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The nitrification inhibitor 1,9-decanediol from rice roots promotes root growth in *Arabidopsis* through involvement of ABA and PIN2-mediated auxin signaling

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ABSTRACT

1,9-decanediol (1,9-D) is a biological nitrification inhibitor secreted in roots, which effectively inhibits soil nitrifier activity and reduces nitrogen loss from agricultural fields. However, the effects of 1,9-D on plant root growth and the involvement of signaling pathways in the plant response to 1,9-D have not been investigated. Here, we report that 1,9-D, in the 100–400 μM concentration range, promotes primary root length in *Arabidopsis* seedlings at 3d and 5d, by 10.1%–33.3% and 6.9%–32.6%, and, in a range of 50–200 μM, leads to an increase in the number of lateral roots. 150 μM 1,9-D was found optimum for the positive regulation of root growth. qRT-PCR analysis reveals that 1,9-D can significantly increase *AtABA3* gene expression and that a mutation in ABA3 results in insensitivity of root growth to 1,9-D. Moreover, through pharmacological experiments, we show that exogenous addition of ABA (abscisic acid) with 1,9-D enhances primary root length by 23.5%–63.3%, and an exogenous supply of 1,9-D with the ABA inhibitor Flu reduces primary root length by 1.0%–14.3%. Primary root length of the *pin2/eir1-1* is shown to be insensitive to both exogenous addition of 1,9-D and ABA, indicating that the auxin carrier PIN2/EIR1 is involved in promotion of root growth by 1,9-D. These results suggest a novel for 1,9-D in regulating plant root growth through ABA and auxin signaling.

1. Introduction

Nitrate (NO_3^-) and ammonium (NH_4^+) are the most important sources of nitrogen (N) for plant growth in most settings (Kronzucker et al., 1997, 1999). When NH_4^+-N is applied to the soil, NH_4^+ can be converted to NO_3^- via nitrification carried out by soil microbial communities, even in flooded soils otherwise dominated by NH_4^+ (Kirk and Kronzucker, 2005), and the NO_3^- produced can lead to N leaching and runoff and at times severe pollution of groundwater and above-ground waterways (McSwiney and Robertson, 2005; Coskun et al., 2017a, b; Min and Shi, 2018; Min et al., 2021b; Li et al., 2022). Nitrification inhibitors inhibit nitrification in the soil and can effectively reduce the environmental risks of NO_3^- (Sun et al., 2015; Coskun et al., 2017a, b; Min et al., 2021a; Souza et al., 2021). Most of the currently available nitrification inhibitors are chemically synthesized and, thus, are referred to as synthetic nitrification inhibitors (SNIs), and the success of their application is influenced by a variety of environmental factors (Abalos et al., 2014). SNI application can significantly increase the cost of agricultural production but can also pose a potential environmental pollution risk in its own right. (Woodward et al., 2016).3

Biological nitrification inhibitors (BNIs) are a class of substances secreted by plant roots, and several of these can effectively reduce soil nitrification, thus presenting the potential for application in agricultural fields (Subbarao et al., 2006, 2013; Coskun et al., 2017a; Lu et al., 2019). At the same time, BNIs, as naturally produced plant materials, are more environmentally friendly than synthetic nitrification inhibitors (Woodward et al., 2016). Several types of BNIs have been identified from the root exudates of various species in the past two decades. In sorghum,

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three BNIs, such as methyl 3-(4-hydroxyphenyl) propionate (MHPP) (a phenylpropanoid), sakuranetin (a flavanone), and sorgoleone (a benzoquinone) have been reported from root exudates (Zakir et al., 2008; Subbarao et al., 2013). In *Brachiara humidicol*, a cyclic diterpene named brachialactone was isolated from roots (Subbarao et al., 2009). Two BNIs, 1,9-decanediol (1,9-D) (a fatty alcohol) and syringic acid (an aromatic acid), were identified in rice root exudates (Sun et al., 2016; Lu et al., 2022). Recently, 2,7- dimethoxy-1,4-naphthoquinone (Zeanone) and 2-hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4H)-one (HDMBOA) were isolated from maize root systems (Otaka et al., 2022). However, previous studies mainly focused on BNI effects on nitrogen transformation in the soil and not on plant growth and physiology (Subbarao et al., 2009; Byrnes et al., 2017; Wang et al., 2021). Whether BNIs can affect plant root development in any significant manner has not been sufficiently examined. Evidence has been found that root exudates can dramatically affect the growth of plant seedlings and roots (Yang et al., 2013). Thus, it is reasonable to assume that BNIs might also influence plant root growth, for which reason the current study was designed.

It has been noted that MHPP exhibit an inhibitory effect on root length in *Arabidopsis* and in the medicinal *Perilla genus* (Liu et al., 2016; Ma et al., 2020). Another BNI, syringic acid, had no significant effect on root growth in a nutrient-rich substrate, whereas it inhibited root growth in a nutrient-deficient medium (Reigosa and Malvido-Pazos, 2007; Adams et al., 2020). Our previous studies showed that 1,9-D can inhibit nitrification and reduce N_2O emissions in different soil types (Lu et al., 2019) and that the secretion of 1,9-D can be induced by NH_4^+, O_2, and nitrifying microbes (Zhang et al., 2019). Although there have been many studies on the environmental effects of 1,9-D, to our best knowledge, no studies exist that report the outcomes of 1,9-D application on root growth and development.

A variety of hormones and signaling molecules that regulate the growth of plant roots have been reported, including auxin, ethylene, abscisic acid (ABA), cytokinins (CK), gibberellins (GA), salicylic acid (SA), jasmonates (JA), and much is known about their interactions (Van de Poel et al., 2015; Lopez-Ruiz et al., 2020). The auxin indole-3-acetic (IAA) plays a crucial role in root growth, development, and differentiation (Di et al., 2016) and does so under a variety of soil stresses, such as alkaline stress or ammonium toxicity (Zou et al., 2012; Xu et al., 2013). Plants maintain conventional root growth by retaining the maximum auxin concentration in the quiescent center (Laskowski et al., 2008). IAA-deficient *Arabidopsis* mutants exhibit inhibition of lateral root growth at different developmental stages (Celenza et al., 1995), and *pin1* and *pin2* single mutants show reduced root length and root meristem size compared to wild type (Blilou et al., 2005). Exogenous addition of low concentrations of IAA inhibits plant root elongation and stimulates cell differentiation, but low concentrations of IAA promote the formation of adventitious and lateral roots (Lincoln et al., 1990; Di et al., 2021; Wang et al., 2022). Synergy of IAA with various substances can also regulate root growth. IAA can act synergistically with brassinosteroids to promote maize root geotropism (Kim et al., 2000). Ethylene is the smallest plant hormone and has a simple C_2H_4 structure, with functions in the regulation of leaf development, senescence, fruit ripening, and stimulating germination (Dubois et al., 2018). In *Arabidopsis* roots, ethylene and its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were found to induce ectopic root hairs and increased root width (Tanimoto et al., 1995; Smalle and Van Der Straeten, 1997) coupled to an inhibition of plant root cell elongation and growth (Le et al., 2001; Li et al., 2015a, b). In plants, ethylene interacts with other hormones to stimulate plant growth. IAA is a positive regulator of ethylene-induced inhibition of root elongation (Rahman et al., 2001). Ethylene can up-regulate auxin biosynthesis in inhibited root cells to facilitate root extension and growth (Swarup et al., 2007).

ABA also mediates multiple developmental processes in plants. It has been shown that high concentrations of ABA inhibit the growth of primary roots (Thole et al., 2014). On the contrary, low concentrations of ABA can maintain root growth by reducing ethylene synthesis under water stress (Spollen et al., 2000). Under aluminum and ammonium stress, plants increase ABA accumulation to maintain primary root growth (Hou et al., 2009; Sun et al., 2020). The exogenous addition of ABA promoted primary root elongation in *pyl112458* seedlings and the *pyl* duodecuple ABA receptor mutant (Gonzalez-Guzman et al., 2012; Zhao et al., 2018). ABA in roots induces genes in the clade A PP2C family, which may lead to reduced inhibition of primary root growth (Wang et al., 2019).

Other hormones also play an essential role in regulating root growth. When plants are under aluminum stress, for instance, CK and JA promote primary root growth to facilitate acclimation to the stress (Yang et al., 2017; Wang et al., 2020); however, under cadmium stress, JA has been shown to produce the opposite trend in root growth (Lei et al., 2020).

It is unknown whether various BNIs share similar or distinct signaling pathways involved in plant root growth when such effects are observed. Sorghum-secreted MHPP has been shown to regulate root growth through the NO/ROS-mediated auxin response pathway in *Arabidopsis* (Liu et al., 2016). In Perilla, MHPP regulates root growth by changing the expression of genes involved in the phenylpropanoid metabolic pathway, thereby influencing the accumulation of secondary metabolites in roots and leaves (Ma et al., 2020). The signaling pathway through which 1,9-D regulates root growth remains to be determined.

In this study, we attempt to answer the following two questions: (i) Does 1,9-D regulate the growth of primary and lateral roots of plants? (ii) If so, which signaling pathways are principally involved?

2. Materials and methods

2.1. Plant material and growth conditions

The wild-type *Arabidopsis* used in this study was *Col-0*, and the mutant seeds *aba3-1* (CS157) and *pin2/eir1-1* (CS8058) were in the *Col-0* background. Seeds were stored at 4 °C for 48h and then surface-sterilized with 70% (v/v) ethanol and 3% sodium hypochlorite. The sterilized seeds were plated on Murashige and Skoog medium (MS) (adjusted to pH 5.8 with 1M KOH), supplemented with 1% (w/v) agar and 1% (w/v) sucrose. Seedlings were grown vertically under a 16/8h light/dark cycle in a growth chamber maintained at 23 ± 1 °C with illumination at 100 μmol m^{-2} s^{-1}.

2.2. Chemical treatment

The stock solutions of both 1,9-D ($C_{10}H_{22}O_2$) (synthesized by WuXi AppTec, Shanghai, China) and ABA (Sigma-Aldrich) were dissolved in ethanol; a stock solution of the ABA inhibitor 1-methyl-3-phenyl-5-[3-trifluoromethyl)phenyl]-4(1H)-pyridinone (Flu) (Sigma-Aldrich) was dissolved in acetone.

The seeds were germinated and grown on MS medium for five days, and then seedlings were transferred with similar root lengths to fresh MS medium supplemented with 1,9-D at various concentrations (50, 100, 150, 200, 400, and 800 μM) for another 3 or 5 days.

ABA pharmacology experiments were performed by transferring 5-day-old seedlings to an MS medium containing 1,9-D (0 or 150 μM) with ABA (0.1, 0.2, 0.5, and 1.0 μM) or 1,9-D (0 or 150 μM) with Flu (0.2 and 0.5 μM) for three days.

2.3. Phenotypic analysis

Primary root length was measured under different treatments. Measurements were made by marking the apical position at the bottom of the plate every 24 h and scanned for imaging after 3 or 5 days using Image J software. The number of lateral roots was measured as mature lateral roots greater than 0.5 mm in length. At least 12 replicate plants were calculated for each treatment.

2.4. qRT-PCR analysis

Total RNA was extracted from three-day-old seedlings using TRIzol Reagent (TaKaRa). Then DNase-treated total RNA (1 μg) was used for complementary DNA (cDNA) synthesis with a Reverse Transcription System Kit (Promega) and oligo(dT) primers. The diluted cDNA (20 times) was used for qRT-PCR analysis with TB Green Premix Ex Taq (Takara). The cycle threshold $2^{(-\Delta\Delta C(T))}$-based method was used for relative quantitation of gene expression. *Actin2* was used as an internal control for qRT-PCR standardization. For each gene, qRT-PCR was performed on three biological replicates, with six parallel replicates set up for each biological replicate. The specific primers used for each gene are listed in Supplementary Table S1.

2.5. Statistical analysis

Each experiment was repeated at least three times. Data were analyzed by one-way variance analysis (ANOVA, $P < 0.05$), and the results are presented as means ± SD.

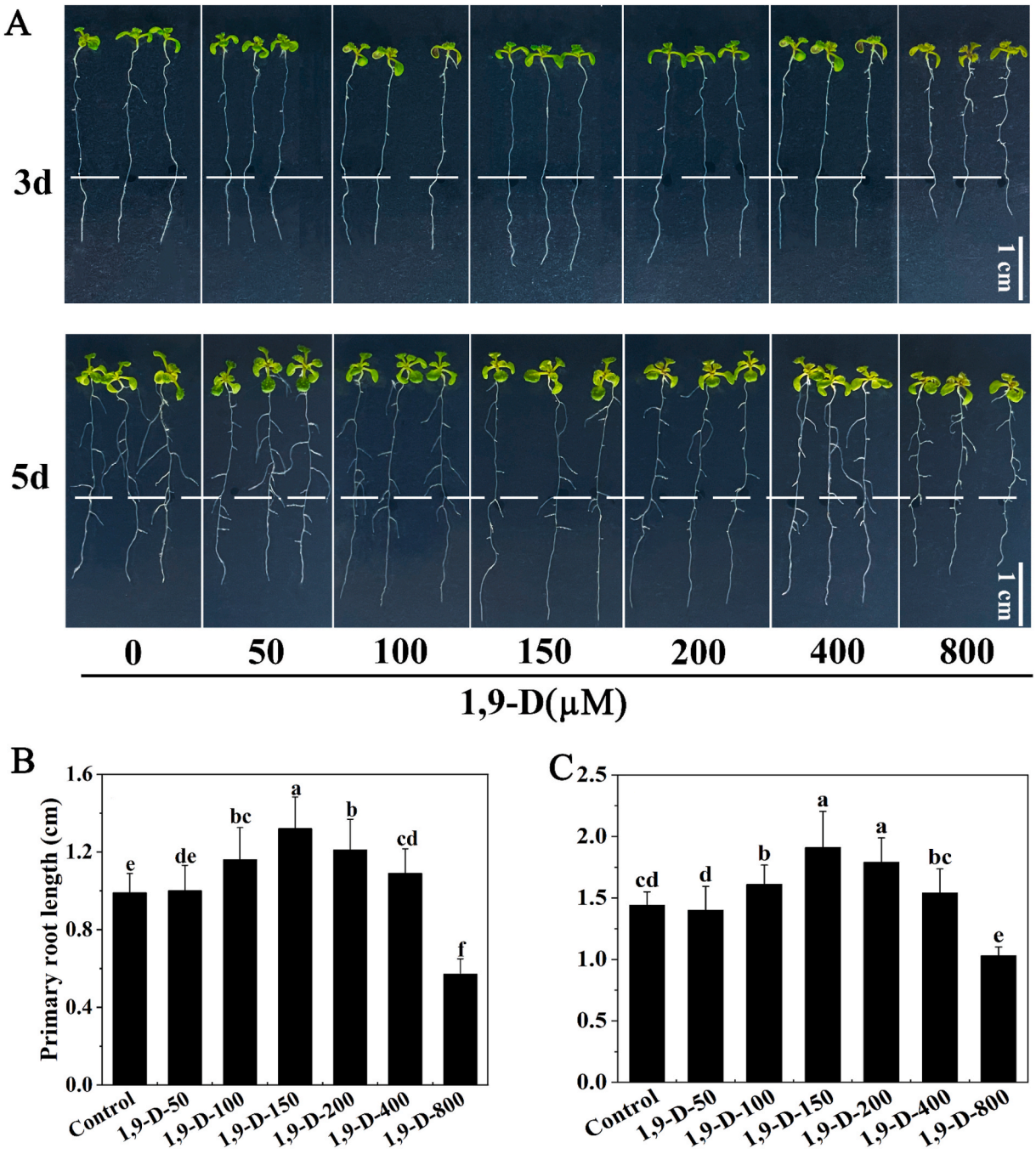

Fig. 1. Effects of 1,9-D on root system development in *Arabidopsis*. (A), Five-day-old *col-0* seedlings grown in MS medium were treated with or without 1,9-D for 3d-5d, the broken line indicates the start point after transfer, bar = 1 cm; (B), the primary root length for 3d seedings, n = 16; (C), the primary root length for 5d seedings, n = 16. Different letters indicate significantly different values ($P < 0.05$).

3. Results

3.1. Effects of 1,9-D on root growth of Arabidopsis

To identify the effect of 1,9-D on the primary root elongation, five-day-old wild-type seedlings were transferred to fresh medium supplemented with different concentrations of 1,9-D for 3 and 5 days. Compared to the control, the primary root length of the seedlings was stimulated by 10.1%–33.3% when exposed to 100–400 μM 1,9-D, but a 42.4% inhibitory effect of 800 μM 1,9-D was found on the primary roots growth of seedlings treated 3d (Fig. 1A and B). Similarly, the primary root length of seedlings was also increased by 6.9%–32.6% after five days of 100–400 μM 1,9-D treatment (Fig. 1A and C).

As shown in Fig. S1, a range of 50–400 μM, 1,9-D promoted the number of lateral roots by 8.4%–6.8%. No significant effect of 800 μM of 1,9-D was found on the lateral root number compared to the control. Thus, 1,9-D tended to promote root growth at low concentrations (100–400 μM) and displayed inhibitory activity at high concentrations (>800 μM). At the same time, 150 μM 1,9-D was the optimum concentration for regulating root growth.

3.2. Effect 1,9-D on the expression of major hormone synthesis genes in Arabidopsis

The content and distribution of hormones are key factors influencing root growth (Zluhan-Martinez et al., 2021). To investigate which hormone regulatory pathways are involved in the 1,9-D regulation of root growth, we determined the expression levels of the major hormone synthesis genes in plants (Lopez-Ruiz et al., 2020). As shown in Fig. 2, 1, 9-D did not significantly increase the transcription levels of leading auxin synthesis genes (*AtYUC9, AtTAA1, AtASA1*) in *Arabidopsis* seedlings. Similarly, although the expression levels of ethylene, JA, CK, SA, and GA synthesis or transport genes in the seedlings treated with 1,9-D showed either higher or lower levels compared to control, none of these differences reached statistical significance. However, the expression of the ABA synthesis gene *AtABA3* was significantly increased in 1, 9-D-treated seedlings. The above data suggest that the *AtABA3* gene may be involved in the process of 1,9-D regulation of root growth.

3.3. ABA is involved in the regulation of primary root length by 1,9-D

In order to explore whether the *AtABA3* gene is involved in the growth promotion by 1,9-D, seedlings of the ABA-synthesis mutant *aba3-1* were treated with or without 1,9-D (Fig. 3A). The addition of 1,9-D significantly increased primary root length in *col-0* seedlings (Fig. 3B). However, the *aba3-1* mutant was insensitive to the treatments, indicating that ABA is a contributing factor in 1,9-D regulation of root growth in *Arabidopsis*.

In order to clarify the role of ABA in the regulation of primary root length by 1,9-D, we performed pharmacological experiments on 3d *col-0* seedlings. As shown in Fig. 4, in treatments without 1,9-D, exogenous addition of ABA promoted primary root length by 39.8%, 61.2%, 67.3%, and 48.0% (Fig. 4A and C), respectively, while Flu reduced primary root length in *col-0* seedlings by 23.5% and 28.6% (Fig. 4B and D).

Exogenous supplementation of ABA to media containing 1,9-D could promote primary root length by 55.1%, 63.3%, 51.0%, and 23.5% (Fig. 4C), respectively; exogenous supply of Flu to media containing 1,9-D could reduce primary root length by 1.0% and 14.3% (Fig. 4D).

3.4. Effect of 1,9-D on the expression of ABA genes

In order to more comprehensively investigate the relationship between 1,9-D and ABA, we analyzed the expression levels of members of ABA synthesis and metabolism gene families in 1,9-D-treated seedlings by qRT-PCR analysis. As shown in Fig. 5B, 1,9-D significantly increased the transcription level of the ABA synthesis genes *AtABA1* and *AtABA3*. Meanwhile, 1,9-D increased the expression of *AtNCED3* and *AtAAO1* and decreased the expression of other synthesis genes, but none of the

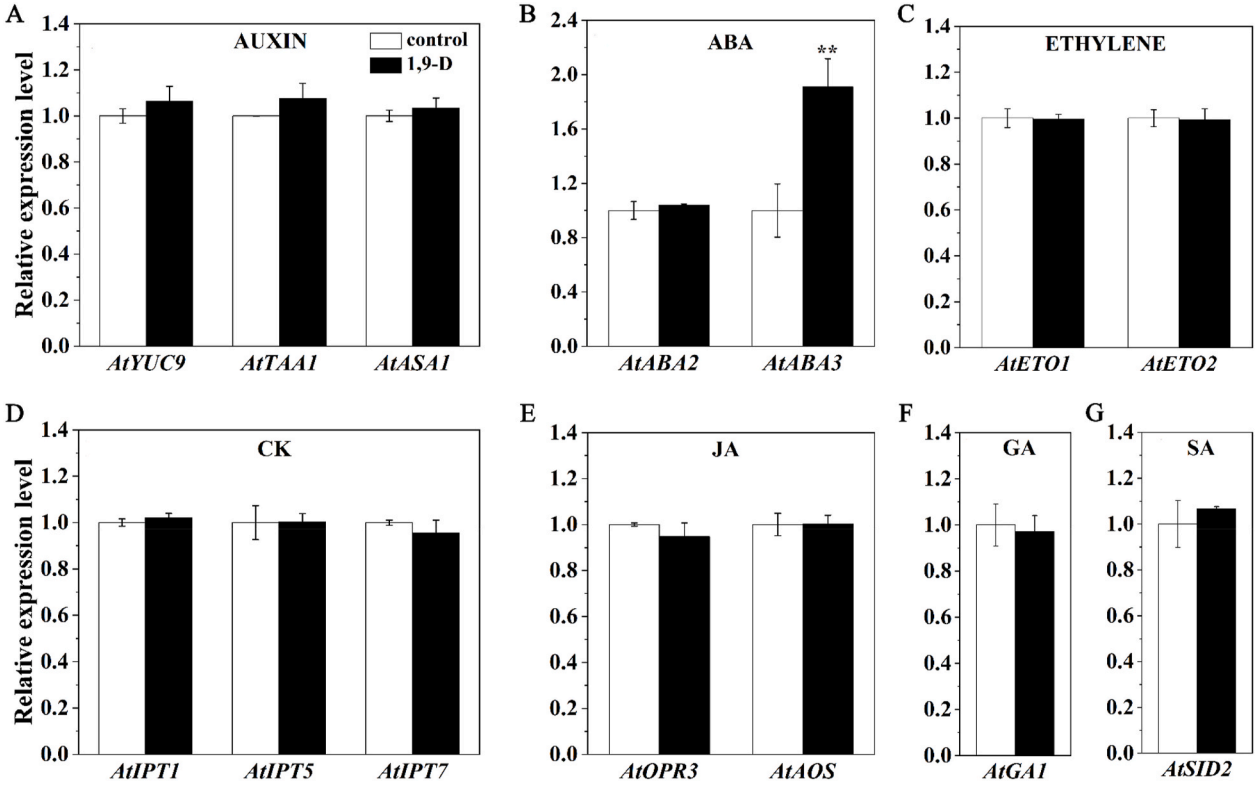

Fig. 2. qRT-PCR analysis of the expression of major hormone synthesis genes in *Col-0* seedlings treated with or without 1,9-D for 3d. (A), auxin; (B), ABA, (C), ethylene; (D), CK; (E), JA; (F), GA; (G), SA synthetic genes. Asterisks indicate a significant difference from the corresponding control (**, $P < 0.05$).

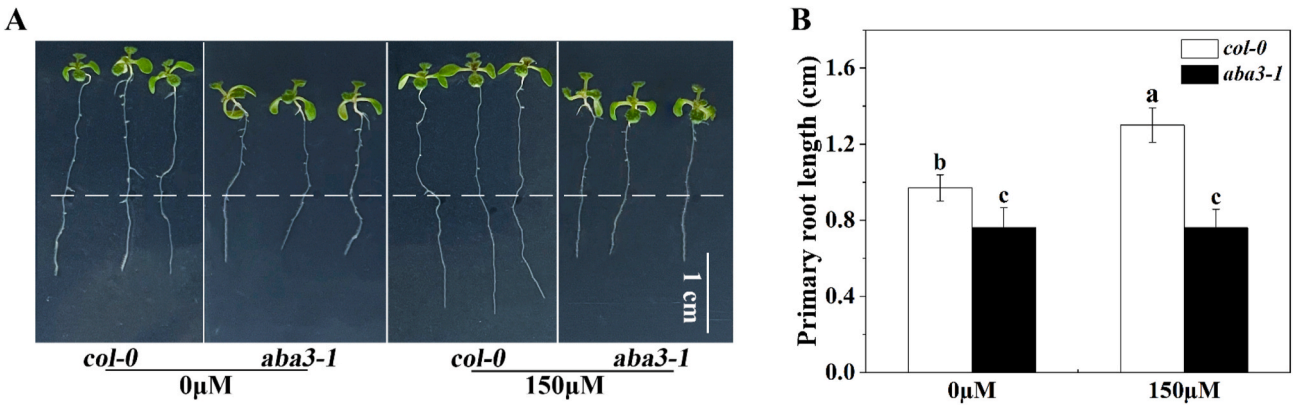

Fig. 3. The *col-0* and *aba3-1* mutant seedlings treated with or without 1,9-D for 3d (A), the broken line indicates the start point after transfer, bar = 1 cm; (B), the primary root length, n = 24. Different letters indicate significantly different values ($P < 0.05$).

differences reached significance compared to the control (Fig. 5A,C). We also found that 1,9-D could significantly increase the expression of the ABA metabolism gene *CYP707A4* (Fig. S2). By contrast, 1,9-D had no significant impact on the expression of the genes *CYP707A1* and *CYP707A2*, while the *CYP707A3* gene was not detected in this experiment.

3.5. Promotion of root growth by 1,9-D requires the auxin carrier PIN2/EIR1

Previous studies have demonstrated that the stimulatory effect of ABA on root growth requires auxin signaling and auxin transport via the PIN2/EIR1 auxin efflux carrier (Li et al., 2017). Therefore, we investigated the effect of 1,9-D on the primary root length of the auxin mutant *pin2/eir1-1* (Fig. 6A). In the treatment without 1,9-D, there was no significant difference in primary root length between *col-0* and *pin2/eir1-1* mutants. However, *col-0* showed significantly higher primary root length than the *pin2/eir1-1* mutant in the treatment with 1,9-D. Similarly, as shown in Fig. 6B, *col-0* seedlings were susceptible to the exogenous addition of ABA, while *pin2/eir1-1* seedlings were not sensitive to ABA.

4. Discussion

Previous studies on 1,9-D, a fatty alcohol and biological nitrification inhibitor isolated from rice root secretions, elucidated its action pathway on microorganisms and its effect on soil N transformation (Sun et al., 2016; Lu et al., 2019; Zhang et al., 2019). However, the impact of 1,9-D on root growth and development had not been examined. Here, we found that 1,9-D can significantly regulate root growth in *Arabidopsis*, with a pronounced dose effect; low concentrations of 1,9-D promoted primary root length and lateral root number, while high concentrations inhibited growth. Studies had been shown that in *Arabidopsis*, fatty alcohols are an important component of root lipids (Delude et al., 2016), and *Arabidopsis* root injury increases fatty alcohol content (Domergue et al., 2010), suggesting that the fatty alcohol 1,9-D may not be inert and exercise regulatory effects on root growth.

Meanwhile, 1,9-D, a secondary metabolite secreted by the root system, may also have effects similar to allelochemicals. Allelochemicals extracted from *C. canadensis* were shown to inhibit root growth in *Arabidopsis* at certain concentrations, and the inhibitory effect became more significant as the concentration increased (Zhang et al., 2022). However, low concentrations were not examined in the latter study, so the possibility that such root-exuded allelochemicals could also promote root growth in different concentration ranges remains unknown. Ferulic acid, also an allelochemical, has been shown to increase root length, fresh weight, and dry weight in soybean at a concentration of 0.1 mM; however, this transitioned to an inhibitory effect at increased concentration (Herrig et al., 2002). The above findings are consistent with a divergent concentration-dependent effect of 1,9-D on the regulation of root growth in *Arabidopsis*.

The role of MHPP as BNI in regulating root growth differs from that of 1,9-D, with MHPP showing an inhibitory effect on primary roots of *Arabidopsis* in the concentration range of 40–80 μM (Liu et al., 2016). The difference in root growth regulation by MHPP and 1,9-D might be due to different modes of action on the plant and their different chemical structures.

In the current study, we present evidence for the possible involvement of ABA signaling during the response of *Arabidopsis* to 1,9-D treatment. We found that the mutant *aba3-1* was insensitive to 1,9-D treatment. When 0.2 or 0.5 μM Flu was included along with 1,9-D treatment, 1,9-D weakened the inhibitory effect of Flu on root growth; meanwhile, 1,9-D could enhance the promotion of root growth at low concentrations (0.1, 0.2, or 0.5 μM) of ABA. These results indicate that 1,9-D may have increases the content of ABA in roots, promoting primary growth. ABA is an essential stress hormone in plant growth and development (Chernys and Zeevaart, 2000; Geiger et al., 2011). So far, most studies have indicated that adding ABA inhibits the growth of primary roots in *Arabidopsis* (Nguyen et al., 2013; Thole et al., 2014). Nevertheless, recent studies showed that low concentrations of ABA could improve root growth in *Arabidopsis* (Li et al., 2017; Xie et al., 2020; Miao et al., 2021).

Up-regulation of the gene for ABA synthase leads to an increase in the ABA content of plants (Liu et al., 2010). The data in Fig. 4 shows that 1,9-D increased the expression of the ABA synthesis genes *AtABA1* and *AtABA3* in *Arabidopsis* seedlings. Induction of ABA-biosynthesis-related gene expression explains the promotion of root development by 1,9-D treatment. *AtABA1* catalyzes the synthesis of violaxanthin by encoding zeaxanthin epoxidase (ZEP), which generates epoxy carotenoid precursors for the ABA biosynthetic pathway and functions early in ABA biosynthesis (Xiong et al., 2002). Increased expression of the *AtABA1* gene by 1,9-D offered the possibility of synthesizing more all-trans-violaxanthin for ABA (Audran et al., 2001). *AtABA3* encodes the sulfurase that produces a functional cofactor and is a critical enzyme in ABA synthesis, and *AtABA3* is a crucial regulator of ABA-stress-responsive gene expression and stress tolerance (Xiong et al., 2001). *Arabidopsis* subjected to salt stress or ABA showed a significantly increased stress response, coincident with an increase in the expression of *AtABA1* and *AtABA3* genes (Barrero et al., 2006). All of the above indicates that 1,9-D might act as a signaling substance to enhance the expression of ABA-synthesis genes. Our data show that the exogenous application of 1,9-D also increased the expression of the ABA-metabolism gene *CYP707A4*. However, the upregulation ratio was less than that for ABA-synthesis genes. This phenomenon might be explained by feedback exerted by ABA-metabolism genes and their

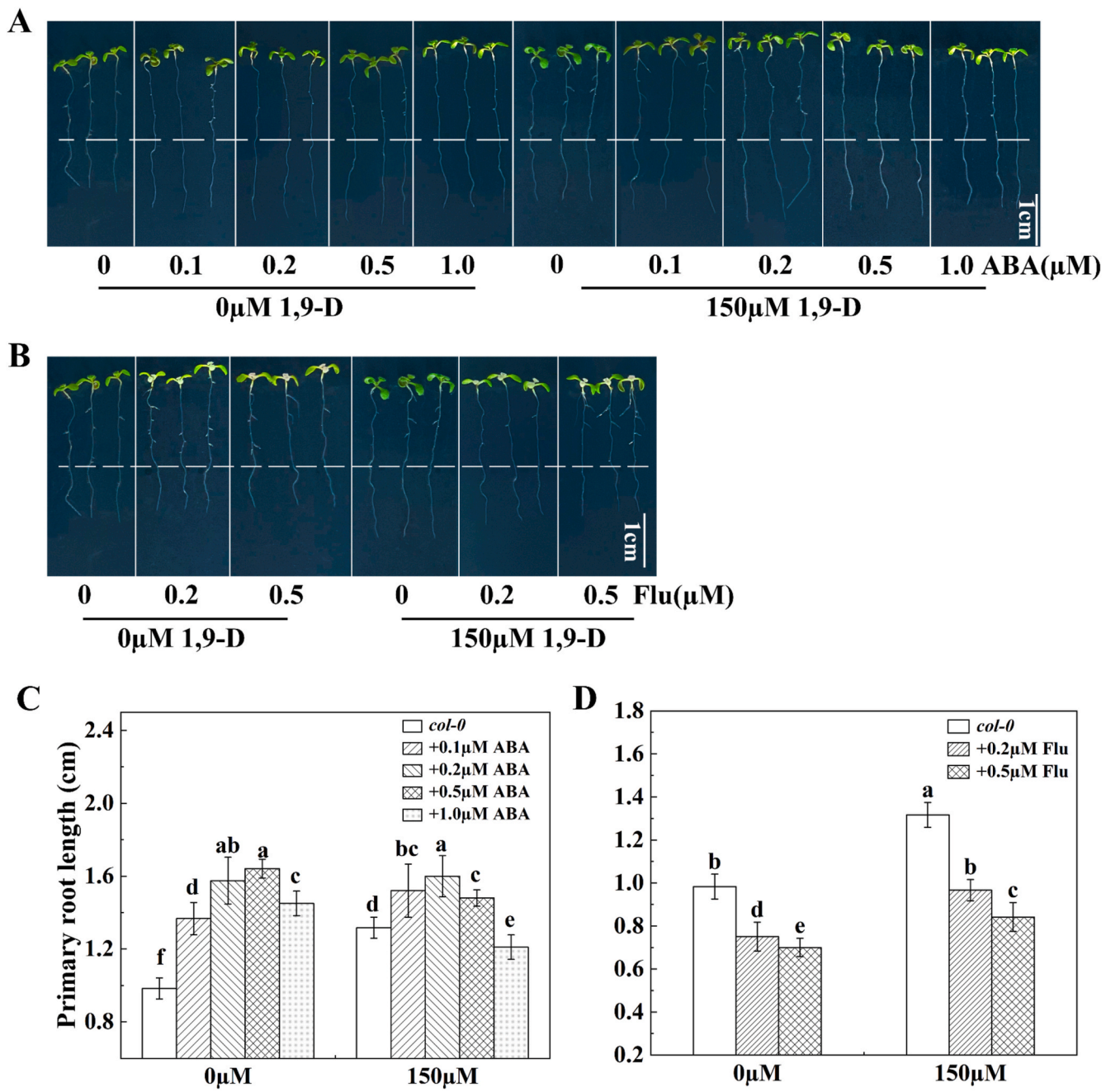

Fig. 4. Primary root length of *col-0* seedlings treated with or without 1,9-D for 3d with ABA (A) or Flu (B), the broken line indicates the start point after transfer, bar = 1 cm; (C), the primary root length following treatment with ABA, n ≥ 1 2; (D), the primary root length following treatment with Flu, n≥ 1 2. Different letters indicate significantly different values ($P < 0.05$).

products on the upregulation of ABA-synthesis genes (Umezawa et al., 2006).

Our experiments with the *pin2/eir1-1* mutant revealed that the addition of 1,9-D did not significantly promote the growth of primary roots in the *pin2/eir1-1* mutant, supporting ABA mediation of the process. Other studies also offer support for this view (Li et al., 2017). The accumulation of auxin in plants is the result of its synthesis, transport, and metabolism (Li et al., 2015). Although 1,9-D did not significantly increase auxin synthesis in this study, it may regulate root growth by altering free IAA content in roots and by targeting auxin transport (Casimiro et al., 2001; Di et al., 2018, 2021). Auxin is transported towards the root meristem, where it modulates root growth, in a balance between environmental stimuli and plant-internal carbohydrate availability. TARGET OF RAPAMYCIN (TOR) is a core hub among signaling cascades that balances cellular responses upon hormonal and nutrient signaling, including root length activity (Retzer and Weckwerth, 2021). Yuan et al. (2020) showed that glucose regulates auxin transport and influences root growth by activating TOR kinase and phosphorylating the auxin transport protein PIN2 resident in the plasma membrane. It is therefore not unreasonable to postulate a specific role of 1,9-decanediol in auxin transport by regulating PIN2 via TOR. However, other response mechanisms, such as those between ABA and auxin (IAA), are not precluded. ABA regulates root growth in *Arabidopsis* by inhibiting the activity of the ABA receptor PP2C, preventing it from phosphorylating downstream targets, and by releasing plasma-membrane H^+-dependent adenosine triphosphatase 2 (AHA2), thereby completing ABA signaling (Miao et al., 2021).

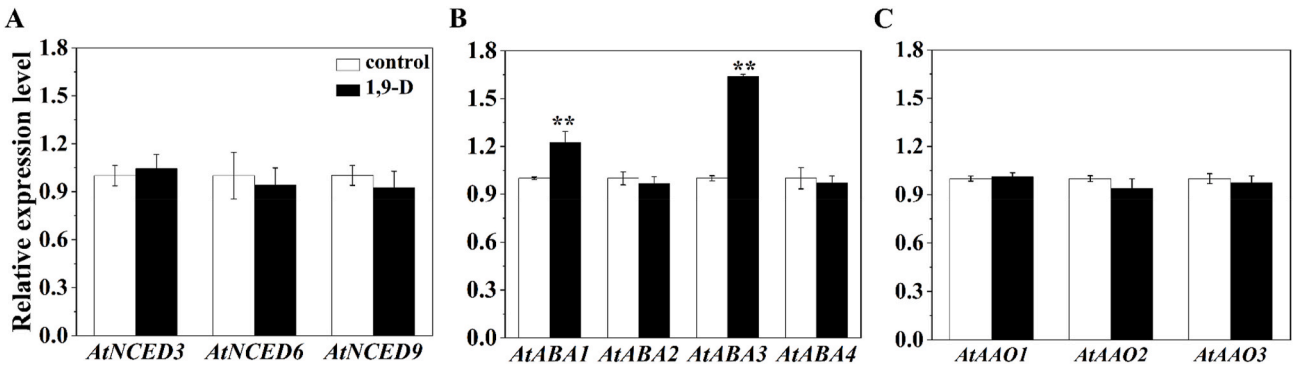

Fig. 5. qRT-PCR analysis of the expression of ABA synthesis genes in *col-0* seedlings treated with or without 1,9-D for 3d. (A), *AtNED* gene family. (B), *AtABA* gene family. (C), *AtAAO* gene family. Asterisks indicate a significant difference in relation to the corresponding control (**, $P < 0.05$).

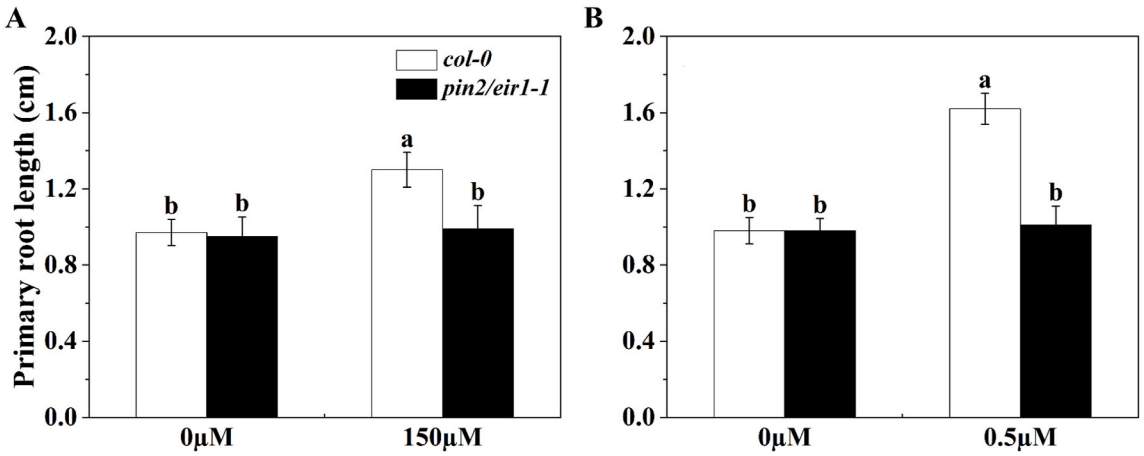

Fig. 6. Primary root length of *col-0* and *pin2* mutant seedlings (3d) treated with different chemicals. (A), treated with or without 1,9-D, n = 20. (B), treated with or without ABA, n = 20. Different letters indicate significantly different values ($P < 0.05$).

5. Conclusion

Our data demonstrate a novel role of the biological nitrification inhibitor 1,9-D in regulating root development for the first time. We show that 1,9-D can stimulate primary root growth in *Arabidopsis* by increasing root ABA content and via targeting the PIN2/EIRI auxin efflux carrier for auxin signaling and transport. The ability of 1,9-D to not only inhibit nitrification in the root zone but also to stimulate plant root growth qualifies it as a potentially environmentally friendly and effective N fertilizer additive and root promoter. In addition, we show that 1,9-D induces ABA synthesis, offering potentially even broader benefits by acting as a signaling additive for general plant resistance to adversity rather than as a simple biological nitrification inhibitor.

CRediT authorship contribution statement

Mingkun Ma: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Visualization. **Yufang Lu:** Project administration, Writing – review & editing. **Dongwei Di:** Methodology, Writing – review & editing. **Herbert J. Kronzucker:** Writing – review & editing. **Gangqiang Dong:** Data curation, Visualization. **Weiming Shi:** Conceptualization, Project administration, Writing – review & editing.

Declaration of competing interest

Author Gangqiang Dong is employed by Amway (China). The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2022.153891.

References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric. Ecosyst. Environ. 189, 136–144. https://doi.org/10.1016/j.agee.2014.03.036.

Adams, E., Miyazaki, T., Moon, J.Y., Sawada, Y., Sato, M., Toyooka, K., Hirai, M.Y., Shin, R., 2020. Syringic acid alleviates cesium-induced growth defect in *Arabidopsis*. Int. J. Mol. Sci. 21 (23), 9116. https://doi.org/10.3390/ijms21239116.

Audran, C., Liotenberg, S., Gonneau, M., North, H., Frey, A., Tap-Waksman, K., Vartanian, N., Marion-Poll, A., 2001. Localisation and expression of zeaxanthin epoxidase mrna in *Arabidopsis* in response to drought stress and during seed development. Aust. J. Plant Physiol. 28 (12), 1161–1173. https://doi.org/10.1071/PP00134.

Barrero, J.M., Rodriguez, P.L., Quesada, V., Piqueras, P., Ponce, M.R., Micol, J.L., 2006. Both abscisic acid (ABA)-dependent and ABA-independent pathways govern the induction of *NCED3* , *AAO3* and *ABA1* in response to salt stress. Plant Cell Environ. 29 (10), 2000–2008. https://doi.org/10.1111/j.1365-3040.2006.01576.x.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., Scheres, B., 2005. The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433 (7021), 39–44. https://doi.org/10.1038/nature03184.

Byrnes, R.C., Nùñez, J., Arenas, L., Rao, I., Trujillo, C., Alvarez, C., Arango, J., Rasche, F., Chirinda, N., 2017. Biological nitrification inhibition by *Brachiaria* grasses mitigates soil nitrous oxide emissions from bovine urine patches. Soil Biol. Biochem. 107, 156–163. https://doi.org/10.1016/j.soilbio.2016.12.029.

Casimiro, I., Marchant, A., Bhalerao, R.P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inze, D., Sandberg, G., Casero, P.J., Bennett, M., 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. Plant Cell 13 (4), 843–852. https://doi.org/10.1105/tpc.13.4.843.

Celenza Jr., J.L., Grisafi, P.L., Fink, G.R., 1995. A pathway for lateral root formation in *Arabidopsis Thaliana*. Genes and Devement 9 (17), 2131–2142. https://doi.org/10.1101/gad.9.17.2131.

Chernys, J.T., Zeevaart, J.A.D., 2000. Characterization of the 9-cis-epoxycarotenoid dioxygenase gene family and the regulation of abscisic acid biosynthesis in avocado. Plant Physiol. 124 (1), 343–354, 0.1104/pp.124.1.343.

Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017a. How Plant root exudates shape the nitrogen cycle. Trends Plant Sci. 22 (8), 661–673. https://doi.org/10.1016/j.tplants.2017.05.004.

Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017b. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. Nature Plants 3 (6), 10.1038/nplants.2017.74.

Delude, C., Fouillen, L., Bhar, P., Cardinal, M.J., Pascal, S., Santos, P., Kosma, D.K., Joubes, J., Rowland, O., Domergue, F., 2016. Primary fatty alcohols are major components of suberized root tissues of *Arabidopsis* in the form of alkyl hydroxycinnamates. Plant Physiol. 171 (3), 1934–1950, 0.1104/pp.16.00834.

Di, D.W., Li, G., Sun, L., Wu, J.J., Wang, M., Kronzucker, H.J., Fang, S., Chu, J., Shi, W. M., 2021. High ammonium inhibits root growth in *Arabidopsis Thaliana* by promoting auxin conjugation rather than inhibiting auxin biosynthesis. J. Plant Physiol. 261, 153415 https://doi.org/10.1016/j.jplph.2021.153415.

Di, D.W., Zhang, C.G., Luo, P., An, C.W., Guo, G.Q., 2016. The biosynthesis of auxin: how many paths truly lead to IAA? Plant Growth Regul. 78 (3), 275–285. https://doi.org/10.1007/s10725-015-0103-5.

Di, D.W., Sun, L., Zhang, X., Li, G., Kronzucker, H.J., Shi, W., 2018. Involvement of auxin in the regulation of ammonium tolerance in rice (*Oryza sativa* L.). Plant Soil 432 (1–2), 373–387. https://doi.org/10.1007/s11104-018-3813-4.

Domergue, F., Vishwanath, S.J., Joubes, J., Ono, J., Lee, J.A., Bourdon, M., Alhattab, R., Lowe, C., Pascal, S., Lessire, R., Rowland, O., 2010. Three *Arabidopsis* fatty acyl-coenzyme a reductases, FAR1, FAR4, and FAR5, generate primary fatty alcohols associated with suberin deposition. Plant Physiol. 153 (4), 1539–1554. https://doi.org/10.1104/pp.110.158238.

Dubois, M., Van den Broeck, L., Inze, D., 2018. The pivotal role of ethylene in plant growth. Trends Plant Sci. 23 (4), 311–323. https://doi.org/10.1016/j.tplants.2018.01.003.

Geiger, D., Maierhofer, T., Al-Rasheid, K.A., Scherzer, S., Mumm, P., Liese, A., Ache, P., Wellmann, C., Marten, I., Grill, E., Romeis, T., Hedrich, R., 2011. Stomatal closure by fast abscisic acid signaling is mediated by the guard cell anion channel SLAH3 and the receptor RCAR1. Sci. Signal. 4 (173) https://doi.org/10.1126/scisignal.2001346 ra32.

Gonzalez-Guzman, M., Pizzio, G.A., Antoni, R., Vera-Sirera, F., Merilo, E., Bassel, G.W., Fernandez, M.A., Holdsworth, M.J., Perez-Amador, M.A., Kollist, H., Rodriguez, P.L., 2012. *Arabidopsis* PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. Plant Cell 24 (6), 2483–2496. https://doi.org/10.1105/tpc.112.098574.

Herrig, V., Ferrarese Mde, L., Suzuki, L.S., Rodrigues, J.D., Ferrarese-Filho, O., 2002. Peroxidase and phenylalanine ammonia-lyase activities, phenolic acid contents, and allelochemicals-inhibited root growth of soybean. Biol. Res. 35 (1), 59–66. https://doi.org/10.4067/s0716-97602002000100009.

Hou, N.N., You, J.F., Pang, J.D., Xu, M.Y., Chen, G., Yang, Z.M., 2009. The accumulation and transport of abscisic acid insoybean (*Glycine max* L.) under aluminum stress. Plant Soil 330 (1–2), 127–137. https://doi.org/10.1007/s11104-009-0184-x.

Kim, S.K., Chang, S.C., Lee, E.J., Chung, W.S., Kim, Y.S., Hwang, S., Lee, J.S., 2000. Involvement of brassinosteroids in the gravitropic response of primary root of maize. Plant Physiol. 123 (3), 997–1004. https://doi.org/10.1104/pp.123.3.997.

Kirk, G.J., Kronzucker, H.J., 2005. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. Ann. Bot. 96 (4), 639–646, 10.1093/aob/mci216.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385 (6611), 59–61, 10.1038/385059a0.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., Kirk, G.J.D., 1999. Nitrate-ammonium synergism in rice. a subcellular flux analysis. Plant Physiol. 119 (3), 1041–1046, 10.1104/pp.119.3.1041.

Laskowski, M., Grieneisen, V.A., Hofhuis, H., Hove, C.A., Hogeweg, P., Maree, A.F., Scheres, B., 2008. Root system architecture from coupling cell shape to auxin transport. PLoS Biol. 6 (12), e307. https://doi.org/10.1371/journal.pbio.0060307.

Le, J., Vandenbussche, F., Van Der Straeten, D., Verbelen, J.P., 2001. In the early response of *Arabidopsis* roots to ethylene, cell elongation is up- and down-regulated and uncoupled from differentiation. Plant Physiol. 125 (2), 519–522. https://doi.org/10.1104/pp.125.2.519.

Lei, G.J., Sun, L., Sun, Y., Zhu, X.F., Li, G.X., Zheng, S.J., 2020. Jasmonic acid alleviates cadmium toxicity in *Arabidopsis* via suppression of cadmium uptake and translocation. J. Integr. Plant Biol. 62 (2), 218–227. https://doi.org/10.1111/jipb.12801.

Li, G.J., Song, H.Y., Li, B.H., Kronzucker, H.J., Shi, W.M., 2015a. Auxin resistant1 and PIN-FORMED2 protect lateral root formation in *Arabidopsis* under iron stress. Plant Physiol. 169 (4), 2608–2623. https://doi.org/10.1104/pp.15.00904.

Li, G.J., Xu, W.F., Kronzucker, H.J., Shi, W.M., 2015. Ethylene is critical to the maintenance of primary root growth and Fe homeostasis under Fe stress in *Arabidopsis*. J. Exp. Bot. 66 (7), 2041–2054. https://doi.org/10.1093/jxb/erv005.

Li, T.L., Wang, Z.G., Wang, C.X., Huang, J.Y., Feng, Y.Y., Shen, W.S., Zhou, M., Yang, L. Z., 2022. Ammonia volatilization mitigation in crop farming: a review of fertilizer amendment technologies and mechanisms. Chemosphere 303 (1), 134944. https://doi.org/10.1016/j.chemosphere.2022.134944.

Li, X.Q., Chen, L., Forde, B.G., Davies, W.J., 2017. The biphasic root growth response to abscisic acid in *Arabidopsis* involves interaction with ethylene and auxin signalling pathways. Front. Plant Sci. 8, 1493. https://doi.org/10.3389/fpls.2017.01493.

Lincoln, C., Britton, J.H., Estelle, M., 1990. Growth and development of the axr1 mutants of *Arabidopsis*. Plant Cell 2 (11), 1071–1080. https://doi.org/10.1105/tpc.2.11.1071.

Liu, Y.Y., Wang, R.L., Zhang, P., Chen, Q., Luo, Q., Zhu, Y.Y., Xu, J., 2016. The nitrification inhibitor methyl 3-(4-hydroxyphenyl)propionate modulates root development by interfering with auxin signaling via the NO/ROS pathway. Plant Physiol. 171 (3), 1686–1703. https://doi.org/10.1104/pp.16.00670.

Liu, Y.G., Ye, N.H., Liu, R., Chen, M.X., Zhang, J.H., 2010. H_2O_2 mediates the regulation of ABA catabolism and GA biosynthesis in *Arabidopsis* seed dormancy and germination. J. Exp. Bot. 61 (11), 2979–2990. https://doi.org/10.1093/jxb/erq125.

Lopez-Ruiz, B.A., Zluhan-Martinez, E., Sanchez, M.P., Alvarez-Buylla, E.R., Garay-Arroyo, A., 2020. Interplay between hormones and several abiotic stress conditions on Arabidopsis Thaliana primary root development. Cells 9 (12). https://doi.org/10.3390/cells9122576.

Lu, Y.F., Zhang, X.N., Ma, M.K., Zu, W.J., Kronzucker, H.J., Shi, W.M., 2022. Syringic acid from rice as a biological nitrification and urease inhibitor and its synergism with 1,9-decanediol. Biol. Fertil. Soils 58 (3), 277–289. https://doi.org/10.1007/s00374-021-01584-y.

Lu, Y.F., Zhang, X.N., Jiang, J.F., Kronzucker, H.J., Shen, W.S., Shi, W.M., 2019. Effects of the biological nitrification inhibitor 1,9-decanediol on nitrification and ammonia oxidizers in three agricultural soils. Soil Biol. Biochem. 129, 48–59. https://doi.org/10.1016/j.soilbio.2018.11.008.

Ma, J.H., Cao, Y.H., Sun, L.L., Li, Y., Ju, Q., Yang, X.H., Xu, J., 2020. Methyl 3-(4-hydroxyphenyl) propionate modulates plant growth and secondary metabolite accumulation by inducing metabolic changes in *perilla frutescens*. Plant Soil 453 (1–2), 577–593. https://doi.org/10.1007/s11104-020-04625-x.

McSwiney, C.P., Robertson, G.P., 2005. Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (*Zea Mays* L.) cropping system. Global Change Biol. 11 (10), 1712–1719. https://doi.org/10.1111/j.1365-2486.2005.01040.x.

Miao, R., Yuan, W., Wang, Y., Garcia-Maquilon, I., Dang, X.L., Li, Y., Zhang, J.H., Zhu, Y. Y., Rodriguez, P.L., Xu, W.F., 2021. Low ABA concentration promotes root growth and hydrotropism through relief of ABA INSENSITIVE 1-mediated inhibition of plasma membrane H^+-ATPase2. Sci. Adv. 7 (12) https://doi.org/10.1126/sciadv.abd4113.

Min, J., Shi, W.M., 2018. Nitrogen discharge pathways in vegetable production as non-point sources of pollution and measures to control it. Sci. Total Environ. 613–614, 123–130. https://doi.org/10.1016/j.scitotenv.2017.09.079.

Min, J., Sun, H.J., Kronzucker, H.J., Wang, Y., Shi, W.M., 2021a. Comprehensive assessment of the effects of nitrification inhibitor application on reactive nitrogen loss in intensive vegetable production systems. Agric. Ecosyst. Environ. 307, 107227 https://doi.org/10.1016/j.agee.2020.107227.

Min, J., Sun, H.J., Wang, Y., Pan, Y.F., Kronzucker, H.J., Zhao, D.Q., Shi, W.M., 2021b. Mechanical side-deep fertilization mitigates ammonia volatilization and nitrogen runoff and increases profitability in rice production independent of fertilizer type and split ratio. J. Clean. Prod. 316, 128370 https://doi.org/10.1016/j.jclepro.2021.128370.

Nguyen, H.N., Kim, J.H., Hyun, W.Y., Nguyen, N.T., Hong, S.W., Lee, H., 2013. TTG1-mediated flavonols biosynthesis alleviates root growth inhibition in response to ABA. Plant Cell Rep. 32 (4), 503–514. https://doi.org/10.1007/s00299-012-1382-1.

Otaka, J., Subbarao, G.V., Ono, H., Yoshihashi, T., 2022. Biological nitrification inhibition in maize-isolation and identification of hydrophobic inhibitors from root exudates. Biol. Fertil. Soils 58 (3), 251–264. https://doi.org/10.1007/s00374-021-01577-x.

Rahman, A., Amakawa, T., Goto, N., Tsurumi, S., 2001. Auxin is a positive regulator for ethylene-mediated response in the growth of *Arabidopsis* roots. Plant Cell Physiol. 42 (3), 301–307. https://doi.org/10.1093/pcp/pce035.

Reigosa, M.J., Malvido-Pazos, E., 2007. Phytotoxic effects of 21 plant secondary metabolites on *Arabidopsis Thaliana* germination and root growth. J. Chem. Ecol. 33 (7), 1456–1466. https://doi.org/10.1007/s10886-007-9318-x.

Retzer, K., Weckwerth, W., 2021. The TOR-Auxin connection upstream of root hair growth. Plants 10 (1), 150. https://doi.org/10.3390/plants10010150.

Smalle, J., Van Der Straeten, D., 1997. Ethylene and vegetative development. Physiol. Plantarum 100 (3), 593–605. https://doi.org/10.1111/j.1399-3054.1997.tb03065.x.

Souza, E.F.C., Rosen, C.J., Venterea, R.T., 2021. Co-application of DMPSA and NBPT with urea mitigates both nitrous oxide emissions and nitrate leaching during irrigated potato production. Environ. Pollut. 284, 117124 https://doi.org/10.1016/j.envpol.2021.117124.

Spollen, W.G., LeNoble, M.E., Samuels, T.D., Bernstein, N., Sharp, R.E., 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. Plant Physiol. 122 (3), 967–976. https://doi.org/10.1104/pp.122.3.967.

Subbarao, G.V., Ishikawa, T., Ito, O., Nakahara, K., Wang, H.Y., Berry, W.L., 2006. A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with *brachiaria humidicola*. Plant Soil 288 (1–2), 101–112. https://doi.org/10.1007/s11104-006-9094-3.

Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F., Yoshihashi, A.T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I.M., Lascano, C.E., Berry, W.L., Ito, O., 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proc. Natl. Acad. Sci. USA 106 (41), 17302–17307. https://doi.org/10.1073/pnas.0903694106.

Subbarao, G.V., Nakahara, K., Ishikawa, T., Ono, H., Yoshida, M., Yoshihashi, T., Zhu, Y., Zakir, H.A.K.M., Deshpande, S.P., Hash, C.T., Sahrawat, K.L., 2013. Biological nitrification inhibition (BNI) activity in sorghum and its characterization. Plant Soil 366 (1–2), 243–259. https://doi.org/10.1007/s11104-012-1419-9.

Sun, H.J., Zhang, H.L., Powlson, D., Min, J., Shi, W.M., 2015. Rice production, nitrous oxide emission and ammonia volatilization as impacted by the nitrification inhibitor 2-chloro-6-(trichloromethyl)-pyridine. Field Crop. Res. 173, 1–7. https://doi.org/10.1016/j.fcr.2014.12.012.

Sun, L., Di, D.W., Li, G.J., Kronzucker, H.J., Wu, X.Y., Shi, W.M., 2020. Endogenous ABA alleviates rice ammonium toxicity by reducing ROS and free ammonium via regulation of the SAPK9-bZIP20 pathway. J. Exp. Bot. 71 (15), 4562–4577. https://doi.org/10.1093/jxb/eraa076.

Sun, L., Lu, Y.F., Yu, F.W., Kronzucker, H.J., Shi, W.M., 2016. Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. New Phytol. 212 (3), 646–656. https://doi.org/10.1111/nph.14057.

Swarup, R., Perry, P., Hagenbeek, D., Van Der Straeten, D., Beemster, G.T., Sandberg, G., Bhalerao, R., Ljung, K., Bennett, M.J., 2007. Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation. Plant Cell 19 (7), 2186–2196. https://doi.org/10.1105/tpc.107.052100.

Tanimoto, M., Roberts, K., Dolan, L., 1995. Ethylene is a positive regulator of root hair development in *Arabidopsis Thaliana*. Plant J. 8 (6), 943–948. https://doi.org/10.1046/j.1365-313X.1995.8060943.x.

Thole, J.M., Beisner, E.R., Liu, J., Venkova, S.V., Strader, L.C., 2014. Abscisic acid regulates root elongation through the activities of auxin and ethylene in *Arabidopsis Thaliana*. G3: Genes, Genomes, Gentics 4 (7), 1259–1274. https://doi.org/10.1534/g3.114.011080.

Umezawa, T., Okamoto, M., Kushiro, T., Nambara, E., Oono, Y., Seki, M., Kobayashi, M., Koshiba, T., Kamiya, Y., Shinozaki, K., 2006. CYP707A3, a major ABA 8'-hydroxylase involved in dehydration and rehydration response in *Arabidopsis Thaliana*. Plant J. 46 (2), 171–182. https://doi.org/10.1111/j.1365-313X.2006.02683.x.

Van de Poel, B., Smet, D., Van Der Straeten, D., 2015. Ethylene and hormonal cross talk in vegetative growth and development. Plant Physiol. 169 (1), 61–72. https://doi.org/10.1104/pp.15.00724.

Wang, X., Bai, J.H., Xie, T., Wang, W., Zhang, G.L., Yin, S., Wang, D.W., 2021. Effects of biological nitrification inhibitors on nitrogen use efficiency and greenhouse gas emissions in agricultural soils: a review. Ecotoxicol. Environ. Saf. 220, 112338 https://doi.org/10.1016/j.ecoenv.2021.112338.

Wang, X.J., Guo, C., Peng, J., Li, C., Wan, F.F., Zhang, S.M., Zhou, Y.Y., Yan, Y., Qi, L.J., Sun, K.W., Yang, S.H., Gong, Z.Z., Li, J.G., 2019. Abre-binding factors play a role in the feedback regulation of ABA signaling by mediating rapid ABA induction of ABA co-receptor genes. New Phytol. 221 (1), 341–355. https://doi.org/10.1111/nph.15345.

Wang, Y.Y., Wang, R., Zhao, S., Lu, C.M., Zhu, Z.Q., Li, H., 2022. Transporter NRT1.5/NPF7.3 suppresses primary root growth under low K^+ stress by regulating the degradation of PIN-FORMED2. BMC Plant Biol. 22 (1), 330. https://doi.org/10.1186/s12870-022-03730-6.

Wang, Z.R., Liu, L., Su, H., Guo, L.Q., Zhang, J.L., Li, Y.F., Xu, J.Y., Zhang, X.C., Guo, Y. D., Zhang, N., 2020. Jasmonate and aluminum crosstalk in tomato: identification and expression analysis of WRKYS and almts during JA/Al-Regulated root growth. Plant Physiol. Biochem. 154, 409–418. https://doi.org/10.1016/j.plaphy.2020.06.026.

Woodward, E.E., Hladik, M.L., Kolpin, D.W., 2016. Nitrapyrin in streams: the first study documenting off-field transport of a nitrogen stabilizer compound. Environ. Sci. Technol. Lett. 3 (11), 387–392. https://doi.org/10.1021/acs.estlett.6b00348.

Xie, Q.J., Essemine, J., Pang, X.C., Chen, H.Y., Cai, W.M., 2020. Exogenous application of abscisic acid to shoots promotes primary root cell division and elongation. Plant Sci. 292, 110385 https://doi.org/10.1016/j.plantsci.2019.110385.

Xiong, L.M., Ishitani, M., Lee, H., Zhu, J.K., 2001. The *Arabidopsis* LOS5/ABA3 locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression. Plant Cell 13 (9), 2063–2083. https://doi.org/10.1105/tpc.010101.

Xiong, L.M., Lee, H.J., Ishitani, M., Zhu, J.K., 2002. Regulation of osmotic stress-responsive gene expression by the LOS6/ABA1 locus in *Arabidopsis*. J. Biol. Chem. 277 (10), 8588–8596. https://doi.org/10.1074/jbc.M109275200.

Xu, W.F., Jia, L.G., Shi, W.M., Baluska, F., Kronzucker, H.J., Liang, J.S., Zhang, J.H., 2013. The tomato 14-3-3 Protein TFT4 modulates H^+ efflux, basipetal auxin transport, and the PKS5-J3 pathway in the root growth response to alkaline stress. Plant Physiol. 163 (4), 1817–1828. https://doi.org/10.1104/pp.113.224758.

Yang, Y., Liu, S.W., Pan, K., Wu, F.Z., 2013. Effects of Chinese onion' s root exudates on cucumber seedlings growth and rhizosphere soil microorganisms. J. Appl. Ecol. 24 (4), 1109–1117.

Yang, Z.B., Liu, G.C., Liu, J.J., Zhang, B., Meng, W.J., Muller, B., Hayashi, K.I., Zhang, X. S., Zhao, Z., De Smet, I., Ding, Z.J., 2017. Synergistic action of auxin and cytokinin mediates aluminum-induced root growth inhibition in *Arabidopsis*. EMBO Rep. 18 (7), 1213–1230. https://doi.org/10.15252/embr.201643806.

Yuan, X., Xu, P., Yu, Y., Xiong, Y., 2020. Glucose-TOR signaling regulates PIN2 stability to orchestrate auxin gradient and cell expansion in *Arabidopsis* root. Proc. Natl. Acad. Sci. USA 117 (51), 32223–32225. https://doi.org/10.1073/pnas.2015400117.

Zakir, H., Subbarao, G.V., Pearse, S.J., Gopalakrishnan, S., Ito, O., Ishikawa, T., Kawano, N., Nakahara, K., Yoshihashi, T., Ono, H., Yoshida, M., 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum Bicolor*). New Phytol. 180 (2), 442–451. https://doi.org/10.1111/j.1469-8137.2008.02576.x.

Zhang, X.N., Lu, Y.F., Yang, T., Kronzucker, H.J., Shi, W.M., 2019. Factors Influencing the release of the biological nitrification inhibitor 1,9-decanediol from rice (*Oryza Sativa* L.) roots. Plant Soil 436 (1–2), 253–265. https://doi.org/10.1007/s11104-019-03933-1.

Zhao, Y., Zhang, Z.J., Gao, J.H., Wang, P.C., Hu, T., Wang, Z.G., Hou, Y.J., Wan, Y.Z., Liu, W.S., Xie, S.J., Lu, T.J., Xue, L., Liu, Y.J., Macho, A.P., Tao, W.A., Bressan, R.A., Zhu, J.K., 2018. *Arabidopsis* duodecuple mutant of PYL ABA receptors reveals PYL repression of aba-independent SnRK2 activity. Cell Rep. 23 (11), 3340–3351. https://doi.org/10.1016/j.celrep.2018.05.044 e3345.

Zluhan-Martinez, E., Lopez-Ruiz, B.A., Garcia-Gomez, M.L., Garcia-Ponce, B., de la Paz Sanchez, M., Alvarez-Buylla, E.R., Garay-Arroyo, A., 2021. Integrative roles of phytohormones on cell proliferation, elongation and differentiation in the *Arabidopsis Thaliana* primary root. Front. Plant Sci. 12, 659155 https://doi.org/10.3389/fpls.2021.659155.

Zou, N., Li, B.H., Dong, G.Q., Kronzucker, H.J., Shi, W.M., 2012. Ammonium-induced loss of root gravitropism is related to auxin distribution and TRH1 function, and is uncoupled from the inhibition of root elongation in *Arabidopsis*. J. Exp. Bot. 63 (10), 3777–3788. https://doi.org/10.1093/jxb/ers068.