Effect of Exogenous Abscisic acid (ABA) on the Morphology, Phytohormones, and Related Gene Expression of Developing Lateral Roots in 'Qingzhen 1'

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Research Article

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Abstract

Lateral roots (LRs) are critical for plant stress tolerance and productivity. Understanding how hormones and genes interact in a fluctuating environment to coordinate LR development is a major challenge. Abscisic acid (ABA) is the primary stress-responsive hormone and mediates LR development in various plant species. However, the effect of exogenous ABA on LR development has not been elucidated in apple. In this study, 'Qingzhen 1' was treated with exogenous 5 μM ABA for 20 days to investigate the regulation mechanism of ABA on LR development. Morphological observations advocated that ABA inhibited both LR and shoot development in 'Qingzhen 1' apple plants, where the root number was 16.94%, the root length was 30.32%, the plant height was 10.88%, and the stem thickness was 8.08% lower than those in the control plants. Meanwhile, the endogenous ABA concentration was significantly increased, but the indole-3-acetic acid (IAA), zeatin riboside (ZR), and jasmonic acid (JA) concentrations were significantly decreased with ABA treatment. Furthermore, the expression levels of ABA-related genes (*MdCYP707A2*, *MdABI1*, *MdAREB2*, and *MdABF3*) were significantly upregulated, while the expression levels of auxin-related genes (*MdYUCCA3*, *MdYUCCA8*, *MdPIN1 MdPIN2, MdPIN3*, and *MdARF19*), root development-related genes (*MdWOX5* and *MdWOX11*), and cell cycle-related genes (*MdCYCD1;1* and *MdCYCD3;1*) were significantly downregulated at the early stage of ABA treatment, which act together on the inhibition of LR development. Taken together, the changes in hormone levels and gene expression resulted in inhibited LR development of apple plants in response to ABA.

Key Message

This study revealed the mechanism that exogenous ABA application inhibits the LR development of 'Qinzhen 1' apple rootstock by affecting the auxin signaling and the expression of growth-related genes.

Introduction

Plant roots function in the critical role of water and nutrient uptake, for responses to abiotic and biotic signals in the soil, and to anchor the plant in the ground (Zobel et al., 2007). Therefore, root morphology is the primary trait that influences plant resource acquisition. To maximize access to soil nutrients, LRs are formed from the primary roots (PRs) and adventitious roots (ARs) to expand the total length and surface area of the actively absorbing root system (Nibau et al., 2008; Ge et al., 2019). LRs are initiated through activation of pericycle cells at the xylem poles (Casimiro et al., 2003), and the development of LRs is known to be a result of the interaction between the plant itself and environmental stimuli (Postma et al., 2014).

Various phytohormones affect LR formation and act as chemical signaling. For example, auxin is a primary signal that promotes lateral root primordia (LRP)/LR initiation, facilitates LR emergence, and regulates LR development (De Smet et al., 2007; Qin and Huang, 2018; Du and Scheres, 2018;). Cytokinins (CTKs) interact with auxin to inhibit LRP/LR initiation (Bielach et al., 2012; Jing and Strader, 2019), reduce LRs number, and stimulate LR elongation (Rani Debi et al., 2005). In Cucumber and Arabidopsis, Gibberellic acids (GAs) positively regulate LR development (Bidadi et al., 2014). However, in Populus, GAs negatively regulate LR development by inhibiting LRP initiation and LR elongation (Gou et al., 2010; Farquharson, 2010). Additionally, the effects of JA on LR development is conflicting; JA was reported to promote LRs formation in Arabidopsis (Cai et al., 2014), increase the number of LRs in rice and Arabidopsis (Wang et al., 2002; Sun et al., 2009), but reduce the LR length and number in sunflower (Corti Monzón et al., 2012). In general, the mechanisms of phytohormone regulation of LR are interactive and could be species-dependent.

The phytohormone ABA functions in many plant developmental processes, such as seed and bud dormancy, stomatal closure, and is especially important for the plants in response to environmental stresses, including water stress, salinity, freezing tolerance, etc. (De Smet et al., 2006). Numerous reports have described the inhibitory effect of ABA on LR formation (Xing, et al., 2016; Lu et al., 2019). However, the effect of ABA on LR development and how ABA interacts with other phytohormones and regulates related gene expressions during LR formation and development is still not fully elucidated in

apple rootstocks. Recent research showed that ABA altered the polar localization of *ZmPIN1* and disrupted the distribution of auxin and thus inhibits LR initiation and development (Lu et al., 2019). ABA-induced LR inhibition could not be rescued by exogenous auxin, indicating that there is an ABA sensitive and auxin-independent checkpoint that may be involved in the post-emergence stage (De Smet et al., 2003). *ABI4* mediates ABA and CTK-dependent inhibition of LR formation by reducing polar auxin transport through the down-regulated expression of the auxin-efflux carrier protein *PIN1* (Shkolnik-Inbar and Bar-Zvi, 2010). These results proposed that ABA-regulated LR development is auxin-dependent in terms of auxin biosynthesis, transport, and signaling.

Apple occupies a dominant position in global fruit production and is considered the most important fruit crop in temperate areas (Zhang et al., 2019; Samnegård et al., 2019). Apple trees mainly grow in the form of a grafted chimera of rootstock and scion. In addition to be capable of reducing tree vigor and having precocious flowering, the ability to adapt stresses which determined mainly by root structure and function were required for apple rootstock (Wang et al., 2019). 'Qingzhen 1' is a kind of apomictic apple rootstock that has been planted in many places in China, with early-flowering habits, high resistance to re-plant disease, and leading to better fruit yield and quality. (Sha et al., 2013). Our previous experiments found that their LRs are well developed and saline-alkali tolerant, but the mechanism of their tolerance to abiotic stress is unclear. To further understand how ABA regulates LRs development in apple rootstocks, the dynamic changes of root growth, phytohormones, and related gene expressions were compared between plants cultured under normal conditions and ABA treatment. This study provides important insights into how ABA regulates LR development in apple rootstock.

Materials And Methods

Plant materials and treatments

The tissue-cultured 'Qingzhen 1'plants (with 6–8 leaves) were transferred and cultivated in plastic containers (42 × 32 × 17 cm) filled with 10 L 1/2-strength Hoagland nutrient solution (composition is listed in supplemental Table S1, 12 plants in each container). The growth conditions are as follows: light was 100 μmol photons m^{-2} s^{-1} (14-h light photoperiods), temperatures were 27 ± 1°C/21 ± 1°C (day/night), and relative humidity was 60–70%. The nutrient solution was aerated hourly with an air pump for 30 mins and the solution was replaced weekly. The pH of the solution was adjusted to 6.0 ± 0.2 with H_3PO_4. After 15 days of pre-culture, a total number of 240 plants were divided into two groups: half of the plants were cultured with 5 μM ABA in solution, the other half of plants continued to receive regular cultivation served as the controls.

Morphological parameters

The LR samples were collected at 5 time points: 0, 5, 10, 15, and 20 days after 5 μM ABA application with three biological replicates. At each time point, randomly collected LR samples were immediately immersed in liquid nitrogen and stored at -80°C for subsequent analysis of hormone levels and gene expressions. Root length, root surface area, and root volume were obtained by analyzing root 2D images with WinRHIZO Pro scanning and analysis systems (WinRHIZO 2003, Quebec, Canada) (Tahir et al., 2021a; b). The number of roots was counted manually. The plant height was measured using a ruler from the stem base to the growth point, and stem diameter was measured using a cursor caliper (0.5 cm upon the stem base).

Hormones Measurement

Multiple hormones in LRs, including IAA, ZR, GA_3, GA_4, JA, and ABA concentrations, were determined using an enzyme-linked immunosorbent assay. The extraction and purification of those hormones were performed according to previously described methods (Wang et al., 2020; Tahir et al., 2021c). The absorbance of IAA, ZR, GA_3, GA_4, JA, and ABA at 490 nm was determined by ELISA spectrophotometer; all antibodies against each hormone were monoclonal and were obtained from the Center of Plant Growth Regulator, China Agricultural University.

RNA extraction and cDNA synthesis from lateral roots of ‘Qingzhen 1’ plants

Total RNAs of LR samples were extracted using RNA prep pure Plant Kit (TianGen, Beijing, China, http://www.tiangen.com) according to the manufacturer’s protocols. Total RNAs were converted into cDNAs using a One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen, Beijing, China, https://www.transgen.com.cn).

Expression analysis by RT-qPCR

The relative expression of genes involved in ABA synthesis (*MdNCED3*, *MdAAO4)*, metabolism (*MdCYP707A2*), and signal transduction (*MdABI1*, *MdAREB2*, and *MdABF3*); IAA synthesis (*MdYUCCA3*, *MdYUCCA8*), transport (*MdPIN1*, *MdPIN2*, *MdPIN3*), and signal transduction (*MdIAA3*, *MdIAA14*, *MdARF7*, and *MdARF19*); cell cycle (*MdCYCD1;1*, *MdCYCD3;1*, *MdCYCP1;1*, and *MdCYCP4;1*); and LR development (*MdSHR*, *MdWOX5*, *MdWOX11*, *MdLBD16*, and *MdLBD29*) were analyzed from LR samples of the plants via RT-qPCR. Primer pairs were designed by Primer 6.0 software utilizing the apple gene sequences that were annotated in GenBank. The sequence information and primers were list in Supplemental Table S2 and Supplemental Table S3, respectively. Real-time qPCR analysis was performed using a Perfect Start™ Green qPCR Super Mix kit (TransGen, Beijing, China, https://www.transgen.com.cn) according to the manufacturer’s instructions. Real-time qPCR procedures were conducted according to a previous study (Li et al., 2021). The relative expressed levels were analyzed using the cycle threshold (Ct) $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). All experiments were repeated three times. Apple *ACTIN* gene was used for normalization (Zhang et al., 2021).

Statistical analysis

All data were statistically analyzed with Excel and SPSS 25.0 (SPSS, Chicago, IL, USA) software. Significant differences between treatments were distinguished by the student’s t-test comparison at $P < 0.05$ and $P < 0.01$, considering each time point separately. Figures were made using Origin 2018 software.

Results

Effect of ABA treatment on root morphology

Four fundamental parameters (root number, root length, root surface area, and root volume) were measured to determine the effects of ABA treatment on root development in hydroponic conditions. It was observed that the 5 μM ABA application inhibited the root growth, especially LR development (Fig. 1a). Compared with control, ABA-treated plants showed a lower average root number per plant during the whole experiment (Fig. 1b). On the contrary, the root number of the control plants increased and peaked at 10 d (Fig. 1b) in control. At 10 d, 15 d, and 20 d during ABA treatment, the root length, root surface area, and root volume in control plants were significantly higher than those in ABA-treated plants (Fig. 1c, 1d, and 1e). As the absorption area of the root system is related to the diameter, according to Mao et al., (2020), roots were classified into four types based on their diameters: <0.5 mm, 0.5-2.0 mm, 2.0–5.0 mm, and > 5.0 mm. The root length, root surface area, and root volume of the control and ABA-treated plants are listed in Table 1. Fine roots (root diameter < 0.2 mm) can confer greater nutrient uptake per unit root mass (Wang et al., 2006), those root parameters of fine root were significantly lower in ABA-treated roots than in control roots at 10 d, 15 d, and 20 d (Table 1).

Table 1
Root length, root surface area, and root volume in control and ABA-treated 'Qingzhen 1' apple plants.

Treatments	The length of different diameters (cm)				The surface area of different diameters (cm^2)				The volume of different diameters (cm^3)			
	0.0-0.5 mm	0.5-2.0 mm	2.0–5.0 mm	> 5.0 mm	0.0-0.5 mm	0.5-2.0 mm	2.0–5.0 mm	> 5.0 mm	0.0-0.5 mm	0.5-2.0 mm	2.0–5.0 mm	> 5.0 mm
CK-0 d	415.68 ± 23.65	262.21 ± 19.27	26.95 ± 34.18	2.01 ± 0.12	28.41 ± 1.22	56.62 ± 3.20	20.67 ± 1.50	3.86 ± 0.11	0.21 ± 0.01	2.60 ± 0.10	1.66 ± 0.05	0.36 ± 0.04
CK-5 d	573.64 ± 17.23	209.51 ± 23.50	25.51 ± 17.34	2.14 ± 0.20	44.71 ± 2.26	58.69 ± 2.86	21.78 ± 2.08	3.75 ± 0.27	0.34 ± 0.03	2.52 ± 0.10	1.56 ± 0.08	0.53 ± 0.02
CK-10 d	559.23 ± 45.87	248.68 ± 12.28	27.06 ± 16.32	1.78 ± 0.09	41.34 ± 1.05	68.11 ± 1.98	22.46 ± 2.15	3.76 ± 0.12	0.32 ± 0.01	2.75 ± 0.12	1.56 ± 0.03	0.41 ± 0.01
CK-15 d	560.13 ± 24.32	291.94 ± 23.16	24.85 ± 21.67	1.86 ± 0.08	41.91 ± 0.89	78.78 ± 3.67	20.22 ± 2.38	3.77 ± 0.25	0.31 ± 0.01	2.96 ± 0.09	1.36 ± 0.04	0.43 ± 0.03
CK-20 d	612.78 ± 31.98	313.78 ± 29.83	23.61 ± 13.34	1.58 ± 0.12	43.10 ± 1.32	83.07 ± 2.95	19.94 ± 1.29	3.78 ± 0.91	0.31 ± 0.03	1.99 ± 0.08	1.40 ± 0.02	0.47 ± 0.01
ABA-0 d	427.78 ± 27.20	266.56 ± 16.23	28.84 ± 16.43	2.10 ± 0.16	29.23 ± 2.47	58.37 ± 1.06	23.07 ± 1.68	3.79 ± 0.24	0.21 ± 0.01	2.66 ± 0.01	1.54 ± 0.03	0.39 ± 0.02
ABA-5 d	436.68 ± 32.78	212.27 ± 15.43	31.54 ± 23.35	4.48 ± 0.32	31.45 ± 1.49	57.21 ± 1.65	27.24 ± 2.46	3.80 ± 0.17	0.19 ± 0.02	1.61 ± 0.13	1.97 ± 0.01	1.32 ± 0.05
ABA-10 d	426.82 ± 30.45	177.07 ± 9.66	23.79 ± 15.21	3.04 ± 0.15	29.11 ± 1.34	49.51 ± 0.81	20.40 ± 1.78	3.81 ± 0.13	0.21 ± 0.01	1.30 ± 0.09	1.45 ± 0.03	1.06 ± 0.06
ABA-15 d	494.92 ± 43.11	210.44 ± 15.98	25.83 ± 10.22	2.89 ± 0.12	35.68 ± 0.76	56.67 ± 2.17	21.97 ± 1.94	3.82 ± 0.20	0.24 ± 0.02	1.43 ± 0.08	1.56 ± 0.02	0.86 ± 0.05
ABA-20 d	480.65 ± 12.39	185.33 ± 17.12	26.56 ± 17.45	4.21 ± 0.11	32.64 ± 0.19	51.49 ± 5.22	22.81 ± 1.88	3.83 ± 0.19	0.23 ± 0.03	1.35 ± 0.02	1.64 ± 0.06	1.13 ± 0.04

Roots were classified into four different size groups based on their diameter; <0.5 mm, 0.5–2.0 mm, 2.0-5.0mm, > 5.0 mm. Values are the means ± SD of three biological replicates

Effect of ABA treatment on plant height and stem diameter

The formation of the root system keeps pace with the development of the shoot (Wang et al., 2006). To explore this phenomenon, the plant height and stem diameter of ABA-treated and control plants were measured. The result showed that the plant height of control plants was significantly higher than ABA-treated plants at 10 d, 15 d, and 20 d (Fig. 2), the stem diameter of control plants was higher than ABA-treated plants at 20 d (Fig. 2). It can be concluded that 5 μM ABA application inhibited the shoot development, which was consistent with the inhibition to roots (Fig. 1 and Table 1).

Effect of ABA treatment on phytohormones

Phytohormones are small chemicals that play a crucial role in plant growth and development. Here, we measured the dynamic changes of endogenous hormones in LR samples, including IAA, ABA, ZR, JA, GA_3, and GA_4, to verify how they

interact with ABA and affect root development (Fig. 3). Results showed that the ABA concentration in ABA-treated plants was 3–4 times significantly higher than the control plants during treatment. The concentration of IAA and ZR were significantly lower in ABA treated plants compared with those in the control at 5 d and 10 d, but then were subsequently higher at 20 d. The concentration of JA was lower in ABA treated plants than in the control plants at all time points. In addition, the data indicated, GA_4 and GA_3 showed the same change trend in control and treatment. GA_4 concentrations in both treatments decreased after arising, and significant differences found between with control were higher at 5 d and 10 d. GA_3 showed an obvious downward trend, and no significant differences were detected between the ABA-treated and control plants, irrespective of sampling time (Fig. 3).

Moreover, previous studies have shown that higher ratios of IAA/ZR, IAA/ABA, and ABA/GA_{1+3} improve root growth (Mao et al., 2020). In our results, (Fig. 3), The IAA/ZR ratio was significantly lower in the ABA-treated plants at 10 d and 15 d than in control. The IAA/ABA ratio was lower, while the ABA/GA_3 ratio was significantly higher in the ABA-treated plants compared to the control plants at all sampling time points. This result indicated that exogenous ABA might inhibit LR development by changing the level of endogenous hormones, especially reducing the concentration of auxin.

Effect of ABA treatment on the expression of ABA-related genes

The relative expressions of genes involved in ABA synthesis, metabolism, and signal transduction related genes in ABA-treated and the control were observed. The expression of *MdNCED3* in the ABA-treated plants was significantly lower than untreated plants at 5 d and 10 d. The expression of *MdAAO4* behaved similarly in both ABA-treated and untreated plants, except for a significantly lower expression at 5 d in ABA-treated plants. The expression of *MdCYP707A2* was almost 10 times higher in ABA-treated samples than in control at 5 d. *MdABI1*, *MdAREB2*, and *MdABF3* were higher in ABA-treated samples than in control at most sampling time points (Fig. 4). Taken together, these results indicated that the plant responds to exogenous ABA application by rapidly reducing the synthesis of endogenous ABA, promoting ABA metabolism, and enhancing the ABA signal output.

Effect of ABA treatment on the expression of IAA-related genes

The expression of genes related to IAA synthesis, transport, and signal transduction was measured (Fig. 5). The relative expressions of *MdYUCCA3* and *MdYUCCA8* in the ABA-treated plants were significantly lower at 5 d while they were greater at 10 d, 15 d, and 20 d than in the control. Compared to the control, the expression of *MdPIN1, MdPIN2, MdPIN3* in the ABA-treated plants was lower at all sample time. Moreover, the relative expression levels of *MdIAA3*, *MdIAA14*, *MdARF7*, and *MdARF19* were repressed at 5 d and 10 d since ABA application. These results indicated that exogenous ABA application significantly repressed the expression of IAA synthesis, transport, and signal transduction-related genes at the early stages of treatment, while the up-regulation of those genes later may contribute to adaptive adjustments of the ABA-treated plants.

Effect of ABA treatment on the expression of genes related to LR development and cell cycle

ABA application inhibited the LR development, so the expression of LR development and cell cycle-related genes was examined (Fig. 6). Exogenous application of ABA resulted in a notable decrease in the expression of *MdWOX5* and *MdWOX11* expression at 5 and10 d (Fig. 6). No significant differences were found between both groups for the relative expression of *MdLBD16* and *MdLBD29* at 5 d. however, the expressions of *MdLBD16* and *MdLBD29* were significantly higher in ABA-treated plants than the control plants at 10 d, 15 d, and 20 d (Fig. 6). Moreover, the relative expression of *MdSHR* was significantly lower at 5 d and higher at 15 d in ABA-treated plants than in control (Fig. 6). In general, the relative expressions of root development related genes in the ABA-treated plants were lower than the control group at the early stages of development.

Moreover, the relative expression level of *MdCYCP1;1* and *MdCYCP4;1* in ABA-treated plants was lower than in control at all sampling points. The relative expression level of *MdCYCD1;1 and MdCYCD3;1* was significantly lower in ABA-treated plants

than in the control at 5 d; however, these genes up-regulated state was observed in one or two time point of 10 d, 15 d, and 20 d (Fig. 6). This result is similar to the expression pattern of genes related to root development.

Discussion

ABA plays a central role in plant response to various stresses. It is also involved seed development, root growth, and stomatal aperture in higher plants (Yu et al., 2020). In our study, exogenous application of ABA clearly showed that the root number, root length, root surface area, and root volume in ABA-treated plants were lower than those of the control, especially, inhibited the growth of the fine roots (Fig. 1 and Table 1). Coincided with the root, the height and diameter of shoot in ABA-treated plants were also lower than control (Fig. 2). The decrease of effective absorbing roots may be the direct factor that inhibits the growth and thickening of shoots. These data suggest that exogenous ABA inhibited root and shoot development of 'Qingzhen 1'apple plants. Similarly, the inhibition of ABA on root and shoot was consistent with those previous studies ((Guo et al., 2009) for *Arachis hypogaea* L; (Lu et al., 2019) for maize; (Sharp and LeNoble, 2002) for tomato). Thus, ABA seems to inhibited root and shoot and those morphological response to ABA appeared robust and stable regardless of the species.

In general, the development of LR system is controlled primarily by auxin, but it is ultimately the result of the joint action of various hormones. Our results showed that exogenous ABA treatment decreases IAA concentrations at the early stage of ABA application (Fig. 3).This result leads to the inhibition of LR development in apple plants and may represent a common physiological response in roots (Casimiro et al., 2003). Our data also indicate that the levels of ABA were significantly higher in ABA-treated plants, which may take an inhibitory effect on LR formation (Guo et al., 2009). In addition, the endogenous concentrations of ZR decreased in ABA-treated plants (Fig. 3). The decreased ZR perhaps limited cell division and reduced the number of LRs, which are in accordance with the previous report (Rani Debi et al., 2005). Studies have demonstrated that a higher IAA/ABA ratio results in the induction of root initiation (Zheng et al., 1999). Consistently, the lower IAA/ABA ratio in ABA-treated plants at all sampling time points was observed in this study (Fig. 3), which may repress the LR initiation. Those results suggest that exogenous ABA treatment inhibits LR development by changing the status of endogenous hormones.

Studies have shown that the process of LR initiation and development is auxin-dependent. For example, *PIN* proteins regulate root growth and development by alert the auxin polar transport (Grieneisen et al., 2007), and *IAA14* inactivates *ARF7* and *ARF19* to block LR formation (Fukaki et al., 2005). Lots of evidence suggests that there is an integration between ABA and auxin signaling pathways in LR developmental process. Such as, overexpression of ABA-insensitive 4 (*ABI4*) impairs LR development by reducing the expression of the auxin-efflux transporter *PIN1* (Shkolnik-Inbar and Bar-Zvi, 2010); the maize *VIVIPAROUS1* (*VP1*) and its Arabidopsis ortholog *ABI3*, which encodes a transcription factor involved in ABA signaling, are auxin-inducible (Suzuki et al., 2003; Brady et al., 2003). To be consistent with these reports (Fig. 4 and Fig. 5), ABA suppressed the transcription of *MdPIN1*, *MdPIN2*, *MdPIN3*, *MdARF7*, and *MdARF19* in our results. Meanwhile, auxin biosynthesis genes (*MdYUCCA3* and *MdYUCCA6*) also exhibited downregulation in response to the ABA treatment (Fig. 5). The downregulation of auxin-related genes implies a decrease in auxin content. Actually, the growth reduction of LR was accompanied by a notable drop of endogenous auxin level in ABA treated apple rootstock (Fig. 1, Fig. 3, and Table 1), which further confirms the interaction between ABA and auxin on the regulation of LR development in plants.

The effect of ABA and auxin on LR development was at least partly implemented through the regulation of the related genes expression. Reports have shown that *ARF7* and *ARF19* regulate LR formation by directly activating *LBD16* and *LBD29* (Wilmoth et al., 2005; Porco et al., 2016). *WOX11/12* promotes the expression of *MdLBD1*6 and *MdLBD29* (Liu et al., 2014). *WOX5* and *LBD29* have also been reported to regulate cell cycle genes, which promote LR initiation and were repressed by ABA (Feng et al., 2012; Forzani et al., 2014; Vergara et al., 2017). In the results of this study, the expressions of *MdWOX5*, *MdWOX11*, *MdLBD16*, *MdLBD29*, *MdCYCD1;1*, *MdCYCD3;1*, *MdCYCDP1;1*, and *MdCYCP4;1* were collectively downregulated at the early stage of ABA application (Fig. 6), which coincided with the quiescence of LR development. Interestingly, we

noticed that the expression of those genes was elevated at the last stage of ABA treatment, which were accompanied with a slight alleviation of root growth inhibition (Fig. 1). This might be a self-adapting adjustment to stress of plants.

In general, the root system architecture is a comprehensive result of complicated crosstalk between developmental and environmental signals. Under various stress, ABA is the most drastic accumulated plant hormone in responding to environmental stimuli. To a certain extent, exogenous ABA can be regarded as a kind of environmental stress. The data presented in this study provides some evidence for the potential involvement of specific genes and pathways in ABA-mediated inhibition of LR development. However, some problems demanding a prompt solution, including the mechanisms associated with crosstalk between ABA and other hormones and the mechanisms of endodermal ABA signaling that promotes LR quiescence. This will be crucial to clarify the tolerance mechanism and improve tolerance breeding of apple rootstock.

Conclusion

This study explains the mechanism that caused inhibition of LR development in apple rootstock under exogenous ABA supply. It was demonstrated that 5 μM ABA application inhibited root and shoot growth in ‘Qingzhen 1’ apple plants by reducing the endogenous IAA, ZR, JA concentrations in LR samples. In addition, an antagonistic relationship between ABA and IAA was observed, ABA reduced the concentration of auxin and downregulated the expression of IAA-related genes, which repressed root development-related genes and cell cycle-related genes expression ultimately resulted in the inhibition of LR development. The finding of the key genes regulating auxin synthesis and transport by ABA has a certain guiding significance. However, the relationship between ABA and other hormones on LR development is still unclear in apple rootstock. Further understanding the regulation mechanism of ABA on LR development is essential for improve tolerance breeding of apple rootstock.

Declarations

Author contributions

Xiaoyun Zhang and Muhammad Mobeen Tahir are the main authors. Dong Zhang and JianXin Niu participated in the experimental design. Xiaoyun Zhang, Muhammad Mobeen Tahir, Shaohuan Li, Ting Tang, Jiangping Mao, and Ke Li conducted experiments. Dong Zhang, Weiwei Yang and Yun Shao revised the article. Xiaoyun Zhang and Muhammad Mobeen Tahir participated in writing and were approved for publication. All the authors read and approved the manuscript for publication.

Competing interest

The authors declare that they have no competing interests.

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Figures

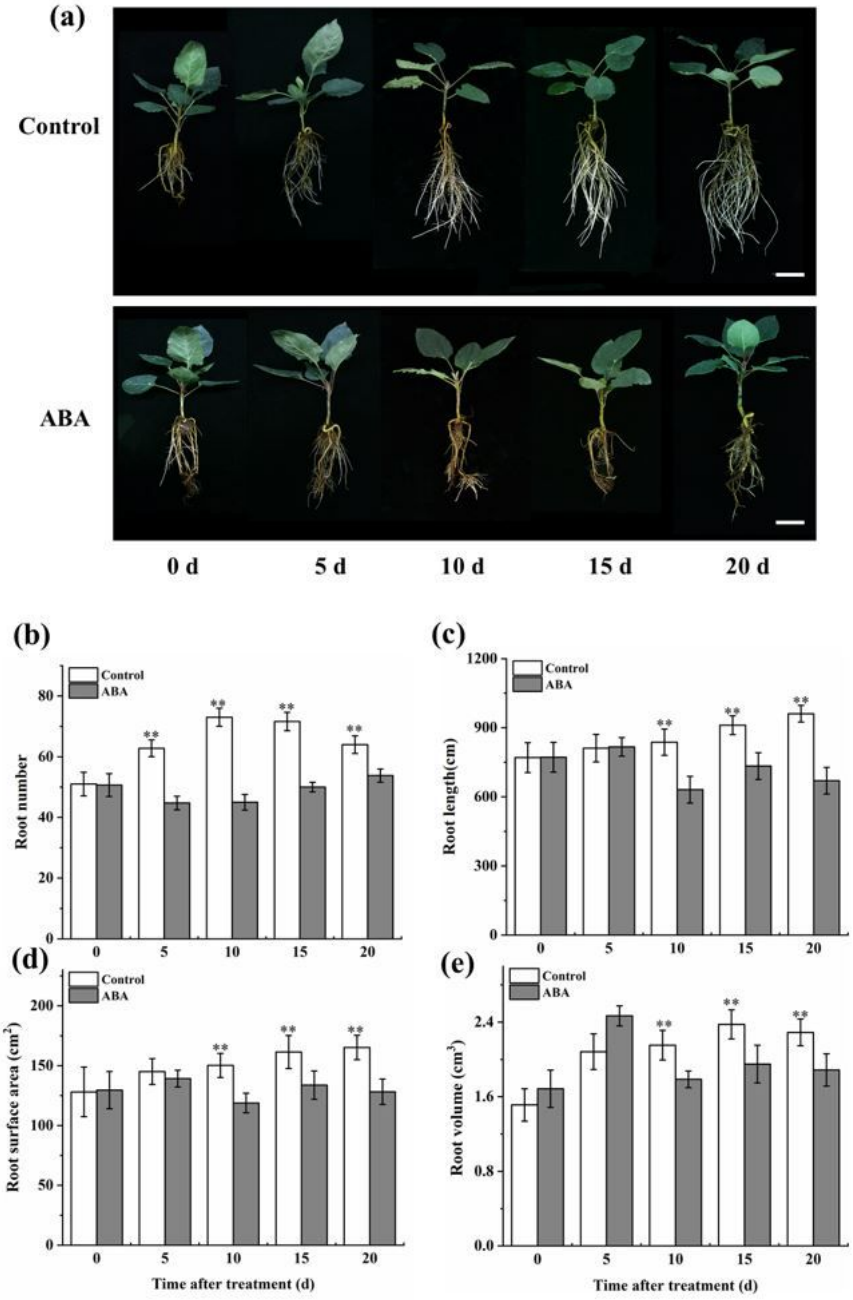

Figure 1

Effect of the exogenous application of 5 μM abscisic acid (ABA) on hydroponically grown 'Qingzhen 1' apple. (a) Control was cultured in 1/2-strength Hoagland nutrient solution without ABA for 20 d; ABA was treated 5 μM ABA for 20 d. (b) root number per plant, (c) root length per plant, (d) root surface area per plant, and (e) root volume per plant. Different asterisks indicate a significant difference at *p < 0.05 and **p < 0.01 using Student's t-test. Scale bar: 3.5 cm

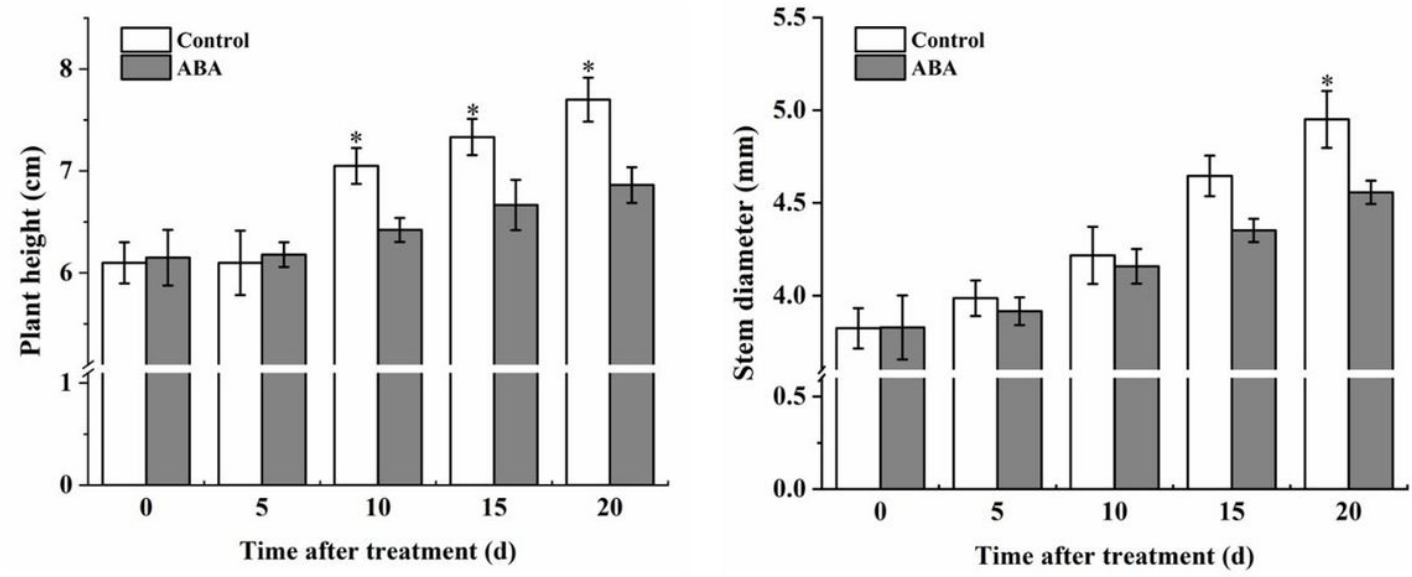

Figure 2

Effect of exogenous application of 5 μM ABA on plant height and stem diameter of hydroponically grown ‘Qingzhen 1’ plants.

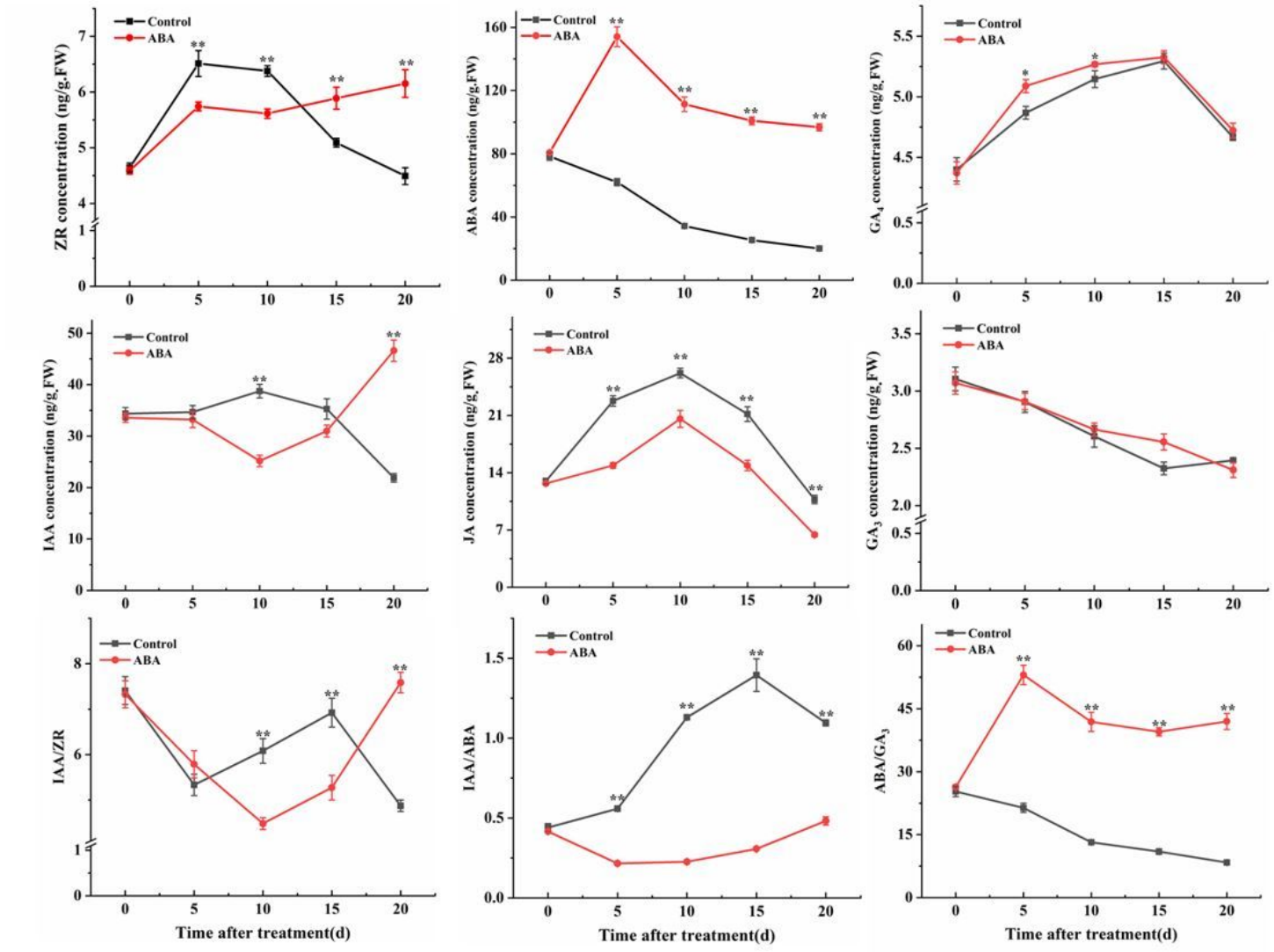

Figure 3

Effect of the exogenous application of 5 μM ABA on the concentration of ZR, IAA, ABA, GA3, GA4, and JA, and the ratios of IAA/ ZR, IAA/ABA, and ABA/GA3 in lateral roots of ‘Qinzheng 1’ plants.

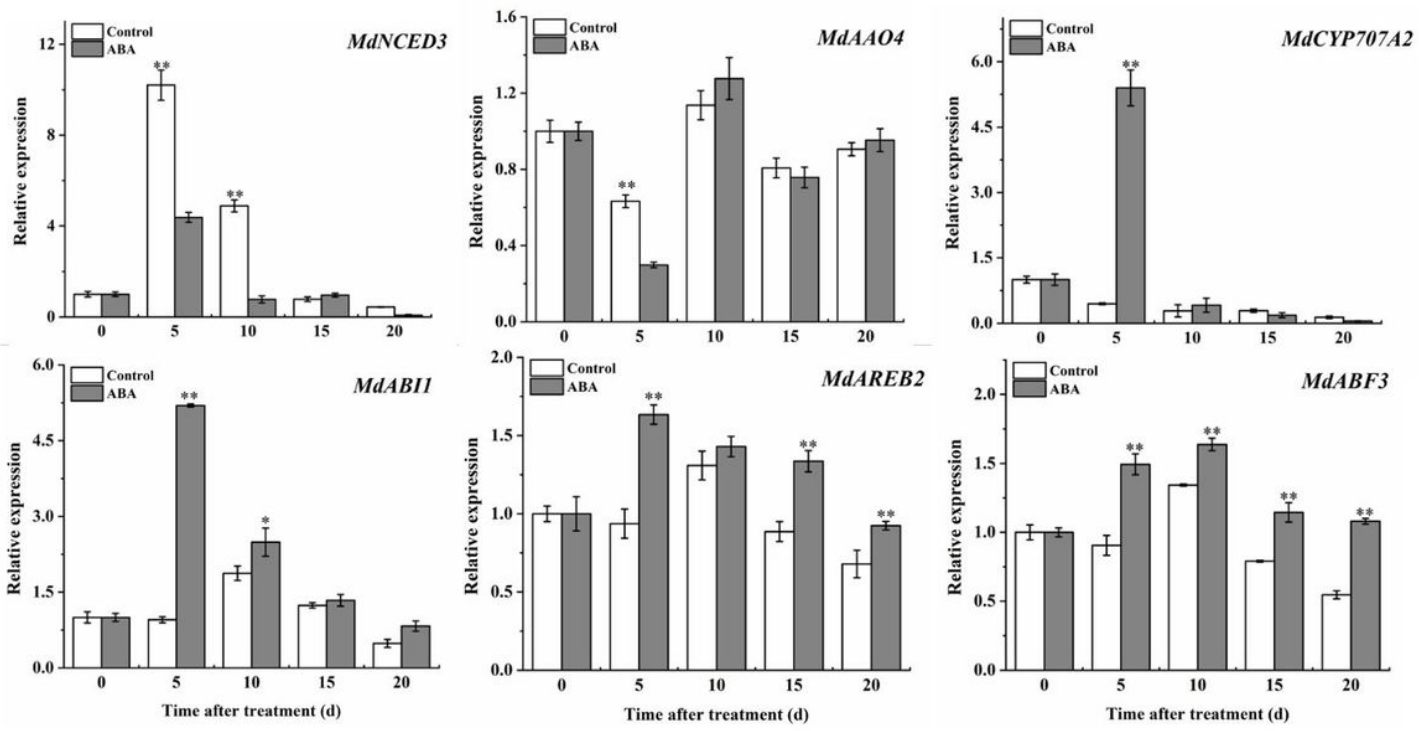

Figure 4

Effect of exogenous application of 5 μM ABA on the relative expression of ABA synthesis, metabolism, and response-related genes in lateral roots of ‘Qinzheng 1’ plants.

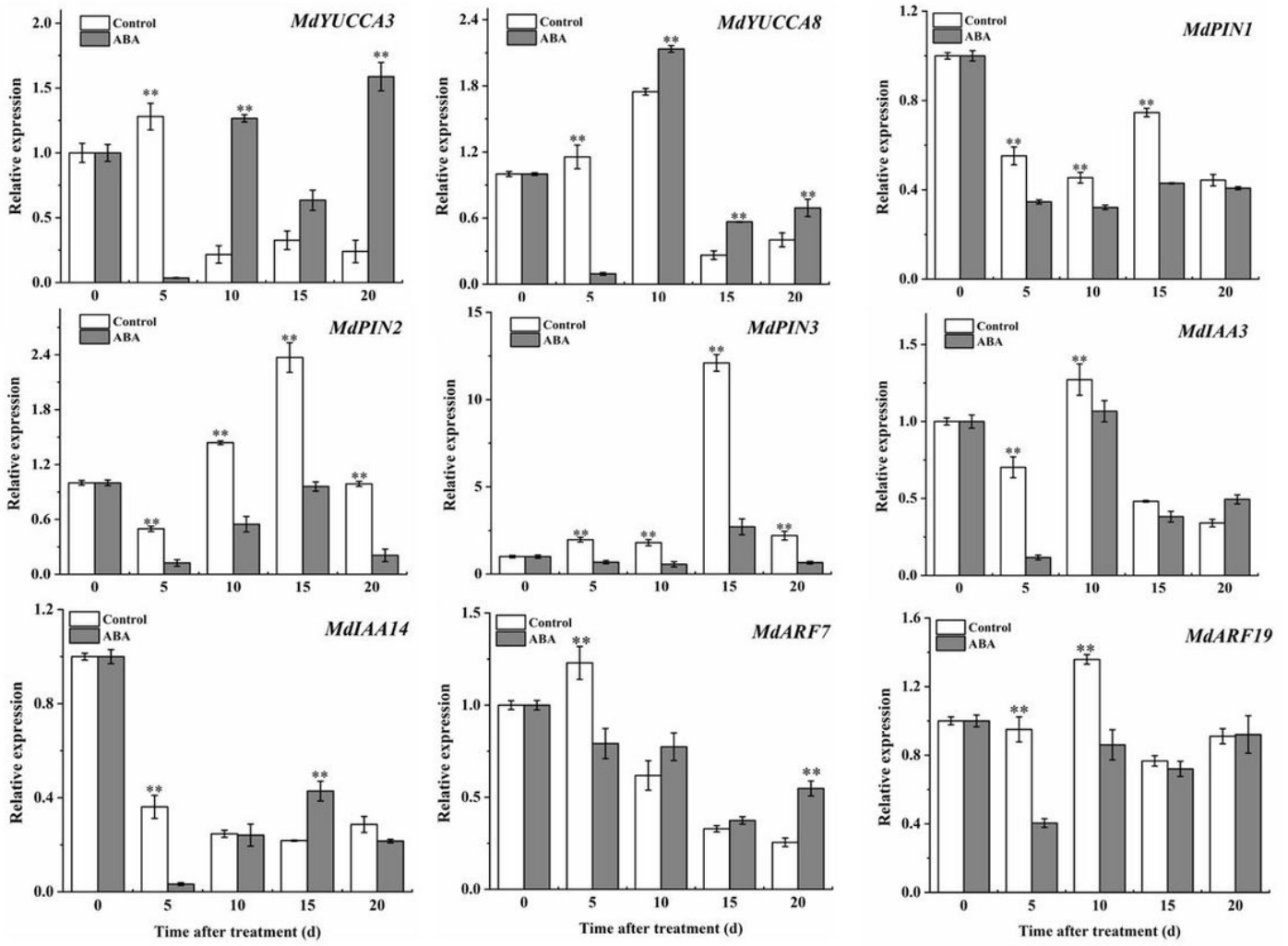

Figure 5

Effect of exogenous application of 5 μM ABA on the relative expression of IAA synthesis, transportation, and signal-related genes in lateral roots of ‘Qinzheng 1’ plants.

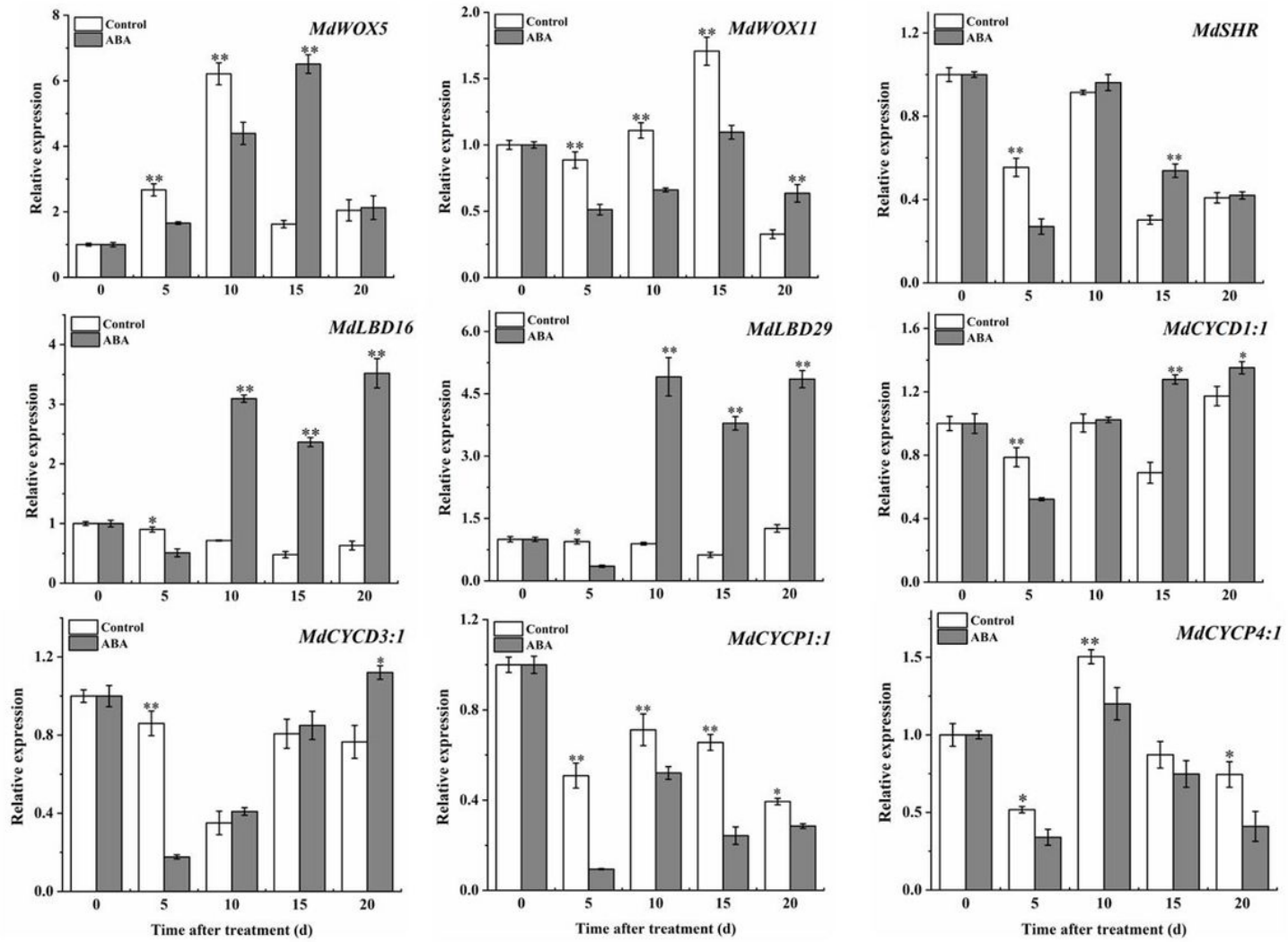

Figure 6

Effect of exogenous application of 5 μM ABA on the relative expression of lateral root development-related genes, and cell cycle-related genes in lateral roots of ‘Qinzheng 1’ plants.

Supplementary Files

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- Supplementarytables.docx