

Ethylene modulates root growth and mineral nutrients levels in trifoliolate orange through the auxin-signaling pathway

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Abstract

Roots are fundamental to plant growth, with their development being profoundly influenced by water, mineral nutrients, and notably, plant hormones. Citrus, a leading horticultural crop cultivated globally, holds immense economic value. This study tried to investigate the roles and mechanisms effect of foliar spray 1.0 $\mu\text{mol/L}$ ethephon (ETH) of ethylene and 5.0 $\mu\text{mol/L}$ AgNO_3 (an ethylene inhibitor) its inhibitor with auxin on root growth and mineral nutrients content levels in trifoliolate orange under sand culture conditions for 45 days. Our findings indicate that ethephon treatment significantly boosted the number of lateral roots, root fresh weight, and enhanced the mineral nutrient content (N, P, K, B, Ca, Mg, Fe, Mn, and Zn, with the exception of Cu) in both root and shoot tissues. In contrast, the inhibitors notably reduced the length of lateral roots and decreased the content of P, and Fe in both root and shoot, whilst elevating Zn contents. Furthermore, ETH incited the up-regulation of auxin biosynthesis and transportation genes, leading to an increased endogenous auxin content in the lateral root-hair region. whereas, the inhibitors produced an opposite effect. We thus conclude that ethylene modulates auxin transportation and biosynthesis. This activity stimulates the auxin signaling pathway locally-specifically, in the root hair zone in the lateral root, which in turn, regulates root growth and mineral nutrient content.

Keywords: auxin; citrus; ethylene; mineral nutrients; root

Introduction

Roots constitute an integral part of the plant axis, typically residing underground (Britannica, 2021). They serve crucial functions such as the absorption of water and nutrients (Lavelly *et al.*, 2020), anchoring plants to the ground (Ian *et al.*, 2021), and storing large quantities of food. Additionally, roots play a significant role in response to abiotic or biotic stress (Xie *et al.*, 2021; Yan *et al.*, 2022). With the expansion of root networks,

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also known as root system architecture that encompasses root length, number, branching, diameter, and surface area (Kochian, 2016), the plant's growth becomes increasingly reliant on the availability of water, mineral nutrients (P, N, and K), and particularly the activation of hormonal pathways (auxin, brassinosteroid, cytokinin, and ethylene) (Zhang *et al.*, 2016, 2018; Xie *et al.*, 2021).

Auxin operates as a paramount hormonal regulator of root development (Vandenbrink *et al.*, 2019; Ding *et al.*, 2021). For instance, in *Arabidopsis*, the enhancement of auxin biosynthesis and endogenous auxin levels significantly increases root branching, while its local accumulation in root pericycle cells triggers lateral root initiation (Dubrovsky *et al.*, 2011). Recent studies have revealed numerous gene families implicated in the regulation of auxin biosynthesis and transport. These include *YUC*, *TAA1/TAR*, and *CYP79B2/B3* families involved in auxin biosynthesis tuning (Cao *et al.*, 2019; Brumos *et al.*, 2014) and the *PIN*, *PILS*, *ABCB*, *AUX/LAX* families which contribute to auxin transportation (Barbez *et al.*, 2012; Zhou *et al.*, 2018; Mellor *et al.*, 2022). Such localized auxin biosynthesis and polar auxin transport create an auxin gradient in tissues and cells, thereby shaping diverse signaling pathways (Cao *et al.*, 2019). Auxin also interacts with other phytohormones, either synergistically or antagonistically, influencing root morphogenesis and development (Saini *et al.*, 2013; Roychoudhry *et al.*, 2022).

Ethylene (ETH) is a gaseous hormone that acts as a central modulator of plant growth. It orchestrates various plant developmental processes, from ripening, flowering, and abscission to dormancy, and also stimulates or inhibits growth while resisting stress (Ruzicka *et al.*, 2007; Jasper *et al.*, 2008; Liu *et al.*, 2022). Ethephon, an ethylene-releasing chemical, overcomes the limitations of ethylene's gaseous nature and is widely used for numerous practical applications (Hu *et al.*, 2017). Overproduction of ethylene also impacts other phytohormones (Li *et al.*, 2018), and biotechnological modifications of ethylene synthesis and sensitivity are commonly employed to improve agricultural production and quality (Blecker *et al.*, 2000; Schaller *et al.*, 2017).

A plethora of evidence has established that hormones operate under a complex interconnected and continually adaptive network (Ruzicka *et al.*, 2007; Zemlyanskaya *et al.*, 2018). This study focuses on the interaction between auxin and ethylene. Application of ethylene significantly inhibits primary root growth (Qin *et al.*, 2019) and synergizes with auxin to control primary root growth and root hair decay, while it exhibits an antagonistic role in lateral root formation (Qin *et al.*, 2018). Ethylene enhances apical hook bending by activating the *HOOKLESS1* (*HLS1*) transcription factor and inhibiting the auxin response factor *ARF2*. This process of apical hook formation illustrates the typical influence of ethylene modulation on auxin response at the response level (Ruzicka *et al.*, 2007). For instance, in *Arabidopsis*, it was demonstrated that the effect of ethylene on root growth primarily transpires through the regulation of local auxin distribution, which is contingent on its biosynthesis and transport (Ruzicka *et al.*, 2007). It either stimulates biosynthesis and transport towards the elongation zone or inhibits auxin influx and dampens auxin signaling, thus inhibiting cell elongation (Strader *et al.*, 2010). A recent study reported that the application of ethylene promotes the expression of auxin reporter genes *PIN2*, subsequently increasing its abundance around the root tip and fostering root hair development (Chen *et al.*, 2022). Furthermore, Vaseva *et al.* (2018) reported that ethylene inhibits vegetative growth by restricting cell elongation, through cross-talk with auxins acting on the epidermis. Moreover, Qin *et al.* (2017) found that root elongation inhibition induced by ethylene is dependent on the biosynthesis of the *YUC*-based auxin signaling pathway. When it comes to ethylene inhibitors, silver ions (Ag) are considered to be potent, specific, non-competitive inhibitors of ethylene binding and are commonly utilized to study ethylene action in plants (Strader *et al.*, 2009; Li *et al.*, 2015; Malambane *et al.*, 2018).

In natural environments, root growth and development are regulated not only by growth-regulating substances, but also by mineral nutrients, with the primary macro-elements being nitrogen (N), phosphorus (P), and potassium (K). Most plants primarily source their nitrogen (N) from nitrate (NO_3^-). The nitrate transporter sensor *NRT1.1* has been shown to modulate auxin transport, which can either inhibit or stimulate

root growth, thereby aiding roots in colonizing nitrate-rich patches (Gojon *et al.*, 2011; Bouguyon *et al.*, 2016). Moreover, in *Arabidopsis*, nitrate has been demonstrated to induce the transcriptional expression of TAR2 and LAX3, thereby influencing root branching development (Mounier *et al.*, 2014; Maghiaoui *et al.*, 2020). Phosphorus (P) is a crucial element for all life forms and is often one of the most limiting nutrients for plant growth and reproduction (Hou *et al.*, 2020). Root morphogenesis is influenced by factors such as P concentration, P availability, and P distribution. P notably impacts the initiation and growth of cluster roots (Shane *et al.*, 2005; Liu *et al.*, 2022). In the context of citrus cultivation, Liu *et al.* (2017) reported that varying P concentrations significantly inhibited plant growth and root development. In *C. grandis*, P deficiency has been found to reduce photosynthetic performance and impair nutrient absorption, leading to the production of reactive oxygen species (ROS) that subsequently inhibit growth (Meng *et al.*, 2021). Potassium (K⁺) is another essential macronutrient for plant growth and development. It is involved in numerous physiological processes, such as osmoregulation, photosynthesis, assimilate transport, and enzyme activation within plant cells (Pettigrew *et al.*, 2008; Du *et al.*, 2017; Carmeis *et al.*, 2017). In rice, Chen *et al.* (1997) found that K fertilization increased the number of roots, enhanced root regrowth capacity, and improved the absorption of other nutrients (such as N, P, K, Ca, Mg). Previous studies have indicated that, on acid upland soil, potassium fertilization can enhance available soil K and crop root growth, thereby increasing nutrient uptake and yield, and promoting root growth and grain yield (Almeida *et al.*, 2016). In addition to the aforementioned elements, other key elements such as calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and boron (B) play essential roles in plant growth, development, and reproduction due to their availability and content (Ruiz *et al.*, 2015).

Citrus fruits, representing one of the foremost horticultural crops cultivated globally, generate considerable economic returns (Matheyambath *et al.*, 2016; Hu *et al.*, 2022). However, citrus plants are usually shallow-rooted and highly dependent on mycorrhizas (Wu *et al.*, 2016). Therefore, the regulation of citrus roots has become an urgent task in current citrus cultivation. Therefore, advancements in the yield and quality of citrus orchards hold significant implications for the economic landscape. Despite the importance of exogenous growth regulators, the precise mechanisms underlying the impact of ethylene on the expansion of auxin-mediated citrus roots and nutrient absorption remain elusive. Gaining a more profound understanding of how plants govern their root system architecture (RSA) is imperative, as this knowledge can be harnessed to augment crop yields and enhance nutrient acquisition efficiency. The objective of this study was to examine the influence and mechanisms of ethylene and its interplay with auxin on the growth of trifoliolate orange roots from a phytophysiological and molecular biology standpoint. These findings may shed light on the pivotal role ethylene plays in root development, thereby enhancing our understanding of root growth regulation.

Materials and Methods

Experimental design

The study engaged seedlings that were subjected to three distinct growth regulators: a control treatment (CK), 5.0 μmol/L AgNO₃ (Ag) (Bais *et al.*, 2000), and 1.0 μmol/L ethephon (ETH) (Zhang *et al.*, 2013). Each treatment was administered in triplicate, resulting in a total of nine pots, each containing three seedlings.

Plant material and growth conditions

Sand, initially cleansed with tap and pure water thrice, was subsequently sterilized at 121 °C and 0.1 MPa. Uniform and healthy seeds of *Poncirus trifoliata*, sourced from the Wuhan Academy of Agricultural Science and Technology in China, were sterilized using 75% ethyl alcohol for 15 minutes. The seeds were then placed in a constant-temperature incubator set at 28 °C/80% relative humidity, under dark conditions, for pre-germination treatment. Upon the growth of 1 cm buds, the seeds were sown in pots (30 cm high x 20 cm

diameter) filled with sand, and were placed under a 28/22 °C day/night and 16/8 h light/dark cycle in an incubator (HP400G-C, Wuhan Ruihua Instrument and Equipment Co., Ltd., China). Every three days, seedlings were irrigated with the respective growth regulators (100 mL). After 45 days of treatment, the roots of the seedlings were collected, the pots were disassembled, and the sand was gently washed off with pure water three times before the samples were stored at -80 °C. The Hoagland nutrient solution formulation (Zhang *et al.*, 2016) used in this experiment included the following components and their respective concentrations: 6.00 mM KNO₃, 4.00 mM Ca(NO₃)₂·4H₂O, 2.00 mM MgSO₄·7H₂O, 1.00 mM NH₄H₂PO₄, 46.00 μM H₃BO₃, 9.20 μM MnCl₂·4H₂O, 0.77 μM ZnSO₄·7H₂O, 0.32 μM CuSO₄·3H₂O, 0.12 μM H₂MoO₄, 50 mM EDTA-Fe, with pH adjusted to 5.5-6.00.

Phenotypical analysis

Upon harvesting, each group was photographed and the plants were separated into shoots and roots. The collected roots were scanned using an Epson Perfection V700 Photo Dual Lens System (J221A, Indonesia), and the morphological characteristics were analyzed using WinRHIZO software 2007b (Regent Instruments Inc., Quebec, Canada). The samples were then promptly frozen at -80°C for subsequent analysis.

Mineral nutrients analysis

Both shoot and root materials from each treatment were oven-dried to a constant weight at 105 °C for 48 hours. Approximately 0.5 g of the dried material was pulverized using liquid nitrogen. A 0.2 g sample of the powder was then ashed in a muffle oven at 500 °C for 10 hours. The resultant white ash was dissolved in 0.1 mol/L HCl and subsequently submitted to the Nanjing Institute of Soil Sciences, Chinese Academy of Sciences, for the determination of elemental content (N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, and B) using an Atomic Absorption Spectrometer (SPECTR AA220, Varian Co., USA). The total nitrogen content was ascertained using ICP-MS (Spectrometer, type, American Thermoelectric Company, USA), in accordance with the method described by Lu *et al.* (2004).

Endogenous auxin measurements

An Enzyme-Linked Immunosorbent Assay (ELISA) was performed on lateral root-tip and root hair region, the stele and epidermis of taproot root hair region, to ascertain the content of endogenous auxin within the root system. This process was conducted in triplicate.

RNA extraction, RT-PCR and qRT-PCR

Total RNA from the root hair zone (2-3 cm from root tip, approximately 0.5 g) of the lateral root was extracted using Trizol reagent (Invitrogen, USA), following the provided product instructions. The PrimeScript RT reagent kit (TaKaRa, Japan) was used to synthesize first-strand cDNA from the total RNA, with DNase I (TaKaRa, Japan) used to eliminate genomic DNA. PCR primers for auxin synthesis and transport carrier genes were designed based on *Citrus sinensis* cDNA sequences (<http://citrus.hzau.edu.cn/orange/>, Xu *et al.*, 2013) using Primer 5.0. Actin was employed as an internal reference gene to standardize the data of the quantitative RT-PCR (Table 1, Huang *et al.*, 2013). The relative expression was determined by qRT-PCR, with the intercalation dye SYBR Green used as the fluorescent reporter in an ABI Q7 real-time PCR system (Applied Biosystems, USA). Final reaction mixtures were 10 μL, comprising 4.2 μL cDNA template, 0.4 μL of each primer, and 5 μL of SYBR Premix Ex TaqTM (2×) (TaKaRa, Japan). The qRT-PCR was carried out with an initial denaturation at 95 °C for 10 minutes, followed by 40 amplification cycles at 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s. The 2^{-ΔΔC_t} method was employed to compare the relative expression levels of the amplified products. qRT-PCR results are expressed as mean ± SD of four independent biological replicates.

Table 1. Compilation of the gene-specific primer sequences employed in this study for quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Gene	Accession No.	Forward primer (5'-3')	Reverse primer (5'-3')	Amplification size (bp)
<i>ABCB1</i>	Ciclev10010916m	GAGCCATTCAC GCCACTTC	TCTTGTAACCG AGCCTTTGAGC	186
<i>ABCB19</i>	Ciclev10010931m	GCATGAGTTTG GGTCAGTCTTT	CATCTTCCATT TGTTGGGTCTT	127
<i>AUX1</i>	Ciclev10011596m	CTTGACTCTGC CCTATTCAATTCT C	TGGACCCAGTA ACCCATCAAGC	205
<i>LAX1</i>	Ciclev10031413m	TTGGCGGACAT GCAGTGAC	CAGCGGCAGCA GAAGGAAT	123
<i>LAX2</i>	Ciclev10028271m	TGTGGGAAGAT GGGTAGGGAC	TAGTCATGCTC GCCACCC	98
<i>LAX3</i>	Ciclev10001072m	ATCACTTTCGC TCCTGCTGC	CAAACCCAAAT CCCACCACTA	133
<i>PIN1</i>	Ciclev10007787m	GCTTTGGCAAC AGAAGAGGATT	ATTACACTTGT CGGCCGCATA	94
<i>PIN3</i>	orange1.1g006199m	CATGCCTCCAG CGAGTGTAT	TGCCACCTGAA AGCGATTAGA	126
<i>PIN4</i>	Ciclev10012938m	ATGGGGTTGAA AACGAAGGG	CCTGATAAGTT TCCCTCCACACC A	167
<i>TAR2</i>	Ciclev10020085m	CACACACGGCA CACCCCTA	GCCTCCCCTC CCCAGATC	137
<i>YUC3</i>	Ciclev10006828m	CCTTCAGTTTT AGCCGTTGC	GGAAGTTTGGA AGTTGGCAGA	157
<i>YUC4</i>	Ciclev10008466m	GACCATCTGGG TTAGCCGTTT	GTATTTTGGGA AGTTTTCAGGG A	185
<i>YUC6</i>	Ciclev10008473m	GTGGTTGCTAA AGTGGCTGC	GTTGAAGGGGA CCCAAAGA	122
<i>YUC8</i>	Ciclev10020503m	GTGATAATGGTA GGGGCAGGA	GAATGGCAGGT GAGGGAGC	183
β -actin	Ciclev10025866m	CCGACCGTATG AGCAAGGAAA	TTCTGTGGAC AATGGATGGA	190

Statistical analysis

Statistical analysis was conducted using an Analysis of Variance (ANOVA) with SAS software (version 8.1). Data processing and graphing were facilitated by Microsoft Excel 2003 and Photoshop 7.0.1 software, respectively. Duncan's multiple range test was employed to identify significant differences between treatments at $P < 0.05$.

Results

Observations on plant and root growth

Figure 1 demonstrates the impact of ETH and Ag treatments on the shoot and root development of *Poncirus trifoliata*. Notably, ETH significantly increased the density of lateral roots. In comparison to the control group (CK), the Ag treatment also led to a rise in the length and density of lateral roots (Figure 1). Further data on root fresh weight, taproot length, lateral root length, and lateral root number are displayed in Table 2. In contrast to CK, ETH treatment significantly augmented the number of lateral roots, whereas Ag resulted in a reduction of root fresh weight and lateral root length. Comparable outcomes regarding taproot length were obtained for ETH, Ag, and CK treatments.

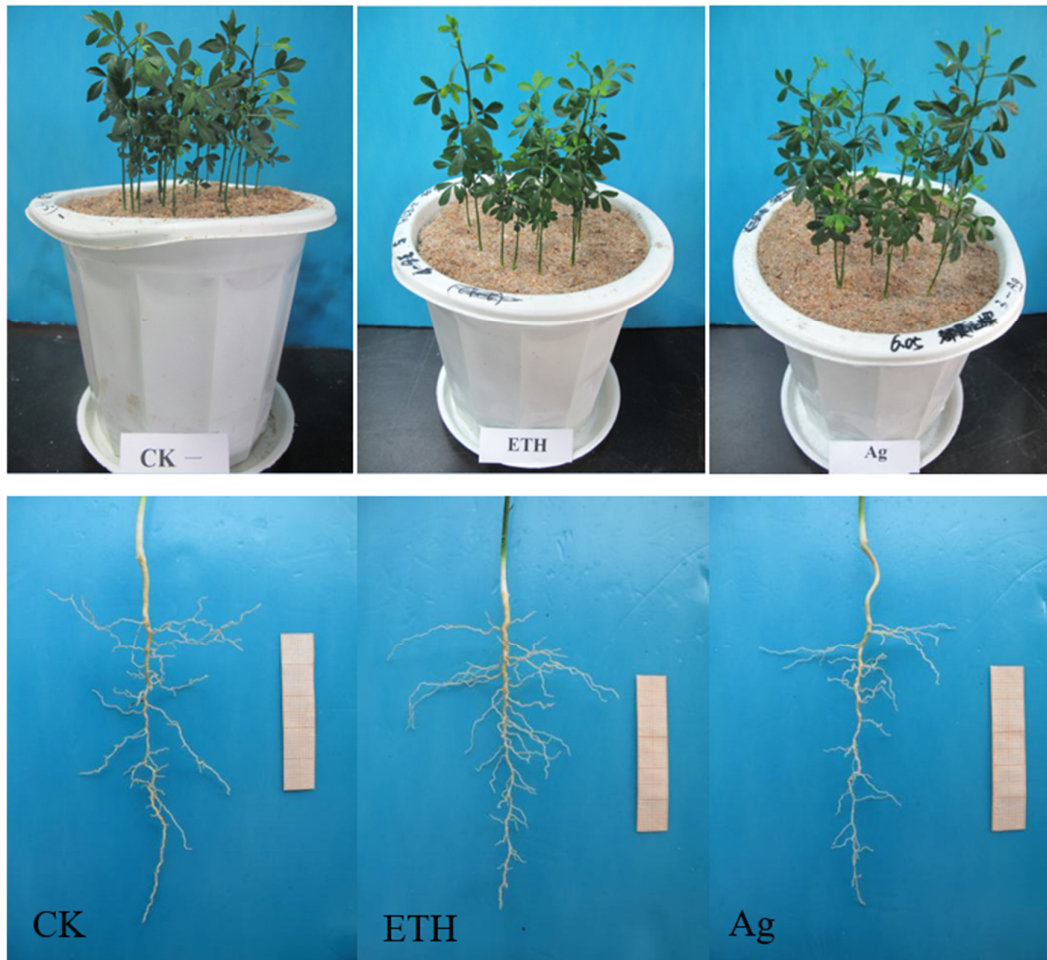


Figure 1. Depiction of *Poncirus trifoliata* seedlings subjected to treatment with ethylene (ETH) and its inhibitors (Ag)
 Note: bar=5.0 cm.

Table 2. Quantification of the effects of ethylene and its inhibitors on the growth of tap and lateral roots of *Poncirus trifoliata*, presented as mean±standard deviation (SD)

Treatments	Root fresh weight (g)	Tap-root length (cm)	Lateral root number (#)	Lateral-root length (cm)
CK	0.16± 0.04 ab	10.3± 1.5 a	23.1± 2.3 bc	4.2± 0.6 a
ETH	0.17± 0.03 a	10.9± 1.2 a	28.3± 2.5 a	4.5± 0.5 a
Ag	0.12± 0.02 c	9.7± 0.9 a	22.3± 2.2 c	3.2± 0.4 b

Variations in plant mineral nutrient composition

Figure 2 illustrates the influence of ETH and Ag (an inhibitor of ETH) on the concentrations of N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, and B in *Poncirus trifoliata* seedlings. As indicated in Figure 2, ETH heightened P, K, Mg, Fe, and B levels in the root by 18%, 3%, 19%, 9%, 18% respectively. It also elevated Mg, Fe, Zn, and B concentrations in the shoot by 7%, 27%, 39%, 14%, respectively. Simultaneously, ETH caused a minor decrease in Cu levels in the root but did not significantly impact other elements in the root or shoot. On the other hand,

compared with CK, Ag significantly diminished P and Fe contents in the root by 6% and 14%, respectively, and P, Mg, and Fe in the shoot by 5%, 4%, and 29%. It also moderately decreased N, K, Ca, and Cu concentrations in the root and K, Ca, Cu concentrations in the shoot. Conversely, Ag amplified Zn contents in the root by 57%, and Zn and B contents in the shoot by 42% and 7%, respectively. No noteworthy difference was found in root Mg, B levels, and in shoot N levels (Figure 2).

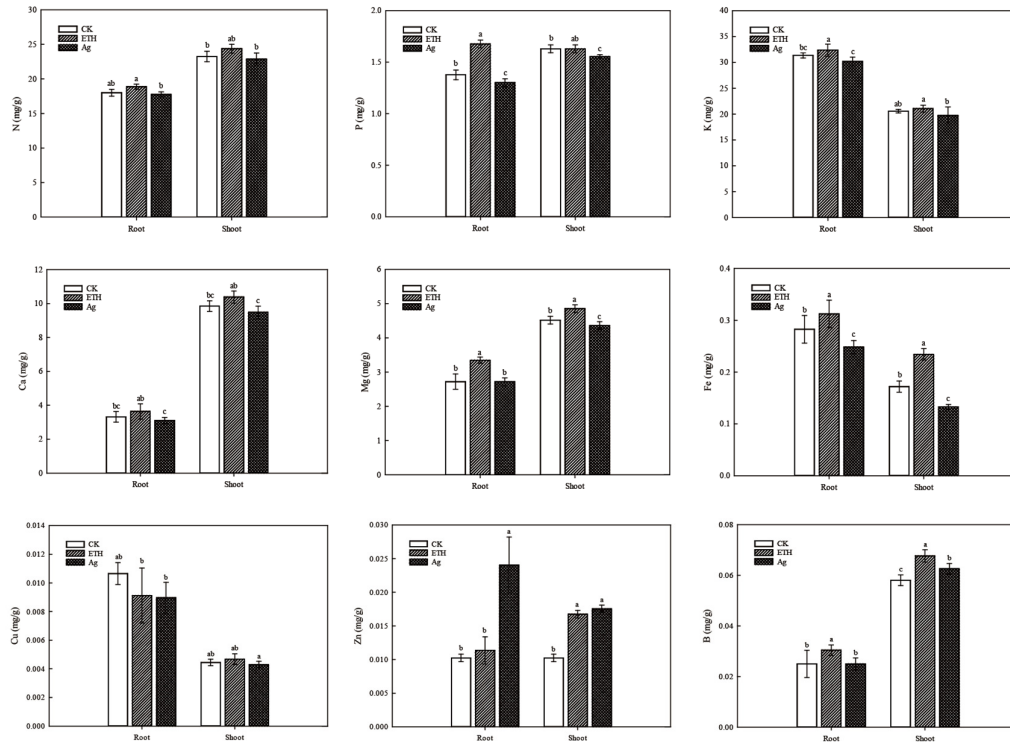


Figure 2. Graphical illustration of the impact of ethylene and its inhibitors on the concentrations of P, K, Ca, Mg, Fe, Mn, Cu, Zn, and B in *Poncirus trifoliata* seedlings
Data (means \pm SD, n = 4) followed by different letters in the column indicate significant ($P < 0.05$) differences.

Measurement of root endogenous auxin content

Endogenous auxin content was evaluated through ELISA, with the results presented in Figure 3. In lateral roots, ETH considerably increased the auxin level in the root hair zone (by 12%) while only subtly influencing root tips (4%). Intriguingly, in comparison to CK, Ag markedly decreased the auxin content in the root hair zone with no significant effects detected on the root tips of the lateral root (Figure 3A). Within the taproot's root hair zone, ETH demonstrated significant variations in the stele, but had a minor effect in the epidermis. Conversely, Ag noticeably decreased the IAA level in the epidermis of the root hair zone, but barely affected the IAA content in the stele of the root hair zone on the taproot (Figure 3B).

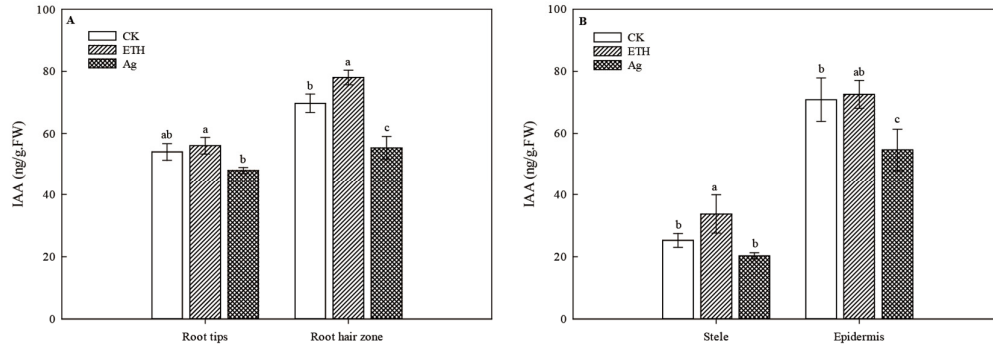


Figure 3. Examination of the effects of ethylene and its inhibitors on indole-3-acetic acid (IAA) content in the root hair zone on the lateral root-tip (A), as well as the stele and epidermis of the taproot root hair region (B) of *Poncirus trifoliata*. Data (means \pm SD, n = 4) followed by different letters in the column indicate significant ($P < 0.05$) differences.

Expression levels of root auxin biosynthesis and transport genes

We examined the gene expression of four YUCCAs family genes and one TARs gene, which are known to regulate auxin biosynthesis in the lateral root tip. We also studied previously identified auxin transport genes family (AUX1, LIKE AUX1 (LAX), PINs, ABCBs). The relative expression analysis of auxin biosynthesis genes indicated that ETH treatment notably elevated the relative expression level of YUC4 and YUC8, while decreasing YUC3. In contrast, Ag treatment lowered the expression levels of YUC4, YUC8, and TAR2. The effect of both treatments on YUC6 was not significant (Figure 4). Furthermore, as shown in Figure 5, AUX1, LAX1, LAX2, PIN1, and PIN4 exhibited increased relative expression levels when treated with ETH (Figure 5), while the expression of PIN3 was downregulated. In comparison to CK, Ag treatment significantly induced the relative expression of LAX1, LAX2, LAX3, ABCB1, ABCB19, whereas AUX1, PIN1, PIN3, and PIN4 were upregulated.

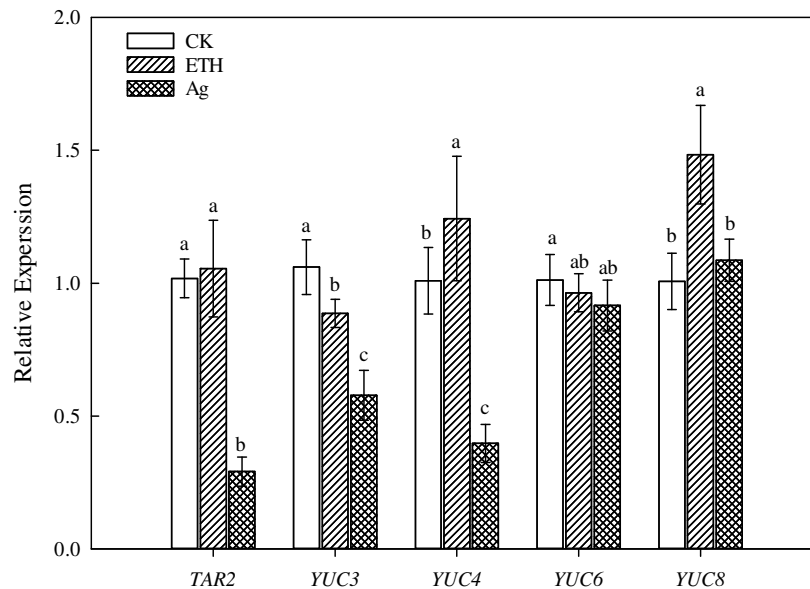


Figure 4. Assessment of the relative expression of auxin biosynthesis genes in the root hair zone of the lateral root of *Poncirus trifoliata*, in response to ethylene and its inhibitors

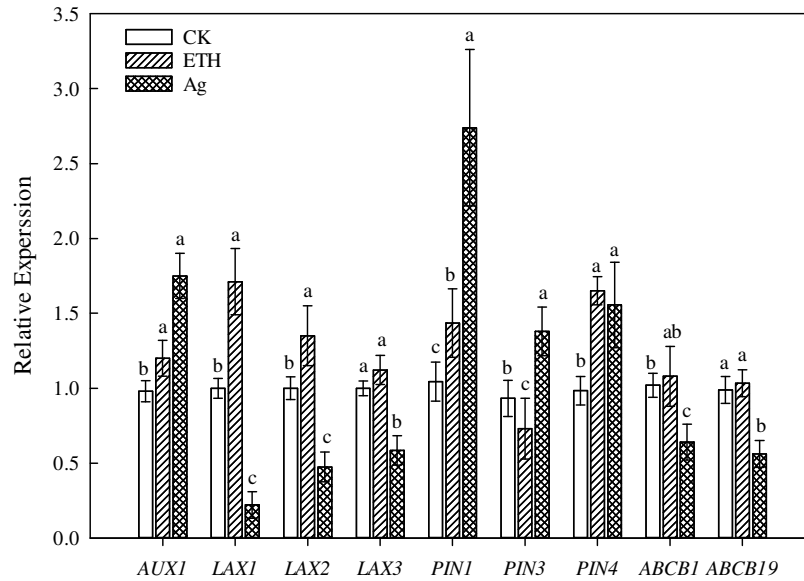


Figure 5. Analysis of the relative expression of auxin transport genes in the root hair zone of the lateral root of *Poncirus trifoliata*, following treatment with ethylene and its inhibitors

Discussion

Ethylene exerts influence on root growth and mineral nutrient uptake

The primary factors influencing root growth are plant hormones and the external environment, with particular focus herein on Ethylene. Prior research noted that the application of 1.0 $\mu\text{mol/L}$ ethephon yielded optimal results in citrus (Zhang *et al.*, 2016). This aligns with our observations, where the same concentration of ethephon treatment considerably augmented the number of lateral roots and root fresh weight, as well as improved mineral nutrient content in both roots and shoots, excluding Cu. Studies on *Trifolium repens* L. reported that lower concentrations of ethylene (100 nM ACC) stimulate root growth, and low P treatment amplifies root-derived ethylene receptivity (Dinh *et al.*, 2012). Similar findings by Daniel *et al.* (2011) in *Arabidopsis* indicated that ACC treatment enhanced auxin transport, causing localized accumulation of auxin and subsequently inhibiting lateral root growth, a phenomenon congruent with our observation that ethephon treatment did not extend the length of lateral roots. Research on *Phaseolus vulgaris* also implied that exogenous ethylene has a role in regulating the extension of the main root and lateral roots (Borch *et al.*, 1999). In general, ethylene is involved in the regulation of physiological and morphological reactions initiated by nutrient deficiencies and abiotic stress (Romera *et al.*, 2016).

Ethylene's interaction with nitrogen (N) can affect plant leaf gas exchanges, root architecture, and the development of leaves, fruits, and flowers (Khan *et al.*, 2015). However, our study did not reveal a significant difference in N content in either root or shoot. This may be due to the low concentration of ethylene applied or differences in mineral element detection methodologies. Chen *et al.* (2021) demonstrated that root-derived ethylene signals, modulated via NRT1.5, influence root-to-shoot K⁺ translocation, contributing to the mutual interaction between N form and K⁺. This finding may explain why N and K exhibited non-significant differences in our study and suggests potential spatial variations. Ethylene is considered an activator in several studies focusing on mineral nutrition such as P, S, Fe, K, Mg, S, N, and others (Romera *et al.*, 2016; García *et*

al., 2021a; García *et al.*, 2021b). In this study, we aim to further validate the hypothesis that there are no species differences in citrus.

Ethylene is known to modulate plant root growth by influencing auxin biosynthesis and transport, leading to basipetal auxin accumulation that restricts cell elongation (Ruzicka *et al.*, 2007; Xie *et al.*, 2021). We analyzed root endogenous auxin content in both lateral roots and taproots and observed differences in the root hair zone following ethylene treatment. Subsequently, we chose to investigate auxin biosynthesis and transport genes' expression in the root-tip of the lateral root, aiming to clarify the relationship between ethylene and auxin. We found that YUC4 and YUC8 were significantly upregulated with ethylene treatment, aligning with Ranjan *et al.* (2007) reported that ethylene constrains root cell expansion by increasing auxin biosynthesis. In contrast, Ag treatment led to their downregulation. Additionally, in *Arabidopsis*, AUX1, LAX3, and PIN3 had a positive role in ACC transport, similar to our findings. However, PIN2 and ABCB19 did not influence transport, showing a nonsignificant difference in this study (Daniel *et al.*, 2011; Gloria *et al.*, 2012).

These findings indicate that ethylene modulates auxin content in the root hair zone, leading to changes in root growth and mineral nutrient absorption. Therefore, Ethylene may affect auxin-dependent root growth and mineral nutrient uptake by modifying auxin signaling, synthesis, or transport (Ruzicka *et al.*, 2007; Strader *et al.*, 2010; Gloria *et al.*, 2012). Future investigations are needed to determine whether this type of signaling pathway regulates ethylene responses and exhibits non-specificity in citrus.

Conclusions

Exogenous ethylene enhances the number of lateral roots, improves mineral nutrient absorption, and marginally affects root fresh weight. Conversely, its inhibitor (Ag) has an inhibitory effect. Furthermore, the application of exogenous ethylene stimulates auxin biosynthesis and regulates auxin transport, activating the auxin signaling pathway regionally. This regulation in turn affects root growth and alters the mineral nutrient content of the plant.

Authors' Contributions

Conceptualization, D.J.Z.; Data curation, S.D.Y. and C.H.H.; Formal analysis, S.D.Y. and C.H.H.; Funding acquisition, D.J.Z.; Investigation, C.L.T. and S.D.Y.; Project administration R.H.H.; Supervision, D.J.Z.; Writing, C.H.H. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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