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Study on the Effect of Indolebutyric Acid-Lanthanum Chelate on Vegetable Growth and Its Molecular Mechanism

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Abstract: In order to reduce the non-point source pollution caused by the large or excessive application of chemical fertilizers and pesticides, this study was aimed to develop new alternative functional substances. The effects of different concentrations of indolebutyric acid-lanthanum chelate on seed germination and tomato seedling growth were studied by germination test of cucumber and tomato seeds and pot experiment of tomato seedlings. The results showed that the optimum concentration of indolebutyric acid-lanthanum chelate could promote the germination and growth of tomato and cucumber seeds. Among them, 0.02 mg/L of indolebutyric acid-lanthanum chelate is the optimum concentration for tomato and cucumber seed germination. When the concentration is 0.1 mg/L, it is the optimum concentration for radicle and hypocotyl growth. In the pot experiment, when the optimum concentration of indolebutyric acid-lanthanum chelate on tomato seedlings was 0.5 mg/L, the plant height, stem diameter, aboveground biomass, underground biomass and leaf area of tomato seedlings could be significantly promoted. At the same time, the absorption of the main elements P, K, Mg and trace elements Fe, Mn, Cu, Zn and Na and their transfer from the underground part to the aboveground part of the seedlings were enhanced. The relative expression of the tomato expansion protein genes *leEXP2*, *leEXP18* and *leEXP5* was also significantly increased. Therefore, the suitable concentration of indolebutyric acid-lanthanum chelate has the effect of promoting root growth and promoting the quality of tomato seedlings.

Key words: Indolebutyric acid-lanthanum chelate, promote root growth, fertilizer reduction, cucumber, tomato.

1. Introduction

With the first article published in 1968 by Indian scientists N. K. Dutt and K. Nag about the lanthanide complexes with Schiff base (disalicylidene ethylenediamine) as ligands, scholars from various countries have begun research on rare earth chelates [1]. In 1996, Wang *et al.* [2] synthesized 1-naphthaleneacetic acid (HNAA), 2,4-dichlorophenoxyacetic acid (HDAA), three complexes of indole acetic acid (HIAA) and rare earth cerium (Ce) in water: $Ce(NAA)_3$, $Ce(DAA)_3$, $Ce(IAA)_3$, by observing their effects on the growth of wheat coleoptiles, it was found that the growth promoting effects of the three complexes on wheat coleoptiles were related to their solubility in water. In 2001, Teng *et al.* [3] found that low concentrations of ruthenium-gibberellin complex (< 250 ppm) can increase the germination rate of mung bean and green bean by 50%-100%, and the root length and stem length of its seedling growth increased by 15% and 20%, respectively; at high concentrations (> 250 ppm), the germination rate and seedling growth were inhibited. Subsequent studies have also shown that certain concentrations of rare earths and their compounds can promote plant seed germination, seedling growth and increase crop yield, while also increasing the absorption of nutrients by crops, thereby improving crop quality, and when the concentration is too high, plant growth produces inhibition [4-9].

At present, the research on rare earth complexes mainly focuses on the research of synthesis methods

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and physical and chemical properties [10, 11], and its application in agriculture is less and more superficial. However, according to its physicochemical properties, rare earth complexes are promising materials, which may have the role of several raw materials for synthetic complexes, and even exhibit synergistic effects. In order to respond to the zero-growth action of chemical fertilizers and pesticides vigorously carried out by the Ministry of Agriculture, and further promote quality agriculture and green agriculture, this study was aimed to develop new alternative functional substances. Therefore, based on the previous studies, the rare earth lanthanum chloride and organic acid were chelated, and the growth promoting effect and mechanism were studied from the molecular biology level [12-16]. The mechanism was studied to determine the expression of the promoting function of the indolebutyric acid-lanthanum chelate.

2. Materials and Methods

2.1 Materials

Preparation method of indolebutyric acid-lanthanum chelate (RI): dissolve indolebutyric acid with 95% ethanol, add a certain concentration of NaOH solution, and stir to fully react the two substances. Then, the above solution was slowly added to a lanthanum chloride solution dissolved in 80% ethanol under stirring to form a precipitate which was RI. It was filtered, washed with distilled water, and the Cl^- in the precipitate was removed, followed by vacuum drying. Two kinds of raw materials for synthesizing RI: indolebutyric acid (99.7% purity) and lanthanum chloride (99.7% purity) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. The seeds used in the germination test were tomato (Zhongza No. 9) and cucumber (Biqiu) from Beijing Yanheyu Technology Development Co., Ltd. The plastic pot used in the pot experiment was opened above and the bottom was closed. The bottom of the pot was covered with 1 cm thick sand layer, and each pot was filled with 3 kg of soil. The soil was sandy loam soil collected from the Hulufa vegetable production base in Fangshan District, Beijing. The soil sample was air dried and passed through a 2 mm sieve. The basic physical and chemical properties of the tested soil are shown in Table 1.

2.2 Methods

2.2.1 Germination Test

The experiment was conducted in the laboratory of the west campus of China Agricultural University from March to June 2017. Select full-bodied, basically the same size of tomato and cucumber seeds, soak in hot water at 55 °C for 10 min, stir constantly; then rinse with distilled water for three times, return to normal temperature in the shade and air dry to the initial water content [17]. Seed soaking treatment was carried out with RI solutions at concentrations of 0.002, 0.02, 0.1, 1 and 2 mg/L, respectively. The distilled water was used as a control and all soaking time was 4 h. Then place the seeds in a clean glass culture dish with two layers of qualitative filter paper and a diameter of 18 cm. The filter paper in the dish is moistened with about 30 mL of distilled water, and 80 seeds per dish. Each concentration was repeated three times. The culture dishes were then placed in a constant temperature incubator, germinated for 24 h in the dark at 25 °C, and the number of seeds germinated in each dish was recorded. Then continue the dark culture, record the number of seeds germination every day, weigh and replenish the evaporated water, and keep the humidity of each dish consistent.

Table 1 Basic physical and chemical properties of soil.

Physicochemical properties	pH	EC (μS/cm)	Organic carbon (g/kg)	Total nitrogen (g/kg)	Available phosphorus (mg/kg)
Contents	8.01	145.3	10.22	1.15	9.24

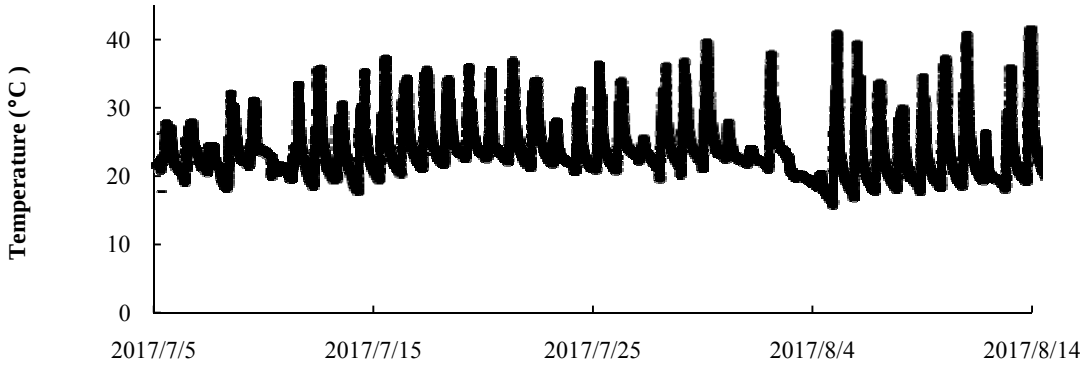

Fig. 1 Change of temperature in greenhouse during the tomato growth period (year/month/day).

2.2.2 Pot Experiment

The experiment was conducted in the solar greenhouse and laboratory of the China Agricultural University west campus from June to August 2017. The tomato seeds were immersed in hot water at 55 °C and placed in a constant temperature incubator at 25 °C for dark germination. After the seeds were white, they were sown in a 32-well plate containing a mixed substrate (peat and vermiculite *v*/*v* = 2:1, stirred with carbendazim 1,000 times solution). After the seeds are germinated, they are sown in a 32-well tray filled with a mixed substrate (pick and vermiculite *v*/*v* = 2:1, stirred with carbendazim 1,000-fold diluted solution), and placed in an artificial climate chamber. The photoperiod is 12 h/12 h, the light intensity is about 600 μmol/m^2/s, and the temperature is 26 °C/18 °C. After the seedlings' emergence, the clear water and Hoagland's nutrient solution are used alternately. When the tomato seedling grows to two leaves and one heart, the plants with the same growth were selected and planted in plastic pots. The temperature recorder of model TH22R was placed at a height of about 1.5 m in the middle of the greenhouse to measure the temperature change inside the greenhouse, and the data were recorded every 10 min. After 7 d of colonization, rooting treatment was carried out using RI solutions with concentrations of 0.05, 0.2, 0.5, 1 and 2 mg/L, respectively, with clear water as a blank control. The specific test concentrations are shown in Table 2. Each concentration has three replicates, root treatment was performed every 5 d, and 200 mL of each solution was applied to each root. The test period was 27 d, and each solution was applied four times. The temperature change in the greenhouse during the growth period of the tomato is shown in Fig. 1.

2.3 Measurement Indicators

2.3.1 Germination Index of Tomato and Cucumber Seeds

Germination means that the radicle is longer than 1/2 of the length of the seed, and the number of seeds germinated was recorded every day (the end of the test was: the number of germinations did not change for two consecutive days. The germination number of more than 80% of the treatments in this test remained substantially stable after 5 d of culture). The germination potential was counted on the second day after the treatment, and the germination rate was counted on the fifth day.

Germination potential (*GP*) = (Number of seeds germinated on Day 2/Total number of seeds tested) × 100% (1)

Germination rate (*GR*) = (Number of seeds germinated on Day 5/Total number of seeds tested) × 100% (2)

Germination index (*GI*) = Σ(*Gt*/*Dt*) (3)

where *Gt* is the number of seeds germinated on Day *t*, and *Dt* is the number of days when the corresponding seeds are germinated.

Vigor index (VI) = $GI \times S$ (4)

where S is the average radicle length. Radicle and hypocotyl length, are measured directly with a ruler.

2.3.2 Growth Index of Tomato Seedlings

Growth index of tomato seedlings are given below:

Plant height (cm): tape measurement, measuring the distance from the seedling cotyledon to the growth point.

Stem diameter (cm): vernier caliper measurement, measuring the thickness of the stem at 1 cm below the cotyledonary node.

Leaf area (cm^2): the leaves were scanned with an Epson scanner and the area of the second true leaves was analyzed using Win RHIZO root analysis software.

Fresh weight of the aboveground and underground part (g): analytical balance weighing. First rinse the tomato seedlings with deionized water, and blot the surface with absorbent paper, then weigh the aboveground and underground biomass (fresh weight).

Dry weight of the aboveground and underground part (g): the sample was placed in an oven, firstly blanched at 105 °C for 30 min, then dried at 75 °C to constant weight, and the aboveground and underground biomass (dry weight) was weighed using an analytical balance.

Total dry weight of plant (g): dry weight of the aboveground part + dry weight of the underground part.

Root-to-crown ratio: dry weight of the underground part/dry weight of the aboveground part.

Strong seedling index: (stem diameter/plant height + dry weight of the underground part/dry weight of the aboveground part) × dry weight of whole plant.

Determination of element content (inductively coupled plasma atomic emission spectroscopy (ICP-AES) method [18]) (g/kg): first, the dried plant samples are ground, then, the aboveground biomass of each treated plant sample is weighed about 0.3 g, and the underground biomass is weighed about 0.15 g and 6 mL of HNO_3 (99.8% purity) is added for overnight, then 2 mL of H_2O_2 (99.8% purity) is added. Dissolve in a closed microwave digestion system (CEM, Matthews, NC, USA), transfer the sample to a 50 mL volumetric flask after the digestion, and make up the volume, then take 10 mL of the sample in the centrifuge tube. The contents of P, K, Mg, Fe, Mn, Cu, Zn and Na in the digestion liquid are determined by inductively coupled plasma optical emission spectrometer ICP-AES (OPTIMA 3300 DV, Perkin-Elmer, USA). A standard sample verification procedure is added to each batch.

2.3.3 Determination of the Tomato Expansion Protein Genes *leEXP2*, *leEXP5* and *leEXP8* (Real-Time (RT)-PCR Method [19, 20])

The day after planting tomato seedlings with 0.5 mg/L RI roots was determined as Day 0, and the total RNA of the second true leaves of tomato was extracted on Days 0, 1, 2, 3, 5 and 7 after treatment. RT-PCR was used to detect the effect of RI on the expression of the expansion protein gene [21-23] *leEXP2*, *leEXP5* and *leEXP8* in tomato. The *EF1a* gene was used as an internal reference, and the primer was synthesized by Shanghai Jierui Bioengineering Co., Ltd. The primer sequences of the respective genes are shown in Table 3. The expression of genes in different treatment samples was calculated by $2^{-\Delta\Delta Ct}$ method [24], ΔCt = Ct (target gene) – Ct (internal reference gene), $\Delta\Delta Ct = \Delta Ct$ (test group) – ΔCt (control group), relative expression amount = $2^{-\Delta\Delta Ct}$.

3. Results

3.1 Effects on the Germination of Tomato Seeds

Table 2 shows that different concentrations of RI have different effects on the germination and growth of tomato seeds. With the increase of RI solution concentration, the germination index and growth index of tomato seeds firstly increased and then decreased.

Compared with the blank treatment, RI with a concentration of 0.02 mg/L significantly promoted the

Table 2 Effects of different concentrations of indolebutyric acid-lanthanum chelate application on seed germination of tomato and cucumber.

Items	Tomato						Cucumber					
	CK	0.002 mg/L	0.02 mg/L	0.1 mg/L	1 mg/L	2 mg/L	CK	0.002 mg/L	0.02 mg/L	0.1 mg/L	1 mg/L	2 mg/L
Germination potential (%)	54b	61.33ab	63.33a	62ab	59.33ab	57.33ab	74c	82bc	92.67a	90ab	91.33ab	84abc
Germination rate (%)	88.67b	94ab	96a	94ab	92.67ab	92.67ab	90a	92a	95.33a	94.67a	93.33a	90a
Germination index	50.7b	55.65a	59.57a	58.32a	57.43a	56.49a	162.42c	166.34b	176.88a	176.24a	174.39a	170.39ab
Vigor index	338c	384.29b	432.89a	436.73a	371.72bc	359.65bc	1,666.58c	1,732.7bc	2,088.14a	2,144.31a	1,792.33b	1,704.84bc
Hypocotyls length (mm)	41.89d	45.89c	51.33b	55.94a	50.89bc	47.89c	70.78c	83.33b	86.56a	88.72a	78.5b	79.72b
Radicle length (mm)	66.67cd	69.06b	72.67a	74.89a	64.72c	63.67d	102.61b	104.17b	118.06a	121.67a	102.78b	100.06b

Different lowercase letters after the same rows indicate significant differences ($p < 0.05$).

Table 3 Primers of real-time PCR.

Gene names	GenBank No.	Sequences
leEXP2	AF096776	5'-ccgaacccgtctctacctaa-3' 5'-tctcccaaacctactcaccc-3'
leEXP5	AF059489	5'-tcatttcctaactataccctcg-3' 5'-tacatcccctgaacctccaaca-3'
leEXP18	AJ004997	5'-ccttccccctttcacctccaa-3' 5'-cttaaggcagaacgtgagcg-3'
EF1a	X14449	5'-cttaaggcagaacgtgagcg-3' 5'-tgaagtggaagacggagggg-3'

germination potential, germination rate, germination index and vigor index of tomato seeds, which increased by 17.28%, 8.27%, 17.5% and 28.07%, respectively. It not only improved the vigor of tomato seeds, but also significantly promoted germination. In the range of RI concentration of 0.002~0.1 mg/L, the radicle length increased significantly by 3.58%~12.33%. Compared with the control, the RI used in this paper has a significant effect on the growth of hypocotyls of tomato seeds under different concentration conditions.

3.2 Effects on the Germination of Cucumber Seeds

As shown in Table 2 that with the increase of RI concentration, the germination index and growth index of cucumber seeds both increased first and then decreased, and the optimal concentration of germination index and growth index were different.

When the RI concentration was 0.02 mg/L, the germination potential of cucumber seeds was the most significant, which was 25.23% higher than that of the control; in addition, the five concentrations of RI compared with the control both have significant effects on the germination index of cucumber seeds. However, its germination rate does not constitute a significant difference, probably because the germination rate of cucumber seeds used in the experiment is originally high; in the range of RI concentration of 0.02~0.1 mg/L, the vigor index, hypocotyl length and radicle length of cucumber seeds were significantly increased to different extents, and the effect of 0.1 mg/L RI was the best, which increased by 28.67%, 25.35% and 18.58%, respectively.

3.3 Effects on the Growth of Tomato Seedlings

3.3.1 Effect on the Apparent Growth of Tomato Seedlings

It can be seen from Figs. 2 and 3 that, similar to the results of the germination test, with the increase of RI

concentration, the growth of tomato seedlings is first promoted and then inhibited. The RI concentration of 0.5 mg/L significantly promoted the plant height and stem diameter of tomato seedlings, which increased by 39.9% and 10.2%, respectively, compared with the control, and the plant height and stem diameter of 2 mg/L RI on tomato seedlings did not show a significant promotion.

3.3.2 Effect on the Biomass of Tomato Seedlings

The results in Fig. 4 are the biomass of tomato seedlings after RI treatment. It can be seen that within a certain concentration range, as the RI concentration increases, the harvested tomato biomass first increases and then decreases. Among them, when the concentration of RI was 0.5 mg/L, the biomass of the aboveground and underground parts of tomato seedlings increased the most, which was 1.38 (fresh weight), 1.48 (dry weight), 1.52 (fresh weight) and 1.61 times (dry weight).

3.3.3 Effect on Leaf Area of Tomato Seedlings

Fig. 5 shows that the 0.5 mg/L RI promoted the growth of tomato leaves most significantly, which was 140.5% higher than the control.

3.3.4 Effects on Root-Shoot Ratio and Seedling Index of Tomato Seedlings

As shown in Fig. 6, compared with the control, the root-shoot ratio of the tomato seedlings under the five concentrations of RI treatment was not significantly different. However, the effect on the strong seedling index increased first and then decreased with the increase of concentration. When the RI concentration was 0.5 mg/L and 1 mg/L, the strong seedling index increased by 13.1% and 13%, respectively, compared with the control, indicating that applying this concentration of RI can improve the quality of tomato seedlings.

3.3.5 Effects on Nutrient Uptake of Tomato Seedlings

The content of each element in the blank control tomato seedlings was taken as the standard 1. The relative content of each element in the treatment group

Fig. 2 Tomato seedlings of different concentrations of indolebutyric acid-lanthanum chelate.

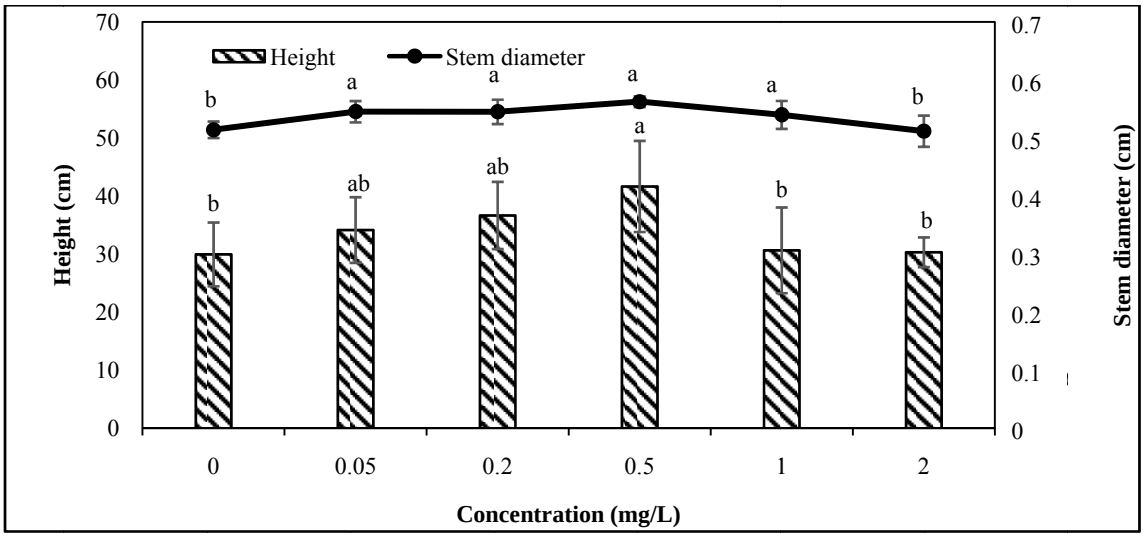

Fig. 3 Effects of different concentrations of indolebutyric acid-lanthanum chelate on height and stem diameter of tomato plant.

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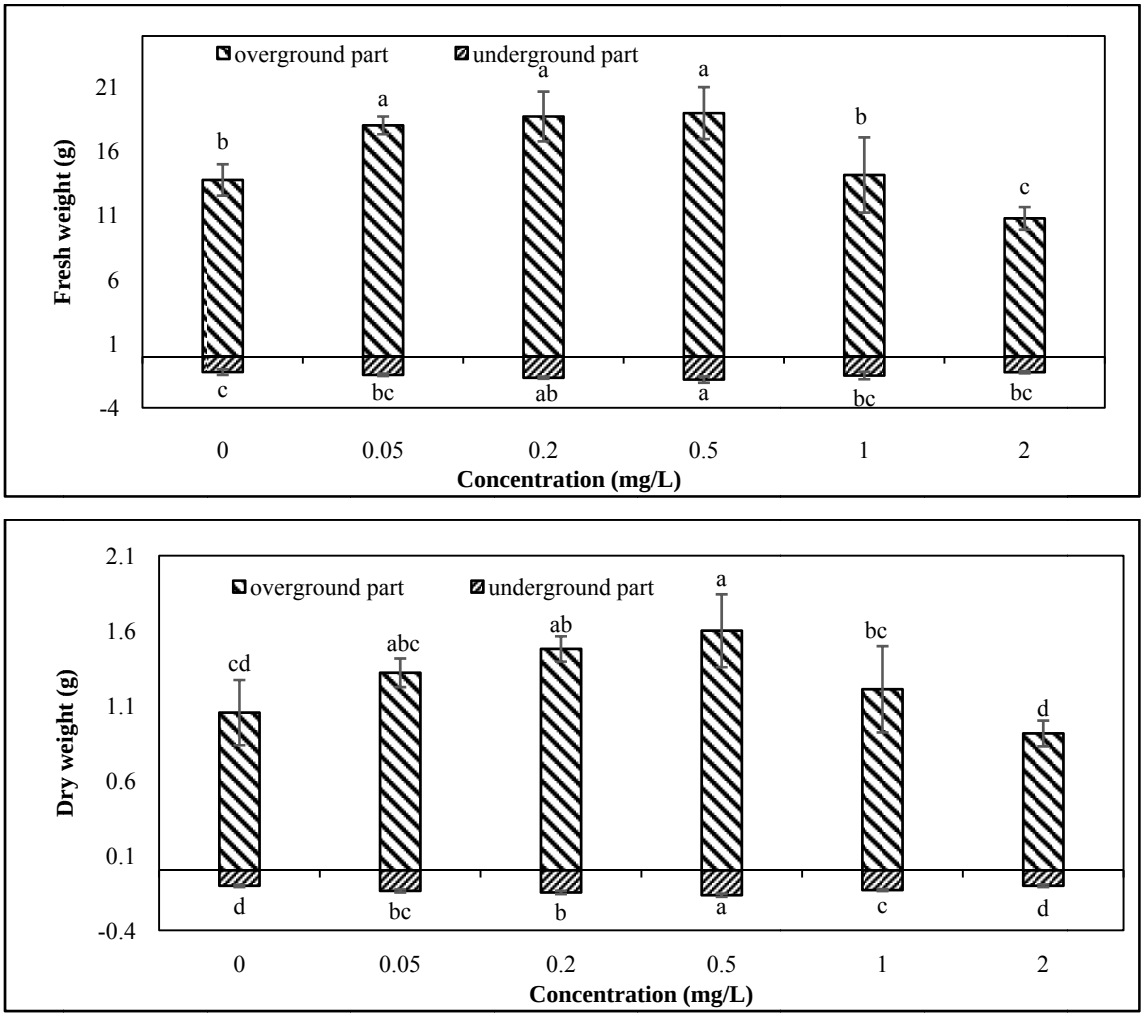

Fig. 4 Effect of different concentrations of indolebutyric acid-lanthanum chelate on fresh weight and dry weight of tomato plant.

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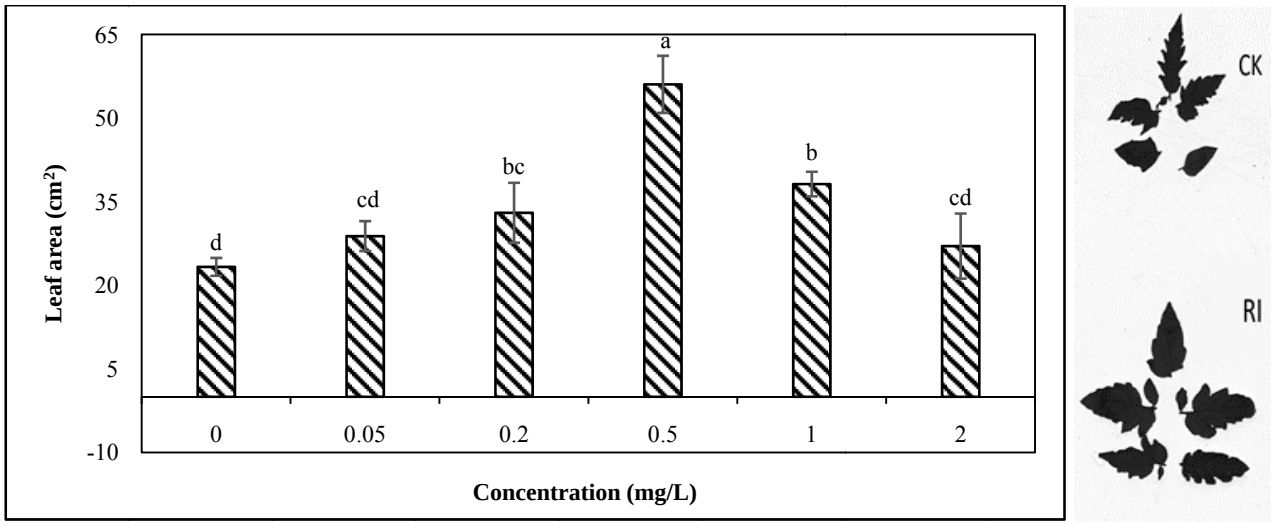

Fig. 5 Effect of different concentrations of indolebutyric acid-lanthanum chelate on leaf area of tomato plant and scanning of the second true leaves of tomato seedlings treated with 0.5 mg/L indolebutyric acid-lanthanum chelate.

The same letters present the non-significant difference at 95% confidential level.

and the control was used to characterize the elemental absorption of tomato seedlings. It can be seen from Fig. 7 that RI can increase the absorption of some elements in the upper and lower parts of tomato seedlings, and the types and levels of elemental absorption are slightly different in each part.

The results show that RI increases the absorption of Mn, P, and K in the aboveground part of the plant. When the RI concentration is 1 mg/L, the absorption of Mn increases by 131.4% compared with the control. At the concentration of 0.5 mg/L, the absorption of P and K increased by 48.1% and 46.5%, respectively, and the absorption of Mn increased by 111.1%. Moreover, RI can inhibit the absorption of Na in the aboveground part of tomato seedlings. After 0.5 mg/L RI treatment, the uptake of Na by tomato was only 57.4% of the control, and the absorption of Mg, Fe, Cu and Zn by RI-treated tomato did not show significant difference.

RI can promote the absorption of trace elements Fe, Mn, Cu, Zn and macroelements K and Mg in the underground part of tomato seedlings, and it will increase first and then decrease with the increase of RI concentration. Moreover, the absorption increment of trace elements Fe, Mn, Cu and Zn is higher than that of the constant elements K and Mg, but there is no significant difference in the absorption of P and Na elements. Compared with the control and other concentrations, the 0.5 mg/L RI increased the absorption of Fe, Mn, Cu and Zn in the underground part of tomato to the maximum, which increased by 87.7%, 66.6%, 58.7% and 36.1%, respectively, followed by K and Mg elements, which increased by 13.1% and 12.9%, respectively, compared with the control. However, for the Mg element, the content was the highest when the RI concentration was 1 mg/L, which was 19.1% higher than the control.

3.3.6 Effect on the Expression of Tomato Expansion Protein

In order to further understand the mechanism of RI promoting tomato seedling growth, this study selected the expansion protein genes *leEXP2*, *leEXP18* and *leEXP5* from the molecular biology level (Fig. 8), and used RT-PCR to detect the effect of different treatments on its expression. An expanded protein is a cell wall protein that causes the polysaccharide complex to slide by weakening non-covalent bonds between cell wall polysaccharides (such as cellulose, cellulose microfibrils, etc.), causing cell wall elongation and pressure relaxation, thereby promoting plant growth [14-16].

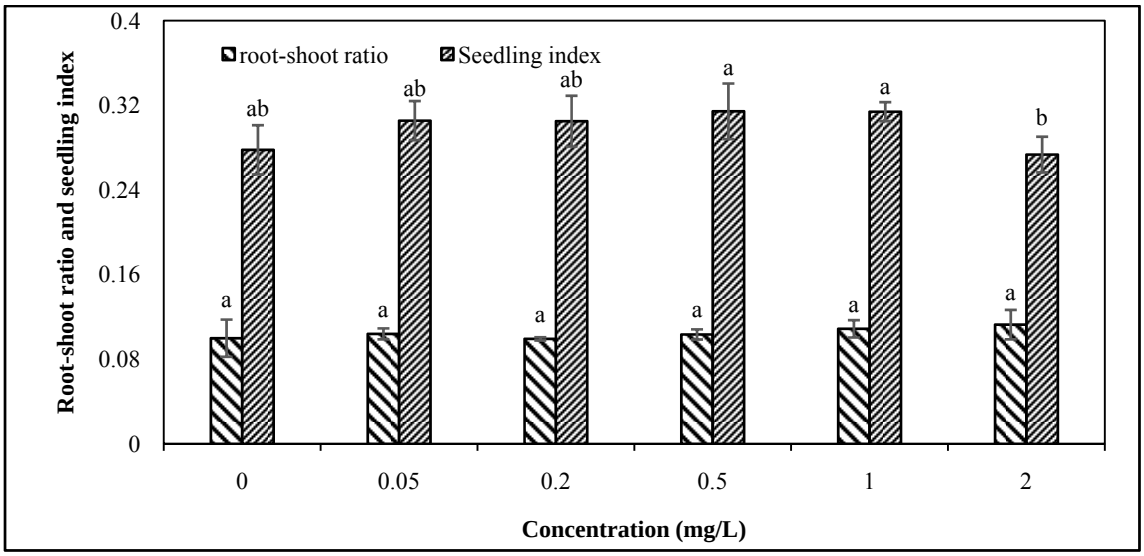

Fig. 6 Effect of different concentrations of indolebutyric acid-lanthanum chelate on root-shoot ratio and seedling index of tomato plant.

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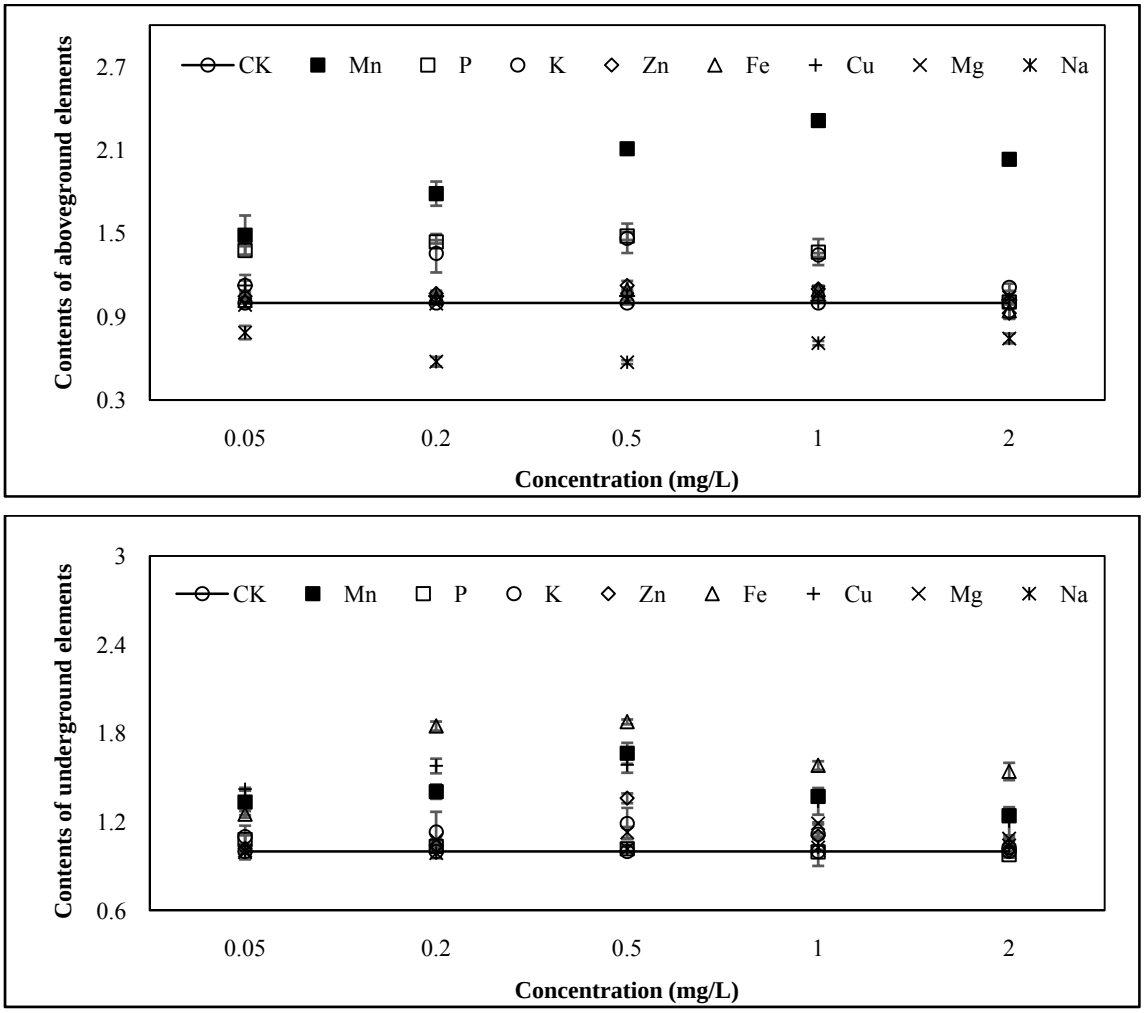

Fig. 7 The contents of elements in the aboveground and underground parts of tomato seedlings which are treated with different concentrations of indolebutyric acid-lanthanum chelate.

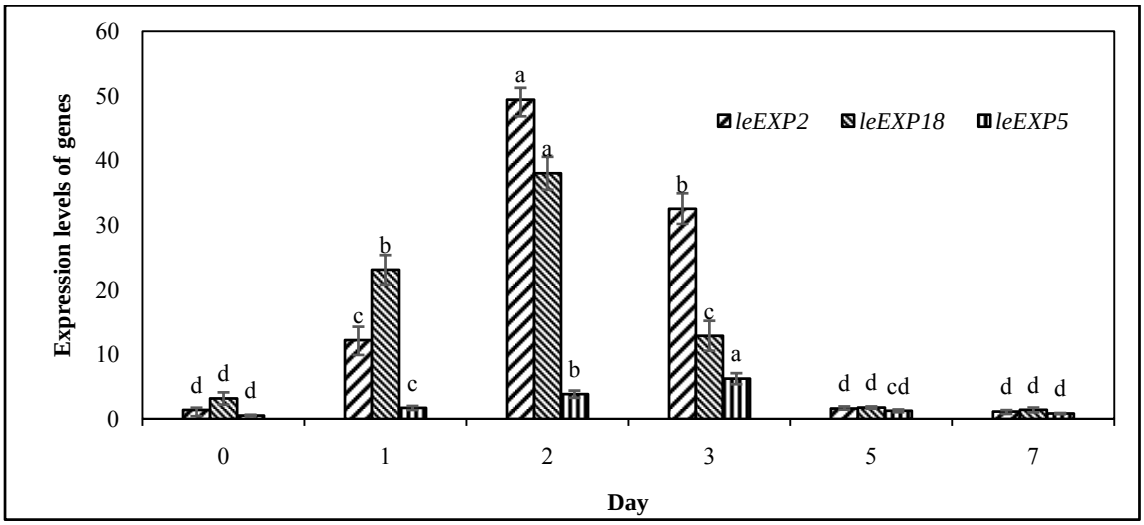

Fig. 8 Effect of 0.5 mg/L of indolebutyric acid-lanthanum chelate on the expression levels of genes *leEXP2, leEXP18* and *leEXP5.*

The same letters present the non-significant difference at 95% confidential level.

It can be seen from Fig. 8 that after RI-treated tomato, the genes *leEXP2* and *leEXP18* were induced to be expressed on the day of treatment, and the gene expression amount reached a large value on the second day, and then began to decrease; *leEXP5* was also induced on the day of treatment, but the maximum was reached on the third day. Therefore, it can be concluded that RI can promote the growth of tomato seedlings by inducing the expression of the expansion protein genes *leEXP2*, *leEXP18* and *leEXP5*, which mainly contribute to tomato growth by inducing the expression of the genes *leEXP2* and *leEXP18*.

4. Discussion

Replacing traditional fertilizers with functional water-soluble fertilizers is one of the main ways to avoid agricultural pollution and achieve sustainable agricultural development. Therefore, it is of great practical significance to develop new types of active substances that promote growth and improve quality. A large number of previous studies had shown that rare earths and organic acids had the functions of promoting crop growth and development, increasing yield and quality, and improving stress resistance, and the complexes produced by the reaction of rare earths with certain organic acids could promote the germination and growth of plant seeds [2]. At present, most researches on RI focus on synthesis methods, thermal stability and structure, and few studies on the growth-promoting functions of plants.

In the germination test, through the determination of the germination index and growth index, it was found that RI had a promoting effect on the germination and growth of tomato and cucumber seeds (Table 2). Teng *et al.* [3] showed that the germination test of mung beans and green beans showed that the low concentration of lanthanum-gibberellin complex could increase the germination rate by 50%-100%, and increased the length of radicle and hypocotyl by 15%-20%. And high concentration would be suppressed. This result was consistent with the results of the RI test in this study, indicating that the active substance RI had a biological stimulating effect, which provided a basis for pot experiments.

In the pot experiment, the measurement of growth indexes such as plant height, stem diameter, leaf area, dry weight of the aboveground and underground part, root-to-crown ratio and strong seedling index showed that a certain concentration of RI had a significant effect on the growth of tomato seedlings. This study further studied the mechanism of action of this active substance and found that the appropriate concentration of RI could promote the absorption of trace elements Fe, Mn, Cu, Zn and macroelements K, P, and Mg in the roots of tomato seedlings, and accelerated the transfer of nutrients from the roots to the ground, and then promoted the growth and development of tomato seedlings, and improved seedling quality. At the same time, it was further verified from the molecular biology level that the appropriate concentration of RI could stimulate the expression of tomato expansion protein genes *leEXP2*, *leEXP18* and *leEXP5* (Fig. 8) to increase, thereby increasing the expansion protein in tomato plants, promoting cell division, elongation, and making tomato plants grow tall and strong.

5. Conclusions

A suitable concentration of RI can promote the germination and growth of tomato and cucumber seeds. The 0.02 mg/L RI is the optimum concentration for tomato and cucumber seed germination. When the concentration is 0.1 mg/L, it is the most suitable for tomato and cucumber radicle and hypocotyl growth.

The RI of 0.5 mg/L can promote the plant height, stem diameter, biomass and leaf area of tomato seedlings, and other seedling stage index increased significantly. At the same time, the absorption and the transfer of some nutrients from the underground part to the aboveground part of the tomato seedlings were increased, and the relative expression of the tomato

expansion protein genes *leEXP2*, *leEXP18* and *leEXP5* was significantly increased, thereby improving the quality of tomato seedlings.

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