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Exogenous Glutathione enhances tolerance of the potato (*Solanum tuberosum* L.) to cadmium stress by regulating the biosynthesis of phenylpropanoid and the signal transduction of plant hormones

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Abstract

Background Cadmium (Cd) pollution has brought harm to the growth and development of potato. Glutathione (GSH) is an important antioxidant that may play an active role in the response of a potato to Cd stress. However, how GSH influences the effect of Cd on potatoes is unknown. In this study, we investigated the effects of exogenous GSH on the phenylpropanoid biosynthesis pathway and plant hormone signal transduction pathway in potatoes under Cd stress to explore new ideas for how potatoes respond to Cd stress. We cultured 21-day-old 'Atlantic' plantlets in Murashige and Skoog (MS) medium supplemented with 500 μmol/L $CdCl_2$ or 500 μmol/L $CdCl_2$ + 400 μmol/L GSH. We then investigated the activities of key enzymes in the phenylpropanoid biosynthesis pathway, hormone levels, and the expression levels of related genes at different time points.

Results Analysis showed that 96 h of treatment with glutathione led to an increase in the expression levels of genes encoding phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD); an increase in the enzymic activities of PAL, CAD and POD; and an increase in the content of lignin. The content of lignin was positively correlated with the expression levels of several genes (PAL: PG0031457, CAD: PG0005359, POD: PG0011640 and PG0015106). In addition, the levels of Salicylic acid (SA) and Jasmonic acid (JA) increased significantly, the expression levels of the genes encoding transcription factor TGA (PG2023696), pathogenesis-related protein 1 (PR1) (PG0005111), and the transcription inhibitor Aux/IAA (PG0006093) all increased while the expression levels of jasmonate ZIM domain-containing protein (JAZ) (PG0004367), auxin influx carrier (AUX) (PG0006550) and auxin response factor (ARF) (PG0005794) all decreased. We also observed a reduction in the content of IAA.

Conclusion Exogenous GSH improved the tolerance of potato, Atlantic cv. to Cd stress by regulating the phenylpropanoid biosynthesis pathway and the plant hormone signal transduction pathway.

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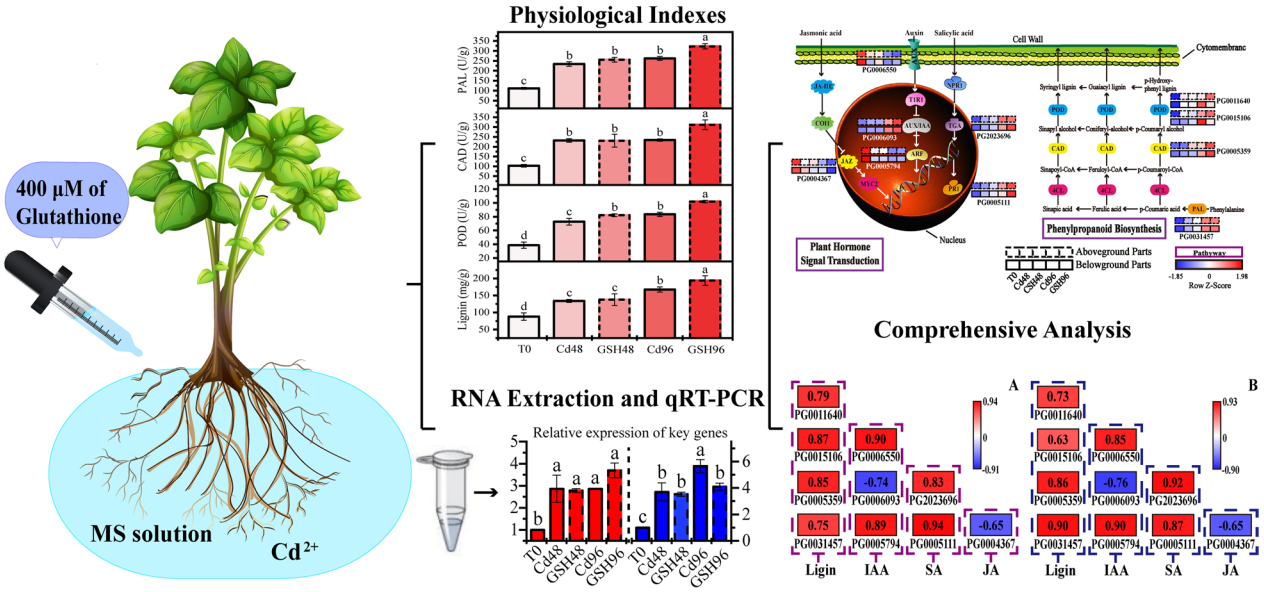
Highlights

1. Glutathione affected the synthesis of phenylpropanoid in the potato.
2. Glutathione affected the levels of plant hormone and the expression of related genes in potato.
3. Glutathione enhanced the tolerance of potato to cadmium stress.
4. Some genes play an important role in potato response to cadmium stress.

Keywords Glutathione, Cadmium stress, Lignin synthesis, Endogenous plant hormones, Gene regulation

Graphical Abstract



Background

Due to the large amount of industrial waste produced by the development of industry, the use of chemical fertilizers and pesticides in agricultural production, etc., may carry Cd and lead to its large release, which increases the Cd content in the surface soil. The pollution of soil by cadmium (Cd) has become increasingly serious over recent years [1]. Cd pollution can reduce seed germination, induce chromosomal aberration, affect the normal growth pattern of plants, and reduce crop quality [2–4]. Previous studies have shown that plants can produce a series of stress responses to Cd pollution, including oxidative stress, an imbalance in enzymatic activity and plant signaling molecules (hormones and calcium ions), thus resulting in damage to the photosynthetic system, plasma membrane peroxidation, cell damage, changes in enzymatic activity, endoplasmic reticulum stress, protein degradation, or DNA damage or mutation. These factors can have an adverse effect on physiological and biochemical metabolism and can ultimately inhibit plant growth, or even lead to death [5–7]. With regards to human health, eating crops grown in soil contaminated by Cd can lead to cancer, bone lesions, lung dysfunction, renal dysfunction, anemia and other diseases [8–10]. As a result, there is an urgent need to explore ways for investigating Cd's damaging effects on plants.

Glutathione (GSH) is a tripeptide composed of glutamic acid, cysteine, and glycine. GSH is an important non-enzymatic antioxidant in plants and can directly eliminate various reactive oxygen species (ROS)-free radicals such as singlet oxygen and H_2O_2 [11, 12]. GSH predominantly scavenges H_2O_2 via the AsA-GSH (ascorbic acid-glutathione) cycle pathway. The ratio of GSH/GSSG is regulated by the glutathione reductase (GR) and plays an important role in signal transduction [13, 14]. GSH is also the precursor of phytochelatins (PCs) and the

substrate of glutathione S-transferase (GST), which plays an important role in the resistance of plants to heavy metal toxicity [15]. Previous studies, involving stocks of barley, showed that exogenous GSH reduced the absorption of Cd, restored the damaged plasma membrane and chloroplast structure, improved the photosynthetic rate under Cd stress, and promoted the growth of aboveground biomass [16]. In addition, GSH regulates the harm caused by external stress to plants by synergistic effects with other pathways. For example, a plant's tolerance to stress can be regulated by both the phenylpropanoid biosynthesis pathway and the plant hormone signal transduction pathway. Previous studies have shown that GSH improved the activity of POD and other antioxidant enzymes in rice under heavy metal stress [17]. Evidence also suggests that there may be multidirectional interactions between plant hormone signal transduction pathways and GSH to protect plants against stress [18, 19].

The phenylpropane biosynthesis pathway is considered to play an important role in a plant's response to abiotic stress. Lignin is the end-product of this pathway and an important component of the cell wall, the first line of defense for plants against external stress [20, 21]. Previous studies showed that Cd exists in the form of ions or combines with certain components of the cell wall, including glue, cellulose, hemicellulose, and lignin, so that Cd is limited by the cell wall. This mechanism prevents Cd from entering the cytoplasm, thereby protecting intracellular metabolic activities [22, 23]. In addition, several of the enzymes involved in this pathway also play an important role in the tolerance of plants to stress. For example, POD is an antioxidant enzyme that is closely associated with the biosynthesis of phenylpropanoid [24]. An increase in the activity of POD can remove excessive ROS and free radicals from plants, thus reducing the damage incurred by plant tissues in response to a stressful environment [25, 26].

Plant hormones are certain organic signaling molecules that are produced by plants by metabolic activity; these hormones can exert specific physiological effects at very low concentrations [27]. Plant hormones regulate various processes in plants, including growth development [28, 29], and adaptation to biotic and abiotic stressors [30, 31]. Plant hormones can regulate such processes in both an independent and cooperative manner. For example, the deletion of JA in the tomato was previously shown to reduce photosynthesis, weaken the defense ability of the antioxidant system, and enhanced sensitivity to Cd [32]. Zhou et al. carried out experiments in potatoes and demonstrated that the promotion of SA biosynthesis can improve the capacity of potatoes to defend against diseases [33]. Consequently, our understanding of a plant's ability to respond to stress would be significantly enhanced by investigating the pathways responsible for the phenylpropanoid biosynthesis and plant hormone signal transduction.

The potato is a major global food crop and plays an important role in human life [34]. Over recent years, and owing to an increase in Cd pollution, an increasing number of studies have begun to investigate the impact of Cd on the growth and development of the potato by applying biochemical and molecular genetics methods [35]. Additionally, how to alleviate the negative impact of cadmium pollution on potato growth is imperative. Our previous studies have found that glutathione may play a positive role in potato response to cadmium stress [36]. Previous studies have demonstrated that exogenous GSH has a positive impact on the physiology and metabolism of potatoes under Cd stress [37], although the precise mechanisms involved have yet to be elucidated. In the present study, we used potato tissue culture plantlets to investigate alterations in the important pathways following Cd stress and exogenous GSH treatment. Our findings may provide a solid foundation for the alleviation of Cd stress and identify a detoxification mechanism in the potato during Cd pollution.

Materials and method methods

Plant materials and growth conditions

We used potato, Atlantic cv. tissue culture plantlets in these experiments. The plantlets were purchased from Gansu Ailan Potato Seed Industry Co., Ltd. (Dingxi, China). Plantlets were grown in MS medium in a culture box, at a temperature of 21 °C, a photoperiod of 16 h light: 8 h dark, and a light intensity of 3000 Lx for 20 days of treatment.

Experimental design

After 20 days of growth, tissue culture plantlets were divided into five groups for experimental treatment: (1) T0 (untreated), (2) Cd48 (48 h of growth following the addition of 500 μmol/L of $CdCl_2$ to MS solution), (3) GSH48 (48 h of growth following the addition of 500 μmol/L of $CdCl_2$ and 400 μmol/L of GSH to MS solution), (4) Cd96 (96 h of growth following the addition of 500 μmol/L of $CdCl_2$ to MS solution), and (5) GSH96 (96 h of growth following the addition of 500 μmol/L of $CdCl_2$ and 400 μmol/L of GSH to MS solution). At the end of the experiment, we collected the aboveground and underground parts of the plants in each group. These plant tissues were washed three times with deionized water, snap frozen in liquid nitrogen, and then stored at − 80 °C freezer to await subsequent analysis. Each group featured three biological repetitions.

The measurement of physiological indexes

Physiological measurements were carried out for each group of samples. The activity of phenylalanine ammonia lyase (PAL) was determined by a specialist kit (BC0210) provided by Beijing Solebao Science Technology Co., Ltd. (Beijing, China) and ultraviolet spectrophotometry at a wavelength of 290 nm, in accordance with the manufacturer's instructions. Finally, the specific activity of PAL in a given sample was calculated by the formula provided in the kit instructions. PAL (U/g) $39 = 17.3 \times \Delta A \div W$. ΔA is a dynamic parameter based on the spectrophotometric value of different samples. W is the sample weight. The activity of cinnamyl alcohol dehydrogenase (CAD) was determined by a specialist kit (BC4170) provided by Beijing Solebao Science Technology Co., Ltd. (Beijing, China) and ultraviolet spectrophotometry at a wavelength of 340 nm, in accordance with the manufacturer's instructions. The specific activity of CAD in a given sample was then calculated by the formula provided in the kit instructions. CAD (U/g) $= 321.54 \times \Delta A \div W$. ΔA is a dynamic parameter based on the spectrophotometric value of different samples. W is the sample weight.

The activity of peroxidase (POD) was determined by a specialist kit (BC0090) provided by Beijing Solebao Science Technology Co., Ltd. (Beijing, China) and ultraviolet spectrophotometry at a wavelength of 470 nm, in accordance with the manufacturer's instructions. Then, we determined the specific activity of POD in a given sample using an established formula; the formula: provided in the kit instructions. POD (U/g) $= 7133 \times \Delta A \div W$. ΔA is a dynamic parameter based on the spectrophotometric value of different samples. W is the sample weight.

Lignin content was determined by a kit (BC4200) provided by Beijing Solebao Science Technology Co., Ltd. (Beijing, China) and ultraviolet spectrophotometry. Samples from each treatment were dried to a constant weight, and a 5 mg sample was used for analysis. Tests were carried out as described by the manufacturer's instruction. The absorbance of each sample was measured at 280 nm and the lignin content of each sample was calculated according to an established formula. The formula: provided in the kit instructions. Lignin content (mg/g) $= 2.184 \times \Delta A \div W$. ΔA is a dynamic parameter based on the spectrophotometric value of different samples. W is the sample weight.

Levels of IAA and SA were determined by HPLC [38]. The mobile phase was methanol and 0.1% phosphoric acid (9:1), the flow rate was 0.8 ml/min, the injection volume was 10 µl, and the column temperature was 30 °C. Each sample was analyzed three times and hormone levels were calculated by referring to a standard curve.

Levels of Jasmonic acid (JA) were measured by liquid chromatography–mass spectrometry (LC–MS) with a mobile phase of 0.1% formic acid and acetonitrile, a flow rate of 0.3 mL/min, an injection volume of 5 µl, and a column temperature of 30 °C. Each sample was analyzed three times and the level of JA was calculated by referring to a standard curve.

RNA extraction and reverse transcription

The collected plant tissues were cleaned three times with deionized water, rapidly frozen in liquid nitrogen, and then stored in a − 80 °C refrigerator for RNA extraction. RNA was then extracted using a SteadyPure Universal RNA Extraction Kit (Accurate, Hunan, China) in accordance with the manufacturer's instructions. RNA concentration was measured using NanoDrop 2000 and RNA integrity was assessed by agilent Bioanalyzer 2100. Then, we reverse transcribed RNA using FastKing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China).

Quantitative real-time polymerase chain reaction (qRT-PCR)

In accordance with previous studies [36], we identified 10 mRNAs for real-time fluorescent quantitative PCR (qRT-PCR) verification (Additional file 1). Actin was used as an internal reference gene. In accordance with the manufacturer's instructions, we used a TB Green Premix Ex Taq II Kit to detect the cDNA obtained on a fluorescent qPCR instrument. The relative expression levels of each gene being tested were calculated according to the $2^{-\Delta\Delta Ct}$ method [39]. Three biological replicates for each group were tested and each reaction was performed with three technical replicates. The primers used for the qRT-PCR experiment are listed in Additional file 1. The mRNA data were uploaded to the National Center for Biotechnology Information (NCBI; SRP314907).

Data analysis

Microsoft Excel 2016 software was used to collate data. SPSS version 19.0 software, Origin 2018 software, and Adobe Illustrator CS6 software were used for data analysis. Differences between groups were detected by performing analysis of variance (ANOVA) using the LSD and SNK methods. Pearson's correlation coefficient was used to identify correlations between data. $P < 0.05$ was considered to be statistically significant.

Results

The effects of different treatments on the levels of PAL, CAD, POD, and lignin in the potato

Following treatment, the levels of PAL increased significantly; an increase in treatment time led to an increase in PAL level. When the plants were treated with GSH, the levels of PAL in the GSH96 group were significantly higher than those in plants undergoing other treatments;

compared with T0, the levels of PAL increased by 189%. When comparing the GSH48 and GSH96 groups, the levels of PAL increased by 37.89% and 23.21%, respectively. There was no significant difference in the levels of PAL when compared between the Cd48 and Cd96 groups following Cd stress; the levels of PAL in the Cd96 group were significantly lower than those in the GSH96 group. The activity of CAD activity also increased significantly after treatment. After 48 h of treatment, there was no significant difference between the Cd48 and GSH48 groups with regards to CAD activity. After 96 h of treatment, the CAD activity in the GSH96 group was significantly higher than that in the CD96 group, by 33.1% (Fig. 1). The levels of POD were significantly higher after treatment. Following Cd stress, the levels of POD in the Cd96 group were significantly higher than those in the Cd48 group, by 14.79%. Following GSH treatment, the levels of POD in the GSH96 group were significantly higher than those in the GSH48 group and were also significantly higher than those in any of the other treatment groups. When the plantlets were treated with GSH or GSH and CD together, the levels of lignin increased significantly. As treatment time increased, the levels of lignin increased continuously. Following Cd stress, the levels of lignin in the CD96 group were significantly higher than those in the Cd48 group, by 25.02%. When GSH was added, the levels of lignin in the GSH96 group were significantly higher than those in the GSH48 group; the levels of lignin of the GSH96 group were significantly higher than those in other treatments.

The effects of different treatments on hormone levels in the potato

The levels of IAA decreased significantly after 96 h of treatment. The IAA levels in the Cd96 group decreased by 36.58% when compared with T0 group while the levels of IAA in the GSH96 group decreased by 42.59% when compared with the T0 group. The levels of IAA in the Cd96 group were significantly lower than those in the Cd48 and GSH48 group (Fig. 2). The levels of SA in the Cd96 group were 360% higher than those in the T0 group while the levels of SA in the GSH96 group were significantly higher those in any other treatment groups (504% and 31.3% higher than the levels in the T0 and CD96 groups, respectively). When plants were treated for 48 h, there was no significant difference in the levels of JA when compared between the Cd48 and GSH48 groups. When plants were treated for 96 h, the levels of JA in the Cd96 and GSH96 groups were significantly higher than those in the T0, Cd48 and GSH48 groups.

The effects of different treatments on gene expression in the potato

The expression levels of POD-related genes (PG0011640 and PG0015106), key genes in the phenylpropanoid biosynthetic pathway, were significantly upregulated in both the aboveground and underground parts of the plants following treatment. The expression levels of PG0005359 were the highest in the GSH treatment group at 96 h; these levels were significantly higher than those in the other treatment groups (both

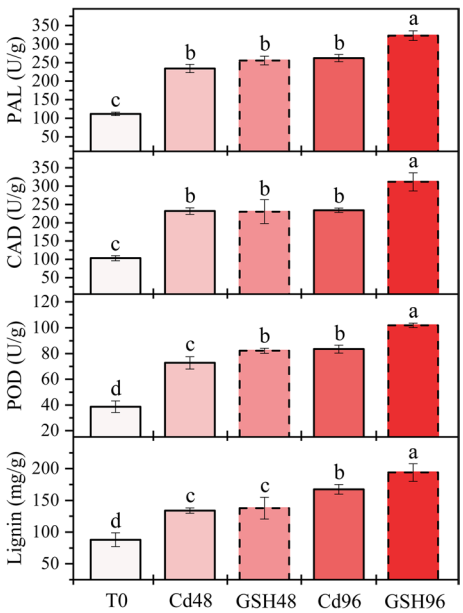


Fig. 1 The effects of different treatments on the levels of PAL, CAD, POD, and lignin in the Potato, Atlantic cv. The error bar refers to the standard error. Different letters indicate significant differences at the $P < 0.05$ level

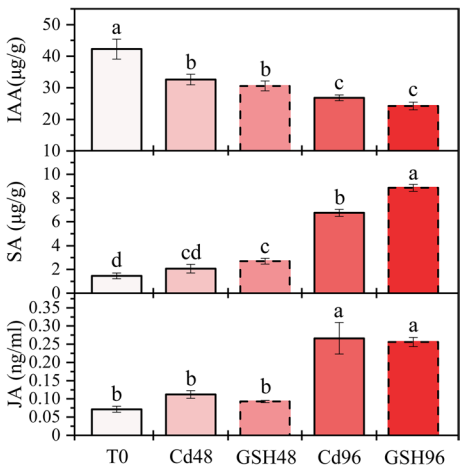


Fig. 2 The effects of different treatments on hormone levels in the potato. Error bars refer to the standard error. Different letters indicate significant differences at the $P < 0.05$ level

in the aboveground and underground parts of the plant). The expression levels of PG0031457 increased was the treatment time increased; the levels in the CD96 and GSH96 groups were significantly higher than those in the T0 group. We also found that the levels of PG0006550 and PG0005794 genes related to IAA were significantly downregulated after treatment; the expression levels of PG0006550 and PG0005794 in the CD96 group were significantly lower than those in the T0 group (both for the aboveground and underground parts of the potato). The expression levels of PG2023696 and PG0005111, genes related to SA, increased after treatment and reached the highest level 96 h after GSH treatment; these levels were significantly higher than seen in the other treatment groups. The expression levels of PG0004367, a gene related to JA, decreased significantly after treatment (Fig. 3).

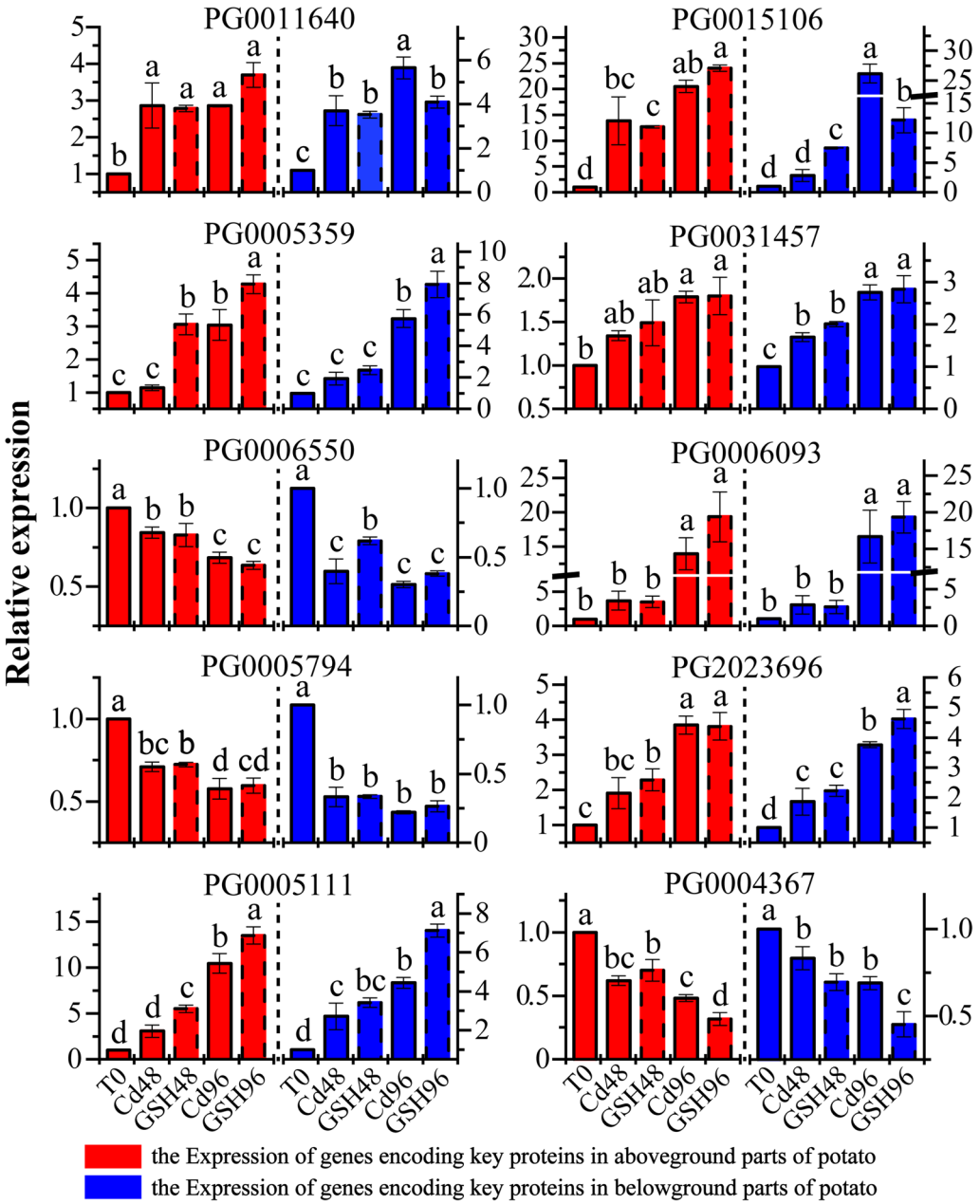


Fig. 3 The expression of genes encoding key proteins in aboveground **A** and belowground **B** parts of the potato

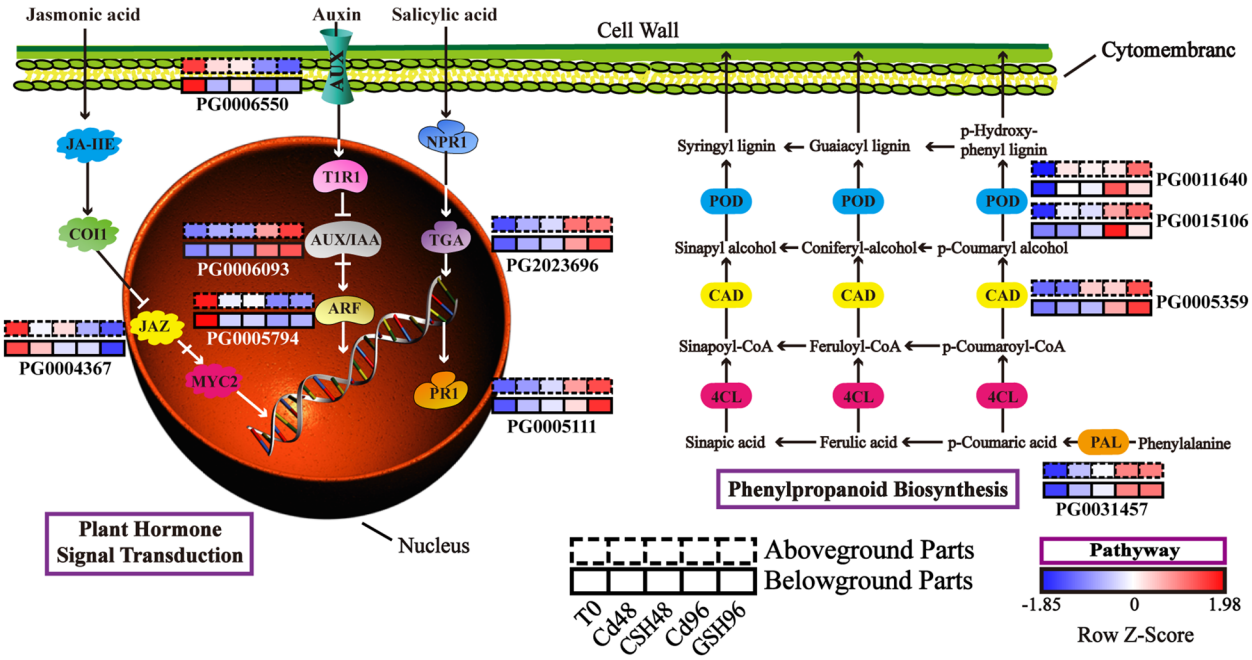


Fig. 4 A model illustrating changes in phenylpropanoid biosynthesis and plant hormone signal transduction pathways that are regulated by exogenous glutathione in potatoes under cadmium stress

Glutathione regulated key pathways in response to Cd stress

Next, we constructed a pathway model to investigate the regulatory effects of GSH on potato plantlets under Cd stress (Fig. 4). When plants were subjected to Cd stress, we found that PAL-related genes in the phenylpropanoid biosynthesis pathway were upregulated, thus resulting in an increase in the levels of PAL. This activated the phenylpropanoid biosynthesis pathway, leading to the upregulated expression of CAD, POD and other related genes. Following the addition of GSH, we found that the expression levels of PAL, CAD and POD-related genes were all upregulated. The activities of PAL, CAD, and POD all increased accordingly, thus accelerating the synthesis of lignin; this led to enhanced resistance to external stress. Under Cd stress and GSH treatment, we found that genes related to the transcription factor TGA, and PR1-related genes were upregulated, thus enhancing salicylic acid signal transduction. Genes related to the auxin carrier were downregulated after treatment, while the transcription inhibitory factor AUX/IAA was upregulated; this inhibited the expression of auxin-responsive transcription factor ARF, thereby inhibiting the transduction of auxin signals. After treatment, genes related to the transcription inhibitor JAZ were downregulated in the JA signal transduction pathway; this activated MYC2 and enhanced the transduction of JA signaling.

Correlation between gene expression and physiological indices

The expression levels of PG0031457, PG0005359, PG0011640 and PG0015106 were positively correlated with lignin levels. The correlation coefficient between gene expression and lignin content in the shoots of the potatoes was 0.75–0.85 while the correlation coefficient between gene expression and lignin content in the underground parts of the potatoes was 0.63–0.9. The expression levels of PG0006550 and PG0005794 were positively correlated with IAA content (the correlation coefficients were 0.9 and 0.89, respectively) and the expression levels of PG0006093 in the shoots of the plant were negatively correlated with IAA levels (the correlation coefficient was − 0.74). The expression levels of PG0006550 and PG0005794 were positively correlated with IAA levels (the correlation coefficients were 0.85 and 0.9, respectively) and the expression levels PG0006093 in the underground parts of the plant were negatively correlated with IAA levels (the correlation coefficient was − 0.76). The correlation coefficients between the expression levels of PG2023696 and PG0005111 in the shoots of the potatoes and the SA levels were 0.83 and 0.94, respectively; those for the underground parts of the potatoes were 0.92 and 0.87, respectively. The expression levels of PG0004367 were negatively correlated with JA levels; the correlation coefficients for the aboveground and underground parts of the potatoes were − 0.65 (Fig. 5).

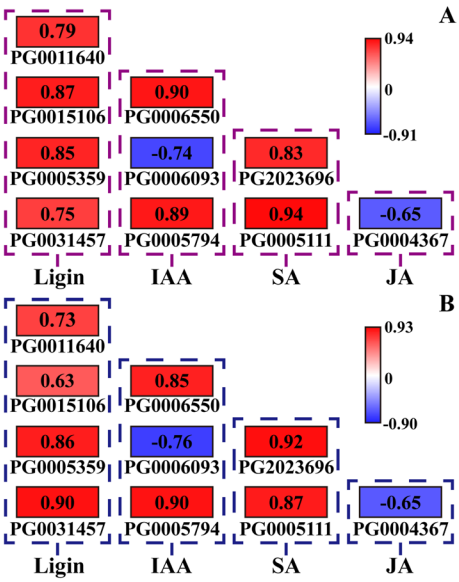


Fig. 5 Correlation between aboveground gene expression and certain indices **A** and the correlation between underground gene expression and certain indices **B**

Discussion

Adverse environment exerts a serious negative impact on plant growth [40]. Reducing the harm caused by abiotic stress to plants by the exogenous application of specific treatments has gradually become a key hotspot for research [41]. Glutathione (GSH), as an important exogenous substance, can improve the antioxidant defense ability of cells and plays an important role in regulating intracellular REDOX signal transduction [42]. GSH may balance the content of ROS in cells and catalyze the transformation of H_2O_2 by exerting influence on the GSH/GSSG ratio and protecting cells from oxidative damage, thereby improving the ability of a plant to resist external stress [43]. In addition, GSH plays an important role in a plant's response to stress by coordinating with other pathways. There is evidence that the accumulation of GSH in *Arabidopsis* under Cd stress is related to enhanced levels of LcGSHS expression and that the SA signaling cascade is involved in this accumulation. Moreover, the overexpression of LcGSHS in transgenic *Arabidopsis* was shown to lead to a greater tolerance to Cd stress than in wild type plants [44]. In addition, GSH participates in the enzymatic reactions of POD and other antioxidant enzymes via the regulatory action of GST so as to achieve the purpose of eliminating multiple reactive oxygen species in plants [45]. In this study, we found that GSH enhanced the self-protection mechanisms of potatoes under Cd stress by exerting influence on the activities of key enzymes, the levels of certain hormones, and the expression levels of genes related to the phenylpropanoid biosynthesis pathway.

Changes in key enzymes in the phenylpropanoid biosynthesis pathway (such as POD, CAD, and PAL) can influence the activity of the entire pathway. PAL acts as the switch for the phenylpropanoid biosynthesis pathway and plays an important role in resisting biotic and abiotic stressors [46, 47]. For example, poplar and cotton have been shown to induce PAL activity in response to mechanical damage and pest infection [48, 49]. Under high temperatures, exogenous SA has been shown to induce the gene expression of PAL and lead to the accumulation of polyphenols in grapes, thus improve resistance to high temperatures [50]. In a previous study, Huang et al. reported that PAL mutants of *Arabidopsis* were more sensitive to ultraviolet light than wild type plants but were more tolerant to drought [51]. In the present study, we found that treatment with exogenous GSH activated the genes encoding PAL and increased the levels of PAL.

Cinnamyl alcohol dehydrogenase (CAD) is an important rate-limiting enzyme in the plant lignin synthesis pathway and plays an important role in a plant's resistance to stress [52]. Studies have shown that the overexpression of a CAD coding gene SaCADCAD in Sedum could improve tolerance to Cd in *Arabidopsis thaliana* [53]. In another study, a gene encoding CAD was found to be highly expressed in sweet potato under low temperature stress [54]. Furthermore, Park et al. reported that CAD activity was significantly induced in rice in response to UV treatment, thus suggesting that CAD plays an important role in the resistance of rice to UV radiation [55], our present results compare with these earlier findings.

Peroxidase (POD) is an important antioxidant enzyme in organisms. When a plant experiences stress from the external environment, it can remove more ROS in vivo. The improvement of antioxidant responses in plants has been shown to be related to the reduction of oxidative damage and improved plant tolerance [56, 57]. A high degree of lignification was observed in soybean roots and wheat under salt stress; these observations were accompanied by high levels of total phenols and the lignin precursors (hydroxyphenyl and syringyl), thus promoting the activities of POD and PPO [58, 59]. Previous studies have demonstrated that the levels of POD increase in plants under Cd and Zn stress [60]. Furthermore, studies involving wheat by Yu et al. showed that POD could alleviate the oxidative damage induced by aluminum stress [61]. In the present study, after treatment, we found that POD-related genes were upregulated and the levels of POD increased.

Lignin is the final product of the phenylpropanoid biosynthesis pathway and the first line of defense for plants against external stress. When plants are subjected to heavy metal stress, the root organs can resist the invasion of heavy metals via cell wall fixation and root efflux. Increased levels of lignin helps to fix the cell wall and represents a detoxification mechanism induced by heavy metal stress over long-term evolution in plants [62]. Previous studies in rice showed that under Cu stress, the expression of genes related to the lignin biosynthesis pathway in the roots were upregulated and that the levels of lignin in the roots increased significantly [63]. Increased expression levels of the CAD gene were observed in maize under drought stress; this promoted the synthesis and accumulation of lignin [64]. Recent studies have shown that the accumulation of lignin may contribute to the improvement of Cd tolerance in plants [65, 66]. In the present study, the activities of PAL, CAD, and POD were found to increase after the treatment of potatoes with GSH. We also found that the expression levels of related genes were also upregulated; this promoted the synthesis of lignin and protected the plants from Cd toxicity.

The plant hormone signal transduction pathway plays an important role in a plant's resistance to stress conditions. The regulatory effect of auxin on the growth and development of plants occurs mainly via auxin signal transduction. The core members of the auxin signal transduction mechanism are composed of three protein families which are used as auxin coreceptors: transport inhibitor response1/auxin signaling F-box protein (TIR1/AFB), auxin/indole-3-acetic acid transcriptional repressor (Aux/IAA) and auxin response factor (ARF) [67]. As the main auxin input vector, AUX/LAX (auxin1/like Aux1) is involved in the regulation of auxin-mediated plant development. For example, AUX1 and LAX3 play an important role in the development of the lateral roots in rice [68, 69]. Previous studies involving rice have shown that the induction of auxin production activates the expression of expandins, thus leading to relaxation of the cell wall; this renders plants more vulnerable to the external environment [70]. For example, the application of exogenous auxin can promote the occurrence of diseases caused by *Agrobacterium tumefaciens*, *Pseudomonas savastanoi* and Pst DC3000 [71–73]. Our present results demonstrate that auxin participates in the weakening of the defense response in plants and that blocking the auxin response helps to increase plant resistance. In this study, the addition of GSH downregulated the expression of AUX-related genes, activated the transcription inhibitor Aux/IAA, upregulated the expression of related genes, and inhibited the auxin response factor ARF, thereby inhibiting auxin signal transduction. Similarly, in this study, the addition of GSH caused auxin input vector AUX related genes to be downregulated, transcription suppressor Aux/IAA to be activated, related genes to be upregulated, and auxin response factor ARF to be inhibited, thus inhibiting auxin signal transduction and improving cadmium tolerance of potato.

Salicylic acid (SA) is a small molecule phenolic compound that can activate plant resistance-related metabolism; improve plant resistance or tolerance to drought, salinity, plant diseases and insect pests; and alleviate the damage caused by environmental stress [74]. The signaling system that regulates SA is closely related to plant resistance [75]. SA forms an active monomeric form by regulating the depolymerization of NPR1. The monomer NPR1 has been shown to move to the nucleus and interact with TGA transcription factors to promote the gene expression of PR and initiate the defense response [76]. Under conditions of NaCl stress, spraying grapes with SA was shown to reduce the accumulation of Na^+ and Cl^- in grape leaves and roots, and increase the concentration of K^+ and Ca^{2+}, thus reducing the damage caused by salt accumulation and improving the absorption of beneficial ions [77]. In another study, Metwally et al. reported that SA could alleviate the inhibition of Cd stress on the growth of barley [78]. There is evidence that SA can regulate the accumulation of ROS such as H_2O_2 in plants by regulating the activity of antioxidant enzymes, thus improving the resistance or tolerance of plants to stress [79, 80]. In addition, SA has been shown to stabilize auxin repressor protein AUX/IAA by inhibiting the expression of TIR1/AFB transport inhibitor resistant1)/(auxin signaling F-BOX), thereby inhibiting auxin response and enhancing plant resistance [81]. In the present study, we found that after treatment, the genes encoding TGA (PG2023696) and PR1 (PG0005111) were upregulated, thus promoting the signal transduction of SA. In this study, it was found that genes encoding TGA and PR1 were upregulated after plant treatment, promoting salicylic acid signal transduction, which may help to break the inhibition effect of cadmium stress on potato growth.

Jasmonic acid (JA) plays an important role in a plant's response to abiotic stress. During signal transduction, the JAZ repressor protein, as a negative regulator, affects the transduction of JA signals by interacting with the MYC2 transcription factor [82]. Previous studies showed that JA signaling is involved in the regulation of temperature stress [83]. For example, Hu et al. found that the JA pathway inhibited the transcriptional

activity of ICE1/2 via the JAZ protein and regulated the freezing tolerance of *Arabidopsis thaliana* [84]. In another study, Wang et al. found that increasing the levels of JA in plants could effectively increase resistance to cold stress [85]. JA also plays an important role in plants under osmotic stress. When cotton was subjected to drought stress, researchers found that the leaves synthesized a large amount of JA to improve the roots ability to absorb water [30]. Zhao et al. reported that the alpha-linolenic acid metabolism pathway is involved in a plant's response to salt stress by regulating the levels of JA [86]. JA can also regulate the response of plants to heavy metal stress. Previous studies by Li et al., carried out in wheat, showed that JA significantly increased the transcription of the glutathione S-transferase gene and enhanced the resistance of wheat to copper stress [87]. Li et al. also increased the concentration of JA by altering the transcription levels of some genes encoding JA-responsive proteins in rice, thereby enhancing tolerance to low potassium or potassium deficiency [88]. In the present study, exogenous GSH enhanced JA signal transduction by inhibiting the expression of genes related to the JAZ protein, thus increasing the levels of JA. In our study, exogenous GSH enhanced JA signal transduction by inhibiting the expression of JAZ protein-related genes. At the same time, JA content also significantly increased, and finally enhanced the resistance of potato to cadmium stress.

Conclusions

The phenylpropanoid biosynthesis pathway of potato was activated under cadmium stress, and the levels of PAL, POD, CAD and the expression of related genes were significantly increased, which may be an important strategy for potato to cope with cadmium stress. After exogenous GSH was applied, this process was further strengthened, resulting in a significant increase in lignin accumulation and enhanced potato tolerance to cadmium stress. In addition, potato endogenous hormone levels and hormone signal transduction pathways responded to cadmium stress. Interestingly, exogenous CSH can regulate this process, the level of auxin and its signal transduction are inhibited, and the level of salicylic acid and jasmonic acid and signal transduction are further enhanced. This study explored and obtained a new method based on exogenous GSH that can be used to regulate potato cadmium stress tolerance, which also has important reference value for the in-depth study of potato cadmium stress response mechanism. At the same time, the important pathways and key genes involved in this study can provide new ideas for potato heavy metal tolerance breeding in the future.

Abbreviations

GSH	Glutathione
PAL	Phenylalanine ammonia-lyase
CAD	Cinnamyl alcohol dehydrogenase
POD	Peroxidase
PG	PGSC0003DMG40
SA	Salicylic acid
IAA	Indole-3-acetic acid (auxin)
JA	Jasmonic acid
qRT-PCR	Quantitative real-time polymerase chain reaction

Supplementary Information

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Additional file 1: Table S1. Gene IDs and primer sequences for the genes used for qPCR verification.

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Author contributions

YK: conceptualization, original draft preparation. Yanhong Yao: investigation. YL: formal analysis. MS: data curation. WZ: visualization. RZ: validation. HL: supervision. SQ: funding acquisition, project administration. XY: software, methodology, review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analysed during the current study are available in the NCBI repository, https://www.ncbi.nlm.nih.gov/sra/?term=SRP314907, the accession number is SRP314907. The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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