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RESEARCH PAPER

Abscisic acid homeostasis is mediated by feedback regulation of *MdMYB88* and *MdMYB124*

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Abstract

The phytohormone abscisic acid (ABA) is involved in various plant processes. In response to drought stress, plants quickly accumulate ABA, but the regulatory mechanism of ABA accumulation is largely unknown, especially in woody plants. In this study, we report that MdMYB88 and MdMYB124 are myeloblastosis (MYB) transcription factors critical for ABA accumulation in apple trees (*Malus* x *domestica*) following drought, and this regulation is negatively controlled by ABA. MdMYB88 and MdMYB124 positively regulate leaf water transpiration, photosynthetic capacity, and stress endurance in apple trees under drought conditions. MdMYB88 and MdMYB124 regulate the expression of biosynthetic and catabolic genes of ABA, as well as drought- and ABA- responsive genes. MdMYB88 associates with promoter regions of the ABA biosynthetic gene 9-*cis*-epoxycarotenoid dioxygenase 3 (*NCED3*). Finally, expression of *MdMYB88* and *MdMYB124* is repressed by ABA. Our results identify a feedback regulation of MdMYB88 and MdMYB124 in modulating ABA homeostasis in apple trees.

Keywords: Abscisic acid homeostasis, apple, MdMYB88/MdMYB124, drought tolerance

Introduction

Abscisic acid (ABA) is a phytohormone that regulates various plant growth and development processes, including seed germination, seed maturation, dormancy, stomatal movement, senescence, and root growth. ABA also regulates plant responses to both biotic and abiotic stimuli. Under drought stress, the concentration of ABA in leaves increases rapidly to induce stomatal closure, so as to avoid water loss (Shinozaki and Yamaguchi-Shinozaki, 2000; Wilkinson and Davies, 2002; Shinozaki *et al.*, 2003; Chinnusamy *et al.*, 2004; Zhu, 2016). Besides ABA, the hydraulic function of plants also regulates stomatal movements (Hernandez-Santana *et al.*, 2016). It has been reported that the increase in root ABA concentration under drought

stress is correlated with increased root hydraulic conductivity (Thompson *et al.* 2007). Exogenous application of ABA to roots or ABA-overproducing transgenic plants increases root hydraulic conductance (Hose *et al.* 2000; Thompson *et al.* 2007). In barley (*Hordeum vulgare*), the roots of wild-type plants accumulate more ABA than the ABA-deficient barley mutant *Az34*, resulting in increased root hydraulic conductivity of wild-type plants and higher water movement from the roots. Therefore, wild-type plants are capable of maintaining higher leaf water potential and higher transpiration rates to respond to increases in air temperature (Veselov *et al.*, 2018).

The regulation of the concentration of ABA is mediated by a balance of ABA biosynthesis and catabolism. ABA is primarily produced by the *de novo* biosynthetic pathway from carotenoids, in which zeaxanthin is converted to all-*trans*-violaxanthin catalyzed by zeaxanthin epoxidase (ZEP/ABA1; Marin *et al.*, 1996). All-*trans*-violaxanthin is subsequently catalyzed sequentially by an unknown enzyme, 9-*cis*-epoxycarotenoid dioxygenase (NCED), dehydrogenase/reductase (SDR/ABA2) and aldehyde oxidase (AAO/AO), to ABA (Tan *et al.*, 1997; Burbidge *et al.*, 1999; Chernys and Zeevaart, 2000; Iuchi *et al.*, 2001; Qin and Zeevaart, 2002; Cheng *et al.*, 2002; Gonzalez-Guzman *et al.*, 2002; Seo *et al.*, 2004). A second biosynthetic pathway occurs via two glucosidases in Arabidiopsis, β-glucosidase homolog 1 and 2 (BG1 and BG2), which catalyze the hydrolysis of Glc-conjugated ABA (abscisic acid-glucose ester [ABA-GE]) to ABA (Lee *et al.*, 2006; Xu *et al.*, 2012; Chen *et al.*, 2020). In Arabidopsis, ABA catabolism is regulated by hydroxylation and conjugation. Hydroxylation is mediated by the members of cytochrome P450 protein family (CYP707A1 to CYP707A4). Conjugation is regulated by uridinediphosphate glucosyltransferases (UGT71B6, UGT71C5, UGT71B7, and UGT71B8; Xu *et al.*, 2002; Kushiro *et al.*, 2004; Saito *et al.*, 2004; Okamoto *et al.*, 2006; Priest *et al.*, 2006; Dong *et al.*, 2014; Liu *et al.*, 2015).

A few transcription factors have been identified to transcriptionally regulate genes involved in ABA metabolism. The bZIP transcription factor VirE2-interacting protein 1 (VIP1) directly binds to DNA fragments of the *CYP707A1* and *CYP707A3* promoters and enhances their expression (Tsugama *et al.*, 2012). Another transcription factor, basic helix-loop-helix 122 (bHLH122), directly represses *CYP707A3* (Liu *et al.*, 2014). SHORT VEGETATIVE PHASE (SVP), a MADS-box transcription factor, negatively regulates the expression of *CYP707A1* and *CYP707A3*, but positively controls the *AtBG1* gene by associating with their promoter regions (Wang *et al.*, 2018).

Drought stress is one of the adverse environmental conditions restricting fruit crop production and quality. To breed drought-tolerant fruit crops, traditional and biotechnological approaches (e.g., marker-assisted selection and genetic transformation) have been applied (Marguerit *et al.*, 2012; Wang *et al.*, 2012; Cao *et al.*, 2013; Wang *et al.*, 2014; Li *et al.*, 2015; Virlet *et al.*, 2015; Liu *et al.*, 2018; Sun *et al.*, 2018). However, traditional breeding is time- and labor-consuming due to the long juvenile period of fruit trees. Biotechnological approaches have proved to be feasible for improving drought resistance in perennial woody plants (Cao *et al.*, 2013; Wu *et al.*, 2016; Liao *et al.*, 2017; Sun *et al.*, 2018; Ma *et al.*, 2019); however, a thorough understanding of molecular responses to drought stress is still needed here.

MYB transcription factors are reportedly involved in various plant processes, including primary and secondary metabolism, cell fate and identity, developmental processes, and responses to biotic and abiotic stresses (Dubos *et al.*, 2010). Numerous *MYB* genes have been characterized for their roles in response to drought stress (Baldoni *et al.*, 2015). The apple tree (*Malus* x *domestica*) genome contains 229 *MYB* genes, many of which are responsive to various abiotic stresses, indicating the potential participation of these genes in apple stress resistance. Overexpression of one of these *MYB* genes, *MdoMYB121*, remarkably enhances apple tree resistance to high salinity, drought, and cold stress (Cao *et al.*, 2013). In addition, overexpression of *MdSIMYB1* increases apple tree resistance to polyethylene glycol treatment (PEG; Wang *et al.*, 2014). Previously, we characterized the positive roles of MdMYB88 and its paralog MdMYB124 in improving freezing tolerance of apple trees (Xie *et al.*, 2018). We further revealed that both MdMYB88 and MdMYB124 mediate the drought resistance of apple roots by regulating root xylem development and secondary cell wall formation (Geng *et al.*, 2018). However, it is less certain whether both genes are involved in ABA homeostasis.

Here, we provide evidence that MdMYB88 and MdMYB124 enhance ABA accumulation under control and drought conditions, and this accumulation is negatively mediated by ABA. Our results highlight the roles of MdMYB88 and MdMYB124 in ABA homeostasis in perennial apple trees, thereby providing genetic determinants for apple breeding in the future.

Materials and methods

Plant materials, growth conditions, and stress treatment

For gene cloning, domesticated *M.* x *domestica* 'Golden Delicious' apple trees grown in a greenhouse were used for RNA extraction. *MdMYB88/124* RNAi plants, *MdMYB88* or *MdMYB124*-overexpressing plants were previously generated (Xie *et al.*, 2018). GL-3, a seedling selected from *M.* x *domestica* 'Royal Gala', was used as the genetic background to generate transgenic apple plants (Dai *et al.*, 2013). Because of the sequence similarity of MdMYB88 and MdMYB124, transcripts of both *MdMYB88* and *MdMYB124* were reduced in *MdMYB88/124* RNAi plants (Xie *et al.*, 2018).

Transgenic apple and GL-3 plants were rooted in half-strength Murashige and Skoog (MS) medium (2.215 g l^{-1} MS salts, 20 g l^{-1} sucrose, and 7 g l^{-1} agar, pH 5.8), supplemented with 0.5 mg l^{-1} indole-3-butytric acid (IBA) and 0.5 mg l^{-1} indoleacetic acid (IAA) under dark conditions for 3 d, and then held under long-day conditions (14 h light/ 10 h dark) for an additional 45 d. Then they were transplanted into soil and grown in a light growth chamber with 60% humidity and light intensity of 8000 lux under long-day conditions. After two months, transgenic apple and GL-3 plants were transplanted to garden pots (43.5 cm × 20 cm × 11 cm,

length × width× depth) with 4.5 kg soil (peat to vermiculite in a ratio of 3:1) for an additional month. Overexpression or RNAi plants were grown together with GL-3 in the same pots, and the position of each plant in each pot was random. Alternatively, GL-3, overexpression, and RNAi plants were grown individually in separate pots. Drought treatment was carried out by withholding water for 30 d. The soil relative water content (SRWC) was measured every two days at 18:00, gravimetrically (Li *et al.*, 2019) (Supplementary Fig. S1 at *JXB* online).

SRWC (%) = 100 × [(Maximum water content – Pot weight) / (Maximum water content – Dry weight of soil)]

The photosynthetic parameters (Gs, E, An, and WUEi) were measured with a LiCOR-6400 portable photosynthesis system (LI-COR, Nebraska USA) when SRWC was 75–85% (day 0), 45–55% (day 12), and 25%-35% (day 18), respectively. The environmental conditions were as follows: light intensity was 1000 μmol m^{-2} s^{-1}, CO_2 concentration was 450±10 cm^3 m^{-3}, the leaf temperature was 25±2 °C, and the relative humidity of the sample cell was 22±2%. Fifteen plants (a pair of transgenic/non-transgenic plants planted in the same pot) or 21 plants (plants of each treatment planted in single pots) of each genotype were used for measurement of photosynthetic parameters.

To examine the survival rate, GL-3 and transgenic plants were treated with drought stress with the methods described above. After treatment, plants were re-watered and allowed to recover for 11 d to calculate survival rate. Thirty-six plants (a pair of transgenic/non-transgenic plants planted in the same pot) or 21 plants (plants of each treatment planted in single pots) of each genotype were used and each 12 (a pair of transgenic/non-transgenic plants planted in the same pot) or seven (plants of each treatment planted in single pots) plants were used as a biological replication, respectively.

PEG treatment was carried out using hydroponically cultured plants (Geng *et al.*, 2019). Briefly, rooted plants were transferred to soil for two months and then to 1/2 Hoagland solution. After one month, PEG6000 (Sigma, USA) was added to the solution to a final concentration of 20% (w/v) for 6 h. Leaves and roots were collected for RNA (27 plants were collected and each nine plants were used as a biological replication, three replicates were used) or ABA (five plants of each genotype were collected) extraction.

To examine the expression of *MdMYB88* and *MdMYB124* under ABA treatment, 'Golden Delicious' plants were sprayed with 100 μM ABA for 0, 1, or 3 h, and the leaves were collected for RNA extraction. Twenty-seven plants were collected and from this nine plants were used as a biological replication (three replicates were used). For expression of *MdMYB88* and *MdMYB124* under drought treatment, water was withheld for 6 d from 'Golden Delicious' plants. The mature leaves (fourth, fifth and sixth leaves) were collected on 0, 2, 4, and 6 d after drought and used for RNA extraction. For water loss experiments, leaves of three-month-old plants were detached and air dried. Water loss was calculated based on the weight after dehydration for 30, 60, 120, 240, and 360 min. Fifteen plants of each genotype were collected. Ion leakage of leaves under control and drought (withholding water for 7 d) conditions was measured using the methods described previously (Xie *et al.*, 2018).

Measurement of leaf relative water content

The leaf relative water content (LRWC) was determined as described previously (Li *et al.* 2019). Plant leaf water status was measured when SRWC was 75–85% (control), 45–55% (moderate), 25%-35% (severe), or after dehydration for 0 h and 2 h. Nine leaves from three plants were collected randomly, weighed quickly and then transferred to deionized water overnight to measure turgid weight. Leaves were then dried and weighed to measure dry weight.

LRWC (%) = 100 × [(fresh weight – dry weight) / (turgid weight – dry weight)].

Measurement of leaf water potential

Leaf water potential (Ψ_{leaf}) was measured with young leaves (one leaf per plant was collected, and nine plants of each genotype were used) using a Model 600 Pressure Chamber, as described by the manufacturer (PMS Instrument Company, USA).

Measurement of leaf hydraulic conductivity

Leaf hydraulic conductivity (K_{leaf}) of both transgenic and non-transgenic plants was performed with a high pressure flow matter (HPFM; Dynamax, Houston, USA) as described by Geng *et al.* (2018). In brief, after drought treatment, leaves were soaked in de-gassed water and connected to HPFM. Leaf hydraulic conductivity was measured using a quasi steady-state method in accordance with the HPFM manual. Nine plants of each genotype were used.

RNA extraction and qRT–PCR analysis

Total RNA from apple leaves was extracted by the cetyltrimethylammonium bromide (CTAB) method (Chang *et al.*, 1993), and then treated with RNase-free DNase I (Fermentas, USA) at 37 °C for 30 min to eliminate residual DNA. About 2 μg RNA was then used for reverse transcription with a RevertAid First Strand cDNA Synthesis Kit (K1622; Thermo Fisher Scientific, MA, USA). Quantitative reverse transcription PCR (qRT–PCR) was performed on an Applied Biosystem Step One Plus™ instrument (Life Science, USA), using a ChamQ™ SYBR® qPCR Master Mix (Vazyme, China) according to the manufacturer's instructions. The malate dehydrogenase (*MdMDH*) gene in apple trees was used as a reference gene. Primers used for qRT–PCR analysis are listed in Supplementary Table 1 (available at *JXB* online). Three replicates were used for each sample and relative quantification was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

RNA-seq analysis

Plant leaves were collected from two-month-old GL-3 and transgenic apple plants and dehydrated for 0 h and 2 h. Three biological replicates were performed for each experiment, with five leaves used per replicate per treatment. Total RNA was used for qRT–PCR and library construction, according to the manufacturer's instructions [NEBNext® UltraTM RNA Library Prep Kit for Illumina® (#E7530L, NEB, USA)]. Libraries were sequenced in an Illumina NovaSeq 6000 platform with a PE 150 sequencing run and the pair-end data of 44.5 million reads per sample were generated (Annoroad, Beijing, China). After removing the adaptors and low-quality reads, the remaining reads were mapped to the reference genome of *Malus* × *domestica* 'Golden Delicious' (GDDH13 v1.1, https://iris.angers.inra.fr/gddh13/) using HISAT2 version 2.1.0 (Kim *et al.*, 2015) with default parameters. The HTSeq version 0.11.0 (Anders *et al.*, 2015) was then used to quantify the read counts per gene. Differentially expressed genes (DEGs) between the drought-treated and control samples were identified using DEseq2 R packages (Love *et al.*, 2014) based on the read counts with the adjusted Q value <0.05 and 1.5-fold change in gene expression. Gene ontology (GO) annotation and enrichment analyses were conducted using the online tools agriGO (http://bioinfo.cau.edu.cn/agriGO/; Tian *et al.*, 2017) and KOBAS (http://kobas.cbi.pku.edu.cn/index.php; Ai and Kong, 2018).

Measurement of stomatal apertures

For stomatal aperture measurements, we used leaves of transgenic apple and GL-3 plants grown in soil for two months. Leaves were cut off and plunged into stomatal opening solution (30 mM KCl, 0.1 mM $CaCl_2$, and 10 mM MES-KOH, pH 6.15) under light (120 μmol m^{-2} s^{-1}) for 2 h,

as described previously in Arabidopsis, to induce stomatal opening (Kwak *et al.*, 2001). Subsequently, ABA was added to the stomatal opening solution to a final concentration of 5 μM. Stomata were observed in leaf strips (obtained by using tweezers) with an EX30 microscope (SDPTOP, China) after ABA treatment for 1 h. Stomatal apertures were measured using ImageJ (National Institutes of Health, USA) software.

ChIP–qPCR

ChIP–qPCR assay was performed as previously described (Xie *et al.*, 2018). Leaves from GL-3 plants with or without drought treatment for 0 d and 6 d were used for cross-linking, and the ChIP assay was performed with an anti-MdMYB88/MdMYB124 antibody (Genscript, USA). The antibody specificity is provided in Supplementary Fig. S2 and a specific 53 kDa protein band was detected using anti-MdMYB88/MdMYB124 antibody in immunoblots of plant extracts (An *et al.*, 2017). Primers used for ChIP-qPCR are listed in Supplementary Table S1.

Electrophoretic mobility shift assay (EMSA)

A MdMYB88 protein was previously generated (Xie *et al.*, 2018). An EMSA assay was performed according to the manual of LightShift Chemiluminescent EMSA Kit (#89880; Thermo Scientific, Waltham, MA, USA). The oligonucleotide probes labeled with biotin are listed in Supplementary Table S1.

Measurement of abscisic acid content

ABA was extracted as described (Müller and Munné-Bosch, 2011). About 100 mg of fresh leaves or roots was ground in liquid nitrogen into a powder and then extracted with 500 μl of cold extraction buffer (methanol: isopropanol: acetic acid=20: 79: 1, v: v: v), followed by vortexing for 5 min. After centrifugation at 4 °C at 18 514 × *g* for 10 min, the supernatant was collected and the pellet was re-extracted with 500 μl of cold extraction buffer. The extraction process was repeated three times and a constant amount of internal standard ([$^2H_6$](+)-*cis*, *trans*-ABSCISIC ACID (^{2}H-ABA)) was added. Finally, the combined supernatant was filtered through a 0.22 μm PTFE filter (Shimadzu, Kyoto, Japan). Eight standard ABA (Sigma-Aldrich, Steinheim, Germany) solutions were prepared ranging from 0.5 to 100 ng ml^{-1}. Samples were then analyzed by QTRAP® 5500 LC-MS/MS (AB SCIEX, Redwood City, USA). Gradient elution was performed with solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid) at the following flow rate: 0–2 min, B=20%; 2–6 min, B increased to 90%; 6.1–11 min, B=90%; 11–12 min, B decreased to 20%; 12–15 min, B=20%. Experiments were conducted in negative ionization mode. The capillary voltage was −4.5 kV and temperature was 400°C. The parameter of de-clustering potential was −60V and collision energy was −14 V (153.3) and −27 V (204.2). Five independent replicates were used for each treatment. Data were analyzed and processed using Multiquant software (AB SCIEX, USA).

Dual-luciferase assay

The assay was carried out as described previously (Xie *et al.*, 2018). The CDS of *MdMYB88* was cloned into pGreen II 62-SK (Hellens *et al.*, 2005), then the vector was transformed into *Agrobacterium tumefaciens* GV3101 (effector). The promoter of *MdNCED3* was cloned into a pGreen II 0800 vector (Hellens *et al.*, 2005), to drive the expression of the firefly luciferase reporter gene. The vectors were each co-transformed with the helper plasmid pSoup19 into GV3101 (reporter). The reporter and effector were then mixed together in a 2:3 volume ratio to transform *Nicotiana benthamiana* leaves. The empty pGreen II 62-SK vector was used as a negative control. The constitutive 35S promoter driving the expression of Renilla luciferase was used as an internal reference. Ten biological repeats were measured for each sample. A dual-luciferase assay was performed using a Dual-Luciferase® Reporter (DLR™) assay system kit according to the manufacturer's instructions (Promega, USA). Primers used for dual-luciferase assay are listed in Supplementary Table S1.

Quantification and statistical analysis

For all experiments, results are shown as means±SD and statistical significance was determined by one-way ANOVA (Tukey's test) analysis using SPSS (version 21.0, USA). Variations were considered significant if $P<0.05$, 0.01, or 0.001.

Accession numbers

Sequence data can be found under the following accession numbers at NCBI: MdMYB88 (KY569647), MdMYB124 (KY569648), MdNCED3 (XM_008380174.2), MdCYP707A1 (XM_008383813.2), MdCYP707A2 (XM_008358695.2), MdCYP707A4 (XM_008395589.3). MdUGT71B6 (NM_001328974.1), SPOTTED LEAF3 (SPL3) (XM_008385482.3), nuclear factor Y, subunit A7 *(NF-YA7)* (XM_029105023.1), homeobox protein 6 (*HB6*) (XM_008340338.3), homeobox protein 7 (*HB7*) (MG149566.1), plant U-box 9 (*PUB9*) (XM_008350751.3), histidine kinase 3 (AHK3) (XM_029096232.1), outer plastid envelope protein 16 (*OEP16*) (XM_029088417.1).

Results

MdMYB88 and MdMYB124 act as positive regulators for drought tolerance in apple trees

Previously, we characterized the function of MdMYB88 and MdMYB124 in response to drought stress in apple roots (Geng *et al.*, 2018). We observed that *MdMYB88* and *MdMYB124* were also drought-inducible in apple leaves (Fig. 1A). This led us to examine the involvement of MdMYB88 and MdMYB124 in drought tolerance by examining phenotypes of the aboveground portions of apple trees. We first measured the relative water content of GL-3 and *MdMYB88/124* RNAi plants that we generated previously (Xie *et al.*, 2018). We found that *MdMYB88/124* RNAi plants lost more water under dehydration conditions, especially 6 h after dehydration (Fig. 1B). We then investigated their ability to survive under drought stress. After 30 d of drought treatment followed by 11 d of recovery, only 6–8% of *MdMYB88/124* RNAi plants survived, while 24% of non-transgenic GL-3 plants were still alive (Fig. 1C, D). Additionally, compared to GL-3 plants, *MdMYB88/124* RNAi plants had lower photosynthetic rate (An), stomatal conductance (Gs), rate of transpiration (E) and instantaneous water-use efficiency (WUEi) when treated with drought for 12 d and 18 d (Fig. 1E-H). Furthermore, the extent of ion leakage in *MdMYB88/124* RNAi plants was markedly higher than that of GL-3 plants after 7 d of drought exposure (Supplementary Fig. S3A).

We also tested the drought tolerance of *MdMYB88*-overexpressing (OE) plants and *MdMYB124* OE plants. Compared to GL-3 plants, *MdMYB88* OE and *MdMYB124* OE plants were more tolerant to drought when irrigation was withheld for 30 d (Fig. 2A). After a 11 d re-watering period,

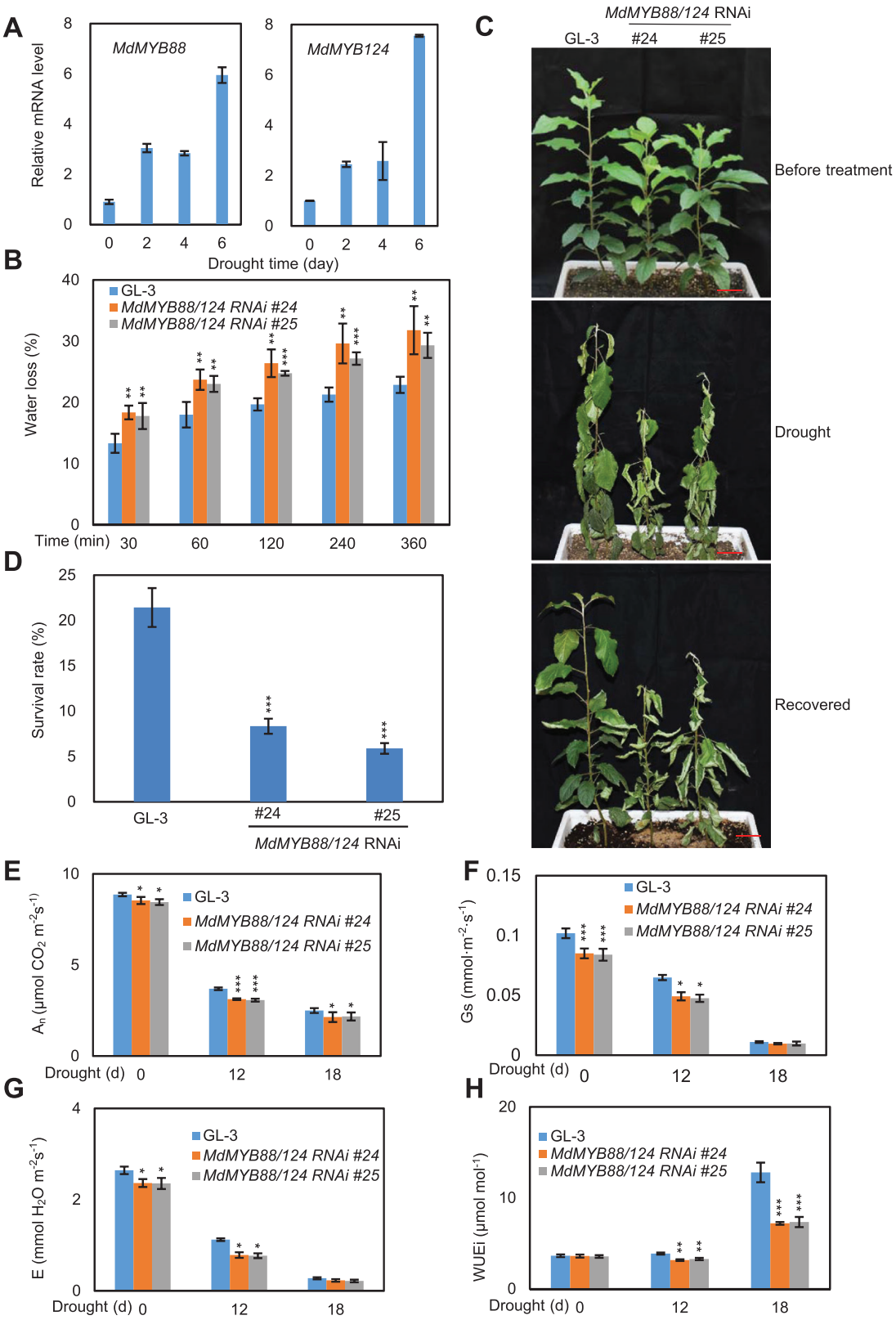

Figure 1. *MdMYB88/124* RNAi plants are sensitive to drought. (A) Expression of *MdMYB88* and *MdMYB124* in 'Golden Delicious' (*Malus* x *domestica*) under drought stress for 0, 2, 4, and 6 d. Data are mean ±SD (n=3). (B) Water loss of detached leaves at 25°C . Data are mean±SD (n=15). Leaves were detached from fifteen plants. (C) Drought tolerance of GL-3, two independent *MdMYB88/124* RNAi lines. Bars=5 cm. Five-month-old plants were treated with drought for 30 d, and then re-watered for 11 d. (D) Survival rate of GL-3 and two independent *MdMYB88/124* RNAi lines under drought stress. Thirty-six plants were collected and 12 plants were used in a biological replication, for three replicates. (E-H) The rate of photosynthesis (E), stomatal conductance (F), rate of transpiration (G), and instantaneous water-use efficiency (H) of GL-3 and two independent *MdMYB88/124* RNAi lines under drought stress. Data are mean±SD (n=15). One-way ANOVA (Tukey's test) was performed and statistically significant differences are indicated by * (P<0.05), ** (P<0.01) or *** (P<0.001). (This figure is available in color at *JXB* online.)

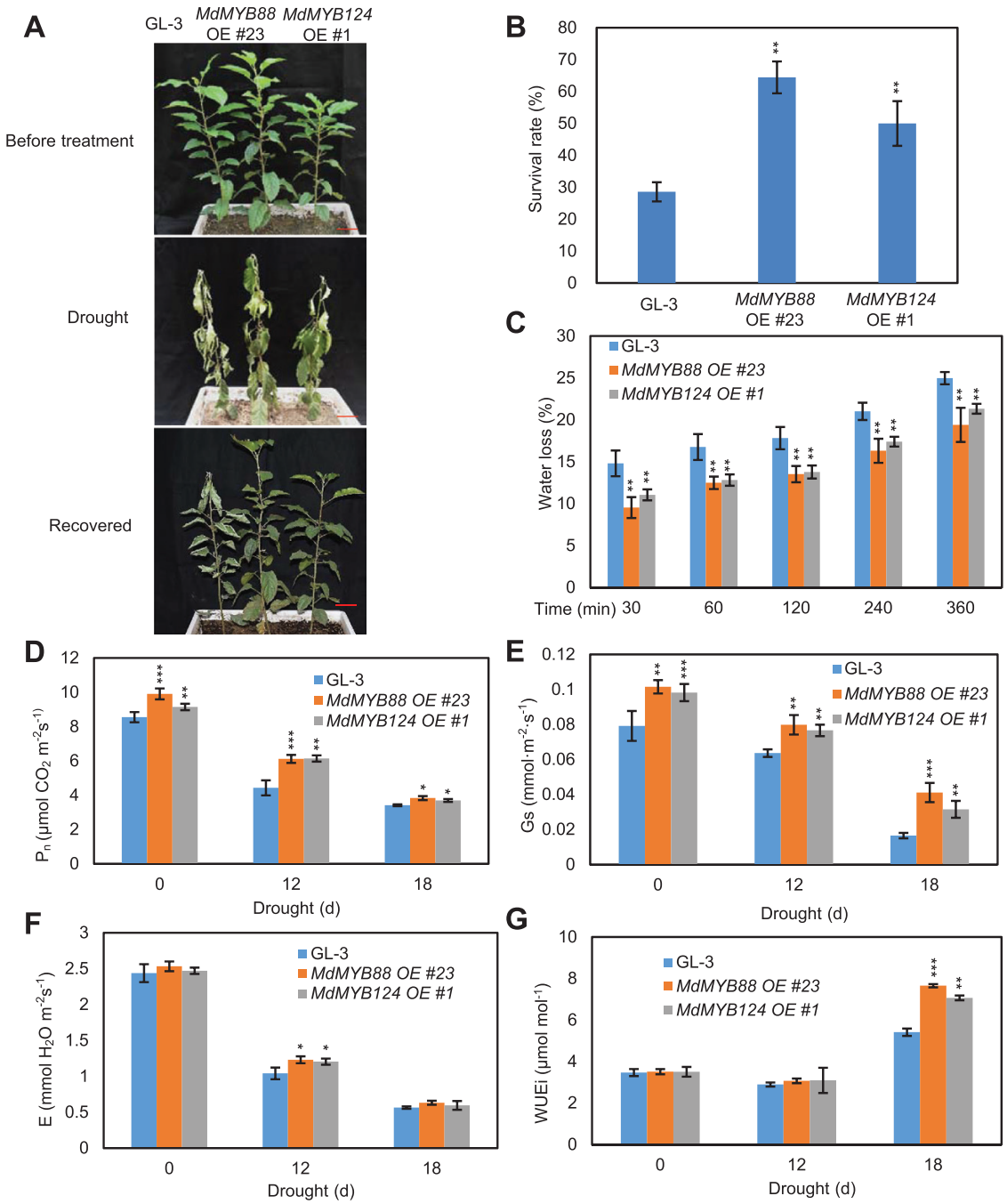

Figure 2. *MdMYB88* and *MdMYB124* OE plants are tolerant to drought. (A) Drought resistance of GL-3 and transgenic *MdMYB88, MdMYB124* OE plants. Bars=5 cm. Five-month-old plants were treated with drought for 30 d, and then re-watered for 11 d. (B) Survival rate of GL-3 and transgenic *MdMYB88, MdMYB124* OE plants shown in (A). Thirty-six plants were collected and 12 plants were used in a biological replication, for three replicates. (C) Water loss of detached leaves from GL-3 and transgenic *MdMYB88, MdMYB124* OE plants. Data are mean±SD (n=15). Leaves were detached from fifteen plants. (D-G) The rate of photosynthesis (D), stomatal conductance (E), rate of transpiration (F), and instantaneous water-use efficiency (G) of GL-3 and transgenic *MdMYB88, MdMYB124* OE plants under drought stress. Data are mean±SD (n=15). One-way ANOVA (Tukey's test) was performed and statistically significant differences are indicated by * (P<0.05), ** (P<0.01) or *** (P<0.001). (This figure is available in color at *JXB* online.)

50–60% *MdMYB88* OE and *MdMYB124* OE plants recovered, while only 28% GL-3 plants survived (Fig. 2B). Dehydration experiments showed that detached leaves of *MdMYB88* OE and *MdMYB124* OE plants lost less water than GL-3 plants (Fig. 2C; Supplementary Fig. S3B). Moreover, *MdMYB88* OE and *MdMYB124* OE plants performed better than GL-3 plants when examining An, Gs, E and WUEi, after being deprived of water for 12 d and 18 d (Fig. 2D-G). In addition, ion

leakage assays revealed that the cell membranes of *MdMYB88* OE and *MdMYB124* OE plants were less damaged than those of GL-3 plants under drought stress (Supplementary Fig. S3A). To further confirm our results, we planted the individual non-transgenic and transgenic plants (different lines) in different pots and examined their drought responses under the same soil water content. The results were similar to those shown in Figs. 1 and 2 (Supplementary Figs. S4, S5).

To further validate the drought responses, we measured the leaf relative water content (LRWC) in GL-3 and transgenic plants when SRWC was 75–85% (control), 45–55% (moderate), and 25%-35% (severe). Results showed that LRWC of the overexpression plants was higher than that of GL-3, while LRWC of RNAi plants was lower under drought (Supplementary Fig. S6). We also measured the leaf water potential (Ψ_{leaf}) and leaf hydraulic conductance (K_{leaf}) of GL-3 and transgenic plants under drought (SRWC was 45–55%; Supplementary Fig. S7). The K_{leaf} and Ψ_{leaf} of the *MdMYB88* OE lines were higher than those of GL-3, while *MdMYB88/124* RNAi lines had lower K_{leaf} and Ψ_{leaf} than GL-3 plants, under control and drought conditions (Supplementary Fig. S7).

Together, our results suggest that MdMYB88 and MdMYB124 play positive roles in drought tolerance in apple trees.

MdMYB88 and MdMYB124 regulate the expression of drought-responsive genes

To further understand the molecular function of MdMYB88 and MdMYB124 in drought tolerance of apple trees, we performed RNA-seq analysis on dehydrated GL-3 and *MdMYB88/124* RNAi leaves. RNA-seq data showed that the expression of 4554 genes were induced, while 3101 genes were repressed in GL-3 leaves after 2 h of dehydration (using both Q value of <0.05 and 1.5-fold as a cutoff; Supplementary Table S2). Compared with GL-3 leaves, the expression of 273 genes increased, while the expression of 276 genes decreased in *MdMYB88/124* RNAi plants under dehydration conditions (Supplementary Table S3). Under control conditions, only 52 genes were up-regulated by MdMYB88 and MdMYB124, while 69 genes were down-regulated (Supplementary Table S4). Additionally, 55 out of 276 genes were dehydration-inducible, whereas 15 out of 273 genes were repressed by dehydration. These data indicate that MdMYB88 and MdMYB124 regulate the expression of drought-responsive genes. A gene ontology (GO) enrichment analysis suggested that the differentially expressed genes (DEGs) in *MdMYB88/124* RNAi plants were remarkably enriched with those that respond to stimuli and ABA, and those involved in the phenylpropanoid biosynthetic process were enriched under control and dehydration conditions. These results suggest the potential roles of MdMYB88 and MdMYB124 in the ABA response and secondary metabolite accumulation (Fig. 3; Supplementary Fig. S8).

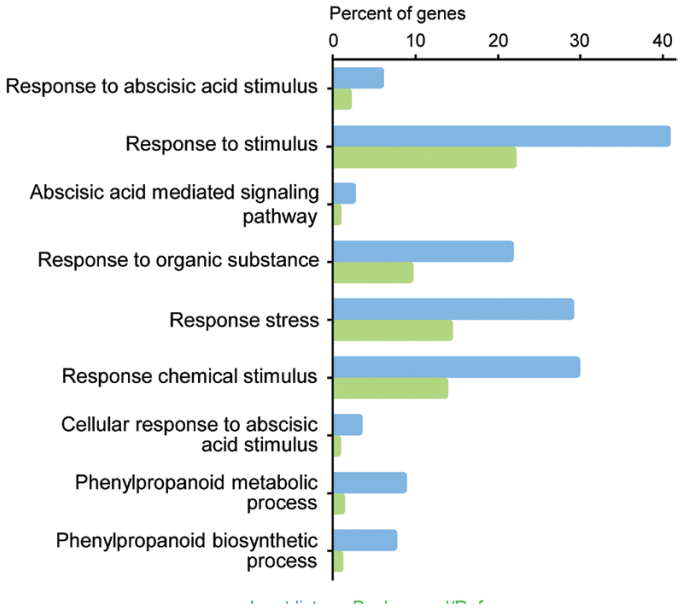

Figure 3. GO enrichment analysis of differently expressed genes in *MdMYB88/124* RNAi plants versus GL-3 plants under dehydration conditions. The percentage of genes is mapped by the GO term, and represents the abundance of the term. Blue bars are the percentage for the input list which is calculated by the number of genes mapped to the GO term divided by the number of all genes in the input list. The green bars are the same calculation applied to the background list percentage. (This figure is available in color at *JXB* online.)

To verify the genes regulated by MdMYB88 and MdMYB124, we selected nine genes and performed qRT–PCR analysis using GL-3, *MdMYB88/124* RNAi, *MdMYB88* OE, and *MdMYB124* OE plants under control and dehydration conditions. The expression patterns for seven out of nine genes were confirmed (Fig. 4; Supplementary Table S3), suggesting the reliability of the RNA-seq data. Our RNA-seq and qRT–PCR data suggest that MdMYB88 and MdMYB124 positively regulate the expression of *MdSPL3, MdNF-YA7, MdHB6, MdHB7, MdOEP16,* and *MdPUB9*, but negatively modulate the expression of *MdAHK3* under dehydration conditions (Fig. 4; Supplementary Table S3). Among these seven genes, *SPL3*, *HB7*, *NF-YA7*, and *OEP16* have been identified as positive regulators for drought stress resistance in Arabidopsis or wheat, while AHK3 is a negative regulator (Tran *et al.*, 2007; Pudelski *et al.*, 2012; Valdés *et al.*, 2012; Lee *et al.*, 2015; Wang *et al.*, 2015; Zang *et al.*, 2017).

MdMYB88 and MdMYB124 mediate abscisic acid accumulation following drought

ABA content is regulated by both ABA biosynthetic genes and catabolic genes. *NCED3* catalyzes the rate-limiting step in *de novo* ABA biosynthesis (Tan *et al.*, 1997; Burbidge *et al.*, 1999; Chernys and Zeevaart, 2000; Iuchi *et al.*, 2001; Qin and Zeevaart, 2002), whereas *UGT71B6* and *CYP707A1-A4* catalyze ABA to form ABA-GE (Xu *et al.*, 2002; Kushiro *et al.*, 2004; Okamoto *et al.*, 2006; Priest *et al.*, 2006; Dong *et al.*,

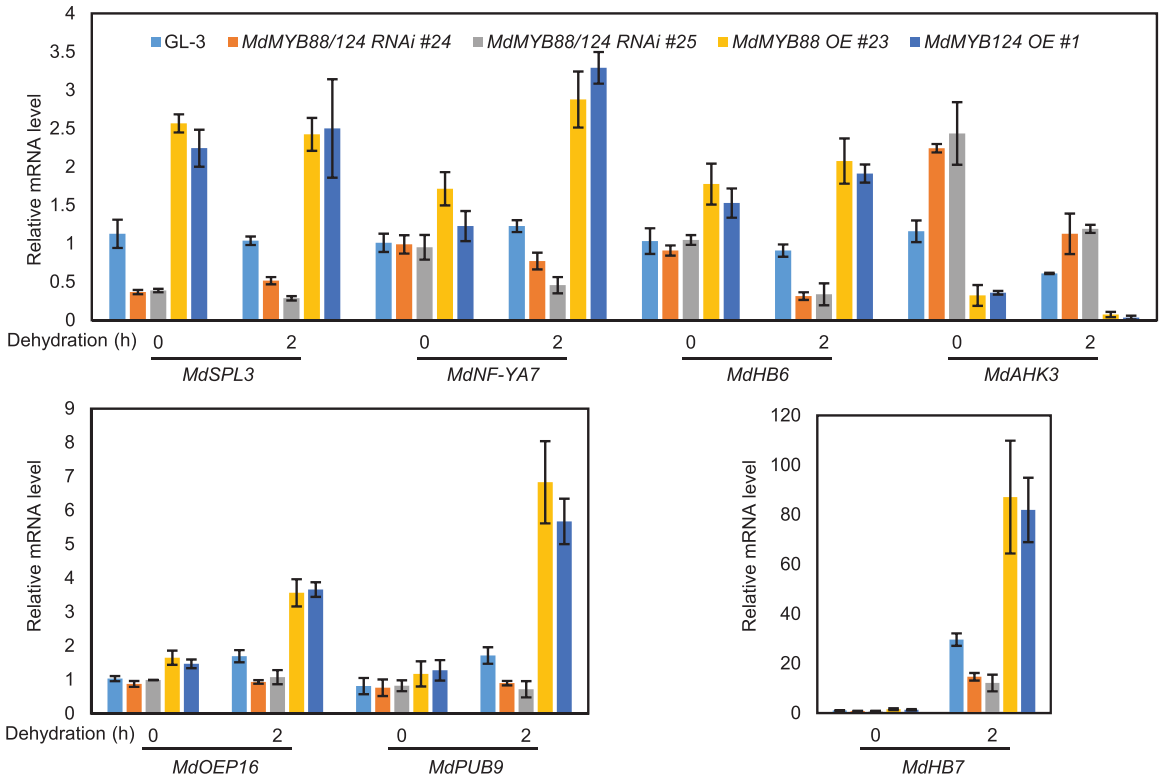

Figure 4. Verification of RNA-seq data. Leaves detached from two-month-old soil-grown GL-3, two independent *MdMYB88/124* RNAi lines , *MdMYB88* OE, and *MdMYB124* OE plants were dehydrated for 0 h or 2 h. Error bars indicate standard deviation (n=3). (This figure is available in color at *JXB* online.)

2014; Liu *et al.*, 2015). From the RNA-seq data, we found that the gene homologous to *UGT71B6* in Arabidopsis was negatively regulated by *MdMYB88* and *MdMYB124* (Fig. 5A, Supplementary Table S3). We also investigated the expression of *MdNCED3*, *CYP707A1*, *A2*, and *A4* in GL-3, as well as in *MdMYB88* and *MdMYB124* transgenic plants. We found that under control and dehydration conditions, expression of *MdNCED3* was much lower in GL-3 and *MdMYB88/124* RNAi plants, but higher in *MdMYB88* OE and *MdMYB124* OE plants (Fig. 5B). *MdCYP707A4* expression was increased in *MdMYB88/124* RNAi plants compared with GL-3 plants, but was reduced in *MdMYB88* OE and *MdMYB124* OE plants under dehydration conditions (Fig. 5C). However, no changes in expression of *MdCYP707A1* and *MdCYP707A2* were detected between GL-3 and transgenic plants under control or dehydration conditions (Supplementary Fig. S9).

We then sought to determine if MdMYB88 and MdMYB124 were involved in ABA accumulation under drought stress. ABA was measured using LC-MS/MS in GL-3 and transgenic plants, under control or dehydration conditions. As shown in Fig. 5D, ABA accumulated rapidly and substantially in leaves after dehydration for 2 h (Fig. 5D). Similarly, severe drought treatment induced ABA accumulation in all plant leaves (Fig. 6). ABA concentrations in *MdMYB88/124* RNAi plant leaves were much lower than those of GL-3 plants under dehydration and drought stress conditions, whereas *MdMYB88* OE and *MdMYB124* OE plant leaves contained more ABA than GL-3 plants under dehydration and severe drought stress (Figs. 5D, 6).

The above data suggest that *MdMYB88* and *MdMYB124* positively regulate ABA accumulation under drought stress, and this regulation could be achieved by the integration of the function of ABA biosynthetic and catabolic genes.

MdMYB88 directly regulates MdNCED3 *expression*

MdMYB88 and MdMYB124, as transcription factors, can bind to *cis*-elements in the promoter regions of their direct target genes. To determine whether MdMYB88 and MdMYB124 directly regulate promoters of these ABA biosynthetic and catabolic genes, we analyzed promoter sequences of *MdNCED3*, *MdUGT71B6* and *MdCYP707A4*. We identified MdMYB88 and MdMYB124 binding sites in three regions of the *MdNCED3* promoter (Supplementary Fig. S10): −1830 bp to −1826 bp, −1368 bp to −1364 bp and −880 bp to −876 bp. A ChIP–qPCR analysis using anti-MdMYB88/124 antibody suggested that MdMYB88 and MdMYB124 can bind to the promoter region of *MdNCED3* from −880 bp to −876 bp. Moreover, after drought stress the enrichment of MdMYB88/MdMYB124 on the *MdNCED3* promoter was

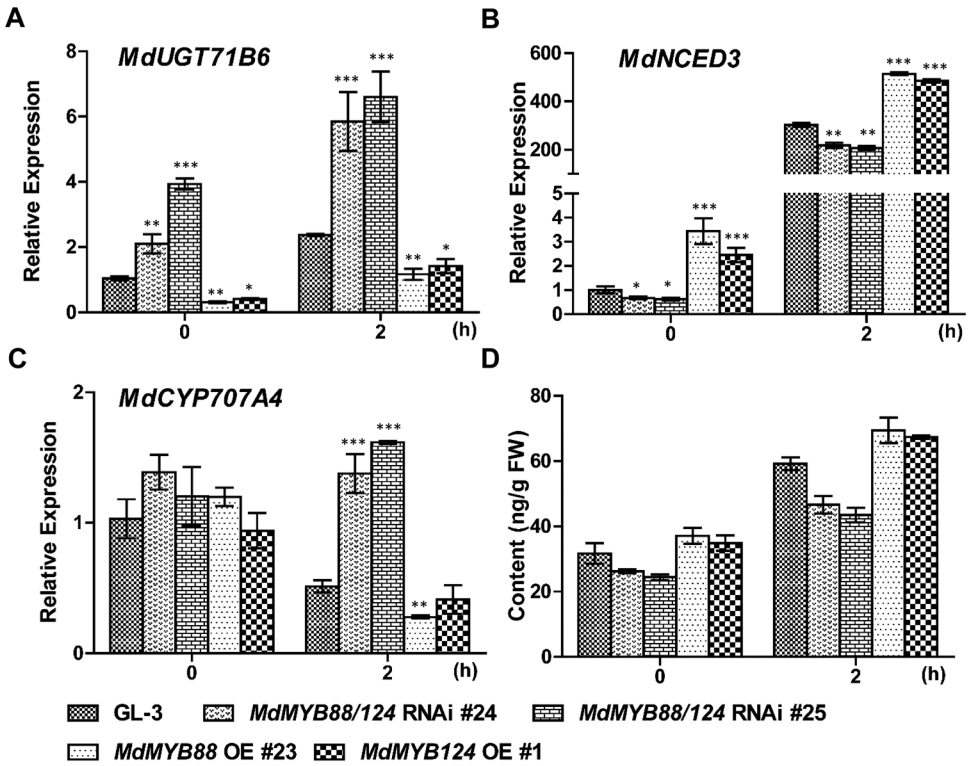

Figure 5. MdMYB88 and MdMYB124 modulate ABA accumulation after dehydration by regulating expression of ABA biosynthetic and catabolic genes. (A-C) Relative expression of *MdUGT71B6*, *MdNCED3*, and *MdCYP707A4* in GL-3, *MdMYB88/124* RNAi, and overexpressing (OE) plants under control or dehydration conditions. (D) ABA concentrations in GL-3 and transgenic plants under control or dehydration conditions. One-way ANOVA (Tukey's test) was performed and statistically significant differences were indicated by ** (P<0.05), ** (P<0.01) or *** (P<0.001). Error bars indicate standard deviation (n=3 in A-C; n=5 in D).

enhanced (fragment c in Fig. 7A, B). An EMSA assay confirmed this direct binding by MdMYB88 on fragment c (Fig. 7C). Dual-luciferase assay further verified the positive regulation of *MdNCED3* by MdMYB88 (Fig. 7D). However, we did not identify MdMYB88 and MdMYB124 binding sites in the promoter regions of the two genes (*MdUGT71B6* and *MdCYP707A4*) we tested (Supplementary Fig. S11). These results suggest that MdMYB88 and MdMYB124 regulate ABA accumulation under drought conditions through direct activation of *MdNCED3* transcription, while they may indirectly repress the expression of *MdUGT71B6* and *MdCYP707A4*.

Abscisic acid-induced stomatal responses in MdMYB88 *and* MdMYB124 *transgenic plants*

The involvement of MdMYB88 and MdMYB124 in ABA accumulation suggested their involvement in stomatal movements. To test this hypothesis, we examined the sensitivity to ABA-induced stomatal closure in GL-3, *MdMYB88/124* RNAi, *MdMYB88* OE, and *MdMYB124* OE transgenic plants. When treated with 5 μM ABA for 1 h, *MdMYB88/124* RNAi plants were slightly less sensitive to ABA-induced stomatal closure, while *MdMYB88* OE and *MdMYB124* OE plants displayed a strong sensitivity to ABA-induced stomatal closure, compared with GL-3 plants (Supplementary Fig. S12.

Drought-simulated abscisic acid accumulation predominantly occurs in apple leaves

In response to drought stress, ABA accumulates rapidly in plants (Zhu, 2016). To investigate ABA accumulation in apple trees in response to drought stress, we measured ABA concentrations from leaves and roots of hydroponically grown GL-3, *MdMYB88*, and *MdMYB124* transgenic plants, under control conditions and following PEG treatment (used to simulate drought). LC-MS/MS measurements revealed that, after simulated drought conditions for 6 h, ABA rapidly accumulated, in apple tree leaves and roots. However, the accumulation was markedly lower in the roots than leaves of the apple tree after PEG treatment, though a slight accumulation was seen, compared to control treatment (Fig. 8A). Although MdMYB88 and MdMYB124 positively regulate *MdNCED3* expression in apple tree roots, the fold change in expression of *MdNCED3* in response to simulated drought in GL-3 roots was notably lower than that in leaves (Fig. 8B, C; Supplementary Fig. S13), which is also consistent with lower ABA concentrations in

apple tree roots than that in leaves in response to simulated drought (Fig. 8A). These results demonstrate that both leaves and roots can biosynthesize ABA, although this capacity is higher in leaves than in roots.

MdMYB88 and MdMYB124 are repressed by ABA

The above data showed that MdMYB88 and MdMYB124 regulate ABA accumulation by integrating its biosynthesis and catabolism. Next we investigated the expression of *MdMYB88* and *MdMYB124* expression by exogenous ABA treatment. We found that the expression of *MdMYB88* and *MdMYB124* were repressed by ABA treatment (Fig. 9), indicating that MdMYB88 and MdMYB124 may participate in the negative feedback regulation of ABA.

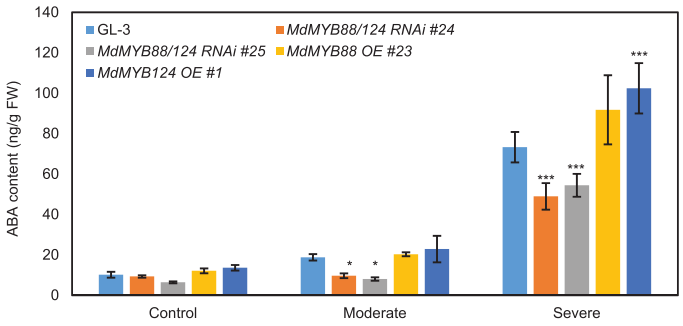

Figure 6. ABA concentration in leaves of GL-3, *MdMYB88*, and *MdMYB124* transgenic plants under drought stress. Error bars indicate standard deviation (n=5). One-way ANOVA (Tukey's test) was performed and statistically significant differences are indicated by ** (P<0.05), ** (P<0.01) or *** (P<0.001). Control: SRWC was 75–85%; moderate: SRWC was 45–55%; severe: SRWC was 25%-35%. This figure is available in color at *JXB* online.

Discussion

In this study, we illustrate the feedback regulation of ABA by MdMYB88 and MdMYB124 under control and drought conditions in apple plants. *MdMYB88* and *MdMYB124* are induced by drought stress and they positively regulate ABA accumulation which in turn represses expression of both genes (Figs. 1 A; 9).

Regulation of stomatal conductance is one of the early responses to drought. Stomatal control is regulated by plant hydraulic function, as well as ABA. Increase of root ABA concentrations under drought is observed to be correlated with increased root hydraulic conductivity (Thompson *et al.* 2007). Exogenous ABA application to roots results in increased root hydraulic conductance (Hose *et al.* 2000; Thompson *et al.* 2007). It is possible that the higher root hydraulic conductivity of *MdMYB88* or *MdMYB124* OE plants results in higher

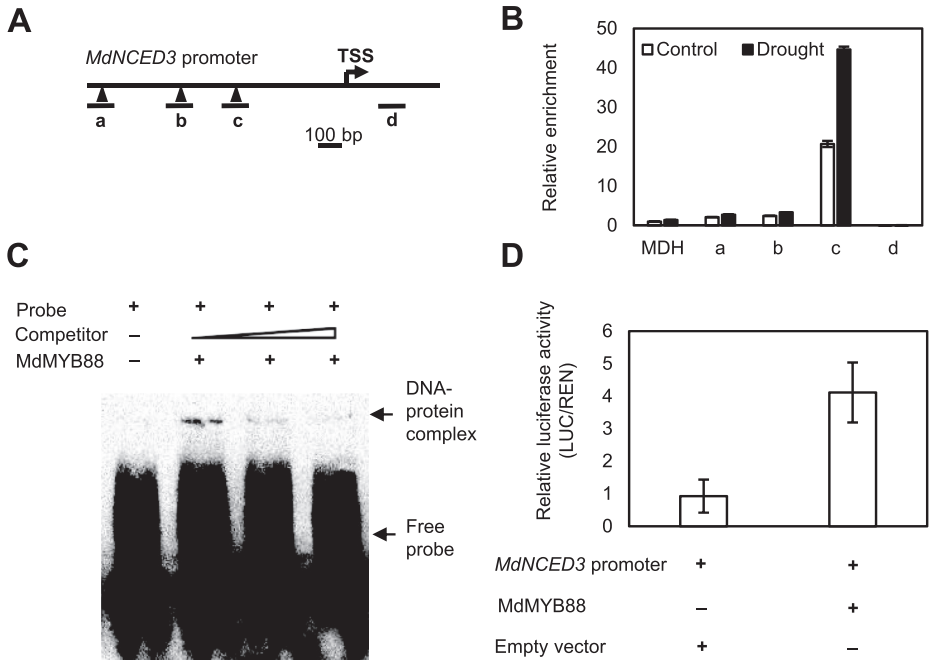

Figure 7. MdMYB88 directly binds to the promoter region of *MdNECD3*. (A) Diagram of *MdNCED3* promoter regions. Fragments a and b contain *cis*-element AGCCG from –1830 bp to –1826 bp, –1368 bp to –1364 bp. Fragment c contains a *cis*-element of CGCGG from –880 bp to –876 bp, fragment d serves as a negative control. TSS, transcription start site. (B) ChIP-qPCR analysis of *MdNCED3* using GL-3 plants drought treated for 0 and 12 d. *MdMDH* serves as the reference gene. Data are mean±SD (n=3). (C) MdMYB88-His is able to bind the promoter region of *MdNECD3* as determined by EMSA analysis. Arrowheads indicate protein-DNA complex or free probe. *MdNCED3* probe contains CGCGG element. (D) The regulation of *MdNCED3* by MdMYB88 as detected by dual-luciferase assay. Effects of MdMYB88 on *MdNCED3* promoter activation. *MdNCED3* promoter was fused to the LUC reporter and the promoter activity was determined by a transient dual-LUC assay in tobacco. The relative LUC activity was normalized to the reference Renilla (REN) luciferase. Error bars indicate SD (n=10).

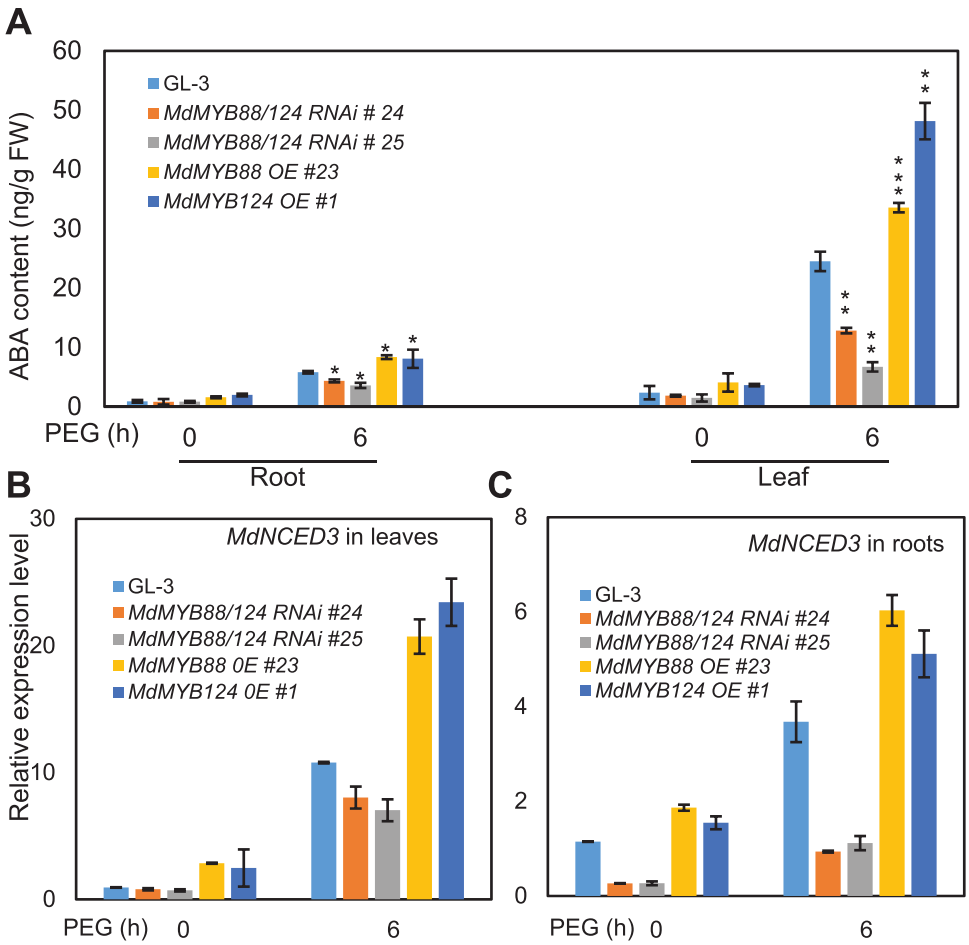

Figure 8. ABA concentration and expression of *MdNCED3* in leaves and roots of GL-3, *MdMYB88*, and *MdMYB124* transgenic plants in response to simulated drought stress. Two-month-old plants were cultured hydroponically for one month and then treated with 20% PEG8000 for 0 h and 6 h. Error bars indicate standard deviation (n=5). One-way ANOVA (Tukey's test) was performed and statistically significant differences are indicated by * (P<0.05), ** (P<0.01) or *** (P<0.001). (This figure is available in color at *JXB* online.)

water flow from the roots; therefore, higher LRWC and higher stomatal conductance (Supplementary Fig. S6; Fig. 2E). In contrast, lower root hydraulic conductivity of the *MdMYB88/124* RNAi plants leads to lower LRWC and stomatal conductance under control and drought conditions.

ABA plays a positive role in root and leaf hydraulic conductivity (Morillon and Chrispeels 2001). Exogenous ABA application or genetic manipulation to increase endogenous ABA concentrations results in increased leaf hydration (Thompson *et al.* 2007; Parent *et al.* 2009), and can increase leaf expansive growth under water deficit, via improvement of plant-water relations (Sansberro *et al.*, 2004). Moreover, ABA can also induce the mRNA and protein expression of leaf aquaporin prolactin induced proteins (PIPs), which play a key role in regulating water transport in roots and leaves (Morillon and Chrispeels 2001; Aroca *et al.* 2006; Parent *et al.* 2009; Tardieu *et al.* 2010), thereby contributing to leaf hydraulic conductivity (Morillon and Chrispeels 2001). We previously found that root hydraulic conductivity (K_{root}) is lower in *MdMYB88/124* RNAi plants but higher in *MdMYB88* or *MdMYB124* OE plants under drought stress (Geng *et al.*, 2018). In this study, we found that the leaf relative water content (LRWC) of overexpression lines of *MdMYB88* and *MdMYB124* was higher than that of GL-3 plants, while LRWC of RNAi lines was lower under control and drought conditions (Supplementary Fig. S6). To understand the coordination of leaf water status, we measured the leaf hydraulic conductance (K_{leaf}) of transgenic and GL-3 plants (Supplementary Fig. S7). The results showed that K_{leaf} of *MdMYB88*-overexpressing lines was higher than that of GL-3 plants under control and drought conditions (SRWC was 45–55%) while *MdMYB88/124* RNAi lines had lower K_{leaf}. Given that MdMYB88 and MdMYB124 positively regulated ABA biosynthesis, our results support the notion that ABA positively correlated with K_{leaf} and K_{root}, as well as LRWC, which is consistent with previous observations (Morillon and Chrispeels 2001; Thompson *et al.* 2007).

The detached leaves of *MdMYB88/124* RNAi plants lost more water than GL-3 plants under dehydration conditions,

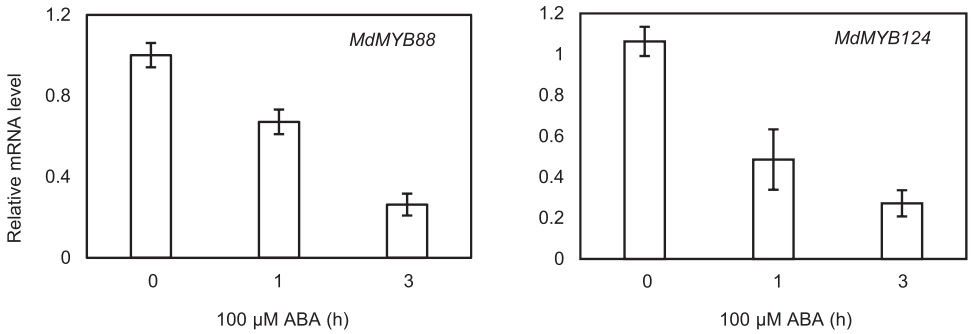

Figure 9. *MdMYB88* and *MdMYB124* are repressed by ABA. Relative expression of *MdMYB88* and *MdMYB124* in *M.* × *domestica* 'Golden Delicious' sprayed with 100 μM ABA for 0, 1, and 3 h. Data are mean±SD (n=3). One-way ANOVA (Tukey's test) was performed and statistically significant differences are indicated by ** (P<0.01) or *** (P<0.001).

while both *MdMYB88* and *MdMYB124* OE plants maintained more water after dehydration (Figs 1B, 2C). However, stomatal conductance under drought stress was lower in the *MdMYB88/124* RNAi plants, but higher in the *MdMYB88* or *MdMYB124* OE plants (Figs 1, 2). In detached leaves, the leaf hydraulic conductance still exists (Coupel-Ledru *et al.*, 2017). In such detached leaves, almost all water evaporates through stomata; therefore, stomatal apertures regulated by ABA must be an important factor during this process. Thus, we infer that the detached leaves of *MdMYB88* and *MdMYB124* OE plants, and *MdMYB88/124* RNAi plants regulated stomatal closure by ABA accumulation.

We also observed that *MdMYB88/124* RNAi plants have lower stomatal conductance compared to GL-3 and OE plants, which have higher stomatal conductance at the beginning of drought treatment (day 0; Figs 1, 2). Previously, we found that MdMYB88 and MdMYB124 regulate stomatal development, as stomatal clusters are observed in the *MdMYB88/124* RNAi plants (Xie *et al.*, 2018). This abnormal stomatal patterning might contribute to a basal lower stomatal conductance in the *MdMYB88/124* RNAi plants. In addition, the root hydraulic conductivity of *MdMYB88* or *MdMYB124* OE plants is higher compared to GL-3 plants under control conditions (Geng *et al.*, 2018), which could result in higher water flow from the roots, therefore, higher LRWC and higher stomatal conductance.

Endogenous ABA concentrations are determined by ABA biosynthesis and catabolism. In response to drought stress, ABA accumulates quickly to induce stomatal closure and to avoid water loss through transpiration (Zhu, 2016, Umezawa *et al.*, 2010). The primary pathway to biosynthesize ABA in plants is *de novo* biosynthesis from carotenoids. Many genes are identified in this pathway, such as *ABA1*, *NCED*, and *AAO3* genes (Zhu, 2016). In Arabidopsis, NCED3 has a major role in ABA biosynthesis. Overexpression of *NCED3* in transgenic Arabidopsis leads to an increase in endogenous ABA concentrations and improves drought resistance (Luchi *et al.*, 2001). In addition, NCED5 also contributes to ABA biosynthesis in response to dehydration (Frey *et al.*, 2012). In our study, we found that MdMYB88 and MdMYB124 positively regulate the expression of *MdNCED3* by associating with its promoter regions (Fig. 7). In contrast to the dramatic change in *MdNCED3* expression, no transcript changes in *MdAAO3* were observed in *MdMYB88* and *MdMYB124* transgenic plants under dehydration or control conditions (Supplementary Fig. S14).

The catabolic pathway of ABA involves ABA hydroxylation, mediated by cytochrome P450 members (*CYP707A1* to *CYP707A4*), and ABA conjugation mediated by UGTs (*UGT71B6*, *UGT71C5*, *UGT71B7*, *UGT71B8;* Kushiro *et al.*, 2004; Okamoto *et al.*, 2006; Priest *et al.*, 2006; Dong *et al.*, 2014; Liu *et al.*, 2015). Arabidopsis UGT71B6, together with its two homologs (UGT71B7 and UGT71B8), contributes to endogenous ABA concentrations (Priest *et al.*, 2006; Dong *et al.*, 2014). UGT71C5 itself regulates ABA homeostasis, implicating a major role of UGT71C5 in ABA accumulation (Liu *et al.*, 2015). Suppression of tomato *SlUGT71C5* also leads to elevated ABA concentrations (Sun *et al.*, 2017). Our results show that both MdMYB88 and MdMYB124 negatively regulate the expression of *MdUGT71B6* and *MdCYP707A4* under dehydration stress (Fig. 5A, C; Supplementary Table S3). Interestingly, there was no altered expression of *MdCYP707A4* between non-transgenic and transgenic plants under control conditions (Fig. 5C). Besides the *de novo* biosynthetic pathway of ABA, *BG1* and *BG2* genes are also responsible for ABA accumulation by catalyzing the conversion of ABA-GE to ABA (Xu *et al.*, 2012). However, we could not identify the exact *BG* genes, *UGT71B7, UGT71B8,* and *UGT71C5* in the apple genome (Velasco *et al*, 2010; Li *et al.*, 2016; Daccord *et al.*, 2017), which might be due to the incomplete assemblies and annotation of the apple genome. Hence, our current results suggest that MdMYB88 and MdMYB124 promote ABA accumulation in response to drought in apple trees, possibly by activating the expression of an ABA biosynthetic gene (*MdNCED3*) and repressing ABA catabolic genes (*MdCYP707A4* and *MdUGT71B6*). These three genes are closely related to their homologs in Arabidopsis (Supplementary Fig. S15). This sequence homology further suggests that they play the same role in ABA signaling pathways in apple trees, as in Arabidopsis.

Altered ABA homeostasis results in altered expression of drought- and ABA-responsive genes (Nambara and Marion-Poll, 2005; Tuteja, 2007). Among the DEGs from the RNA-seq data under control and dehydration conditions, we identified 55 drought-inducible genes which were down-regulated in *MdMYB88/124* RNAi plants in response to air drying (Supplementary Tables S2, S3); these include *MdHB7*, *MdMYB102*, *MdPUB9*, and *MdOEP16*. This number is close to one-fifth of the 276 down-regulated genes in the *MdMYB88/124* RNAi plants. These 55 genes might be positive drought regulators and be positively regulated by MdMYB88/MdMYB124; therefore they could play important roles towards drought tolerance regulated by MdMYB88 and MdMYB124. Some genes that are repressed by drought were up-regulated in *MdMYB88/124* RNAi plants after dehydration treatment. Among the 273 up-regulated genes in *MdMYB88/124* RNAi plants, we identified 15 drought-repressed genes such as *MdAHK3* (Supplementary Tables S2, S3). These genes could be negative drought factors, and be negatively regulated by MdMYB88 and MdMYB124. The 15 genes could also contribute to drought tolerance via MdMYB88 and MdMYB124, though it is possible that their roles might be not as critical as the 55 genes that were up-regulated. Interestingly, homologs of *HB7* and *OEP16* in Arabidopsis and wheat, respectively, are positive regulators for drought tolerance improvement, whereas AHK3 is a negative regulator (Söderman *et al.*, 1996; Tran *et al.*, 2007; Zang *et al.*, 2017).

Phenylpropanoids are a class of plant secondary metabolites, which are activated under abiotic stress, including drought stress (Sharma *et al.*, 2019). Plant phenylpropanoids have a prominent role in reactive oxygen species (ROS) scavenging (Agati *et al.*, 2012). GO enrichment analysis of DEGs in *MdMYB88/124* RNAi plants revealed the involvement of genes in phenylpropanoid metabolism regulated by MdMYB88 and MdMYB124 (Fig. 3). Our recent study also revealed the accumulation of metabolites in the phenylpropanoid biosynthesis pathway in *MdMYB88* or *MdMYB124* OE plants under drought stress (Geng *et al.*, 2020). Therefore, it is possible that the stronger ROS scavenging ability of *MdMYB88* or *MdMYB124* OE plants with higher phenylpropanoid content contributed to their stronger ability to endure drought.

Collectively, we have demonstrated that, in response to drought stress, MdMYB88 and MdMYB124 modulate ABA accumulation by activating ABA biosynthetic genes and repressing ABA catabolic genes in apple leaves, thus regulating the drought response. However, *MdMYB88* and *MdMYB124* expression was repressed by ABA (Fig. 9). Overaccumulation of ABA is not always good, as ABA can induce senescence (Becker and Apel, 1993), pollen sterility and reduce growth directly and indirectly (Blum, 2015). In our study, the *MdMYB88* or *MdMYB124* OE plants accumulated more ABA than GL-3 plants, and had higher LRWC, to maintain the balance between drought tolerance and plant biomass. Future studies will aim to test the field performance of our transgenic plants to evaluate the positive and negative effects of ABA on drought tolerance and plant production .

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Measurement of soil relative water content during drought treatment.

Fig. S2. The antibody specificity of MdMYB88.

Fig. S3. Drought tolerance of GL-3, and *MdMYB88/124* transgenic plants determined by ion leakage (A) or water loss (B).

Fig. S4. Drought tolerance of *MdMYB88/124* RNAi plants.

Fig. S5. Drought tolerance of *MdMYB88* and *MdMYB124* OE plants.

Fig. S6. Leaf relative water content of GL-3, *MdMYB88/124* RNAi, *MdMYB88* OE, and *MdMYB124* OE plants under drought stress.

Fig. S7. Leaf water potential and leaf hydraulic conductivity of GL-3, *MdMYB88* OE, *MdMYB88/124* RNAi plants under control and drought conditions.

Fig. S8. GO enrichment analysis of differentially expressed genes in *MdMYB88/124* RNAi plants versus GL-3 plants under control conditions.

Fig. S9. Relative expression of *MdCYP707A1* and *MdCYP707A2* in GL-3, *MdMYB88/124* RNAi, and overexpression (OE) plants under control or dehydration conditions.

Fig. S10. MdMYB88/MdMYB124 core binding sites (CGCGG) in the promoter region of *MdNECD3*.

Fig. S11. Promoter region of *MdCYP707A4* and *UGT71B6*.

Fig. S12. ABA stomatal sensitivity of GL-3, *MdMYB88/124* RNAi, *MdMYB88* OE, and *MdMYB124* OE plants.

Fig. S13. Expression of *MdCYP707A4*, *MdCYP707A1* and *MdUGT71B6* in roots of GL-3, *MdMYB88*, and *MdMYB124* transgenic plants in response to simulated drought stress.

Fig. S14. *MdAAO3* expression in GL-3 and transgenic apple plants after detached leaves were dehydrated for 2 h.

Fig. S15. Comparisons of MdNCED3, UGT71B6, and MdCYP707A4 sequences with their close homologs in Arabidopsis.

Table S1. Primers used in this study.

Table S2. Differentially expressed genes in GL-3 plants under drought stress.

Table S3. Differentially expressed genes in *MdMYB88/124* RNAi plants under drought stress.

Table S4. Differentially expressed genes in *MdMYB88/124* RNAi plants under control conditions.

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Conflicts of interest

The authors have no conflict of interest to declare.

Author contributions

QG designed the experiments. YX, CB, PC, XWL, DG, XFL, YY, NH, and FZ performed the experiments. QG and CB wrote the manuscript. ZL analyzed RNA-seq data. SZ, FM, XZ, and CN analyzed the data.

Data availability

The RNA-seq data is deposited at NCBI under BioProject accession PRJNA529852.

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