitamin C synthesis of pakchoi. With the increase of Pb dose, the oxidative stress marker MDA continued to increase, and the activities of antioxidant enzymes continued to decrease. This shows that the dose dependent Pb stress causes the decrease of the antioxidant capacity and the increase of oxidative stress level. From another angle, we found that the tolerance of pakchoi to adverse environment is reduced from another angle through the ratio of GSH/GSSG. With the help of APX and DHAR activity abnormalities, we can summarize the above results as: Pb stress dose-dependently causes AsA-GSH circulatory system imbalance, which reduces the tolerance of pakchoi to oxidative stress.

Chloroplast is the place where plants photosynthesize. The chlorophyll in the chloroplast absorbs light energy to participate in the normal progress of photosynthesis. The net photosynthetic rate is a key indicator for evaluating the photosynthesis efficiency of plants. Under high-dose metal accumulation stress (60 mg/kg Cd + 90 mg/kg Cu), the photosynthetic characteristics (chlorophyll a and b content, Pn, Tr, Gr and Ci) and nutrients of pea are reduced (Lei, Zhu et al. 2021). This experiment found that medium and high doses of Pb stress reduced the values of Pn, Tr and Gs, and high doses of Pb treatment significantly reduced the values of Ci, which directly explained the negative effects of Pb stress on the photosynthesis of pakchoi. In addition, PSII photoreaction is an important stage of photoreaction (Ci, Jiang et al. 2009), and Fv/Fm and Fv/Fo can be used to measure the original light energy conversion efficiency and maximum light energy conversion potential of PSII system in pakchoi. Research by Li et al.

confirmed that under 100 μmol/L Cd hydroponic conditions, the photosynthetic parameters Fv/Fo and Fv/Fm of elsholtzia serrata were significantly reduced (Li, Yang et al. 2015). This study found that as the Pb dose increased, Fv/Fm continued to decrease, and showed a dose-dependent effect. This shows that the negative effect of Pb stress on the photosynthesis of pakchoi via the abnormality of PSII light response system. In addition, the effects of medium and high doses of Pb on the contents of chlorophyll a and chlorophyll b were obviously reduced in a dose-dependent manner. Studies have found that GLK expression leads to the increased levels of chlorophyll and LHC (Li, Wang et al. 2020), and genes such as PIF and HCF are also involved in chloroplast development and chlorophyll synthesis (Schmitz, Schöttler et al. 2012, Zhang, Xiong et al. 2021). The EGY1 (Ethylene-dependent gravitropism-deficient and yellow-green 1) gene encodes a thylakoid membrane-localized protease involved in chloroplast development in mesophyll cells (Sanjaya, Muramatsu et al. 2021). Our research also found that after Pb stress, the expression of chlorophyll synthesis genes (HCF and PIF) and chloroplast development-related genes (GLK, GLN2 and EGY1) of pakchoi were down-regulated to varying degrees, this further confirms that Pb stress may affect the photosynthesis of pakchoi through the development of chloroplast and the downregulation of chlorophyll synthesis. The above results indicate that Pb exposure affects the PSII photoresponse system of photosynthesis by affecting the development of chloroplasts and the synthesis of chlorophyll, which also presents a dose-dependent effect.

In conclusion, we found that Pb stress dose-dependently has an adverse effect on the mineral content of pakchoi, AsA-GSH and photosynthesis. In detail, Pb induces oxidative stress in pakchoi, and the AsA-GSH cycle and photosynthesis are weakened, which further leads to abnormal chlorophyll content and decreasing chloroplast development gene expression. Because heavy metals accumulate in plants through the food chain in the environment, they will eventually endanger humans and animals, and even the entire ecological environment. The results of this study supplement the toxicology of heavy metals and provide instructions for the planting of pakchoi and warnings of heavy metal hazards.

Declarations

Ethical Approval

Not applicable.

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Competing Interests

The authors have announced no conflict of interest. All authors have read the manuscript and consented to submit it in its current form for consideration for publication in the Journal.

Author Contributions

Zhanming Tan: Visualization, Investigation, Writing-original draft. **Cuiyun Wu**: Manuscript revision, Formal analysis. **Zhengying Xuan**: Software, Formal analysis. **Yunxia Cheng**: Formal analysis. **Renci Xiong**: Software, Investigation. **Zhihang Su**: Software, Investigation. **Desheng Wang**: Conceptualization, Resources, Supervision, Validation, Writing-review & editing.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Consent to Participate

All authors agree to participate in the submission of the manuscript in its current form.

Consent to Publish

All authors have read the manuscript and consented to submit it in its current form for consideration for publication in the Journal.

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Figures

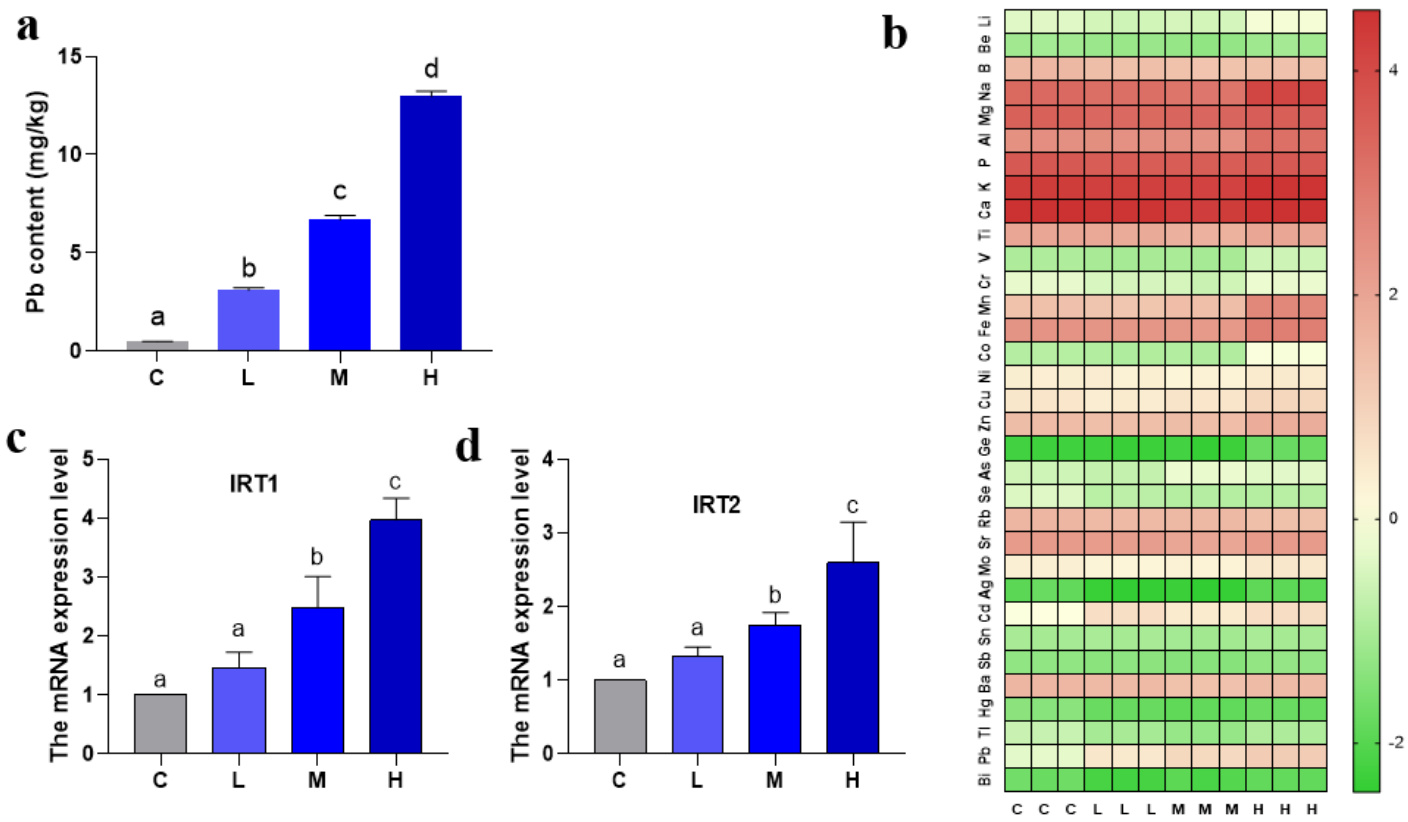

Figure 1

Pb content and total element content of leaves of pakchoi. (a) Pb content (mg/kg) in the leaves of pakchoi. (b) After Pb exposure in pakchoi, the heat map of total element content. (c) The mRNA level of IRT1 (n=3). (d) The mRNA level of IRT2 (n=3). The same letter indicates no significant difference ($P > 0.05$), completely different letter indicates significant difference ($P < 0.05$).

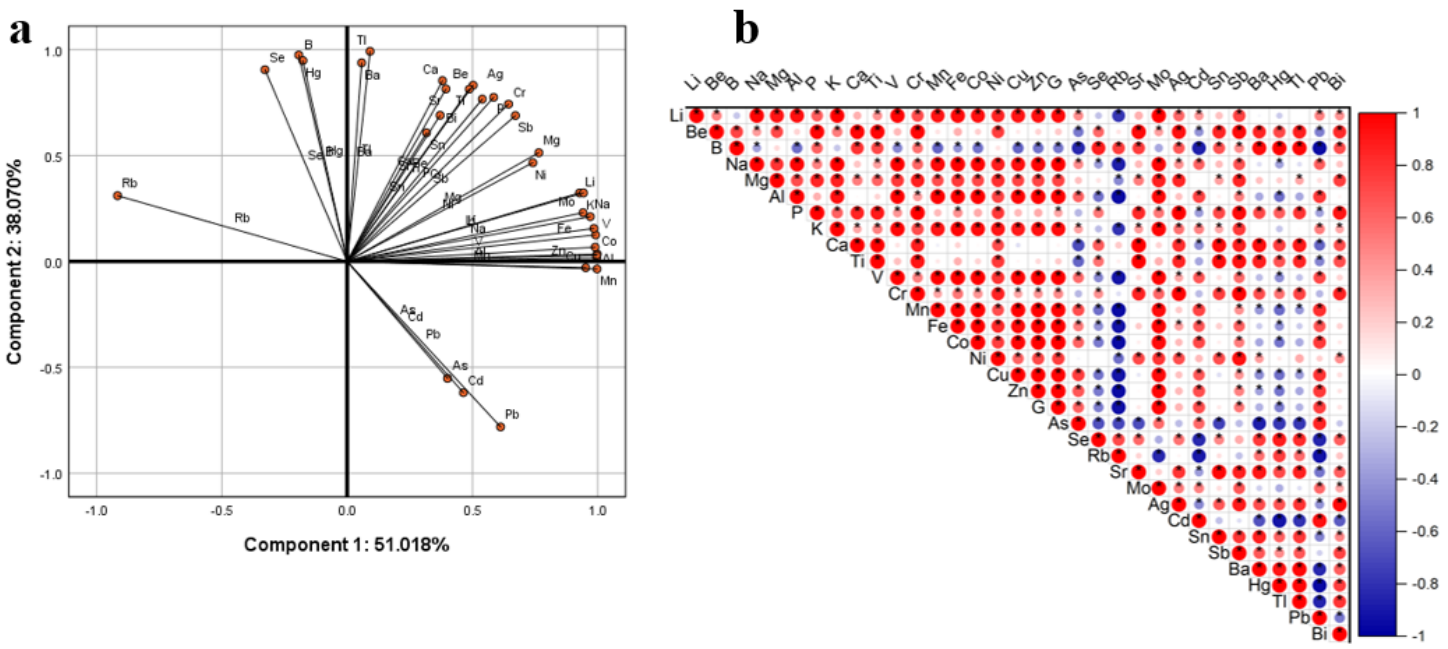

Figure 2

Principal component analysis and correlation analysis. (a) Use SPSS (version 25.0) software to carry out the PCA of total element content (logarithm based on 10). The first component (x axis) is 51.018 %, and the second component (y axis) is 38.070 %. (b) Use Origin software to perform correlation analysis on the rotated score matrix output by PCA. Positive correlation (red), negative correlation (blue). The color depth and the size of the circle are related to the strength of the correlation. The "*" sign indicates significant difference.

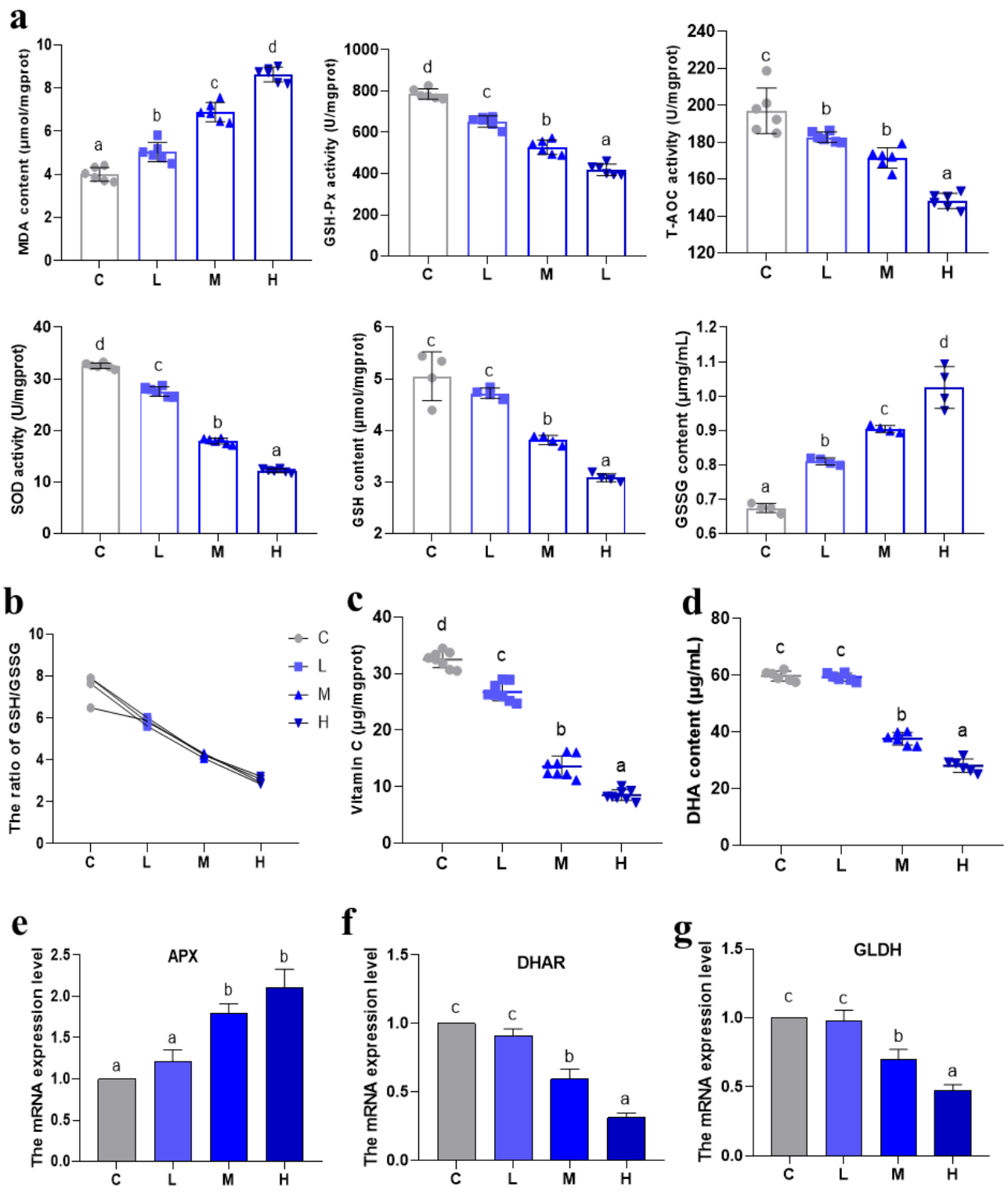

Figure 3

The effect of Pb stress on the AsA-GSH cycle of pakchoi. (a) The content and activity of MDA, GSH-Px, T-AOC, SOD, GSH and GSSG (n=4). (b) The ratio of GSH/GSSG (n=4). (c) The content of AsA (Vitamin C) (μg/mgprot) (n=8). (d) The content of DHA (μg/mL) (n=8). (e) The mRNA level of APX (n=3). (f) The mRNA level of DHAR (n=3). (g) The mRNA level of GLDH (n=3). The same letter indicates no significant difference ($P > 0.05$), completely different letter indicates significant difference ($P < 0.05$).

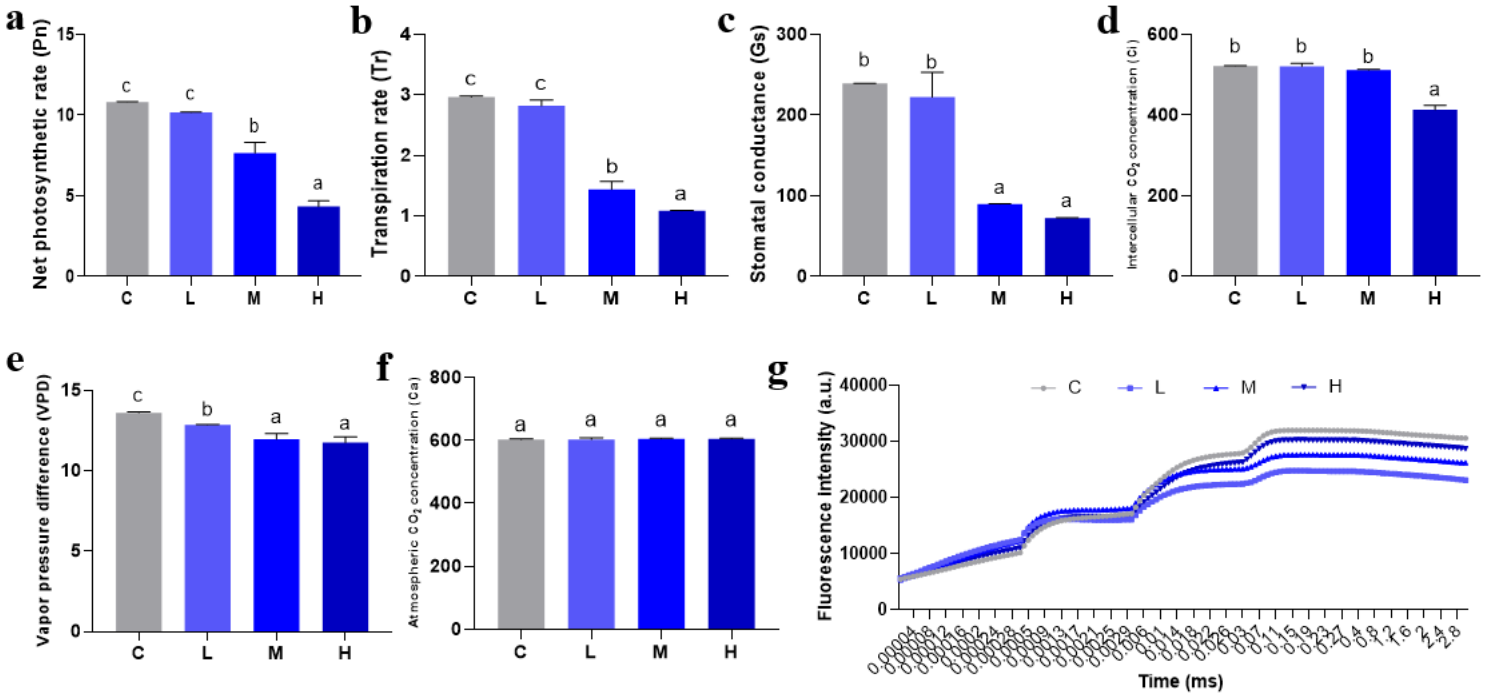

Figure 4

Effect of Pb stress on the dose-dependent effect of photosynthesis of pakchoi. (a) The net photosynthetic rate (Pn) (n=3). (b) The transpiration rate (Tr) (n=3). (c) The stomatal conductance (Gs) (n=3). (d) The intercellular CO_2 concentration (Ci) (n=3). (e) The vapor pressure difference (VPD) (n=3). (f) The atmospheric CO_2 concentration (Ca) (n=3). (g) Over time, the fluorescence intensity (a. u.) of pakchoi (n=3). The same letter indicates no significant difference ($P > 0.05$), completely different letter indicates significant difference ($P < 0.05$).

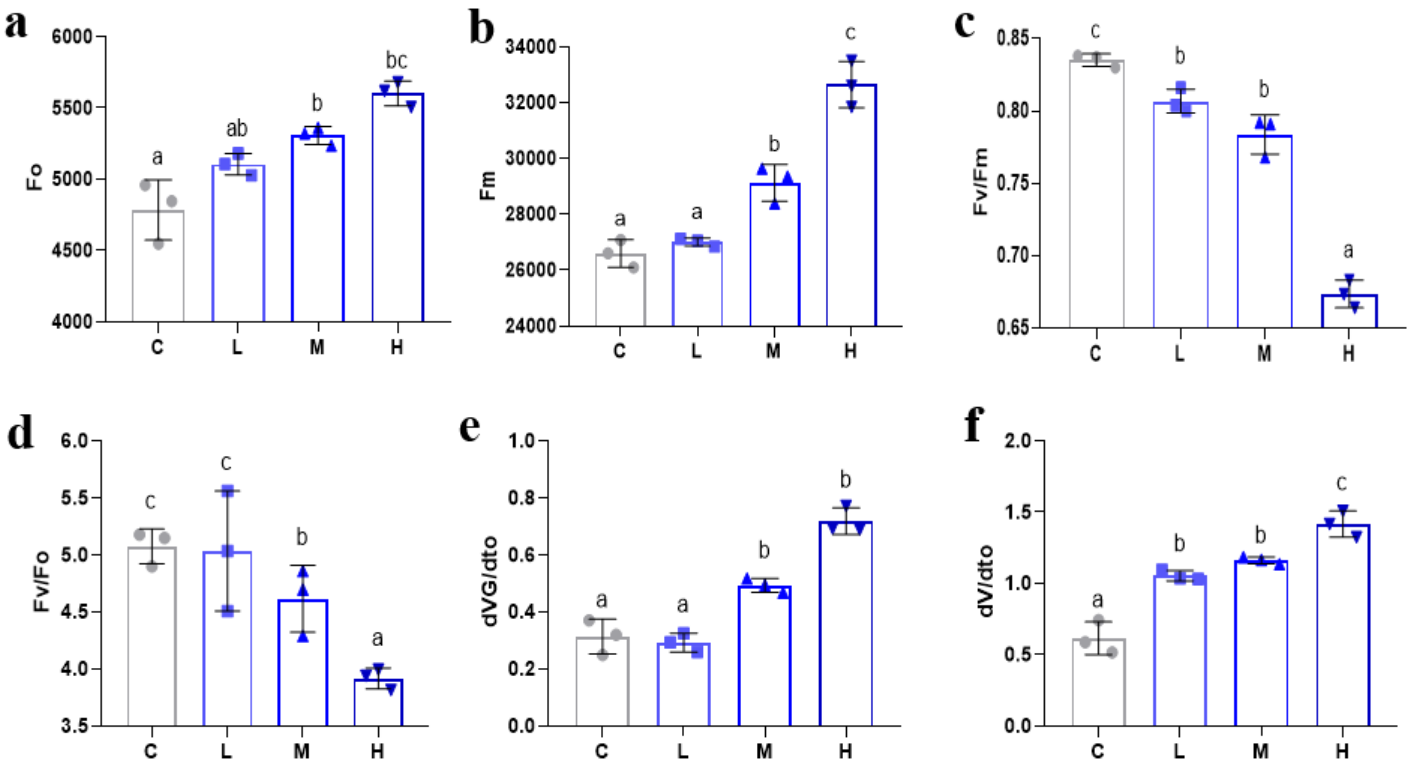

Figure 5

Pb stress has a dose-dependent effect on the PS response system of pakchoi. (a) The initial fluorescence (Fo) of Pb stress pakchoi (n=3). (b) The maximum fluorescence (Fm) of Pb stress pakchoi (n=3). (c) The maximum photochemical efficiency of PS (Fv/Fm) (n=3). (d) The potential of PS Photochemical activity (FV/Fo) (n=3). (e) The 100 μs photoreaction center closed purification rate (dVG/dto) in the C, L, M and H groups (n=3). (f) The 300 μs photoreaction center closed purification rate (dV/dto) (n=3). The same letter indicates no significant difference ($P > 0.05$), completely different letter indicates significant difference ($P < 0.05$).

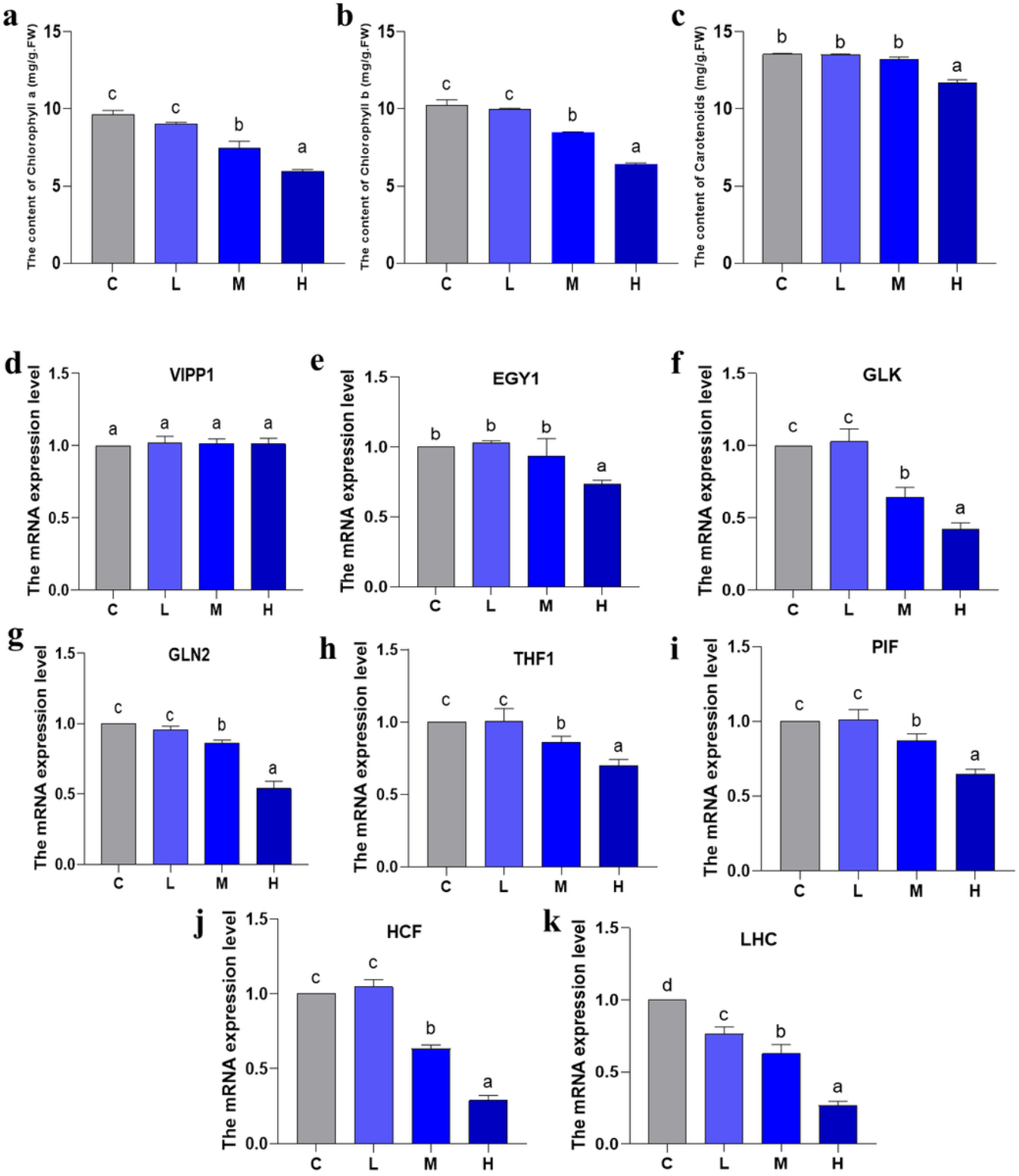

Figure 6

The effect of Pb stress on the chlorophyll content and chloroplast formation of pakchoi. (a) The content of chlorophyll a (n=3). (b) The content of chlorophyll b (n=3). (c) The content of carotenoids (n=3). (d) The mRNA level of VIPP1 (n=3). (e) The mRNA level of EGY1 (n=3). (f) The mRNA level of GLK (n=3). (g) The mRNA level of GLN2 (n=3). (h) The mRNA level of THF1 (n=3). (i) The mRNA level of PIF (n=3). (j)

The mRNA level of HCF (n=3). (k) The mRNA level of GLDH (n=3). The same letter indicates no significant difference ($P > 0.05$), completely different letter indicates significant difference ($P < 0.05$).

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