

Article

Effects and Mechanism of Auxin and Its Inhibitors on Root Growth and Mineral Nutrient Absorption in Citrus (Trifoliate Orange, *Poncirus trifoliata*) Seedlings via Its Synthesis and Transport Pathways

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Abstract: As an endogenous hormone, auxin plays a crucial role in regulating plants' growth and development, and also in the responses to abiotic stresses. However, the effects and mechanism of auxin and its inhibitors on plant growth and mineral nutrient absorption in citrus have not been thoroughly studied. Therefore, we used trifoliate orange (citrus's rootstock, *Poncirus trifoliata*) as the experimental material to supplement the research content in this area. The trifoliate orange seedlings were treated with exogenous auxin (indolebutyric acid, IBA) and auxin inhibitor (2-naphthoxyacetic acid, 2-NOA) in a sand culture system. The results showed that compared to the control, exogenous auxin ($1.0 \mu\text{mol L}^{-1}$ IBA) significantly enhanced the taproot length, lateral root length, and lateral root number by 17.56%, 123.07%, and 88.89%, respectively, while also markedly elevating the levels of nitrogen (N), phosphorus (P), potassium (K), copper (Cu), and zinc (Zn) by 14.29%, 45.61%, 23.28%, 42.86%, and 59.80%, respectively. Again compared to the control, the auxin inhibitor ($50.0 \mu\text{mol L}^{-1}$ 2-NOA) dramatically reduced the taproot length, lateral root length, and lateral root number by 21.37%, 10.25%, and 43.33%, respectively, while also markedly decreasing the levels of N, magnesium (Mg), iron (Fe), Cu, and Zn by 7.94%, 10.42%, 24.65%, 39.25%, and 18.76%, respectively. Furthermore, IBA increased auxin accumulation in the root hair, stele, and epidermal tissues of citrus taproots, and promoted the up-regulation of auxin synthesis genes (*TAR2*, *YUC3*, *YUC4*, *YUC6*, *YUC8*) and transport genes (*ABCB1*, *ABCB19*, *AUX1*, *LAX1*, *LAX2*, *PIN1*, *PIN3*, *PIN4*). In contrast, 2-NOA decreased auxin levels in the root hair, stele, and epidermal tissues of citrus taproots, and was involved in the down-regulation of auxin synthesis genes (*TAR2*, *YUC3*, *YUC4*, *YUC6*) and transport genes (*ABCB1*, *AUX1*, *LAX1*, *LAX2*, *LAX3*, *PIN3*). Interestingly, 2-NOA dramatically elevated auxin level specifically in the root tip of citrus taproot. Therefore, 2-NOA disrupts auxin reflux from the root tip to root hair and epidermal tissues in citrus taproot through down-regulation of auxin transport genes, thereby creating localized (i.e., root hair zone and epidermal tissues) auxin deficiencies that compromise root system architecture and nutrient acquisition capacity. According to the results of this study, exogenous auxin analogs could regulate citrus growth and mineral nutrient absorption through the auxin synthesis and transport pathways.

Keywords: 2-naphthoxyacetic acid; GC-MS; indolebutyric acid; mineral nutrition



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1. Introduction

During plant growth and development, auxin is involved at nearly every stage, from seed germination to aging and death. Auxin biosynthesis pathways are primarily classified into tryptophan (Trp)-dependent and Trp-independent mechanisms. The predominant routes of auxin production in plants occur through Trp-dependent pathways, which encompass four major enzymatic routes: the indole-3-acetamide (IAM) pathway, indole-3-pyruvate (IPA) pathway, tryptamine (TAM) pathway, and the indole-3-acetaldoxime (IAOx) pathway [1]. The auxin synthesis and transport pathway is an extremely complex system that plays a crucial role in plant growth and development and their responses to stress [2,3]. However, current research on the regulation of plant roots through signaling pathways remains incomplete.

The citrus fruit, renowned for its distinctive flavor and abundant nutritional content, is extensively cultivated in 140 countries worldwide, making it the most widely produced fruit globally [4]. However, soil erosion leads to the loss of organic matter, which in turn reduces the water and nutrient retention capacity of the soil, thus adversely affecting citrus production [5]. Therefore, understanding the effects of auxin on citrus roots is crucial to enhancing the mineral element absorption level and stress resistance of citrus plants.

In general, roots are mainly divided into two types: primary roots formed during embryo development and secondary roots formed after embryo development [6]. These secondary roots include lateral roots (LRs), which branch from the main root, and adventitious roots (ARs), which develop on non-root tissues such as hypocotyls, stems, and leaves [7]. Auxin, as the main plant hormone, plays an important role in root development. Its ability to drive root growth and development in response to the regulatory networks of related genes has been well characterized [8].

Auxin synthesis genes, as well as input and output vectors, play an important role in auxin signal transmission. The regulation of the plant root structure mainly depends on the active auxin-synthesizing genes *tryptophan aminotransferase-related* (TAR) and *yucca* (YUC) in the apex, which induce cells to produce auxin through specific signaling pathways [9,10]. *AUX1* plays an important role in regulating the asymmetric distribution of auxin in root tips, especially in synergistic action with *LIKE AUX3* (*LAX3*) to regulate plant auxin levels [11,12]. The directed flow of auxin between cells is closely related to PIN efflux transporters. In the *PIN* gene family, *PIN1* is a target regulating auxin action, while *PIN3* is an auxin response factor in ARF7-mediated lateral root development [12,13]. In addition, SWI/SNF chromatin also promotes root stem cell niche formation by targeting *PIN4* [13]. As a novel vector, the ABCB protein family plays a regulatory role in maintaining auxin levels during plant tissue mitosis [14].

Mineral nutrients are selectively absorbed through the root system to regulate plant growth and development [15,16]. Mineral elements play a variety of important roles in plants. For example, phosphorus (P) and potassium (K) regulate the sugar metabolism pathway of the plant, while magnesium (Mg) influences photosynthesis and respiration [17,18]. It is worth noting that zinc (Zn) has a very important relationship with the synthesis and transport of auxin [19].

Although the effects of auxin on plant growth and mineral nutrient absorption in plants have been thoroughly studied, the effects of auxin and its inhibitors on root growth and mineral nutrient absorption in citrus have not been entirely elucidated. For example, it is not clear whether auxin and its inhibitors regulate citrus root growth and nutrient absorption through auxin synthesis and transport. Therefore, we conducted this study to explore the regulatory mechanisms of IBA and 2-NOA on the root growth and mineral nutrient absorption of citrus seedlings. By analyzing the effects of exogenous treatment on taproot elongation, lateral root density, mineral element content, and related gene

expression, the key role of auxin in root development and nutrient absorption was clarified. This study further revealed the internal molecular mechanism of auxin's influence on root structure and function by regulating the expression dynamics of synthesis and transport genes, in order to provide a theoretical basis for optimizing root management and mineral nutrition regulation in citrus cultivation, and to help improve citrus stress resistance and production efficiency.

2. Materials and Methods

2.1. Materials and Growth Conditions

In this study, trifoliate orange (*Poncirus trifoliata*) seeds purchased from the experimental base of the Wuhan Forest Fruit Promotion Station were selected as experimental materials. First, the river sand used for planting materials was washed three times with tap water and pure water, and then sterilized using a high-temperature autoclave to ensure cleanliness. The high temperature autoclave (121 °C, 15 psi) lasted for 60 min, and the time count began when the center of the log reached 121 °C. The sterilization process was carried out in two high-temperature cycles, with cooling during the cycle interval. Then, full and consistent seeds were selected, soaked, and disinfected with 75% (*v/v*) ethanol for 15 min, and washed 4–5 times with sterile water. After disinfection, the seeds were placed in a sterilized tray and placed in a dark incubator at 28 °C for germination. When the germinated seeds were 1 cm in length, they were sown in round pots (height × length = 30 cm × 20 cm) filled with sand and placed in a greenhouse.

The transplanted potted seedlings were cultured under 16 h of light (28 °C) and 8 h of darkness (22 °C). Nutrient solution was applied from the day of transplantation and the seedlings were watered every 3 days with a dosage of 100 mL each time. After the growth of three true leaves, the treatment began. All seedlings were irrigated with full Hoagland solution (100 mL) once every 3 days.

The basic culture Hoagland solution was composed of the following: 6.00 mM KNO₃, 4.00 mM Ca (NO₃)₂·4H₂O, 1.00 mM NH₄H₂PO₄, 2.00 mM MgSO₄·7H₂O, 9.40 μM MnCl₂·4H₂O, 0.34 μM CuSO₄·3H₂O, 0.74 μM ZnSO₄·7H₂O, 48.00 μM H₃BO₃, 0.14 μM H₂MoO₄, and 50.00 mM EDTA-Fe, at 5.84–6.02 pH.

2.2. Experiment Design

The seedlings were first treated with growth regulators (Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China) on 26 February 2024 in three different ways: the control group (CK), 1.0 μmol L⁻¹ IBA, and 50.0 μmol L⁻¹ 2-NOA. Four pots were treated, each containing 10 seeds, and the process was repeated three times. Following 10 treatment applications, the test material was removed, and the root morphology was determined. The cultivation substrate was stripped away, and the fine sand on the root surface was washed with water to determine the root morphology. After 15 treatments, the same method was used to remove the test material for subsequent analysis.

2.3. Study of Root Morphology

After 10 treatments of the transplanted test material, 3 seedlings with more consistent growth were selected from the pot seedlings, and the length of the taproot and lateral root, the diameter of the taproot, and the number of lateral roots were measured with a ruler. Each index was measured three times.

2.4. Mineral Nutrient Content Analysis

The test materials of the aboveground and underground parts of each treatment were packaged and dried in an oven at 105 °C to constant weight (about 48 h). After

crushing, 0.5 g of the test materials was ground into a powder with liquid nitrogen. After weighing, 0.2 g of the sample was placed into a muffle furnace (500 °C) for ashing for 10 h, then removed and dissolved with 0.1 mol L⁻¹ HCl. The dissolved powder was sent to the Institute of Soil Science, Nanjing, Chinese Academy of Sciences, where the contents of phosphorus, potassium, calcium, magnesium, iron, copper, zinc, boron, and other mineral nutrients were determined using an atomic absorption spectrometer (SPECTR AA220, Varian Co., Palo Alto, CA, USA). The determination method followed that of Borah et al. [20]. The total nitrogen content was determined using a plasma emission spectrometer (ICP Spectrometer, Model: IRIS Advantage, Edison, NJ, USA) and analyzed following Kramberger's method [21].

2.5. Root Auxin Content

The auxin content of four regions of the taproot was separately quantified using gas chromatography–mass spectrometry (GC-MS): root tips (0–0.5 cm from the apex), root hair zones (2–3 cm from the apex), epidermis (4–5 cm from the apex), and stele (4–5 cm from the apex).

The relevant test materials from each treatment were collected, frozen, and stored in dry ice. These samples were sent to the Engineering Research Center of the Ministry of Education of Plant Growth Regulators, China Agricultural University, for IAA determination. Each treatment was repeated three times, and the determination followed Ali et al.'s method [22].

2.6. Analysis of Gene Expression

Gene selection: Auxin synthesis and transport genes were screened using the Sweet Orange whole genome databases (<http://citrus.hzau.edu.cn/index.php>, accessed on 10 February 2025) [23].

Primer design and synthesis: Gene primers were designed using Primer 5, and the housekeeping gene β -actin was selected as the internal reference gene to amplify with other target genes and correct differences in their relative expression levels across each sample (Table 1, [24]). The primers were synthesized by BGI Co., Ltd., Shenzhen, China. Before use, the primers were centrifuged at 10,000× g at room temperature for 1 min. RNase-free H₂O was added according to the corresponding concentration of the respective primers, diluted to 10 µmol L⁻¹, and the solution was stored in a refrigerator at 4 °C after gentle mixing.

Table 1. Compilation of the gene-specific primer sequences employed in this study for qRT-PCR.

Gene	Accession No.	Forward Primer (5'-3')	Reverse Primer (5'-3')	Amplification Size (bp)
ABCB1	Ciclev10010916m	GAGCCATTCACGCCACTTC	TCTTGTAACCGAGCCTTTGAGC	186
ABCB19	Ciclev10010931m	GCATGAGTTTGGGTCAGTCTTT	CATCTTCCATTTGTTGGGTCTT	127
AUX1	Ciclev10011596m	CTTGACTCTGCCCTATTCATTCTC	TGGACCCAGTAACCCATCAAGC	205
LAX1	Ciclev10031413m	TTGGCGGACATGCAGTGAC	CAGCGGCAGCAGAAGGAAT	123
LAX2	Ciclev10028271m	TGTGGGAAGATGGGTAGGGAC	TAGTCATGCTCGCCACCC	98
LAX3	Ciclev10001072m	ATCACTTTCGCTCCTGCTGC	CAAACCCAAATCCCACCACTA	133
PIN1	Ciclev10007787m	GCTTTGGCAACAGAAGAGGATT	ATTACACTTGTCGGCGGCATA	94
PIN3	orange1.1g006199m	CATGCCTCCAGCGAGTGTTAT	TGCCACCTGAAAGCGATTAGA	126
PIN4	Ciclev10012938m	ATGGGGTTGAAAACGAAGGG	CCTGATAAGTTTCCTCCACACCA	167
TAR2	Ciclev10020085m	CACACACGGCACACCCCTA	GCCTCCCACTCCCCAGATC	137
YUC3	Ciclev10006828m	CCTTCAGGTTTAGCCGTTGC	GGAAGTTTGGAAGTTGCGAGA	157
YUC4	Ciclev10008466m	GACCATCTGGGTAGCCGTTT	GTATTTTGGGAAGTTTTCAGGGA	185
YUC6	Ciclev10008473m	GTGGTTGCTAAAGTGGCTGC	GTTGAAGGGGACCCAAAAGA	122
YUC8	Ciclev10020503m	GTGATAATGGTAGGGGCAGGA	GAATGGCAGGTGAGGGAGC	183
β -actin	Ciclev10025866m	CCGACCGTATGAGCAAGGAAA	TTCTGTGGACAATGGATGGA	190

RNA extraction: Root segments 0–1 cm and 2–3 cm away from the lateral root tip were frozen in liquid nitrogen for 1 min and then stored in an ultra-low temperature refrigerator at -80°C for total RNA extraction. The extraction method followed the product manual of the kit.

Synthesis of first strand cDNA: cDNA was synthesized using PrimeScript[®]RT reagent Kit (TaKaRa, Chiyoda, Japan) kit, and gDNA was removed using the gDNA Eraser in the kit.

Fluorescence quantitative PCR: The relative expression levels of each gene in each sample were determined using the SYBRGreenI incorporation method on a Q7 real-time quantitative PCR instrument (ABI, Los Angeles, CA, USA). The fluorescent quantitative PCR reagent was SYBR[®]PreMix Ex Taq[™] (TaKaRa, Japan). Relative quantitative PCR was calculated using the Livak and Schmittgen [25] $2^{-\Delta\Delta\text{CT}}$ method, and each reaction was repeated four times.

2.7. Statistical Analysis

Data and graphics were processed using Microsoft Excel 2010 and Photoshop 7.0.1 software. All test data were analyzed using SAS mathematical statistics analysis software (version 8.1) to perform an ANOVA for testing significant differences ($p < 0.05$).

3. Results

3.1. The Morphology of Trifoliate Orange Seedlings

As shown in Figures 1 and 2, IBA and 2-NOA treatments had a significant effect on trifoliate orange seedlings. Although exogenous IBA treatment did not cause significant changes in the aboveground part of the plant, root development in the underground part was noticeably more robust. Compared with the control, the length of the taproot and lateral root density increased significantly after IBA treatment. In contrast, the potted plants treated with 2-NOA were shorter, with sparse and yellow leaves. The root system after 2-NOA treatment was weaker than that of the control group, with shorter taproots and shorter lateral roots.



Figure 1. Depiction of trifoliate orange seedlings subjected to treatment with IBA and 2-NOA. Note: (a,d)—CK, (b,e)— $1.0\ \mu\text{mol L}^{-1}$ IBA, (c,f)— $50\ \mu\text{mol L}^{-1}$ 2-NOA; bar = 5.0 cm.

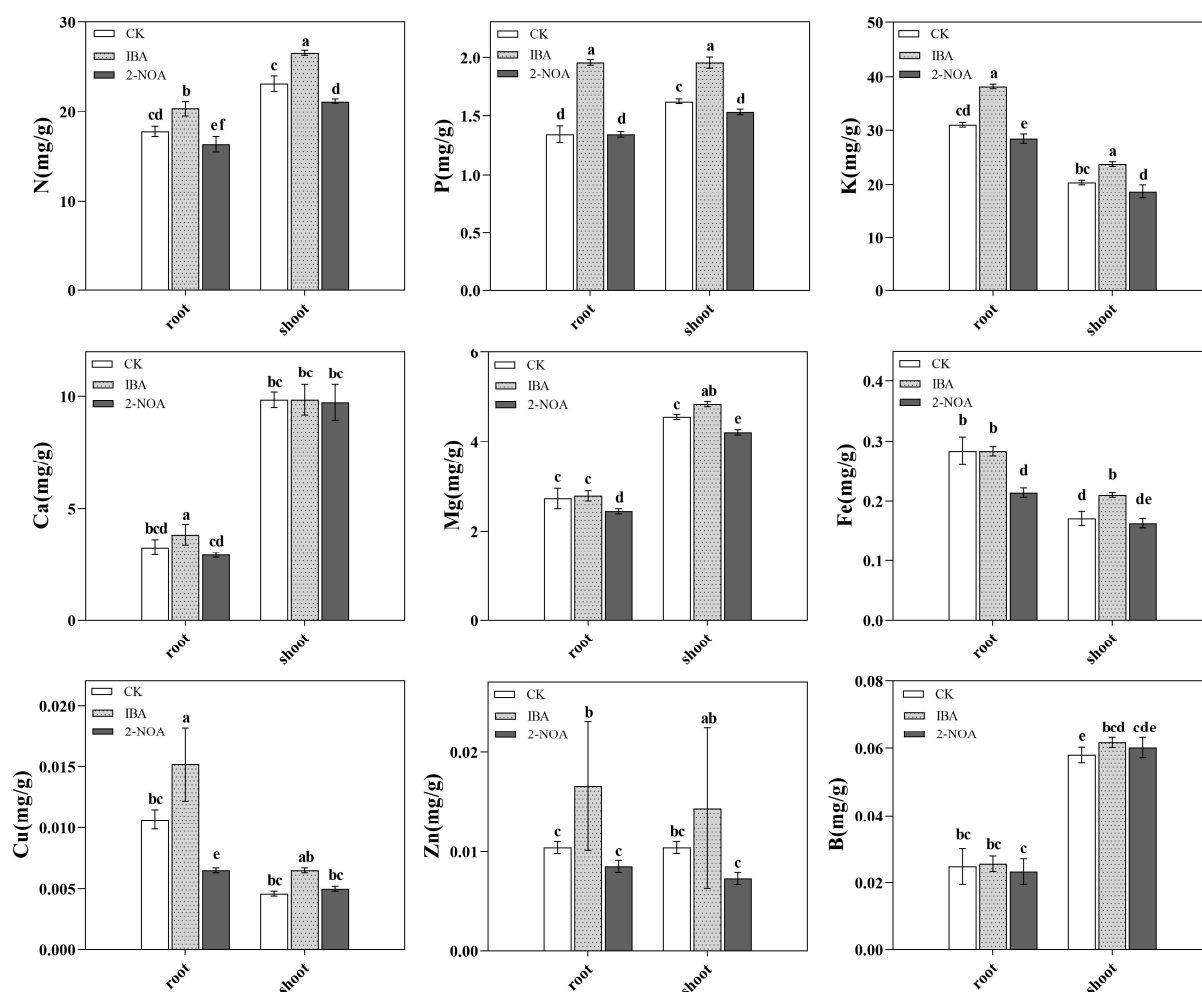


Figure 2. Effects of IBA and 2-NOA on the concentrations of N, P, K, Ca, Mg, Fe, Cu, Zn, and B in trifoliate orange seedlings. Different letters in the column indicate significant ($p < 0.05$) differences.

3.2. The Growth of Main and Lateral Roots

To quantitatively evaluate the effects of auxin and its inhibitors on taproot and lateral root development of trifoliate orange seedlings, we analyzed the root phenotype data of trifoliate orange seedlings in each treatment group. As shown in Figure 1, after IBA treatment, the length and diameter of the taproot significantly increased by 17.56% and 18.18% compared with the control group, respectively. After IBA treatment, the lateral root length and number increased by 123.08% and 88.89%, respectively, compared with the control. In contrast, compared with the control group, 2-NOA treatment reduced the taproot length and diameter by 21.37% and 9.00%, respectively. After 2-NOA treatment, the lateral root length and number were reduced by 10.26% and 88.89%, respectively, compared with the control. These results indicate that auxin promoted the growth of citrus roots, while auxin inhibitors showed obvious inhibition (Table 2).

Table 2. Effects of IBA and 2-NOA on the growth of the tap and lateral roots of trifoliate orange (mean $\pm$ SD).

Treatment	Tap Root Length (cm)	Tap Root Diameter (cm)	Lateral Root Length (cm)	Lateral Root Number (#)
CK	5.24 $\pm$ 1.48 b	0.11 $\pm$ 0.01 b	0.39 $\pm$ 0.08 b	1.80 $\pm$ 0.44 b
1.0 $\mu\text{mol}\cdot\text{L}^{-1}$ IBA	6.16 $\pm$ 0.39 a	0.13 $\pm$ 0.01 a	0.87 $\pm$ 0.22 a	3.40 $\pm$ 1.63 a
50 $\mu\text{mol}\cdot\text{L}^{-1}$ 2-NOA	4.12 $\pm$ 0.14 c	0.10 $\pm$ 0.01 b	0.35 $\pm$ 0.03 c	1.02 $\pm$ 0.10 c

Note: # represents the number of lateral roots. Data (means $\pm$ SD, $n = 4$) followed by different letters in the column indicate significant ($p < 0.05$) differences—the same as below.

3.3. Variations in Plant Mineral Nutrient Composition

According to Figure 2, treatment with IBA and its inhibitor 2-NOA had varying effects on the aboveground and root mineral concentrations of trifoliolate orange seedlings. Compared with the control, IBA treatment had the greatest effect on the content of P, Cu, and Zn in orange root, which increased by 45.61%, 42.86%, and 59.80%, respectively. Additionally, after IBA treatment, the contents of N, K, and Ca in orange root were increased by 14.29%, 23.28%, and 17.24%, respectively. In aboveground tissues, the effect of IBA treatment on the Zn concentration was 38.14% higher than that of the control. Similarly, after IBA treatment, the concentrations of N, P, K, Mg, Fe, and B in aboveground tissues increased by 14.63%, 20.29%, 16.67%, 6.25%, 22.73%, and 6.49%, respectively, while the concentrations of Ca did not change. In contrast, 2-NOA treatment significantly reduced the Cu content in orange root by 39.29% compared with the control. After 2-NOA treatment, the contents of N, K, Mg, and Fe in orange root decreased by 7.94%, 8.21%, 10.42%, and 24.65%, respectively, while the content of P did not change. In the aboveground tissue, the concentrations of N, P, K, and Mg after 2-NOA treatment decreased by 8.54%, 5.80%, 8.33%, and 7.50%, respectively, compared with the control group. Notably, after 2-NOA treatment, the concentration of Cu and B in aboveground tissues increased by 8.33% and 3.9%, respectively.

3.4. Measurement of Endogenous Auxin Content in Roots

To study the effects of exogenous auxin and its inhibitors on endogenous auxin, we compared the concentrations of endogenous IAA in the tissues of the root tip, root hair, epidermis, and stele of the taproot. Compared with the control group, IBA markedly increased the auxin concentration in the root hair and the epidermis by 23.58% and 88.64%, respectively, while its effect on the root tip and epidermis was relatively minor, increasing the auxin concentration by 6.45% and 7.94%, respectively (Figure 3). Interestingly, after treatment with 2-NOA, the auxin concentration in the root tip increased considerably, by 30.32%. In contrast, the concentrations in the root hair and the stele in 2-NOA treatment were decreased significantly by 18.87% and 24.60%, respectively. Remarkably, the concentrations of endogenous IAA in the taproot epidermis remained largely unaffected while treated by 2-NOA.

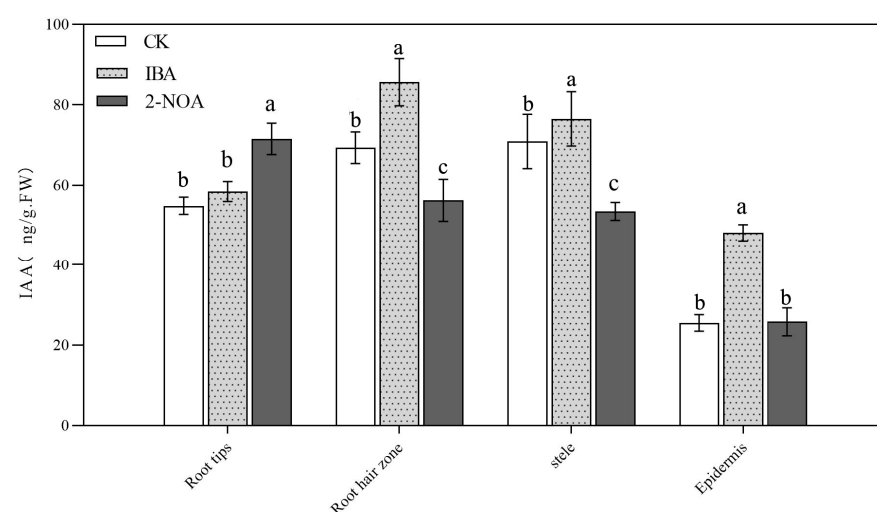


Figure 3. Effects of IBA and 2-NOA on indole-3-acetic acid (IAA) concentrations in the taproot of trifoliolate orange. Different letters in the column indicate significant ($p < 0.05$) differences.

3.5. The Expression Levels of Root Auxin Biosynthesis and Transport Genes

Auxin synthesis is regulated by related genes, such as the *TAR* gene, which regulates the conversion of tryptophan to indole 3-pyruvate (IPA), while the *YUCCA* family genes primarily promote the conversion of IPA to IAA. It is generally believed that the root tip is a key site for plant auxin synthesis; therefore, the root tip was selected for expression analysis. In this experiment, the relative expression levels of auxin synthesis-related genes (*TAR2*, *YUC3*, *YUC4*, *YUC6*, *YUC8*) in lateral root tips were cloned and measured under exogenous auxin and its inhibitor treatments (Figure 4). As shown in Figure 4, *TAR2*, *YUC3*, *YUC4*, *YUC6*, and *YUC8* were positively regulated by IBA, with *TAR2* exhibiting the highest expression level, while 2-NOA markedly reduced its relative expression. In contrast, *YUC8* was positively regulated by IBA, and 2-NOA treatment had minimal effect on its expression.

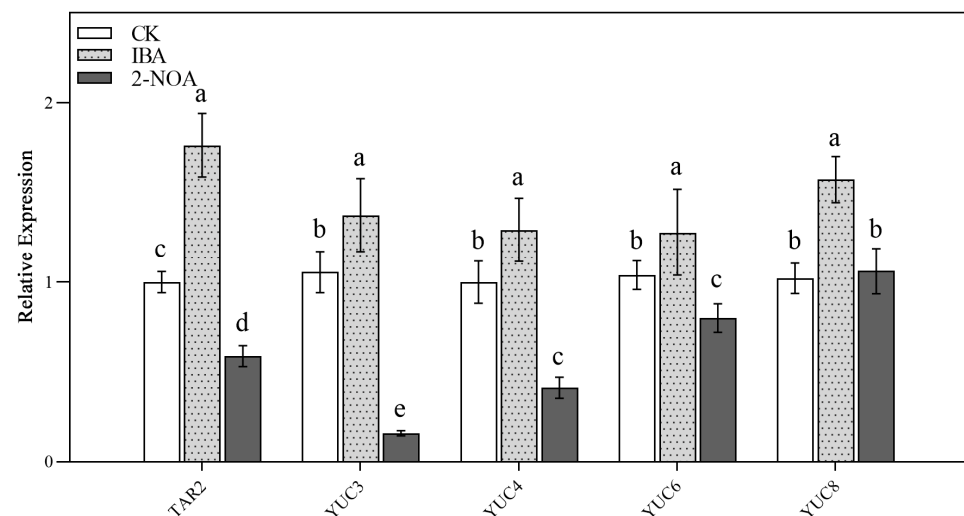


Figure 4. Relative expression of auxin biosynthesis genes in the root hair zone of the lateral roots in trifoliate orange. Different letters in the column indicate significant ($p < 0.05$) differences.

Auxin transport occurs through two modes: fast, long-distance, non-specific non-polar transport, and slower polar transport regulated by transport carriers. In this experiment, the relative expression levels of trifoliate orange auxin transport vector genes (*AUX1*, *LAX1*, *LAX2*, *LAX3*, *PIN1*, *PIN3*, *PIN4*, *ABCB1*, *ABCB19*) in lateral root tips were cloned and measured (Figure 5). As shown in Figure 5, IBA treatment strikingly up-regulated the expression levels of *AUX1*, *LAX1*, and *LAX2* in the input vector, while 2-NOA treatment significantly down-regulated their expression levels. The relative expression of *LAX3* was less pronounced than that of *AUX1*, *LAX1*, and *LAX2*; however, 2-NOA treatment still led to down-regulated expression. As shown in Figure 5, IBA treatment markedly up-regulated the expression levels of *PIN1*, *PIN3*, and *PIN4* in root tips within the output vector. In contrast, 2-NOA treatment observably down-regulated the expression levels of *PIN3* in root tips but had no significant effect on *PIN1* and *PIN4*. The effects of the *ABCB* family on auxin transport in lateral root apex were analyzed by examining the *ABCB1* and *ABCB19* genes. IBA treatment prominently up-regulated the expression levels of both *ABCB1* and *ABCB19*. However, 2-NOA treatment markedly down-regulated the expression level of *ABCB1* but had no significant effect on *ABCB19*.

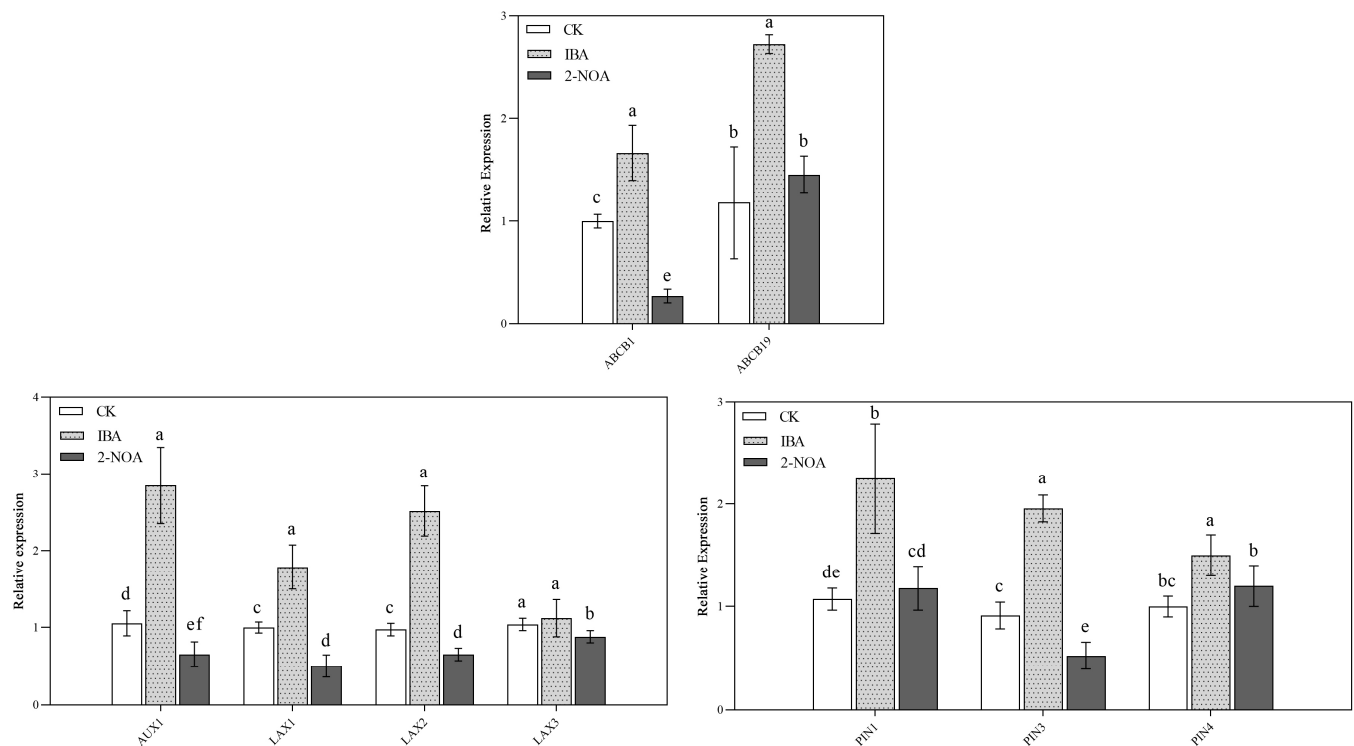


Figure 5. Relative expression levels of auxin transport genes in the root hair zone of the lateral roots in trifoliate orange. Different letters in the column indicate significant ($p < 0.05$) differences.

4. Discussion

4.1. Effect of Auxin on the Root Growth of Trifoliate Orange

The results showed that IBA promoted the growth and development of the roots of trifoliate orange seedlings. Roychoudhry and Kepinski [26] demonstrated that auxin plays an important role in triggering and supporting plant root development, as well as in molecular regulatory pathways, which is consistent with our results. Previous studies have shown that auxin synthesis, transport, and signaling have significant effects on root growth and development [27]. This was consistent with our observation that exogenous auxin significantly promoted the development of the trifoliate orange root system, increasing taproot length, taproot diameter, lateral root length, and lateral root number to varying degrees. In particular, the lateral root growth was markedly enhanced. In contrast, the results indicated that 2-NBA had an inhibitory effect on root growth, particularly on taproot length and the number of lateral roots. This is likely because 2-NOA affects the transport of auxin from the root tip to the base and the root hair area, leading to a reduced auxin concentration in the root hair area [28].

4.2. Effect of Auxin on Mineral Nutrients of Trifoliate Orange

As a major regulatory factor in root development, auxin can promote root growth, thereby increasing root surface area and mineral absorption [29]. Minerals are important nutrient regulators in plant root development, which can promote taproot elongation and lateral root formation by activating auxin-mediated signaling pathways [30–32]. Through the joint regulation of mineral nutrition, root growth and development are further promoted [33,34]. After treating plants with the auxin inhibitor 2-NOA, 2-NOA inhibited root growth by inhibiting auxin inflow, thus affecting nutrient uptake [35,36]. Our results showed that after IBA treatment, the concentrations of N, P, K, Ca, Cu, and Zn in the root system observably increased, while the concentrations of Mg, Fe, and B remained almost unchanged. The two nutrients K and N both play key roles in the photosynthesis

process and the subsequent long-distance transport of photoassimilates [37,38]. This means that auxin promotes the absorption of mineral nutrients from the roots of the plant, and indirectly promotes the progress of photosynthesis. This indicated that IBA could enhance the efficiency of mineral nutrient uptake by plant roots. In contrast, 2-NOA significantly decreased the concentrations of N, K, Mg, Fe, and Cu in roots, slightly decreased the concentrations of B and Ca, and had minimal effects on P and Zn. Previous studies have shown that N is an essential component of plant metabolic activities, and P plays a key role in important metabolic processes. Photosynthesis and respiration rely heavily on K and Cu, which play particularly important roles in these processes. Therefore, it can be inferred that auxin enhances the growth capacity of trifoliate orange. However, after treatment with auxin inhibitors, the growth potential of the plants was markedly reduced [39–44]. After treatment with IBA, the contents of N, P, K, and Fe in trifoliate orange shoots were significantly increased, while the concentrations of Mg, Cu, Zn, and B slightly increased, with no change observed in the Ca concentration. In the aboveground tissues treated with 2-NOA, the concentrations of N, P, K, and Mg decreased notably, while Fe, Zn, and B showed smaller decreases, and the concentrations of Cu and Ca remained unchanged. The changes in mineral concentrations in the aboveground tissues were generally consistent with those in the root system, except the concentration of Ca, which remained unaffected under any treatment. Based on the research of Peaucelle et al., this stability in Ca concentration may be attributed to its primary role in maintaining cell wall stability, which is not influenced by auxin in aboveground tissues [45].

4.3. Expression of Auxin and Its Related Genes

In this study, the expression of several auxin synthesis genes and transport vector genes in the apex of trifoliate orange roots was analyzed. According to the experimental results, IBA treatment substantially increased the expression levels of auxin synthesis genes, including *TAR2*, *YUC3*, *YUC4*, *YUC6*, and *YUC8*. Moreover, the expression levels of auxin transport genes *AUX1*, *LAX1*, *LAX2*, *PIN1*, *PIN3*, *PIN4*, *ABCB1*, and *ABCB19* were also up-regulated. This may be because exogenous auxin treatment significantly improved auxin synthesis and transport in trifoliate orange, consistent with the findings of Du et al. [46] and Leftley et al. [47]. Our study further demonstrated that exogenous auxin can stimulate the expression of *PIN*, *YUC*, *LAX*, and *AUX* family genes in plants, thereby promoting the growth and development of plants, consistent with previous studies [48,49]. Under 2-NOA treatment, the expression levels of auxin synthesis genes (*TAR2*, *YUC3*, *YUC4*, and *YUC6*) and auxin transport genes (*AUX1*, *LAX1*, *LAX2*, *LAX3*, *PIN3*, and *ABCB1*) were reduced, whereas the auxin output vectors *PIN1* and *PIN4* were unaffected. These results align with the findings of Zhang et al. [50] and Xi et al. [51]. *PIN1* primarily regulates the polar transport of auxin to the root cap in mid-column tissues, promoting taproot elongation, while *PIN4* mainly influences the polar transport of auxin to the root tip, thereby regulating root tip growth [50,51]. Therefore, it is speculated that 2-NOA does not impede auxin transport from the apex to the root tip but inhibits auxin transport from the base to the root hair region by down-regulating the expression of related auxin transport vector genes. This markedly reduces the auxin concentration in the root hair region, thereby inhibiting the normal development of the root hair in this region.

5. Conclusions

In this study, exogenous auxin (IBA) was shown to promote citrus growth, especially the root architecture and nutrient levels, while the auxin inhibitor (2-NOA) produced the opposite effects. Further, the auxin concentrations in different parts of the taproot and the expression of auxin synthesis and transport genes were analyzed. It was found that

IBA increased auxin accumulation in the root hair, stele, and epidermal tissues of citrus taproots through the up-regulation of auxin synthesis genes (*TAR2*, *YUC3*, *YUC4*, *YUC6*, *YUC8*) and transport genes (*ABCB1*, *ABCB19*, *AUX1*, *LAX1*, *LAX2*, *PIN1*, *PIN3*, *PIN4*). In contrast, 2-NOA decreased auxin levels in the root hair, stele, and epidermal tissues of citrus taproots through the down-regulation of auxin synthesis genes (*TAR2*, *YUC3*, *YUC4*, *YUC6*) and transport genes (*ABCB1*, *AUX1*, *LAX1*, *LAX2*, *LAX3*, *PIN3*). Interestingly, 2-NOA dramatically and specifically elevates the auxin levels in the root tip of the citrus taproot. These findings suggest that 2-NOA disrupts auxin reflux from the root tip to the root hair and epidermal tissues in the citrus taproot through the down-regulation of auxin transport genes, thereby creating localized (i.e., the root hair zone and epidermis) auxin deficiencies that compromise the root system architecture and nutrient acquisition capacity.

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