The promotion of Festuca sinensis heavy metal stress tolerance mediated by Epichloë endophyte

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The promotion of *Festuca sinensis* heavy metal stress tolerance mediated by *Epichloë* endophyte

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Abstract

Background: *Festuca sinensis* is a perennial grass of the genus *Festuca*, which has strong stress tolerance and high adaptability. *F. sinensis* normally symbiotic with *Epichloë* endophyte. In order to evaluate the possibility of *F. sinensis*-endophyte association as bioremediation grass in heavy metal polluted soils, the effects and mechanism of the *F. sinensis*-endophyte interaction under heavy metal stress was investigated.

Results: The growth performance and physiology variations of *F. sinensis* with (E+) and without endophyte (E-) were evaluated after they were subjected to Zn^{2+} and Cd^{2+} treatments. The results showed that heavy metal treatments had significant effects on plants as the growth indices of plants under Zn^{2+} and Cd^{2+} treatments had significant differences compared with plants under control treatment (P<0.05). Zn^{2+} treatment had positive effects on plants whereas Cd^{2+} treatment had negative effects. The plants under

Cd^{2+} treatment produced more lolitrem B (*P<0.05*). Endophyte increased host heavy metal stress tolerance by promoting host growth as the E+ plants had significantly higher plant height, tiller number, root length (*P<0.05*). Endophyte also promoted host Zn^{2+} ion absorbing and induced more endogenous hormone production (*P<0.05*).

Conclusions: These results suggested that *Epichloë* regulated host growth and physiology to improve association tolerance to environmental conditions.

Key words: *Festuca sinensis*; *Epichloë sinensis*; Zn^{2+} treatment; Cd^{2+} treatment; growth, ion absorbing, hormone, alkaloids.

Background

Festuca sinensis, as native cool-season perennial grass species, distributed across the cold and semi-arid regions of China. This species grazed by cattle and sheep, is widely utilised in grassland production on the Qinghai–Tibet Plateau of China[1]. It is also important for grassland establishment, restoration of degraded grassland and ecological management [2]. *F. sinensis* is frequently infected with an asexual, symptomless *Epichloë* species [3-7]. This endophyte has been isolated and identified by morphology with colony, texture, conidia and conidiophore, and phylogene with house-keeping gene, which confirmed that the strain is new species name after *Epichloë sinensis* [8]. *Epichloë* endophytes interact mutualistically with their host plant, mainly by enhancing the fitness of the grass host and protection from both biotic and abiotic stresses [9,10]. Research to reveal the relationship between *F. sinensis* and *Epichloë* endophyte showed that associations between *F. sinensis* and endophytes produce alkaloids [11], and endophyte could increase *F. sinensis* seed germination and seedling growth [12], competition in mix-sowing grassland [13], enhance host cold-stress resistance [5,12], and improve host drought and waterlogged resistance [14]. However, the effects of endophyte on host tolerance to other stress such as heavy metal and salt have not been clarified.

At present, soils polluted by heavy metals occur more frequently across the globe, which has serious impacts on the survival of plants and ecosystem. Bioremediation is an effective method of treating heavy metal polluted soils. *Epichloë* endophytes can also increase the host stress tolerance of heavy metals such as cadmium (Cd), aluminum (Al), zinc (Zn)and copper (Cu) tolerance [15-19]. The relationship between *E. sinensis* endophyte and *F. sinensis* under heavy metal stress is unknown. The aims of this study were to investigate the effects and mechanism of *F. sinensis*-endophyte interaction under heavy metal stress, and to evaluate the possibility of *F. sinensis*-endophyte association as bioremediation in heavy metal polluted soils.

RESULTS

Plant growth

Plant height (Fig. 1A) and tiller numbers (Fig. 1B) of E+ plants were significantly higher ($P < 0.05$) than those of E- plants under two heavy metal (Zn^{2+} and Cd^{2+}) treatments and control (except for tiller numbers). The heavy metal treatments had significant effects on plant height (Fig. 1A). For both E+ and E- plants, the plant height was highest under Zn^{2+} treatment and lowest under Cd^{2+} treatment ($P < 0.05$). However, heavy metal treatments had no significant effects on tiller numbers for both E+ and E- plants (Fig. 1B).

The root length of E+ plants was significantly higher ($P < 0.05$) than that of E- plants under control and Zn^{2+} treatment (Fig. 2A). Heavy metal treatments only had significant effects on root length of E+ plants as the E+ plants had significantly longer root length under control and Zn^{2+} treatment than under Cd^{2+} treatment. The plant biomass (Fig. 2B) of E+ plants was significantly higher ($P < 0.05$) than those that of E- plants under Zn^{2+} treatment. The heavy metal treatments had significant effects on plant biomass. For both E+ and E- plants, the plant biomass was highest under Zn^{2+} treatment and lowest under Cd^{2+} treatment ($P < 0.05$).

Cd^{2+} and Zn^{2+} ion content

Both aboveground (Fig. 3A) and underground (Fig. 3B) Cd^{2+} ion contents were significantly higher ($P < 0.05$) in E+ plants than in E- plants under Cd^{2+} treatment. There was no significant difference between E+ and E- plants under control and Zn^{2+} treatment. For both E+ and E- plant, aboveground and underground Cd^{2+} ion contents were significantly higher ($P < 0.05$) under Cd^{2+} treatment than under control and Zn^{2+} treatments. There was no significant difference between control and Zn^{2+} treatment.

The aboveground Zn^{2+} ion content (Fig. 3C) of E+ plants were significantly higher ($P < 0.05$) than that of E- plants only under Zn^{2+} treatment. There was no difference in Zn^{2+} ion content between E+ and E- plants under control and Cd^{2+} treatment. For both E+ and E- plant, aboveground Zn^{2+} ion contents were significantly higher under Zn^{2+} treatment than under control and Cd^{2+} treatment. There was no difference in aboveground Zn^{2+} ion contents between control and Cd^{2+} treatments. The underground Zn^{2+} ion content (Fig. 3D) of E+ plants were significantly higher ($P < 0.05$) than that of

E- plants under control and Zn^{2+} treatment. There was no significant difference in underground Zn^{2+} ion content between E+ and E- plants under Cd^{2+} treatment. For E+ plant, the underground Zn^{2+} ion contents were highest under Zn^{2+} treatment and lowest under Cd^{2+} treatment ($P < 0.05$). For E- plant, the underground Zn^{2+} ion contents were significant higher under Zn^{2+} treatment than under control and Cd^{2+} treatments. There was no significant difference in the underground Zn^{2+} ion contents between control and Cd^{2+} treatments.

Plant hormone contents

The gibberellin (GA_3) contents (Fig. 4A) in E+ plants were consistently higher ($P < 0.05$) than in E- plants for all 3 treatments. For E+ plants, GA_3 contents highest under Zn^{2+} treatment were and lowest under Cd^{2+} treatment ($P < 0.05$). However, for E- plants, GA_3 contents were highest under control treatment and lowest under Cd^{2+} treatment ($P < 0.05$). The cytokinins (CTK) contents (Fig. 4B) in E+ plants were consistently higher ($P < 0.05$) than in E- plants for all 3 treatments. For both E+ and E- plants, CTK contents were highest under Zn^{2+} treatment and lowest under Cd^{2+} treatment ($P < 0.05$). The indole-3-acetic acid (IAA) contents (Fig. 4C) in E+ plants were significantly higher ($P < 0.05$) than those in E- plants for all 3 treatments. For both E+ and E- plants, IAA contents under control and Zn^{2+} treatments were significantly higher ($P < 0.05$) than those under Cd^{2+} treatment. The abscisic acid (ABA) contents (Fig. 4D) in E- plants were significantly higher ($P < 0.05$) than those in E+ plants under Zn^{2+} treatment. For both E+ and E- plants, ABA contents were highest under Cd^{2+} treatment and lowest under Zn^{2+} treatment ($P < 0.05$).

Alkaloids

E+ plants produced both tested alkaloids - peramine and lolitrem B whereas E- plants did not produce any alkaloids (Fig. 5). The heavy metal treatments only had significant effects on lolitrem B contents. There was no significant difference in peramine contents amongst these 3 treatments . The lolitrem B contents in E+ plants under Cd^{2+} treatment were significantly higher than those in E+ plants under control and Zn^{2+} treatments.

Discussion

Zn, a necessary trace element of plant, can promote plants growth. However, excessive Zn in soil will cause heavy metal pollution and reduce plants growth [20]. A previous study has shown that 300 mg/L Zn^{2+} in soil inhibited tillering and leaf extension and reduced biomass of *Achnatherum sibiricum* [21]. Another study showed that 20 $mg{\cdot}L^{-1}$ Zn^{2+} promoted *F. arundinacea* seed germination and biomass accumulation whereas 50 $mg{\cdot}L^{-1}$ Zn^{2+} inhibited seed germination [222]. In the present study, 500 $mg{\cdot}L^{-1}$ Zn^{2+} significantly increased ($P<0.05$) plant height and biomass of *F. sinensis* which suggest that *F. sinensis* has stronger resistance to Zn^{2+} and can grow in soil with high concentration of Zn^{2+}. Cd is one of the heavy metals that are most toxic to plants. It disturbs plant physiological processes, including photosynthesis, respiration and nutrient element absorption, and seriously inhibits plant growth and development [23]. Cd inhibited the germination and growth of *A. inebrians*, *Elymus dahuricus* and *Hordeum brevisubulatum*, leading to leaf yellowing and radicle browning, and biomass reduction [24,25]. Cd has a sustained inhibitory impact on seed germination and seedling growth of *A. sibiricum* [22]. The present study found that 100 mg/L Cd^{2+} inhibited plant height, biomass accumulation, and tillering, which were consistent with the findings of other researches that Cd usually inhibited plant growth.

In the present study, *Epichloë* endophyte significantly increased plant height, tiller number and biomass of *F. sinensis* under Zn^{2+} stress, and significantly increased tiller number and root growth under Cd^{2+} treatment. Similar results were found from previous researches which suggested that endophyte increased host tolerance to heavy metal and alleviate the toxicity. Bonnet et al.[16] also found that endophyte increased perennial ryegrass tolerance to Zn with increased aboveground biomass. Endophyte promote the performance of *Lolium perenne*, *F. arundinacea* and *A. sibiricum* tillering under Cd^{2+} treatment [19, 21]. Endophyte can alleviate the toxicity of Cd to *F. arundinacea* and *F. pratensis* as E+ plants have more biomass compared with E- plants. *Epichloë* endophytes can also increase host heavy metal stress tolerance such as Cd, Al, Zn and Cu [15-18].

In the present study, endophyte improve the absorption of $Zn^{2+}$and Cd^{2+} ion in plants which suggested that endophyte may not reduce host toxicity. The changes of ion absorption and distribution may have relationship with root exudate. The previous researches showed that endophyte improve the phenolics contents of *F. arundinacea* and *A. sibiricum* which reduce the toxicity of heavy metals [26-28]. Phenolics in root exudate of *F. arundinacea* could chelate with some heavy metal ions which reduced heavy metal activity and toxicity [29]. However, the ions improvement in plants need more explanation.

Heavy metal stress can disturb plant physiological processes, including photosynthesis, photoelectronic transfer and mineral nutrition absorption [30]. The response of host to abiotic stress such as heavy metal is very complicated. The endogenous hormones variation is one of the direct responses. Plant endogenous hormones are organic substances that regulate plant growth and development which may be part of a signal-transduction pathway and stimulate signal reactions for stress responses[31, 32]. Studies[33, 34] revealed that endogenous regulations (e.g. biosynthesis, transport, redistribution, and conjugation of plant hormones) play a crucial role during the acclimation process against stress. Exogenous application of plant hormones has also been reported to enhance stress tolerance in plants affected by heavy metals [35-37]. In the present study, the four tested hormone (GA_3, CTK, IAA and ABA) contents had significant variations under heavy metal stress. Zn^{2+} treatment increased the contents of GA_3 and CTK and reduced the contents of ABA. Cd^{2+} treatment reduced the contents GA_3 and IAA. Previous studies also revealed that Cd^{2+} treatment reduced the contents of IAA, ethylene and GA_3 in *Oryza sativa* which suggest that Cd^{2+} stress disturb the biosynthesis of endogenous hormones [38]. These changes of endogenous hormones confirmed that the plants utilize hormones during stress response. *Epichloë* endophyte also have significant effects on endogenous hormones in plants and increased GA_3, CTK and IAA contents and reduced ABA contents. These results were consistent with the previous researches that *Epichloë* endophyte change hormones to improve host stress tolerance [9,10, 39].

The benefits that endophytes confer on plant health, and conversely, detrimental effects on animal health, are partially due to the production of biologically active alkaloids [40-42]. Two such important alkaloid classes are the ergots and lolitrems (indole diterpenes) which cause neurotoxic effects on grazing and granivorous vertebrates, two other classes of endophyte-derived alkaloids, peramine and lolines, are known to be highly active against invertebrates, yet have little or no activity against mammalian species [43]. Alkaloids may play roles in host biotic stress tolerance such as pathogen and insects [44-74]. The contents of alkaloids varied with many factors including endophyte, host genotype and environmental conditions [40, 48]. In the present study, the contents of lolitrem B increased under heavy metal stress which suggest its association with stress tolerance. However, the mechanism needs more clarification.

CONCLUSION

Endophyte can promote the growth and development of *F. sinensis* under heavy metal stress. The mechanism that endophyte employed in improving host heavy metals stress tolerance include increasing the content of growth hormones such as IAA and GA_3, reducing the content of ABA and adjusting the alkaloid contents. This study has hence provided more evidence about the *Epichloë* endophyte relationship with hosts and extended the symbiosis research to more native species.

Methods

Plant materials

F. sinensis seeds were collected from endophyte infected (E+) or endophyte free (E-) plants in summer, 2016 in experimental field blocks (104°39′ E, 35°89′ N, Altitude 1653 m) at the College of Pastoral Agriculture Science and Technology (CPAST), Yuzhong campus of Lanzhou University [11]. The plants were grown from seed collected in Hongyuan, Sichuang (102°33'E, 32°48'N, Altitude 3491 m) in 2013.

Endophyte viability in seeds was assessed by aniline blue staining and microscopic examination [3]. After assessment, the seeds were stored in 4℃ until utilization. In August 2017, the well filled, healthy-looking E+ and E- seeds were planted in plastic trays (30 cm × 25 cm × 8 cm) filled with 1.5 kg soil (commercial fine sandy soil, Lanzhou) which had been sterilized in an oven at 130℃ for 30 min. Five rows with 10 seeds were planted per tray at a depth of 1 cm. Two trays per E+ and E- seeds were placed in a temperature controlled greenhouse (18℃ - 24℃) with 10 h of illumination per day in Yuzhong campus of Lanzhou University. After plants had 3 tillers, endophyte viability in E+ and E- populations seedlings were determined by microscopic examination of the host leaf sheath pieces after they had been stained with aniline blue [4]. The seedlings germinated from E+ seeds with characteristic longitudinally-orientated hyphae of the endophyte were marked as E+ and the seedlings germinated from E- seeds without hyphae were marked as E-.

Experimental design

The marker seedlings were transplanted into round pots (upper diameter 15.5 cm × lower diameter 11.5 cm × height 14 cm) containing the same amount of media (sterilized commercial vermiculite and black soil in a w/w ratio 3:1). Each pot had only one similar growth seeding and equal initial water treatment. After one-month stabilization with the same irrigation, three different treatments were established, which included control treatment, Zn^{2+} treatment and Cd^{2+} treatment. Each treatment has 5 replicates which were randomly placed in greenhouse maintained at a constant condition (temperature: 25 ± 2 ℃, humidity: 42 ± 5%). During the experimental period, the plants were watered 100 mL every 3 days, control treatment were watered as normal, Zn^{2+} and Cd^{2+} treatments were watered with 100 mL $ZnCl_2$ solution of 500 $mg \cdot L^{-1}$ and 100mL $CdCl_2$ solution of 100 mg/L at 1st and 14th day, respectively.

Experimental evaluations

Determination of endogenous phytohormones

After 28 days growth, 2 gram fresh leaves were collected from each plant for gibberellin (GA_3), indole-3-acetic acid (IAA), cytokinins (CTK) and abscisic acid (ABA) contents test using enzyme-linked immunosorbent assay (Danshi biology, Shanghai, China).

Plant growth

After 28 days growth, plant height and tiller number of each plant were recorded. The whole plants were then carefully removed from pots, washed with distilled water and dried on a filter paper. The root length per plant was determined and all harvested plants were separated into roots and shoots and their fresh weight recorded. Dry weight was obtained after oven-drying the tissue at 60°C until a constant weight was reached. The dry aboveground and underground parts from each treatment were weighed separately to determine total dry matter per plant. After weighting, the plant materials were ground twice using a mixer mill (Retch 400MM, German) at 30 Hz for 2 min for analysis of alkaloid, Zn^{2+} and Cd^{2+} ions contents.

Measurements of Zn^{2+} and Cd^{2+} ions contents

Zn^{2+} and Cd^{2+} contents were analysed by using atomic absorption spectrometry (M6AA system, Thermo, USA) after mineralization in mixture of acids [49, 50].

Measurements of alkaloid contents

Concentrations of peramine and lolitrems B were measured using high performance liquid chromatography (HPLC) [51-53].

Statistical analyses

All averages and Standard error of the difference (SE) of measurements were recorded in Excel software, and statistical analysis was performed using SPSS software (version 18.0, Chicago, IL, USA). Two-way ANOVA at the 95% confidence level was used to estimate the effects of endophyte and treatments on host plants. A repeated-measures ANOVA with Fisher's least signififficant differences (LSD) test was used to determine whether differences between means were statistically significant.

Abbreviations

Cd: cadmium; Al: aluminum; Zn: zinc; Cu: copper; GA_3: gibberellin; CTK: cytokinins; IAA: indole-3-acetic acid; ABA: abscisic acid

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

PT and MNW conceived and designed the experiments. MNW contributed reagents, materials, and analysis tools. MML contributed taking care of plants. PT, MNW and MG wrote the manuscript. All authors contributed to the manuscript and approved the submitted version.

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Figure captions

FIG. 1 Effect of Epichloë endophyte and Cd^{2+}, Zn^{2+} on plant height (A) and tiller number (B) of *F. sinensis*

Note: Different lowercase letters indicate significant differences between treatments

($P<0.05$).

FIG. 2 Effects of *Epichloë* endophyte and Cd^{2+}, Zn^{2+} on plant root length (A) and biomass (B) of *F. sinensis*

Note: same as for Fig. 1.

FIG. 3 Effect of Epichloë endophyte and Cd^{2+}, Zn^{2+} on the aboveground and underground content of Cd and Zn ions in F. sinensis. A: aboveground Cd ions, B: underground Cd ions, C: above ground Zn ions, D: underground Zn ions.

Note: same as for Fig. 1.

FIG. 4 Effect of Epichloë endophyte and Cd^{2+}, Zn^{2+} on the contents of GA_3 (A), CTK(B), IAA(C) and ABA (D) in F. sinensis

Note: same as for Fig. 1.

FIG. 5 Peramine (A) and lolitrem B(B) content of F. sinensis E+ plants under Zn2+, Cd2+ treatment.

Note: The left is alkaloids peak. Different lowercase letters indicate significant differences between treatments ($P<0.05$).

Figures

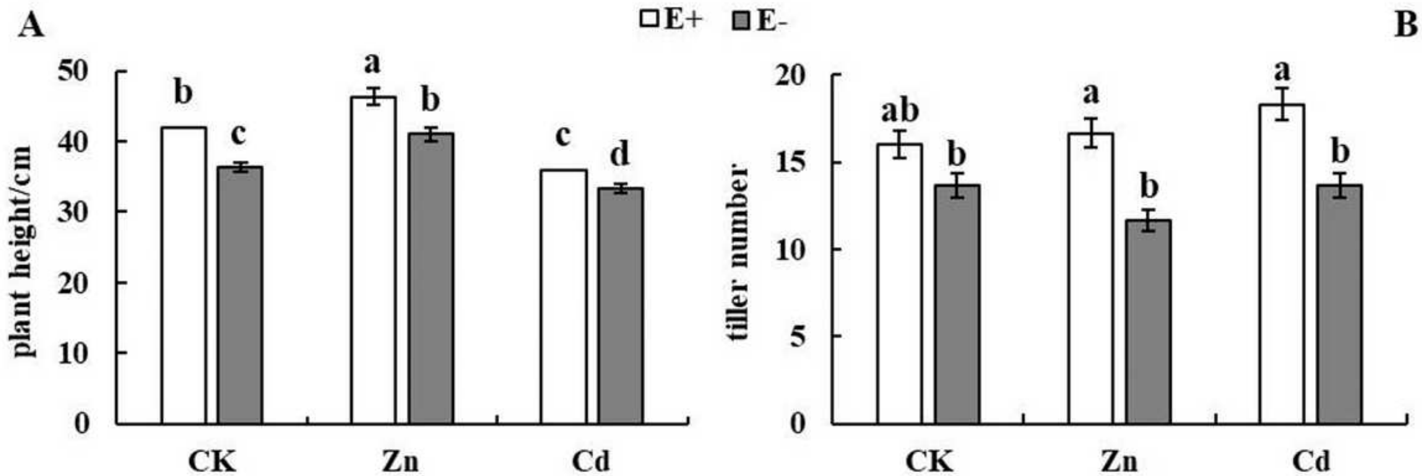

Figure 1

Effect of Epichloë endophyte and Cd2+, Zn2+ on plant height (A) and tiller number (B) of F. sinensis Note: Different lowercase letters indicate significant differences between treatments (P<0.05).

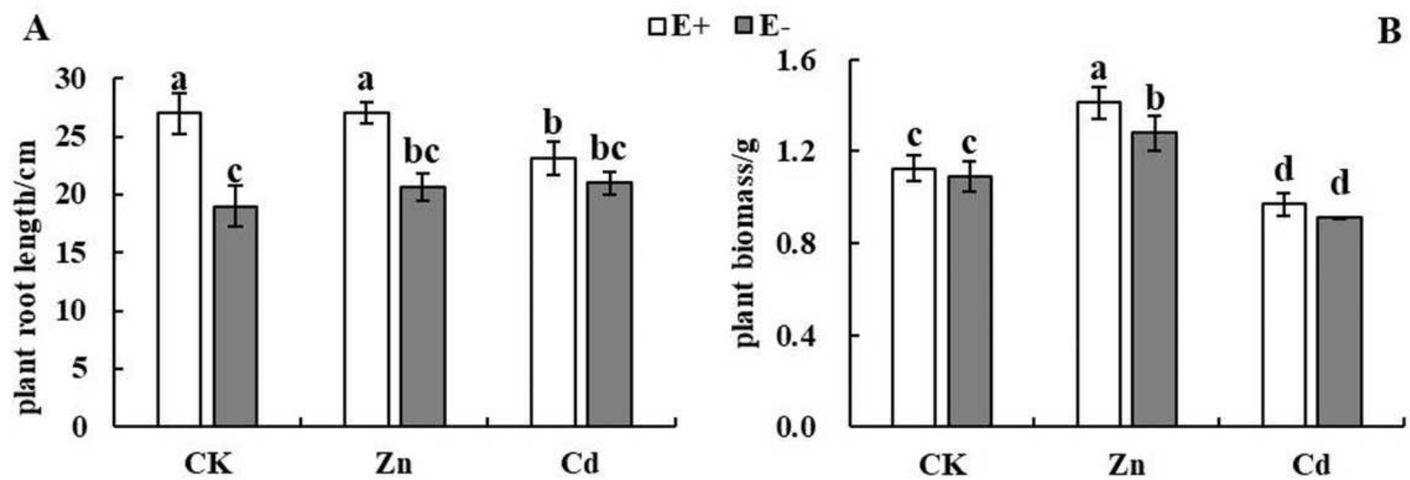

Figure 2

Effects of Epichloë endophyte and Cd2+, Zn2+ on plant root length (A) and biomass (B) of F. sinensis Note: same as for Fig. 1.

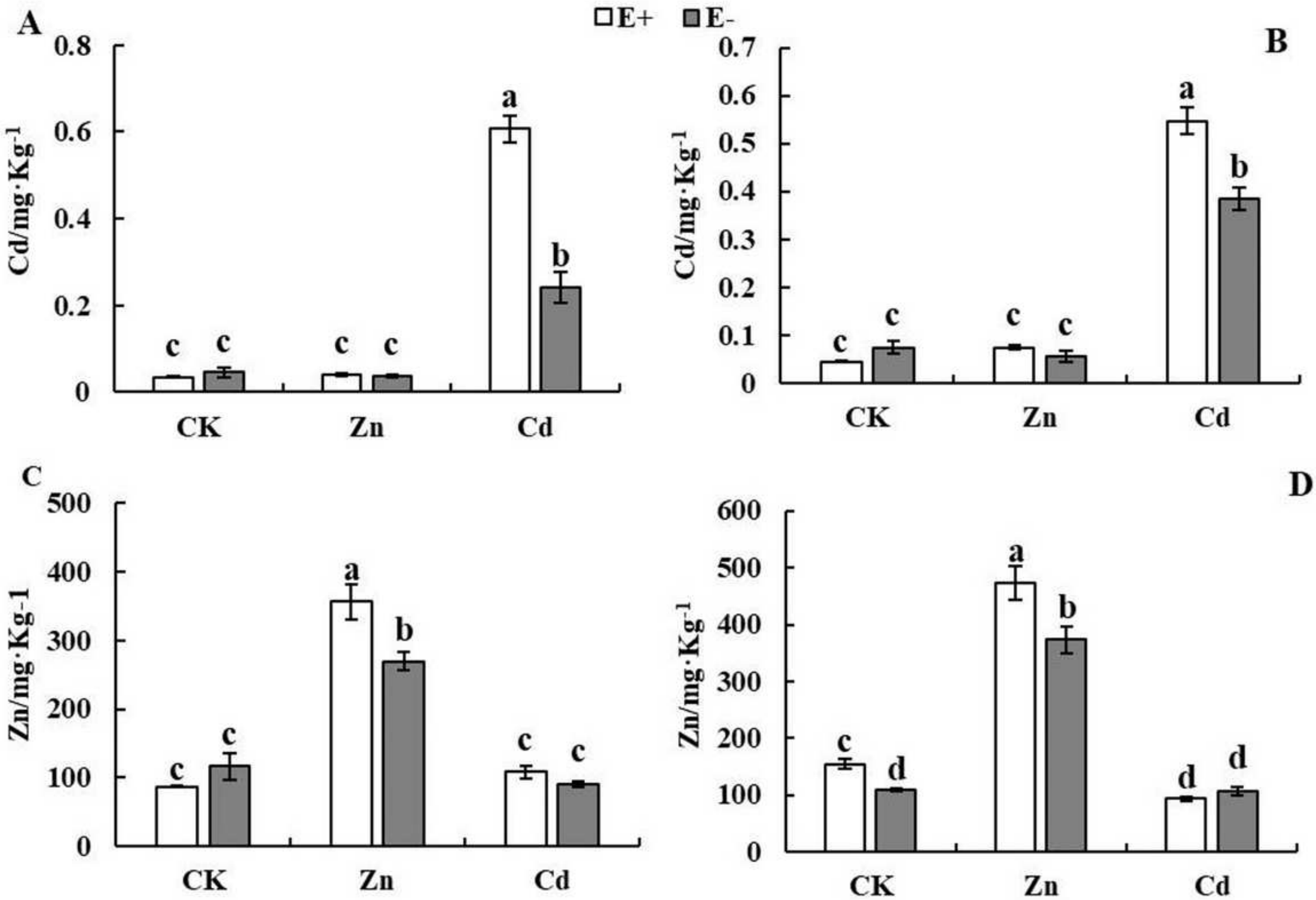

Figure 3

Effect of Epichloë endophyte and Cd2+, Zn2+ on the aboveground and underground content of Cd and Zn ions in F. sinensis. A: aboveground Cd ions, B: underground Cd ions, C: above ground Zn ions, D: underground Zn ions. Note: same as for Fig. 1.

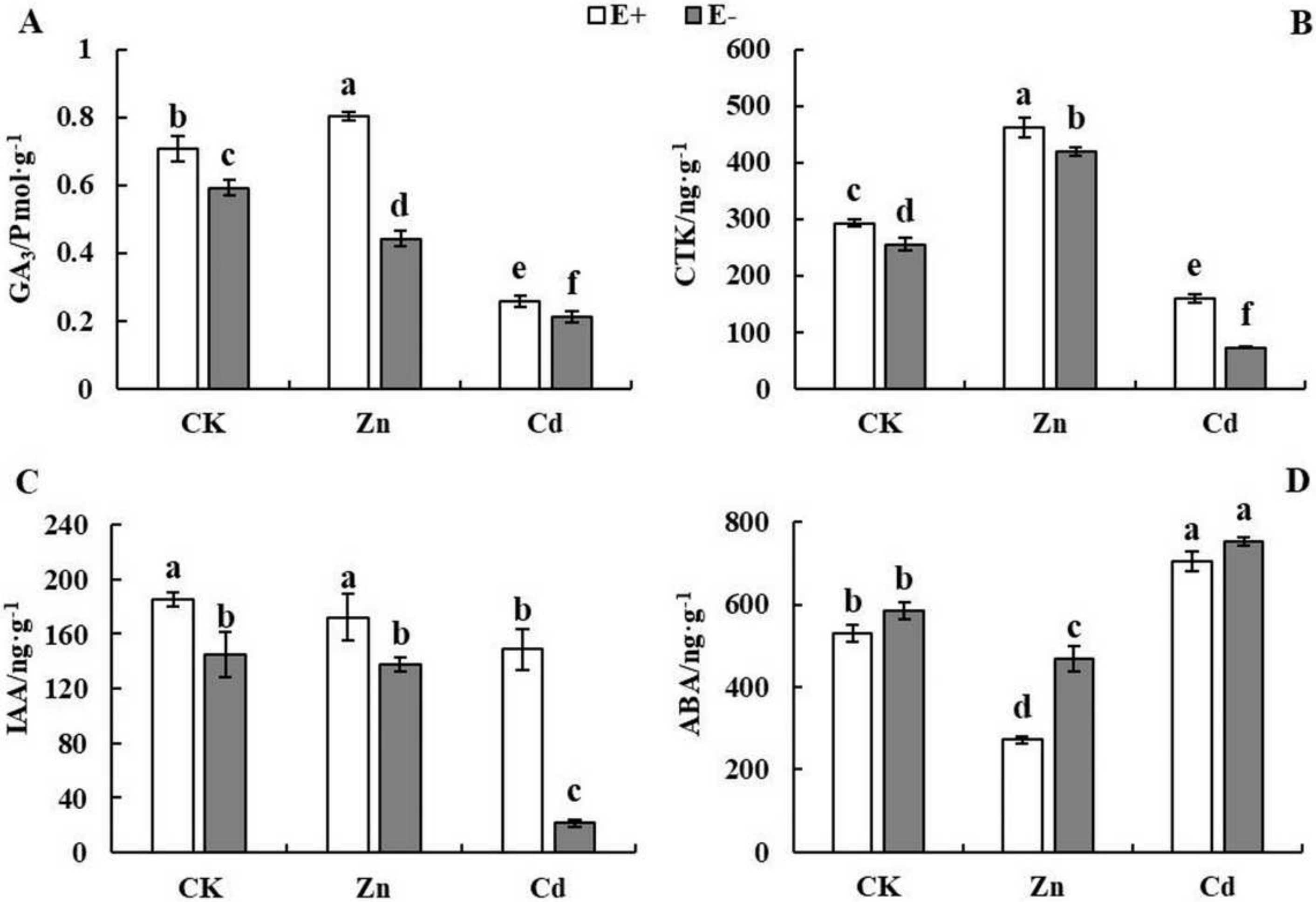

Figure 4

Effect of Epichloë endophyte and Cd2+, Zn2+ on the contents of GA3 (A), CTK(B), IAA(C) and ABA (D) in F. sinensis Note: same as for Fig. 1.

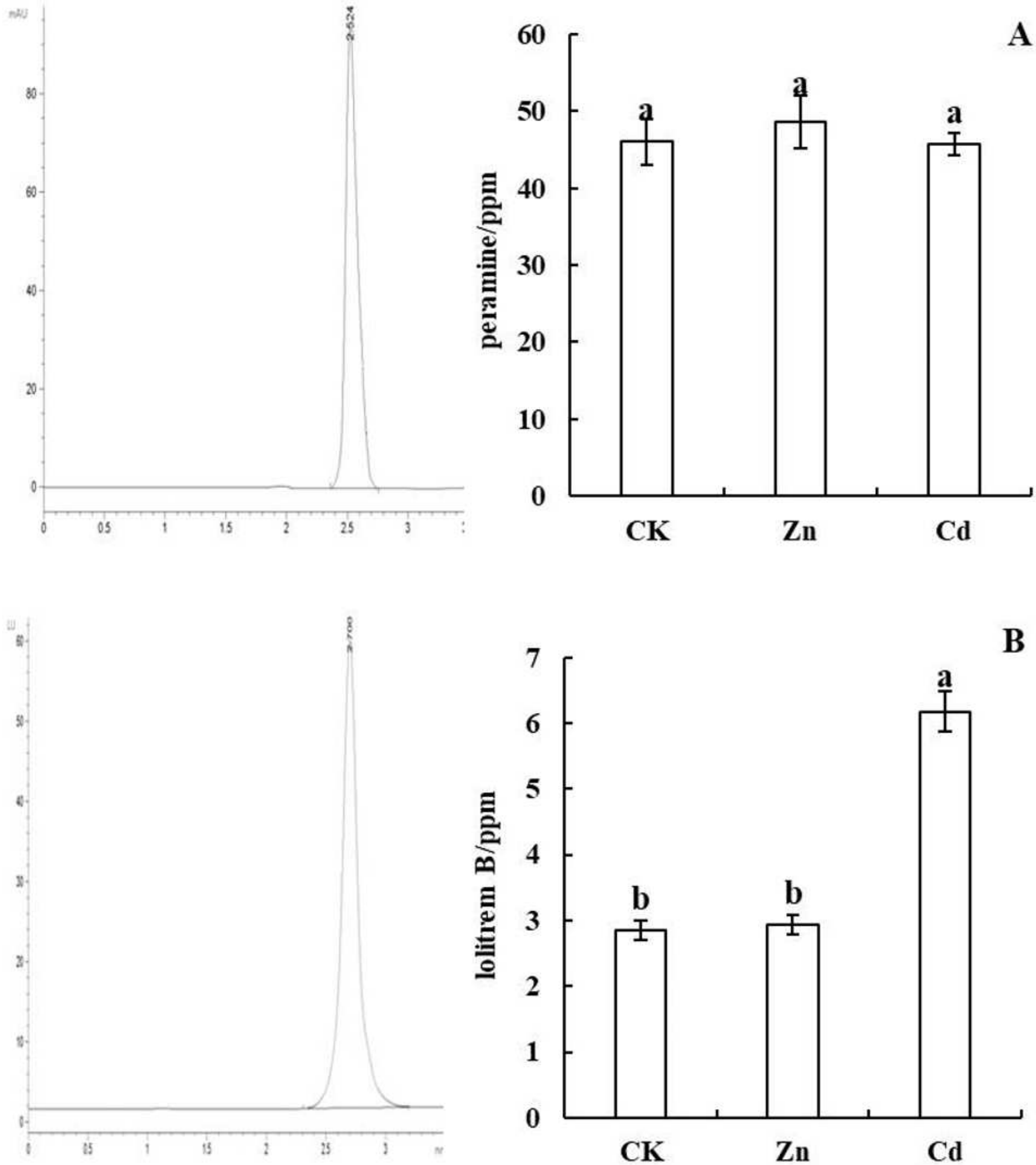

Figure 5

Peramine (A) and lolitrem B(B) content of F. sinensis E+ plants under Zn2+, Cd2+ treatment. Note: The left is alkaloids peak. Different lowercase letters indicate significant differences between treatments (P<0.05).