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Effects of Auxin at Different Concentrations on the Growth, Root Morphology and Cadmium Uptake of Maize (*Zea mays* L.)

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ABSTRACT

Indoleacetic acid (IAA) is an important regulator that plays a crucial role in plant growth and responses to abiotic stresses. In the present study, a sand cultivation experiment was carried out to investigate the effects of IAA at different concentrations (0, 0.01, 0.1, 0.5, 1, and 2.5 mmol/L) on maize growth, root morphology, mineral elements (Ca, Mg) and Cd uptake under 20 mg/kg Cd stress. The results showed that 0.01 mmol/L is the optimal IAA concentration for enhancing the Cd tolerance of maize. Compared with the control treatment, 0.01 mmol/L IAA promoted maize growth, with significant increases in the height, shoot and root biomass by 34.6%, 25.0% and 16.3%; altered the root morphology, with increases in root length, root tip number, and root tip density by 8.9%, 31.4% and 20.7%, respectively; and enhanced the mineral element uptake of maize, resulting in significant increases in the Ca content in shoots and roots by 640.6% and 1036.4% and in the Mg content in shoots by 205.8%, respectively. In addition, 0.01 mmol/L IAA decreased the Cd content and uptake in the shoots by 51.9% and 39.6%, respectively. Furthermore, the Cd content and uptake exhibited a significant negative correlation with Ca content in roots and a significantly positive correlation with root morphology, and the Cd content in shoots was significantly and negatively correlated with root tip number. Thus, 0.01 mmol/L IAA was effective in enhancing the Cd tolerance and plant growth of maize.

KEYWORDS

Abiotic stress; mineral nutrient; plant growth; phytohormone; root trait

1 Introduction

Cadmium (Cd) is an unnecessary element for plants. After being absorbed by the soil, Cd is transported and taken up by plants [1]. When Cd uptake occurs to a certain extent, it will inhibit plant growth, damage the photosynthetic system, interfere with the metabolism of mineral elements, induce oxidative damage, accelerate plant senescence, and even cause plant death [2,3]. In plants that live in Cd stress environments for a long time, a series of adaptive mechanisms can form, including changes in plant root morphology, nutrient absorption, reactive oxygen levels, hormone homeostasis, and gene expression [4].

Auxin is an important plant growth regulator that can promote cell division and elongation and the differentiation and formation of new organs, change the morphology of plant roots, and regulate the absorption of nutrients by plants. It plays an important role in plant Cd tolerance [5,6]. Under Cd stress, 40 mg/L IAA can significantly increase the total root length, root surface area and root tip number of



Solanum nigrum [7], and 10^{-9} mol/L IBA can significantly increase the content of nutrient elements such as nitrogen, phosphorus, potassium, calcium, sulfur, magnesium, iron, manganese, zinc, copper and other elements [8]. While auxin affects plant root development and nutrient absorption, auxin affects the absorption, transfer and uptake of Cd by plants. Zhang et al. [9] reported that 2 and 10 $\mu\text{mol/L}$ IAA can inhibit the absorption and uptake of Cd and reduce the Cd content in the roots, stems, mature leaves and tender leaves of tea seedlings (*Camellia sinensis*). Ran et al. [7] reported that 20 mg/L IAA significantly increased the Cd content of the Cd hyperaccumulator *Solanum nigrum* and the nonhyperaccumulator *Solanum melongena* plants, and 40 mg/L IAA significantly increased the Cd content of *Solanum nigrum* but significantly decreased the Cd content of *Solanum melongena*. The effects of IAA on the Cd uptake characteristics of plants are related to the concentration of IAA and plant species.

Maize has a large biomass, is easy to harvest, and is a widely planted crop worldwide [10]. Maize has a certain ability to absorb, transfer and uptake Cd, is a good material studying plant Cd resistance mechanism. In recent years, there have been studies that IAA can change the transport pathway or as a signal substance to enhance the stress resistance of maize [11], but has less related studies of maize root morphology and nutrient elements. Most of maize Cd resistance mechanism was carried out at a single IAA concentration. But the influence mechanism of IAA on plant growth, physiology and Cd tolerance is related to the concentration of IAA and plant species. Therefore, it is necessary to study the effects of different concentrations IAA on the growth and Cd uptake of maize. We used low-Cd maize cultivar maize Huidan No. 4 as the material to study the effects of IAA on plant height, biomass, root morphology, root traits, mineral element content and Cd uptake characteristics of maize under 20 mg/kg Cd stress, to provide basic data and theoretical support in IAA alleviate the toxicity of Cd to maize. We hypothesize that IAA can inhibit the absorption and uptake of Cd in maize by promoting the development of maize roots and the absorption of mineral elements to enhance maize Cd tolerance and promote maize growth.

2 Materials and Methods

2.1 Materials

The test material was a native low-Cd maize cultivar (Huidan No. 4) from Yunnan Province, China. The culture substrate was quartz sand (the main component of which is SiO_2), with a particle size of 0.5–1 mm, white powder. IAA (3-indoleacetic acid, $\text{C}_{10}\text{H}_9\text{NO}_2$) is a white crystal that is poorly soluble in water. IAA was purchased from Beijing Coollab Technology Co., Ltd. (China) (Coolaber RT Purity > 99%). The culture vessel was an uncovered cylindrical sealed tank with a diameter of 12 cm and glass bottles with a diameter of 6.5 cm and a height of 25 cm.

2.2 Experimental Treatments

First, the seeds of Huidan No. 4 were surface-sterilized by immersion in 75% ethanol for 10 min and in 10% sodium hypochlorite for 10 min, followed by rinsing with water until colorless. The seeds were germinated in a petri dish (150 mm) at 25°C for 3 days. After germination to about 1 cm, two seedlings with uniform growth were transplanted to a glass bottle, which 0.4 kg quartz sand and 40 ml of 50% Hoagland nutrient solution were added. Seedlings were cultured for 14 days in a greenhouse (average temperature 25°C, average humidity 56%).

Second, after 14 days, two maize seedlings with the same growth were transplanted to a sealable tank. To each sealed tank, 1.475 kg of quartz sand and 100 ml of 50% Hoagland nutrient solution were added. The nutrient solution contained 20 mg/kg Cd and the corresponding concentration of IAA (0, 0.01, 0.1, 0.5, 1, 2.5 mmol/L IAA), Cd was added in the form of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$, 4 replicates per treatment. Seedlings were grown for 30 days and harvested in greenhouse.

2.3 Determination of Plant Height and Biomass

After the maize seedlings were harvested, the plant height was measured with a measuring tape, and then the seedlings were divided into shoots and roots. The samples were soaked in ethylenediaminetetraacetic acid solution (EDTA) for 15 min. The shoots and roots soaked in EDTA were rinsed with tap water and deionized water 3 times and then dried in an oven at 105°C for 30 min and then at 75°C. The dried shoots and roots were weighed to a constant weight to obtain the shoot and root biomass [12].

2.4 Determination of Root Morphology

The root was rinsed with tap water and then with distilled water 2–3 times, and the water was absorbed with filter paper. The root morphology was scanned with an Epson Perfection V700 scanner (Seiko Epson, Japan), the scanned image was saved, and the root length, root surface area, root volume, root average diameter, root tip number, and branch number were analyzed with Win RHIZO PRO STD4800 (Regent, Canada) software [13].

According to the root length, root surface area, root tip number and branch number recorded by WinRHIZO and the root biomass, the specific root length (SRL), specific surface area (SRA), root tip density (RTD) and root branching intensity (RBI) of the root system were calculated. The calculation formula was as follows: specific root length (m/g) = root length (m)/biomass (g), specific surface area (cm²/g) = root surface area (cm²)/biomass (g), root tip density (No./cm) = root tip number(number)/root length (cm), branch density (No./cm) = branch number(number)/root length (cm) [14].

2.5 Determination of Plant Cd, Ca and Mg Contents

Plant samples were dried, ground and passed through a 100-mesh sieve. A 0.5-g sample was transferred to polyethylene digestion irrigation, concentrated HNO₃ and H₂O₂ (5:3) were added to the sample, and the sample was digested in a pressure digestion tank and measured by flame atomic absorption spectrophotometry (AAS ICE 3000 Series, Thermo Scientific, Franklin, USA) [15,16].

2.6 Determination of Biological Transport Factors and Translocation Factors

Plant Cd uptake characteristics are expressed by the biological transfer factor (BTF) and the transfer factor (TF) [17] with the following formula: Cd uptake (μg/plant) = Cd content (mg/kg) × plant biomass (g/plant); biological transport factor (BTF) = shoot Cd content (mg/kg) × shoot biomass (g)/root Cd content (mg/kg) × root biomass (g); transfer factor (TF) = shoot Cd content (mg/kg)/root Cd content (mg/kg).

2.7 Statistical Analysis

The test data were the average of 4 replicates, and all data were sorted and analyzed in Excel. The least significant difference method (LSD) has a relatively simple method, in all possible pairwise comparison of mean of multiple treatments. LSD overcomes some drawbacks of the *T*-test method. Therefore, the LSD method was used to mean analysis [18]. Analysis of variance, significance test (LSD method, significance level *P* = 0.05) and correlation analysis (Pearson method, significance level *P* = 0.05) were performed by IBM SPSS Statistics 21 software. Origin (Version 9.0 Pro, Origin Lab Company, Massachusetts, USA) was used for charting.

3 Results

3.1 The Effect of IAA on Maize Growth

Under 20 mg/kg Cd stress, 0.01 mmol/L IAA resulted in a significant increase in maize plant height and shoot and root biomass, which increased by 34.6%, 25.0%, and 16.3%, respectively. IAA (0.1 mmol/L) resulted in a significant increase in plant height by 21.4% but resulted in a significant decrease in root biomass by 20.4%. IAA (0.5 mmol/L) resulted in significant decreases in plant height and shoot and root

biomass, which were decreased by 24.3%, 13.9%, and 18.4%, respectively. IAA at 1 and 2.5 mmol/L resulted a significant decrease in root biomass of 26.5% and 12.2%, respectively. Under Cd stress, 0.01 mmol/L IAA significantly promoted maize growth (Table 1).

Table 1: Effect of difference concentrations of IAA treatment on plant height and biomass of maize under Cd stress

IAA concentration (mmol/L)	Plant height (cm)	Shoot biomass (g/plant)	Root biomass (g/plant)
0	23.33 ± 2.98 b	0.36 ± 0.01 b	0.49 ± 0.03 b
0.01	31.42 ± 4.21 a	0.45 ± 0.04 a	0.57 ± 0.02 a
0.1	28.33 ± 1.13 a	0.38 ± 0.03 b	0.39 ± 0.02 cd
0.5	17.67 ± 1.60 c	0.31 ± 0.02 c	0.4 ± 0.0 cd
1	27.08 ± 1.74 ab	0.37 ± 0.02 b	0.36 ± 0.03 d
2.5	22.17 ± 2.62 b	0.37 ± 0.03 b	0.43 ± 0.01 c

All values represent the means ± standard deviations; different lowercase letters within a column indicate significant differences based on one-way analysis of variance in SPSS 21 followed by the least significant differences at the 5% confidence level.

3.2 The Effects of IAA on Root Morphology and Traits of Maize

Under 20 mg/kg Cd stress, 0.01 mmol/L IAA resulted in a significant increase in root length of maize by 8.9%. IAA at 0.1 and 2.5 mmol/L resulted in significant decreases in root length of 14.4% and 12.8%, respectively. IAA at 0.01, 0.1, 0.5, 1, and 2.5 mmol/L resulted in significant decreases in root surface area and root volume; the root surface area was decreased by 11.1%, 20.2%, 12.5%, 16.9% and 17.8%, the root volume was decreased by 11.1%, 20.2%, 11.1%, 16.9%, 17.8%, and 0.5 mmol/L AA resulted in a significant decrease in the average root diameter by 20.0%. IAA (0.01 mmol/L) resulted in a significant increase in the number of root tips by 31.4%. IAA (1 mmol/L) resulted in a significant decrease in the number of root tips by 12.9%. There was no significant effect on the number of branches treated with IAA (Fig. 1).

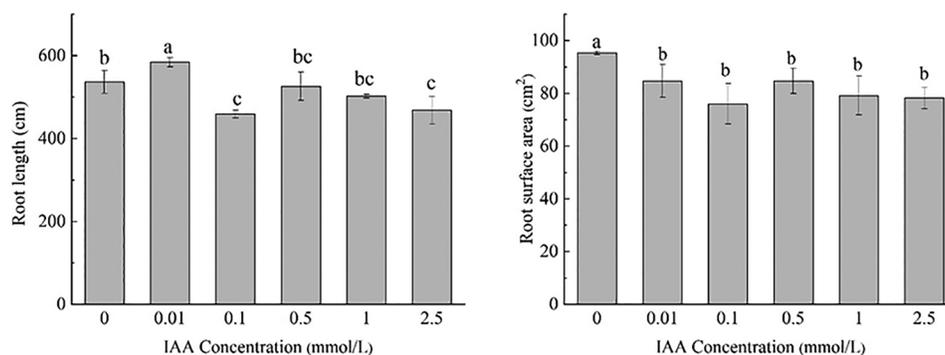


Figure 1: (Continued)

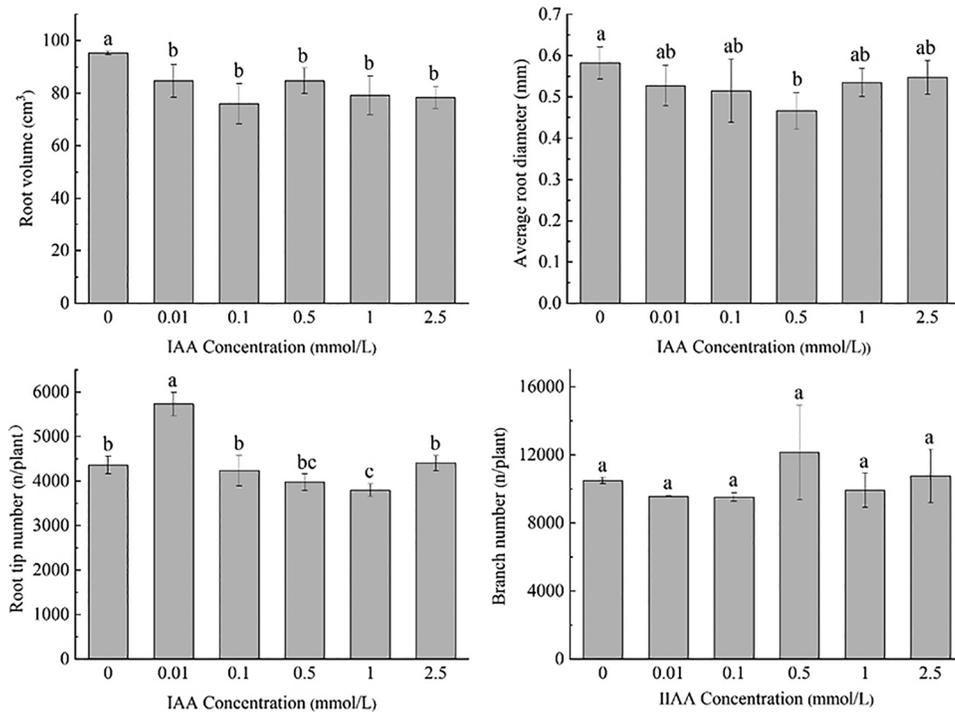


Figure 1: Effect of difference concentrations of IAA treatment on root morphology of maize under Cd stress; different little letters refer to $P < 0.05$ according to LSD test

Under 20 mg/kg Cd stress, 0.5 and 1 mmol/L IAA resulted in significant increases in the specific root length of maize of 19.2% and 28.4%, respectively. IAA (0.01 mmol/L) resulted in a significant decrease in the specific surface area by 24.7%. IAA at 0.01, 0.1, and 2.5 mmol/L resulted in a significant increase in root tip density, which was increased by 20.7%, 13.7%, and 16.2%, respectively. IAA (2.5 mmol/L) resulted in a significant increase in root branch density by 16.8% (Fig. 2). In general, under Cd stress, exogenous application of IAA has significant effects on maize root morphology and traits.

3.3 The Effects of IAA on the Contents of Ca and Mg in Maize

Under 20 mg/kg Cd stress, 0.01, 0.1, 0.5, 1, and 2.5 mmol/L IAA resulted in significant increases in the Ca content of maize of 640.6%, 241.8%, 371.2%, and 387.5%. In addition, the root Ca content increased by 1036.4%, 1136.3%, 1265.4%, 902.1%, and 668.1%, respectively, and 0.01, 0.1, 0.5, 1, and 2.5 mmol/L IAA resulted in significant increases in shoot Mg content, of 205.8%, 126.2%, 162.9%, 156.5%, and 155.5%, respectively. IAA had no significant effect on the root Mg content. In general, under Cd stress, applying IAA can promote the absorption of Ca and Mg in maize (Fig. 3).

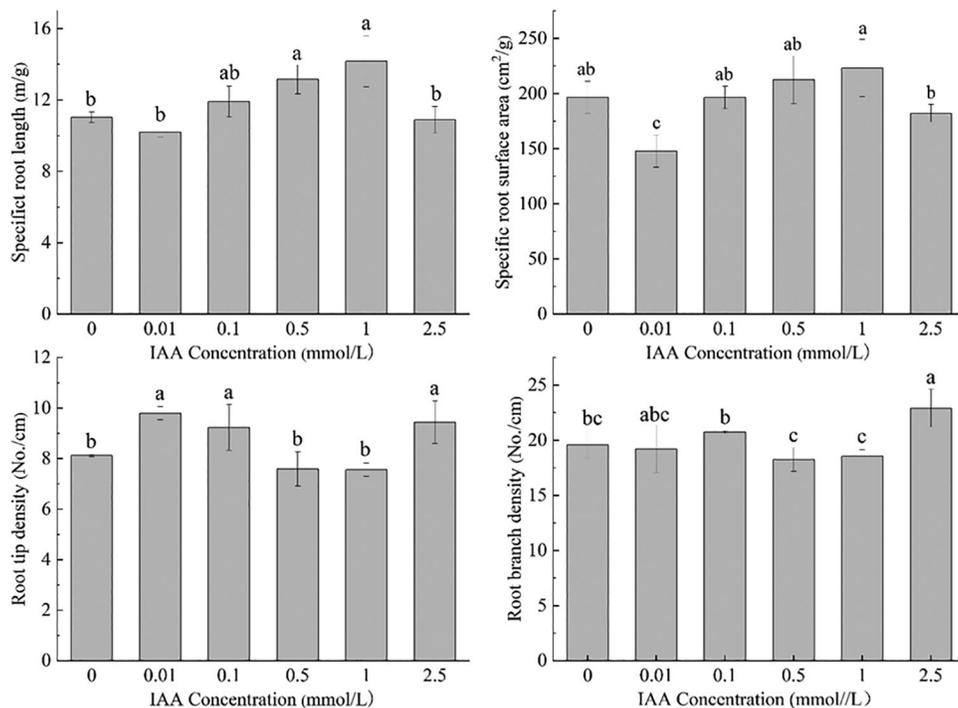


Figure 2: Effect of difference concentrations of IAA treatment on root traits of maize under Cd stress; different little letters refer to $P < 0.05$ according to LSD test

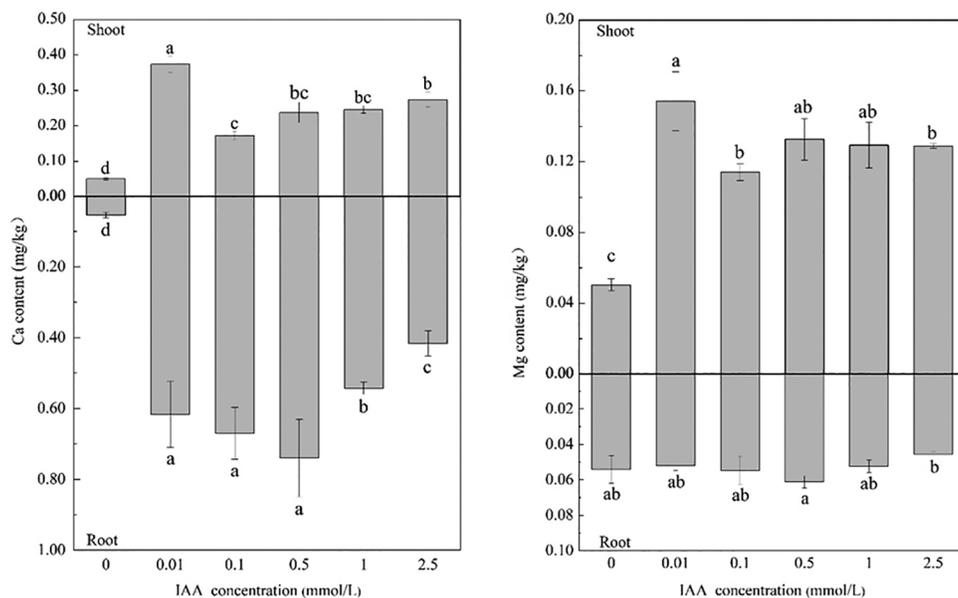


Figure 3: Effect of difference concentrations of IAA treatment on Ca and Mg content of maize under Cd stress; different little letters refer to $P < 0.05$ according to LSD test

3.4 The Effects of IAA on Maize Cd Content and Uptake

Under 20 mg/kg Cd stress, 0.01 mmol/L IAA resulted in significant decreases in the Cd content, and the uptake of maize shoots decreased by 51.9% and 39.55%, respectively. IAA at 0.5 and 2.5 mmol/L resulted in significant increases in Cd content and shoot uptake. The Cd content increased by 141.5% and 36.3%, respectively, and the uptake increased by 108% and 41.9%, respectively. IAA at 0.1, 0.5, 1 and 2.5 mmol/L resulted in significant decreases in Cd content and uptake of maize roots; the Cd content decreased by 58.0%, 30.0%, 32.0%, and 47.4%, respectively, and the uptake decreased by 67.2%, 42.2%, 49.9%, and 53.4%, respectively. It can be seen that 0.01 mmol/L IAA can significantly reduce the Cd content and uptake of maize shoots (Fig. 4).

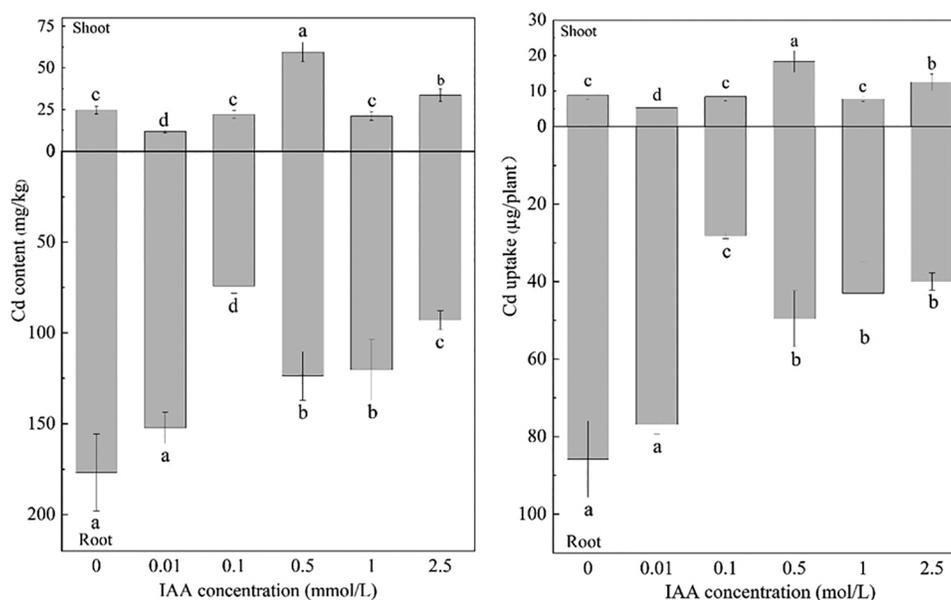


Figure 4: Effect of difference concentrations of IAA treatment on Cd content and uptake of maize; different little letters refer to $P < 0.05$ according to LSD test., Cd: cadmium

3.5 The Effects of IAA on the Biological Transport Factor and Transfer Factor of Cd in Maize

Under 20 mg/kg Cd stress, 0.1, 0.5, 1 and 2.5 mmol/L IAA resulted in significant increases in the biological transport factor of maize Cd of 113.8%, 244.5%, 27.3% and 159.2%, respectively. IAA (0.1, 0.5 and 2.5 mmol/L) resulted in significant increases in transfer factor of 189.3%, 258.3% and 204.7%, respectively. In general, the application of IAA can promote maize Cd from root transfer shoots (Table 2).

Table 2: Effects of difference concentrations of IAA on biological transfer factor (BTF) and transfer factor (TF) of maize

IAA concentration (mmol/L)	BTF	TF
0	0.10 ± 0.02 c	0.14 ± 0.01 cd
0.01	0.07 ± 0.01 c	0.08 ± 0.01 d
0.1	0.30 ± 0.04 a	0.30 ± 0.04 b
0.5	0.37 ± 0.04 a	0.48 ± 0.04 a
1	0.19 ± 0.05 b	0.18 ± 0.04 c
2.5	0.32 ± 0.07 a	0.36 ± 0.06 b

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance in SPSS 21 followed by the least significant differences at the 5% confidence level.

3.6 Correlation Analysis

Correlation analysis indicated that the Cd content of the shoots was significantly negatively correlated with the number of root tips. The Cd content of the roots was extremely negatively correlated with the IAA concentration, extremely positively correlated with root length and root surface area, and positively correlated with root volume. The Cd uptake of roots was extremely negatively correlated with IAA concentration, extremely positively correlated with root length and root surface area, and significantly positively correlated with root volume, root tip number and branch number (Table S1). In addition, the root Ca content was significantly negatively correlated with the Cd content ($r = -0.490$, $P < 0.05$) and the uptake amount ($r = -0.528$, $P < 0.05$).

4 Discussion

Growth status is an effective indicator of plant resistance. Cd can inhibit plant seed germination, photosynthesis, respiration, nutrient absorption, and other processes, cause plant metabolism disorders, slow growth, and reduce biomass [19,20]. IAA can alleviate the inhibitory effect of Cd on plant growth. Khan et al. found that under 100 $\mu\text{mol/L}$ Cd stress, 5 $\mu\text{mol/L}$ IAA can significantly increase the fresh weight of tomato (*Lycopersicon esculantum* L.) shoots [21]. In this experiment, 0.01 mmol/L IAA resulted in significant increases in maize plant height and shoot biomass. This may be because IAA regulates the plant's antioxidant system, reduces the uptake of malondialdehyde, and enhances plant photosynthesis to alleviate the damage caused by heavy metals to plants, thereby promoting plant growth [7,22]. IAA (0.5 mmol/L) resulted in significant decreases in the plant height and shoot biomass of maize seedlings. This may be due to the high concentration of IAA, which inhibited plant growth. Other studies have shown that an IAA concentration that is too high will destroy the membrane structure of plant cells, and the toxicity of IAA modulators is greater than that of naturally synthesized IAA at the same concentration [23].

The root is the first organ in which plants perceive soil abiotic stress. When roots are subjected to environmental stress, it usually manifests as a decrease in root biomass and a change in root morphology [24,25]. For example, under Cd stress, the root length, root surface area, root volume and total root tip number of peanuts (*Arachis hypogaea* L.) were significantly decreased, but the average root diameter was significantly increased [26]. Studies have found that IAA can activate the entire pericycle to form a lateral root primordium, split the lateral root primordium, and release pectin lyase and other substances to modify the cell wall of its surrounding cortex or epidermal cells so that the lateral roots can break through the cortex and epidermal cells for exposure, which plays important roles in plant root development and the response to environmental stress [27,28]. Under Pb stress, 10 and 100 mmol/L IAA can significantly increase the root length, root surface area and root biomass of *Picris divaricata* Vant [29]. IAA (0.1 nmol/L) can significantly increase sunflower (*Helianthus annuus* L.) root volume, root surface area, and average root diameter [30]. In this experiment, 0.01 mmol/L IAA resulted in significant increases in maize root biomass, root length, root tip number, and root tip density. This may be because IAA promoted cell division and differentiation, thereby promoting the growth of plant roots [31]. However, the regulatory effect of IAA on roots is closely related to the concentration of IAA. Under Cr stress, 10 $\mu\text{mol/L}$ IAA can significantly increase pea (*Pisum sativum* L.) root biomass, but 100 $\mu\text{mol/L}$ IAA significantly reduces root biomass [32]. In this experiment, 0.1, 0.5, 1, and 2.5 mmol/L IAA resulted in significant reductions in maize root biomass, root surface area and root volume. This may be due to the high concentration of IAA, which inhibited root development. The decrease in root biomass may be indirectly caused by the decreases in root surface area and root volume.

Both Ca and Mg are trace elements that are necessary for plant growth and are the same divalent ions as Cd, Ca and Mg have ionic radii similar to those of Cd, and the ions will compete for absorption and transport sites [33]. Under heavy metal stress, Ca or Mg can enhance cell antioxidant enzyme activity, affect root secretion of low molecular weight organic acids, and help root cells maintain a normal osmotic system [34–36]. Exogenous Ca or Mg can attenuate the inhibitory effect of Cd on plant growth [37]. Recent studies have shown that exogenous Ca mitigates Cd toxicity in plants by alleviation of growth inhibition, regulation of metal uptake and translocation, improvement of photosynthesis, mitigation of oxidative damages and the control of signal transduction in the plants [38]. Kikuchi et al. [39] reported that MgO suppression of transfer of Cd into the edible part of rice plants (*Oryza sativa* L. cv. Kinuhikari) from the contaminated soils. But there are few studies on the interaction mechanism between plant exogenous Mg and Cd, most studies are about the effect of magnesium deficiency on plant Cd accumulation. Wu et al. [37] review that deficient Mg facilitates Cd accumulation is beneficial for Cd phytoextraction plants. Studies have found that IAA is directly involved in the transportation of nutrient elements. For example, changes in plant endogenous IAA content will affect the absorption of nitrogen by plant roots [40]. In this experiment, IAA treatment significantly increased the plant Ca and shoot Mg contents. The increase in Ca content may be related to the regulation of Ca^{2+} -ATPase by IAA [41]. Dindas et al. [42] performed measurements with scanning Ca^{2+} -selective extracellular electrodes in *Arabidopsis thaliana* root hairs, to test whether IAA triggers a calcium influx. The study suggested that IAA provoked a transient influx of Ca^{2+} , IAA triggers elevation of cytosolic calcium in root hairs. IAA triggers cytoplasmic Ca^{2+} transients, which require $\text{SCF}^{\text{TIR1/AFB}}$ -based auxin signaling, and transcriptional analyses revealed that Ca^{2+} -dependent, feedback control circuit within a functional signaling pathway consisting of AUX1 mutants, $\text{SCF}^{\text{TIR1/AFB}}$ receptor and CNGC14 Ca^{2+} channel. But the mechanism by which IAA increases the Mg content of maize shoots still needs to be studied.

In this study, 0.01 mmol/L IAA resulted in significant decreases in the Cd content and uptake of maize shoots, while 0.5 and 2.5 mmol/L IAA resulted in significant increases in the Cd content and uptake of maize shoots. Correlation analysis indicated that the Cd content of the shoots was significantly negatively correlated with the number of root tips. This may be because under 0.01 mmol/L IAA, the number of root tips increased significantly, which promoted maize growth, diluted the Cd in the plant, increased the content of hemicellulose 1, and prevented the transfer of Cd from roots to shoots [43]. While 0.5 and 2.5 mmol/L IAA significantly inhibited the growth of maize, the increase in Cd content may be due to the enhancement of transpiration or stomatal conductance of plants by IAA, which promotes the Cd absorption of roots and the transfer from roots to shoots [44]. This study also found that 0.1, 0.5, 1, and 2.5 mmol/L IAA resulted in significant decreases in the Cd content and uptake of maize roots. The root Cd content was significantly or extremely negatively correlated with the IAA concentration and root Ca content and was significantly or extremely positively correlated with root length, root surface area, and root volume. Root Cd uptake was significantly or extremely negatively correlated with IAA concentration, and root Ca content was significantly or extremely negatively correlated with root length, root surface area, root volume, root tip number, and branch number. This shows that IAA inhibits root development and promotes the absorption of the nutrient element Ca, which is one of the main reasons why IAA reduces the absorption and uptake of Cd in maize roots.

Biological transport factors and transfer factors are indicators to measure the ability of plants to transport heavy metals from roots to shoots after absorbing heavy metals [45,46]. In this study, 0.1, 0.5, 1, and 2.5 mmol/L IAA resulted in a significant increase in the biological transport factor, and 0.1, 0.5, and 2.5 mmol/L IAA resulted in a significant increase in the transfer factor. However, the Cd biological transport factor and the transfer factor of maize under the six IAA concentrations were less than 0.5, indicating that the ability of maize to transport from roots to shoots was weak. This may be related to the low-uptake maize variety Huidan No. 4 used as the experimental material in this experiment.

5 Conclusion

Under 20 mg/kg Cd stress, 0.01 mmol/L IAA changed maize root morphology and promoted mineral element uptake in maize, decreased the Cd content and uptake in shoots, and promoted maize growth. In conclusion, the application of optimal concentrations can help maize adapt to cadmium stress. Therefore, this study may help to better understand the role of phytohormones in promoting Cd tolerance in host plants.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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