Plant Molecular Biology (2024) 114:86
https://doi.org/10.1007/s11103-024-01479-0

1-Butanol treatment enhances drought stress tolerance in *Arabidopsis thaliana*

Thi Nhu Quynh Do[1,2] · Daisuke Todaka[1] · Maho Tanaka[1,3] · Satoshi Takahashi[1,3] · Junko Ishida[1,3] · Kaori Sako[1,4] · Atsushi J. Nagano[5,6] · Yumiko Takebayashi[7] · Yuri Kanno[8] · Masanori Okamoto[8] · Xuan Hoi Pham[2] · Motoaki Seki[1,3,9,10]

Received: 8 January 2024 / Accepted: 14 June 2024 / Published online: 18 July 2024

Abstract

Abiotic stress is a major factor affecting crop productivity. Chemical priming is a promising strategy to enhance tolerance to abiotic stress. In this study, we evaluated the use of 1-butanol as an effectual strategy to enhance drought stress tolerance in *Arabidopsis thaliana*. We first demonstrated that, among isopropanol, methanol, 1-butanol, and 2-butanol, pretreatment with 1-butanol was the most effective for enhancing drought tolerance. We tested the plants with a range of 1-butanol concentrations (0, 10, 20, 30, 40, and 50 mM) and further determined that 20 mM was the optimal concentration of 1-butanol that enhanced drought tolerance without compromising plant growth. Physiological tests showed that the enhancement of drought tolerance by 1-butanol pretreatment was associated with its stimulation of stomatal closure and improvement of leaf water retention. RNA-sequencing analysis revealed the differentially expressed genes (DEGs) between water- and 1-butanol-pretreated plants. The DEGs included genes involved in oxidative stress response processes. The DEGs identified here partially overlapped with those of ethanol-treated plants. Taken together, the results show that 1-butanol is a novel chemical priming agent that effectively enhances drought stress tolerance in *Arabidopsis* plants, and provide insights into the molecular mechanisms of alcohol-mediated abiotic stress tolerance.

Key message

1-Butanol priming enhances drought tolerance via mechanisms that include stimulating stomatal closure and delaying leaf water loss in *Arabidopsis thaliana*

Keywords 1-Butanol · Drought stress tolerance · *Arabidopsis thaliana* · Chemical priming · Stomatal closure · Transcriptome

✉ Motoaki Seki
motoaki.seki@riken.jp

1 Plant Genomic Network Research Team, RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-Cho, Tsurumi-Ku, Yokohama, Kanagawa 230-0045, Japan

2 National Key Laboratory for Plant Cell Biotechnology, Agricultural Genetics Institute, Pham Van Dong Road, Bac Tu Liem District, Hanoi, Vietnam

3 Plant Epigenome Regulation Laboratory, RIKEN Cluster for Pioneering Research, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

4 Department of Advanced Bioscience, Faculty of Agriculture, Kindai University, Nara 631-8505, Japan

5 Faculty of Agriculture, Ryukoku University, Shiga 520-2194, Japan

6 Institute for Advanced Biosciences, Keio University, Yamagata 997-0017, Japan

7 Mass Spectrometry and Microscopy Unit, RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan

8 Plant Chemical Genomics Research Team, RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan

9 Kihara Institute for Biological Research, Yokohama City University, 641-12 Maioka-Cho, Totsuka-Ku, Yokohama, Kanagawa 244-0813, Japan

10 Graduate School of Science and Engineering, Saitama University, Saitama, Saitama 338-8570, Japan

Introduction

As the world's population continues to grow and the world's arable land continues to shrink (Potapov et al. 2021), finding strategies to increase crop productivity per cropland unit has become critical to future global food security. Crop productivity can be increased by boosting crop production gains and reducing crop production losses. With regard to the latter, many biotic and abiotic factors can negatively impact the production of crop plants. Among these, drought is a detrimental factor that affects crop growth at various stages of development, hinders water-nutrient circulation and photosynthetic process, and causes crop production losses (Raza et al. 2023). Drought alone resulted in 34% of total crop and livestock production losses in the least developed nations and low- to middle-income countries during the 2008–2018 period (FAO 2021). Moreover, while both crop and livestock production are affected by drought, 82% of the overall drought impact has affected the agricultural sector (FAO 2021). Therefore, development of crops with enhanced drought tolerance is a priority to mitigate crop production losses.

Various approaches have been employed to develop crop plants tolerant to biotic and abiotic stress factors, including drought (Guzmán et al. 2021). Although traditional approaches, such as conventional plant breeding, are time-consuming, other more modern methodologies, such as those of plant genetic modifications, incur concerns regarding the long-term safety for consumers and detrimental impacts on the environment (de Vendômois et al. 2010; Savvides et al. 2016). Under these circumstances, chemical priming, which is more time-efficient and may be environmentally friendly, has emerged as an important alternative approach to tackle the challenges of improvement in the tolerance of crop plants to biotic and abiotic stress factors (Savvides et al. 2016; Mauch-Mani et al. 2017; Kerchev et al. 2020; Sako et al. 2021).Under the chemical priming approach, crop plants are treated with either naturally occurring or synthetic chemical priming agents prior to exposure to environmental stress factors, so as to enhance the tolerance of the treated plants to the corresponding stresses (Savvides et al. 2016; Mauch-Mani et al. 2017; Kerchev et al. 2020; Sako et al. 2021; Guzmán et al. 2021; Chakraborti et al. 2022). Chemical priming agents of different types have been used, including (i) reactive chemical species, such as hydrogen peroxide (H_2O_2), (ii) metabolites and phytohormones, such as ethanol, (iii) compounds involved in epigenetic regulation, such as SAHA, and (iv) nanoparticles, such as CeO_2 (Sako et al. 2021). In addition, various abiotic stress factors have been treated effectively using chemical priming strategies, such as drought (Farooq et al. 2009; Alonso-Hernández et al. 2011; Ha et al. 2014; Ziogas et al. 2015; Kim et al. 2017; Djanaguiraman et al. 2018; Utsumi et al. 2019), high-intensity light (Xu et al. 2010; Jin et al. 2015; Wu et al. 2017), high temperature (Yamauchi et al. 2015; Wu et al. 2017; Tsai et al. 2019; Vaidya et al. 2019; Zhang et al. 2019), low temperature (Jin et al. 2015; Wu et al. 2017; Cofer et al. 2018; Min et al. 2018; Fu et al. 2019; Ahmadi Soleimanie et al. 2020), and salinity (Alonso-Hernández et al. 2011; Hasanuzzaman et al. 2011; Nguyen et al. 2017, 2018). We previously showed that ethanol treatment reduced heat stress damage and enhanced fruit quality in tomato through the increased expression of stress-related genes encoding late embryogenesis abundant (LEA) protein, reactive oxygen species (ROS) elimination enzymes, and activated gluconeogenesis (Todaka et al. 2024). Ethanol-treated Arabidopsis plants also showed enhanced drought tolerance through activated stomatal closure and gluconeogenesis (Bashir et al. 2022). Furthermore, the application of ethanol leads to stomatal closure and regulates protein folding by activation of the HSP chaperon network, then increases drought avoidance in cassava (Vu et al. 2022). In addition, ethanol treatment enhanced drought tolerance in wheat and rice (Bashir et al. 2022). However, the molecular mechanisms regulating the enhancement of ethanol-induced drought stress tolerance are still not fully understood. Furthermore, it is currently unknown whether other alcoholic compounds can produce priming effects similar to ethanol.

Inspired by these open questions, we report here the investigation of a set of other alcoholic compounds for their priming effects against drought stress. The results showed that pretreatment of *Arabidopsis* plants with 1-butanol induced the stomatal closure, decreased cell membrane damage and leaf water loss, and enhanced their tolerance to drought stress. Furthermore, we performed transcriptome analysis to investigate the dynamics of differentially expressed genes (DEGs) associated with 1-butanol pretreatment in *Arabidopsis*. This study provides exploratory insights into the priming mechanism of 1-butanol.

Materials and methods

Plant growth conditions

Arabidopsis thaliana ecotype Col-0 (wild type), *abscisic acid insensitive 1–1* (*abi1-1*) mutant (Leung et al. 1994; Meyer et al. 1994; Bertauche et al. 1996) and *9-cis-epoxycarotenoid dioxygenase 3-2* (*nced3-2*) mutant (Urano et al. 2009) were used in the experiments. The seeds were sown directly on plates containing Dio propagation mix No. 2 soil

(DIO Chemicals, Tokyo, Japan) and grown in a growth room for 7 days at 22 °C under an 16 h/8 h (light/dark) photoperiod with ~ 100 µmol m^{-2} s^{-1} photon flux density. The seedlings were then transferred to pots (Plant pot No. 2, φ70 mm, H60 mm, yamato plastic Co., Ltd., Nara, Japan) containing horticultural clay granules soil (SERAMIS, Westland horticulture Ltd. Tyrone, UK) and grown for 13 days under the same conditions.

Chemical priming treatments

Twenty-day-old wild type plants were treated for 4 days with or without 0.3% (50 mM) solution of isopropanol, methanol, 1-butanol, or 2-butanol. In a separate experiment, 20-day-old wild type plants were treated for 3 days with one of an additional concentration of 1-butanol (10, 20, 30, or 40 mM). In the case of experiments using *abi1-1* and *nced3-2*, the plants were treated for 3 days with 20 mM 1-butanol. Specifically, the plants in pots (Plant Pot No. 2, φ70 mm, H60 mm, Yamato Plastic Co., Ltd., Nara, Japan) were placed in trays (60 × 40 cm H10cm), and alcohol treatment was initiated by replacing water supply with one of the aforementioned alcohol solutions in those trays at a ratio of 2L of solution per tray volume/surface area, which was retained for either 3 or 4 days according to the experiment. For each treatment, six pots each containing four plants (24 plants in total) were used with three experimental replications (a total set of 72 plants per treatment).

Drought stress tolerance test

Drought stress treatment was performed mostly in accordance with the procedure described by Kim et al. (2017). Specifically, after the chemical priming treatments, plants were subjected to drought stress by removing all solutions from the trays. After withholding water for 2 weeks, water was resupplied in the trays for 5 days and the percentage survival of the drought-treated plants was recorded. For each treatment, six pots each containing four plants (24 plants in total) were used with three experimental replications (a total set of 72 plants per treatment).

Stomatal aperture analysis

Three fully expanded rosette leaves, one leaf per plant, were detached; the epidermis from the abaxial leaf side was peeled from each leaf and placed immediately in the observation solution that comprised 10 mM KCl, 10 mM $CaCl_2$, 5 mM MES-NaOH (pH 6.0), and a drop of SilwetL-77 per 50 mL solution volume. The stomata were observed and photographed using a digital microscope (VB-7010, Keyence). The stomatal aperture (50 stomata per treatment) was measured and analyzed using ImageJ.

Relative water content analysis

Samples for determination of the relative water content (RWC) were collected at 11 days of drought treatment. The entire aboveground portion of each plant was collected and immediately weighed to determine the fresh weight (FW). The samples were then dried at 80 °C for 24 h and the dry weight (DW) was recorded. The RWC was calculated using the following formula: RWC (%) = (FW − DW)/FW × 100. For each treatment, 24 plants in total were sampled with three experimental replications (a total set of 72 plants per treatment).

Histochemical staining for H_2O_2

Four-day-old *Arabidopsis* plants treated with or without 0.05% 1-butanol for 24 h, with or without subsequent treatment with 100 mM NaCl for 12 h, were stained using a modified version of a published method (Kumar et al. 2014; Mostofa et al. 2015). To detect H_2O_2, plants were stained for 5 h with 0.1% 3,3'-diaminobenzidine (DAB) in 10 mM potassium phosphate (pH 7.0) following a previously described method (Nguyen et al. 2017).

ABA measurement

The ABA content in shoots was measured in Col-0 and both mutant backgrounds: *abi1-1, nced3-2*. We collected samples for ABA measurement at 3 time points: before pretreatment; 3 days after water-treated plants or 20 mM 1-butanol-treated plants; and drought treatment after water pretreated or 20 mM 1-butanol pretreated. For each time point, the shoot tissues were collected from three plants for each treatment and for each plant group with six replications (a total set of 18 plants per treatment). Sample (∽5 mg dry weight) was homogenized in 500 µL of 80% acetonitrile containing d6-ABA (Icon Isotopes). After 3 min incubation, the supernatant was collected by centrifugation, and the pellet was rinsed again with 80% acetonitrile. The combined supernatant was dried using a SpeedVac (Thermo Fisher Scientific). The sample was dissolved in 500 µL of 1% acetic acid water. Oasis WAX 30 mg solid-phase extraction cartridge (Waters) was washed with 100% acetonitrile and was conditioned with 1% acetic acid water. The sample was loaded and followed by a first wash with 500 µL of 1% acetic acid water and a second wash with 80% acetonitrile. The ABA fraction was eluted with 80% acetonitrile containing 1% acetic acid and dried with N_2 gas. Finally, sample was dissolved in water and analyzed using an ultra-high-performance liquid chromatography (UHPLC)-electrospray interface (ESI) and a quadrupole-orbitrap mass spectrometer (Vanquish-Q Exactive, Thermo Fisher Scientific). The LC equipped with a 1.7 µm, 2.1 mm × 50 mm, ACQUITY UPLC BEH C18

column (Waters) was used with a binary solvent system comprising water containing 0.06% acetic acid (A) and acetonitrile containing 0.01% formic acid (B). Separation was performed using a gradient of increasing solvent B content with a flow rate of 0.25 mL min^{-1} at 40 °C. The gradient was increased linearly from 5% B to 27% B over 11 min and then 99% B at 11.1 min. After 11.9 min of 99% B, the initial condition was restored and allowed to equilibrate for 4 min. The retention times of ABA and d6-ABA were 9.88 and 9.82 min, respectively. MS conditions were follows; spray voltage = 2.5 kV, S-lens RT level = 50, resolution = 70,000, isolation window = 0.4 (*m/z*), and ion mode = negative. ABA and d6-ABA were detected 263.1289 and 269.1665 by selected ion monitoring (SIM), respectively.

Ion leakage analysis

Electrolyte leakage was determined from the detached aboveground parts of drought-stressed plants at the following time points: before drought stress (D0), 6 days of drought stress (D6), 11 days of drought stress (D11), and 12 days of drought stress (D12). The detached aboveground parts ($n = 6$ per each experimental group) were individually placed in 2 mL tubes containing 2 mL deionized water and gently shaken for 3 h, then treated at 60 °C for 30 min and kept for 30 min at room temperature. The percentage electrolyte leakage was determined as the percentage conductivity before and after heat treatment of the detached aboveground parts (Nishiyama et al. 2011).

RNA extraction and RNA-sequencing analysis

To identify the genes differentially regulated in response to 1-butanol treatment, we performed RNA-sequencing (RNA-seq) analysis of shoots and roots. A plant growth system using ceramic-based granular soil to facilitate the isolation of shoots and roots separately was adapted (Rasheed et al. 2016). Specifically, we performed RNA-seq analysis to assess the transcriptomic changes in shoots and roots at four time points: before pretreatment (W0), at water (W3D0) or 1-butanol (B3D0) pretreatment for 3 days, drought treatment for 6 days after water pretreatment (W3D6), drought treatment for 6 days after 1-butanol pretreatment (B3D6), drought treatment for 10 days after water pretreatment (W3D10), and drought treatment for 10 days after 1-butanol pretreatment (B3D10). For each time point, the shoot tissues and root tissues were collected separately from three plants for each treatment with six replications (a total set of 18 plants per treatment). Total RNA was extracted using the Plant RNA Purification reagents (Invitrogen, Thermo Fisher scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions. The quality of the total RNA extracts was evaluated using a Bioanalyzer system (Agilent, Santa Clara, CA, USA).

The sequencing library for the RNA-seq analysis was prepared using the Lasy-Seq method (Kamitani et al. 2019). Specifically, 110 ng total RNA was used per sample. The sequencing libraries were prepared using the 151 bp paired-end mode of the HiSeq X Ten platform (Illumina, San Diego, CA, USA). The RNA-seq analyses were performed with R1 reads. Low-quality reads and adapters were trimmed using Trimmomatic version 0.39 (http://www.usadellab.org/cms/?page=trimmomatic) with the 'ILLUMINACLIP:TruSeaq3-SE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36' options. HISAT2 (http://daehwankimlab.github.io/hisat2/) version 2.2.1 was used to map the reads to the *Arabidopsis thaliana* reference genome (TAIR10) with the '–max-intronlen 5000' option. Aligned reads within gene models were counted using featureCounts version 2.0.1 (http://subread.sourceforge.net/) with the '–fracOverlap 0.5–O–t gene–g ID–s 1–primary' options. DEGs were identified using R version 4.0.4 (https://www.r-project.org/) and the DESeq2 version 1.30.1 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html) package. Genes with a false discovery rate < 0.05 in each comparison were identified as DEGs. A gene ontology (GO) analysis was performed using the PANTHER knowledgebase (https://www.pantherdb.org). The RNA-seq data has been deposited in the genbank database under the accession number GSE248433.

Reverse transcription–quantitative PCR (RT-qPCR) analysis

To confirm the changes in gene expression under drought stress as well as to evaluate the reliability of the RNA-seq data, we performed an RT-qPCR analysis. The cDNA synthesis was performed using the ReverTra Ace™ qPCR RT kit (Toyobo, Osaka, Japan) in accordance with the manufacturer's instructions. The RT-qPCR analysis was performed as previously described (Matsui et al. 2017). For each treatment, three plants were used as one sample with six independent biological replications (a total set of 18 plants per treatment). Details on the primers used in the RT-qPCR analysis are provided in Supplementary Table S1.

Results

Enhanced drought stress tolerance by 1-butanol treatment

Previous reports have shown that pretreatment of *Arabidopsis*, rice, wheat, and cassava plants with ethanol improves their tolerance to drought stress (Bashir et al. 2022; Vu et al.

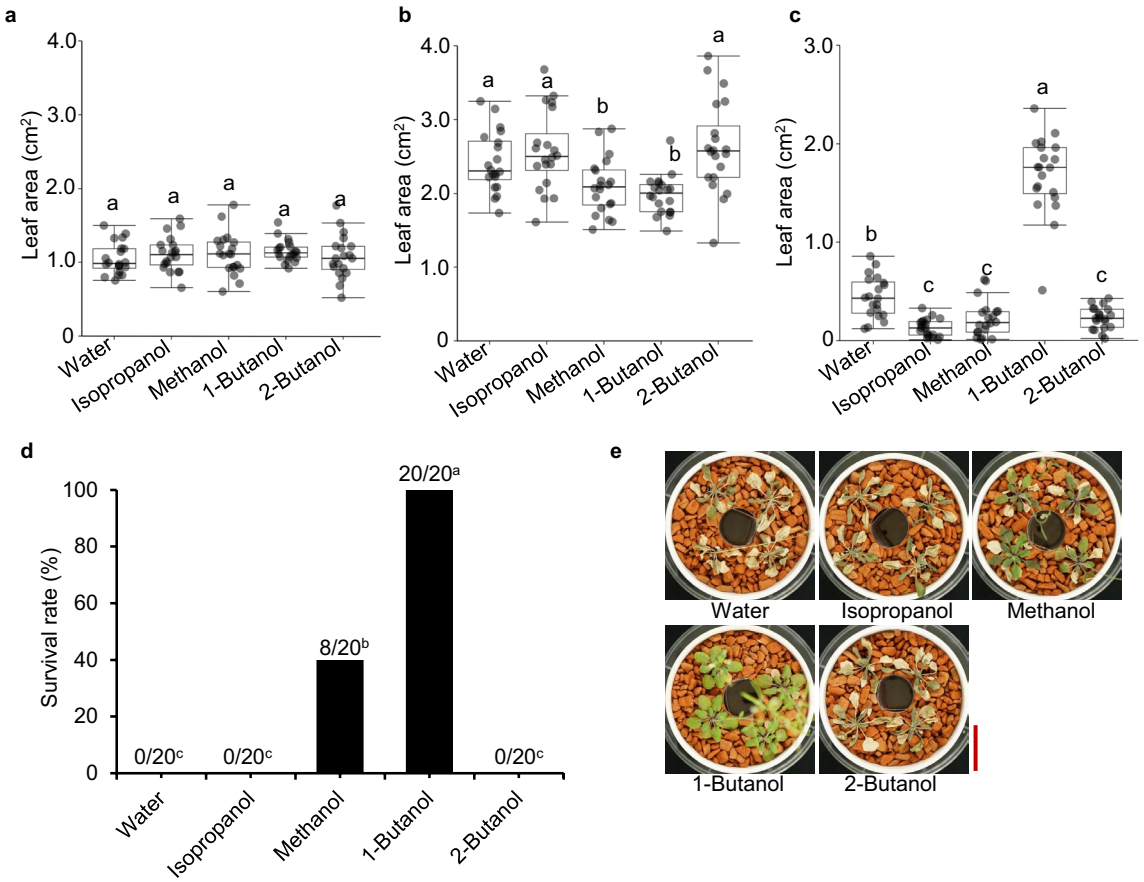


Fig. 1 Effects of alcohol treatments on growth and drought tolerance of *Arabidopsis*. **a** Leaf area of seedlings grown under the well-watered condition for 20 days. **b** Leaf area of seedlings grown under the well-watered condition for 20 days and pretreated with 50 mM isopropanol, methanol, 1-butanol, or 2-butanol, or water for 4 days. **c** Leaf area of seedlings grown under the well-watered condition for 20 days, pretreated with alcohol or water for 4 days, and then subjected to drought stress for 13 days. **a**–**c** Different lowercase letters above boxes indicate statistically significant differences ($p < 0.05$, Tukey's test in R; $n = 20$). **d** Survival of plants grown under the well-watered condition for 20 days, pretreated with alcohol or water for 4 days, and then treated with drought stress for 13 days. Survival was recorded after rewatering for 5 days. Different lowercase letters above the bars indicate statistically significant differences ($p < 0.05$, Fisher's exact test in R; $n = 20$). **e** Plants at the stage that survival was evaluated. Scale bars: 3.0 cm

2022). Inspired by these results, we investigated the priming effects of a set of other alcohols, comprising isopropanol, methanol, 1-butanol, and 2-butanol, on *Arabidopsis* for the enhancement of drought stress tolerance. Twenty-day-old plants were pretreated with 50 mM solution of one of the aforementioned alcohols for 4 days. Before the pretreatments, the leaf areas were not different between in the plants pretreated with all the alcohols and in the plants pretreated with water (Fig. 1a). After the pretreatments, the leaf in the plants pretreated with methanol or 1-butanol was lower compared with those of plants pretreated with water, isopropanol, or 2-butanol (Fig. 1a, b). At the day of the drought treatment for 13 days, the leaf area of 1-butanol-pretreated plants was significantly higher than those of water-, isopropanol-, methanol-, and 2-butanol-pretreated plants (Fig. 1c). The plants pretreated with 1-butanol showed 100% (20/20) survival and the methanol-pretreated plants exhibited 40% (8/20) survival under the same drought treatment (Fig. 1d, e). By contrast, no plants pretreated with isopropanol, 2-butanol, or water survived after exposure to the drought treatment. These results indicated that, among the tested alcohols, 1-butanol had the strongest priming effect on *Arabidopsis* for the enhancement of drought stress tolerance.

We also compared the priming effect of 50 mM 1-butanol with that of 50 mM ethanol (Supplementary Fig. S1). The decrease in leaf area was similar in plants pretreated with 1-butanol or ethanol (Supplementary Fig. S1a, b). After drought treatment for 13 days and at 5 days after the start of rewatering, the leaf areas of the 1-butanol- and ethanol-pretreated plants were higher than those of the water-pretreated plants (Supplementary Fig. S1c–f). All alcohol-pretreated plants survived the drought treatment, whereas no water-pretreated plants were alive after rewatering (Supplementary

Fig. S1g). These results suggested that the effectiveness of 1-butanol for priming was similar to that of ethanol.

A high concentration of a chemical priming agent can adversely affect plant growth (Kim et al. 2017; Matsui et al. 2022). As shown in Fig. 1b and Supplementary Fig. S1b, 50 mM 1-butanol pretreatment for 4 days slightly but significantly decreased leaf growth. Therefore, we evaluated whether 1-butanol pretreatment for a shorter period and at a lower concentration could affect leaf growth and increase drought tolerance. The plants were subjected to drought stress after pretreatment with 0, 10, 20, 30, 40, or 50 mM 1-butanol solution for 3 days. The leaf area did not differ significantly among plants after pretreatment with the different concentrations of 1-butanol (Fig. 2a, b) nor among plants before pretreatment (Supplementary Fig. S2). In contrast to the results of the initial experiment (Fig. 1b), the 50 mM 1-butanol pretreatment for 3 days did not decrease leaf growth (Fig. 2a, b). This was most likely due to the shortened pretreatment period. After drought treatment for 6 days, the leaf area of the plants pretreated with 10 mM or 20 mM 1-butanol was not significantly different from that of plants pretreated with 0 mM 1-butanol (Fig. 2c, d). By contrast, inhibition of plant growth was increasingly evident in plants treated with 30, 40, or 50 mM 1-butanol solutions for the same period (Fig. 2c, d). These results suggested that 30 mM or higher concentrations of 1-butanol adversely affected plant growth. Interestingly, 20–50 mM concentrations of 1-butanol resulted in 100% survival of the 1-butanol-pretreated plants under the drought treatment (Fig. 2e, f). We further confirmed that 20 mM 1-butanol did not inhibit shoot growth during the pretreatment period and increased the percentage survival under drought stress (Supplementary Fig. 3). Collectively, the results indicated that 20 mM 1-butanol was the optimal concentration for use as a chemical priming agent for drought stress tolerance, and thus 20 mM 1-butanol pretreatment for 3 days was used for the subsequent analyses.

Effects of 1-butanol pretreatment on stomatal aperture under the well-watered condition, and on shoot relative water content and electrolyte leakage under drought stress

The leaf stomatal aperture was decreased by 1-butanol pretreatment (Fig. 3a, b). To characterize the effects of 1-butanol treatment on the water content status in plants under drought stress, the relative water content of the above-ground parts was assessed (Fig. 3c, d). At drought treatment for 11 days, the relative water content in the plants pretreated with 1-butanol was 2.5-fold higher than that of plants pretreated with water (Fig. 3c).

To characterize differences in cell membrane damage under drought stress between the water- and 1-butanol-treated plants, we measured electrolyte leakage before and after exposure to drought stress. Significant differences in electrolyte leakage between the water and 1-butanol pretreatments were not observed in the plants subjected to drought treatment for 0 days (D0) and 6 days (D6) (Fig. 3e). However, in the plants subjected to drought treatment for 11 days (D11) and 12 days (D12), the electrolyte leakage of 1-butanol-treated plants was significantly lower than that of water-treated plants (Fig. 3e). These data suggested that 1-butanol pretreatment could alleviate cell membrane damage under drought stress.

Collectively, these results suggested that 1-butanol could enhance drought stress tolerance in *Arabidopsis* plants through stimulating closure of the stomatal aperture, which in turn led to improved shoot water content and decreased cell membrane damage in 1-butanol-pretreated plants under drought stress.

Pretreatment with 1-butanol did not enhance drought stress tolerance in *abi1-1* and *nced3-2* mutants

The *ABI1* and *NCED3* genes play crucial roles in abscisic acid (ABA)-dependent regulatory mechanisms, including drought tolerance, and therefore we next investigated whether 1-butanol-mediated drought tolerance was observed in the corresponding *Arabidopsis* mutants. In the Col-0 plants, 1-butanol pretreatment increased significantly the survival ratio after drought treatment while in the *abi1-1* and *nced3-2* mutants, 1-butanol pretreatment did not significantly enhance the survival ratio after the same treatment (Fig. 4a–d). Furthermore, we also measured the ABA content in WT, *abi1-1*, and *nced3-2* mutants pretreated with or without 1-butanol (Fig. 4e–g). We found that the ABA level was higher after drought stress in 1-butanol-treated WT plants than in 1-butanol-untreated WT plants. In the *abi1-1* and *nced3-2* mutants, however, the ABA level was found to be less after drought stress in 1-butanol-treated plants than in 1-butanol-untreated plants (Fig. 4e–g). Collectively, our data suggest that ABA-dependent ABI1- and/or NCED3-mediated regulatory mechanisms could be, at least partially, involved in the 1-butanol-enhanced drought tolerance.

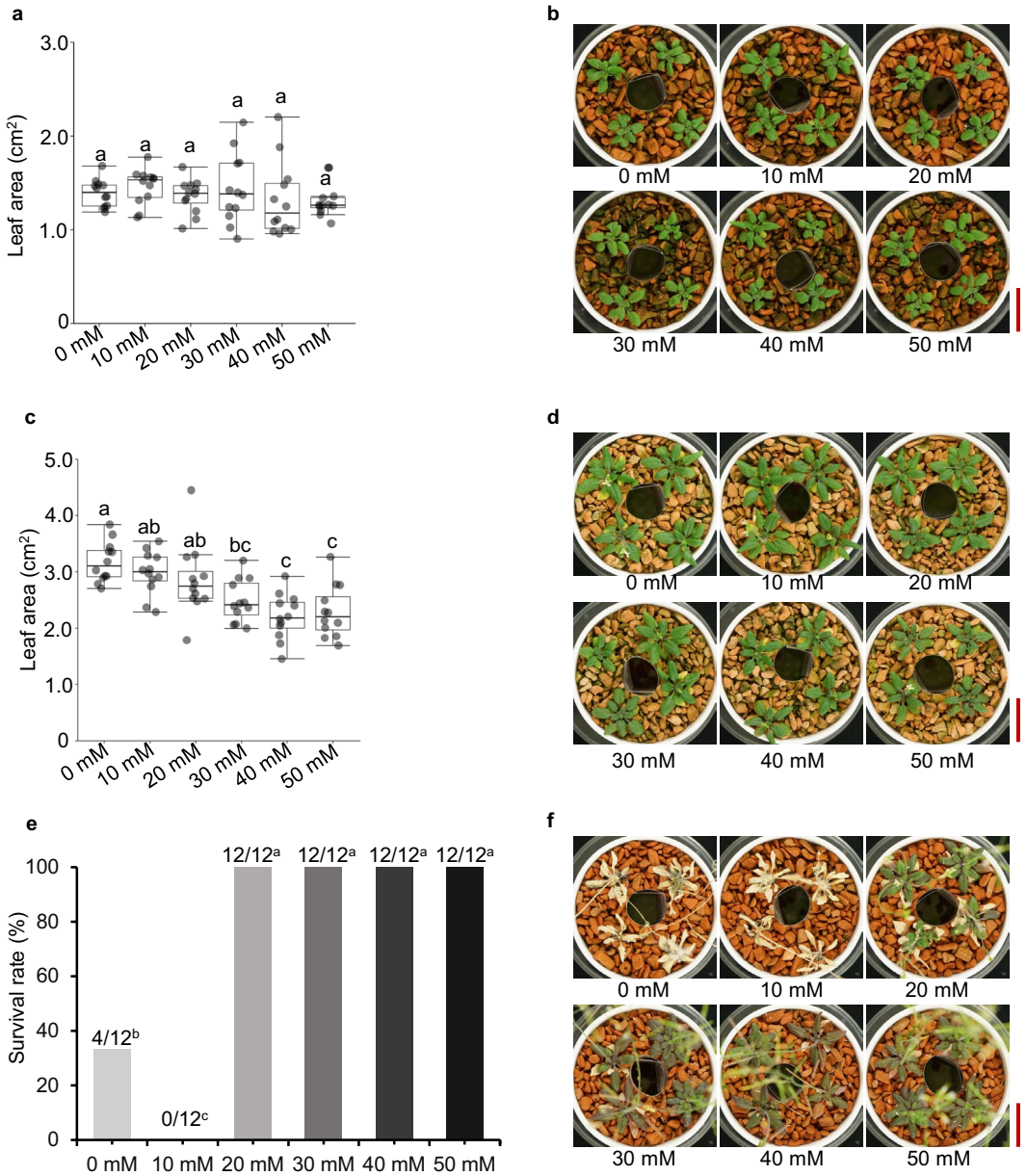


Fig. 2 Effects of 1-butanol treatments at different concentrations on growth and drought tolerance of *Arabidopsis*. **a** Leaf area of seedlings grown under the well-watered condition for 20 days and pretreated with 1-butanol at 0, 10, 20, 30, 40, or 50 mM for 3 days. **b** Plants at the stage that leaf area was evaluated in (**a**). **c** Leaf area of seedlings grown under the well-watered condition for 20 days, pretreated with 1-butanol at 0, 10, 20, 30, 40, or 50 mM for 3 days, and then treated with drought stress for 6 days. **a**, **c** Different lowercase letters above boxes indicate statistically significant differences ($p < 0.05$, Tukey's test in R; $n = 12$). **d** Plants at the stage that leaf area was evaluated in (**c**). **e** Survival of plants grown under the well-watered condition for 20 days, pretreated with 1-butanol at 0, 10, 20, 30, 40, or 50 mM for 3 days, and then treated with drought stress for 13 days. Survival was recorded after rewatering for 10 days. Different lowercase letters above the bars indicate statistically significant differences ($p < 0.05$, Fisher's exact test in R; $n = 12$). **f** Plants at the stage that survival was evaluated in **e**. Scale bars: 3.0 cm

Identification of genes regulated by 1-butanol treatment

We performed RNA-seq analyses to comprehensively examine the changes in gene expression of *Arabidopsis* plants subjected to 1-butanol or water pretreatment and drought stress exposure (Fig. 5). In this experiment, we used wild type plants. The sampling time points for the RNA-seq analysis are shown in Fig. 5a. From principal component analysis (PCA) of the data for each time point, it was expected that the expression profile of W3D10 differed strongly from that of B3D10 both in shoots and roots (Fig. 5b). Volcano

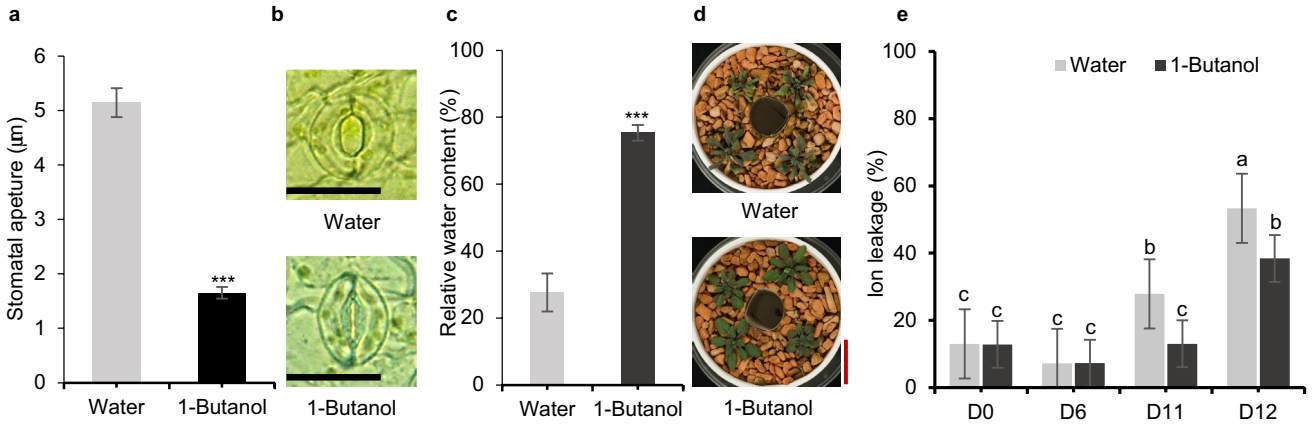


Fig. 3 Effects of 1-butanol treatment on stomatal aperture, relative water content, and electrolyte leakage of *Arabidopsis*. **a** Stomatal aperture on rosette leaves of 20-day-old seedlings grown under the well-watered condition and then treated with 20 mM 1-butanol or water for 3 days. Fifty stomata per seedling were measured ($n=3$). **b** Representative images of stomata at the stage that the stomatal aperture was measured. Scale bars: 10 μm. **c** Relative water content of the shoot of 20-day-old plants grown under the well-watered condition, pretreated with 20 mM 1-butanol or water for 3 days, and then subjected to drought treatment for 11 days ($n=24$). **a**, **c** Asterisks above bars indicate statistically significant differences between water-treated and 1-butanol-treated plants (*** $p<0.001$, Student's *t*-test). **d** Plants at the stage that the relative water content was quantified. Scale bars: 3.0 cm. **e** Electrolyte leakage from the shoot of 20-day-old plants grown under the well-watered condition, pretreated with 20 mM 1-butanol or water for 3 days, and then electrolyte leakage was quantified immediately (D0) or after drought treatment for 6 days (D6), 11 days (D11), or 12 days (D12). Different lowercase letters above the bars indicate statistically significant differences ($p<0.05$, Tukey's test in R; $n=6$)

plots showed that a marked number of DEGs were detected in the comparison between B3D10 and W3D10 in shoots and roots (Fig. 5c), supporting the results of the PCA analysis. Regarding up-regulated DEGs, 6, 583, and 3401 genes in shoots and 30, 669, and 3154 genes in roots were identified in the comparisons between B3D0 and W3D0, between B3D6 and W3D6, and between B3D10 and W3D10, respectively (Fig. 5d; Supplementary Tables S2, S3, S4, S6, S8, and S10). The number of down-regulated DEGs was 6, 65, and 2978 in shoots and 9, 9, and 2104 in roots in the same comparisons, respectively (Fig. 5d; Supplementary Tables S2, S3, S5, S7, S9, and S11). Venn diagrams showed that most DEGs did not overlap among the drought stress periods (Fig. 5d). In the comparisons of shoots and roots, the D0 and D6 drought treatments did not overlap in the detected DEGs (Fig. 5d). Interestingly, at 10 days of drought stress exposure, the 1166 up-regulated DEGs and 1183 down-regulated DEGs overlapped between shoots and roots (Fig. 5d).

A GO enrichment analysis was performed (Supplementary Table S12–20). The results showed that the up-regulated DEGs at D6 in shoots were enriched in GO terms associated with the cell wall, glucosinolate metabolism, and oxidative and hypoxic stress (Supplementary Table S12). Among the up-regulated DEGs at D10 in shoots, GO terms associated with photosynthesis and glucosinolate metabolism were enriched (Supplementary Table S14). In roots, for example, GO terms associated with tissue development and glucosinolate metabolism were enriched in the up-regulated DEGs at D10 (Supplementary Tables S16, 17).

RT-qPCR analysis of the expression of genes associated with oxidative stress

Although enriched GO terms were not detected in the up-regulated DEGs at D0, we noted that some of the DEGs were associated with oxidative stress, and we considered that these genes might be important for 1-butanol-enhanced drought tolerance. Therefore, we confirmed the expression profiles by RT-qPCR analysis. The results showed that the expression levels of *RS6* and *OXS3* in the shoots, and *HRG1*, *HRG2*, *HSP23.5*, and *UGT74E2* in the roots were up-regulated (Fig. 6a–f). These up-regulated expression patterns were consistent with the RNA-seq data, thus validating the results of the RNA-seq analysis.

Comparative analysis of transcriptome data from 1-butanol-treated and ethanol-treated plants

To examine the regulatory mechanisms for the enhancement of drought stress tolerance by alcoholic compounds, we compared transcriptome data from 1-butanol application with those from ethanol application. When the DEGs from the plants pretreated with water or 1-butanol for 3 days (the data presented herein) were compared with the DEGs from the plants pretreated with water or ethanol for 3 days (GenBank accession GSE201380; Bashir et al. 2022), no overlap of up- and down-regulated DEGs was observed (Fig. 7a). In addition, we compared the DEGs from the plants exposed to drought stress after 1-butanol or ethanol pretreatment. For

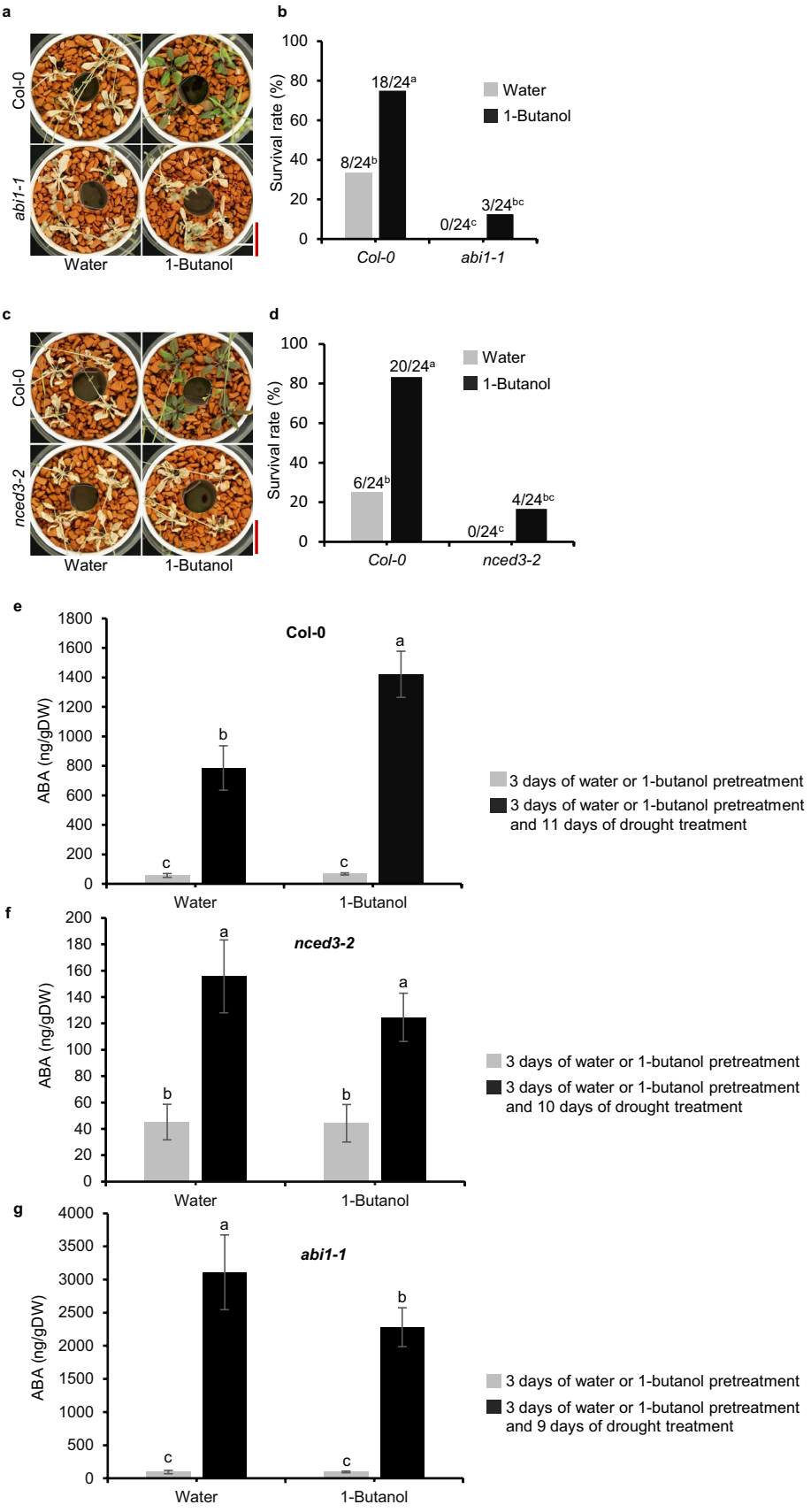
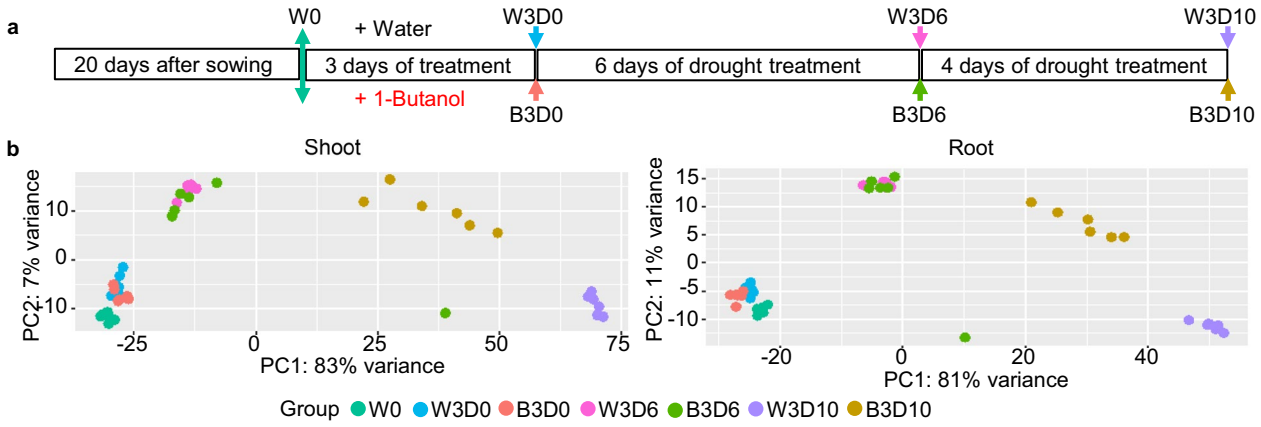
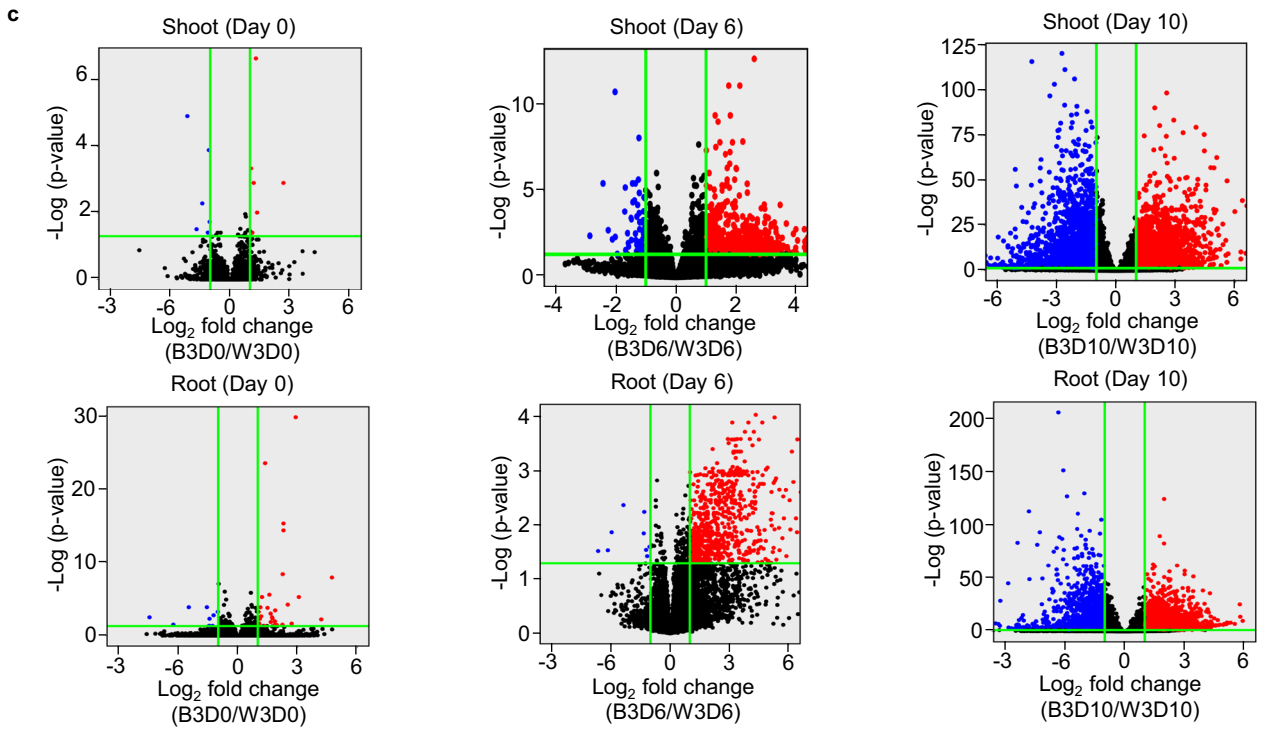
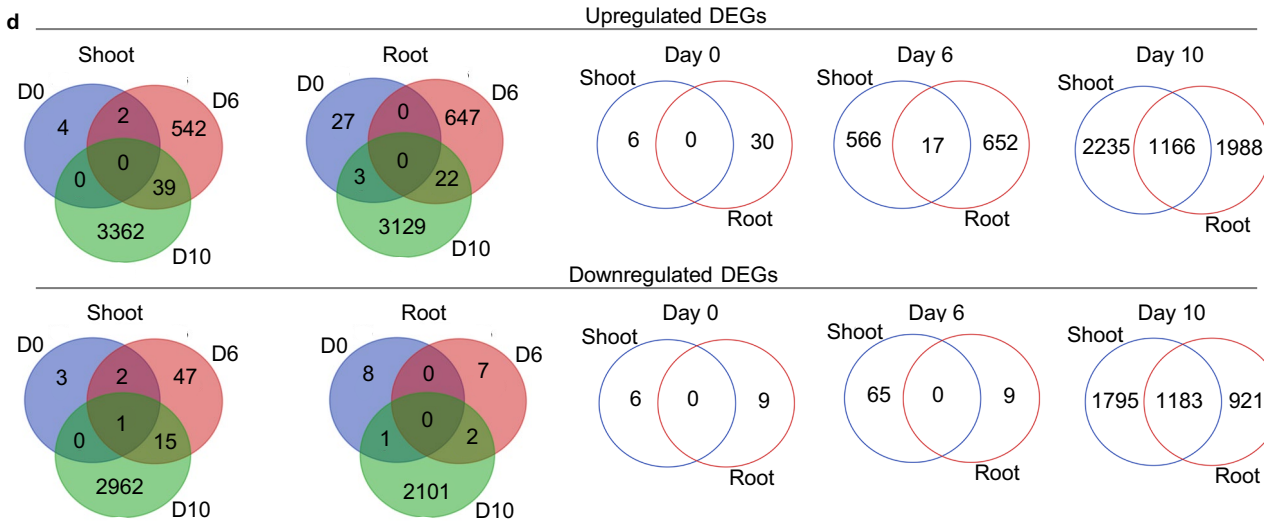


Fig. 4 Effects of 1-butanol treatment on drought tolerance of *Arabidopsis abi1-1* and *nced3-2* mutants. **a** Plants of the wild type (Col-0) and *abi1-1* mutant grown under the well-watered condition for 20 days, pretreated with either 20 mM 1-butanol or water for 3 days, subjected to drought treatment for 12 days (Col-0) or 9 days (*abi1-1*), and then rewatered for 5 days. **b** Survival of plants in (**a**). **c** Plants of the wild type (Col-0) and *nced3-2* mutant grown under the well-watered condition for 20 days, pretreated with either 20 mM 1-butanol or water for 3 days, subjected to drought treatment for 12 days (Col-0) or 10 days (*nced3-2*), and then rewatered for 5 days. **d** Survival of plants in (**c**). **e** ABA content in the shoot of Col-0 plants pretreated with either 20 mM 1-butanol or water for 3 days and subjected to drought stress treatment for 11 days. **f** ABA content in the shoot of *nced3-2* mutant plants pretreated with either 20 mM 1-butanol or water for 3 days and subjected to drought stress treatment for 10 days. **g** ABA content in the shoot of *abi1-1* mutant plants pretreated with either 20 mM 1-butanol or water for 3 days and subjected to drought stress treatment for 9 days. Different letters above the bars indicate statistically significant differences ($p < 0.05$) by Fisher's exact test in (**b**, **d**) (n = 24), and by Tukey's test in (**e–g**) (n = 6). Scale bars: 3.0 cm


a
W0
+ Water
W3D0
W3D6
W3D10
20 days after sowing
3 days of treatment
6 days of drought treatment
4 days of drought treatment
+ 1-Butanol
B3D0
B3D6
B3D10

b
Shoot
Root
PC2: 7% variance
PC1: 83% variance
PC2: 11% variance
PC1: 81% variance
Group W0 W3D0 B3D0 W3D6 B3D6 W3D10 B3D10


c
Shoot (Day 0)
Shoot (Day 6)
Shoot (Day 10)
Root (Day 0)
Root (Day 6)
Root (Day 10)
-Log (p-value)
Log2 fold change
(B3D0/W3D0)
(B3D6/W3D6)
(B3D10/W3D10)


d
Upregulated DEGs
Shoot
Root
Day 0
Day 6
Day 10
D0
D6
D10
Downregulated DEGs

◂**Fig. 5** Transcriptome analysis for detection of differential gene expression in response to drought treatment in shoots and roots of 1-butanol-treated or water-treated *Arabidopsis* plants. **a** Schematic diagram of sampling time points for transcriptome analysis. Twenty-day-old plants grown under the well-watered condition were pretreated with either 20 mM 1-butanol or water for 3 days, and then subjected to drought treatment for up to 10 days. W0: samples were collected immediately before the onset of 1-butanol or water treatment; W3D0 and B3D0: samples were collected immediately after treatment with water or 1-butanol for 3 days, respectively; W3D6 and B3D6: samples were collected after drought treatment for 6 days; W3D10 and B3D10: samples were collected after drought treatment for 10 days. **b** Scatter plots of the first and second principal components (PC1 and PC2, respectively) for transcriptome data for shoots and roots. **c** Volcano plots of transcriptome data for shoots and roots at each sampling time point. The horizontal green line indicates the threshold -log (*p*-value) ($p=0.05$). Vertical green lines indicate the threshold fold-change interval (the value was set to 2.0). Red and blue data points indicate up- and down-regulated differentially expressed genes (DEGs), respectively. Black data points indicate genes either with no differential expression (i.e. $p>0.05$) or with differential expression at levels below our chosen threshold fold-change. **d** Venn diagrams showing overlap of up-regulated DEGs (upper row) and down-regulated DEGs (lower row) in shoots and roots at each sampling time point

the comparison, the DEGs at D10 (the data presented herein) and the DEGs from the shoots of plants subjected to drought stress for 13 days (GenBank accession GSE201380; Bashir et al. 2022) were used because the magnitudes of drought stress were similar (the stress magnitudes were between the initial leaf wilting point and permanent leaf wilting point in the water-pretreated plants). Venn diagrams showed that more than half of the up- and down-regulated DEGs of ethanol-pretreated plants overlapped with those of 1-butanol-pretreated plants (Fig. 7b). A GO enrichment analysis using the overlapping DEGs was performed (Supplementary Table S21 to S26). The enriched GO terms for up-regulated overlapping DEGs included NADH dehydrogenase complex (plastoquinone) assembly (GO:0010258), cellular response to sulfur starvation (GO:0010438), positive regulation of auxin-mediated signaling pathway (GO:0010929), photosynthetic electron transport in photosystem I (GO:0009773), glucosinolate biosynthetic process (GO:0019761), and flavonoid biosynthetic process (GO:0009813) (Supplementary Table S21). The enriched GO terms for down-regulated overlapping DEGs included UDP-L-arabinose biosynthetic process (GO:0033358), capsule organization (GO:0045230), proline metabolic process (GO:0006560), glutamine family amino acid catabolic process (GO:0009065), and mitochondrial ATP synthesis coupled electron transport (GO:0042775) (Supplementary Table S22).

1-Butanol treatment enhances high-salinity tolerance by neutralizing the salinity-induced accumulation of reactive oxygen species

Ethanol has been reported to enhance high-salt tolerance in *Arabidopsis* plants by detoxification of reactive oxygen species (ROS) (Nguyen et al. 2017). Inspired by this report, we investigated the effects of 1-butanol on high-salinity tolerance. *Arabidopsis* plants were pretreated with or without 10 mM 1-butanol prior to exposure to 100 mM NaCl. Pretreatment with 1-butanol resulted in the survival of more than 80% of plants under exposure to high salinity, whereas pretreatment with water led to survival of less than 10% of plants under the same condition (Supplementary Fig. S4a, b). Next, we analyzed the accumulation of H_2O_2 indicated by DAB staining in 1-butanol- and water-pretreated plants under high salinity. In the absence of 100 mM NaCl, the cotyledons of 1-butanol-pretreated plants were weakly stained by DAB (Supplementary Fig. S4b). In the presence of 100 mM NaCl, the cotyledons of water-pretreated plants were intensely stained by DAB. However, the cotyledons of 1-butanol-pretreated plants were not stained by DAB. These data suggested that 1-butanol treatment could enhance high-salinity stress tolerance through a decrease in ROS accumulation, similar to the effectiveness of ethanol treatment (Nguyen et al. 2017).

Discussion

The aim of this study was to investigate whether alcoholic compounds other than ethanol enhance drought stress tolerance in *Arabidopsis*. The results showed that 1-butanol was the most effective compound among the tested alcohols for improvement in the drought stress tolerance of *Arabidopsis*, with 100% survival observed (Fig. 1). Previously, ethanol treatment was reported to enhance high-salinity, heat, and drought stress tolerance in *Arabidopsis* (Nguyen et al. 2017; Bashir et al. 2022; Matsui et al. 2022) and drought stress tolerance in cassava (Vu et al. 2022). These reports linked such enhancement of drought stress tolerance by ethanol treatment to its effects on stomatal closure and water retention. In the present study, we showed that 1-butanol treatment led to increased stomatal closure and enhanced water retention (Fig. 3). These results raised the possibility that the mechanism for drought stress tolerance induced by ethanol and that induced by 1-butanol might act on similar pathways. Previous studies have indicated that ethanol fails to enhance drought tolerance in the *Arabidopsis abi1-1* mutant (Bashir et al. 2022), a mutant defective in the ABA signaling pathway (Gosti et al. 1999), suggesting

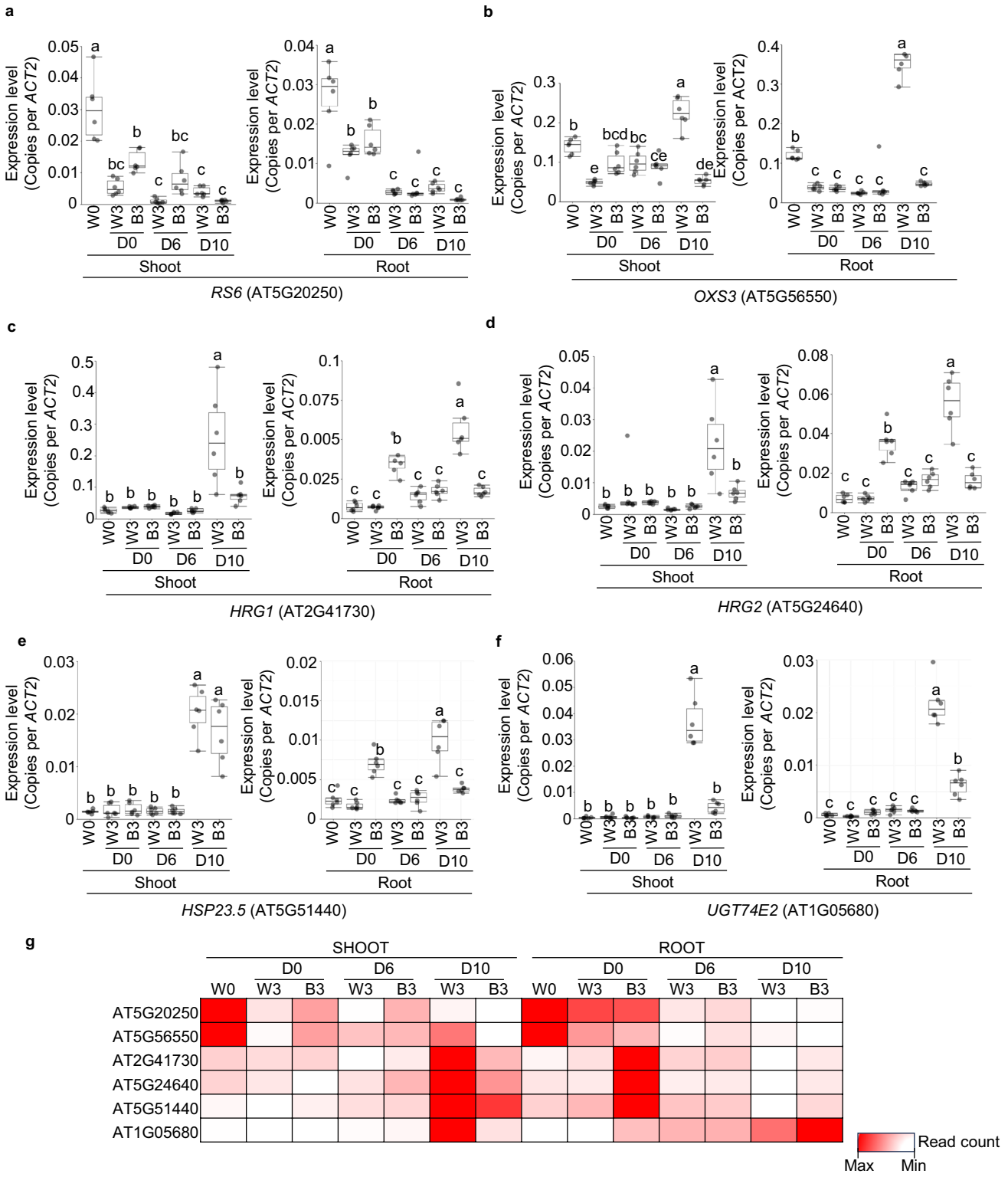


Fig. 6 Expression profiles of reactive oxygen species-related genes as estimated by reverse transcription–quantitative PCR (RT-qPCR) and transcriptome analysis. Samples used for transcriptome analysis were also used for RT-qPCR analysis. **a**–**f** Results of RT-qPCR analysis. Different lowercase letters above the boxes indicate statistically significant differences ($p < 0.05$, Tukey's test in R; $n = 6$). **a** AT5G20250 (*RS6*). **b** AT5G56550 (*OXS3*). **c** AT2G41730 (*HRG1*). **d** AT5G24640 (*HRG2*). **e** AT5G51440 (*HSP23.5*). **f** AT1G05680 (*UGT74E2*). **g** Heat map of RNA-sequencing gene expression read count

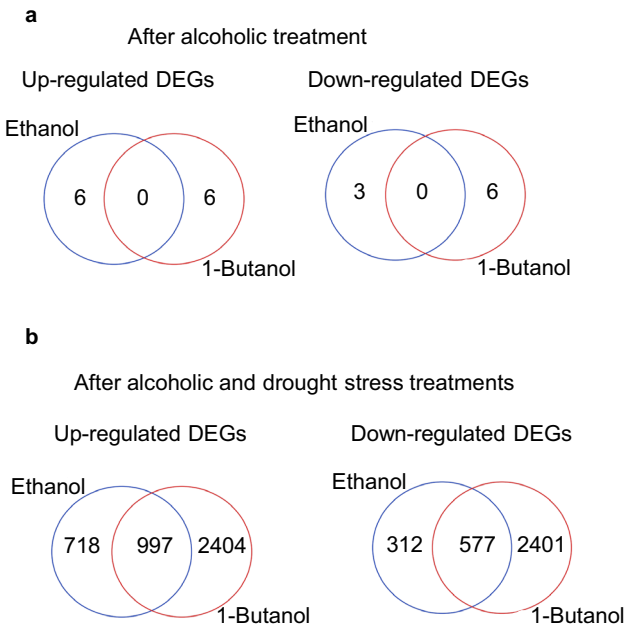


Fig. 7 Comparative analysis of differentially expressed genes (DEGs) between 1-butanol- and ethanol-treated *Arabidopsis* plants. **a** Venn diagrams showing overlap of up-regulated DEGs (left) and down-regulated DEGs (right) in seedlings treated with 1-butanol or ethanol for 3 days. **b** Venn diagrams showing overlap of up-regulated DEGs (left) and down-regulated DEGs (right) in seedlings pretreated with 1-butanol for 3 days and then subjected to drought treatment for 10 days, and seedlings pretreated with ethanol for 3 days and then subjected to drought treatment for 13 days

the involvement of ABA signaling in the drought tolerance mediated by ethanol. In the present study, we showed that 1-butanol treatment led to little improvement in percentage survival of drought-exposed *abi1-1* plants (Fig. 4a). Therefore, it is suggested that 1-butanol-mediated drought tolerance also acts through the ABA signaling pathway, similar to that mediated by ethanol. Moreover, we observed that the percentage survival of *nced3* mutant plants, which are defective in ABA biosynthesis during drought stress (Iuchi et al. 2001; Endo et al. 2008), was not significantly enhanced by 1-butanol treatment (Fig. 4b). This finding supported the hypothesis that both ABA signaling and the ABA biosynthesis pathway are involved in drought tolerance mediated by 1-butanol.

The current results also showed that methanol effectively increased drought stress tolerance, as illustrated by the 40% survival of methanol-pretreated plants exposed to drought stress (Fig. 1). This finding might be consistent with a previous report of the positive effects of methanol treatment on the growth and development of crop plants grown in irrigated agricultural fields in the desert region of southwestern USA (Nonomura and Benson 1992). Further analysis is needed to elucidate the molecular mechanisms that govern methanol-enhanced drought tolerance.

Through a comparative transcriptome analysis of ethanol- and 1-butanol-treated plants, we observed overlap in the drought-responsive pathways between the two treatment groups (Fig. 7, Supplementary Tables S21, 22). Numerous up- and down-regulated DEGs were observed in both the ethanol- and 1-butanol-treated groups under drought stress (Fig. 7). The GO enrichment analysis showed that genes with a function in NAD(P)H dehydrogenase complex assembly (GO:0010275) and photosynthetic electron transport in photosystem I (GO:0009773) were among the top-ranked mutually up-regulated DEGs (Supplementary Table S21). While photosynthetic electron transport in photosystem I functions in generating NAD(P)H (Yamori and Shikanai 2016), the NAD(P)H dehydrogenase complex assembly acts to concentrate CO_2 in the bundle sheath cells (Peterson et al. 2016). These functions suggest that one possible mechanism for the drought stress responses induced by ethanol and 1-butanol is enhanced efficiency of carbon capture during photosynthesis to compensate for the reduction in stomatal opening under drought stress. In addition, the expression of genes with a function in the positive regulation of the auxin-mediated signaling pathway (GO:0010929) and the glucosinolate biosynthetic process (GO:0019761 and GO:0019758) was up-regulated in both ethanol- and 1-butanol-treated plants (Supplementary Table S21). Glucosinolates enhance drought tolerance in *Arabidopsis* (Salehin et al. 2019) by promoting stomatal closure (Endo et al. 2008; Khokon et al. 2011). Furthermore, auxin-sensitive Aux/IAA proteins regulate glucosinolate contents during drought tolerance in *Arabidopsis* (Salehin et al. 2019). These findings suggest that ethanol and 1-butanol likely improve drought stress tolerance in *Arabidopsis* through induction of the auxin-signaling-mediated regulation of glucosinolate biosynthesis. Furthermore, significant up-regulation of genes functioning in the biosynthesis of anthocyanin compounds and in the catabolism of serine-family amino acids was detected in the present GO enrichment analysis (Supplementary Table S21). Anthocyanins can control plant water loss via reduction of stomatal transpiration and density, and are considered crucial regulators of drought tolerance in tobacco (Cirillo et al. 2021). Metabolism of amino acids is critical to maintain energy generation during drought in sugarcane (Diniz et al. 2020). In addition, the expression of genes with a function in the biosynthetic and metabolic processes of other flavonoids was up-regulated in the present GO enrichment analysis (Supplementary Table S21). In general, flavonoids participate in antioxidant systems that respond to various abiotic stress factors in different plant species (Shomali et al. 2022). An increase in the antioxidant capacity induced by an increase in the content

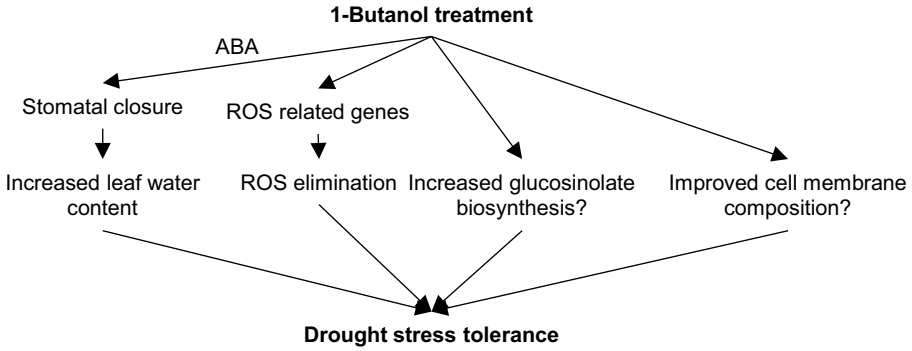


Fig. 8 Proposed mechanism underlying 1-butanol-enhanced drought tolerance of *Arabidopsis* plants. The 1-butanol treatment primes the relevant mechanisms to enhance drought tolerance

of flavonoid compounds leads to improved tolerance of ultraviolet-B, salinity, drought, and water stresses (Hernandez et al. 2004; Hodaei et al. 2018; Gao et al. 2019; Khalil et al. 2022; Gourlay et al. 2022). Furthermore, an increase in flavonoid content is associated with a lower degree of lipid peroxidation and a higher capacity for ROS detoxification, which in turn improves plant tolerance of cadmium and lead stresses (Yang et al. 2011; Javad et al. 2022). Therefore, the present data suggested that these biosynthetic and metabolic processes of flavonoids induced by ethanol or 1-butanol likely play a role in the drought stress response in *Arabidopsis*.

The present GO enrichment analysis showed that the overlapping down-regulated DEGs included genes with a function in UDP-L-arabinose biosynthesis (GO:0033358) (Supplementary Table S22). L-Arabinose is a component of α-1,5-arabinan (Takahashi et al. 2023). Increase in the content of specific arabinans in the cell wall of guard cells confers enhanced flexibility, which leads to wider stomatal opening (Carroll et al. 2022). Therefore, ethanol and 1-butanol may induce stomatal closure by limiting the arabinan-dependent flexibility of the guard cell wall.

Previously, we reported that external ethanol molecules could be metabolized into various molecules including sugars (Bashir et al. 2022). We consider that this is one of the reasons why alcohol-treated plants increase abiotic stress tolerance. The accumulated sugars can be used as an alternative energy source when stomata are closed under drought conditions. It has been well established that *Clostridium acetobutylicum,* a Gram-positive anaerobic microorganism have the pathway for butanol production (Foulquier et al. 2022). Although plants do not positively produce butanol, they might have a metabolic pathway related to butanol. If so, external butanol molecules might be metabolized and led to the accumulation of metabolites including sugars. To investigate this hypothesis, experiments including metabolome and NMR using radioisotope-labeled butanol will be required. In the green alga *Chlamydomonas eugametos*, it has been reported that 1-butanol can serve both as an activator of phospholipase (PLD) and as a substrate in PLD catalyzed transphosphatidylation reactions (Munnik et al. 1995). In plants, PLDs are enzymes involved in the modification of major membrane phospholipids (Deepika and Singh 2022). Specifically, PLDs can hydrolyze the phosphodiester bond of glycerophospholipids to release free head groups and transfer the phosphatidyl moiety to water to produce phosphatidic acid as an important secondary messenger involved in various signaling pathways (Pokotylo et al. 2018; Kim and Wang 2020). However, in the presence of 1-butanol, PLDs transfer the phosphatidyl moiety to 1-butanol to form phosphatidyl-1-butanol (Munnik et al. 1995). Futhermore, 1-butanol has been shown to be able to activate transphosphatidylation of PLDs, which consequently stimulates further production of phosphatidyl-1-butanol. Because of these reactions, 1-butanol is often used as an effective inhibitor of PLD-catalyzed phosphatidic acid production (Gardiner et al. 2003). Whether phosphatidyl-1-butanol itself has any biological significance is unknown. Therefore, further studies are needed to investigate the possible implications of PLD-catalyzed formation of phosphatidyl-1-butanol.

In conclusion, we propose a model for the mechanisms underlying 1-butanol-mediated drought stress tolerance in *Arabidopsis* (Fig. 8). Specifically, 1-butanol pretreatment causes stomatal closure, which leads to reduced water loss from the leaves under drought stress. Furthermore, 1-butanol pretreatment increases the expression levels of ROS-related genes that are important for elimination of ROS molecules that accumulated excessively under abiotic stress. Finally, our data implicate the ABA-dependent ABI1- and/or NCED3-mediated regulatory mechanisms in the enhancement of drought stress tolerance induced by 1-butanol pretreatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11103-024-01479-0.

Acknowledgements We thank Chieko Torii, Kayoko Mizunashi, Kyoko Y. Mogami, Minoru Ueda, Yoshinori Utsumi, Akihiro Ezoe, and Anh Thu Vu for their comments and technical support. We thank Edanz (https://jp.edanz.com/english-editing-c) for editing a draft of this manuscript. We thank Dr. Kazuo Shinozaki (RIKEN CSRS) for gifting

nced3-2 seeds. We thank Dr. Toshinori Kinoshita (Nagoya University) for providing *abi1-1* seeds.

Author contributions The experiments conducted in this study were designed by DTNQ, DT, and MS. Physiological experiments were performed by DTNQ, DT, MT, and JI. DAB staining was performed by KS. RNA-seq analysis was performed by AJN, ST, DT, MT, and DTNQ. The ABA measurement was performed by YT, YK, and MO. Editing and reviewing were done by DTNQ, DT, KS, XHP and MS. The manuscript was written by DTNQ, DT, and MS.

Funding This work was supported by grants from RIKEN, Japan (to MS), including the RIKEN–AIST Joint Research Fund (full research), Core Research for Evolutionary Science and Technology (JPMJCR13B4 to MS), and A-STEP (JPMJTM19BS to MS) of the Japan Science and Technology Agency (JST), and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (Innovative Areas 18H04791 and 18H04705 to MS).

Data availability The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declarations

Competing interests The authors have not disclosed any competing interests.

References

Ahmadi Soleimanie S, Vafaee Y, Koushesh Saba M (2020) Preharvest application of sodium nitroprusside improves tomato fruit quality and alleviates chilling injury during cold storage. Int J Veg Sci 26:364–378. https://doi.org/10.1080/19315260.2019.1636444

Alonso-Hernández CM, Conte F, Misic C, Barsanti M, Gómez-Batista M, Díaz-Asencio M, Covazzi-Harriague A, Pannacciulli FG (2011) An overview of the gulf of batabanó (Cuba): environmental features as revealed by surface sediment characterisation. Cont Shelf Res 31:749–757. https://doi.org/10.1016/j.csr.2011.01.006

Bashir K, Todaka D, Rasheed S, Matsui A, Ahmad Z, Sako K, Utsumi Y, Vu AT, Tanaka M, Takahashi S, Ishida J, Tsuboi Y, Watanabe S, Kanno Y, Ando E, Shin K-C, Seito M, Motegi H, Sato M, Li R, Kikuchi S, Fujita M, Kusano M, Kobayashi M, Habu Y, Nagano AJ, Kawaura K, Kikuchi J, Saito K, Hirai MY, Seo M, Shinozaki K, Kinoshita T, Seki M (2022) Ethanol-mediated novel survival strategy against drought stress in plants. Plant Cell Physiol 63:1181–1192. https://doi.org/10.1093/pcp/pcac114

Bertauche N, Leung J, Giraudat J (1996) Protein phosphatase activity of abscisic acid insensitive 1 (ABI1) protein from Arabidopsis thaliana. Eur J Biochem 241:193–200. https://doi.org/10.1111/j.1432-1033.1996.0193t.x

Carroll S, Amsbury S, Durney CH, Smith RS, Morris RJ, Gray JE, Fleming AJ (2022) Altering arabinans increases arabidopsis guard cell flexibility and stomatal opening. Curr Biol 32:3170-3179.e4. https://doi.org/10.1016/j.cub.2022.05.042

Chakraborti S, Bera K, Sadhukhan S, Dutta P (2022) Bio-priming of seeds: plant stress management and its underlying cellular, biochemical and molecular mechanisms. Plant Stress 3:100052. https://doi.org/10.1016/j.stress.2021.100052

Cirillo V, D'Amelia V, Esposito M, Amitrano C, Carillo P, Carputo D, Maggio A (2021) Anthocyanins are key regulators of drought stress tolerance in tobacco. Biology (basel) 10:139. https://doi.org/10.3390/biology10020139

Cofer TM, Engelberth M, Engelberth J (2018) Green leaf volatiles protect maize (Zea mays) seedlings against damage from cold stress. Plant Cell Environ 41:1673–1682. https://doi.org/10.1111/pce.13204

de Vendômois JS, Cellier D, Vélot C, Clair E, Mesnage R, Séralini G-E (2010) Debate on GMOs health risks after statistical findings in regulatory tests. Int J Biol Sci 6:590–598. https://doi.org/10.7150/ijbs.6.590

Deepika D, Singh A (2022) Plant phospholipase D: novel structure, regulatory mechanism, and multifaceted functions with biotechnological application. Crit Rev Biotechnol 42:106–124. https://doi.org/10.1080/07388551.2021.1924113

Diniz AL, da Silva DIR, Lembke CG, Costa MD-BL, ten-Caten F, Li F, Vilela RD, Menossi M, Ware D, Endres L, Souza GM, (2020) Amino acid and carbohydrate metabolism are coordinated to maintain energetic balance during drought in sugarcane. Int J Mol Sci 21:9124. https://doi.org/10.3390/ijms21239124

Djanaguiraman M, Boyle DL, Welti R, Jagadish SVK, Prasad PVV (2018) Decreased photosynthetic rate under high temperature in wheat is due to lipid desaturation, oxidation, acylation, and damage of organelles. BMC Plant Biol 18:55. https://doi.org/10.1186/s12870-018-1263-z

Endo A, Koshiba T, Kamiya Y, Nambara E (2008) Vascular system is a node of systemic stress responses. Plant Signal Behav 3:1138–1140. https://doi.org/10.4161/psb.3.12.7145

FAO (2021) The impact of disasters and crises on agriculture and food security: 2021. Rome. https://doi.org/10.4060/cb3673en

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant Drought Stress: Effects, Mechanisms and Management. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C (eds) Sustainable Agriculture. Springer, Netherlands, Dordrecht

Foulquier C, Rivière A, Heulot M, Dos Reis S, Perdu C, Girbal L, Pinault M, Dusséaux S, Yoo M, Soucaille P, Meynial-Salles I (2022) Molecular characterization of the missing electron pathways for butanol synthesis in *Clostridium acetobutylicum*. Nat Commun 13:4691. https://doi.org/10.1038/s41467-022-32269-1

Fu J, Sun P, Luo Y, Zhou H, Gao J, Zhao D, Pubu Z, Liu J, Hu T (2019) Brassinosteroids enhance cold tolerance in elymus nutans via mediating redox homeostasis and proline biosynthesis. Environ Exp Bot 167:103831. https://doi.org/10.1016/j.envexpbot.2019.103831

Gao L, Liu Y, Wang X, Li Y, Han R (2019) Lower levels of UV-B light trigger the adaptive responses by inducing plant antioxidant metabolism and flavonoid biosynthesis in medicago sativa seedlings. Funct Plant Biol 46(10):896–906. https://doi.org/10.1071/FP19007

Gardiner J, Collings DA, Harper JDI, Marc J (2003) The effects of the phospholipase D-antagonist 1-butanol on seedling Development and microtubule organisation in arabidopsis. Plant Cell Physiol 44(7):687–696. https://doi.org/10.1093/pcp/pcg095

Gosti F, Beaudoin N, Serizet C, Webb AAR, Vartanian N, Giraudat J (1999) ABI1 protein phosphatase 2C Is a negative regulator of abscisic acid signaling. Plant Cell 11:1897–1909. https://doi.org/10.1105/tpc.11.10.1897

Gourlay G, Hawkins BJ, Albert A, Schnitzler J, Peter Constabel C (2022) Condensed tannins as antioxidants that protect poplar against oxidative stress from drought and UV-B. Plant Cell Environ 45:362–377. https://doi.org/10.1111/pce.14242

Guzmán A, Barragán S, Cala Vitery F (2021) Dropout in rural higher education: a systematic review. Front Educ (lausanne) 6:727833. https://doi.org/10.3389/feduc.2021.727833

Ha G, Roth A, Khattra J, Ho J, Yap D, Prentice LM, Melnyk N, McPherson A, Bashashati A, Laks E, Biele J, Ding J, Le A, Rosner J, Shumansky K, Marra MA, Gilks CB, Huntsman DG,

McAlpine JN, Aparicio S, Shah SP (2014) TITAN: inference of copy number architectures in clonal cell populations from tumor whole-genome sequence data. Genome Res 24:1881–1893. https://doi.org/10.1101/gr.180281.114

Hasanuzzaman M, Hossain MA, Fujita M (2011) Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. Plant Biotechnol Rep 5:353–365. https://doi.org/10.1007/s11816-011-0189-9

Hernandez I, Alegre L, Munne-Bosch S (2004) Drought-induced changes in flavonoids and other low molecular weight antioxidants in Cistus clusii grown under Mediterranean field conditions. Tree Physiol 24:1303–1311. https://doi.org/10.1093/treephys/24.11.1303

Hodaei M, Rahimmalek M, Arzani A, Talebi M (2018) The effect of water stress on phytochemical accumulation, bioactive compounds and expression of key genes involved in flavonoid biosynthesis in chrysanthemum morifolium L. Ind Crops Prod 120:295–304. https://doi.org/10.1016/j.indcrop.2018.04.073

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9- *cis* -epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. Plant J 27:325–333. https://doi.org/10.1046/j.1365-313x.2001.01096.x

Javad S, Shah AA, Ramzan M, Sardar R, Javed T, Al-Huqail AA, Ali HM, Chaudhry O, Yasin NA, Ahmed S, Hussain RA, Hussain I (2022) Hydrogen sulphide alleviates cadmium stress in *Trigonella foenum-graecum* by modulating antioxidant enzymes and polyamine content. Plant Biol 24:618–626. https://doi.org/10.1111/plb.13393

Jin SH, Li XQ, Wang GG, Zhu XT (2015) Brassinosteroids alleviate high-temperature injury in Ficus concinna seedlings via maintaining higher antioxidant defence and glyoxalase systems. AoB Plants. https://doi.org/10.1093/aobpla/plv009

Kamitani M, Kashima M, Tezuka A, Nagano AJ (2019) Lasy-Seq: a high-throughput library preparation method for RNA-Seq and its application in the analysis of plant responses to fluctuating temperatures. Sci Rep 9:7091. https://doi.org/10.1038/s41598-019-43600-0

Kerchev P, van der Meer T, Sujeeth N, Verlee A, Stevens CV, Van Breusegem F, Gechev T (2020) Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. Biotechnol Adv 40:107503. https://doi.org/10.1016/j.biotechadv.2019.107503

Khalil R, Yusuf M, Bassuony F, Haroun S, Gamal A (2022) Alpha-tocopherol reinforce selenium efficiency to ameliorates salt stress in maize plants through carbon metabolism, enhanced photosynthetic pigments and ion uptake. S Afr J Bot 144:1–9. https://doi.org/10.1016/j.sajb.2021.08.033

Kim SC, Wang X (2020) Phosphatidic acid: an emerging versatile class of cellular mediators. Essays Biochem 64:533–546. https://doi.org/10.1042/EBC20190089

Kim J-M, To TK, Matsui A, Tanoi K, Kobayashi NI, Matsuda F, Habu Y, Ogawa D, Sakamoto T, Matsunaga S, Bashir K, Rasheed S, Ando M, Takeda H, Kawaura K, Kusano M, Fukushima A, Endo TA, Kuromori T, Ishida J, Morosawa T, Tanaka M, Torii C, Takebayashi Y, Sakakibara H, Ogihara Y, Saito K, Shinozaki K, Devoto A, Seki M (2017) Acetate-mediated novel survival strategy against drought in plants. Nat Plants 3:17097. https://doi.org/10.1038/nplants.2017.97

Kumar D, Yusuf MA, Singh P, Sardar M, Sarin NB (2014) Histochemical detection of superoxide and h2o2 accumulation in *brassica juncea* seedlings. Bio protoc. https://doi.org/10.21769/BioProtoc.1108

Leung J, Bouvier-Durand M, Morris P-C, Guerrier D, Chefdor F (1979) Giraudat J (1994) arabidopsis ABA response gene ABI1: features of a calcium-modulated protein phosphatase. Science 264:1448–1452. https://doi.org/10.1126/science.7910981

Matsui A, Iida K, Tanaka M, Yamaguchi K, Mizuhashi K, Kim J-M, Takahashi S, Kobayashi N, Shigenobu S, Shinozaki K, Seki M (2017) Novel stress-inducible antisense RNAs of protein-coding loci are synthesized by RNA-dependent RNA polymerase. Plant Physiol 175:457–472. https://doi.org/10.1104/pp.17.00787

Matsui A, Todaka D, Tanaka M, Mizunashi K, Takahashi S, Sunaoshi Y, Tsuboi Y, Ishida J, Bashir K, Kikuchi J, Kusano M, Kobayashi M, Kawaura K, Seki M (2022) Ethanol induces heat tolerance in plants by stimulating unfolded protein response. Plant Mol Biol 110:131–145. https://doi.org/10.1007/s11103-022-01291-8

Mauch-Mani B, Baccelli I, Luna E, Flors V (2017) Defense priming: an adaptive part of induced resistance. Annu Rev Plant Biol 68:485–512. https://doi.org/10.1146/annurev-arplant-042916-041132

Mdar K, Mds J, Rahman T, Ma H, Muroyama D, Minami I, Munemasa S, Ic M, Nakamura Y, Murata Y (2011) Allyl isothiocyanate (AITC) induces stomatal closure in *Arabidopsis*. Plant Cell Environ 34:1900–1906. https://doi.org/10.1111/j.1365-3040.2011.02385.x

Meyer K, Leube MP Grill E (1994) a protein phosphatase 2C Involved in ABA Signal Transduction in Arabidopsis thaliana. Science 264:1452–1455. https://doi.org/10.1126/science.8197457

Min K, Showman L, Perera A, Arora R (2018) Salicylic acid-induced freezing tolerance in spinach (Spinacia oleracea L.) leaves explored through metabolite profiling. Environ Exp Bot 156:214–227. https://doi.org/10.1016/j.envexpbot.2018.09.011

Mostofa MG, Rahman A, Ansary MMU, Watanabe A, Fujita M, Tran LSP (2015) Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. Sci Rep 5:14078. https://doi.org/10.1038/srep14078

Munnik T, Steven Arisz IA, Vrije T, Musgravea A (1995) G Protein Activation Stimulates Phospholipase D Signaling in Plants. Plant Cell. https://doi.org/10.1105/tpc.7.12.2197

Nguyen HM, Sako K, Matsui A, Suzuki Y, Mostofa MG, Van HC, Tanaka M, Tran L-SP, Habu Y, Seki M (2017) Ethanol enhances high-salinity stress tolerance by detoxifying reactive oxygen species in arabidopsis thaliana and rice. Front Plant Sci 8:1001. https://doi.org/10.3389/fpls.2017.01001

Nguyen HM, Sako K, Matsui A, Ueda M, Tanaka M, Ito A, Nishino N, Yoshida M, Seki M (2018) Transcriptomic analysis of *Arabidopsis thaliana* plants treated with the Ky-9 and Ky-72 histone deacetylase inhibitors. Plant Signal Behav 13:e1448333. https://doi.org/10.1080/15592324.2018.1448333

Nishiyama R, Watanabe Y, Fujita Y, Le DT, Kojima M, Werner T, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, Kakimoto T, Sakakibara H, Schmülling T, Tran L-SP (2011) Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis. Plant Cell 23:2169–2183. https://doi.org/10.1105/tpc.111.087395

Nonomura AM, Benson AA (1992) The path of carbon in photosynthesis: improved crop yields with methanol. Proc Natl Acad Sci 89:9794–9798. https://doi.org/10.1073/pnas.89.20.9794

Peterson RB, Schultes NP, McHale NA, Zelitch I (2016) Evidence for a Role for NAD(P)H dehydrogenase in concentration of CO_2 in the bundle sheath cell of *zea mays*. Plant Physiol 171:125–138. https://doi.org/10.1104/pp.16.00120

Pokotylo I, Kravets V, Martinec J, Ruelland E (2018) The phosphatidic acid paradox: too many actions for one molecule class? Lessons from plants. Prog Lipid Res 71:43–53

Potapov P, Turubanova S, Hansen MC, Tyukavina A, Zalles V, Khan A, Song X-P, Pickens A, Shen Q, Cortez J (2021) Global maps of cropland extent and change show accelerated cropland expansion

in the twenty-first century. Nat Food 3:19–28. https://doi.org/10.1038/s43016-021-00429-z

Rasheed S, Bashir K, Matsui A, Tanaka M, Seki M (2016) Transcriptomic analysis of soil-grown arabidopsis thaliana roots and shoots in response to a drought stress. Front Plant Sci 7:00180. https://doi.org/10.3389/fpls.2016.00180

Raza A, Mubarik MS, Sharif R, Habib M, Jabeen W, Zhang C, Chen H, Chen ZH, Siddique KHM, Zhuang W, Varshney RK (2023) Developing drought-smart, ready-to-grow future crops. Plant Genome 16(1):e20279. https://doi.org/10.1002/tpg2.20279

Sako K, Nguyen HM, Seki M (2021) Advances in chemical priming to enhance abiotic stress tolerance in plants. Plant Cell Physiol 61:1995–2003. https://doi.org/10.1093/pcp/pcaa119

Salehin M, Li B, Tang M, Katz E, Song L, Ecker JR, Kliebenstein DJ, Estelle M (2019) Auxin-sensitive Aux/IAA proteins mediate drought tolerance in arabidopsis by regulating glucosinolate levels. Nat Commun 10:4021. https://doi.org/10.1038/s41467-019-12002-1

Savvides A, Ali S, Tester M, Fotopoulos V (2016) Chemical priming of plants against multiple abiotic stresses: mission possible? Trends Plant Sci 21:329–340. https://doi.org/10.1016/j.tplants.2015.11.003

Shomali A, Das S, Arif N, Sarraf M, Zahra N, Yadav V, Aliniaeifard S, Chauhan DK, Hasanuzzaman M (2022) Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. Plants 11:3158. https://doi.org/10.3390/plants11223158

Takahashi D, Soga K, Sasaki K, Kikuchi T, Kutsuno T, Nishiyama Y, Johnson KL, Sampathkumar A, Bacic A, Kotake T (2023) Cell wall pectic β-1,4-galactan contributes to increased plant freezing tolerance induced by cold acclimation. Biorxiv. https://doi.org/10.1101/2023.05.31.542803

Todaka D, Quynh DTN, Tanaka M, Utsumi Y, Utsumi C, Ezoe A, Takahashi S, Ishida J, Kusano M, Kobayashi M, Saito K, Nagano AJ, Nakano Y, Mitsuda N, Fujiwara S, Seki M (2024) Application of ethanol alleviates heat damage to leaf growth and yield in tomato. Front Plant Sci 15:1325365. https://doi.org/10.3389/fpls.2024.1325365

Tsai W-A, Weng S-H, Chen M-C, Lin J-S, Tsai W-S (2019) Priming of plant resistance to heat stress and tomato yellow leaf curl thailand virus with plant-derived materials. Front Plant Sci 10:00906. https://doi.org/10.3389/fpls.2019.00906

Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2009) Characterization of the ABA-regulated global responses to dehydration in arabidopsis by metabolomics. Plant J 57:1065–1078. https://doi.org/10.1111/j.1365-313X.2008.03748.x

Utsumi Y, Utsumi C, Tanaka M, Van HC, Takahashi S, Matsui A, Matsunaga TM, Matsunaga S, Kanno Y, Seo M, Okamoto Y, Moriya E, Seki M (2019) Acetic acid treatment enhances drought avoidance in cassava (manihot esculenta crantz). Front Plant Sci 10:00521. https://doi.org/10.3389/fpls.2019.00521

Vaidya AS, Helander JDM, Peterson FC, Elzinga D, Dejonghe W, Kaundal A, Park S-Y, Xing Z, Mega R, Takeuchi J, Khanderahoo B, Bishay S, Volkman BF, Todoroki Y, Okamoto M, Cutler SR (2019) Dynamic control of plant water use using designed ABA receptor agonists. Science 1979:366. https://doi.org/10.1126/science.aaw8848

Vu AT, Utsumi Y, Utsumi C, Tanaka M, Takahashi S, Todaka D, Kanno Y, Seo M, Ando E, Sako K, Bashir K, Kinoshita T, Pham XH, Seki M (2022) Ethanol treatment enhances drought stress avoidance in cassava (Manihot esculenta Crantz). Plant Mol Biol 110:269–285. https://doi.org/10.1007/s11103-022-01300-w

Wu H, Tito N, Giraldo JP (2017) Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. ACS Nano 11:11283–11297. https://doi.org/10.1021/acsnano.7b05723

Xu Y, Sun X, Jin J, Zhou H (2010) Protective effect of nitric oxide on light-induced oxidative damage in leaves of tall fescue. J Plant Physiol 167:512–518. https://doi.org/10.1016/j.jplph.2009.10.010

Yamauchi Y, Kunishima M, Mizutani M, Sugimoto Y (2015) Reactive short-chain leaf volatiles act as powerful inducers of abiotic stress-related gene expression. Sci Rep 5:8030. https://doi.org/10.1038/srep08030

Yamori W, Shikanai T (2016) Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. Annu Rev Plant Biol 67:81–106. https://doi.org/10.1146/annurev-arplant-043015-112002

Yang Y, Zhang Y, Wei X, You J, Wang W, Lu J, Shi R (2011) Comparative antioxidative responses and proline metabolism in two wheat cultivars under short term lead stress. Ecotoxicol Environ Saf 74:733–740. https://doi.org/10.1016/j.ecoenv.2010.10.035

Zhang J, Xing J, Lu Q, Yu G, Xu B, Huang B (2019) Transcriptional regulation of chlorophyll-catabolic genes associated with exogenous chemical effects and genotypic variations in heat-induced leaf senescence for perennial ryegrass. Environ Exp Bot 167:103858. https://doi.org/10.1016/j.envexpbot.2019.103858

Ziogas V, Tanou G, Belghazi M, Filippou P, Fotopoulos V, Grigorios D, Molassiotis A (2015) Roles of sodium hydrosulfide and sodium nitroprusside as priming molecules during drought acclimation in citrus plants. Plant Mol Biol 89:433–450. https://doi.org/10.1007/s11103-015-0379-x