Reasonable mowing and nitrogen fertilization promote chromium remediation by the hyperaccumulator Leersia hexandra Swartz

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Abstract

Leersia hexandra Swartz, is a chromium (Cr) hyperaccumulator, but the potential for continuous remediation or reasonable mowing and nitrogen fertilization pattern has not been fully characterized. This study aimed to investigate the Cr remediation by *L. hexandra* under reasonable mowing and nitrogen fertilization patterns using hydroponic experiments. The mowing heights (no mowing, CK; the shoots were reserved for 0, 5 and 10 cm), mowing time (CK; the shoots were mowing after 30, 60, 90 and 120 days), mowing frequencies (CK; the shoots were mowing for 1, 2 and 3 times) and concentrations (42, 84 and 168 mg/L) of nitrogen fertilization (calcium nitrate, ammonium sulfate, and urea) have effects on biomass, Cr concentration and accumulation in shoots of *L. hexandra*. The results show that the highest biomass (67.0 g) and Cr accumulation (5.33 mg/pot) of *L. hexandra* found at mowing pattern (10 cm, 90 days, three times), which were increased by 91.4%, and 36.0% compared to CK, respectively. The addition of 84 mg/L ammonium sulfate significantly increased $(p < 0.05)$ the biomass and Cr accumulation of *L. hexandra*. In addition, the contents of hormones GA3, 6-BA, 6-KT, TZR and JA in leaves were significantly increased under reasonable mowing and nitrogen fertilization treatments, while IPA and JA in stems. In conclusion, the study results have shown that reasonable mowing and nitrogen fertilization are effective options for improving the continuous phytoremediation of Cr-contaminated.

1. Introduction

Chromium (Cr) is widely used in the industry as a plating or alloying agent, pigment, and tanning agent, as well as in manufacturing textile dyes, mordants, pigments, ceramic glazes, refractory bricks, and pressure-treated lumber, and as an anti-corrosive material (Pushkar et al. 2021, Shanker 2019). The wide use of Cr has aggravated the consequent environmental contamination, becoming a cause for increasing concern in recent years (Adhikari et al. 2022, Sharma et al. 2021). Therefore, how to remediate chromium pollution has become a recent research topic (Ali et al. 2022, Bao et al. 2022, Liu et al. 2018). Phytoremediation, a green remediation technology, is more efficient and cheaper than traditional physicochemical remediation techniques and does not cause secondary pollution (Ali et al. 2013, Malaviya et al. 2020, Padmavathiamma &Li 2007). The hyperaccumulator is the core vehicle of phytoremediation technology (Li et al. 2018).

Leersia hexandra Swartz is the first chromium hyperaccumulator discovered in China (Zhang et al. 2007). The *L. hexandra* has a high capacity for Cr uptake and tolerance, accumulating Cr in the stems and leaves of the plant, thus showing great potential for phytoremediation of Cr contamination (Liu et al. 2014, Wang et al. 2020). However, the phytoremediation efficiency of Cr by *L. hexandra* is still constrained because of factors, such as the growth cycle and biomass, despite the high levels of Cr uptake in *L. hexandra*. Increasing Cr accumulation in plants is a decisive factor in promoting phytoremediation efficiency (Yang et al. 2020). Many planting methods have been used to improve the accumulation of heavy metals in plants, such as plant-growth stimulating bacteria (Chen et al. 2017), intercropping (Deng et al. 2016), seedling establishment methods (Deng et al. 2021), etc. Some studies show that because of the own growth cycle of plants, they could adopt the mowing method involving multiple harvests a year at different stages to increase the biomass and thus increase Cr accumulation (Dou et al. 2022, Wei et al. 2006).

L. hexandra has a strong regenerative capacity and fast growth rate and can be mowed several times without affecting its growth (Zhang et al. 2009). Therefore, mowing the shoots of *L. hexandra* not only removes a significant amount of Cr, but also potentially promotes its growth to improve chromium removal. The regeneration of plants after mowing is related to mowing patterns (Kubo et al. 2010). The mowing patterns include mowing height, mowing time, and mowing frequency. It has been shown that low-intensity mowing patterns cannot stimulate plant regeneration, while high-intensity mowing patterns can change plant resource allocation and inhibit plant shoot growth (Achichi et al. 2021, Bunnell et al. 2005, Zhao et al. 2019). Therefore, it is critical to determine the reasonable mowing patterns. However, it is unclear what mowing patterns will promote the regeneration of *L. hexandra* and thus promote the remediation of Cr.

Nitrogen (N) is the most essential nutrient that affects plant growth and development (Gao et al. 2021, Hessini et al. 2019). Nitrogen fertilization application speeds up the uptake of micronutrients and thus increases plant biomass and does not affect the chromium enrichment capacity of *L. hexandra* (Zhou et al. 2021, Zhu et al. 2016). Therefore, it is feasible to increase the amount of material in *L. hexandra* by applying nitrogen and promoting the remediation of Cr. Common plant nitrogen fertilizations include nitrate (NO_3^-), ammonium nitrogen (NH_4^+), urea and amino acids (Hu et al. 2014), etc. The different nitrogen fertilization forms affect plant biomass and metal uptake (Hassan et al. 2005, Nagar 2019). Therefore, it is vital to choose the proper nitrogen fertilization for the growth and development of plants. In addition, some studies have shown that the average nitrogen content in plants is about 1.5% (DW) and that plants cannot absorb and use excessive nitrogen fertilization (Yang et al. 2019). Unreasonable nitrogen application pollutes local soil, water and air, accelerates global climate change, and affects human health (Nasiroleslami et al. 2021, Sainju et al. 2019). Therefore, it is necessary to find a reasonable concentration of nitrogen fertilization to apply to plants. However, it is unclear precisely what nitrogen fertilization application pattern will promote the regeneration of *L. hexandra*, thus the remediation of Cr.

Plant growth is highly regulated by hormones (Zhang et al. 2019), including heteroauxin (IAA), indolebutyric acid (IBA), jasmonic acid (JA), gibberellin (GA) (Cui et al. 2020, Parwez et al. 2022, Wu et al. 2022, Zhou et al. 2019). Different phytohormones play essential roles in diverse growth and developmental processes and various biotic and abiotic stress responses in plants (Bari &Jones 2009, Hayashi et al. 2021). For instance, GA can promote plant growth by stimulating cell division and cell elongation, whereas JA can increase plant resistance to ecological stresses (Jiang et al. 2021, Novakova et al. 2014). In the present study, we hypothesized that reasonable mowing and nitrogen fertilization application might enhance the remediation of chromium by *L. hexandra*, inducing hormone level changes and resulting in biomass and Cr accumulation capacity alterations. The effects of mowing pattern and nitrogen fertilization application on biomass, chromium concentration and chromium accumulation of *L. hexandra* were thus investigated in a hydroponic experiment. The phytohormone concentration of the leaves and stems of *L. hexandra* was also measured. Specifically, this study aimed to find the reasonable mowing pattern and nitrogen fertilization application pattern to promote regeneration and chromium remediation in *L. hexandra*, and to identify the phytohormone that plays a vital role in the regeneration of *L. hexandra* leaves and stems.

2. Materials And Methods

2.1 Plant preculture and treatment

Seedlings of *L. hexandra* were collected from a paddy filed in Guilin, China (110°06'~ 110°41' E, 24°18'~ 24°46' N). The seedings were washed with deionized water three times. 20 ~ 30 healthy plants of *L. hexandra* with uniform growth were selected for one pot, and the fresh weight difference between pots was controlled at 4 g. Then, pots of *L. hexandra* in a round plastic drum made of polyethylene with a specification of 210mm×190mm. Each pot of *L. hexandra* seedlings was precultured for seven days in a modified Hoagland solution, the same formula as (Liu et al. 2020). All plants were grown under controlled environmental conditions with a 14h (6:00 ~ 20:00) photoperiod, a 30 ℃/25 ℃ day/night temperature regime, 70%~75% relative humidity, and light source of white light plus violet light.

2.2 Mowing experiments

Mowing height experiment

To assess the efficiency of chromium (Cr) remediation by *L. hexandra*, the Cr treatment of precultured *L. hexandra* with 0, 40 and 80 μmol/L of potassium dichromate solution ($K_2Cr_2O_7$) in the Hoagland solution. The nutrient solution was renewed every three days, followed by the Cr treatment. The mowing height experiment included the control treatment (no mowing), and the shoots of the plant were mowed at 0 cm (H0), 5 cm (H5) and 10 cm (H10) above the culture solution with scissors. The shoots of *L. hexandra* were mowed, as described above, after 30 days of Cr treatment. The root was left intact to allow for the re-growth of shoots. After the re-growth of shoots for following 30 days, the shoots of *L. hexandra* were harvested. The total period of the mowing height experiment is 60 days. Each treatment was performed in triplicates.

The mowed and harvested shoots were rinsed three times in deionized water. The washed shoots were oven-dried at 70 ℃ for two days to determine the dry weight (DW). Each dried sample (about 0.2g) was digested with concentrated nitric acid and perchloric acid (5:3, v/v) in a block heater (Skrzydlewska et al. 2003). The Cr concentrations in digestates were quantified using inductively coupled plasma mass spectrometry (ICP-MS, NexION 2000b, Perkin-Elmer, USA). Quality control for Cr analysis was performed by a certified reference material (GBW07603). The recovery of Cr in all cases was 90 ~ 108%. The Cr accumulation equals the biomass multiplied by the Cr concentration (Yalcin et al. 2020).

Mowing time experiment

The Cr treatment of *L. hexandra* was the same as mentioned above. The mowing time experiment included the control treatment (no mowing), and the shoots of the plant were mowed at 10 cm (the reasonable mowing height of 2.2.1) above culture solution with scissors after 30, 60, 90 and 120 days of Cr treatment, and used for biomass and Cr concentration measurement. The roots were left intact to allow for the re-growth of shoots the following 150, 120, 90 and 60 days. After the re-growth of shoots, the shoots of the plant were mowed as described above. The total period of the mowing time experiment is 180 days. Each treatment was performed in triplicates. The shoots biomass and Cr concentration were also measured by the same methods used in the mowing height experiment.

Mowing frequency experiment

The Cr treatment of *L. hexandra* was the same as mentioned above. The shoots of plant were mowed at 10 cm (the reasonable mowing height of 2.2.1) above culture solution with scissors. The mowing frequency experiment included the control treatment (no mowing), one time (mowing after 90 days, the reasonable mowing time of 2.2.2), two times (mowing after 90, 180 days) and three times (mowing after 90, 180, 270 days). After each mowing, the underground rhizomes and roots were left intact to allow for the re-growth of shoots. After the re-growth of shoots, the shoots of *L. hexandra* were mowed as described above, and the biomass and Cr concentration were also measured by the same methods used in the mowing height experiment. The total period of the mowing frequency experiment is 360 days.

2.3 Nitrogen fertilization experiment

To evaluate the effect of nitrogen fertilization forms and concentration on the Cr accumulation of *L. hexandra*, nitrogen fertilizations were added at 42, 84 and 168 mg/L as calcium nitrate [$Ca(NO_3)_2$], ammonium sulfate [$(NH_4)_2SO_4$] and urea [$CO(NH_2)_2$] to the nitrogen-free Hoagland nutrient solution (Table 1) of Cr treatment. The Cr treatment of *L. hexandra* was added to 80 μmol/L as $K_2Cr_2O_7$. After 90 days of Cr and nitrogen fertilization application, the shoots of the plant were mowed at 10 cm above the culture solution with scissors. And then, the shoots of the plant were harvested after 30 days of cutting for biomass and Cr concentration measurement.

Table 1
The formula of nitrogen-free Hoagland’s nutrient solution

Drug Name	Concentration (mg/L)
Monopotassium phosphate (KH_2PO_4)	136
Magnesium sulfate ($MgSO_4$)	493
Iron sulfate heptahydrate ($FeSO_4·7H_2O$)	2.78
Ethylenediamine tetraacetic acid disodium ($C_{10}H_{16}N_2Na_2O_8$)	3.73
Potassium iodide (KI)	0.83
Boric acid (H_3BO_3)	6.2
Manganese sulfate ($MnSO_4$)	22.3
Zinc sulfate ($ZnSO_4$)	8.6
Sodium molybdate (Na_2MoO_4)	0.25
Cupric sulfate ($CuSO_4$)	0.025
Cobalt chloride ($CoCl_2$)	0.025

2.4 Measurement of phytohormone concentration

L. hexandra in chromium environment (80 μmol/L as $K_2Cr_2O_7$) has two treatments: 1) in the mowing pattern (10 cm, 90 days, 3 times) and the addition of 84 mg/L ammonium sulfate, 2) no mowing, no nitrogen fertilization. And then, the *L. hexandra* stems and leaves were harvested after 30 days of mowing for hormone determination. The solid phase extraction (SPE), and rapid and sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) were used to analyze and determine 18 phytohormones in the final harvested stems and leaves of *L. hexandra* (Yalcin et al. 2020).

Samples were weighed, ground and put into a 10 mL centrifuge tube (with plug); 5 mL extracting solution (Methanol: water: formic acid, 15:4:1, with 0.5% BHT) was added into the tube. After the mixture had been mixed with the Vortex oscillator and ultrasonic crushed for 30 minutes, it was placed under − 40 ℃ for 60 min. After that, the mixture was centrifuged (4℃, 12000rpm, 10min), and the supernatant was moved into new clean centrifuge tubes. Solid phase extraction: (1) Activation: wash the extraction column with 3mL water and 3mL methanol, sequentially. (2) Adsorption: pump the supernatant through the column (the flow rate should be less than 1 mL/min). (3) Washing: wash the column with 3mL water and an aqueous solution with 10% methanol. (4) Elution: pump 2 mL methanol through the column and collect the liquid. The eluent was dried with an evaporation concentrator, then 200μL 80% methanol was added, and the solution was whirled and centrifuged (4℃, 12000rpm, 10min) once again. The supernatant could now be analyzed with LC-MS/MS.

2.5 Statistical analyses

All data were summarized, and mean and variance were found using Excel 2019. One-way analysis of variance (ANOVA) was performed using SPSS 23.0. When ANOVA showed significant differences ($p < 0.05$), a posteriori comparison of means was made using the Fisher's least significant difference (LSD) test at the 95% confidence level. Use Origin 2021 to plot bar and box plots.

3. Results

3.1 Effect of different mowing and nitrogen fertilization patterns on biomass

The effects of different mowing heights on the shoot biomass of *L. hexandra* are shown in Fig. 1(a). At the chromium treatment of 0, 40 and 80 μmol/L, the shoot biomass of *L. hexandra* at the mowing height of 10 cm was the greatest, increasing by 23.0%, 28.2% and 28.9% than the control, respectively. However, at chromium concentration of 80 μmol/L, the shoot biomass of *L. hexandra* in the 0 cm mowing height treatment was lower than the control, which suggested excessive mowing suppresses the growth of *L. hexandra*.

The effects of different mowing times on the shoot biomass of *L. hexandra* at a mowing height of 10 cm are shown in Fig. 1(b). At 0, 40, and 80 μmol/L Cr concentration treatments, the shoot biomass accumulation of *L. hexandra* was most significant at mowing time treatment of 90 days, increased by 30.0%, 27.0% and 21.0% compared to the control, respectively. However, before mowing, under 40 μmol/L Cd treatment, the shoot biomass of 30 ~ 60 days, 60 ~ 90 days, and 90 ~ 120 days increased by 17.9%, 56.3%, and 21.6%. The above results showed that *L. hexandra* grows fastest at 60 ~ 90 days and slowly at 30 ~ 60 days and 90 ~ 120 days.

Based on a mowing height of 10 cm and a mowing time of 90 days, the total shoot biomass of *L. hexandra* increased with the number of mowing as shown in Fig. 1(c). The shoot biomass of all mowing frequency treatments (one, two and three times mowing) increased significantly over the control (CK) biomass by 48.1%, 62.1% and 91.4%, respectively. The total biomass of *L. hexandra* was greatest (67.0 g/pot) in three mowing treatments and significantly increased compared to one and two mowing treatments. However, there were no significant differences in biomass between the one time mowing and the two times mowing.

The effect of different nitrogen forms and concentration on the shoot biomass of *L. hexandra* is shown in Fig. 1(d). At N concentration of 42 mg/L, the shoot biomass was greatest with the addition of $(NH_4)_2SO_4$, which was 8.00 g/pot. At N concentration of 84 mg/L and 168 mg/L, the shoot biomass was greatest with the addition of $Ca(NO_3)_2$, 11.5 and 10.6 g/pot, respectively. However, there were no significant differences between the biomass of the $(NH_4)_2SO_4$ and $CO(NH_2)_2$ at an N concentration of 168 mg/L.

3.2 Effect of different mowing and nitrogen fertilization patterns on chromium concentration

The effects of different mowing heights on Cr concentration in shoots of *L. hexandra* are shown in Fig. 2 (a) and (b). Before mowing, under the 0, 40 and 80 μmol/L Cr concentration treatments, there were no significant differences in Cr concentration in shoots at different mowing heights treatments (H0, H5 and H10). After mowing, under 80 μmol/L Cr concentration treatments, there were no significant differences in Cr concentration in shoots at different mowing heights treatments (H0, H5 and H10). However, under the 40 μmol/L Cr concentration treatments, the highest Cr concentration in shoots was found in the 0 cm mowing height treatment, which was 90.0 mg/kg and 123 mg/kg, respectively. The probable reason is that the stems and leaves were in direct contact with the chromium-containing culture for foliar uptake after regeneration.

The effects of different mowing time on Cr concentration in shoots of *L. hexandra* based on a mowing height of 10 cm are shown in Fig. 2 (c) and (d). Before mowing (Fig. 2c), the Cr concentration in shoots of 30 ~ 60 days, 60 ~ 90 days, and 90 ~ 120 days increased by 78.5%, 34.4%, 14.0% under 40 μmol/L Cd treatment, which indicated that the fastest rate of Cr uptake by *L. hexandra* at 30 to 60 days. After mowing (Fig. 2d), under 40 and 80 μmol/L Cd treatment, the highest Cr concentration in shoots was found at 90 ~ 180 days, were 139 mg/kg and 198 mg/kg. The results indicated the fastest Cr uptake rate by *L. hexandra* 90 days after mowing.

The effects of different mowing frequencies on Cr concentration in shoots of *L. hexandra* are shown in Fig. 2 (e). The results showed no significant differences in Cr concentration at different mowing frequency treatments (one, two and three times mowing).

The effects of different nitrogen forms and concentration on Cr concentration of *L. hexandra* are shown in Fig. 2(f). At N concentrations of 42, 84 and 168 mg/L, the Cr concentration in shoots of *L. hexandra* with the addition of $(NH_4)_2SO_4$ was higher than the addition of $Ca(NO_3)_2$ and $CO(NH_2)_2$, by 203 mg/kg, 255 mg/kg and 143 mg/kg, respectively. The Cr concentration in shoots of *L. hexandra* with the addition of $(NH_4)_2SO_4$ at the N concentration of 84 mg/L was higher than at the N concentration of 42 and 168 mg/L.

3.3 Effect of different mowing and nitrogen fertilization patterns on chromium accumulation

The effect of different mowing heights on chromium accumulation of in shoots *L. hexandra* is shown in Fig. 3(a). The highest chromium accumulation in shoots of *L. hexandra* at a mowing height of 10 cm was observed under the treatment of 40 and 80 μmol/L chromium concentration, increasing 23.4% and 31.8%, respectively, compared with the control (CK). However, the accumulation of chromium was lower in *L.*

hexandra at a mowing height of 0 cm under 0, 40, and 80 μmol/L chromium concentration treatments than the control, which indicated that over-mowing is not conducive to the accumulation of chromium in *L. hexandra*.

The effects of different mowing times on chromium accumulation in shoots of *L. hexandra* based on a mowing height of 10 cm are shown in Fig. 3(b). The accumulation of Cr in *L. hexandra* increased and then decreased after mowing at different mowing times under the treatment of 40 and 80 μmol/L Cr concentration. The maximum accumulation of Cr at the mowing time of 90 days increased by 57.1% and 40.2%, respectively, compared to the control. However, the Cr accumulation at the mowing time of 120 days was lower than that at the mowing time of 90 days.

The effects of different mowing frequencies on the accumulation of Cr accumulation in shoots of *L. hexandra* based on a mowing height of 10 cm and a mowing time of 90 days are shown in Fig. 3(c). Mowing three times of *L. hexandra* has the most significant accumulation of Cr, which was 5.33 mg/pot, increased by 36.0% compared to the control. The mowing one and two times all showed a more significant increase in Cr accumulation than the control (CK) by 12.4% and 28.3%, respectively. However, no significant difference in Cr accumulation between mowing two and three times.

The effect of nitrogen fertilization form and concentration on Cr accumulation in shoots of *L. hexandra* is shown in Fig. 3(d). The application of 84 mg/L of $(NH_4)_2SO_4$ *L. hexandra* showed the highest accumulation of chromium at 1.54 mg/pot, which was significantly different ($p < 0.05$) from the application of the same concentration of $Ca(NO_3)_2$ and $CO(NH_2)_2$. The results indicated that the application of 84 mg/L of $(NH_4)_2SO_4$ facilitates the accumulation of chromium by *L. hexandra*.

3.4 Effect of reasonable mowing and nitrogen application on phytohormone concentration

Based on the MRM method (Sibel et al. 2019), a phytohormone-targeted metabolomes analysis was carried out to compare the 18 phytohormones concentration changes in the stems and leaves of *L. hexandra* in the control group (no mowing and no nitrogen application) and the assay group (optimal mowing and nitrogen application), as shown in Figs. 4 and 5. As shown in Fig. 4, the concentration of ABA, GA3, NAA, KT, 6-BA, IBA, 6-KT, 2-IP, GA7, SA and TZR was up-regulated in the regenerated leaves of *L. hexandra* in the assay group compared to the control. Compared with the control group, the concentration of ABA, GA3, 6-BA, 6-KT, and TZR in the assay group was highly significant ($p < 0.001$) and increased by 1.38, 1.42, 1.52, 1.12, and 1.25 times, respectively, compared with the control. However, the concentration of NAA, KT, IBA, GA7 and SA was not significantly different from the control. The concentration of IPA, GA1, GA4, IAA, tZT, JA, and MT in regenerated leaves of *L. hexandra* was down-regulated after mowing and nitrogen application to 72.0%, 97.0%, 82.0%, 97.0%, 86.0%, 55.0%, and 82.0% of the control, respectively. The concentration of JA and MT was significantly reduced compared to the control ($p <$ 0.001), the concentration of GA4 and tZT was strongly and significantly lower compared to the control (*p*

< 0.01). However, the concentration of IPA, GA1 and IAA was not significantly different compared to the control.

As shown in Fig. 5, only five phytohormones were up-regulated in the stems of *L. hexandra* after mowing compared with the control, and they were ABA, GA3, IPA, GA7 and tZT. The concentration of the remaining 13 phytohormones was down-regulated, which differed significantly from the changes in hormone dynamics in the leaves. Among the up-regulated hormones, the concentration of IPA was significantly higher than the control by 3.24-fold ($p < 0.001$). The concentration of ABA and GA3 was significantly increased ($p < 0.05$) compared to the control, and 1.06 and 1.71 times higher than the control, respectively. The concentration of GA7 and tZT was not significantly different from the control. However, the concentration of 2-IP, GA1, GA4, IAA, JA, TZR, and MT was all highly significant ($p < 0.001$) lower than the control. 2-IP, GA1, GA4, IAA, TZR, and MT decreased in their concentration, which are phytohormones that promote cell division and growth.

4. Discussion

4.1 Reasonable mowing and nitrogen fertilization promote *L. hexandra* regeneration

The ability to regenerate plants determines whether it can promote the efficiency of chromium remediation. The regenerate capacity affects the plant and whether it accumulates sufficient carbohydrates to ensure Cr uptake after reaching a basal growth state. And shoots biomass accumulation responds directly to the ability of plants to regenerate. The present study showed that the highest of shoots biomass accumulation of *L. hexandra* was found at reasonable mowing (10 cm, 90 days, three times); the biomass accumulation increased by 31.8%, 40.2% and 36.0% compared with the control experiment (no mowing), respectively (Fig. 1). The treatment with a mowing height of 10 cm showed the highest biomass accumulation, because a mowing height of 10 cm leaving more stubble will allow the meristematic tissue to absorb carbohydrates and keep more actively dividing cells, promoting plant regeneration (Cui et al. 2021). However, because of the average height of *L. hexandra* cultured in this experiment being less than 15 cm, whether the mowing height range of > 10 cm has a greater biomass accumulation needs to be further investigated. The above results showed that *L. hexandra* grows fastest at 60 ~ 90 days and grows slowly at 30 ~ 60 days and 90 ~ 120 days. And based on the 10 cm mowing height, the highest biomass accumulation was observed at 90d mowing time due to the fast growth rate. This result indicates that the treatment of 90d mowing time promotes the regeneration of *L. hexandra*. Based on 10 cm and 90 days, the highest biomass accumulation was observed in the mowing frequency of 3 times treatment because of the long total growth period and the high number of harvests. The above results indicated that the mowing frequency of 3 times promoted biomass accumulation in shoots, probably because the damage to the above-ground parts of plants after mowing stimulated the optimal distribution of assimilate functioning in plants and promoted photosynthesis, thus promoting compensatory above-ground growth (Zhao et al. 2022). Under the reasonable mowing patterns, the application of $Ca(NO_3)_2$ at 84 mg/L had the greatest biomass accumulation of 11.5 g/pot for *L. hexandra* (Fig. 1d), indicating that $Ca(NO_3)_2$ application had the best effect on the regeneration of *L. hexandra*. Because nitrate nitrogen promotes the uptake of cations such as K^+, Ca^{2+}, and Mg^{2+} in *L. hexandra*, and these cations increase the osmotic potential of the cells and facilitate cell regeneration (Engels &Marschner 1993). To summarize the above, the combined reasonable mowing and nitrogen fertilizer application can significantly promote the regenerative capacity of *L. hexandra*.

The regulation of endogenous phytohormones is important in the process of plant regeneration. The concentration of ABA, GA3, 6-BA, 6-KT, and TZR were highly significantly increased in the leaves of *L. hexandra* treated with reasonable mowing and nitrogen fertilization compared with the control (no mowing, no nitrogen fertilization) (Fig. 5). In particular, the content of 6-BA increased by 1.52-fold compared to the control, which can plant cell growth, inhibiting the degradation of plant chlorophyll and delaying leaf senescence (Fang et al. 2020). Similarly, GA3 is also a growth-promoting hormone and contributes to the regeneration of plants (Singh et al. 2011). Meanwhile, the up-regulation of ABA content can improve the adaptability and tolerance of regenerative leaves to Cr environment after mowing of the plant. Therefore, the up-regulation of ABA, GA3, 6-BA, 6-KT, and TZR contents could effectively promote cell division and proliferation, stimulate stem bud proliferation, and promote regeneration of leaves in *L. hexandra* (Ahmed et al. 2014, Fang et al. 2020, Yaronskaya et al. 2007). In addition, the IPA content in the stems of *L. hexandra* was highly significantly increased, and the 2-IP, GA1, GA4, IAA, JA, TZR and MT contents were highly significantly decreased compared with the control (Fig. 6). IPA is a growth hormone with biologically active. Thus, the up-regulation of IPA content can promote the growth of *L. hexandra*. However, the down-regulation of 2-IP, GA1, GA4, IAA, JA, TZR and MT may be detrimental to the regeneration of *L. hexandra*. Because in the regeneration process, the stem mainly helps to construct the leaf morphology and distribute leaves regularly for adequate photosynthesis, thus promoting more leaf regeneration. The stem also transfers organic matter made by the leaves to the roots and other parts of the plant for regeneration and use by the plant. To summarize the above, reasonable mowing and nitrogen fertilizer application can alter the phytohormone content and increase biomass in shoots, thereby promoting the regeneration of shoots in *L. hexandra*.

4.2 Reasonable mowing and nitrogen fertilization promote the efficiency of chromium remediation by *L. hexandra*

Likewise, the ability to absorb and accumulate Cr of plants also determines the efficiency of chromium remediation by plants. Because the more Cr is absorbed and accumulated by the plant shoots, the more Cr is taken away from the Cr-contaminated environment. The Cr accumulation in shoots directly responds to the chromium accumulation capacity of the plant, which is the product of plant shoots biomass and the Cr concentration in shoots (Luo et al. 2016). In this study, the highest chromium concentration in shoots was found at 90 ~ 180 days, indicating that mowing promoted chromium absorption on the 90th day. Because mowing on the 90th day promoted the large absorption of water and inorganic salts by roots, Cr elements compete with K, Mg, and Ca in this process, stimulating the significant absorption of Cr by roots (Ullah et al. 2014). After calculating the product of biomass and chromium content, the highest accumulation of chromium in shoots was obtained at 90 days of mowing. However, mowing heights might not affect the Cr absorption capacity of *L. hexandra* in a highly Cr-contaminated environment (80μmol/L). A similar study was found by Qi et al. (Qi et al. 2019) who reported that mowing height did not affect the uranium absorb of *L. perenne*. Similarly, the present study showed that mowing frequencies might not affect the Cr absorption capacity of *L. hexandra* (Fig. 2e), which is similar to the study reported by Xue et al. (Xue et al. 2005) regarding mowing frequency not affecting arsenic absorb of *L. Pteris vittate*. By calculating, the highest Cr accumulation was found at the treatment of 10 cm and three times, which were 1.61 mg/pot and 5.33 mg/pot. Therefore, with constant Cr absorb capacity, the enhancement of Cr phytoremediation with *L. hexandra* was feasible by increasing shoots biomass. Because of the long period of phytoremediation with three times mowing, further improvements are necessary to make phytoremediation a feasible option for remediation of a Cr-contaminated environment. As mentioned above, nitrogen fertilization can increase plant shoots' biomass, thus promoting plant regeneration. In addition, nitrogen fertilization can enhance chromium phytoremediation efficiency (Liao et al. 2007). In this study, 84mg/L $(NH_4)_2SO_4$ has the highest chromium concentration, indicating that 84mg/L $(NH_4)_2SO_4$ promoted the Cr absorption capacity of *L. hexandra*. Because the hydrolysis of NH_4^+ in ammonium nitrogen decreases the pH of the nutrient solution, a lower pH value can enhance the reaction of Cr^{6+} being reduced to Cr^{3+} (Liu et al. 2014, Patterson et al. 1997). Moreover, Calba et al. (Calba et al. 2004) showed that the reduction of pH can increase the solubility of heavy metal compounds, thus causing the enhanced absorption of heavy metals in plants. Liu et al. (Liu et al. 2014)showed that *L. hexandra* more readily absorbed the trivalent chromium (Cr^{3+}). Therefore, the decrease in the pH of the nutrient solution may increase the solubility of chromium compounds in the nutrient solution, increasing chromium uptake by the roots of *L. hexandra*. Although the application of 84 mg/L $(NH_4)_2SO_4$ was not the highest biomass, it was calculated to have the highest chromium accumulation, which was 1.54 mg/pot. To summarize the above, reasonable mowing and nitrogen fertilizer application can increase Cr accumulation in shoots and thus improve the efficiency of phytoremediation of chromium in *L. hexandra*.

5. Conclusion

Overall, the high accumulation of Cr and biomass production suggest that *L. hexandra* has a great potential for Cr phytoremediation. Our study revealed that reasonable mowing (10 cm, 90 days, 3 times) and the application of 84 mg/L $(NH_4)_2SO_4$ can alter the phytohormone content and increase biomass in shoots, promoting regeneration of shoots in *L. hexandra*. Based on the quantitative analysis of phytohormone targeted metabolomes, GA3, 6-BA, 6-KT, TZR, and JA may be the main hormones involved in the leaf regeneration process of *L. hexandra*, and IPA and JA may be the main hormones in the stem regeneration process of *L. hexandra*. The 90 days mowing treatment and the application of 84 mg/L $(NH_4)_2SO_4$ increased the Cr concentration in shoots, indicating that mowing at 90th day and the application of 84 mg/L $(NH_4)_2SO_4$ can promote the ability of Cr absorption. However, mowing heights and frequencies might not affect the Cr absorption capacity of *L. hexandra*. Reasonable mowing (10 cm, 90 days, 3 times) and the application of 84 mg/L $(NH_4)_2SO_4$ increased Cr accumulation in shoots, which demonstrated that reasonable mowing and nitrogen fertilizer application can promote the efficiency of phytoremediation of chromium in *L. hexandra* is feasible.

Declarations

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Authorship contribution statement

J. Liu and X. Jiang: conceived the study. S. Ma and Z. Ding: collected data and prepared the data for analysis. X. Jiang and S. Ma: performed statistical analyses and literature review. S. Ma and X. Jiang: wrote the main manuscript text. D. Dai, S. Chen and J. Zhao: improved the draft. All authors contributed to the interpretation of results and revised the manuscript critically. All authors approved the manuscript.

Data availability

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

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors declare no competing interests.

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Figures

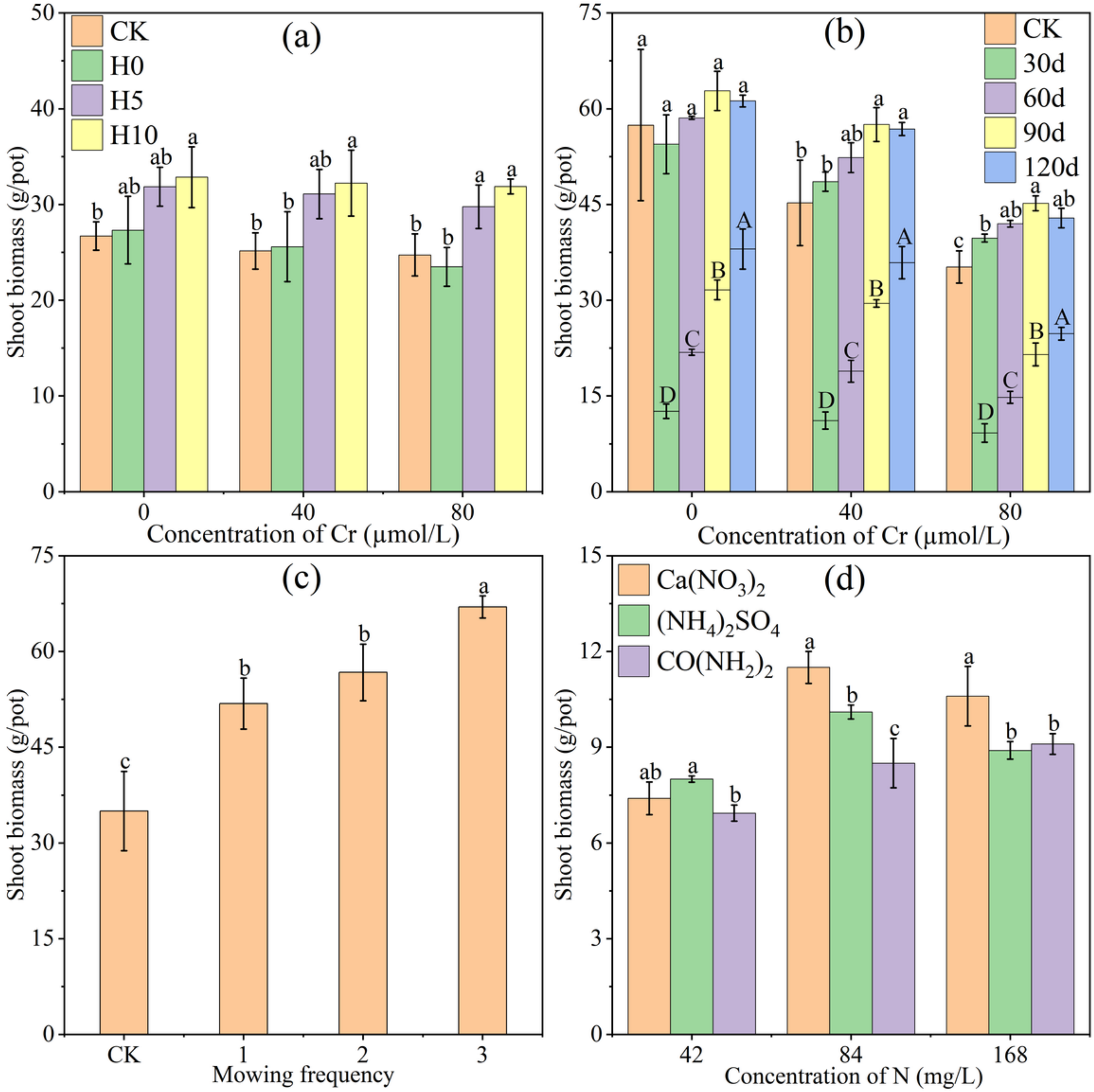

Figure 1

Effects of different mowing heights (a), mowing time (b), mowing frequency (c) and nitrogen fertilization (d) on shoot biomass accumulation of *Leersia hexandra* Swartz. In Fig. (a), CK represents no mowing, H0, H5, and H10 represent mowing at 0 cm (H0), 5 cm (H5) and 10 cm (H10) above culture solution, respectively. In Fig. (b), CK represents the plant grown in culture solution for 180 days, 30d, 60d, 90d, and 120d represent mowing on the 30th, 60th, 90th and 120th day. The different capital letters indicate there are significant differences among the values on shoot biomass before mowing according to the LSD test

($p < 0.05$,n = 3). The different lowercase letters indicate there are significant differences among the values on shoot biomass accumulation according to the LSD test ($p < 0.05$,n = 3).

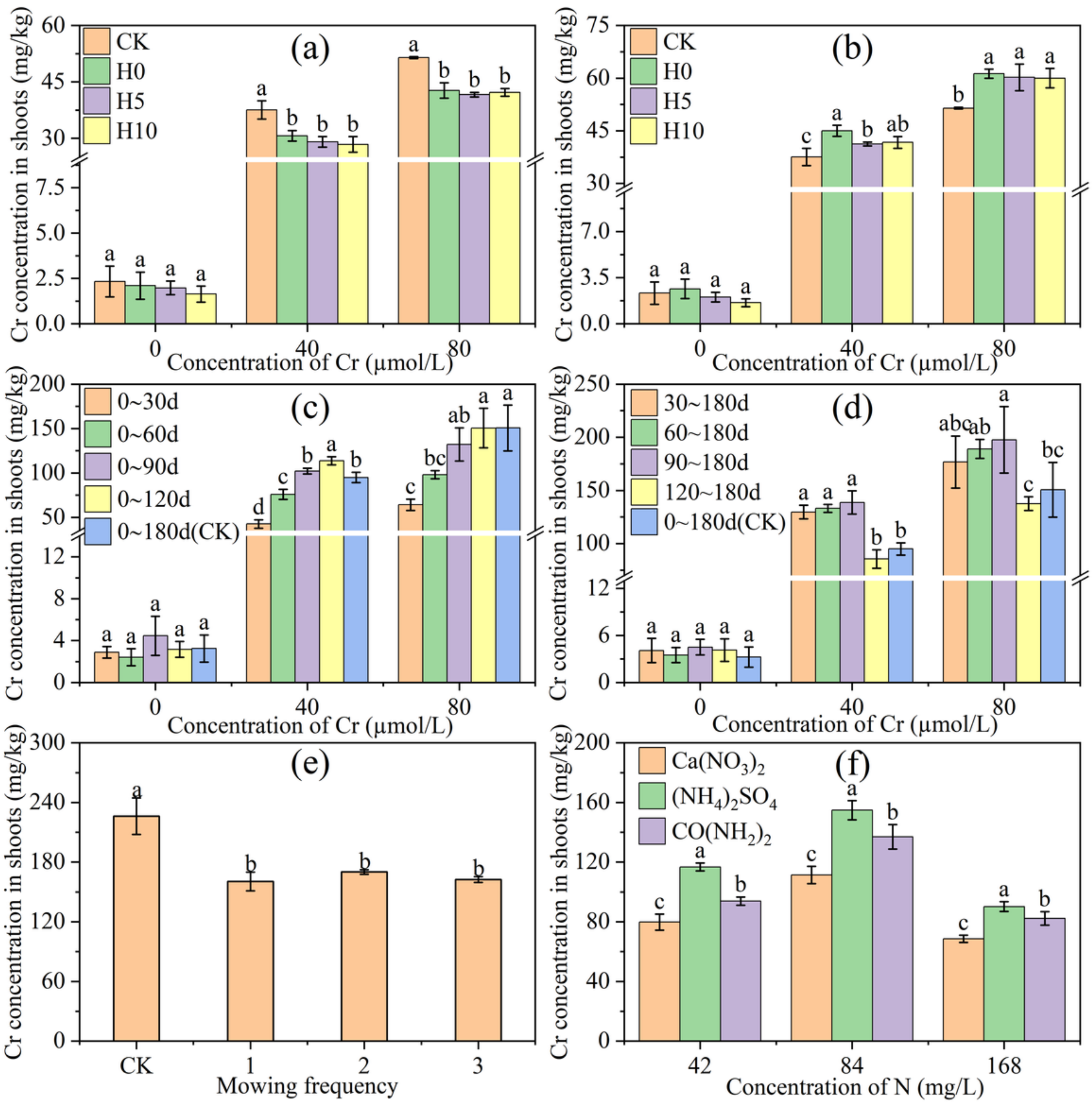

Figure 2

Effects of different mowing patterns (a), (b), (c), (d), (e) and nitrogen fertilization patterns (f) on chromium concentration in shoots of *Leersia hexandra* Swartz. (a) and (c) represent the chromium concentration in shoots of *L. hexandra* before mowing, (b) and (d) represent the chromium concentration in shoots of *L. hexandra* after mowing. Among (a) and (b), CK represents no mowing, H0, H5, and H10 represent mowing treatment 0cm, 5cm, and 10cm, respectively. Among (c) and (d), CK represents no mowing, 30d, 60d, 90d, and 120d represent mowing on the 30th, 60th, 90th and 120th day. The different letters indicate significant differences among the values according to the LSD test (p< 0.05, n = 3).

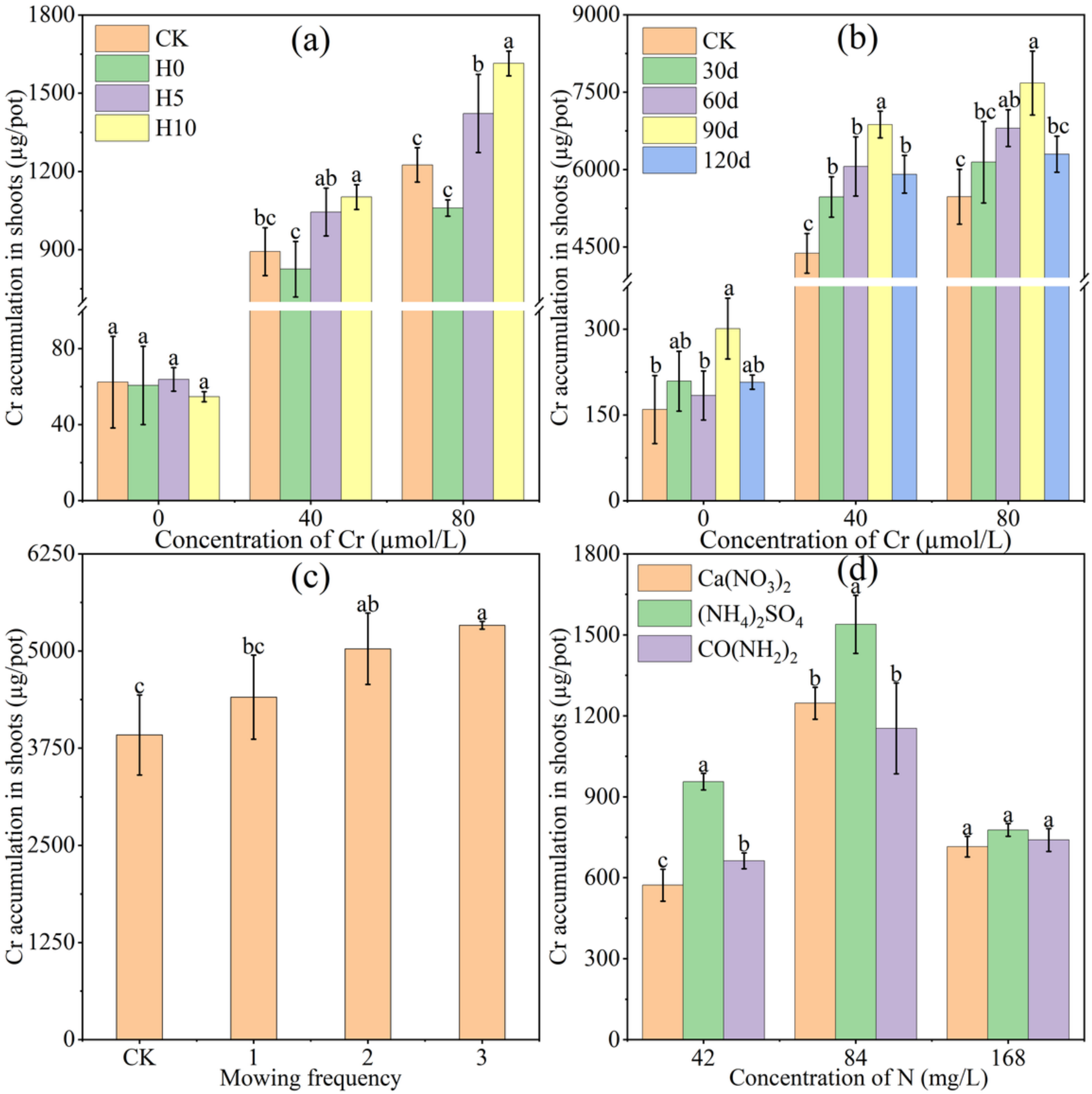

Figure 3

Effects of different mowing heights (a), mowing time (b), mowing frequency (c) and nitrogen fertilization (d) on chromium accumulation in shoots of *Leersia hexandra* Swartz. CK represents no mowing. In (a), H0, H5, and H10 represent mowing treatment 0cm, 5cm, and 10cm, respectively. In (b), 30d, 60d, 90d, 120d represent mowing at 30th, 60th, 90th and 120th day. The different letters indicate significant differences among the values according to the LSD test (p< 0.05, n = 3).

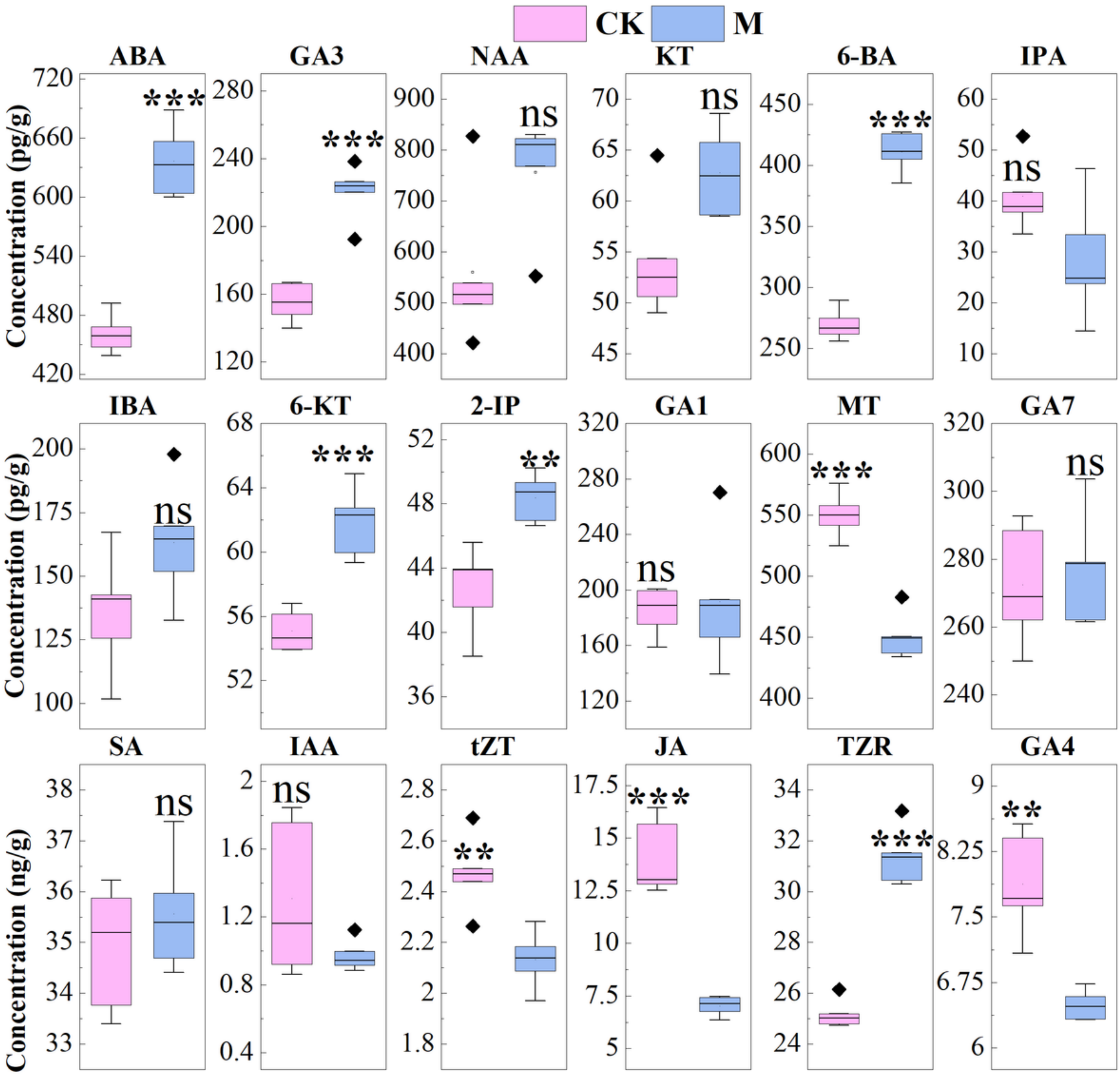

Figure 4

The contents of 18 phytohormones in leaves of *Leersia hexandra* Swartz. M represents reasonable mowing and nitrogen application treatment, and CK represents the control (no mowing, no nitrogen fertilization). Boxes include the 25th to 75th percentiles, horizontal lines within boxes represent median values, horizontal lines outside of boxes represent maximum and minimum values, and black diamonds represent outliers. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, no statistically significant difference.

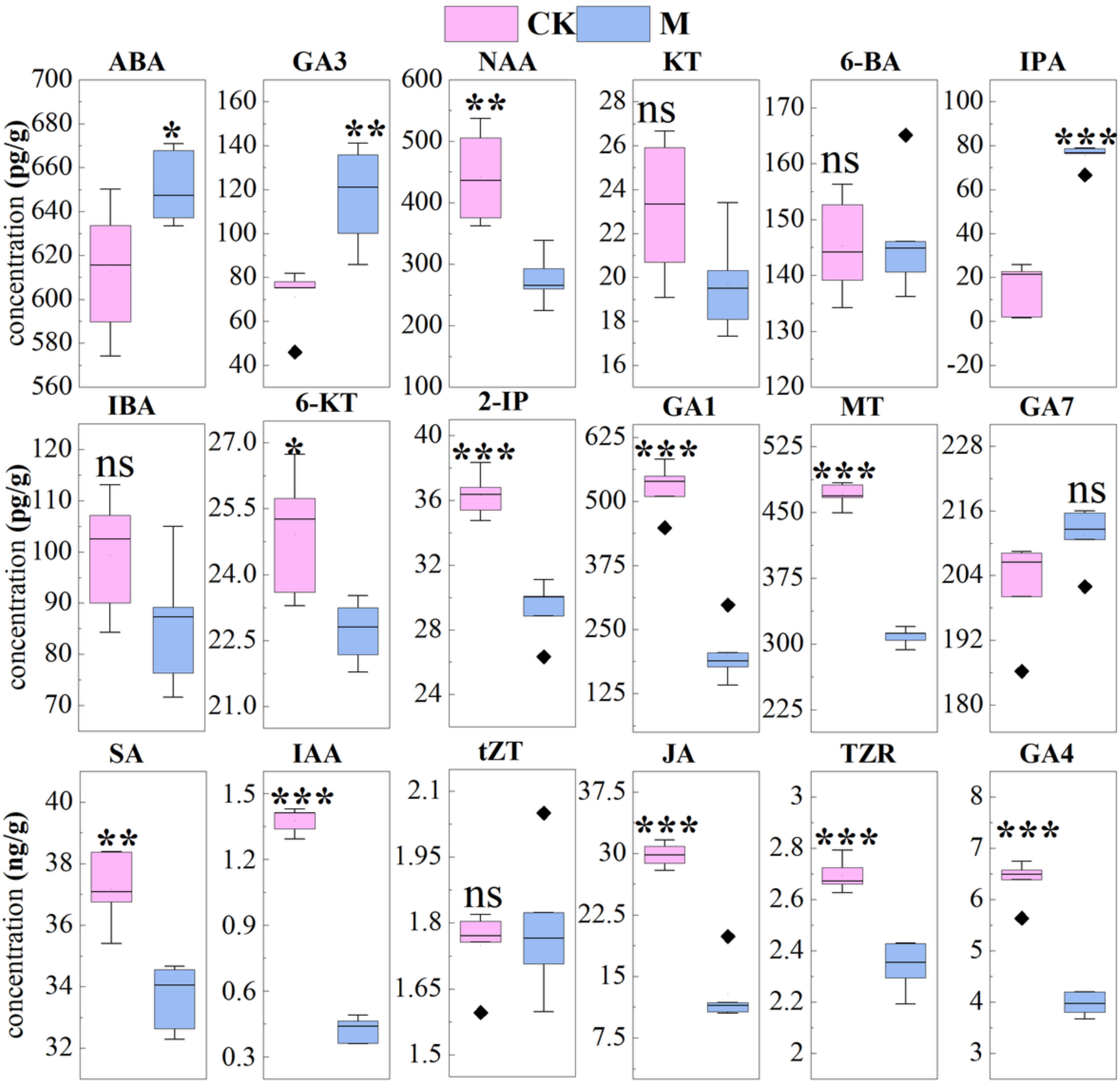

Figure 5

The contents of 18 phytohormones in stems of *Leersia hexandra* Swartz. M represents reasonable mowing and nitrogen application treatment, and CK represents the control (no mowing, no nitrogen fertilization). Boxes include the 25th to 75th percentiles, horizontal lines within boxes represent median values, horizontal lines outside boxes represent maximum and minimum values, and black diamonds represent outliers. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, no statistically significant difference.