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Article

Pb Accumulation Related to Plant Hormones and Its Mechanism Based on Genes Expression and Transporter Activities in Hyperaccumulator *Arabis alpina*

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Abstract: Plants endogenous hormones play an important role in resistance to soil lead (Pb) contamination. In order to explore the response of plant hormones to Pb stress, pot experiment was conducted to analysis the hormones contents, related gene expressions and Pb accumulation of *Arabis alpina* under Pb treatment and transporter (CAX(cation exchangers), HMA(heavy metal ATPase) and ABC(ATP-binding cassette transporter)) activities under foliage spraying auxin (IAA). The results showed that contents of total Pb and soluble components Pb (vacuoles) in roots and leaves of *Arabis alpina* increased with increase of Pb treatment concentrations. Compering to 100 mg·kg^{-1} Pb^{2+} treatment, Pb contents in soluble components of roots and leaves under 300 mg·kg^{-1} Pb^{2+} treatment increased by 896.3% and 238.8%, respectively. The contents of endogenous hormones in leaves and roots increased under Pb treatment concentrations. Comparing to control (0 mg·kg^{-1} Pb^{2+} treatment), contents of auxin in roots and leaves under 100 mg·kg^{-1} Pb treatment increased by 176.19% and 585.29%, respectively. Auxin contents in xylem saps under 100 and 300 mg·kg^{-1} Pb treatments increased by 283.14% and 100.30%, respectively. Gene expression related to auxin transport was up-adjusted. The expressions of three gene related to auxin-repressed 12.5 kDa protein and auxin-responsive GH3 family were down-adjusted. Under foliage spraying IAA, Pb contents in leaves increased by 29.81% and Pb content in symplast sap was higher than that with non spraying IAA treatment. The activities of CAX and HMA in roots of *A. alpina* increased by 9.62% and 8.79% with foliage spraying IAA treatment, while activity of ABC decreased by 21.94%. In general, contents of auxin and related genes expressions of *A. alpina* increased under Pb treatment, which enhanced activities of CAX and HMA resulting in Pb translocation through symplast pathway.

Keywords: plant hormones; hyperaccumulator; Pb translocation; IAA

1. Introduction

Soil heavy metal pollution has attracted much attention worldwide. Soil Pb pollution not only affects the seed germination and growth of plants, but also threats the human body through the food chain [1]. Plant endogenous hormones involved in the regulation of plants growth and development could reduce the toxicity of heavy metals to plants and enhance the tolerance and resistance of plants [2–4]. Plants endogenous hormones and synthetic growth regulators play a vital role in plants resistance to heavy metals [5–7]. Resistance of plants *Atractylodes macrocephala* to Pb could be improved by ABA[8]. Exogenous application of abscisic acid (ABA) and cytokinin could promote the growth of plants and improve resistance to heavy metals, reduce Cd contents in shoot and root of wheat [9,10].

Auxin (IAA) is a substance synthesized in plants to regulate plant growth and development, which can promote cell division, elongation, the differentiation and formation of new organs, and has an important influence on plant growth and development, physiology and biochemistry[11,12]. Application for IAA could promote the absorption of Pb in *Zea Mays*, *Picris divaricata*, *Helianthus annuus* and *Sedum alfredii* [13–15], and enhance the growth and Cd accumulation of *Solanum nigrum*

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[16]. Pb could be transported into root cells through membranes transporters with energy-dependence, and transfer from surface to xylem trough apoplast and symplast pathways and from root to shoot through xylem [17]. Inner flow rate in root and translocation rate from root to shoot of Cd^{2+} in *Malus hupehensis* seedling decreased with foliage spraying ABA[18]. The transporters related to symplast pathway in Pb hyperaccumulators mainly include CAX (cation exchangers), HMA (heavy metal ATPase) and ABC (ATP-binding cassette transporter). CAXs are kind of transporters of divalent ion coordinating the redistribution of calcium ions (Ca^{2+}) and other cations dependent on the energy generated by the proton gradient [19]. HMAs are one of proton pumps that use the energy released by intracellular ATP to reversely transport protons out of the cell across the plasma membrane. ABC is widely located in plasma membrane involved in the transport of chelates PCs-heavy metal into vacuole of cell to detoxification of heavy metal and regulation of auxin dynamic balance [20,21]. ABC participated in regulating auxin transport and distribution in *Malus domesica* and *Passiflora edulis*[20,22]. Under Cu stress, the expression of auxin reporter gene *DR5: GUS* in cotyledons of *Arabidopsis thaliana* changed[23,24]. Exogenous application of IAA may increase the expression of auxin synthesis related genes in plants [25]. Heavy metals may affect the expression of auxin metabolism related genes, such as *GH3* gene family. *GH3* gene family is regulated on Golgi apparatus, combining IAA with amino acids to reduce the activities of IAA[26,27]. Under heavy metal stress, plants can convert free IAA into bound IAA, avoiding excessive IAA degradation[25].

Arabis alpina Var. parviflora Franch belongs to the Arabis genus of Cruciferous family,which is one of hyperaccumulators to Pb. *A. alpina.* was used as the experimental material to investigate the responses of plant endogenous hormones to Pb and its regulation mechanism. The aims are to understand: (1) responses of plant hormones and related gene expression to Pb in *A. alpina* under Pb stress; (2) responses of the activities of heavy metal transporters to foliage spraying IAA under Pb stress. It is important to further explore the plant hormones mechanism of hyperaccumulating Pb of *A. alpina*.

2. Results

2.1. Effect of Pb on Contents of Pb and Endogenous Hormones in Arabis alpina

Contents of total Pb and Pb in subcellular distribution in roots and leaves of *A. alpina* increased with increase in Pb treatment concentrations (Table 1).

Among Pb contents in subcellular distribution, the Pb content in cell wall of roots and leaves was the highest and kept stable under 100 and 300 $mg \cdot kg^{-1}$ Pb^{2+} treatments. Compering to 100 $mg \cdot kg^{-1}$ Pb^{2+} treatment, Pb contents in organelle components of leaves under 300 $mg \cdot kg^{-1}$ Pb^{2+} treatment increased by 115.0%. Pb contents in soluble components (vacuoles) of roots and leaves increased with increase in Pb treatment concentrations. Compering to 100 $mg \cdot kg^{-1}$ Pb^{2+} treatment, Pb contents in soluble components of roots and leaves under 300 $mg \cdot kg^{-1}$ Pb^{2+} treatment increased by 896.3% and 238.8%, respectively. The BCF under 100 and 300 $mg \cdot kg^{-1}$ Pb^{2+} was 2.48 and 2.05, while TF 0.74 and 1.02, respectively.

Table 1. The contents of Pb in subcellular distribution ($mg \cdot kg^{-1}$ FW) and total Pb ($mg \cdot kg^{-1}$ DW) of *Arabis alpina.*

Parts	Pb^{2+} treatment concentration ($mg \cdot kg^{-1}$)	Pb content($mg \cdot kg^{-1}$)			
		F1-Pb	F2-Pb	F3-Pb	Total
Leaves	0	5.38±1.41b	4.42±1.39b	3.39±1.43b	17.55±7.17c
	100	44.77±7.27a	6.07±0.12b	4.43±1.41b	248.22±29.59b
	300	42.28±13.58a	13.02±1.26a	15.01±4.65a	614.32±64.68a
Roots	0	3.62±0.98b	3.09±0.25b	1.53±0.56b	22.23±1.22c
	100	67.52±13.98a	5.78±1.55a	2.15±0.91b	337.86±95.59b
	300	66.10±24.80a	5.41±1.06a	21.42±0.58a	600.09±33.09a

Note: F1:Cell wall components. F2:Organelle components (nucleus, chloroplast and mitochondria). F3:Soluble components (vacuoles). The data was expresses as mean±standard deviation. The different lowercase letters in the same plant part indicate significant difference at *P*<0.05 level (n=3). The same as below.

The contents of endogenous hormones in leaves and roots increased under Pb treatment concentrations. The contents of auxin, abscisic acid and cytokinin in leaves and roots with 100 and 300 mg·kg^{-1} Pb^{2+} treatments were significantly higher than those with control (0 mg/kg Pb^{2+} treatment). Comparing to control, contents of auxin in roots and leaves under 100 mg·kg^{-1} Pb treatment increased by 176.19% and 585.29%, respectively. Contents of abscisic acid in roots and leaves under 100 mg·kg^{-1} Pb treatment increased by 96.91% and 44.83%, respectively. Gibberellin contents in leaves treated 300 mg·kg^{-1} Pb^{2+} were significantly higher than that with control (Table 2).

Table 2. Effect of Pb on endogenous hormones contents (ng·g^{-1}) in leaves and roots of *Arabis alpina.*

Parts	Pb treatment concentration (mg·kg^{-1})	Auxin	Gibberellin	Abscisic acid	Cytokinin
Leaves	0	82.25±38.74c	2.04±0.43b	3.86±0.34b	183.31±14.38b
	100	563.68±96.60a	3.40±0.74a	5.59±1.18a	340.72±63.48a
	300	331.08±13.10b	4.04±0.52a	3.34±0.82b	347.16±61.24a
Roots	0	237.42±17.78b	2.31±0.62b	3.12±0.35b	238.79±32.83b
	100	655.73±278.92a	3.27±0.45b	6.14±2.36a	366.17±21.51a
	300	413.70±46.53a	3.77±0.83a	3.42±0.56b	421.63±33.02a

Auxin contents in xylem saps under 100 and 300 mg·kg^{-1} Pb treatments were 0.89±0.15 ng·L^{-1} and 0.47±0.07 ng·L^{-1}, respectively, which increased by 283.14% and 100.30%, compared to control (0.23±0.01 ng·L^{-1}).

2.2. Effect of Pb on Gene Expression Related to Plant Hormones Dased on RNA-seq analysis

Compared with CK (0 mg·kg^{-1} Pb^{2+}), there were four different expression genes related to plant hormones in roots of *A. alpina* under 100 mg·kg^{-1} Pb^{2+} treatment. Gene expression of Unigene0116550 (*AaGDCST*) related to auxin transport was up-adjusted, which was homologous to *Coccomyxa subellipsoidea* C-169 (Log2(fc)=5.82, *FDR*<0.01). Gene expressions of Unigene0020496 and Unigene0086759 in root, which were related to auxin-repressed 12.5 kDa protein (Log2(fc)=-3.89, *FDR*<0.05) and auxin-responsive GH3 family protein (Log2(fc)=-1.14, *FDR*<0.01) homologous to *Dorcoceras hygrometricum* and *Arabidopsis thaliana*, respectively, were down-adjusted . Gene expressions of Unigene0020496 in leaf homologous to *Dorcoceras hygrometricum* were down-adjusted (Log2(fc)=-3.13, *FDR*<0.01)(Figure 1). Compared with CK (0 mg·kg^{-1} Pb^{2+}), there was one different expression gene Unigene0020496 down-adjusted related to auxin-repressed 12.5 kDa protein in leaves of *A. alpina* under 100 mg·kg^{-1} Pb^{2+} treatment.

Gene expression of Unigene0004954 related to cytokinin hydroxylase-like in roots of *A. alpina* under 100 mg·kg^{-1} Pb^{2+} treatment was down-adjusted, which was homologous to *Brassica napus* (Log2(fc)=-3.81, *FDR*<0.01).

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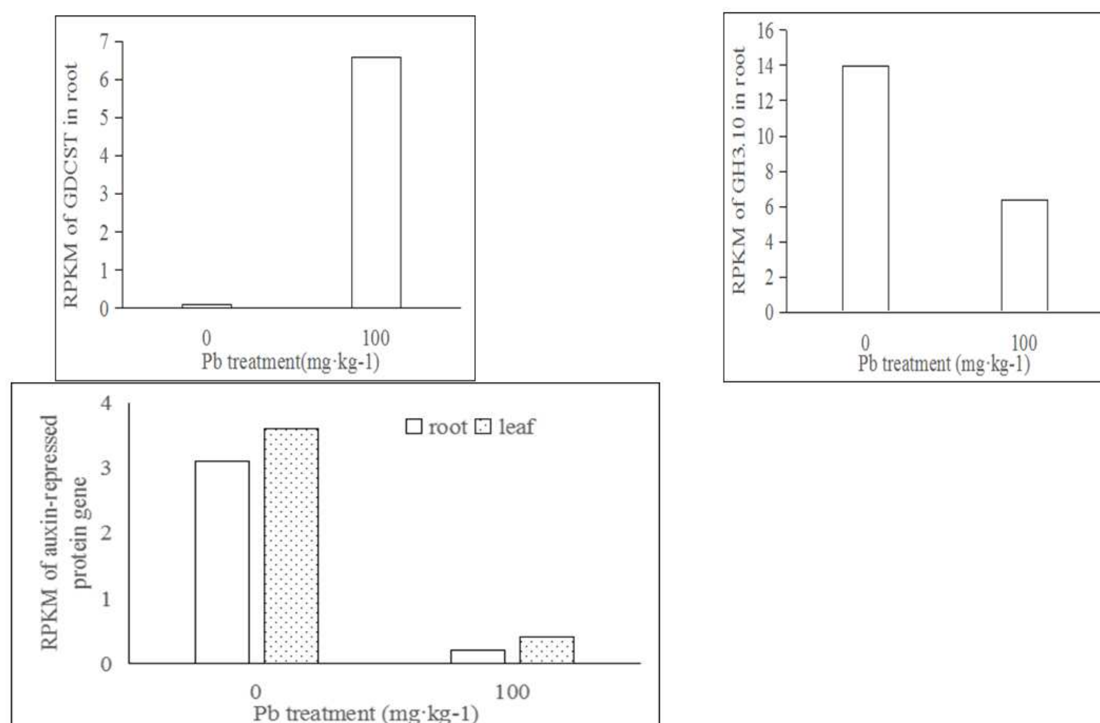

Figure 1. Different expression genes related to plant hormones in roots and leaves of *A. alpina* under 100 mg·kg^{-1} Pb^{2+} treatment.

2.3. Effect of foliage spraying IAA on Pb contents and activities of transporters in Arabis alpina

Pb contents in leaves under foliage spraying IAA treatment increased by 29.81% comparing to non-spraying IAA treatment (Table 3). Pb contents in symplast sap under foliage spraying IAA treatment was 4.64 mg/kg, which was higher than that with non spraying IAA treatment. Pb contents in apoplast, xylem and phloem saps under foliage spraying IAA treatment did not significantly change comparing with non spraying IAA treatment (Table 4).

Table 3. Pb contents of leaves, apoplast, symplast, xylem and phloem saps of *A. alpina* under Pb stress with foliage spraying IAA.

Treatments	Leaves (mg·kg^{-1})	Apoplast sap(mg·kg^{-1})	Symplast sap(mg·kg^{-1})	Xylem sap(μg·L^{-1})	Phloem sap(mg·L^{-1})
Pb	30.36±1.64b	0.11±0.01a	0.19±0.06b	6.94±2.10a	0.21±0.04a
Pb+IAA	39.41±1.20a	0.10±0.01a	4.64±0.21a	7.29±1.99a	0.16±0.03a

Under Pb treatment, the activities of CAX and HMA in roots of *A. alpina* increased by 9.62% and 8.79% with foliage spraying IAA, while activity of ABC decreased by 21.94%. Under Pb treatment, the activities of CAX and HMA in leaves of *A. alpina* decreased by 13.04% and 7.19% with foliage spraying.

Table 4. Activities of CAX, HMA and ABC in leaves and roots of *A. alpina* under Pb stress with foliage spraying IAA.

Parts	Treatments	CAX(U·mL^{-1})	HMA(ng·mL^{-1})	ABC(U·mL^{-1})
Leaves	Pb	888.02±24.14a	501.75±0.48a	1797.23±53.13a
	Pb+IAA	764.87±16.85b	464.04±18.57b	1756.26±79.18a
Roots	Pb	862.05±97.89b	478.36±7.58b	1846.90±26.66a
	Pb+IAA	961.31±23.13a	521.45±8.90a	1420.23±104.61b

3. Discussion

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Under Pb stress, the contents of Pb in vacuole increased and transported from root to shoot, which suggested the strong detoxication of *A. alpina* to Pb. The reason should be related to Pb transportation ability and signaling conductive. Plant hormone contents in *A. alpina* leaves and roots significant were increased under Pb treatment. In order to response to environmental stress, the contents of endogenous hormones in plants increased, which can induce plant resistance and maintain the growth of plants, enhance nutrients transportation and participate in heavy metal stress signaling conductive in plants [16,28–31]. Contents of auxin and ABA in leaves and roots of *A. alpina* increased under Pb treatment to maintain the growth of *A. alpina* and reduced the toxicity of Pb. Auxin join the enhancement of plant tolerance to heavy metals [32] and decrease the toxicity of heavy metals, increase biomass and promote the Pb absorption of *Zea mays* and *Picris dirvaricata* [13,15] found that contents of ascorbic acid, catalase and ascorbate peroxidase activities in *Kandelia candel* leaves increased under the application of exogenous methyl jasmonic acid, which could enhance the resistance of plants to Cd stress[33]. The expression of genes related to auxin synthesis can be stimulated under low concentration of Cu to increase the IAA content, such as amino synthase gene *AUR3*, anthranilic acid synthase gene (*ASA* and *ASB*) and tryptophan synthesis related genes (*TSA1* and *TSB1*) [25]. Hyperaccumulator or tolerant plants could synthesize more IAA than ordinary plants under heavy metal stress, which maybe due to mechanism of rapidly self-regulate endogenous auxin levels [34]. The active auxin level, which is suitable for plant growth and conducive to heavy metal absorption, can be rapidly activated under the heavy metal stress.

One of important physiological responses of plants to adversity environment is an increase in ABA content [35](Liu and Zhang, 2017). The contents of ABA and gibberellin GA3 in the seedling leaves of *Cucumis sativus* increased with the increase of Pb treatments concentration. IAA and cytokinin promote the elongation and division of plant cells, respectively. The contents of IAA and cytokinin increased, which promoted the growth of *Cucumis sativus* seedling leaves under 100mg·L^{-1} Pb treatment. The role of gibberellin in promoting plant growth, breaking dormancy, improving tolerance has been paid more and more attention [36,37]. The content of gibberellin in root increased gradually with the increase of Pb treatments concentration. There is a certain relationship between the resistance of *A. alpina* to Pb and gibberellin. The response of plant hormones to environmental stress may also be related to the balance between different hormones [30]. In terms of endogenous hormone balance, it is possible that *A. alpina* can maintain higher levels of auxin IAA under Pb stress, which is conducive to maintaining the growth of plants. The possible reason is that as one of Pb hyperaccumulator, *A. alpina* has a strong detoxification ability to Pb, which can isolate or detoxify Pb and even stimulate the growth of plants. The reason may be that compared with the increase of IAA, the increase of CTK in *A. alpina* is less, slowing down the rate of cell division, which may lead to a decrease of plant growth rate and the increase of plant leaves or roots density[28].

Plant hormones may be the initiating factors of resistance gene expression. The balance of plant hormones under environmental stress leads to changes in metabolic pathways, including affecting the expression of genes related to auxin metabolism [38,39]. The genes expression of plant hormone are related to the difference of Pb treatment intensity and plant tissue. Auxin response proteins are classified into three groups: auxin/indole acetic acid binding protein(TIR1 and ABP1), AUX/IAA repressed protein (ARP) and auxin response factor (ARF) [40–43]. Auxin-binding protein ABP1 located in cytomembrane. The surface receptor of cytomembrane recognizes the auxin signal and binds with auxin molecules to conduct the auxin into the cell and finally into the nucleus through the endoplasmic reticulum system. In the nucleus, the TIR1 receptor recognizes auxin signals and binds to auxin molecules to activate the auxin response factor (ARF) to regulate the expression of auxin response genes. Auxin response genes mainly include those activated by auxin signal (*GH3* (Gretchen Hagen 3), *SAUR*(small auxin up RNA) and *AUX/IAA*(auxin/indole-acetic acid-inducible)) and those inhibited by auxin (*ARP* and *DRM*(dormancy related protein gene))[44–46]. The overexpression of *MdIAA24* was able to enhance root tolerance to Cd of apple [47]. There are auxin-related gene expressions differences, which are down or up regulated. Under Pb treatment, the expression of Unigene0116550(*AaGDCST*) in root of *A. alpina* was up-adjusted, which was related to auxin transporter. The auxin transporter family located in cytomembrane includes: AUX1/LAX family,

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auxin output vector PIN family and ATP-binding cassette B/P-glycoprotein(ABCB) family[48–50]. The Unigene0116550 gene (*AaGDCST*) expression of auxin transporter was up-adjusted, which indicates that cell membrane receptors with foliage spraying IAA should be stimulated to recognize auxin, transmit auxin signals into cells, and induce increase of the activity of transporters on the cell membrane. Auxin regulates cytomembrane proton pump HMA to activate extracellular acidification, causes plasma membrane ion channel opening or activate plasma membrane ion movement, and promotes the migration of Pb symplast pathway.

The expression of *ARP* gene was up-regulated by biological and abiotic stresses. Overexpression of *OsARP1* gene enhanced resistance of rice to *Magnaporthe oryzae*[51]. The up-regulated expression of *ARP* gene in pepper was affected by abiotic stress [52]. The *ARP* gene is down-regulated by auxin signal. The expression of Unigene0020496 (auxin-repressed 12.5 kDa protein, *AaARP*) in root and leaves were down-adjusted, which should be due to the expression of *AaARP* gene in roots of *A. alpina* was inhibited by leaf auxin application.

In order to maintain the homeostasis of IAA under heavy metal stress, plants quickly transform IAA into a bound state. The function of the IAA binding state is to transport and store excess IAA, so that it is not decomposed. The IAA binding state can be hydrolysis and released when necessary to maintain the normal physiological function of the plant. GH3 protein can promote the binding of various hormones to amino acids and regulate the homeostasis of different hormones in the body. GH3 protein can improve plant resistance by activating salicylic acid (SA), jasmonic acid (JA) and ethylene signaling pathways[53]. The *MdGH3* gene (*MdGH3-5, MdGH3-6, MdGH3-7and MdGH3-8*) of apple was significantly up-regulated under different plant hormones and biological/abiotic stresses, especially drought, low temperature and salt treatment[34]. The expression of Unigene0086759 (auxin-responsive GH3 family, *AaGH3.10*) in root was down-adjusted. That indicates that plants do not need to convert free IAA into bound IAA, but use IAA to activate the activities of heavy metal transporters and migrate heavy metals to the aboveground part with foliage spraying IAA under Pb stress.

The difference of cytokinin related genes is only shown in the root of *A.alpina*. Application of cytokinin can increase biomass of *Helianthus annuus* in the leaf under Pb treatment [54]. Exogenous application of cytokinin (6-BA) increased the aboveground biomass and Cd content, alleviated the toxicity of Cd leaves of *Sedum alfredii* [55]. Cytokinin can improve the activity of plant antioxidant enzymes, alleviate the production of reactive oxygen species free radical, regulate the expression of chloroplast protein genes, and increase protein content [56,57].

The absorption, transport and accumulation of heavy metals in plants include some steps, for example, absorbed by the root system through apoplast and symplast, transported to the phloem through the xylem, and redistributed to the stem, leaf and grain [58]. The relationship between IAA content and Pb content in xylem was inconsistent, which suggested that the xylem transport of Pb was not directly related to auxin content. With foliar spraying of IAA, the migration of Pb symplast pathway increased. The mechanism of IAA is to stimulate the perception of hormone molecular receptors on the cell membrane, thus activating signal transduction pathways and up-regulating related transduction genes. Numerous plant proteins are involved in the transport and uptake of heavy metals, including CAX, HMA and ABC [27,55,59,60]. The activities of HMA and CAX in root of *A. alpina* increased with foliage spraying IAA. The reason might be that auxin regulates HMA to activate extracellular acidification, causing plasma membrane ion channel opening or activating plasma membrane ion movement[16,61]. The CAXs depended on HMA to apply energy to transfer heavy metals into vacuoles or excreted to outside of cell [47,62]. Overexpression of *MdIAA24* decreased the expression of Cd absorption related genes *MdCAX2* in apple, resulting in reduced Cd accumulation and enhancing Cd resistance[47]. The cell membrane was sensitive to auxin signal transduction, and showed an increase in Pb content in the symplast pathway, but no change in Pb content in other pathways. The Pb content in apoplast, xylem and phloem remained unchanged, which was not regulated by IAA on transporters [63,64](Fang and Shen, 2003; Zhou et al., 2007). ATP-binding cassette B (ABC) is one of the auxin transporter family located in cytomembrane and one of Pb transporter located in tonoplast. The activity of ABC decreased under IAA application, which

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resulted in Pb transportation with symplast pathway rather than stored in vacuole. Pb in xylem transfer from root to shoot accompanying with water and nutrient transportation. Hadi et al. (2010) reported that the biomass of maize under the application of IAA increase and thus the accumulation of Pb increase [13]. IAA can promote the transfer of nutrient elements and Cd from roots to the fast-growing parts in the shoots of *Sedum sedum* by regulating the relationship of source-pool[9].

4. Materials and Methods

4.1. Materials

The seeds of *A.alpina* were collected from the Chihong lead-zinc mine district in Huize county, Yunnan Province, China, which located at 103°03′-103°55′E, 25°48′-26°28′N, and 2 494 m above sea level. The basic physical and chemical properties of flue-cured tobacco substrate used in pot experiments were 4.49 pH, organic matter 431.75 $g·kg^{-1}$, available phosphorus content 81.45 $mg·kg^{-1}$, available nitrogen content 437.50 $mg·kg^{-1}$, available potassium content 6220.17 $mg·kg^{-1}$ and Pb content 24.6 $mg·kg^{-1}$.

4.2. Experimental Design

Some 2 kg of flue-cured tobacco substrate were weighed and put into a pot with a length of 33 cm,width 24.5 cm and a height of 13.5 cm. Some 40 seeds of *A.alpina* evenly sown in each pot and sprayed 20 mL of water every three days. After the seed sprouting, thinning was done to 30 plants seedling per pot. Tap water was sprayed every 5 days and the substrate kept loose and moist for three months.

Then Pb^{2+} (0, 100, 300 $mg·kg^{-1}$) prepared with $Pb(CH_3COO)_2$ were added to pots with flue-cured tobacco substrate, and each treatment with three replicates. After 15 days, six seedling of *A.alpina* was replanted. After 20 days, the plants were collected. Pb contents in apoplast and symplast saps in roots, xylem and phloem saps were analyzed. Pb subcellular distribution contents and plant hormones (auxin, abscisic acid, cytokinin and gibberellin) contents in roots and shoots were determined. Some roots and leaves samples of *A.alpina* with 0 and 100 $mg·kg^{-1}$ Pb^{2+} treatments were putted in liquid nitrogen tank rapidly for RNA-seq analysis.

100 $mg·kg^{-1}$ Pb^{2+} prepared with $Pb(CH_3COO)_2$ were added to pots with flue-cured tobacco substrate, and each treatment with three replicates. After 15 days, six seedling of *A.alpina* was replanted. 20 $mg·L^{-1}$ IAA was foliage spraying at 18:00 every two days on the two surface of leaf till forming water drops. After 20 days, Pb contents in apoplast and symplast saps in roots, xylem and phloem saps were analyzed. Pb contents and activities of in CAX, HMA and ABC in roots and leaves were determined.

4.3. Determination of Indicators

Contents of plant hormones： auxin, gibberellin, abscisic acid and cytokinin contents were analyzed with the ELISA assay kit microplate analyzer (Rayto RT-6100). Contents of plant hormones were determined with liquid chromatograph HPLC-MS/MS (LCMS-8040, Shimadzu, Japan). The determination condition was 0.3 $mL·min^{-1}$ flow rate, ODS volume(1.6μm, 75×2mm)(shim-packXR-ODSIII). Solution A(0.05% methanoic acid 5 mM ammonium formate) and solution B(methyl alcohol) were the mobile phase composition to separate based on gradient method.

Pb contents in plant: some 2.000 g of a homogenized plant sample were weighed and placed in 150 mL a triangular flask. 24 mL mixed acid (HNO_3:$HClO_4$=5:1) were added and covered for 12h. Then the mixed solution heated in sand bath at 50°C till the digestive fluid slightly yellowish. After cooling, the volume was adjusted to 50 mL with ultrapure water. Pb content was measured with inductively coupled plasma emission spectrometry (Atomic Absorption Spectrophotometer, PRESEE TAS-990, Beijing).

Subcellular Pb contents: some 0.5000 g of a fresh sample of *A.alpina* roots and leaves were weighed, and putted in an EP tube. The pre-cooled extract (weight: volume 1:50) (250mmol·L^{-1}

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sucrose, 50 mmol·L^{-1} Tris-HCl buffer solution (pH=7.5) and 1mmol/L dithioerythritol) was added and ground into a homogenate in an ice bath, then centrifuged for 15 min at 4°C, 3000 r/min. The residue part mainly contained the cell wall. The supernatant was transferred to a new centrifuge tube and centrifuged for 30 min at 4°C and 10 000 r·min^{-1}. The supernatant from the second centrifugation was the soluble component, and the residue was the organelle component of *A.alpina*.

Apoplast and symplast saps extract and Pb content: Some 0.25 g fresh roots of *A. alpina* was weighted and putted into EP tube. 50 mmol·L^{-1} MES-Tris buffer solution with pH 6.5 was added and reduced pressure 20 min with 0.5 kPa twice. The root samples were taken out and the water were suck dry. The spoplast saps were collected with centrifuged for 15 min at 4°C, 1500 r·min^{-1}. 4°C. Then, the roots were frozen 3 days at -20°C. The symplast sap I was collected with centrifuged for 15 min at 4°C, 3000 r·min^{-1}. 4°C. The roots were ground for 3 min with 1 mL ethyl alcohol added and then centrifuged for 15 min at 4°C, 3000 r·min^{-1}. The residue part were ground for 3 min with 1 mL ethyl alcohol added and then centrifuged for 15 min at 4°C, 3000 r·$min^{-1.}$ The two supernatants were symplast sap II. The symplast saps were the mixture of symplast saps I and symplast saps II(Wu et al., 2015). The apoplast and symplast saps were constant volume to 50 mL with deionized water. Pb content was measured with inductively coupled plasma emission spectrometry (Atomic Absorption Spectrophotometer, PRESEE TAS-990, Beijing).

Xylem and phloem saps extract and Pb content: The plant was moved to the shade. The stem was cut at the 4 cm above the base of root with blade soaked with acetone.The cut of the root side was dried with absorbent cotton dipping in with deionized water. The first drop was discarded with absorbent cotton, then sucked up the spilling saps with 200 μL Eppendorf pipette and transfer to 10 mL Eppendorf tube for 12h. The solution was centrifuged for 10min at 4°C, 3000 r·min^{-1}. The supernatant was xylem sap stored 1.5 mL Eppendorf tube at -70°C(Wu et al., 2015).

The shoots part after cutting was dried with absorbent cotton and inserted into small plastic bottle with 15 mL 25 mmol·L^{-1} EDTA-Na_2. The bottle was put in incubator with closed shading at relative humidity 95% and 20°C for 24 h to collect the phloem sap(Ge et al., 2008).

The xylem and phloem saps were constant volume to 50 mL with deionized water. Pb content was measured with inductively coupled plasma emission spectrometry (Atomic Absorption Spectrophotometer, PRESEE TAS-990, Beijing).

Enzyme extract and activities of CAX, HMA and ABC: Preparation of enzyme extract: some 0.100 g of tissue were weigh, and cut into a mortar. 0.9 mL 0.01mol·L^{-1} of pH=7.2-7.4 PBS buffer was added, and grind into a homogenate in an ice bath. The mixture was centrifuged at 4°C, 5000r·min^{-1} for 15 min with high-speed refrigerated centrifuge (HC-3018R high-speed refrigerated centrifuge, Anhui Zhongke Zhongjia Scientific Instrument Yuanxi Company). The supernatant was taken for determination.

The standard curve of CAX activities: some 150μL of CAX standard dilution (480 ng·mL^{-1}) were pipetted into each tube to produce a 2-fold dilution series (including 240ng·mL^{-1}, 120ng·mL^{-1}, 60ng·mL^{-1}, 30ng·mL^{-1}, 15nng·mL^{-1}). The standard solutions were measured the optical density (OD) at 450 nm with a microplate reader (DNM-9602, Beijing) within 15 minutes.

The standard curve of HMA and ABC activities: some 150μL of HMA or ABC standard dilution (800 U·mL^{-1}) were pipetted into each tube to produce a 2-fold dilution series (including 400 U·mL^{-1}, 200 U·mL^{-1}, 100 U·mL^{-1}, 50 U·mL^{-1}, 25 U·mL^{-1}). The standard solutions were measured the optical density (OD) at 450 nm with a microplate reader (DNM-9602, Beijing) within 15 minutes.

Assay procedure: Some 50μL of standard or sample supernatant were added to the microtiter plate and mixed well, then incubated at 37°C for 30 minutes. 50 μL of biotin antibody IgG was added to the mixture solution, and incubated at 37°C for 30 minutes. 50 μL of streptavidin-HRP was added, and incubated with gentle mixing at 37°C for 15 minutes. Chromogen Solution A 50 μL and Chromogen Solution B 50 μL were added and incubated at 37°C for 15 minutes, then 50 μL stop solution was added to stop the reaction. CAX, HMA and ABC activities were measured the optical density (OD) at 450 nm with a microplate reader (DNM-9602, Beijing) within 15 minutes and calculated with a standard curve of CAX, HMA and ABC. The unit of CAX activities was ng/mL and the unit of HMA and ABC activities was U·mL^{-1}.

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RNA-seq analysis: The total RNA of each sample was extracted and tested for its concentration, purity and integrity. After the sample was qualified, the mRNA was enriched with Oligo (dT) magnetic beads, and chromosome fragmentation buffer (fragmentation buffer) was added to the obtained mRNA. buffer) to make the fragments into short fragments, and then the fragmented mRNA was used as a template to synthesize the first strand of cDNA with random hexamers. Buffer, dNTPs, RNase H and DNA polymerase (polymerase) I was added to synthesize the second strand of cDNA, then purifed by QiaQuick PCR kit and EB buffer added to elution, end repair, base A added, sequencing adapter added, and then to recover the target size fragment by agarose gel electrophoresis, and PCR amplification was carried out in order to complete the entire library preparation work and perform library quality inspection on the prepared library.

Illumina HiSeqTM 2000 was used for sequencing after qualified. The comparison software Bowtie was used to compare the reads obtained by sequencing of each sample with the Unigene library. The expression levels were estimated based on the comparison information from RSEM, andthe RPKM (Reads Per Kilobase of transcript per Million mapped reads) value was calculate to reflect the expression abundance of Unigenes. EBSeq was selected for differential expression analysis, and the FDR method was used to analyze the expression of Unigene. In the differential gene screening, FDR<0.05 and |log2(fc)|>1 were selected as the standard and defined as differential Unigene. fc is the ratio of the average RPKM of the comparative sample. TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was used to predict the topology of the transmembrane helix structure of the transporter, and obtain the corresponding gene base sequence and the amino acid sequence of the transporter.

$$\mathrm{RPKM} = \frac{10^6 C}{NL/10^3} \quad (1)$$

$$\mathrm{fc} = \frac{RPKM(T)}{RPKM(\mathrm{C})} \quad (2)$$

In which, RPKM is the expression level of gene, then C is the number of sequencing reads compared to gene, N is the total number of sequenced fragments compared to the reference gene, and L is the number of bases of gene. RPKM (T) is the expression of related genes under Pb treatment, and RPKM (C) is the expression of related genes under CK.

4.4. Calculation and Statistical Analysis

Data analyzed with Microsoft Excel 2010 and SPSS 18.0 for statistical analysis. The data passed Duncan's multiple comparison test with one-way variance method (ANOVA), and when *P*<0.05, the difference was significant. Microsoft Origin 2021 was used for figure drawing.

5. Conclusion

Pb contents in roots and leaves on *A. alpina* increased with increase of Pb treatment concentrations, especially in vacuole. The contents of plant hormone in leaves and roots increased under Pb treatment. The related gene expression of *AaGDCST* increased resulting in auxin transport and Pb translocation from root to shoot based on RNA-seq analysis. Pb contents in leaves and symplast sap increased under foliage spraying IAA, which were related to activities of CAX and HMA in roots increased and activity of ABC in root decreased. In summary, the Pb translocation and accumulation should be related to IAA content and gene expression of *AaGDCST* as signal transduction for inducing increase in activities of transporter CAX and HMA in root of *A. alpina*. For hyperaccumulation Pb of *A. alpina*, *AaGDCST* has the potential to be utilized as a candidate gene.

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References

1. Zhu, Y.Y.; Dong, Y.W.; Zhu, N.; Jin, H.M. Foliar application of biosynthetic nano-selenium alleviates the toxicity of Cd, Pb, and Hg in *Brassica chinensis* by inhibiting heavy metal adsorption and improving antioxidant system in plant. *Ecotoxicology and Environmental Safety*. **2022**, 240: 113681.
2. Firdaus-e-Bareen; Shafiq, M.; Jamil, S. Role of plant growth regulators and a saprobic fungus in enhancement of metal phytoextraction potential and stress alleviation in pearl millet. J*ournal of Hazardous Materials*. **2012**, 237/238:186-193.
3. Piotrowska-Niczyporuk, A.; Baiquz, A.; Zambrzycka, E.; et al. Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (Chlorophyceae). *Plant Physiology and Biochemistry.* **2012**, 52: 52-65.
4. Abdelgadir, H.A.; Jager, A.K.; Johnson, S.D.; Staden, J.V. Influence of plant growth regulators on flowering, fruiting, seed oil content and oil quality of *Jatropha curcas*. *South African Journal of Botany*. **2020**, 76 (3):440-446.
5. Ahmad, P.; Nabi, G.; Ashraf, M. Cadmium-induced oxidative damage in mustard(*Brassica juncea*(L.)Czern.&Coss. plants can be alleviated by salicylic acid. *South African Journal of Botany*. **2011**, 77(1):36-44.
6. He, J.Y.; Ren, Y.F.; Wang, Y.Y.; Li, Z.J. Root morphological and physiological response of rice seedling with different to tolerance to cadmium stress. *Acta Ecologica Sinica*. **2011**,31(2):0522-0528.
7. Ding, J.H.; Xue, Z.L.; Yang, C.Y. Effect of salicylic acid on membrane lipid peroxidation in rice seedling under copper stress. *HelongjiangAgricultural Science*. **2013**, (1):14-18.
8. Lopez-Orenes, A.; Alba, J.M.; Kant, M.R.; Calderon, A.A.; Ferrer, M.A. OPDA and ABA accumulation in Pb-stressed Zygophyllum fabago can be primed by salicylic acid and coincides with organ-specific differences in accumulation of phenolics. *Plant Physiol Biochem*. **2020**,154:612-621.
9. He, B.; Lu, Q.Y.; Li, Y.Y.; Chen, X.Q.; Wang, X.G.; Gu, M.H. Effects of different growth regulators on cadmium accumulation by *Sedum alfredii* H.. *Journal of Agro-Environmental Science*. **2014**,33(8):1538-1545.
10. Feng, W.J.; Gao, W.; Liu, H.E.; Nie, Z.J.; Qin, S.Y.; Li, C.; Sui, F.Q.; Zhao, P. Effects of plant growth regulator on growth and cadmium uptake and accumulation in wheat seedlings. *Journal of Henan Agricultural University*. **2021**, 55 (6):1036-1044.
11. Ji, P.H.; Jiang, Y.J.; Tang, X.W.; Thanh, H.N.; Tong, Y.A.; Gao, P.C.; Han, W.S. Enhancing of Phytoremediation Efficiency Using Indole-3-Acetic Acid (IAA). *Soil and Sediment Contamination: An International Journal*. **2015**, 24(8):909-916.
12. Li, W.M.; Wang, D.S.; Hu, F.; Li, H.X.; Ma, L.; Xu, L. Exogenous IAA treatment enhances phytoremediation of soil contaminated with phenanthrene by promoting soil enzyme activity and increasing microbial biomass. *Environmental Science and Pollution Research*. **2016**, 23(11):10656-10664.
13. Hadi, F.; Bano, A.; Fuller, M.P. The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.):The role of plant growth regulators(GA3 and IAA) and EDTA alone and in combinations. *Chemosphere.* **2010**, 80(4):457-462.
14. Fässler, E.; Evangelou, M.W.; Robinson, B.H.; et al. Effects of indole-3-acetic acid(IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid(EDDS). *Chemosphere*. **2010**, 80(8):901-907.
15. Du, R.J.; He, E.K.; Tang, Y.T.; Hu, P.J.; Ying, R.R. How Phytohormone IAA and Chelator EDTA affect lead uptake by Zn/Cd hyperaecumulator *Pictis divaricata*. *International Journal of Phytoremediation*. **2011**,13(10):1024-1036.
16. Luo, Y.; Wang, Z.X.; Xiang, Y.Z.; Ren, J.; Luo, G.J.; Liu, F. Effects of Indole-3-acetic Acid and L-glutamic Acid N, N-diacetic Acid on the Growth and Heavy Metal Uptake by *Solanum nigrum* L. in Cd-Pb Contaminated Soil. *Journal of Yunnan Agricultural University (Natural Science)*. **2022**, 37(1):145-151.
17. Uraguchi, S.; & Fujiwara, T. Rice breaks ground for cadmium-free cereals. *Current opinion in plant biology.* **2013**,16(3), 328-334. https://doi.org/10.1016/j.pbi.2013.03.012.
18. Deng, B.; Zhang, W.W.; Yang, H.Q. Abscisic acid decreases cell death in *Malus hupehensis* Rehd. under Cd stress by reducing root Cd^{2+} infux and leaf Transpiration. *Journal of Plant Growth Regulation*. **2022**, 41(2):639-646.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 January 2024 doi:10.20944/preprints202401.1180.v1

19. Liu, M.; Li, Z.R.; Zu, Y.Q. Transport protein CAXs and HMAs related to cadmium absorbing and transferring of plant: A review. *Chinese Agricultural Science Bulletin.* **2020**, 36(30):1-9.
20. Kim, D.Y.; Bovet, L.; Maeshima, M.; Lee, Y. The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. *The plant journal*. **2007**,50(2):207-218.
21. Strader, L.C.; Bartel, B. The Arabidopsis pleiotropicdrug resistance8/abcg36 ATP binding cassette transporter modulates sensitivity to the auxin precursor indole-3-butyric acid. *The Plant Cell.* **2009**, 21(7):1992-2007.
22. Sukumar, P.; Maloney, G.S.; Muday, G.K. Localizes induction of the ATP-binding cassette B19 auxin transporter enhances adventitious root formation in Arabidopsis. *Plant Physiology.* **2013**, 162(3): 1392-1405.
23. Peto, A.; Lehotai, N.; Lozano-Juste, J. Involvement of nitric oxide and auxin in signal transduction of copper-induced morphological responses in Arabidopsis seedlings. *Annals of Botany*. **2011**, 108:449-457.
24. Kolbert, Z.; Pet, A.; Lehotai, N.; Feigl, G.; Erdei, L. Long-term copper(Cu^{2+}) exposure impacts on auxin, nitric oxide(NO) metabolism and morphology of *Arabidopsis thaliana* L. *Plant Growth Regulator*. **2012**, 68:151-159.
25. Zhao, C.R.; Ikka, T.; Sawaki, Y.; et al. Comparative transcriptomic characterization of aluminum, sodium chloride，cadmium and copper rhizotoxicities in *Arabidopsis thaliana*. *BMC Plant Biology,* **2009**, 9,32. https://doi.org/10.1186/1471-2229-9-32
26. Feng, S.; Yue, R.; Tao, S.; et al. Genome-wide identification，expression analysis of auxin-responsive GH3 family genes in maize (*Zea mays* L.) under abiotic stresses. *Journal of Plant Biology*. **2015**, 57: 783-795.
27. Huang, D.; Wang, Q.; Zou, Y.J.; Ma, M.N.; Jing, G.Q.; Ma, F.W.; Li, C. Silencing MdGH3-2/12 in apple reduces cadmium resistance via the regulation of AM colonization. *Chemosphere*. **2021**, 269, 129407.
28. Lin, W.; Zhou, N.N.; Wang, G.; Xiao, L.T.; Zhang, Y.; Li, Z. Effect of Lead Pollution on the content of endogenous hormones in cucumber leaves. *Ecology and Environment*. **2007**, 16(5):1446-1446.
29. He, B.; Chen, X.Q.; Xin, Z.B.; Lu, Q.Y.; Shi, Q.; Gu, M.H.; Wang, Z.L. Effects of four plant growth regulators on growth and cadmium accumulation in rice. *Acta Ecologica Sinica*. **2016**,36(21):6863-6871.
30. Song, J.; Guo, H.C.; Li, J.; Zhang, G.H.; Shi, L.C. Effects of lead stress on growth morphology and endogenous hormone of potato seedlings. *Acta Agricultural Boreali-occidentalis Sinica.* **2019**, 28(11):1830-1835.
31. Zhang, Z.K.; Zhang, C.Y.; Wang, Y.; Ai, Y.M.; Xu, X.Y.; Feng, J.P.; Feng, W.Y.; Song, L.P.; Li, X.P.; Zhou, J.H. Effects of different auxin content on physiological and biochemical characteristics of *Cinnamomum camphora* seedling in Cd polluted soil. *Journal of Northeast Forestry University.* **2023**,51(5):41-48.
32. Sytar, O.; Kumari, P.; Yadav, S.; Brestic, M.; Rastogi, A. Phytohormone priming: regulator for heavy metal stress in plants. *J. Plant Growth Regul.* **2019**, 38 (2), 739-752.
33. Chen, J. Study on the regulatory effect of exogenous methyl jasmonate on heavy metal resistance in mangrove seedlings. *Shanghai: East China Normal University*.**2014**.
34. Zhang, X.M.; Wang, H.J.; Wang, H.B. Advances in biosynthesis and degradation of indoleacetic acid in plants under heavy metal stress. *Chinese Journal of Ecology.* **2017**,36(4) :1097-1105.
35. Liu, Z.Q.; Zhang, C.B. Advance in regulation of heavy metals on nonselective cation channels: A review. *Journal of Agricultural Resources and Environment.* **2017**, 34 (1):1-5.
36. Colebrook, E.H.; Thomas, S.G.; Phillips, A.L.; Hedden, P. The role of gibberellin signalling in plant response to abiotic stress. *Journal of experimental biology*. **2014**,217(1):67-75.
37. Asgher, M.; Khan, MIR.; Anjum, N.A.; Khan, N. Minimising toxicity of cadmium in plants role of plant growth regulators. *Proto plasma*. **2015**,252(2):399-413.
38. Potters, G.; Pasternak, T.P.; Guisez, Y.; Jansen, M.A．Different stresses，similar morphogenic responses: Integrating a plethora of pathways. *Plant, Cell & Environment*. **2009**, 32:158-169．
39. Tognetti, V.B.; Van Aken, O.; Morreel, K. Perturbation in indole-3-butyric acid homeostasis by the UDP-glucosyltransferase UTG74E2 modulates *Arabidopsis* architecture and water stress tolerance. *The Plant Cell.* **2010**, 22:2660-2679.
40. Guilfoyle, T.J.; Ulmasov, T.; Hagen, G. The ARF family of transcription factors and their role in plant hormone-responsive transcription. *Cellular and molecular life sciences.* **1998**,54, 619-627.
41. Jain, M.; Kaur, N.; Garg, R.; Thakur, J.K.; Tyagi, A.K.; Khurana, J.P. Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (*Oryza sativa*). *Funct. Integr. Genom.* **2006**a, 6, 47-59.
42. Jain, M.; Kaur, N.; Tyagi, A.K.; Khurana, J.P. The auxin-responsive GH3 gene family in rice (*Oryza sativa*). *Funct. Integr. Genom.* **2006**b, 6, 36-46.
43. Jain, M.; Tyagi, A.K.; Khurana, J.P. Genome-wide analysis，evolutionary expansion，and expression of early auxin-responsive SAUR gene family in rice (*Oryza sativa*). *Genomics*. **2006**c, 88, 360-371.
44. Gray, W. M.; Kepinski, S.; Rouse, D.; Leyse, O.; Estelle, M. Auxin regulates SCF[TIR_1]-dependent degradation of AUX/IAA proteins. *Nature*. **2001**, 414(6861):271-6.
45. Kumar, R.; Agarwal, P.; Tyagi, A.K.; Sharma, A.K. Genome-wide investigation and expression analysis suggest diverse roles of auxin-responsive GH3 genes during development and response to different stimuli in tomato (*Solanum lycopersicum*). *Molecular Genetics & Genomics*. **2012**, 287(3):221-235.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 January 2024 doi:10.20944/preprints202401.1180.v1

46. Lee, J.; Han, C.T.; Hur, Y. Molecular characterization of the Brassica rapa auxin-repressed, superfamily genes, Br ARP1 and Br DRM1. *Molecular Biology Reports*. **2013**,40(1):197-209.
47. Wang, Q.; Huang, D.; Niu, D.S.; Deng, J.; Ma, F.W.; Liu, C.H. Overexpression of auxin response gene MdIAA24 enhanced cadmium tolerance in apple (*Malus domestica*). *Ecotoxicology and Environmental Safety*. **2021**,225,112734. https://doi.org/10.1016/j.ecoenv.2021.112734.
48. Okada, K.; Ueda, J.; Komaki, M.K.; Bell, C.J.; Shimura, Y. Requirement of auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant Cell*. **1991**,72:523-536.
49. Noh, B.; Murphy, A.S.; Spalding, E.P. Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *Plant Cell*. **2001**, 13:2441-2454.
50. Swarup, K.; Benkova, E.; Swarup, R.; Casimiro, T.; Peret, B.; Yang, Y.; Parry, G.; Nielsen, E.; De Smet, I.; Vanneste, S.; Levesque, M.P.; Carrier, D.; James, N.; Calvo, V.; Ljung, K.; Kramer, E.; Roberts, R.; Graham, N.; Marillonnet, S.; Patel, K.; Jones, J.D.; Taylor, C.G.; Schachtman, D.P.; May, S.; Sandberg, G.; Benfeg, P.; Friml, J.; Kerr, I.; Beeckman, T.; Laplaze, I.; Bennett, M.J. The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology*. **2008**, 10:946-954.
51. Shang, J.; Tao, Y.; Chen, X.; Zou, Y.; Lei, C.; Wang, J.; Li, X.; Zhao, X.; Zhang, M.; Lu, Z.; Xu, J.; Cheng, Z. Identification of a new rice Blast resistance gene, Pid3, by genomewide comparison of paired nucleotide-binding site-Leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics*. **2009**, 182(4):1303-1311.
52. Wang, E.W.; Kim, K.A.; Park, S.C.; Jeong, M.J.; Byun, M.O.; Kwon, H.B. Expression profiles of hot pepper (*Capsicum annuum*) genes under cold stress conditions. *J Biosci*. **2005**, 30(5):657-667.
53. Sun, T.; Chai, T.Y.; Zhang, Y.X. Upstream promoter sequences of Arabidopsis GH3 gene family. *Journal of the Graduate School of the Chinese Academy of Sciences*. **2010**, 27(6):847-852.
54. Tassi, E.; Pouget, J.E.L.; Petruzzelli, G. The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. *Chemosphere*. **2008**,71(1):66-73.
55. He, J.L.; Li, H.; Ma, C.F.; Zhang, Y.L.; Polle, A.; Rennenberg, H.; Cheng, X.Q.; Luo, Z.B. Overexpression of bacterial g-glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. *New Phytol*. **2015**, 205, 240-254.
56. Downes, B.P.; Crowell, D.N. Cytokinin regulates the expression of a soybean β-expansin gene by a post-transcriptional mechanism. *Plant Mol. Biol*. **1998**,37(3):437-444.
57. Zhou, H.W.; Shi, G.X.; Xu, Q.S. Effects of Cd^{2+} polluted water on the activities of antioxidant enzymes and ultrastructure in roots of *Alternanthera philoxeroides*. *Plant Physiology Communication*. **2003**, 9(3):211-214.
58. Uraguchi, S.; Mori, S.; Kuramata, M. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J. Exp. Bot*. **2009**, 60 (9), 2677-2688.
59. Siemianowski, O.; Mills, R.F.; Williams, L.E.; Antosiewicz, D.M. Expression of the P1B-type ATPase AtHMA4 in tobacco modifies Zn and Cd root to shoot partitioning and metal tolerance. *lant biotechnology journal*.**2011**, 9, 64-74.
60. Zhou, J.T.; Wan, H.X.; Qin, S.J.; He, J.L.; Lyu, D.G.; Li, H.F. Net cadmium flux and gene expression in relation to differences in cadmium accumulation and translocation in four apple rootstocks. *Environ. Exp. Bot*. **2016**, 130, 95–105.
61. Yang, Z.B.; Lin, W.W.; Tang, W.X.; Takahashi, K.; Pan, X.; Dai, J.W.; Ren, H.; Zhu, X.Y.; Pan, S.Q.; Zheng, H.Y. TMK based cell surface auxin signaling activates cell wall acidification in Arabidopsis. *Nature*. **2023**, 599: 278-282. https://doi.org/10.1038/s41586-021-03976-4
62. Wu, Q.Y.; Shigaki, T.; Williams, K.A.; Han, J.S.; Kim, C.K.; Hirschi, K.D.; Park, S. Expression of an Arabidopsis Ca^{2+}/H^{+} antiporter CAX1 variant in petunia enhances cadmium tolerance and accumulation. *Plant Physiol*. **2011**, 168 (2): 167-173.
63. Fang, X.R.; Sheng, Q.R. Effects of ABA and IAA on the Behavior of Stomata of Rice Crop Cultivated in Aerobic Soil Condition. *Scientia Agricultural Sinica*. **2003**, 36(12): 1450-1455.
64. Zhou, J.M.; Dang, Z.; Chen, N.C.; Xu, S.G.; Xie, Z.Y. Enhanced phytoextraction of heavy metal contaminated soil by chelating agents and auxin indole-3-acetic acid. *Environmental Science*. **2007**, 28(9): 2085-2088.

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