# ENRICHMENT EFFECT OF PHYTOHORMONES ON ARSENIC UPTAKE AND TOLERANCE MECHANISM OF *LANDOLTIA PUNCTATA*

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**Abstract.** Arsenic (As) contamination of water can pose a significant threat to human health. *Landoltia punctata* is a superior water ecological restoration plant that can absorb As and thus manage the problem of As overload in the aquatic environment. Phytohormones play an important role in regulating plant growth and improving heavy metal resistance. In this study, the enrichment effect of phytohormones on As uptake and tolerance mechanism of *L. punctata* at different concentrations were investigated. The results showed that the application of Indolent-3-acetic (IAA), Abscisic acid (ABA) and Kinetin (KT) were all effective in increasing chlorophyll a (Ca) and carotenoid (Cc) contents. Low concentrations of phytohormones from 0.001 to 1 $\mu mol \cdot L^{-1}$ had a facilitative effect on fluorescence parameters in the order of IAA > ABA > KT. The application of high concentrations of phytohormones inhibited the fluorescence parameters. The application of 0.1 $\mu mol \cdot L^{-1}$ IAA, ABA and KT treatments were the most effective in promoting As uptake in *L. punctata.* The effect sequence of different plant hormone on As enrichment was: ABA > IAA ≥ KT. Low concentrations of IAA, ABA and KT treatments all significantly increased superoxide dismutase (SOD) and glutathione reductase (GR) activities, significantly decreased malondialdehyde (MDA) and $H_2O_2$ contents and promoted proline synthesis in As-stressed *L. punctata.* This indicates that the application of phytohormones can improve the antioxidant activity of As-stressed *L. punctata,* promote the growth of *L. punctata* and improve its As absorption capacity. This study may provide a scientific basis for phytohormones to promote the remediation of As-contaminated waters by *L. punctata,* and can provide scientific basis for phytohormones to promote the remediation of As-contaminated waters by *L. punctata.*
**Keywords:** *phytohormones, arsenic, Landoltia punctata; fluorescence parameters, enrichment effect*

## Introduction

As a naturally widespread and highly toxic metalloid element, arsenic (As) is usually found in the earth's crust and rock formations in the form of sulfide minerals (Majzlan et al., 2014). Due to human activities such as pesticides and mining, As enters groundwater and surface water, causing serious As pollution problems, mainly distributed in major rivers and areas along the Great Delta in South Asia, East Asia, and South America (Kobya et al., 2020). Tens of thousands of people were endangering their health by drinking contaminated water directly or by the transmission of As

through the food chain (Xue et al., 2017). According to the substance priority list of the American Toxic Substances and Disease Registry (ATSDR), As ranks first in the current list of substances that pose the most significant potential threat to human health, and the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (USEPA) have classified As as a Class "1" and Class "A" carcinogen (Yadav al., 2021). Therefore, solving As pollution has become a hot issue of current research.

Phytoremediation of As pollution in water bodies through plants is an economical and efficient green emerging technology. Different species of plants differ greatly in their ability to enrich As, and the selection of plant species is the key to successful phytoremediation (Wani et al., 2017). Aquatic plants are capable of accumulating toxic metals and nutrients in large quantities (Ali et al., 2020). It was reported that *Landoltia punctata*, the common species of duckweed, is a cost-effective, ecologically safe and effective remediation of contaminated mining aquatic plants with the ability to reduce the risk of heavy metal pollution to humans and animals (Sasmaz et al., 2016; Canatto et al., 2021). *L. punctata* was widely found in various freshwater environments in China, with advantages such as fast reproduction speed and strong adaptability to the environment (Wang et al., 2022; Cheng et al., 2009; Zhong et al., 2022). *L. punctata* also is a commonly used plant research material in biology, rich in high starch and high protein, and is a high-quality source of feed and biological substrate (Chakrabarti et al., 2018; Ma et al., 2018).

Plant stress and senescence are closely related to leaf chlorophyll content. Chlorophyll fluorescence parameters can represent the growth potential of plants, and changes in fluorescence parameters induced by photosystem PSII contain much information on photosynthetic activity and therefore can reflect the degree of plant tolerance to the environment (Panda et al., 2008). Moreover, chlorophyll fluorescence parameters can be measured without injuring the plant itself, providing a rapid response to plant photosynthesis (Lv et al., 2018) and reflecting leaf PSII activity (Wang et al., 2010). Therefore, photosynthetic pigments and fluorescence parameters are widely used to determine the physiological response of plants under heavy metal stress. When subjected to abiotic stress, plants are also protected from damage by antioxidant enzyme systems, which mainly include superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and catalase (CAT), which are the main reactive oxygen species (ROS) scavenging systems in plants (Wang et al., 2015a). Malondialdehyde (MDA) is one of the major products of membrane lipid peroxidation (Kumar et al., 2000), and the antioxidant enzyme system in plants can prevent lipid peroxidation to some extent (Chen et al., 2019).

Phytohormones are often used to improve the efficiency of plant remediation of heavy metals, as they can effectively improve plant resistance to heavy metal stress and promote plant growth and development. Phytohormones are small signaling molecules produced in plants at very low concentrations, which move from the site of production to the vicinity of other organs where they function, and regulate cellular processes in certain target cells by binding to specific receptor proteins (Asami et al., 2018), resulting in significant physiological effects on plants. The main hormones that are essential for the development of the plant body include, indoleacetic acid (Indolent-3-acetic, IAA), Abscisic acid (ABA), and Kinetin (KT) (Asami et al., 2018).

It was reported that compared with other varieties of duckweed, *L. punctata* has a higher enrichment capacity and tolerance to As, cadmium and other harmful metals

(Gong et al., 2021). However, there are few reports on the enrichment of plant hormone on As absorption by *L. punctata.* Therefore, the aim of this study is to investigate the effects of IAA, ABA and KT on the physiological response, antioxidant properties, As absorption and mechanism of *L. punctata,* in order to provide scientific guidance for the remediation of As pollution in water.

## Materials and methods

### *Cultivation conditions and experimental design*

*L. punctata* was collected from the unpolluted waters of Nanchang City (China). The newly picked *L. punctata* were cleaned three times with distilled water. *L. punctata* with the same shape and color was selected and domesticated in plastic square basins, which was 36 cm long, 28 cm wide and 8 cm high. Hoagland nutrient solution was added to the basin with a scale of 6 cm. The coverage rate of *L. punctata* in basin was about 80%. After domesticated and cultured at 25°C for one week, the *L. punctata* was subject to the formal cultivation experiment.

The experimental design for formal cultivation was as follows: Each basin contains 400 mL of Hoagland culture nutrient solution, 1.0 g of *L. punctata,* and 3 mg L$^{-1}$ As$^{3+}$. Different concentrations of plant hormones IAA, ABA and KT were then added. Different plant hormones were added at concentrations of 0, 0.001, 0.01, 0.1, 1 and 10 μ mol·L$^{-1}$, respectively, with three replicates of each treatment. Culture experiments were carried out in a constant room (25°C) in 16 h light (incandescent lamp, 100 μmol·m$^{-2}$·s$^{-1}$)/8 h darkness. Photographic observations were made every other day during this period. After 7 days of culture, *L. punctata* was harvested for subsequent determination of relevant indicators. The relative growth rate adopts the method in ISO20079 (2005).

### *Determination of chlorophyll content and chlorophyll fluorescence*

The content of chlorophyll was determined using spectrophotometry as described by Wang et al. (2023). Chlorophyll fluorescence parameters were determined by underwater saturation pulse modulated chlorophyll fluorometer (Diving-PAM) (Lv et al., 2018). After the culture was finished, *L. punctata* were transferred to the dark environment for 20 min, and then were taken in the measurement device. The detection light was turned on to obtain the maximum photochemical photoefficiency ($F_v/F_m$), potential photochemical efficiency ($F_v/F_o$), effective quantum yield $Y$(II), photochemical quenching coefficient ($qP$), non-photochemical quenching coefficient ($qN$), quantum yield of non-regulated energy dissipation $Y$(NO), quantum yield of regulated energy dissipation $Y$(NPQ), etc. The parameters were automatically calculated and generated by the system in the selected mode, and each sample was measured for about 7 min.

### *Index stress markers and antioxidant capacity determination*

The antioxidant enzyme activities of MDA, GR, SOD, H$_2$O$_2$ and Proline were determined in the cellular homogenate using their respective kits by following the manufacturer's instructions from Keming Biotechnology of Suzhou, China (Hu et al., 2020). The MDA content (expressed as mmol g$^{-1}$ FW) was measured by thiobarbituric acid colorimetry. GR activity was calculated by measuring the rate of dehydrogenation

of NADPH by measuring the rate of decrease in absorbance at 340 nm. The SOD activity was assayed by using the photochemical nitrogen blue tetrazolium (NBT) Catalase activity was assayed spectrophotometrically at 25 in 20 mM $KH_2PO4$ buffer (pH 7.0), by measuring the rate of decline in optical density at 240 nm due to decomposition of $H_2O_2$. The proline concentration (expressed as $\mu g \ g^{-1}$ FW) was measured by acid ninhydrin colorimetry. The chemical reagents used are all analytical pure.

### *Determination of As content*

The determination of the total As content of the *L. punctata* was achieved after acid digestion with electrically heated plate and analysis with HG-AFS. Detailed digestion and determination refer to published (Ni et al., 2015). In brief, 0.5 g *L. punctata* was put into the polytetrafluoroethylene crucible, digested by nitric acid and perchloric acid, and then determined.

### *Statistical analysis*

Data were expressed in terms of the mean and standard deviation (SD) with three repetitions, and analyses were statistically analyzed using analysis of variance (ANOVA) with Duncan test at the 95% confidence level using SPSS 19.0 (IBM, USA), and diagrams were made using Origin 10.0 (OriginLab, USA).

## Results and discussion

### *Effect of phytohormones on the biomass of L. punctata under As stress*

In this study, under As stress, the growth status, leaf coverage, and biomass of *L. punctata* treated with IAA, ABA, and KT increased in the culture box (*Fig. A1*). Certain concentrations of IAA, ABA and KT treatments could effectively promote the growth and development of *L. punctata* under As stress. When IAA and $KT \geq 1 \ \mu mol·L^{-1}$ and $ABA \geq 0.1 \ \mu mol·L^{-1}$, biomass began to decline, and when KT was $10 \ \mu mol·L^{-1}$, biomass decreased significantly and was lower than that of control group (CK) ($P < 0.05$) (*Tables A1, A2* and *A3*). It showed that the promotion effect of excessive concentrations of IAA, ABA and KT on the accumulation of biomass of *L. punctata* was reduced, and $KT \geq 10 \ \mu mol·L^{-1}$ directly inhibited the growth and development of *L. punctata*. Faessler et al. (2010) found that $0.0001 \ \mu mol·L^{-1}$ exogenous IAA treatment significantly increased the volume, surface area, length, diameter and density of sunflower under harmful metals stress. This may be due to the fact that IAA belongs to auxin, which can promotes cell division, root and stem growth, and vascular tissue formation, and enhances plant nutrient uptake and accumulation, thereby reducing the toxicity of metal ions to plants and promoting plant growth (He et al., 2022). Li et al. (2014) found that foliar spraying of 10-20 $\mu mol·L^{-1}$ ABA on seedlings significantly increased root number and fresh weight by Cd stress on mung bean seedlings. It was possible that ABA can enhance plant resistance to heavy metals and reduce plant growth inhibition (Han et al., 2016). Wang et al. (2021a) found that under As(III) and As(V) stresses at 2 $mg·L^{-1}$, the application of 0.1~1 $mg·L^{-1}$ of exogenous KT significantly increased the fresh weight of phoenix-tail fern under As(III) stress. It was possible that low concentrations of KT can induce increased root vigor and promote plant root division and differentiation (Werner et al., 2001). One reason why certain

concentrations of IAA, ABA and KT treatments increased the biomass of *L. punctata* was the improvement of photosynthesis, while the decrease in biomass under high concentrations of phytohormone treatments was due to the decrease in photosynthetic pigment content.

### Effect of phytohormones on chlorophyll in As-stressed L. punctata

Under As stress, the magnitude of chlorophyll content in all treatment groups was Ca > Cb > Cc (*Fig. 1*). When plants were subjected to As stress, $Mg^{2+}$ in chloroplasts will be replaced by As and thylakoids will be broken, which will reduce the content of photosynthetic pigments (Li et al., 2011). In this study, IAA treatment increased the content of photosynthetic pigments, and Ca, Cb and carotenoid contents reached their peaks when treated with 1 $\mu mol \cdot L^{-1}$ IAA (*Fig. 1a*). This may be due to the fact that exogenous IAA makes the photosynthetic organ of *L. punctata* develop a defense function and the stimulation makes photosynthesis enhanced (Singh et al., 2015), reducing the toxicity of As. It suggested that IAA treatment significantly increased Ca and Cb in eggplant seedlings and increased the photosynthetic rate, which was 13% higher than that of the control group (Singh et al., 2015). Under the treatment of 0.01 $\mu mol\ L^{-1}$ ABA, Ca reached the peak value (*Fig. 1b*). This indicated that a certain concentration of ABA could increase the photosynthetic pigment content of plants and alleviate the adverse effects of As stress on plant photosynthesis. Ca was sensitive to ABA response and ABA could increase chlorophyllase activity, which was consistent with the results of Zhao et al. (2009).

With the increase of KT concentration, the chlorophyll content first increased and then decreased. When KT concentration was 10 $\mu mol \cdot L^{-1}$, Ca, Cb and Cc decreased significantly (*Fig. 1c*). It indicates that the appropriate concentration of KT helps to reduce the toxicity of As to *L. punctata,* while too high KT impairs the biosynthesis of chloroplasts and photosynthetic pigments. This was in agreement with Wang et al. (2015b), who found that 0.5 $mg \cdot L^{-1}$ KT treatment increased photosynthetic pigment content and 1.5 $mg \cdot L^{-1}$ KT treatment decreased it in maize seedlings under As stress. Photosynthetic pigments showed a decreasing trend when treated with high concentrations of IAA, ABA and KT. This may be due to the high concentration of plant hormones, which will lead to the closure of stomata on the thallus surface, the decrease of transpiration rate, the increase of thallus surface temperature, and the decrease of net photosynthetic rate (Chen et al., 2018).

### Effect of phytohormones on chlorophyll fluorescence parameters of As-stressed L. punctata

The maximum photochemical efficiency ($F_v/F_m$), potential photochemical efficiency ($F_v/F_o$), photochemical fluorescence quenching coefficient ($qP$) and effective quantum yield $Y$(II) of *L. punctata* showed an increasing and then decreasing trend with increasing IAA treatment concentration, while the non-photochemical quenching coefficient ($qN$), non-regulated energy dissipative quantum yield $Y$(NO) and regulated energy dissipative quantum yield $Y$(NPQ) showed a decreasing and then increasing trend (*Table A4*). Low concentrations of IAA can promote the self-regulation ability of As stress in *L. punctata,* while the promotion of photochemical efficiency was weakened at too high concentrations.
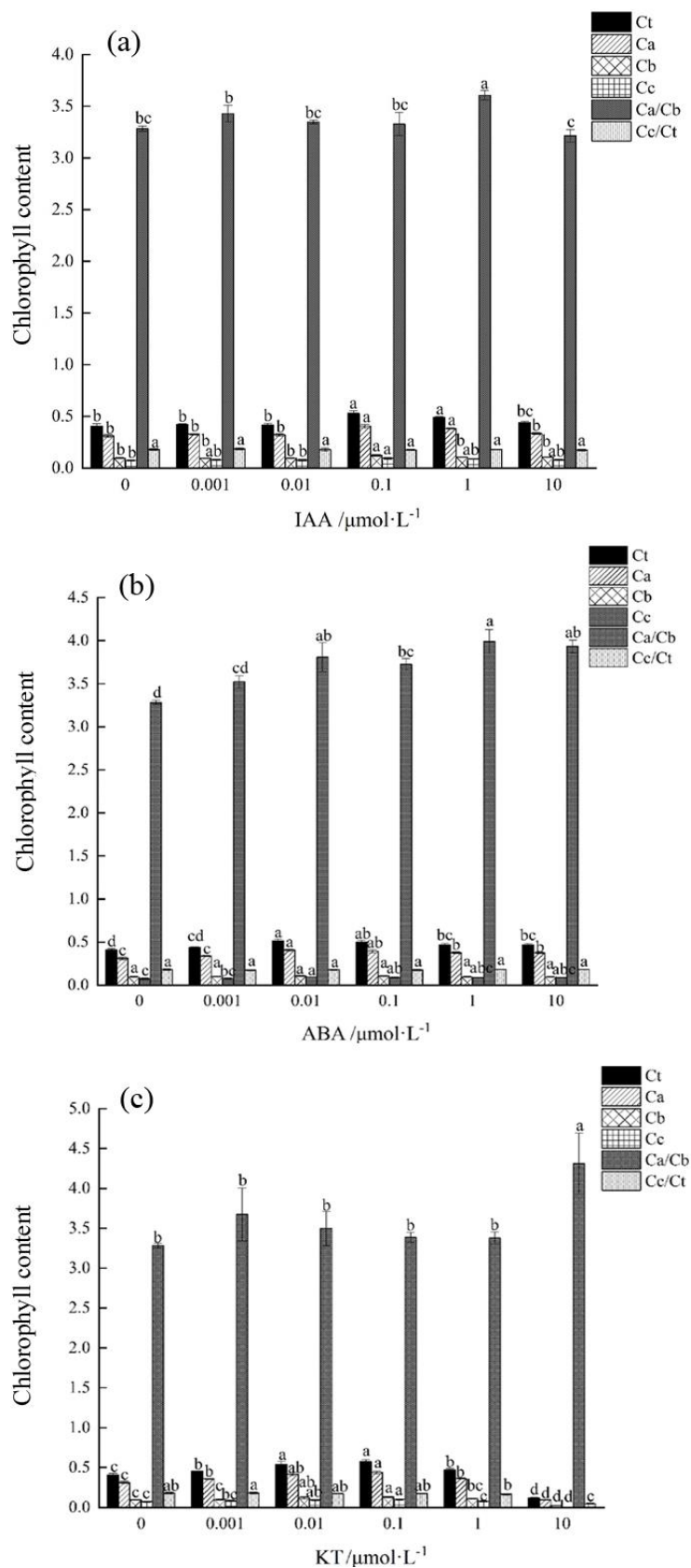
**Figure 1.** *Effects of IAA (a), ABA (b) and KT (c) on chlorophyll content in Landoltia punctata under As stress. Where IAA, ABA, KT are the abbreviation for Indolent-3-acetic, Abscisic acid and Kinetin, respectively*

Both $qP$ and $qN$ indicated that IAA could alleviate the photosynthetic system damage caused by As toxicity. $Y$(NPQ) decreased and then increased because As disrupted the regulatory dissipation mechanism of *L. punctata*, while IAA treatment alleviated the As toxicity. For example, Zhao et al. (2017) found that under 2 mg·L$^{-1}$ As stress, when 10~40 mg·L$^{-1}$ IAA treatments were applied, $qP$ tended to increase, $F_v/F_m$, and $qN$ did not differ significantly in large-leaved wellingtonia, while $qN$ decreased significantly in sword-leaved fenugreek. Under 50 mmol·L$^{-1}$ NaCl-stressed peas, $F_v/F_m$ was significantly increased by 15% for foliar sprays of 30 mg·L$^{-1}$ IAA (Husen et al., 2016). Plants usually have $F_v/F_m$ around 0.8 in the absence of stress, and IAA treatment significantly increased $F_v/F_m$ and $F_v/F_o$ (P < 0.05). However, $F_v/F_m$ was lower than 0.8 in *L. punctata*, indicating that IAA treatment can improve the photochemical efficiency of *L. punctata* and increase photosynthetic activity to increase PSII reaction center activity and alleviate As toxicity to *L. punctata* (Singh et al., 2015). IAA treatment caused a significant increase in $qP$ (p < 0.05) and a significant decrease in $qN$ *(p < 0.05)*, suggesting that IAA enhances the ability of PSII reaction center to capture light quanta and reduces thermal energy dissipation.

$F_v/F_m$, $F_v/F_o$, $qP$ and Y(II) of *L. punctata* showed a trend of increasing and then decreasing with increasing concentration of ABA treatment, while $qN$, $Y$(NO) and $Y$(NPQ) showed a trend of decreasing and then increasing (*Table A5*). When ABA was 0.01 μmol·L$^{-1}$, $Y$(II) increased significantly, which was 30.43% higher than CK. $Y$(NPQ) reached its minimum value when ABA was 0.001 μmol·L$^{-1}$, which was 13.79% lower than CK. For example, 0.05 mmol·L$^{-1}$ ABA significantly increased the $F_v/F_m$ content of drought-stressed maize leaves and alleviated the effects of drought stress on maize leaves (Wang et al., 2021a).

In the ABA treatment group, the value of qP and Y (II) first increased and then decreased, while the value of qN, Y (NO), and Y (NPQ) first decreased and then increased. It may be due to the fact that low concentration (0.001~0.01 μmol·L$^{-1}$) of ABA promotes photosynthesis, while the application of ABA ≥ 0.1 μmol·L$^{-1}$ inhibits electron transfer efficiency.

It shows that a certain concentration of ABA could increase heat dissipation to mitigate the damage of As on *L. punctata*, which was consistent with the findings of Long et al. (2017). This indicates that low concentration of ABA can increase heat dissipation and reduce As damage to *L. punctata*, which was consistent with other research (Long et al., 2017).

In KT group, both $qP$ and $qN$ lower than CK (*Table A6*). When KT was 10 μmol·L$^{-1}$, both $F_v/F_m$ and $F_v/F_o$ reached their minimum values, decreasing by 40.48% and 50.72%, respectively compared with CK. The $qP$ value reached a minimum value at KT of 10 μmol·L$^{-1}$ and decreased by 60.32% compared with CK. The $qN$ value reached a minimum value at KT of 0.1 μmol·L$^{-1}$ and decreased by 45.95% compared with CK. It showed that low concentration of KT treatment could improve photochemical efficiency and electron transfer efficiency, which was consistent with the study of Shao et al. (2012).

### Effects of plant hormones on antioxidant activity of L. punctata

In nature, plants will be subjected to various stresses, which will affect their physiological form and development. Therefore, it was important for plants to adapt to the changing natural environment and to perceive and respond quickly and accurately to each stimulus. ROS are highly responsive to membrane lipids, proteins and DNA,

which were thought to be major contributors to stress damage and rapid cellular injury. Especially when plants are exposed to heavy metals, electron transport chains tend to form $O_2^{\cdot-}$ and the latter disproportionate to form $H_2O_2$. Plants have antioxidant mechanisms that rapidly scavenge excess ROS under abiotic stresses.

With the increase of IAA concentration, the activities of SOD and GR increased first and then decreased, while the content of MDA decreased first and then increased ($P < 0.05$) (*Fig. 2*). The $H_2O_2$ content decreased and the proline content increased first and then decreased ($P < 0.05$) in *L. punctata*. SOD and GR activities increased first and then decreased, while MDA content decreased first and then increased in *L. punctata* (*Fig. 2*). SOD activity was highest when the ABA concentration was 0.1 μmol·L$^{-1}$, which increased by 62.03% compared with the control (CK) treatment (*Fig. 3*). When ABA was 10 μmol·L$^{-1}$, GR activity was lowest and decreased by 13.54% compared with CK. When ABA was 1 μmol·L$^{-1}$, MDA content was the lowest and decreased by 33.97% compared with CK.
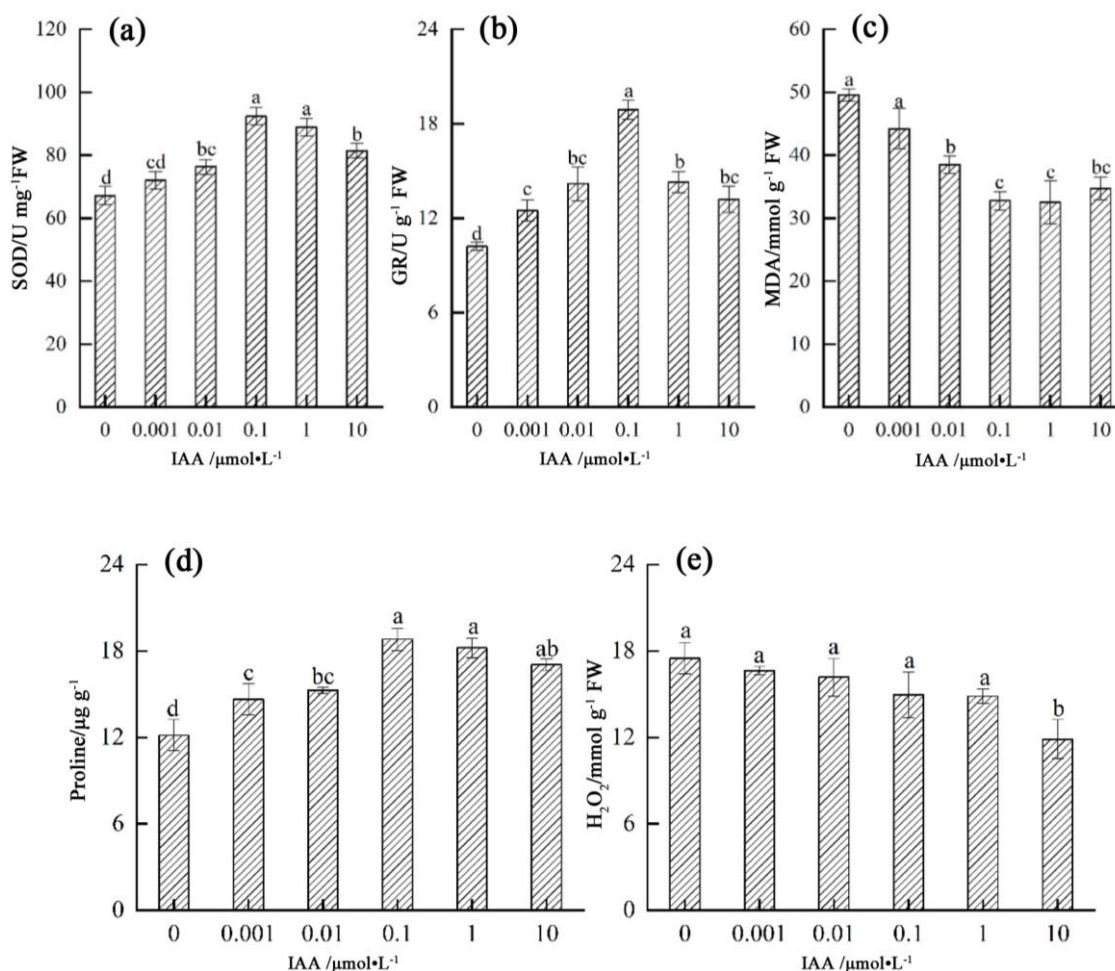


*Figure 2. Effect of IAA on SOD, GR, MDA, Proline and $H_2O_2$ in Landoltia punctata under As stress. Where IAA, SOD, GR and MDA are the abbreviation for Indolent-3-acetic, superoxide dismutase, glutathione reductase and malondialdehyde, respectively*

Under As stress, SOD and GR activities tended to increase and then decrease with increasing KT concentration (*Fig. 4*), and MDA content decreased first and then

increased significantly (P < 0.05). Under normal conditions, ROS of cells in plants maintain a dynamic balance. When subjected to abiotic stress, it will be oxidative stress (Gill et al., 2010), and ROS will increase significantly, leading to plant metabolic disorders and inhibiting growth and development (Hung et al., 2005). While MDA is a product of cell membrane lipid peroxidation, MDA content can reflect the degree of membrane damage. In order to regulate oxidative stress, a variety of antioxidant enzymes and non-enzymatic substances exist in plants to scavenge excess ROS and bring them into dynamic balance.
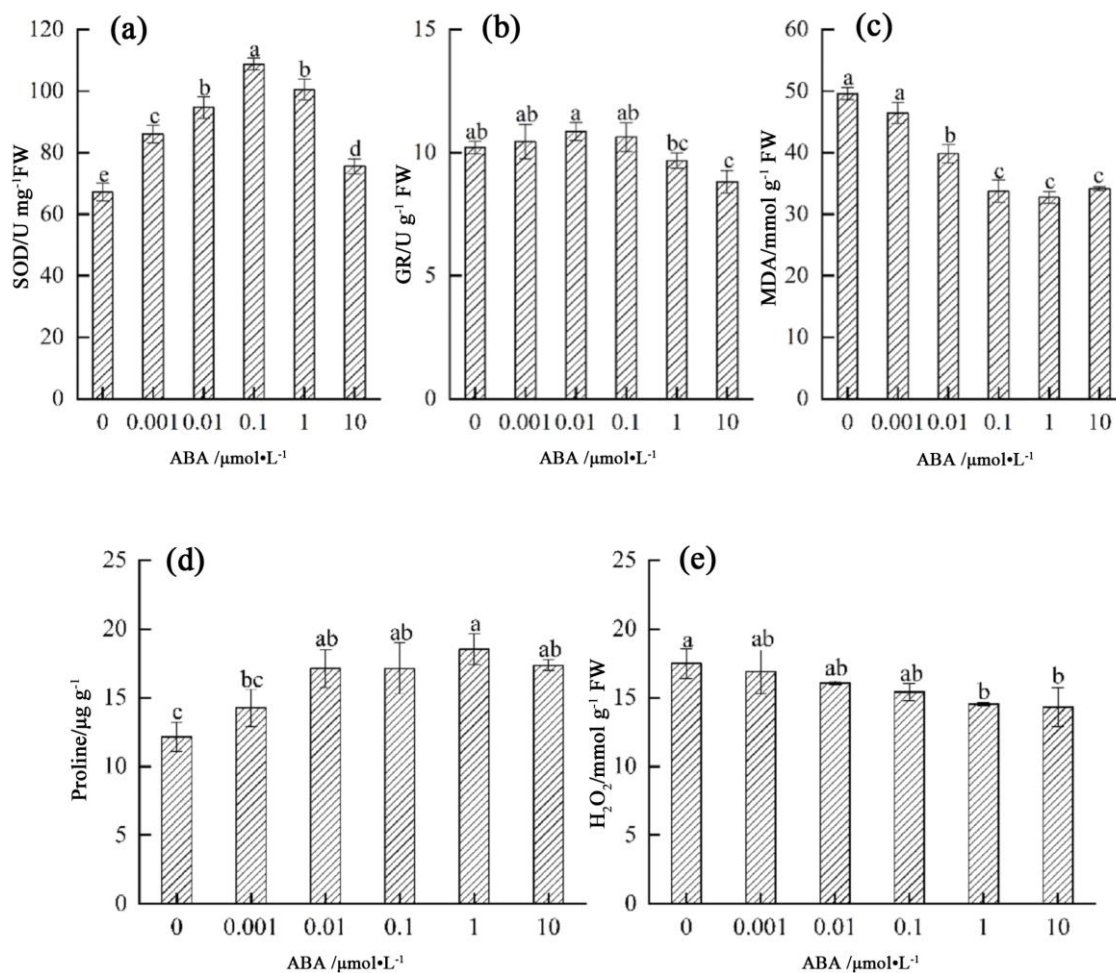


***Figure 3.*** *Effect of ABA on SOD, GR, MDA, Proline and $H_2O_2$ in Landoltia punctata under As stress. Where ABA, SOD, GR and MDA are the abbreviation for Abscisic acid, superoxide dismutase, glutathione reductase and malondialdehyde, respectively*

SOD and GR are one of the important antioxidant enzymes for scavenging ROS. SOD can convert to $H_2O_2$, while excess $H_2O_2$ will be further broken down by peroxidase. GR is an important factor involved in the regulation of the redox state of plant cells. GR can form the ascorbic acid (AsA)-glutathione (GSH) cycle together with reductase II (NADPH), while the reaction of AsA with $H_2O_2$ can occur directly or be catalyzed by APX. The current understanding of stress signaling in plants is that changes in GSH/glutathione disulfide (GSSG) with the synthesis of $H_2O_2$ constitute an early stress signal leading to a physiological response, usually the activation of

antioxidant mechanisms or modification of gene expression. In this study, compared with CK, SOD and GR activities increased, while MDA and $H_2O_2$ content decreased in *L. punctata* for IAA treatment (*Fig. 2*).
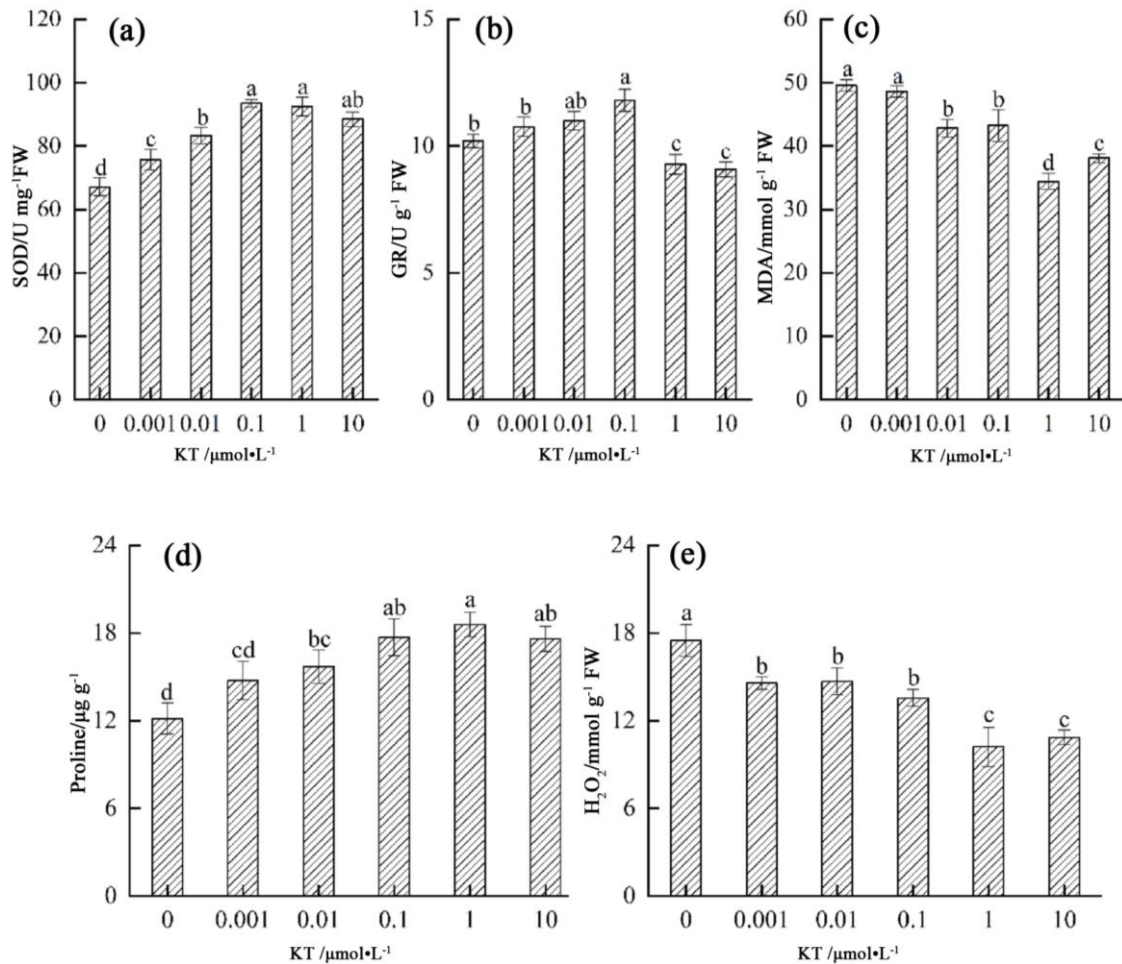


***Figure 4.*** *Effect of KT on SOD, GR, MDA, Proline and $H_2O_2$ in Landoltia punctata under As stress. Where KT, SOD, GR and MDA are the abbreviation for Kinetin, superoxide dismutase, glutathione reductase and malondialdehyde, respectively*

The results showed that IAA could improve the antioxidant enzyme activity, enhance the plant antioxidant system, reduce the formation rate of ROS and significantly reduce oxidative damage (Gill et al., 2010), and alleviate the antioxidant stress of As on *L. punctata.* This was in agreement with the results of Sing et al. (2015), who found that IAA treatment significantly enhanced SOD, POD and CAT enzyme activities in eggplant seedlings (p < 0.05). Studies have shown that ABA suppresses the $O_2^{\cdot-}$ content and attenuates oxidative stress (Yu et al., 2022). In the present study, when treated with 0.1 μmol·L⁻¹ ABA, SOD reached the peak, while GR reached the peak at 0.01 μmol·L⁻¹ ABA treatment (*Fig. 3*). It was shown that ABA application increased SOD and GR activities in *L. punctata* and improved ROS scavenging ability. SOD is directly involved in plant production and scavenging under adversity stress, playing important role in resisting adversity stress and alleviating oxidative stress (Apel et al., 2004). This was consistent with the results of Han et al. (2016) who found that 5 μmol·L⁻¹ of ABA

treatment significantly increased the activity of SOD and APX in poplar cells. In this study, KT treatment caused a significant increase in SOD and GR, and a significant decrease in $H_2O_2$ and MDA ($P < 0.05$) (*Fig. 4*). This was consistent with Wang et al. (2015b) who found that KT can improve As tolerance in maize seedlings by maintaining chlorophyll stability, increasing antioxidant enzyme activity, and inhibiting MDA production. Proline accumulates under stresses such as water deficit, high salt, cold, high temperature and heavy metals, and plays an important role in maintaining osmotic pressure homeostasis in plants and protecting enzymes from inactivation under high salt, heat and cold conditions (Quan et al., 2007). Proline contributes to the stabilization of macromolecular structure and function in plants under heavy metal stress (Gill et al., 2010). Studies have shown that IAA (Wu et al., 2018), ABA (Wang et al., 2021b), and KT (Sun et al., 2018) can increase proline content in plants under adversity stress. In this study, compared with CK, proline content was significantly increased in *L. punctata* under As stress by applying different concentrations of IAA, ABA and KT (*Figs. 2, 3* and *4*). When IAA, ABA and KT $\geq 10$ µmol·L$^{-1}$, the promoting effect was weakened. And proline was more responsive to IAA, and proline synthesis could be enhanced faster at low IAA concentration. It may be due to exogenous phytohormones that reduce proline dehydrogenase (ProDH) activity and increase cellular permeability, favoring proline accumulation (Wang et al., 2014).

### Effect of phytohormones on As enrichment in As-stressed *L. punctata*

Under As stress conditions, with increasing applied concentrations of IAA, ABA and KT, the As unit enrichment, total As enrichment, As removal rate and bioconcentration factor (BCF) all showed an increasing and then decreasing trend (*Tables 1, 2* and *3*). At 0.1 µmol·L$^{-1}$ IAA treatment, As unit enrichment, total As enrichment, As removal rate and BCF reached their maximum values, which increased by 13.74%, 35.35%, 35.10% and 13.14%, respectively, compared with the control group (CK). When ABA was 0.1 µmol·L$^{-1}$, the As unit enrichment, total As enrichment, As removal rate and BCF all reached the maximum value, which increased 56.41%, 67.68%, 67.58% and 56.54%, respectively, compared with CK.

In this study, IAA treatment led to a trend of first increasing and then decreasing As enrichment in the *L. punctata*. When IAA was 0.1 µmol L$^{-1}$, the concentration of As per unit reached its peak (866.8 mg kg$^{-1}$), which was 13.7% higher than the control group (CK) (761.2 mg kg$^{-1}$). When IAA $\geq 10$ µmol·L$^{-1}$, the As unit enrichment, total enrichment, As removal rate and BCF were all lower than those of CK. This indicated that low concentrations (0.001~1 µmol·L$^{-1}$) of IAA had a promotive effect on the As enrichment ability of *L. punctata*, while high concentrations of IAA instead reduced the accumulation of As in the plants. This may be due to the fact that low concentrations of IAA can stimulate plant hemicellulose synthesis, increase the adsorption of heavy metals by the cell wall, and improve the effect of plant transfer of heavy metals (Zhu et al., 2013). It may also be that IAA enhances the antioxidant function of plants and the absorption of As. He et al. (2016) found that the As content in the leaves of both large-leaved wellingtonia and sword-leaved fern reached a maximum at 20 mg·L$^{-1}$ exogenous IAA treatment, and when treated with 60 mg·L$^{-1}$ IAA, the As content in the leaves was not significantly different from the control. In ABA treatment, the enrichment of *L. punctata* increased compared with CK, and the highest As unit enrichment was 1192.0 mg·kg$^{-1}$ at 0.1 µmol·L$^{-1}$ ABA treatment, which was 56.41% higher than that of CK. It showed that ABA was effective in promoting *L. punctata*. It may be because

ABA can promote the development of plant roots under heavy metal stress and significantly improve root vitality (Xiao et al., 2022), while the well-developed root system of *L. punctata* facilitates the uptake of As. Zhao et al. (2009) found a significant increase in Pb content in rice seedlings treated with exogenous ABA (1, 10 mg·dm⁻³). Tang et al. (2019) found that under 5 µmol·L⁻¹ exogenous ABA treatment, Cd content in lettuce roots increased by 29.44% compared with the CK treatment. In KT treatment, the As enrichment of *L. punctata* showed a trend of increasing and then decreasing.

*Table 1. Effect of IAA on arsenic accumulation of Landoltia punctata under As stress*

| IAA (µmol·L⁻¹) | Unit enrichment of arsenic (mg/kg) | Total enrichment of arsenic (mg) | Arsenic removal rate | BCF |
|---|---|---|---|---|
| 0 | 762.1 ± 9.64[b] | 0.099 ± 0.002[cd] | 8.28% | 254.0 ± 3.21[b] |
| 0.001 | 804.7 ± 35.0[ab] | 0.106 ± 0.004[bc] | 8.88% | 268.2 ± 11.67[ab] |
| 0.01 | 824.8 ± 49.8[ab] | 0.115 ± 0.011[b] | 9.61% | 274.9 ± 16.60[ab] |
| 0.1 | 866.8 ± 36.7[a] | 0.134 ± 0.006[a] | 11.18% | 288.9 ± 12.24[a] |
| 1 | 767.6 ± 9.62[b] | 0.105 ± 0.002[bc] | 8.78% | 255.8 ± 3.20[b] |
| 10 | 674.5 ± 13.4[c] | 0.087 ± 0.001[d] | 7.27% | 224.8 ± 4.48[c] |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different IAA treatment at a level of 0.05. Where IAA and BCF are the abbreviation for Indolent-3-acetic and Bioconcentration Factor, respectively

*Table 2. Effect of ABA on arsenic accumulation of Landoltia punctata under As stress*

| ABA (µmol·L⁻¹) | Unit enrichment of arsenic (mg/kg) | Total enrichment of arsenic (mg) | Arsenic removal rate | BCF |
|---|---|---|---|---|
| 0 | 762.1 ± 9.64[d] | 0.099 ± 0.002[c] | 8.28% | 254.0 ± 3.21[d] |
| 0.001 | 924.2 ± 14.7[c] | 0.131 ± 0.002[b] | 10.97% | 308.0 ± 6[c] |
| 0.01 | 1192 ± 25.8[b] | 0.160 ± 0.004[a] | 13.37% | 345.9 ± 8.632[b] |
| 0.1 | 1192 ± 45.8[a] | 0.166 ± 0.005[a] | 13.87% | 397.6 ± 15.26[a] |
| 1 | 884.7 ± 37.8[c] | 0.120 ± 0.004[b] | 10.07% | 294.9 ± 12.60[c] |
| 10 | 780.7 ± 27.3[d] | 0.104 ± 0.004[c] | 8.69% | 260.2 ± 9.118[d] |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different ABA treatment at a level of 0.05. Where ABA and BCF are the abbreviation for Abscisic acid and Bioconcentration Factor, respectively

*Table 3. Effect of KT on arsenic accumulation of Landoltia punctata under As stress*

| KT (µmol·L⁻¹) | Unit enrichment of arsenic (mg/kg) | Total enrichment of arsenic (mg) | Arsenic removal rate | BCF |
|---|---|---|---|---|
| 0 | 762.1 ± 9.64[c] | 0.099 ± 0.002[b] | 8.28% | 254.0 ± 3.21[c] |
| 0.001 | 776.8 ± 22.7[bc] | 0.101 ± 0.003[b] | 8.45% | 258.9 ± 7.57[bc] |
| 0.01 | 805.1 ± 9.56[b] | 0.107 ± 0.003[ab] | 8.95% | 268.3 ± 3.18[b] |
| 0.1 | 850.6 ± 10.7[a] | 0.120 ± 0.000[a] | 10.00% | 283.5 ± 3.57[a] |
| 1 | 770.4 ± 14.9[bc] | 0.108 ± 0.007[ab] | 9.02% | 256.8 ± 4.96[bc] |
| 10 | 739.4 ± 25.3[c] | 0.084 ± 0.008[c] | 7.07% | 246.4 ± 8.439[c] |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different KT treatment at a level of 0.05. Where KT and BCF are the abbreviation for Kinetin and Bioconcentration Factor, respectively

It was shown that suitable concentrations (0.01~0.1 µmol·L$^{-1}$ KT) could promote the As enrichment effect of *L. punctata*. It may be because KT can affect the stomatal opening of plants, thus increasing the transpiration rate and making plants take up more As (Tassi et al., 2008).

## Conclusions

This study was conducted to investigate the enrichment effect of different phytohormones on the As uptake and tolerance mechanism of As polluted waters by *L. punctata*, with the aim of providing scientific basis for phytohormones to promote the remediation of As polluted waters by *L. punctata*. The promoting effect of low concentrations of plant hormones with 0.001~1 µmol L$^{-1}$ on fluorescence parameters and growth of *L. punctata* is in the order of IAA > ABA > KT. Three phytohormones mitigate As damage to *L. punctata* by improving photochemical efficiency, antioxidant enzyme activity, and enhancing plant antioxidant system. However, the comparison of the differences of different exogenous phytohormone addition methods and addition time on As uptake by duckweed and the analysis of the regulation of phytohormone on As uptake by duckweed based on transcriptome and metabolomics need to be studied in depth.

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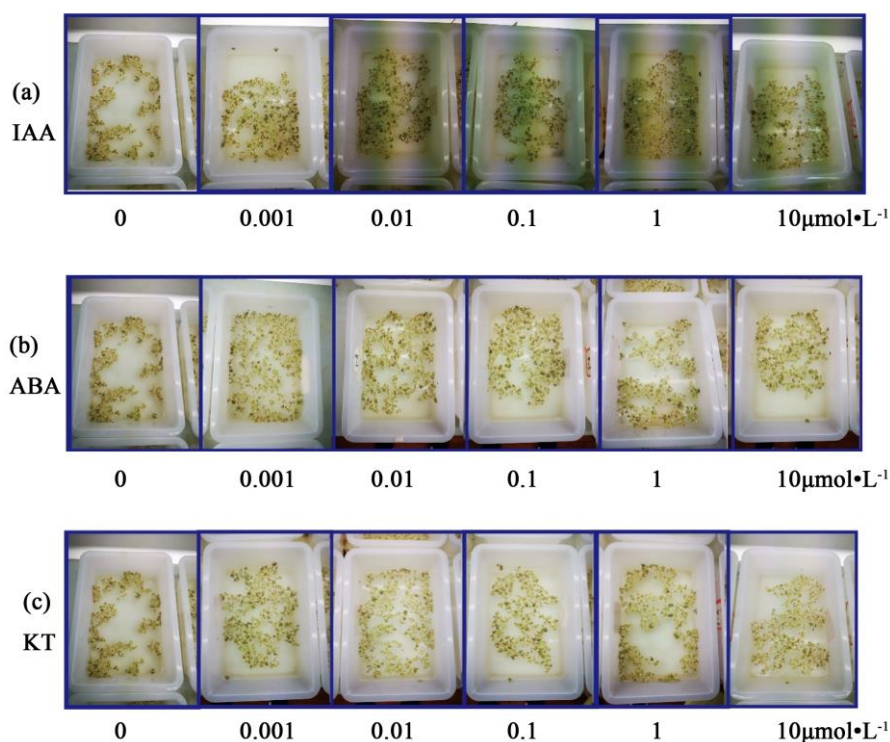
# APPENDIX



***Figure A1.*** *Effects of IAA (a), ABA (b) and KT (c) on the growth of Landoltia punctata under As stress. Where IAA, ABA and KT are the abbreviation for Indolent-3-acetic, Abscisic acid and Kinetin, respectively*

***Table A1.*** *Effect of IAA on the biomass of Landoltia punctata under As stress*

| IAA ($\mu mol \cdot L^{-1}$) | Fresh weight (g FW) | Dry weight (g DW) | Relative growth rate |
|---|---|---|---|
| 0 | $1.185 \pm 0.047^c$ | $0.058 \pm 0.002^d$ | $2.41 \pm 0.57^c$ |
| 0.001 | $1.204 \pm 0.007^{bc}$ | $0.060 \pm 0.001^{cd}$ | $2.65 \pm 0.08^{bc}$ |
| 0.01 | $1.267 \pm 0.052^b$ | $0.064 \pm 0.002^b$ | $3.37 \pm 0.60^b$ |
| 0.1 | $1.407 \pm 0.009^a$ | $0.071 \pm 0.001^a$ | $4.87 \pm 0.09^a$ |
| 1 | $1.248 \pm 0.027^{bc}$ | $0.062 \pm 0.001^{bc}$ | $3.16 \pm 0.30^{bc}$ |
| 10 | $1.175 \pm 0.004^c$ | $0.059 \pm 0.001^d$ | $2.30 \pm 0.04^c$ |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different IAA treatment at a level of 0.05. Where IAA is the abbreviation for Indolent-3-acetic

***Table A2.*** *Effect of ABA on the biomass of Landoltia punctata under As stress*

| ABA ($\mu mol \cdot L^{-1}$) | Fresh weight (g FW) | Dry weight (g DW) | Relative growth rate |
|---|---|---|---|
| 0 | $1.185 \pm 0.047^e$ | $0.058 \pm 0.002^d$ | $2.41 \pm 0.57^d$ |
| 0.001 | $1.294 \pm 0.005^b$ | $0.064 \pm 0.001^b$ | $3.68 \pm 0.05^b$ |
| 0.01 | $1.405 \pm 0.004^a$ | $0.070 \pm 0.001^a$ | $4.86 \pm 0.04^a$ |
| 0.1 | $1.268 \pm 0.005^{bc}$ | $0.064 \pm 0.001^b$ | $3.39 \pm 0.05^b$ |
| 1 | $1.242 \pm 0.022^{cd}$ | $0.062 \pm 0.001^{bc}$ | $3.10 \pm 0.25^{bc}$ |
| 10 | $1.213 \pm 0.014^{de}$ | $0.060 \pm 0.001^{cd}$ | $2.76 \pm 0.17^{cd}$ |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different ABA treatment at a level of 0.05. Where ABA is the abbreviation for Abscisic acid

***Table A3.*** *Effect of KT on the biomass of Landoltia punctata under As stress*

| KT ($\mu mol \cdot L^{-1}$) | Fresh weight (g FW) | Dry weight (g DW) | Relative growth rate |
|---|---|---|---|
| 0 | $1.185 \pm 0.047^a$ | $0.058 \pm 0.002^a$ | $2.41 \pm 0.57^a$ |
| 0.001 | $1.187 \pm 0.018^a$ | $0.059 \pm 0.001^a$ | $2.44 \pm 0.22^a$ |
| 0.01 | $1.212 \pm 0.020^a$ | $0.059 \pm 0.002^a$ | $2.74 \pm 0.24^a$ |
| 0.1 | $1.283 \pm 0.013^a$ | $0.064 \pm 0.000^a$ | $3.55 \pm 0.15^a$ |
| 1 | $1.277 \pm 0.074^a$ | $0.064 \pm 0.003^a$ | $3.46 \pm 0.81^a$ |
| 10 | $1.04 \pm 0.071^b$ | $0.051 \pm 0.002^b$ | $1.17 \pm 0.46^b$ |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different KT treatment at a level of 0.05. Where KT is the abbreviation for Kinetin

***Table A4.*** *Effect of IAA on fluorescence parameters of Landoltia punctata under As stress*

| IAA ($\mu mol \cdot L^{-1}$) | $Y(II)$ | $qP$ | $qN$ | $Y(NO)$ | $Y(NPQ)$ | $F_v/F_m$ | $F_v/F_o$ |
|---|---|---|---|---|---|---|---|
| 0 | $0.23 \pm 0.014^c$ | $0.63 \pm 0.012^c$ | $0.74 \pm 0.031^a$ | $0.48 \pm 0.010^{bc}$ | $0.29 \pm 0.009^a$ | $0.42 \pm 0.011^e$ | $0.69 \pm 0.116^b$ |
| 0.001 | $0.28 \pm 0.007^{bc}$ | $0.82 \pm 0.026^a$ | $0.64 \pm 0.010^b$ | $0.43 \pm 0.003^d$ | $0.27 \pm 0.003^{ab}$ | $0.44 \pm 0.013^{de}$ | $0.76 \pm 0.023^b$ |
| 0.01 | $0.30 \pm 0.014^{ab}$ | $0.75 \pm 0.010^b$ | $0.47 \pm 0.032^{cd}$ | $0.47 \pm 0.007^c$ | $0.22 \pm 0.002^c$ | $0.48 \pm 0.010^{bc}$ | $0.94 \pm 0.040^{ab}$ |
| 0.1 | $0.33 \pm 0.013^a$ | $0.80 \pm 0.017^a$ | $0.44 \pm 0.008^d$ | $0.47 \pm 0.011^c$ | $0.20 \pm 0.015^c$ | $0.50 \pm 0.012^b$ | $1.03 \pm 0.050^{ab}$ |
| 1 | $0.27 \pm 0.039^{bc}$ | $0.70 \pm 0.031^b$ | $0.52 \pm 0.002^c$ | $0.49 \pm 0.013^b$ | $0.26 \pm 0.010^b$ | $0.54 \pm 0.019^a$ | $1.22 \pm 0.337^a$ |
| 10 | $0.25 \pm 0.015^c$ | $0.70 \pm 0.014^b$ | $0.68 \pm 0.020^b$ | $0.52 \pm 0.005^a$ | $0.28 \pm 0.017^{ab}$ | $0.46 \pm 0.017^{cd}$ | $0.88 \pm 0.063^{ab}$ |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different IAA treatment at a level of 0.05. Where IAA is the abbreviation for Indolent-3-acetic. Meaning of relevant indicators: the maximum photochemical photoefficiency ($F_v/F_m$), potential photochemical efficiency ($F_v/F_o$), effective quantum yield $Y(II)$, photochemical quenching coefficient ($qP$), non-photochemical quenching coefficient ($qN$), quantum yield of non-regulated energy dissipation $Y(NO)$, quantum yield of regulated energy dissipation $Y(NPQ)$, etc.

***Table A5.*** *Effect of ABA on fluorescence parameters of Landoltia punctata under As stress*

| ABA ($\mu mol \cdot L^{-1}$) | | $Y(II)$ | $qP$ | $qN$ | $Y(NO)$ | $Y(NPQ)$ | $F_v/F_m$ | $F_v/F_o$ |
|---|---|---|---|---|---|---|---|---|
| 0 | AV | $0.23 \pm 0.014^b$ | $0.63 \pm 0.012^d$ | $0.74 \pm 0.031^a$ | $0.48 \pm 0.010^a$ | $0.29 \pm 0.009^c$ | $0.42 \pm 0.011^b$ | $0.69 \pm 0.116^{ab}$ |
| 0.001 | AV | $0.25 \pm 0.003^b$ | $0.72 \pm 0.001^c$ | $0.51 \pm 0.012^c$ | $0.48 \pm 0.007^a$ | $0.25 \pm 0.006^d$ | $0.42 \pm 0.008^b$ | $0.60 \pm 0.079^{ab}$ |
| 0.01 | AV | $0.3 \pm 0.009^a$ | $0.78 \pm 0.014^b$ | $0.50 \pm 0.014^c$ | $0.43 \pm 0.022^b$ | $0.26 \pm 0.004^d$ | $0.46 \pm 0.011^a$ | $0.86 \pm 0.203^a$ |
| 0.1 | AV | $0.25 \pm 0.006^b$ | $0.84 \pm 0.002^a$ | $0.67 \pm 0.006^b$ | $0.39 \pm 0.009^c$ | $0.34 \pm 0.007^b$ | $0.41 \pm 0.012^{bc}$ | $0.67 \pm 0.011^{ab}$ |
| 1 | AV | $0.20 \pm 0.011^c$ | $0.71 \pm 0.012^c$ | $0.70 \pm 0.009^{ab}$ | $0.47 \pm 0.015^a$ | $0.35 \pm 0.004^b$ | $0.38 \pm 0.020^c$ | $0.64 \pm 0.091^{ab}$ |
| 10 | AV | $0.17 \pm 0.011^d$ | $0.61 \pm 0.003^d$ | $0.73 \pm 0.011^a$ | $0.49 \pm 0.002^a$ | $0.40 \pm 0.008^a$ | $0.32 \pm 0.013^d$ | $0.48 \pm 0.093^b$ |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different ABA treatment at a level of 0.05. Where ABA is the abbreviation for Abscisic acid. Meaning of relevant indicators: the maximum photochemical photoefficiency ($F_v/F_m$), potential photochemical efficiency ($F_v/F_o$), effective quantum yield $Y(II)$, photochemical quenching coefficient ($qP$), non-photochemical quenching coefficient ($qN$), quantum yield of non-regulated energy dissipation $Y(NO)$, quantum yield of regulated energy dissipation $Y(NPQ)$, etc.

***Table A6.*** *Effect of KT on fluorescence parameters of Landoltia punctata under As stress*

| KT ($\mu mol \cdot L^{-1}$) | | $Y(II)$ | $qP$ | $qN$ | $Y(NO)$ | $Y(NPQ)$ | $F_v/F_m$ | $F_v/F_o$ |
|---|---|---|---|---|---|---|---|---|
| 0 | AV | $0.23 \pm 0.014^a$ | $0.63 \pm 0.012^a$ | $0.74 \pm 0.031^a$ | $0.48 \pm 0.010^f$ | $0.29 \pm 0.009^a$ | $0.42 \pm 0.011^a$ | $0.69 \pm 0.116^{ab}$ |
| 0.001 | AV | $0.23 \pm 0.006^a$ | $0.54 \pm 0.022^b$ | $0.45 \pm 0.022^{cd}$ | $0.52 \pm 0.004^e$ | $0.21 \pm 0.007^c$ | $0.43 \pm 0.004^a$ | $0.66 \pm 0.056^{ab}$ |
| 0.01 | AV | $0.17 \pm 0.005^b$ | $0.56 \pm 0.006^b$ | $0.44 \pm 0.007^{de}$ | $0.57 \pm 0.009^d$ | $0.22 \pm 0.009^{bc}$ | $0.45 \pm 0.004^a$ | $0.73 \pm 0.104^a$ |
| 0.1 | AV | $0.16 \pm 0.001^{bc}$ | $0.49 \pm 0.025^c$ | $0.40 \pm 0.009^e$ | $0.60 \pm 0.003^c$ | $0.22 \pm 0.004^b$ | $0.38 \pm 0.002^b$ | $0.60 \pm 0.048^{ab}$ |
| 1 | AV | $0.15 \pm 0.002^c$ | $0.46 \pm 0.032^c$ | $0.49 \pm 0.008^c$ | $0.63 \pm 0.005^b$ | $0.23 \pm 0.005^b$ | $0.34 \pm 0.009^c$ | $0.51 \pm 0.051^{bc}$ |
| 10 | AV | $0.04 \pm 0.001^d$ | $0.25 \pm 0.004^d$ | $0.54 \pm 0.012^b$ | $0.68 \pm 0.000^a$ | $0.30 \pm 0.004^a$ | $0.25 \pm 0.025^d$ | $0.34 \pm 0.044^c$ |

Data are expressed mean ± SD, n = 3; the letters after the numbers indicate the significant differences between different KT treatment at a level of 0.05. Where KT is the abbreviation for Kinetin. Meaning of relevant indicators: the maximum photochemical photoefficiency ($F_v/F_m$), potential photochemical efficiency ($F_v/F_o$), effective quantum yield $Y(II)$, photochemical quenching coefficient ($qP$), non-photochemical quenching coefficient ($qN$), quantum yield of non-regulated energy dissipation $Y(NO)$, quantum yield of regulated energy dissipation $Y(NPQ)$, etc.