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EFFECTS OF CINNAMIC ACID ON THE ROOT GROWTH OF CUCUMBER (*CUCUMIS SATIVUS* L.) AND FIGLEAF GOURD (*CUCURBITA FICIFOLIA* BOUCHÉ) SEEDLINGS

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Abstract

Effects of cinnamic acid (CA) on growth and endogenous hormone content in the roots of cucumber and figleaf gourd seedlings were studied and explained the reason why figleaf gourd was better in resisting CA stress than cucumber using their seedlings as experimental materials. The effect of CA at different concentrations (0, 0.05, 0.10, 0.15, 0.20 and 0.25 mmol/l) was analysed on primary root elongation, total root elongation, average diameter of total roots, total root volume, and total root tips as well as the effects of 0 and 0.25 mmol/l CA on root growth, root density, root hair growth rate, indole acetic acid (IAA) content and ethylene (ET) production rate. The results showed that CA stress decreased the primary root elongation, total root elongation, total root tips, Grade III root and root hair growth rates of both plants. The inhibiting effect of CA was stronger on cucumber than figleaf gourd. Besides, CA stress increased their average diameters of total roots and root hair densities. The increase in the average diameter of total roots of cucumber was larger than that of figleaf gourd while its increase in root hair density was far lower. Apart from that, 0.25 mmol/l CA stress increased the IAA contents and ET production rates in both kinds of roots. The increase in IAA content in cucumber root was 16% higher than in figleaf gourd. However, the increase in the ET production rate of cucumber root was 51% lower than figleaf gourd root. In conclusion, higher concentration IAA could inhibit root growth. Under CA stress, the increase in the IAA content in figleaf gourd root was lower than that in cucumber root, indicating that CA exerted a weaker inhibiting influence on figleaf gourd. Besides, higher ET production rate contributed to the increase in root hair density. Under CA stress, figleaf gourd root had a higher ET production rate than cucumber root, thus having more root hairs.

Introduction

Autotoxicity inhibit or retard growth of other plants by releasing toxic chemical substances (Takako *et al.* 2023). It is a widespread phenomenon in natural ecosystem which had great ecological significance in controlling the density of plant population (Zhang *et al.* 2022). In artificial ecosystem, autotoxicity can decrease the yield by weakening the growth of plants. Therefore, it is believed to be an important cause of continuous cropping obstacle. In agricultural production, autotoxicity generally works through the autotoxins secreted by plants. They can poison the roots of plants directly or indirectly and thus retard the growth of roots and influence the absorption of water and mineral ions (Ren *et al.* 2015).

Root plays an important role in controlling the “source-sink” correlation of plant and is the source for sensing underground semiochemicals (Mirsoleimani *et al.* 2023). Root hair is the most active tissue on the physiological activity in the functional section of root (Wang *et al.* 2009). Under environmental stress, plant hormone controls the quantity and density of root hairs. Ethylene (ET) can regulate the generation and elongation of root hair in *Arabidopsis* (Dolan 2001).

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It has been proved by many studies that ET can promote the growth of roots and root hairs of many higher plants (Dolan 2001). However, due to complex growing environment of root, the knowledge about the changes to the morphology and structure of roots as well as the growth and development processes of root hairs are quite limited. Therefore, using cucumber (sensitive to CA) and figleaf gourd (CA tolerated) as experimental materials, the effect of CA (a typical autotoxin) on the growth of root and the generation of root hair was studied. Through studying the correlation between the generation of root hair and the endogenous hormone of root, this paper attempt to explore the physiological mechanism for the response of roots and root hairs of cucumber and figleaf gourd to CA stress, to provide technical support for autotoxicity.

Materials and Methods

The seeds of cucumber (*Cucumis sativus* L) and figleaf gourd (*Cucurbita ficifolia* Bouché) were disinfected with 70% ethyl alcohol and placed into a 30°C incubator for accelerating germination. After germination, the seeds were sown in plastic cases (25 × 20 × 1.5 cm) with Yamasaki nutrient solution solid media. The concentrations of cinnamic acid (CA) in solid media (pH 6.0) were 0, 0.05, 0.10, 0.15, 0.20, and 0.25 mmol/l. Each plastic case had two cucumber and figleaf gourd seeds and kept in a plant growth chamber with light intensity of 90 μmol $m^{-2}s^{-1}$ and relative air humidity of 60%. The day and night temperatures were 26 and 18°C, respectively, with 12 hrs lighting time. Each treatment had 6 replicates and was repeated 3 times. After 7 d of treatment, the root samples were collected to observe the root morphology and to determine the content of Indole acetic acid (IAA) and ethylene (ET).

The seeds were treated based on the methods proposed by Franklin and Dias (2011). The seeds were sown in a 200 mm culture dish with a thin Yamazaki nutrient solution solid medium. To avoid the medium being reduced for root absorption, a certain amount of medium was replenished every day. After 7 d of treatment, the growth rate and elongation of root hair were observed with a microscope.

The primary root elongation, total root elongation, average diameters of total roots, total root volume and total root tips of cucumber and figleaf gourd seedlings were scanned with a root scanner (Epson Perfection 4900 PHOTO, Japan). Later the parameters were analyzed through the analysis software Win RHIZO.

The roots of cucumber were classified into three grades according to the root diameters, namely, Grade I Root (>2 mm), Grade II Root (1-2 mm) and Grade II Root (<1 mm). Whereas, the roots of figleaf gourd Were Grade I Root (>3 mm), Grade II Root (1.5-3 mm) and Grade III Root (<1.5 mm).

The growth of root hair was observed with the stereoscopic microscope Leica S8AP0 and photographed with Leica DFC450 through Leica Application Suite V4 (Graber *et al.* 2015).

The extraction, purification, and content determination of IAA were based on the method of Kumar *et al.* (2023). The root tip tissue (0.5 g) was kept in low light condition and then precooled with liquid nitrogen. Precooled methyl alcohol (800 ml/l) and antioxidant 1, 2, 6-di-tert-butyl-p-methylphenol (1 mmol/l) were added into the mortar to make the homogenate at 4°C. After 4 hrs, the homogenate was centrifuged (1000 × g) for 15 min. The methyl alcohol was removed in a vacuum drier and then redissolved the extractive with phosphate buffer for enzyme-linked immunosorbent assay. The absorption wavelength was 490 nm.

The determination of ET content was based on the method proposed by Ribaudo *et al.* (2006). The root segments (1 cm) were kept into flasks with 1 mL of 50 mmol/l phosphate buffer (pH 6.8) and sealed the flasks with rubber plugs. The samples were provided with a constant temperature bath (30°C) for 4 hrs. The concentration was determined with gas chromatograph (Shimadzu GC-

14B, Japan), where the chromatographic column was 4 mm× 2m, G.D.X.502 and the carrier gas was hydrogen-nitrogen compound. The column and detector temperature were 60 and 100°C, respectively. All the data were repeated 3 times and the results were expressed as mean ± SD (standard deviation). Statistical analysis was performed using Tukey's test at 5% level.

Results and Discussion

The morphological variations in the roots of cucumber and figleaf gourd seedlings were different after treated with CA. With the increasing concentration of CA, the primary root elongation, total root elongation and total root tips of cucumber and figleaf gourd all declined significantly. The inhibition on the root of cucumber seedling was stronger than on that of figleaf gourd seedling (Figs. 1A, 1B and 1E). The primary roots of cucumber and figleaf gourd were most vulnerable to CA. Treated with 0.25 mmol/l CA, the inhibition ratios of cucumber and figleaf gourd primary roots were 59 and 48%, respectively. After treated with 0.25 mmol/l CA for 7 d, the inhibition ratios of the total root elongation of cucumber and figleaf gourd were 39 and 23%, respectively. With the exposure of cinnamic acid (0.25 mmol/l) , the total root tips of cucumber and figleaf gourd were decreased by 66 and 75%, respectively, than the control. As the CA concentration rose, the total root volume of cucumber was increasing, while that of figleaf gourd decreased slightly (Fig. 1D). On the other hand, the root diameter was increased due to the application of CA. The increase in the root diameter of cucumber was higher than in that of figleaf gourd. After treated with 0.25 mmol/l CA for 7 d, the average root diameters of cucumber and figleaf gourd were increased by 35 and 9%, respectively (Fig. 1C).

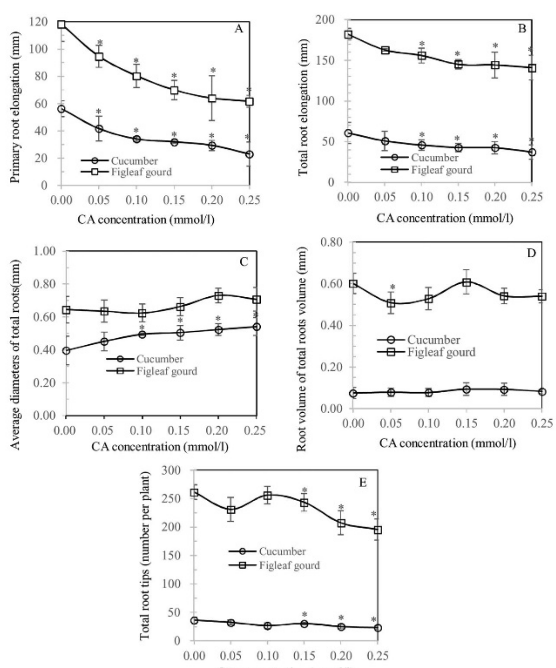

Fig. 1. Effects of CA at different concentrations on primary root elongation (A), total root elongation (B), average diameters of total roots (C), total root volume (D), and total root tips (E) of cucumber and figleaf gourd seedlings.

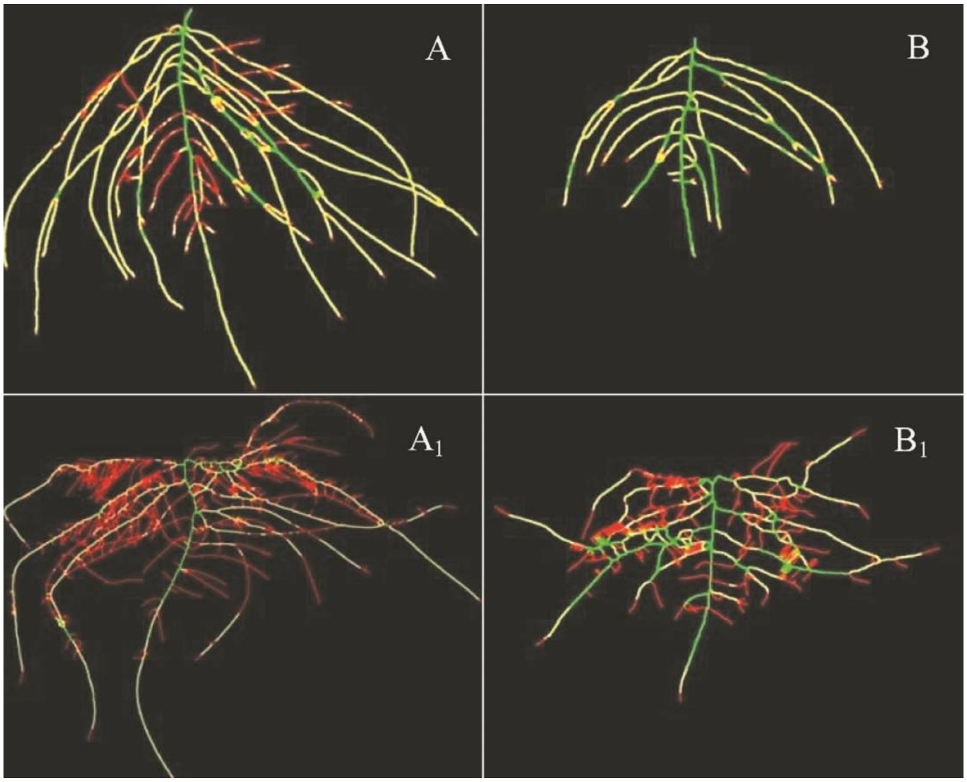

Plate 1. Effects of CA on root growth of cucumber and figleaf gourd seedlings. Root of cucumber (A and B) and Figleaf gourd (A_1 and B_1) were exposured to 0 mmol/l CA (A and A_1), and 0.25 mmol/l CA (B and B_1). Green roots: Grade I roots (thicker roots,); yellow roots: Grade II roots (thick roots); red roots: Grade III roots (fine roots).

The roots were grouped into three classifications according to diameters. They were Grade I root, Grade II root, and Grade III root from thick to thin. After treated with CA, the Grade I and II roots of cucumber and figleaf gourd both were increased while the Grade III roots were decreased significantly (Plate 1). While treated with 0.25 mmol/l CA for 7 d, the Grade III roots of cucumber were lesser than that of figleaf gourd. Thus, in conclusion it could be drawn that CA had a stronger inhibiting effect on cucumber than on figleaf gourd in Grade III roots.

The growth of the primary and total roots of cucumber and figleaf gourd was greatly inhibited by CA and the inhibiting effect on cucumber was stronger than figleaf gourd (Figs 1A and B). Besides, CA could increase the diameter and volume of cucumber root while the increase in those of figleaf gourd root not noticeable (Figs 1C and D). The root became thick (indicating that the lignified degree increased), reducing the root's ability in absorbing water and nutrients (Yu and Matsui 1997, Yu *et al.* 2000, Ye *et al.* 2006). After being exposed to CA for 7 d, the thin roots of cucumber were almost disappeared, yet figleaf gourd still had a considerable amount of thin roots (Plate 1). Therefore, figleaf gourd root was still strong in absorption even under serious autotoxicity. However, cucumber root might lose absorbing ability completely. This paper disagrees with the previous studies study which proposed that autotoxin only had a significant effect on cucumber while figleaf gourd would not be influenced by autotoxin. The root growth of cucumber and figleaf gourd both changed greatly under CA stress, and in that case, the roots grew thick which might result in the early differentiation of vascular bundles. Consequently, it would lead to the early maturity and senescence of roots or the thinning of root cap between the root tip and vascular tissue, which reduced its ability in protecting root tip and thus increased the risk of CA poisoning. Besides, this paper also disagrees with Li *et al.* (2008) who believed that low concentration autotoxin could promote the growth of cotton and high concentration autotoxin would inhibit it.

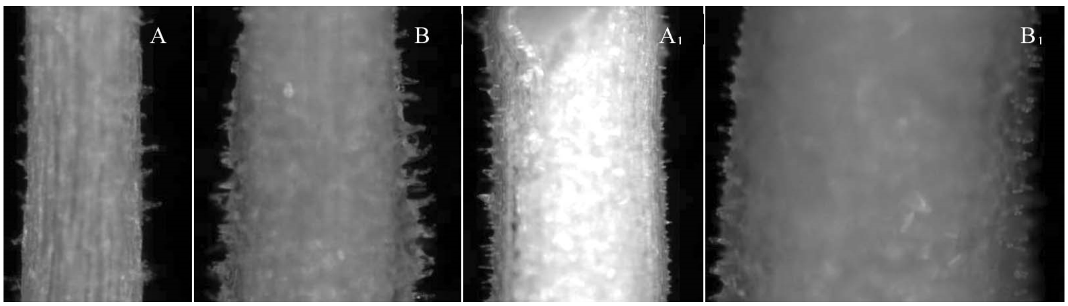

Plate 2. Effects of CA on root density of cucumber and figleaf gourd seedlings. Root of cucumber (A and B) and Figleaf gourd (A_1 and B_1) exposure to 0 mmol/l CA (A and A_1) , and 0.25 mmol/l CA (B and B_1).

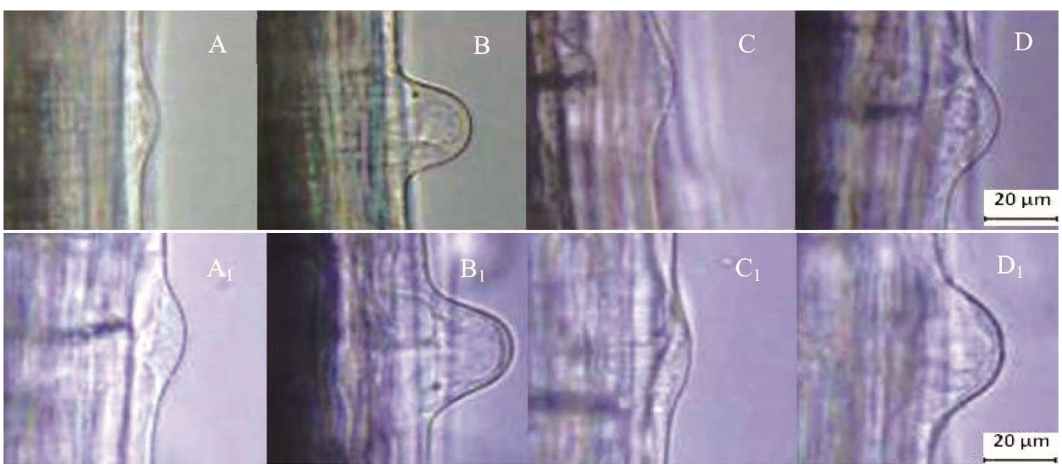

Plate 3. Effects of cinnamic acid (CA) on root hairs growth rate of cucumber and figleaf gourd seedlings. Root hairs growth rate of cucumber (A-D) and figleaf gourd (A1-D1) seedlings exposure to 0 mmol/l CA (A and A1) , 0.25 mmol/l CA (C and C1), 0 mM CA (A and A1) in 60 minutes later (B and B1), and 0.25 mmol/l CA in 60 minutes later (D and D1).

Under 0.25 mmol/l CA stress, the root hair densities of cucumber and figleaf gourd were increased significantly (Plate 2) and the increase in the root hair density of figleaf gourd was higher than in that of cucumber. The root hair density of figleaf gourd and cucumber were increased by 2-fold and 43% compared to the control group, respectively (Fig. 2A). Cinnamic acid (0.25 mmol/l) had a significant inhibiting effect on the root hair growth rates of cucumber and figleaf gourd and were decreased by 64 and 36%, respectively (Fig. 2B). The inhibition rate on the growth of cucumber root hair was higher than on that of figleaf gourd root hair (Plate 3).

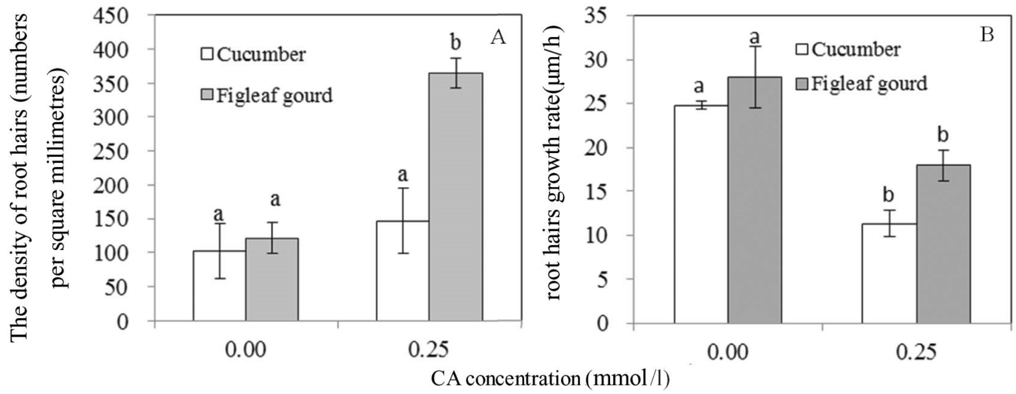

Fig. 2. Effects CA on root density, (A) and root hairs growth rate, (B) of cucumber and figleaf gourd seedlings. Data are the means of three independent replicates with standard errors shown by vertical bars. Different letters mean significant differences between the control and the CA treatment within the species (P <0.05).

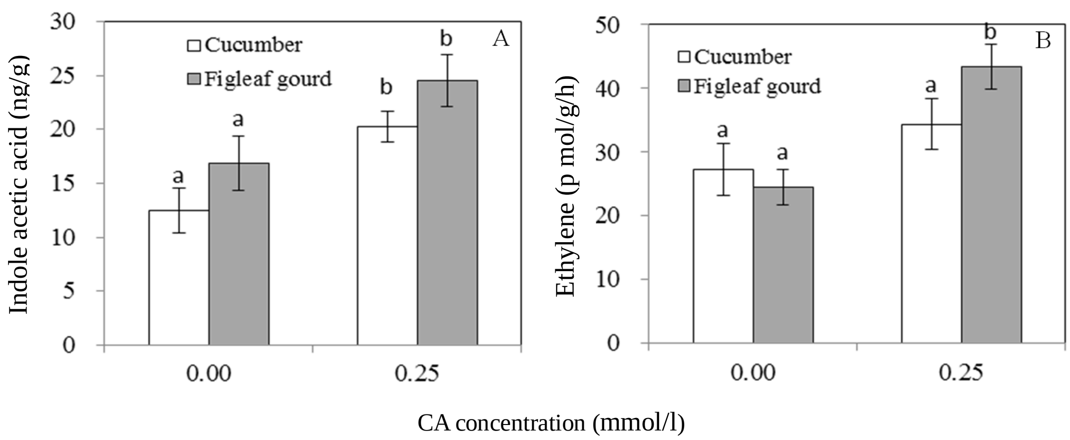

Fig. 3. Effects of CA on indole acetic acid content and ethylene production in roots of cucumber and figleaf gourd seedlings. Data are the means of three independent replicates with standard errors shown by vertical bars. Different letters mean significant differences between the control and the CA treatment within the species (P < 0.05).

Under 0.25 mmol/l CA stress, the IAA content in cucumber and figleaf gourd were increased significantly by 62 and 46% compared with the control group. Whether in the control group or treated group, the IAA content in figleaf gourd roots was higher than that in cucumber roots (Fig. 3A). Similarly, 0.25 mmol/l CA accelerated the production rates of ET in cucumber and figleaf gourd roots by 26 and 77%, respectively, compared with the control group (Fig. 3B).

CA significantly increased the IAA content in cucumber and figleaf gourd roots. On the other hand, CA had a little influence on the ET production rate of cucumber root but significantly increased the ET production rate of figleaf gourd root (Figs 3A and B). It accords with the research findings of Liu *et al.* (2007). The growth of both cucumber and figleaf gourd root were inhibited by CA, which might be caused by the increase of IAA content in roots.

Rahman *et al*. (2002) found that IAA and ET influenced *Arabidopsis thaliana* in the differentiation, growth and development of root hair. On the one hand, IAA could regulate the lateral root generation, root hair elongation, root hair density of *Arabidopsis thaliana* (Ma *et al.* 2001). Therefore, ET and IAA could regulate the generation and development of root hair jointly. The results of the present test have verified those research findings. The test results showed that under CA stress, the IAA content in cucumber and figleaf gourd roots was increased. Higher concentration IAA inhibited the growth of root and meanwhile promoted the generation of a large amount of ET (Fig. 3). And ET induced the generation of considerable root hairs (Plate 2, Fig. 2). Due to the interspecific difference between cucumber and figleaf gourd, the production rate of ET in figleaf gourd was higher than that in cucumber. For this reason, the root hair density of figleaf gourd was higher than that of cucumber under CA stress. Apart from CA, the development of root hair was influenced by many other environmental factors as well and thus root hair was of strong plasticity (Muller *et al.* 2004). As the previous researches mainly focused on the influencing factors of root development and function while those on the active regulating on the root development and physiological function are quite limited, thus, the active regulatory mechanism of root development can be one of the major directions for future researches.

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