ABA regulation of root growth during soil drying and recovery can involve auxin response

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Running head: ABA/IAA mediated adaptation of roots to drought

Abstract

Abscisic acid (ABA) plays the important roles in plant adaptation to water deficits, but its role in regulating root growth (primary root elongation and lateral root number) during different drought-phases remains unclear. Here, we exposed wild-type (WT) and ABA-deficient (*not*) tomato plants to three continuous drought-phases (moderate drying: day 0-21; severe drying: day 22-47; re-watering: day 48-51). It was found that WT increased primary root growth during moderate drying; maintained more lateral roots, and greater primary root and total root length under severe drying; and produced more roots after re-watering. After RNA-Seq analysis, we found that the auxin-related genes in root showed different expression patterns between WT and *not* under drying or re-watering. Further, exogenous supply of IAA partially recovered the root growth of ABA-deficient *not* plants under three continuous drought-phases. Our results suggested that ABA regulation of tomato root growth during soil drying and recovery can involve auxin response.

KEY WORDS: ABA, auxin, drought responses, gene expression, root

1 INTRODUCTION

Drought is responsible for around 40% of crop losses in some agricultural regions and decreases crop yields (Pathan, T., Subudhi, & B., 2004). Roots are often the first organ to respond to environmental fluctuations (Xing, Zhao, Gao, Xiang, & Zhu, 2016) and changes in root architecture can alleviate the effects of various stresses on plants. Roots typically proliferate in moist and nutrient-rich soil while avoiding drought (Galvan-Ampudia et al., 2013). Water deficit usually limits shoot growth more than root growth (R. E. Sharp et al., 2004) and may increase root growth rate compared to well-watered plants (Shaheen, Riaz, & Zafar, 2016). In the field, increased root production was observed when tomato plants were exposed to moderate water deficits (Reid & Renquist, 1997). However, severe water deficit restricts root elongation (Fang & Xiong, 2015). Despite the methodological challenges of measuring root growth *in situ*, the regulation of root architectural changes in response to water deficit has attracted considerable attention.

Drought-induced changes in the accumulation of, and response to, phytohormones mediates changes in crop growth, development and reproduction, including root architecture. Among these hormones, abscisic acid (ABA) has been regarded as most closely related to drought stress (J. Zhang, Jia, Yang, & Ismail, 2006), since ABA accumulates throughout the plant especially in the leaf tissue. ABA alters plant physiological processes by influencing gene expression, which further enables plants to adapt to various conditions (Quach et al., 2014; K. Yamaguchi-Shinozaki & Shinozaki, 2006). A full ABA response in terms of developmental changes requires auxin signaling components (Emenecker & Strader, 2020; Sarah et al., 2018), suggesting ABA is able to integrate auxin signaling to modulate plant performance. Indole-3-acetic acid (IAA), the main auxin in higher plants, is an essential hormone that modulates plant cell division, elongation and differentiation thus controlling almost every aspect of plant growth and development, including lateral root formation and elongation (Perrot-Rechenmann, 2010). In Arabidopsis, *MYB96*-mediated ABA signals are coordinated with IAA signaling pathway including *GH3* genes encoding IAA-amido synthetases that conjugate excess IAA to amino acids. The *MYB96*-knockout mutant produced additional lateral roots and was more susceptible to drought stress, while *MYB96*-overexpressed lines showed enhanced drought resistance (Seo, Xiang, Qiao, Park, & Park, 2009). In rice (*Oryza sativa*), exogenous ABA induced root expression of IAA biosynthesis and efflux genes including *YUC* and *PIN*, suggesting that ABA determines IAA homeostasis through controlling IAA-related gene expression (F. Y. Zhao et al., 2015). Furthermore, ABA can inhibit IAA-mediated lateral root primordia of peanut (*Arachis hypogaea*) by decreasing AUX-dependent auxin transport (Guo et al., 2012), suggesting crosstalk between ABA and IAA signaling pathways. However, many of these gene expression studies have supplied ABA to well-watered plants, or simulated drought by imposing an osmotic stress (C. Li et al., 2019; Rowe, Topping, Liu, & Lindsey, 2016), and it is uncertain

whether this crosstalk regulates plant response to drying soil when substrate water potential decreases and soil strength increases simultaneously (Jin et al., 2013).

Experiments with ABA-deficient mutants have demonstrated that ABA is essential to maintain root growth in both well-watered and drying soil (Fang & Xiong, 2015). ABA-deficient mutants of *Arabidopsis* (*nced3*) and tomato (*not* and *flc*) had longer and more numerous lateral roots when grown *in vitro* without osmotic stress (Belimov et al., 2014; Guo, Liang, & Li, 2009). Osmotic stress (75 mM mannitol applied to MS agar plates) inhibited lateral root length of *Arabidopsis*, but this response was attenuated in the ABA biosynthetic mutant *aba2-1* (Xiong, Wang, Mao, & Koczan, 2006). When grown in both loose and compact soil, *not* had shorter root length, depth and diameter and fewer lateral roots than its WT, revealing that endogenous ABA positively regulates root growth (Tracy, Black, Roberts, Dodd, & Mooney, 2015). Furthermore, reciprocal grafting between wild-type and ABA-deficient mutants demonstrated that translocation of shoot-derived ABA promoted adventitious root growth under well-watered conditions (S. A. McAdam, Brodribb, & Ross, 2016). Localized root ABA accumulation is also essential to maintain root elongation in drying soil, as decreasing ABA concentrations chemically (using the inhibitor fluridone) or genetically (the ABA-deficient maize mutants *vp5* and *vp14*) decreased maize (*Zea mays*) primary root elongation (Robert E Sharp & LeNoble, 2002; Robert E Sharp, Wu, Voetberg, Saab, & LeNoble, 1994). However, to our knowledge there has been no integrated assessment of how ABA status affects both primary and lateral root growth responses to drying soil and thereafter re-watering.

Understanding how water deficit modulates root architecture is critical to understand plant drought resistance. While previous investigations focused on how discrete changes in soil water status affected root growth of wild-type (WT) plants (Dong et al., 2019; Vander Mijnsbrugge et al., 2016; X. Zhang, Lei, Lai, Zhao, & Song, 2018), plants are often exposed to drying soil followed by re-watering in both natural and cultivated conditions (AghaKouchak, Cheng, Mazdiyasni, & Farahmand, 2014; Dodd et al., 2015). Thus, we progressively exposed WT tomato and its ABA-deficient mutant *notabilis* (*not*) to three phases: moderate drying, severe drying and re-watering. At each phase, we analyzed the root architecture, transcript profiling and conducted gene co-expression network analysis, in aiming to unveil the mechanisms by which ABA regulates root architecture. Also, some plants were treated with exogenous ABA or IAA to test how those phytohormones modulate root traits. We hypothesized that endogenous ABA regulated root growth via auxin-dependent processes.

2 MATERIALS AND METHODS

2.1 Plant material and treatments

Tomato (*Solanum lycopersicum* L. cv Lukullus) seeds and its abscisic acid (ABA)-biosynthesis mutant *notabilis* (*not*) were used. *not* is a null mutation in the gene *NCED1*, encoding a 9-cis-epoxycarotenoid dioxygenase involved in ABA biosynthesis (Burbidge, Grieve, Jackson, Thompson, & Taylor, 2010).

For the pot experiments, surface-sterilized seeds were germinated on wet filter paper. Seven days after germination, homogeneous seedlings with one true leaf were transplanted into PVC columns (height 24 cm and diameter 10 cm) filled with sieved sand (diameter ≤ 0.850 mm) maintained at two different soil water contents (see below) under controlled conditions. During treatment, supplementary LED lighting (LPSW-5050LED-304, low intensity white LED lights, Fujian Luopu Biotechnology Co., Ltd., China) supplied 150 μmol m^{-2} s^{-1} at the canopy height for 16 h day^{-1}, with day/night mean temperature of 24/20℃. Greenhouse humidity averaged 65%. To avoid nutrient deficits, the water-washed and air-dried sand was irrigated with half strength of Hoagland solution. In this study, three completely independent experiments were performed using WT and *not* tomato plants: first experiment without the application of exogenous ABA; second experiment with the application of exogenous ABA; and third experiment with the application of exogenous IAA.

To expose WT and *not* plants to similar soil water conditions, seven-day-old seedlings with one true leaf were transplanted to the same pot (one plant of each genotype in each column). In the duration of the experiment, control (well-watered) plants were grown in sand with a water content of 14% (sand water-holding capacity; water potential is -0.01 MPa), which was maintained by weighing (re-irrigated from top and base to the target weight) every day. Seedlings were also transplanted into sand (5% water content with a -0.68 MPa average water potential in the whole pot), with 3 mL of water applied daily to both treatments (to avoid plant dehydration due to soil evaporation) for one week. For the first 21 days, sand water content was maintained at 5% by weighing (re-irrigated from base to the target weight) every day, before plants were exposed to severe drought for another 26 days, with soil evaporation and plant transpiration decreasing water content to 0.4% (with a -7.04 MPa average water potential in the whole pot) by which time leaves of *not* plants were wilting. Then plants were re-irrigated to a water content of 14% and allowed to recover for 4 days. Plants were harvested after 21 (phase I: moderate drying), 47 (phase II: severe drying) and 51 (phase III: re-watering) days of treatment (Fig. S1). To visualize the distribution of water in sand, the column without plants was opened longitudinally at the end of each phase and the derived cross section of sand was photographed. Then the picture was imported into ImageJ software (v2.5.2, NIH) and processed by the Plugins/Bio-Formats function (Rellan-Alvarez et al., 2015). Sand water distribution in the column was visualized by different image colours, with sand water content manually calibrated according to the actual water content in every 2 cm layer (12 layers from each 24 cm column were determined gravimetrically-Table S1).

2.2 Sand water potential determination

The average sand water potential in the pot was assessed by a Dewpoint PotentiaMeter (WP4-T, Decagon, USA) according the manual. Briefly, WP4-T was allowed a warm-up period of 30 min after turning it on (continuous mode; with sample chamber temperature of 25.0 ℃). Then the potential meter

was calibrated with a serial of KCl standard solution (0.05, 0.20, 0.40, 0.60, 0.80 and 1.00 mol kg^{-1}). Each sand sample (pre-incubated at 4 ℃) was loaded into the plastic cup (sample covers the bottom of the cup, but less than half full) and inserted into the chamber drawer to detect water potential.

2.3 Exogenous supply of abscisic acid or indole-3-acetic acid in pot experiments

To investigate abscisic acid (ABA) or indole-3-acetic acid (IAA) effects on WT and *not* plants during three different phases, a completely independent pot experiment was set up. Seedling transplanting procedure and plant growth period for each phase were the same as described above. For the external hormone addition, 2 mL of 1 μM ABA or 5 nM IAA (dissolved in water) was exogenously applied to roots from the bottom of pot at each time, and an equal volume of water was applied to untreated plants, as indicated in Fig. S1. The concentration of ABA (1 μM) used in this study was selected based on an earlier study (Ghassemian et al., 2000), in which the relative root growth of wild-type *Arabidopsis* peaked when exogenous ABA concentration increase to 1 μM in the agar medium; preliminary experiments established that 5 nM IAA was sufficient to promote lateral root number, whereas 10 nM IAA had no effect on lateral root number (Fig. S2). ABA was re-applied at 6 and 7-day intervals during the soil drying and severe drought phases of the experiment, as indicated by arrows (Fig. S1). After re-watering, ABA solution was applied once to roots on the first day of this phase. IAA was re-applied at 3-day intervals throughout the experiment. Plant sampling and root morphological analysis used the same procedure as described above.

2.4 Analysis of plant morphological traits

The sampled shoots were oven-dried at 70°C for 72 h, and the dry weight of each sample was measured. Roots grown in the sand medium were carefully washed out over a sieve with a mesh size of 2 mm, then three roots were oven-dried to determine the dry weight and other roots were scanned in water with a flatbed scanner (Epson Perfection V700 Photo, SEIKO EPSON CORP., Japan). Subsequently, the images were analysed using the software WinRHIZO™ Reg 2016a according to the manufacturer's instructions (Régent Instruments Inc., Canada).

2.5 Abscisic acid quantification

At the end of each phase, the entire roots from pot experiments (with or without the addition of ABA treatment) were sampled for abscisic acid (ABA) determination. For each sample, 0.1 g fresh root was prepared and homogenized in cold buffer (methanol : H_2O : acetic acid = 80 : 20 : 1, v/v/v), after purification by petroleum ether and ethyl acetate, abscisic acid (ABA) was quantified by HPLC (Rigol L3000, RIGOL Technologies, Inc. China) with a reverse-phase C18 Kromasil HPLC column (250 mm × 4.6 mm, 5 μm). The mobile phase consists of an equal volume mixture of methanol and 1% acetic

acid (1: 1, v/v), at a flow rate of 0.8 mL min^{-1}, column temperature at 35°C. Excitation and emission wavelengths were set at 254 nm and 360 nm, respectively. The amount of ABA in the sample was calculated from peak area. The peak area (A_{peak}) at retention time of 10.76 min indicating ABA was quantified, and subsequently ABA concentration (C_{ABA}) in 0.1 g root was calculated against with the linear equation ($C_{ABA} = [A_{peak} + 1.0627] / 59.695$; $R^2 = 0.9997$) from the calibration curve prepared by a serial of ABA standards. To validate the accuracy of HPLC-detected ABA in this study, the ABA concentration of root samples without addition of exogenous ABA treatment was also analyzed by a radio-immunoassay (Quarrie et al. 1988).

2.6 RNA- sequencing and data processing

Total RNA of the entire root, collected from plants at the end of each growth phase, was isolated by the TRIzol® Reagent RNA preparation method (Invitrogen). RNA-sequencing (RNA-Seq) was performed on the BGISEQ-500 platform. Data processing of RNA-Seq experiments raw data in the fastq format, clean reads were obtained by removing reads containing adapter, unknown bases (N) and low-quality reads from raw data (Chen et al., 2018). All the downstream analyses were based on clean data with high quality. The clean reads were mapped to the reference genome (https://www.ncbi.nlm.nih.gov/genome/7?genome_assembly_id=393272) with an average mapping ratio of 94% using HISAT2 (D. Kim, Langmead, & Salzberg, 2015), and then the fragments per kilobase of transcript per million mapped reads (FPKM) were calculated using RSEM (B. Li & Dewey, 2011). The differential gene of RNA-Seq experiments was determined using DESeq (Wang et al., 2010). The resulting *P* values (negative binomial distribution) were adjusted using Benjamini and Hochberg's approach to control the false discovery rate (FDR). Genes with $|\log_2 FC \text{ (Fold change)}| > 1$ and false discovery rate (FDR) value < 0.001 were defined as differentially expressed. The expression dynamics of genes used in this study were visualized using the "ComplexHeatmap" R package. Gene ontology enrichment analysis of the differentially expressed genes (DEGs) was performed using the DESeq (2012) R package based on the hypergeometric distribution. After filtering the low-expressed DEGs, a co-expression network for the remained 5,500 genes was analyzed by weighted gene co-expression network analysis (WGCNA) according to the previous study (Langfelder & Horvath, 2008). The generated co-expression networks were visualized by Cytoscape, and hub genes were identified using the CytoHubba application in Cytoscape (Chin et al., 2014).

2.7 Statistical analysis

Three-way ANOVA (with main factors of experimental phase, genotype and watering treatment) determined treatment effects, with Duncan's multiple range test used to discriminate means across all experimental phases, with all analyses conducted using SPSS (v25). Unless stated otherwise, a statistical significance level of $P < 0.05$ was used.

2.8 Data Availability

The RNA-Seq data have been deposited to the NCBI sequence read archive (SRA) under accession number PRJNA670031. All other data supporting the findings of this study are available within the article and its supplement.

3 RESULTS

3.1 Morphological traits in the three different phases

WT and *not* plants were grown in the same pot to ensure roots of both genotypes were exposed to similar sand moisture (Figs. 1A, S1). Sand water content increased from 9% at the top to 18% at the bottom of the pot even in well-watered plants (Table S1). In drying sand, these gradients were magnified, with evaporation and plant transpiration decreasing sand water content at the top of the pot to 1%, with only 1-2 cm of moist sand (10%) remaining at the bottom of the pot at the end of phase I. At the end of the drought (phase II), there was little water throughout the vertical profile, with dry sand (4%) even at the bottom of the pot. Under these conditions, lateral gradients in sand water content were similar (Fig. 1B), indicating the root system of each plant was exposed to similar conditions.

ABA concentrations of well-watered roots increased throughout the experiment (by 31% when averaged across both genotypes), indicating that ABA status depended on plant development. Throughout the experiment, *not* roots had 67% the ABA concentration of WT roots, with moderate drying increasing ABA concentrations by 38% (averaged across both genotypes) in both genotypes (no significant genotype × treatment interaction – Table S2). Re-watering tended to decrease ABA concentrations of WT roots after 4 days (Fig. 1C). The exposure of *not* to moderate drying resulted in it having the same ABA concentrations as well-watered WT plants at the beginning of the experiment. Thus, the two genotypes differed in their root ABA status, but not in how ABA responded to time or drying. The root ABA concentrations of WT were always higher than those of *not* under well-watered conditions, moderate drying, severe drying and re-watering. The ABA concentration from HPLC was validated by the data obtained from radio-immunoassay (Figs. 1C, S3), suggesting that the ABA concentrations detected by HPLC in this study were accurate.

Under well-watered conditions (14% sand water content), shoot biomass increased throughout the experiment (by 85% when averaged across both genotypes). WT and *not* tomato had similar shoot biomass throughout the experiment when grown in well-watered soil (Figs.1, S1). Under moderate drying (day 0-21 with around 5% sand water content), no significant difference was found in shoot biomass between WT and *not* tomato. During severe drying (day 22- 47 with sand water content from 5% to 0.4% and the average sand water potential in the whole pot dropped from -0.68 MPa to -7.04 MPa), the shoot biomass of *not* tomato was significantly lower than that of WT. Leaf relative water content (RWC) of *not* plants was marginally less at each experimental phase (Fig. S4). At the end of

phase I (moderate drying) and phase III (re-watering), RWC did not significantly differ between WT and *not* tomato. However, at the end of phase II (severe drying), RWC of *not* plants was significantly lower than WT plants, as indicated by a significant phase × treatment interaction (Table S2). Thus, based on these results, we set up these experimental phases (well-watered, moderate drying, severe drying, re-watering).

Moderate drying significantly increased PRL (primary root length) of WT by about 20%, but failed to promote PRL of *not* (Fig. 2A), indicating that ABA-mediated regulation of primary root elongation not just depends on soil moisture but also ABA (significant genotype × water interaction – Table S2). Well-watered WT plants had significantly more (by 27% averaged over the experiment) lateral roots than *not* plants. Moderate drying decreased the number of lateral roots (NLR), and magnified this genotypic difference such that *not* plants exposed to drying soil had 55% fewer lateral roots over the experiment.

Similarly, a greater total root length (TRL) from WT plants compared with *not* was observed especially under water-deficient conditions (Figs. 2B, 2C, S5). Compared to well-watered plants, soil drying at phase I largely decreased NLR and TRL of *not* plants (54% and 45%, respectively), and severe drying (at the end of phase II) further decreased NLR and TRL of *not* by 70% and 50%, respectively. Water deficiency at the first two phases also negatively affected on NLR and TRL of WT plants (-25% of NLR and -21% of TRL, averaged across both phases). Re-watering at phase III promoted NLR and TRL of WT but had very limited effects on *not* plants after 4 days (Fig. 2B, 2C).

Taken together, compared to ABA-deficient tomato (*not*), WT increased primary root growth during moderate drying; maintained lateral root number, primary root and total root length under severe drying, and produced more root number and length after re-watering. Further, endogenous ABA status modulated these various root traits (Table S2).

3.2 Transcriptome analysis of tomato roots

To give insight into molecular aspects of tomato roots responding to moderate drying, severe drying and re-watering, the expressions of large-scale genes from the entire roots were quantified by RNA-Sequencing. A total of 24,226 genes were detected in 36 root samples from the three progressive phases (Fig. S6), of them 7,025 differently expressed genes (DEGs, WT vs *not*) were identified (Table S3) and those DEGs were classified by the enrichment analysis of Gene Ontology (GO). The GO terms GO:0005975 (carbohydrate metabolic process), GO:0055114 (oxidation-reduction process) and GO:0042446 (hormone biosynthetic process) were over-represented (Table S4). Severe drying resulted in larger number of different expressed genes in roots when compared with that of well-watered plants (Fig. 3A, left). The gene expressions including those for ABA biosynthesis and response in WT plants tended to increase in response to soil drying, but decreased in *not* plants (Fig. S7). Besides, overall gene

expression level in *not* plants was relatively lower than WT (Fig. 3A right), indicating ABA may promote gene expression regardless of sand water status.

All the DEGs were subjected to TCseq analysis, which generated 6 gene clusters (Fig. S8). Those clusters were further assigned into 3 different expression patterns and visualized by heatmap (Fig. 3B). The genes from each pattern underwent enrichment analysis of GO (Fig. 3C). At phase I (moderate drying), the GO terms GO:0055114 (oxidation-reduction process) and GO:0009628 (response to abiotic stimulus) were over-represented (Table S5). At phase II (severe drying), a total of 45 GO terms, such as GO: GO:0009734 (auxin-activated signalling pathway), GO:0006833 (water transport), GO:0030104 (water homeostasis) and GO:0009992 (cellular water homeostasis), were significantly enriched (Table S6). At phase III (re-watering), the GO terms GO:0006950 (response to stress), GO:0006952 (defense response), GO:0042446 (hormone biosynthetic process), GO:0042445 (hormone metabolic process), GO:0009692 (ethylene metabolic process) and GO:0009693 (ethylene biosynthetic process) were over-represented (Table S7).

To determine specific genes that are highly associated with plant drought-resistance, we identified 20 distinct modules using weighted gene co-expression network analysis (WGCNA), amongst them the magenta module was closely correlated with endogenous ABA (Fig. S9). Expression of genes in the magenta module was depressed in *not* plants especially with soil drying, but recovered with re-watering. By contrast, WT plants maintained relatively stable gene expression throughout the experiment (Fig. S10), indicating that internal ABA level may affect the expression of genes in magenta module under drought. Genes in this module were over-represented in the GO terms GO:0022613 (ribonucleoprotein complex biogenesis) and GO:0090304 (nucleic acid metabolic process), as shown in Table S8. By using CytoHubba application in Cytoscape, it was predicted that four hub genes *NRP2* (NAP1-related protein 2-like), *NOP6* (nucleolar protein 6), *NOC2* (nucleolar complex protein 2) and *CPN60-2* (chaperonin CPN60-2) might regulate *ABI5L* (ABA-responsive element binding factor) and further influences plant response to drought (Fig. 3D; Tables S9, S10).

3.3 Root morphology as affected by exogenous abscisic acid or indole-3-acetic acid

Throughout the experiment, exogenous ABA enhanced root ABA concentration of *not* plants to a similar level to WT plants (Fig. S11). Applying ABA to water-stressed plants (phase I and II) enhanced PRL and NLR of *not* plants to similar levels as WT plants (Fig. 4), largely independent of soil water status (Table S11). Thus ABA addition phenotypically rescued root growth of *not* plants in drying soil, but had no deleterious impact on WT plants.

Differentially expressed genes involved in the indole-3-acetic acid (IAA) pathway were identified in the three phases (Fig. 5). At phase I (moderate drying), most of the IAA-related genes (24 out of 27) had higher expression levels in WT than *not* plants under the same water conditions. At phase II (severe drying), genes involved in IAA synthesis, homeostasis and response were highly activated in WT plants

but further repressed in *not*, indicating an interaction between endogenous ABA and IAA pathway. At phase III (re-watering), the re-supply of water de-repressed more IAA-related genes in *not* plants when compared to WT. Meanwhile, the expression of genes encoding auxin-induced protein and auxin response factor was maintained at a relatively high level.

The impact of IAA on root growth of WT and *not* was assessed by exogenous application of IAA to plant roots. Moderate drying decreased primary root length (PRL) of *not* compared to WT, while IAA addition increased the PRL of *not* to a level similar with WT throughout the experiment (Fig. 6A), independent of soil water status (Table S12). Furthermore, IAA addition increased the number of lateral roots (NLR) and total root length (TRL) of *not* plants at phase I and II. Re-watering (phase III) could not restore NLR and TRL of *not* regardless of exogenous IAA, with 29% fewer lateral roots and 26 % less TRL than WT plants (Fig. 6B, 6C). Thus, IAA addition phenotypically rescued root growth of *not* plants in drying soil, but had no deleterious impact on WT plants.

4 DISCUSSION

4.1 Responses of root to different levels of drought

In the field, plants regularly face periods of soil drying or even extreme drought followed by rainfall, especially as climatic changes result in more frequent occurrences of drought and flooding events (AghaKouchak et al., 2014). In this study, we mimicked the water changes in pot experiment to understand tomato root growth responses to soil drying and thereafter re-watering (Fig. 1). During Phase I (moderate drying), to alleviate impacts of drying from the upper soil, tomato primary root growth was stimulated to reach water in the deeper soil. Similar results were reported in other plants species, moderate drought (with water potential of -0.51 MPa) largely increased Arabidopsis primary root elongation rate compared with well-watered control (-0.10 MPa) (Van der Weele, Spollen, Sharp, & Baskin, 2000), the stimulation was also shown for rice (Y. Kim et al., 2020). The ability of plant to develop deeper root in respond to water limitation was recognized as an important strategy for plant drought resistance (Fang & Xiong, 2015). In contrast, severe drought often decreased root growth rate, as assessed by the restriction of length of partial/whole root system in Arabidopsis or rice (Y. Kim et al., 2020; Van der Weele et al., 2000). The findings coincide with the results from our study, during Phase II (severe drying), a long-term drought restricted tomato root growth but activated several processes including stress hormone metabolism to cope with severe or extreme drought. The activation of hormonal signaling such as ABA and IAA was found to be associated with enhanced plant drought tolerance in tomato and Arabidopsis (Lee et al., 2012; T. Zhao et al., 2021). It is notable that at the end of phase II it has a -7.04 MPa sand water potential (average water potential in the whole pot), which is much lower than the previously proposed wilting point -1.5 MPa (O'Geen, 2013). The reason is that tomato plants were continuously grown in three progressive phases, with the sand gradually dried

during phase II (the upper sand in the pot firstly dried), tomato plants developed deeper roots and could absorb water at the bottom of pot where there was still 3.0-4.4% water (the lower sand between 20 to 24 cm) at the end of severe drying (phase II), even though the upper sand was very dry (Fig. 1B; Table S1). The substantial water uptake by relatively few deeper roots at base of the pot under drought has been evident by previous study of maize (Sharp & Davies, 1979). During Phase III (re-watering), growth resumed following re-watering via producing new lateral roots in tomato plants. The newly formed lateral roots after re-watering could increase the volume of soil reached by root, and thus confer plant a stronger ability to absorb both nutrient and water for a quick recovery (Carvalho & Foulkes, 2018).

4.2 Roles of ABA in root growth adaptation to drought

Comparing root architecture traits and gene expression profiles between wild-type (WT) and the ABA-synthesis mutant (*not*) revealed that low endogenous ABA concentrations profoundly restricted overall gene expression and reshaped root architecture in the three progressive phases, suggesting that ABA is a key regulator of plant drought resistance by modulating gene expression and root architecture. In *not* plants, the lacking of *NCED1* led to a decrease in ABA concentration especially under drought, the less accumulation of ABA subsequently restricted shoot growth partially via affecting water loss from stomatal (Fig. 1). Previous studies unraveled that ABA is predominantly synthesized in leaves in respond to soil drying, the foliar ABA could be transported via the phloem to roots and alters plant root growth (Scott AM McAdam & Brodribb, 2015; S. A. McAdam et al., 2016; Scott AM McAdam, Manzi, Ross, Brodribb, & Gómez-Cadenas, 2016). Deeper roots in soil were recognized as a desirable trait for better acclimation to drought (Fang & Xiong, 2015; Mohamed, Keutgen, Tawfika, & Noga, 2002). During moderate (phase I) and severe drying (phase II), water limitation highly repressed the number of lateral roots and total root length, particular in the ABA-biosynthesis mutant; while it promoted primary root elongation of WT plants. However, other factors may also contribute to the increased root growth under soil drying. Earlier study found that a better aeration caused by partial drying could increase root elongation, the reason is that a better aeration in soil will provide more O_2 for root respiration and thus support the energy demand of root growth toward water (Liang, Zhang, & Wong, 1996). Under well-watered conditions, the primary root length of *not* was similar or slightly greater than WT (Fig. 2), indicating that the effects of endogenous ABA concentrations on root growth depend on soil water availability (Table S2). Similarly, increasing root tip ABA content of well-watered plants greatly inhibited root elongation, but in drying soil chemically (inhibitor) or genetically (*vp5* or *vp14*) decreasing ABA content restricted root growth (Robert E Sharp & LeNoble, 2002; Robert E Sharp et al., 1994). With greater root extension, plants can take up water and water-soluble nutrients from deeper soil and thus maintain turgor, transpiration and photosynthesis for longer under drought, thereby prolong plant survival (Fang & Xiong, 2015; Rellán-Álvarez et al., 2015; Uga et al., 2013). ABA-

mediated root growth promotion under water-limited conditions were confirmed in the second experiment, the application of 1 μM ABA to an ABA-biosynthesis mutant phenotypically rescued root growth under drought (Fig. 4). Similarly, exogenous application of ABA to ABA-deficient mutants (*Arabidopsis thaliana*) rescued the ability of roots to grow towards higher moisture (Takahashi, Goto, Okada, & Takahashi, 2002). Low exogenous ABA doses (100 nM) promotes root growth rate in the wild-type *Arabidopsis* but not *snrk2.2 snrk2.3* (mutant in ABA perception), which was accompanied by an increase in cell division and mature cell length (Dietrich, Pang, Kobayashi, Fozard, & Bennett, 2017). Four days after re-watering, the primary root growth and root branching of *not* tomato were partially restored compared with WT at the end of phase III (re-watering), while the combination of re-watering and ABA application fully recovered those variables to WT levels (Figs. 2, 4), suggesting an indispensable role of ABA in plant drought recovery.

The less accumulated endogenous ABA, in particular under drought, significantly down-regulated thousands of genes in roots (Figs. 1, 2A, 2B). In the comparison with WT plants, the repressed genes in *not* were overrepresented in the gene ontology (GO) terms related to carbohydrate metabolism, oxidation-reduction and response to the abiotic stimulus at phase I (moderate drying); carbohydrate metabolism, auxin-activated signaling pathway, water transport and water homeostasis at phase II (severe drying); regulation of the metabolic process, carbohydrate metabolic, response to stress, defense response, hormone biosynthesis/metabolism and ethylene biosynthesis/metabolism at phase III (re-watering); the activation of auxin-signaling pathway in wild type tomato but not in the ABA-deficient plants under drought suggested the necessity of ABA signaling for the auxin-dependent root growth (Fig. 3C; Tables S4-S6), effects of ABA and IAA interaction on root architecture were also evident by an earlier study that loss-of-function *abi3* Arabidopsis reduced numbers of lateral roots in the presence of auxin (Brady, Sarkar, Bonetta, & McCourt, 2003). Among the enriched biological processes at phase I and II, some of the GO terms agree with earlier findings that mild and severe drought limited metabolic process but activated defense response (Chaves, Maroco, & Pereira, 2003; Fang & Xiong, 2015). A co-expression network from DEGs responsive to drought was analysed by weighted gene co-expression network analysis (WGCNA). Four hub genes including *NRP2* (NAP1-related protein 2-like), *NOP6* (nucleolar protein 6), *NOC2* (nucleolar complex protein 2) and *CPN60-2* (chaperonin CPN60-2) with a potential target gene *ABI5L* (ABA-responsive element binding factor) were predicted by WGCNA (Fig. 3D). ABI5 is a member of basic leucine zipper (bZIP) transcription factor and it has been recognized as a major ABA signaling component. In Arabidopsis, the homologue of *SlABI5* functions as an activator in the expression of ABA-responsive element (ABRE) via the binding specifically to the *LET65*/*RD29B* gene promoter (Uno et al., 2000), which enables plants respond to abiotic stress such as drought (Kazuko Yamaguchi-Shinozaki & Shinozaki, 1993). Among those hub genes, the nucleosome assembly protein-related protein (NRP) was recently evident that it is associated with plant drought tolerance, *NRP1*-overexpressing *Arabidopsis* showed a better drought tolerance than the wild-type and

the *nrp1-1 nrp2-1* mutants through some unknown mechanisms (Barna, Gémes, Domoki, Bernula, & Fehér, 2018). The results raise the possibility that *NRP* likely alter plant drought resistance by modulating *ABI5L*. However, further experiments are needed to validate the interaction between the hub genes and their target.

4.3 Roles of IAA in root growth adaptation to drought

Prolonged soil drying significantly enriched genes involved in auxin-activated signalling pathways (Fig. 3C), suggesting indole-3-acetic acid (IAA) signalling participates in regulating plant drought resistance. Increased drought intensity gradually activated the IAA signalling pathway at the transcriptional level in WT plants, while re-watering decreased the number of highly expressed genes. In contrast, *not* plants showed an opposite response in the three phases (Fig. 5), suggesting the IAA pathway was activated by drought in an ABA-dependent manner, especially under extreme drought conditions. Indeed, drought induced the flavin monooxygenase gene *YUC7* in *Arabidopsis* roots (belonging to IAA biosynthetic pathway) is an ABA-dependent manner (Lee et al., 2012). The plant hormone IAA, a predominant endogenous form of auxin, regulates plant primary and lateral root growth and development by controlling cell division and elongation (Perrot-Rechenmann, 2010; Stefan & Peter, 2016), thus it was presumed that endogenous IAA may contribute to the drought-induced primary root elongation. Besides, elevated IAA also positively correlated with several stress-related gene expressions and antioxidant enzyme activities, which decreased reactive oxygen species and thus enhanced drought tolerance (Kim et al., 2013; Shi et al., 2014). Re-watering partially eliminated the repression of polar auxin transport and auxin-dependent signaling processes in the ABA-biosynthesis mutant (Figs. 3, 5), which might lead to an enhanced IAA movement and the subsequent restoration of lateral root growth (Qin & Huang, 2018). Moreover, suppressing ethylene biosynthetic and metabolic processes after re-watering may also associate with the regrowth of primary and lateral roots (Fig. 3B, C), as evident by the finding that the accumulation of internal ABA maintains maize (*Zea mays*) primary root elongation by restricting ethylene synthesis (Spollen, LeNoble, Samuels, Bernstein, & Sharp, 2000).

4.4 Crosstalk of ABA and IAA in response to external water change in three drought phases

The addition of 5 nM IAA to *not* partially or fully rescued primary and lateral root growth when compared with WT in three progressive phases (Figs. 4, 6). Either exogenous ABA or IAA tended to recover root growth in *not* plants, likely via ABA-IAA interactions in roots. ABA together with its receptor PYL8 can promote lateral root formation and elongation in an auxin-dependent manner. Briefly, PYL8 could induce MYB77-dependent *ARF7* expression, which further increases the lateral organ boundaries domain transcription factor LBD16 and LBD29 to promote lateral root growth (Xing et al., 2016). Plants respond to drought by multiple mechanisms at morphological, biochemical and molecular levels, ABA and its interactions with IAA was regarded as important. Earlier studies (Boyer, 1982;

Kramer & Boyer, 1995; Levitt, 1980) proposed that plant drought resistance involves several mechanisms: drought avoidance (the ability of plant to maintain high water potential through reducing water loss and enhancing water uptake), drought tolerance (the ability of plant to sustain basic level of physiological activities through maintaining cell turgor pressure and reducing harmful metabolites), drought escape (the artificial or natural adjustment to avoid the seasonal drought stress), and drought recovery (the ability of plant to resume growth after re-watering). In the present study, we analyzed plant root responses to different levels of drought: moderate drying, severe drying and re-watering. In the three progressive phases, plant showed the abilities to alter root structure and modulate the expression of hundreds of genes in respond to external water fluctuations, the adjustments at morphological and molecular level confer plant drought resistance via different mechanisms.

In conclusion, our study provides an insight into the function of ABA and its coordination with IAA, to modulate plant drought resistance by dynamically reshaping root architecture and influencing large-scare of root gene expressions in the three progressive phases, which are important for our understanding of plant adaption to continuously-changed soil water status.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Weifeng Xu, Qian Zhang and Wei Yuan designed the experiments. Wei Yuan, Qianwen Wang, Yiying Cao, Feiyun Xu and Qian Zhang performed the experiments. Qian Zhang, Weifeng Xu, Ian C. Dodd and Wei Yuan interpreted the data and drafted the manuscript.

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Figures and Legends

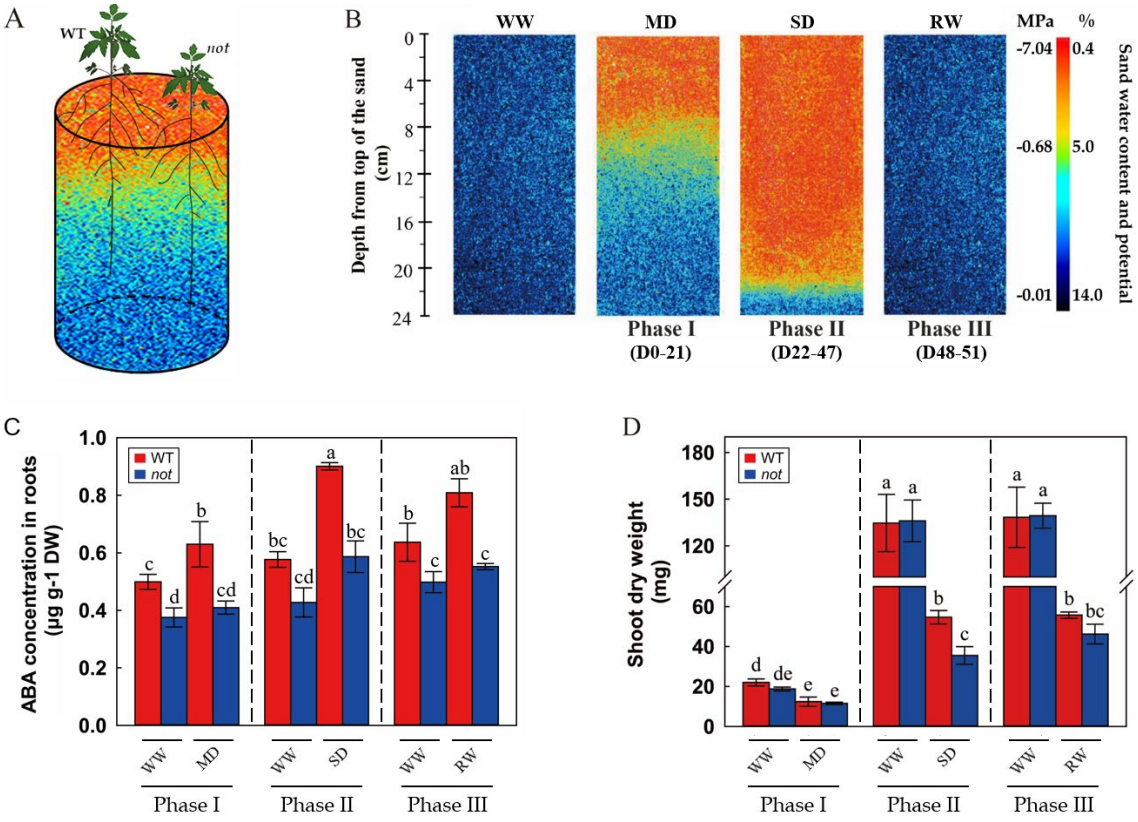

Fig. 1. Experimental setup and plant growth of wild-type (WT) and ABA-biosynthesis mutant (*not*) tomato in three progressive phases of drought stress and recovery. At day 0, seven-day-old seedlings with one true leaf were transplanted to the pot (one plant of each genotype WT or *not* in each column) filled by sand, and sand water-holding capacity is 14%. Phase I: moderate drying (MD, day 0-21; 5% sand water content with water potential of -0.68 MPa); Phase II: severe drying (SD, day 22-47; sand water content gradually dropped from 5% at day 22 to 0.4% at day 47 with water potential of -7.04 MPa); Phase III: re-watering (RW, day 48-51; 14% sand water content with water potential of -0.01 MPa). (**A**) WT and *not* plants are grown in the same pot to minimize the difference in growth conditions. (**B**) Water profile in the pot at drying, drought and re-watering phases. Red color represents low water content in the sand, while blue represents high water content as shown by the color legend. Root ABA concentration (**C**) and shoot dry weight (**D**) of WT and *not* during the three experimental phases. At each phase, WW in the X-axis label means well-watered conditions. Bars in C and D represent means ± SE of three plants, with different letters indicating significant difference between means across all three phases according to Duncan's multiple range test ($P < 0.05$).

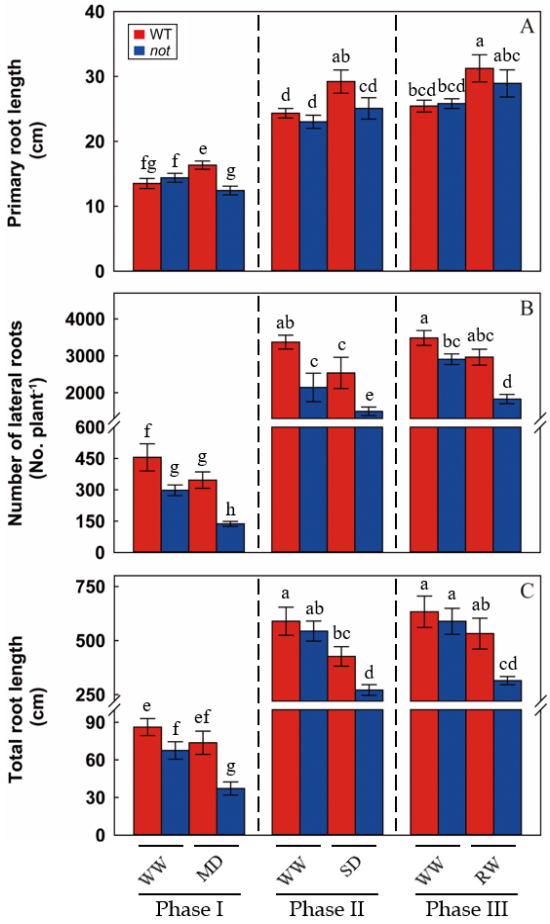

Fig. 2. Root traits of wild-type tomato (WT) and ABA-biosynthesis mutant (*not*) at the end of Phase I: moderate drying (MD); Phase II: severe drying (SD); Phase III: re-watering (RW). Primary root length (**A**), lateral root number (**B**) and total root length (**C**) of WT and *not* plants during the three experimental phases. Bars represent means ± SE of three plants, with different letters indicating significant difference between means across all three phases according to Duncan's multiple range test ($P < 0.05$).

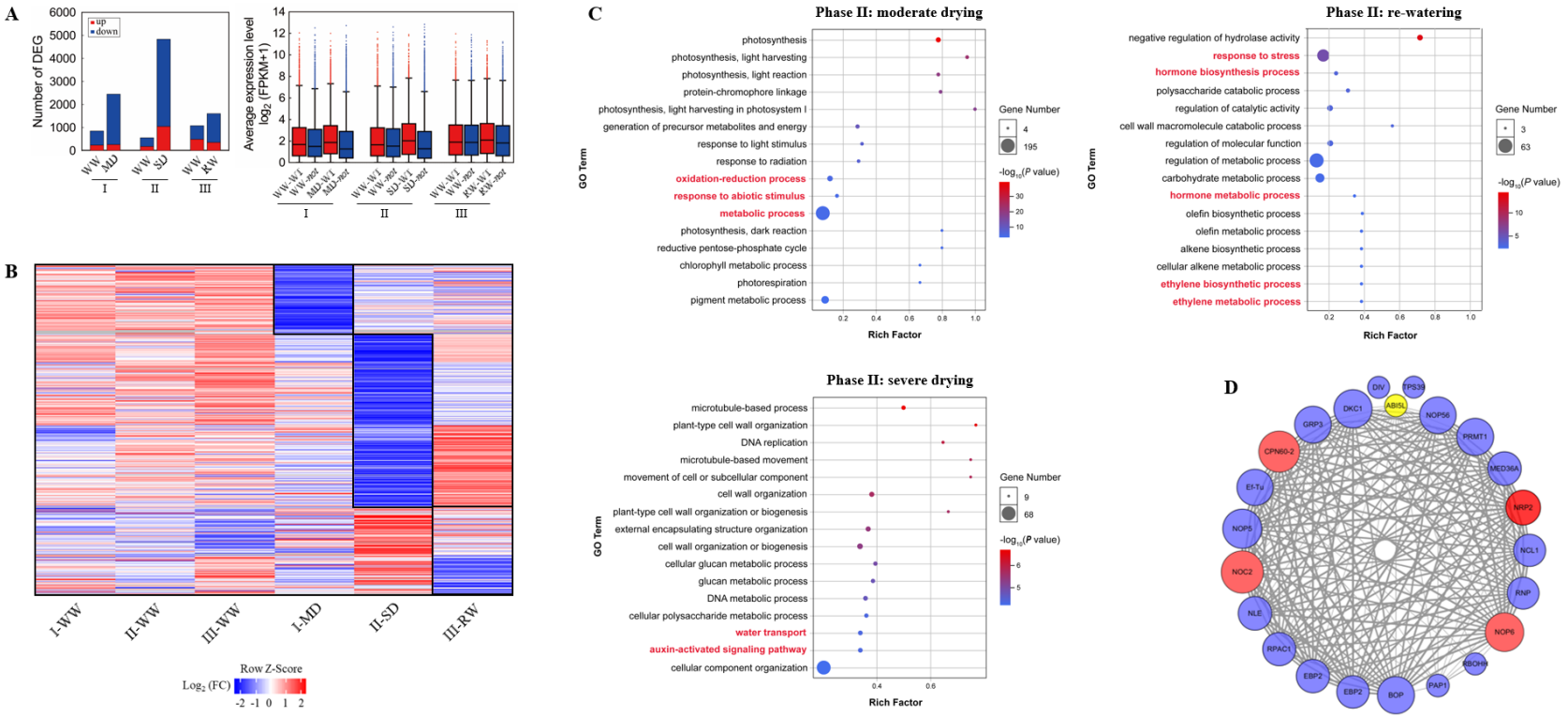

Fig. 3. Gene expression pattern and co-expression network analysis. (**A**) The number of differently expressed genes (DEG, WT vs *not* and fold change > 2) and expression level of wild-type (WT) and ABA-biosynthesis mutant (*not*) at the end of Phase I: moderate drying (MD); Phase II: severe drying (SD); Phase III: re-watering (RW), FPKM represents Fragments per Kilobase Million. (**B**) Heatmap of DEGs during the three experimental phases. (**C**) Significantly enriched GO terms during the three experimental phases. (**D**) Identification of hub genes involved in plant drought responses by using weighted gene co-expression network analysis (WGCNA), red color represents four hub genes, while yellow indicates the target gene.

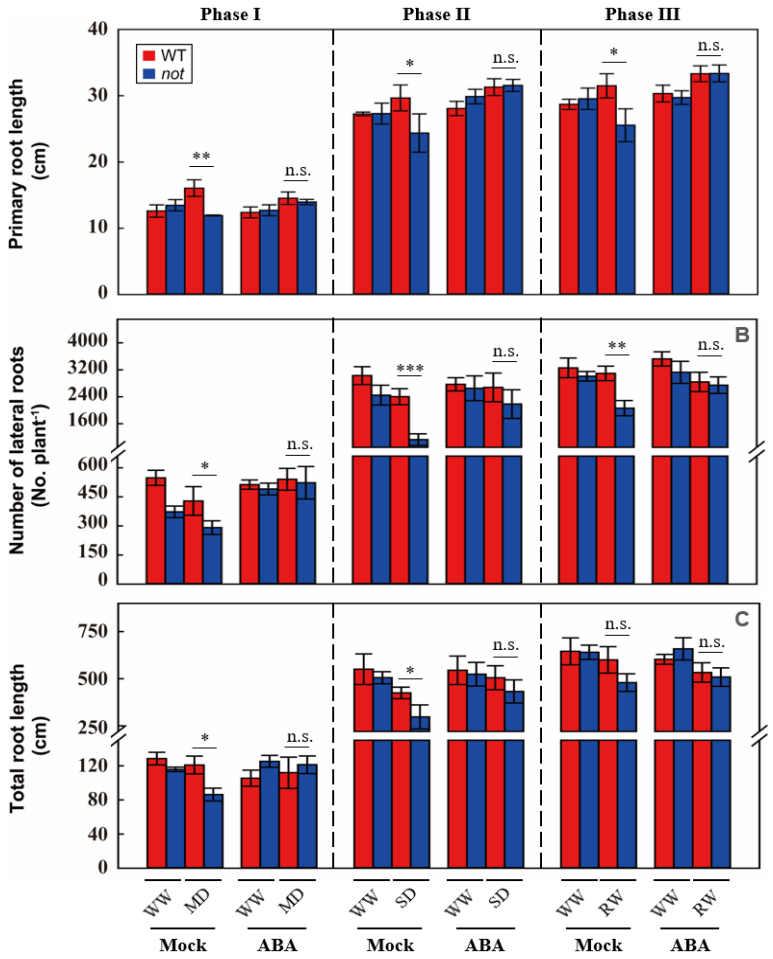

Fig. 4. Exogenous supply of ABA recovered root growth of ABA-biosynthesis mutant (*not*) in three progressive phases under drought. Phase I: moderate drying (MD); Phase II: severe drying (SD); Phase III: re-watering (RW). Primary root length (**A**), lateral root number (**B**) and total root length (**C**) of WT and *not* plants during the three experimental phases. Mock plants received the same volume of water as the ABA-treated plants. Bars represent means ± SE of three plants, with asterisk indicating a significant difference between means according to Duncan's multiple range test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. indicates no significant difference at $P < 0.05$.

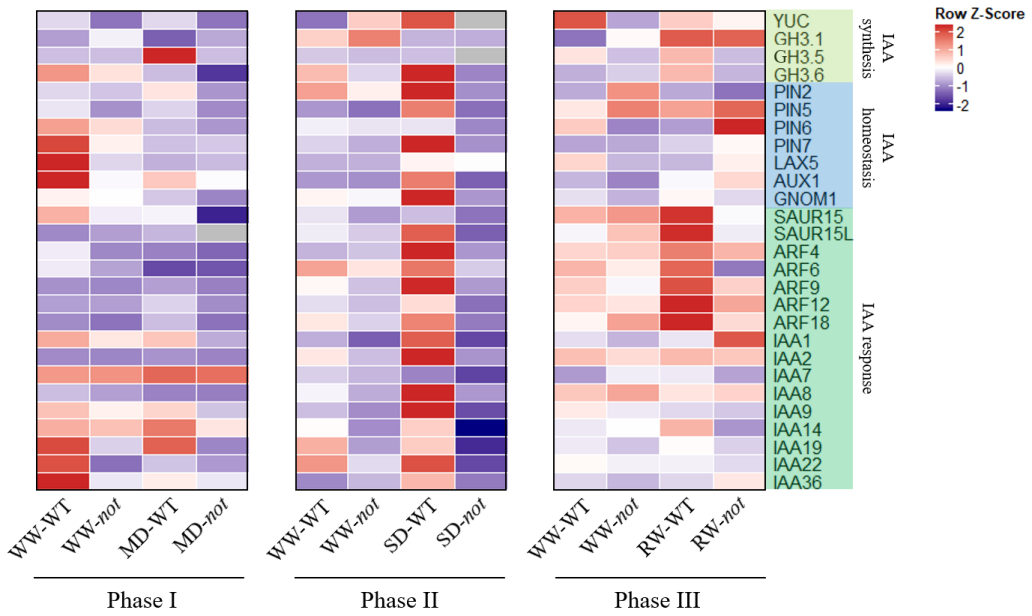

Fig. 5. Heatmap of IAA-related genes expression in wild-type (WT) tomato and ABA-biosynthesis mutant (*not*) at the end of Phase I: moderate drying (MD); Phase II: severe drying (SD); Phase III: re-watering (RW). At each phase, WW in the x-axis label means well-watered conditions. YUC: indole-3-pyruvate monooxygenase; GH: indole-3-acetic acid-amido synthetase; PIN: auxin efflux facilitator; LAX: auxin influx carrier, LAX family; AUX: auxin influx carrier; GNOM1: ARF guanine-nucleotide exchange factor GNOM-like; SAUR: auxin-induced protein; ARF: auxin response factor; IAA1-36: auxin-responsive protein.

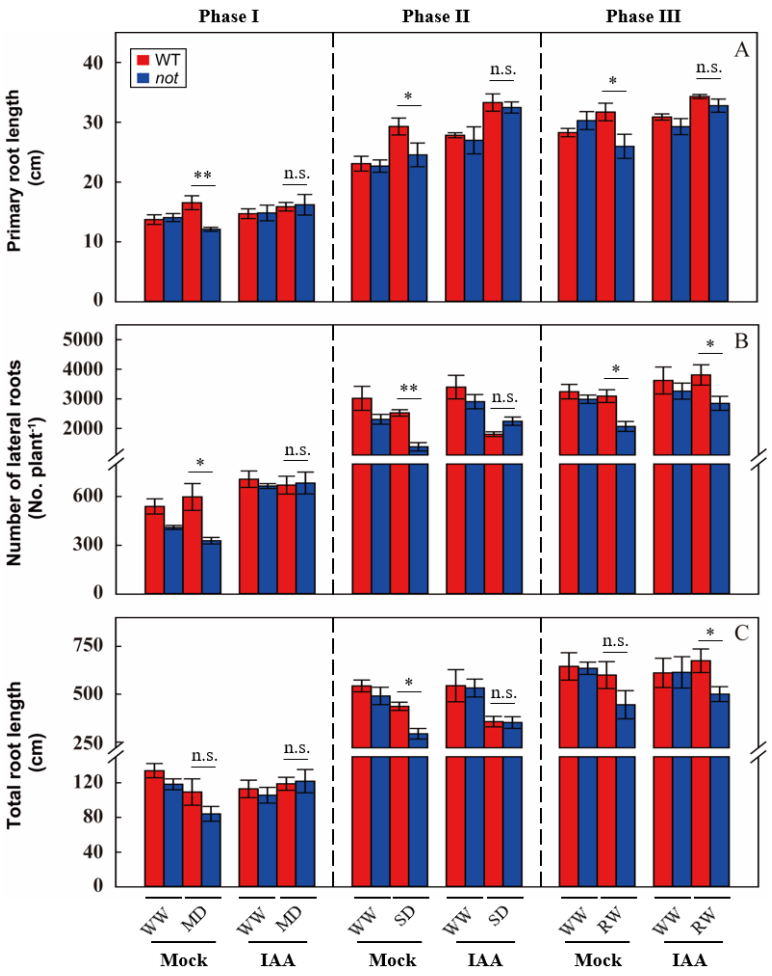

Fig. 6. Exogenous supply of IAA partially recovered the root growth of the ABA-biosynthesis mutant (*not*) in three progressive phases under drought. Phase I: moderate drying (MD); Phase II: severe drying (SD); Phase III: re-watering (RW). Primary root length (**A**), lateral root number (**B**) and total root length (**C**) of WT and *not* plants during the three experimental phases. Mock plants received the same volume of water as the IAA-treated plants. Bars represent means ± SE of three plants, with asterisk indicating a significant difference between means according to Duncan's multiple range test. * $P < 0.05$, ** $P < 0.01$, n.s. indicates no significant difference at $P < 0.05$.