Pak. J. Bot., 52(3): 1045-1054, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-3(25)

TRICHODERMA HARZIANUM IMPROVES DROUGHT RESISTANCE IN MAIZE BY MEDIATING ACETIC ACID-ETHANOL METABOLIC PATHWAYS

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Abstract

Drought stress adversely inhibits plant growth and causes yield loss worldwide. Beneficial *Trichoderma* species have recently been shown to improve drought resistance in different plant species, whereas the underlying mechanisms of *Trichoderma*-induced drought resistance of host plants remain largely elusive. Herein, the effects of a *Trichderoma harzianum* isolate on maize's responses to drought stress were investigated. Inoculation with *T. harzianum* significantly promoted the growth and enhanced drought tolerance of maize plants. The whole genome expression profiles of the *Trichoderma*-inoculated plants were examined by RNA-sequencing, showing that several differentially expressed genes were positively associated with the process of ethanol-acetic acid metabolism. Compared with non-inoculated (control) plants, colonization of maize plant by *T. harzianum* exhibited the increased abscisic acid (ABA) levels. Drought stress induced a further increase of ABA content in the plants, while the levels of ABA were markedly higher in the inoculated plants than the controls. Virus-mediated silencing of *ZmADH1* or *ZmALDH12*, which was involved in the ethanol-acetic acid metabolic pathways, largely weakened the *Trichoderma*-induced effects in the drought-treated plants, such as lower leaf relative water content (RWC) and higher reactive oxygen species (ROS) levels. Collectively, our results suggested that the *Trichoderma*-induced changes of ethanol-acetic acid metabolic pathways played a vital role in regulation of maize adaptation to drought stress.

Key words: Drought stress, *Trichderoma harzianum*, Abscisic acid, Ethanol-acetic acid metabolism.

Introduction

Dramatic climatic changes of global scale, such as elevation of temperature and decrease of rainfall, result in an increasing occurrence of water shortage in arid and semiarid areas. Drought stress is one of the most constraining factors that adversely crop growth, biomass and yields (Bouman *et al.*, 2005, Daryanto *et al.*, 2016). During long-term evolution, a wide range of mechanisms has been developed to counteract negative impacts imposed by drought stress at the morphology, physiology, and molecule levels (Zhu, 2002). Plants can also mediate dynamic changes of several important metabolites such as polyamine, acetic acid and ethanol to regulate a subset of classical stress signaling pathways, such as abscisic acid (ABA)- or jasmonic acid (JA)-mediated stress responses (Minocha *et al.*, 2014, Shi *et al.*, 2017, Rasheed *et al.*, 2018).

Several family members of alcohol dehydrogenases (ADHs), pyruvate decarboxylases (PDCs) and aldehyade dehydrogenases (ALDHs) are involved in the regulation of ethanol-acetic acid metabolism in plants (Rasheed *et al.*, 2018). ADHs can catalyze the interconversion of acetaldehyde to ethanol in plants (Chang *et al.*, 1986, Chung *et al.*, 1994, Yang *et al.*, 2014). Overexpression of *AtADH1* improves abiotic stress adaptation in transgenic *Arabidopsis* plants by activation of several ABA-responsive gene transcripts (Shi *et al.*, 2017). Pyruvate can be converted into the acetyl-Co enzyme A (acetyl-CoA) by pyruvate dehydrogenase (PDH). However, the biosynthesis of acetyl-CoA is also an alternative pathway. In this case, pyruvate can be converted into acetaldehyde by the PDCs, which is then converted into acetate by the ALDHs (Lin & Oliver, 2008, Wei *et al.*, 2009). The gene family of ALDHs contains diverse members for controlling the metabolism of aldehyde and utilizes NAD^+ or $NADP^+$ as a cofactor to convert aldehydes into carboxylic acids (Brocker *et al.*, 2013). In plants, different members of the ALDHs exhibited diverse functions in the control of glycolysis and carnitine biosynthesis (Wei *et al.*, 2009, Brocker *et al.*, 2013, Stiti *et al.*, 2011). The transcription of *ALDH* genes can be induced by various stress factors. Moreover, overexpression of the *ALDH* genes can enhance abiotic stress tolerance in transgenic *Arabidopsis* plants (Kirch *et al.*, 2005, Kotchoni *et al.*, 2006). In *Arabidopsis*, the *PDC1* and *ALDH2B7* genes are involved in the regulation of acetic acid biosynthetic pathways, and the increased transcription of the two acetic acid pathway genes can improve drought tolerance in transgenic *Arabidopsis* plants (Kim *et al.*, 2017). Recently, a positive association has been found between the acetic acid biosynthesis of *Arabidopsis* and plant drought tolerance (Kim *et al.*, 2017). In plants, the whole pathway of ethanol-acetic acid is greatly activated by drought stress, oxygen limitation as well as ABA treatment (Kürsteiner *et al.*, 2003, Rasheed *et al.*, 2013, Papdi *et al.*, 2015). Exogenous acetic acid induces increases of endogenous hormones and thereby increases drought resistance in various plant species such as rice, maize, wheat and cassava (Kim *et al.*, 2017, Papdi *et al.*, 2019). Acetic acid can promote the accumulation of JA and provoke the COI1-mediated signaling pathways, thereby activating downstream genes that are required for drought tolerance (Papdi *et al.*, 2019). In the course of plant adaptation to drought stress in which diverse phytohormones are involved, ABA is the key stress phytohormone related to plant defense against abiotic stress (Vishwakarma *et al.*, 2017). Acetic acid treatment has also been shown to promote the biosynthesis of ABA in cassava plants and further enhance drought tolerance, indicating that acetic acid can mediate drought stress

responses, involving regulation of the ABA-dependent pathways (Papdi *et al*., 2019).

It is increasingly aware that soil microorganisms can induce abiotic stress resistance in different plant species (Shukla *et al.,* 2012, Pandey *et al.,* 2016, Fu *et al.,* 2017, Anam *et al.,* 2019). Several *Trichoderma* species can enhance the adaptation of plants to drought stress (Bae *et al.,* 2009, Shukla *et al.,* 2012, Pandey *et al.,* 2016). Inoculation of rice with *Trichoderma* strains delays drought-induced responses such as stomatal closure and photosynthesis (Pandey *et al.,* 2016). Some strains of *Trichoderma harzianum* can increase the levels of endogenous hormones, the activity of antioxidant systems and compatible solutes, which confer increased drought resistance (Bae *et al.,* 2009, Shukla *et al.,* 2012). However, it remains largely elusive whether *Trichoderma* strains mediate changes of cellular metabolites for plant defense against drought stress and that the molecular mechanisms of *Trichoderma*-induced host resistance.

In this study, we explored the effects of *T. harzianum* on the molecular and physiological responses of maize plants to drought stress. *T. harzianum* markedly enhanced drought resistance in maize plants. Transcriptome analyses showed that several genes involved in the ethanol-acetic acid metabolic process were greatly up-regulated in the *Trichoderma*-inoculated maize plants. Furthermore, virus-mediated silencing of *ZmADH1* or *ZmALDH12*, which are involved in the ethanol-acetic acid metabolic pathways, largely weakened the *Trichoderma*-induced drought resistance in maize plants. Therefore, these findings provided novel insights into the mechanisms underlying the process by which *T. harzianum* mediated the acetic acid-ethanol metabolic pathways, which at least partially contributed to increasing drought resistance in maize plants.

Materials and Methods

Plant materials and growth conditions: The seeds of drought-susceptible maize (*Zea mays* inbred line Anke35) were sterilized with 75% ethanol for 2 min, and were then treated with 2.5% NaClO for 8 min. Subsequently, the seeds were washed at least three times. The treated seeds were germinated under the wetted conditions at 30°C for 72 h in dark. The seedling with uniform size were cultured in soil and placed in growth chamber with a photoperiodic cycle (16 h light at 26°C, 8 h dark at 24°C), about 60% relative humidity, and 180 μmol m^{-2} s^{-1} light intensity. Each pot contained five maize seedlings, and were watered with 1/4 Hoagland' solution every four days.

Microbial inoculation and drought treatments: A *Trichoderma harzianum* strain (accession No. 1250, China General Microbiology Culture Collection Center) was used this experiment. The fungal strain was cultured at 28°C on PDA medium before soil inoculation. The collected spores were sieved through a three layers of gauze and were added to sterile water. 10-day-old maize seedlings were inoculated with or without the concentration of 1×10^8 spores g^{-1} soil for 7 days (d). These plants were then subjected to progressive drought stress by stopping irrigation.

RNA-sequencing (RNA-Seq) and quantitative polymerase chain reaction (qPCR): For RNA-sequencing, 10-day-old maize seedlings were inoculated with the spore suspensions at the concentration of 1×10^8 spores g^{-1} soil for 7 d. Total RNAs were extracted from leaves of non-inoculated and inoculated maize seedlings for cDNA synthesis. Three biological replicates were performed from each group of samples. Then, cDNA libraries were constructed based on the Illumina Hiseq 2500 platform (Illumina, USA). The raw data were deposited in the NCBI SRA database (accession No. PRJNA594751). Transcriptome analyses were performed according to the method (Zhou *et al.,* 2019). Gene Ontology (GO) analysis was further assigned to all the differentially expressed genes (DEGs).

To perform qPCR analyses, total RNAs were extracted from leaf samples and were reversely transcribed into cDNA. The cDNA samples were used as the templates of qPCR reactions in an Applied Biosystems (ABI) 7500 PCR machine. The qPCR reactions were carried out according to the method (Zhou *et al.,* 2016). The maize *ACTIN2* was used as the internal inference to normalize the expression levels of targeted genes. Values for gene expression were calculated from the data of three biological replicates.

Construction of virus-mediated gene silencing (VIGS): A VIGS system was constructed in maize seedlings based on the method (Zhang *et al.,* 2017). The vectors pTRV1 and pTRV2 were used in our experiments. To generate the recombinant vectors *TRV2::ZmADH1* and *TRV2::ZmALDH12*, the partial coding sequence of *ZmADH1* (F: 5'-TTTGCGTCCTTAGCTGTGGT-3'; R: 5'-CTCAGCGTCCTTATGTGGCA-3') and *ZmALDH12* (F: 5'-CACGCACGTCTAATGGTTC-3'; R: 5'-TGTAGTGCAGCGTTGTC-3') were amplified and inserted into the pTRV2, respectively. In the VIGS system, the seeds of maize were sterilized using 2.5% NaClO for 5 min and then washed with at least three times. *Agrobacterium tumefaciens* carrying the pTRV1 and above recombinant vectors was cultured in LB liquid medium at OD600 of 0.5, respectively. The culture was then mixed in 1:1 ratio and was supplemented with Tween 20 (5 mL L^{-1}), acetosyringone (20 mg L^{-1}), and cysteine (500 mg L^{-1}). Subsequently, the germinated seeds were incubated in the mixed culture for 16 h, and were then planted in soil.

Measurement of physiological parameters: Leaf water potential (LWP) of the uppermost fully expanded leaves was determined as described by Lu *et al*., (2013). Leaf samples were harvested to measure relative water content (RWC) using a previously described protocol (Li *et al.,* 2014). Maize leaves were harvested to measure transpirational water loss as reported by Chen *et al*., (2006). The levels of H_2O_2 and O_2^- were quantified as reported by Chen *et al*., (2016a). MDA levels were analyzed as reported by Zhang *et al*., (2013). The electrolyte leakage (EL) of leaves was determined as described by Fan *et al.* (2015). The maximum efficiency of PSII photochemistry (*Fv/Fm*) was imaged using an imaging of chlorophyll fluorescence (FluorCam 7; Photon

Systems Instruments, Brno, Czech Republic) after adaption in the dark for 30 min (Zhou *et al.,* 2019). In addition, endogenous ABA and JA levels in leaves were measured as reported by Kanno *et al*., (2016).

Statistical analysis: All assays were repeated three independent experiments. Data were analyzed via one-way ANOVA, and Duncan's multiple range tests. All the data were expressed as means ± standard deviation (SD). Different letters above the bars represent significant difference at $p<0.05$.

Results

***T. harzianum* enhanced drought resistance in maize plants:** To examine whether soil inoculation with *T. harzianum* affected the growth and drought resistance of maize plants, 10-d-old maize seedlings grown in soil were inoculated with *T. harzianum* for 7 d. Subsequently, these plants were subjected to drought stress imposed by stopping water irrigation (Fig. 1). After 7 d of drought treatment, the leaves of non-inoculated (control) plants were notably wilted, while the leaves of inoculated plants were slightly wilted (Fig. 1a). After 12 d of drought treatment, the leaves of inoculated plants displayed less wilting than that of the controls (Fig. 1a). After re-watering 3 d, the inoculated plants exhibited higher survival rates than the controls (Fig. 1b). Under well-watered conditions, the inoculation with *T. harzianum* increased plant fresh weights compared with the controls (Fig. 1c). Fresh weights were also markedly higher in the *Trichoderma*-treated plants than the controls under drought stress. Furthermore, the inoculation with *T. harzianum* did not impact the values of *Fv/Fm* under well-watered conditions (Fig. 1d). After drought treatments, the values of *Fv/Fm* were notably decreased, while the values of *Fv/Fm* were much higher in the inoculated plants than the controls (Fig. 1d).

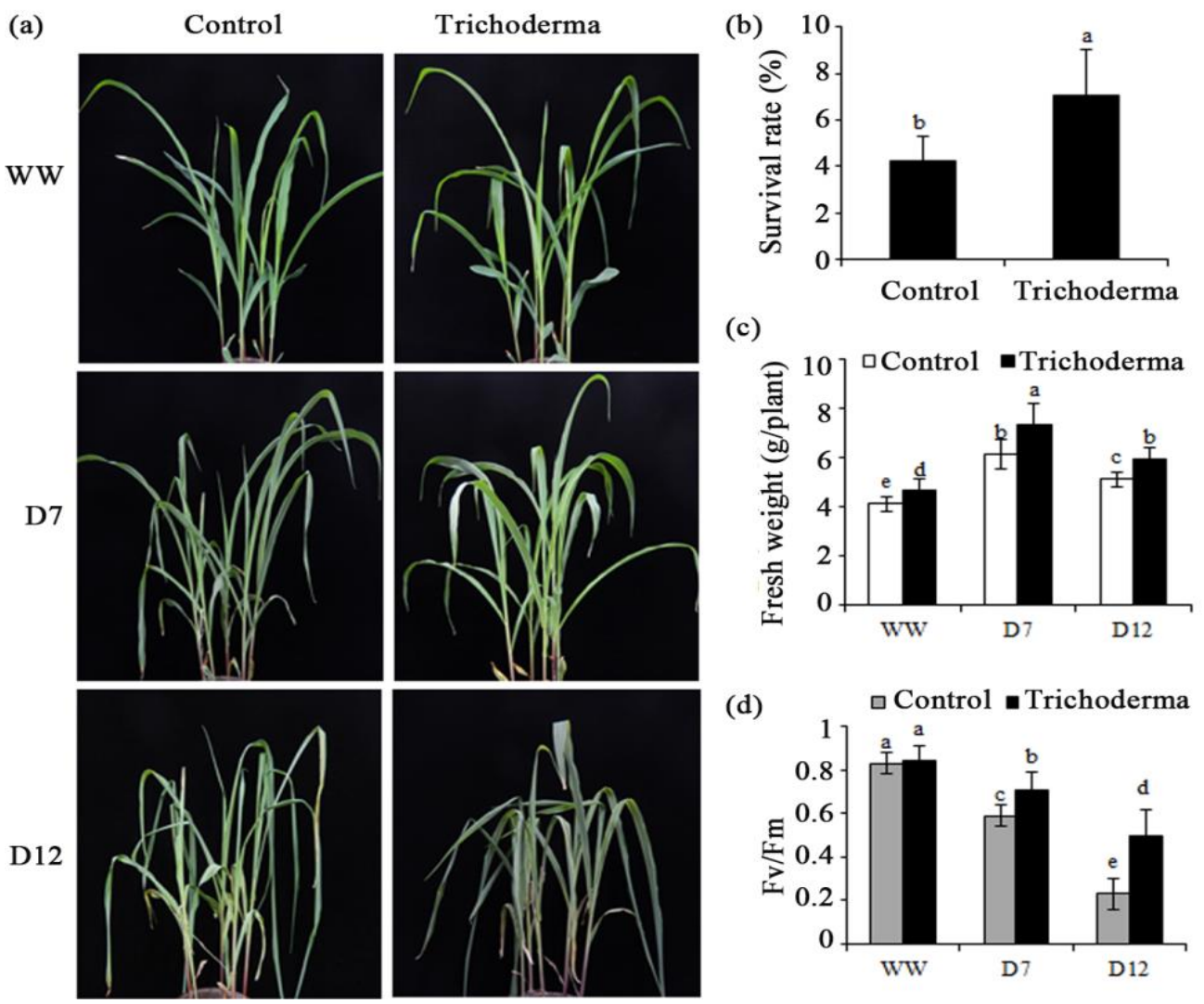

Fig. 1. The effects of drought stress and *Trichoderma* inoculation on the growth of maize seedlings. (a) Phenotype of the non-inoculated (control) and inoculated plants under well-watered (WW) and drought stress conditions. 10-d-old maize seedlings were inoculated with or without *T. harzianum* for 7 d under well-watered conditions. Then, the control and inoculated plants were subjected to drought stress by withholding irrigation for 12 d. D7, withholding irrigation for 7 d; D12, withholding irrigation for 12 d. (b) Survival rates of inoculated plants. Fifty control or inoculated plants were exposed to drought stress for 12 d, and then watering was resumed for 8 d. Each value represents the mean of three replicates. (c) Plant fresh weights of control and inoculated plants. (d) Fv/Fm in leaves of control and inoculated plants. Values represent the mean ± SD (n = 3).

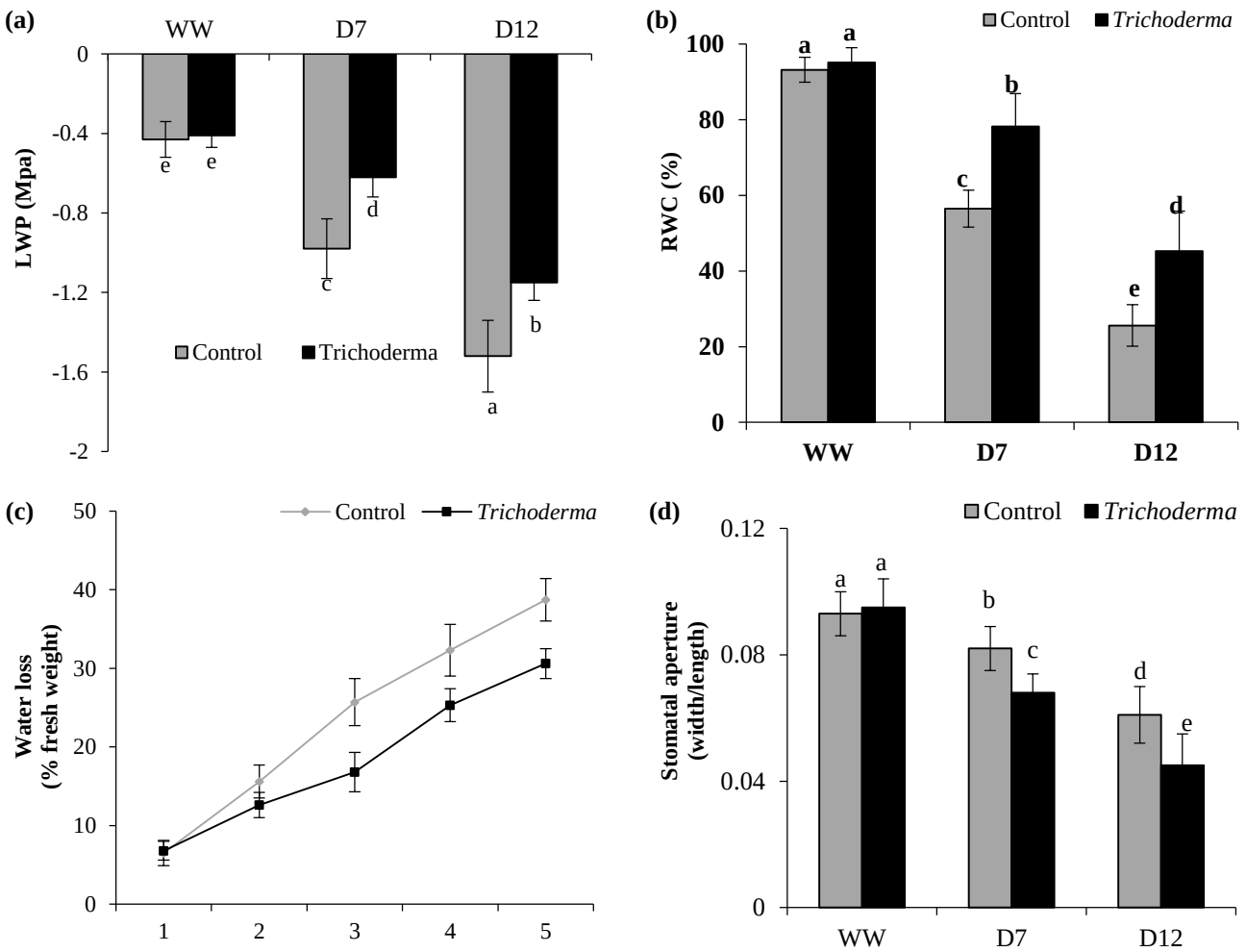

Fig. 2. Determination of leaf water status. Changes in LWP (a), RWC (b), transpirational water loss of detached leaves (c) and stomatal apertures (d) in non-inoculated (control) and inoculated plants exposed to drought stress. Drought stress was imposed as described in Fig. 1(a).

***T. harzianum* increased LWP and RWC in leaves of maize plants:** There was no significant difference in the LWP between the control and inoculated plants under well-watered conditions, whereas that of the inoculated plants was significantly higher than the controls after 7 d and 12 d of drought treatments (Fig. 2a). As shown in Fig. 2b, leaf RWC maintained relatively higher in the inoculated plants than the controls under drought stress. However, leaf RWC was relatively similar between the control and inoculated plants under well-watered conditions. Furthermore, transpirational water loss of inoculated plants was significantly less plants than that of the controls under drought stress (Fig. 2c). Less water loss of the inoculated plants indicated that the changes of stomatal action might be induced by *T. harzianum*. As shown in Fig. 2d, stomatal apertures of the inoculated plants were similar to that of the controls under well-watered conditions. However, the stomatal apertures of inoculated plants were markedly lower than that of the controls after 7 d and 12 d of drought treatments.

***T. harzianum* alleviated adverse effects of ROS on maize plants:** Drought stress-induced oxidative damages to maize plants can be evaluated by cellular ROS levels, EL and MDA content. The levels of two major species of ROS including H_2O_2 and O_2^- were measured in leaves of non-inoculated and inoculated plants. As shown in Fig. 3a, no significant difference was observed between the control and inoculated plants under well-watered conditions. After 7 d and 12 d of drought treatments, the accumulation of H_2O_2 and O_2^- was significantly increased in the maize leaves, while the inoculation with *T. harzianum* markedly lowered the production of ROS in the drought-treated leaves (Fig. 3a,b). Moreover, the values of MDA and EL in the leaves were not impacted by *T. harzianum* under well-watered condition, although their values was increased greatly in the drought-treated leaves (Fig. 3c,d), indicating that drought stress destroyed cell membrane systems. However, the inoculation with *T. harzianum* distinctly reduced the levels of MDA and EL in maize leaves under drought stress.

Transcriptome analyses of *Trichoderma*-inoculated maize plants: To explore the mechanisms underlying the *T. harzianum* enhanced the adaptation of maize plants to drought stress, differential gene expression between the control and inoculated maize leaves was analyzed by RNA-sequencing. 10-d-old maize seedlings grown on soil were treated with *T. harzianum* for 7 d. Then, leaf samples from both the control and inoculated plants were

collected to extract total RNAs for the RNA-sequencing. To investigate the biological processes of these DEGs induced by *T. harzianum*, gene ontology (GO) analyses were assigned to all the DEGs. These DEGs were categorized into biological process (BP), cellular component (CC), and molecular function (MF) (Fig. 4a). These DEGs were associated with diverse biological pathways such as cellulose biosynthetic process, oxidation-reduction, metabolic process and response to oxidative stress in the BP category. In the CC category, the majority of DEGs was related to anaphase-promoting complex, membrane and apoplast. In the MF category, most the DEGs were involved in heme binding, cellulose synthase and iron ion binding oxidoreductase activity. It's worth noting that the inoculation with *T. harzianum* affected the metabolic process of acetic acid-ethanol metabolism in maize leaves, which was as reflected by higher expression of *ZmADH1* and *ZmALDH12*. qPCR analyses further showed that the transcription levels of *ZmADH1* and *ZmALDH12* were obviously induced in maize plants after exposure to *T. harzianum* (Fig. 4b,c).

Regulation of *ZmADH1* and *ZmALDH12* by *T. harzianum* affects the ABA levels in maize plants: To explore the roles of *T. harzianum* in regulating drought responses of maize plants, the content of ABA and JA were measured in plants. As shown in Fig. 5a,b, the levels of ABA in the inoculated leaves was slightly increased compared with the controls under well-watered conditions, but the levels of JA were not changed. Under drought stress, the inoculated leaves accumulated higher ABA content than the controls, while the levels of JA were less in the inoculated plants than the controls.

To examine whether ABA participated in the *Trichoderma*-induced maize drought resistance, we analyzed the impacts of *T. harzianum* on maize drought tolerance upon exposure to 10 μM fluridone (FLU), an inhibitor of ABA biosynthesis. Enhanced drought tolerance in the inoculated plants was not observed in the FLU-treated plants (Fig. 6a). Under drought stress, the inoculation with *T. harzianum* increased leaf RWC and reduced the accumulation of ROS compared with the controls, but that was almost abolished in the FLU-treated plants (Fig. 6b-d). Hence, the roles of *T. harzianum* in the enhanced host drought resistance were positively attributable to the actions of ABA.

To further examine if up-regulation of *ZmADH1* and *ZmALDH12* transcripts by *T. harzianum* was involved in regulation of the ABA biosynthesis, we measured the ABA levels in the leaves of *TRV::ZmADH1* and *TRV::ZmALDH12* plants under drought stress in the presence of *T. harzianum*. There were no significant differences in phenotypes among the control and silenced plants (Fig. 6a). However, upon exposure to *T. harzianum*, the levels of ABA were obviously lower in the silenced plants compared with the controls under drought stress (Fig. 7). The inoculation with *T. harzianum* failed to improve drought tolerance in the inoculated plants after 12 d of drought treatment, as evidenced by lower leaf RWC and higher ROS levels (Fig. 6b-d). These data indicated that the increased transcription of *ZmADH1* and *ZmALDH12* was responsible for the *Trichoderma*-induced maize drought tolerance.

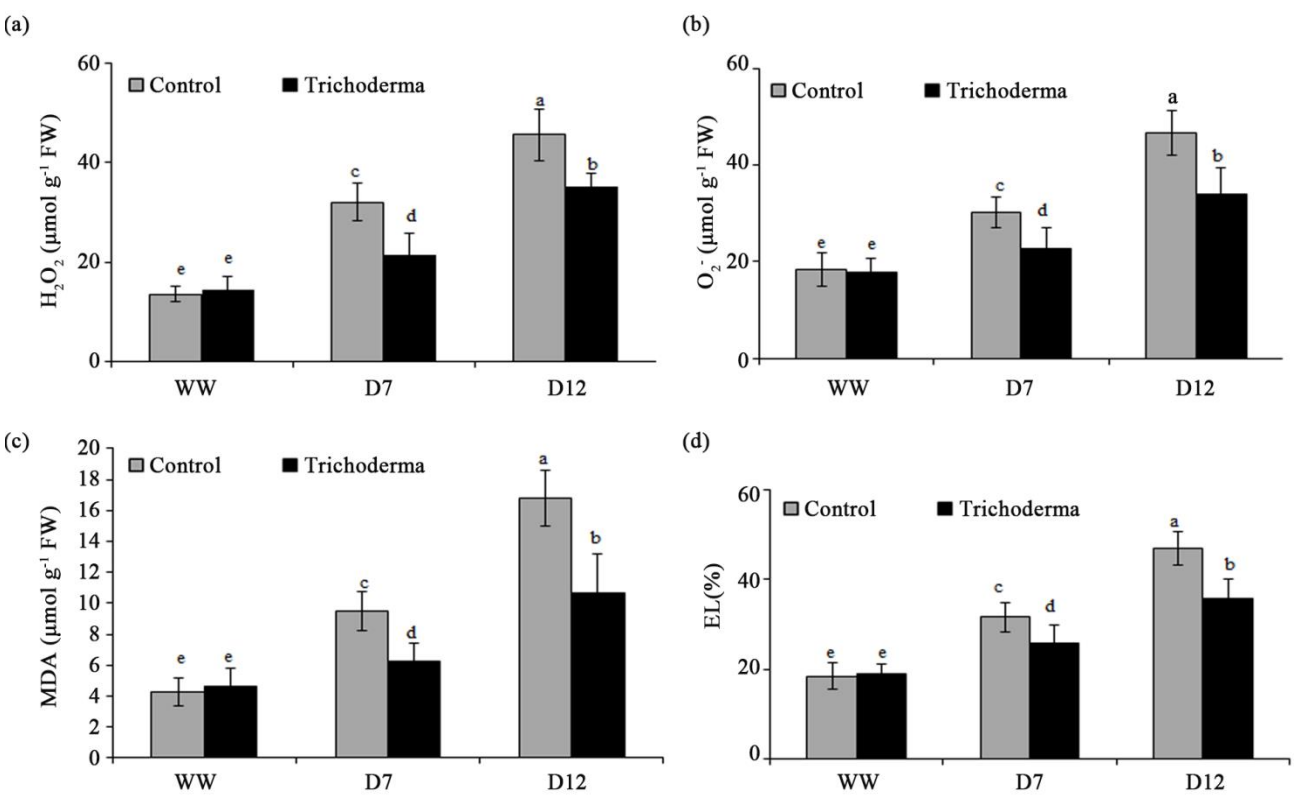

Fig. 3. Cell membrane damage imposed by drought stress. Changes in H_2O_2 (a) and O_2^- content (b), MDA content (c), EL (d) in leaves of non-inoculated (control) and inoculated plants exposed to drought stress. Drought stress was imposed as described in Fig. 1 (a) above.

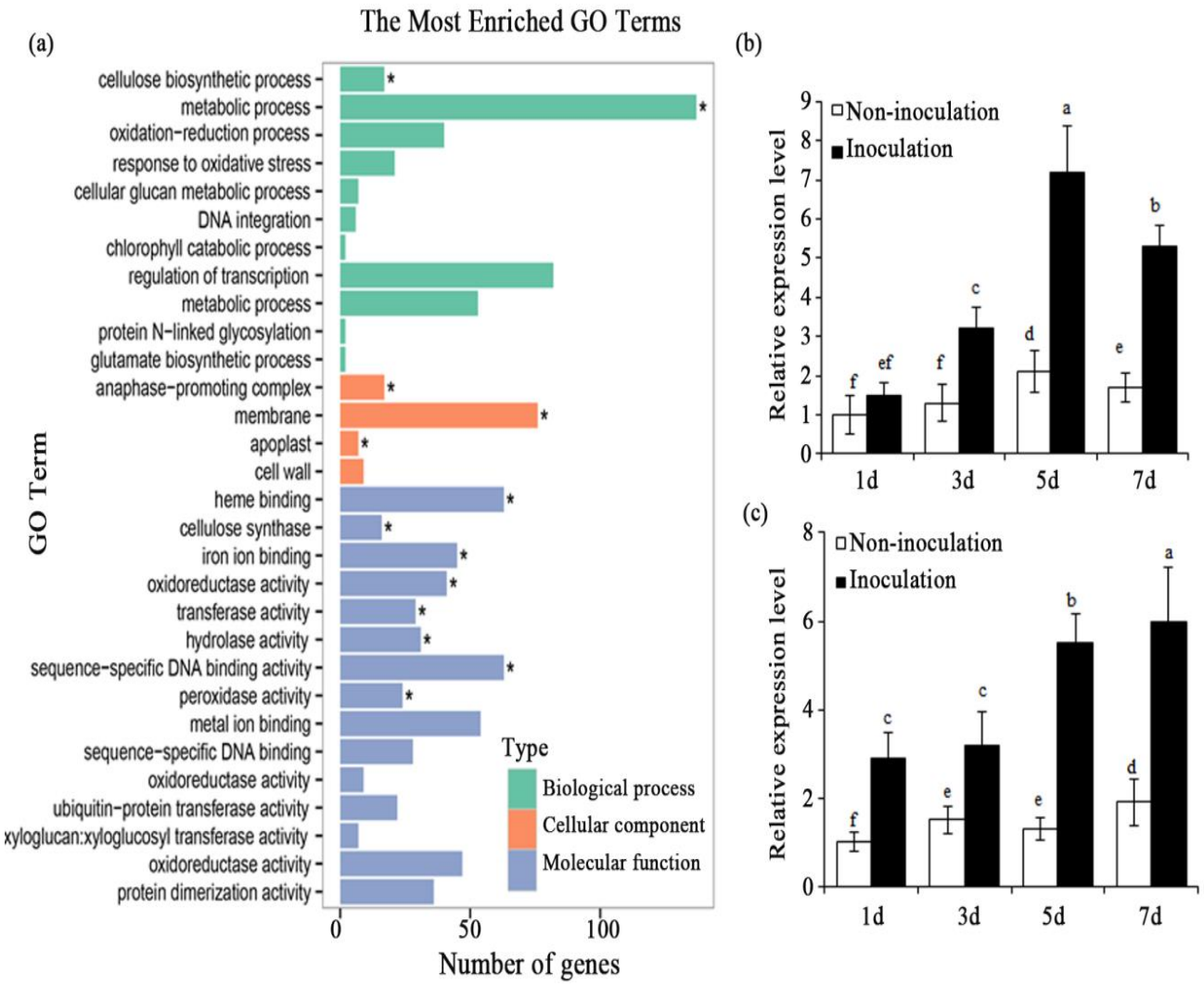

Fig. 4. RNA-sequencing analyses of *Trichoderma*-inoculated maize plants. 10-d-old maize seedlings were inocuated with or without *T. harzianum* for 7 d. Then, leaf samples were harvested to extract total RNA for transcriptome analyses. (a) List of Gene Ontology (GO) terms for differentially expressed genes (DEGs) based on GO classifications. All of the DEGs were categorized into biological process, cellular component and molecular function. Asterisks represent significant differences of the comparison between the control and inoculated plants. Moreover, qPCR analyses of the expression of *ZmADH1* (b) and *ZmALDH12* (c) in maize plants at different inoculation time.

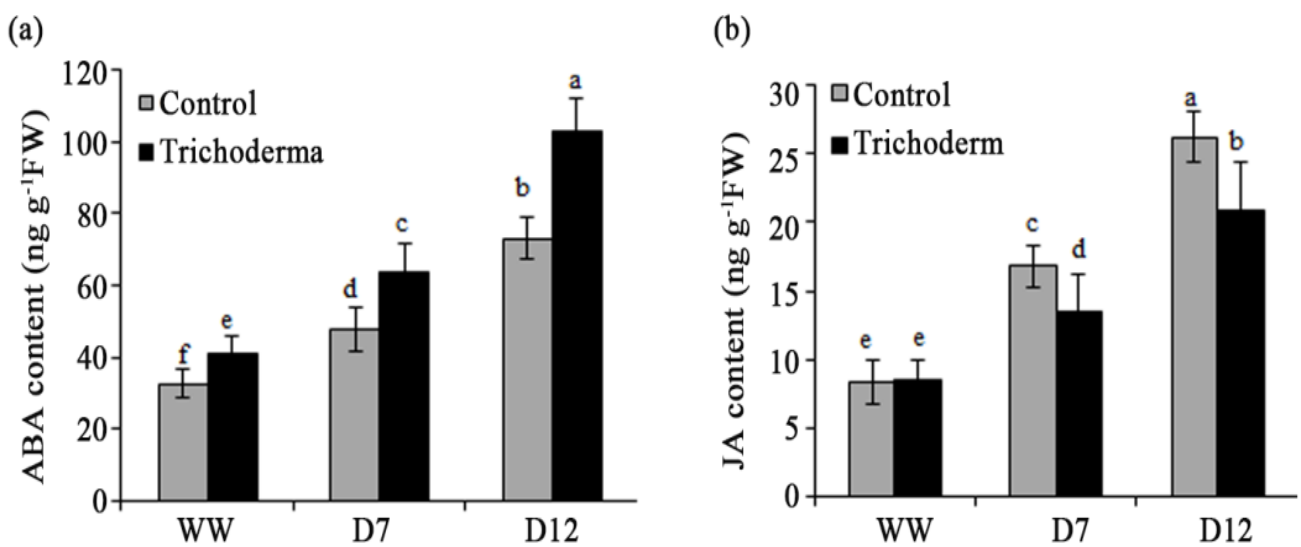

Fig. 5. The effects of ABA (a) and JA (b) levels in leaves of non-inoculated (control) and inoculated plants exposed to drought stress. Drought stress was imposed as described in Fig. 1 (a) above.

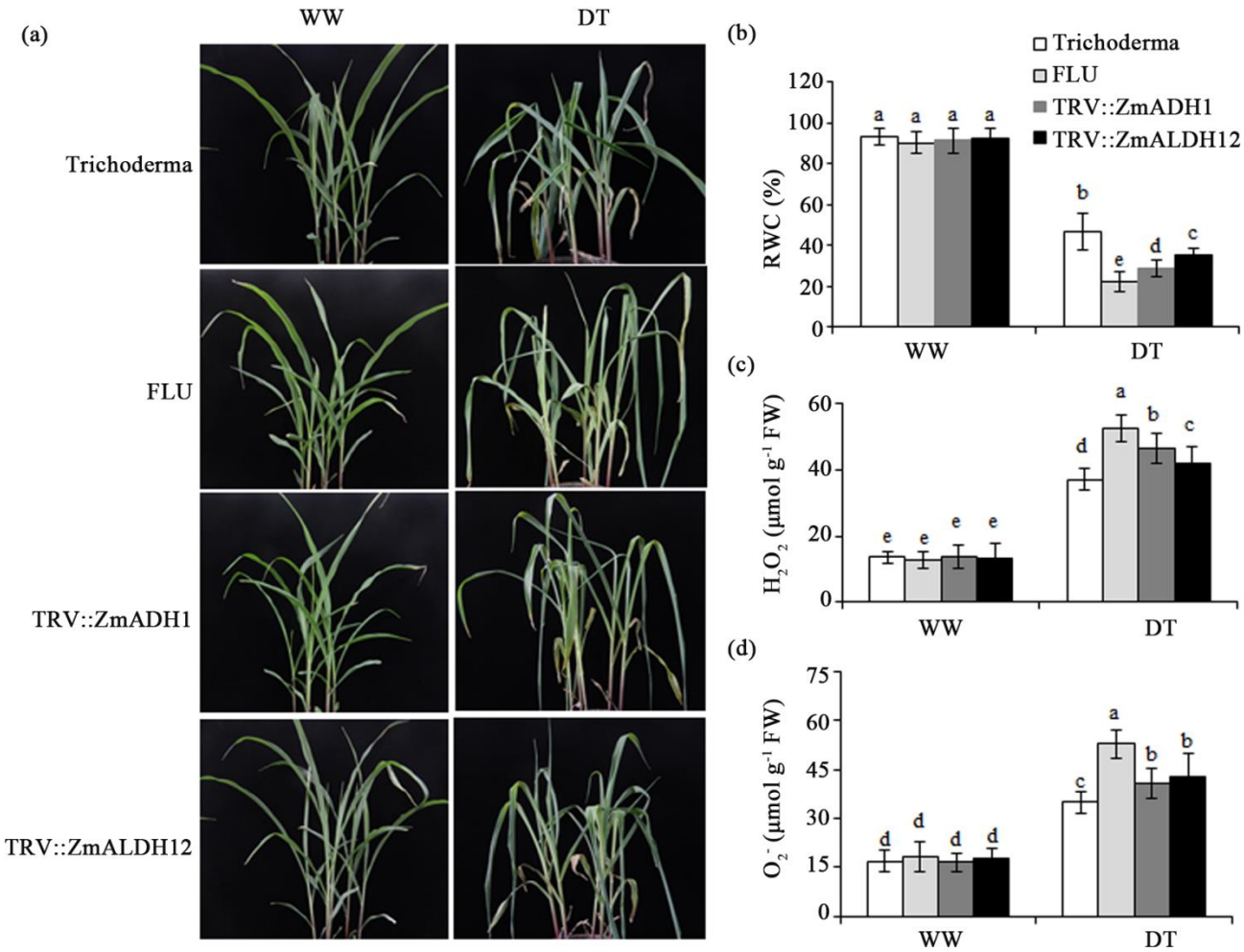

Fig. 6. The effects of ABA levels on the *Trichoderma*-induced maize drought resistance. 10-d-old maize seedlings were inoculated with *T. harzianum* for 7 d under well-watered conditions. Then, the inoculated plants were treated with or without FLU and exposed to drought stress for 12 d. Moreover, the expression of *ZmADH1* and *ZmALDH12* was silenced in the *Trichoderma*-inoculated plants. Then, the silenced plants grown under well-watered conditions for 7 d were also subjected to drought stress for 12 d. These plants were photographed (a) and used to measure leaf RWC (b), H_2O_2 (C) and O_2^- (D). WW, well-watered; DT, drought treatments.

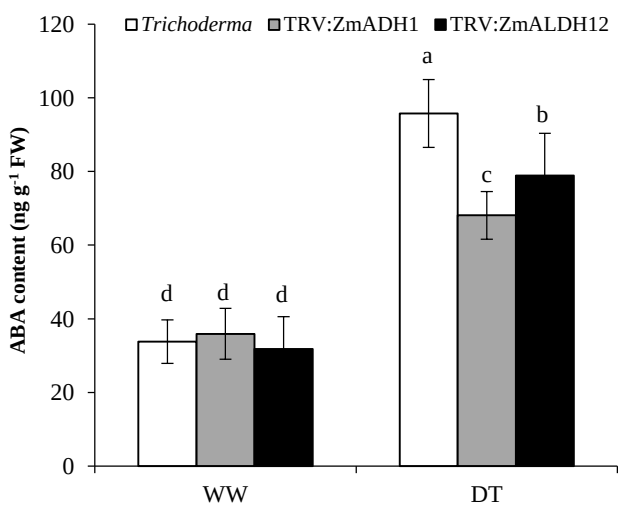

Fig. 7. Virus-mediated silencing of *ZmADH1* or *ZmALDH12* reduced ABA levels in the *Trichoderma*-inoculated maize plants under drought stress. 10-d-old maize WT and silenced seedlings were inoculated with *T. harzianum* for 7 d under well-watered conditions. Then, the inoculated plants were exposed to drought stress for 12 d. These plants were used to measure leaf ABA content. WW, well-watered; DT, drought treatments.

Discussion

Drought stress is a major abiotic stress that increasingly threatens agricultural production. Plants have also evolved a wide range of physiological, biochemical and molecular mechanisms to tolerate water deprivation (Bouman *et al.*, 2005, Gharbi *et al.*, 2019, Shafiq *et al.*, 2019, Wang *et al.*, 2019). Recently, many studies have shown that soil microbes colonized in plant rhizosphere can assist the host in tolerating drought stress (Bae *et al.*, 2009, Shukla *et al.*, 2012, Pandey *et al.*, 2016). *Trichoderma* species can employ diverse strategies such as morphological adaptations and delay of drought responses to improve plant drought resistance (Bae *et al.*, 2009, Shukla *et al.*, 2012). However, it remains largely unknown about the molecular mechanisms of *Trichoderma*-induced host drought resistance. Herein, the beneficial fungi *T. harzianum* mediated the metabolic pathways of acetic acid-ethanol in maize plants with key ramifications for plant-microbe interactions under drought stress. Specifically, the transcription levels of *ZmADH1* and *ZmALDH12*, which was involved in the acetic acid-ethanol pathways, were higher in the *Trichoderma*-inoculated plants than that of the non-inoculated plants.

However, virus-mediating silencing of *ZmADH1* or *ZmALDH12* genes obviously weakened the *Trichoderma*-induced effects in maize plants with reduction of ABA levels. Thus, *T. harzianum* induced the metabolic and molecular alterations in the host with significant ramifications for improving drought adaptation.

Abiotic factors such as dehydration, salinity and heavy metal stresses often lead to overproduction of ROS in different plant species (Choudhury *et al.,* 2017, Huang *et al.,* 2019). Adverse stress-induced repression of CO_2 fixation contributes to excess PSI reduction and high-level ROS accumulation (Arora *et al.,* 2016). The accumulative ROS in the chloroplasts can subsequently trigger the generation of aldehydes, which amplify the degree of oxidative injury (Del Río *et al.,* 2015). Therefore, oxidative stress imposed by harmful conditions is considered as a major cause of cellular damage, and even cell death. The production of cellular ROS can directly react with nucleic acids and proteins, and oxidize membrane lipids, thereby causing the generation of chemically reactive degradation products such as hydroxy acids, alkenes and aldehydes (Sunkar *et al.,* 2003, Mano *et al.,* 2009). Due to their potential toxicity, plants detoxify the generation of ROS and toxic products by antioxidant systems, including enzymatic scavengers and antioxidant substances (Gill & Tuteja, 2010). However, low levels of ROS are also key signaling molecules that activate the ABA-mediated stress responses (Noctor *et al.,* 2018). Therefore, fine regulation of cellular ROS levels is required for plant survival under various stress conditions. Herein, the *Trichoderma*-inoculated maize plants exhibited stronger drought tolerance than the non-inoculated plants. The photosynthesis is often taken as an important indicator of drought tolerance in plants (Su *et al.,* 2015). The inoculated plants had higher values of *Fv/Fm* than the non-inoculated plants under drought stress, indicating that the photosynthetic systems of inoculated plants experienced less drought-induced oxidative damages. The changing tendency of these physiological parameters may be positively related to the capability of maize plants to ameliorate adverse effects imposed by drought stress. Our data further indicated that the values of IL and MDA, which are key indicators of membrane damage degree, were markedly less in the inoculated plants than in the non-inoculated plants under drought stress. Therefore, *T. harzianum* can help the host mitigate the ROS-induced oxidative damages effectively.

In this study, the inoculation of maize plants with *T. harzianum* reduced transpirational water loss and stomatal apertures under drought stress. The levels of ABA were significantly higher in the inoculated plants than the non-inoculated plants. ABA is the most important hormone that plays a core role in regulating the adaptive responses of plants to drought stress (Yoshida *et al.,* 2014). Furthermore, the inhibitor of ABA biosynthesis FLU treatments almost abolished the *Trichoderma*-induced effects observed in the maize plants, indicating that the inoculation with *T. harzianum* conferred higher RWC in the maize leaves under drought stress, at least partially involving activation of the ABA-mediated stomatal closure. Our transcriptome analyses revealed that *T. harzianum* markedly activated the metabolic process of acetic acid-ethanol fermentation, as evidenced by up-regulation of *ZmADH1* and *ZmALDH12* transcripts. ADHs are the putative Zn-binding enzymes that can interconvert ethanol and acetaldehyde (Chung *et al.,* 1994, Yang *et al.,* 2014). The activities of ADH can be induced by various abiotic or biotic stresses (Yang *et al.,* 2014). ADHs are involved in the fermentative metabolism that reduce acetaldehyde to ethanol, and produce NAD^+ and 2 ATP, which protects plant cells from cytoplasmic acidosis (Chung *et al.,* 1994). Metabolomic studies further indicate that ADHs participate in mediating the synthesis of several aroma volatiles such as aldehydes and alcohols, thereby regulating stress responses and cellular ABA levels (Song *et al.,* 2017). Overexpression of *ADH1* in *Arabidopsis* plants alleviates salt, drought and cold stresses by promotion of sugar accumulation and activation of ABA-mediated signaling pathways (Shi *et al.,* 2017). Furthermore, several family members of ALDHs have recently been found to regulate the biosynthesis of acetic acid in plants (Rasheed *et al.,* 2018). Some members of ALDH family can convert the acetaldehyde to acetate during ethanol fermentation, and acetate can be subsequently converted into acetyl-CoA (Wei *et al.,* 2009, Brocker *et al.,* 2013, Stiti *et al.,* 2011). Activation of acetate-mediated pathways has been shown to enhance drought tolerance in various plant species by regulation of ABA- or JA-mediated signaling pathways (Rasheed *et al.,* 2018, Utsumi *et al.,* 2019). Regulation of JA signaling pathways is also an important mechanism in enhancing drought tolerance in plants (Ullah *et al.,* 2018). However, we found here that the *Trichoderma*-treated maize plants accumulated less JA levels than the non-inoculated plants under drought stress, suggesting that induction of ABA biosynthesis by *T. harzianum* primarily contributed to increasing drought resistance in maize plants. Moreover, virus-mediated silencing of *ZmADH1* or *ZmALDH12* notably weakened the effects of *T. harzianum* on drought resistance in the inoculated plants. Therefore, the *Trichoderma*-induced changes of acetic acid-ethanol pathways conferred the increased tolerance of host plants to drought stress.

Conclusion

In summary, the inoculation of maize plants with *T. harzianum* enhanced drought tolerance with higher ABA levels. *T. harzianum* induced the expression of *ZmADH1* and *ZmALDH12* in the maize plants, thereby effectively provoking the ABA-mediated signaling pathways. Moreover, virus-mediated silencing of these genes obviously reduced the levels of ABA, and thus weakened drought tolerance in the *Trichoderma*-inoculated plants. Collectively, our findings indicated that modulation of ethanol-acetic acid metabolic pathways by *T. harzianum* played a vital role in enhancing maize drought resistance.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (31600210), the China Postdoctoral Science Foundation (2017M620214), the Key Research Project of the Anhui Science and

Technology Committee (16030701102) and the Natural Science Foundation of Education Department of Anhui province (KJ2018ZD051).

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(Received for publication 5 December 2018)