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Acetate-mediated novel survival strategy against drought in plants

Jong-Myong Kim[1,2*†], Taiko Kim To[3†], Akihiro Matsui[1], Keitaro Tanoi[4,5], Natsuko I. Kobayashi[4], Fumio Matsuda[6,7], Yoshiki Habu[8], Daisuke Ogawa[9], Takuya Sakamoto[10], Sachihiro Matsunaga[10], Khurram Bashir[1], Sultana Rasheed[1], Marina Ando[1,11], Hiroko Takeda[11], Kanako Kawaura[11], Miyako Kusano[7,12], Atsushi Fukushima[13], Takaho A. Endo[14], Takashi Kuromori[15], Junko Ishida[1], Taeko Morosawa[1], Maho Tanaka[1], Chieko Torii[1], Yumiko Takebayashi[16], Hitoshi Sakakibara[16], Yasunari Ogihara[11], Kazuki Saito[7,17], Kazuo Shinozaki[15], Alessandra Devoto[18] and Motoaki Seki[1,2,11*]

Water deficit caused by global climate changes seriously endangers the survival of organisms and crop productivity, and increases environmental deterioration[1,2]. Plants' resistance to drought involves global reprogramming of transcription, cellular metabolism, hormone signalling and chromatin modification[3–8]. However, how these regulatory responses are coordinated via the various pathways, and the underlying mechanisms, are largely unknown. Herein, we report an essential drought-responsive network in which plants trigger a dynamic metabolic flux conversion from glycolysis into acetate synthesis to stimulate the jasmonate (JA) signalling pathway to confer drought tolerance. In *Arabidopsis*, the ON/OFF switching of this whole network is directly dependent on histone deacetylase HDA6. In addition, exogenous acetic acid promotes *de novo* JA synthesis and enrichment of histone H4 acetylation, which influences the priming of the JA signalling pathway for plant drought tolerance. This novel acetate function is evolutionarily conserved as a survival strategy against environmental changes in plants. Furthermore, the external application of acetic acid successfully enhanced the drought tolerance in *Arabidopsis*, rapeseed, maize, rice and wheat plants. Our findings highlight a radically new survival strategy that exploits an epigenetic switch of metabolic flux conversion and hormone signalling by which plants adapt to drought.

A genetic screen of the drought stress response using mutants of histone-modifying enzymes identified mutants of *Arabidopsis* histone deacetylase HDA6 (refs 9,10) with strong drought tolerance without growth retardation under normal conditions (Fig. 1a,b, Supplementary Figs 1 and 2). Prior to drought stress treatments, the *hda6* mutant plants showed similar growth to wild-type plants under well-watered conditions (Supplementary Fig. 1). HDA6 is a homologue of human HDAC1 and yeast RPD3 (ref. 11), and has wide-ranging functions, for example gene silencing[12–14]. The *hda6* mutants showed enhanced drought tolerance; however, the expression of drought and ABA-responsive genes, water loss and accumulation of osmolytes, *myo*-inositol and trehalose were similar to wild-type plants[3,5,15,16] (Supplementary Fig. 3). Note that the *hda6* mutant exhibited greatly reduced proline levels at day 14 of soil drying (Supplementary Fig. 3). Herein, we show that HDA6 has a fundamental role in regulating a novel drought response pathway.

To identify drought tolerance genes that are regulated by HDA6, we first profiled the genome-wide expression patterns of *hda6* during drought stress. The analysis showed that the acetate biosynthesis pathway was upregulated in wild-type plants under drought stress and that this upregulation was greatly enhanced in the *hda6* mutant (Fig. 1c, Supplementary Table 1 and 2). The acetate biosynthesis pathway mediated by pyruvate decarboxylase PDC1 and acetaldehyde dehydrogenase ALDH2B7, correlated with anoxia in *Arabidopsis*[17]. PDC1 is the key regulator initiating the first step to redirect the metabolic flux from pyruvate in glycolysis to acetaldehyde for the fermentation process under anoxia. ALDH2B7 detoxifies acetaldehyde into acetate and is upregulated during the recovery from anoxia. The expression levels of *PDC1* and *ALDH2B7* are repressed in the absence of stress, and were gradually increased (*PDC1*, by eightfold; *ALDH2B7*, by threefold)

[1]Plant Genomic Network Research Team, RIKEN Centre for Sustainable Resource Science (CSRS), 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. [2]CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan. [3]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. [4]Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. [5]PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan. [6]Metabolic Engineering Laboratory, Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, 1-5, Yamadaoka, Suita, Osaka 565-0871, Japan. [7]Metabolomics Research Group, RIKEN CSRS, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. [8]Plant Physiology Research Unit, Division of Plant and Microbial Sciences, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan. [9]Breeding Strategies Research Unit, Division of Basic Research, Institute of Crop Science, National Agriculture and Food Research Organization, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan. [10]Department of Applied Biological Science, Graduate School of Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan. [11]Kihara Institute for Biological Research, Yokohama City University, 641-12 Maioka, Totsuka, Yokohama 244-0813, Japan. [12]Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan. [13]Metabolome Informatics Research Team, RIKEN CSRS, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. [14]Laboratory for Integrative Genomics, RIKEN Centre for Integrative Medical Sciences, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. [15]Gene Discovery Research Group, RIKEN CSRS, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. [16]Plant Productivity System Research Group, RIKEN CSRS, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. [17]Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan. [18]School of Biological Sciences, Plant Molecular Sciences, Centre for Systems and Synthetic Biology, Royal Holloway University of London, Egham TW20 0EX, UK. †These authors contributed equally to this work. *e-mail: motoaki.seki@riken.jp; jongmyong.kim@riken.jp

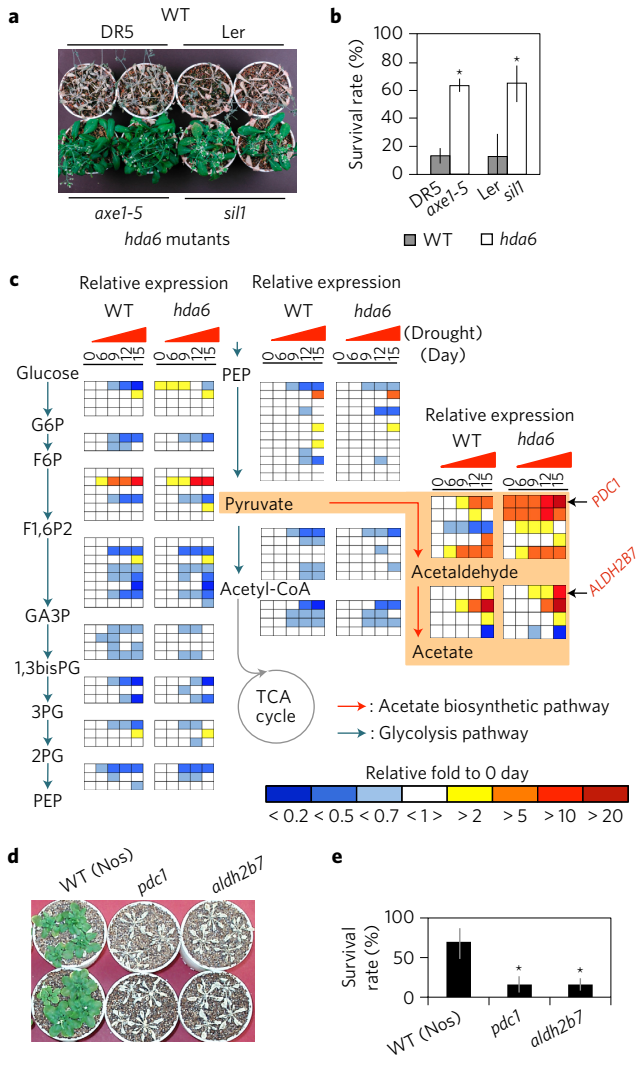

Figure 1 | HDA6 correlates with the activation of the acetate biosynthetic pathway, which is essential for plant drought tolerance. a, Drought tolerance of *hda6* mutants (*hda6*). **b**, Survival rate of *hda6* in the drought stress test. Asterisks indicate significant differences between wild-type (WT) and *hda6* plants (mutants *axe1-5* and *sil1* and their respective WT plants, DR5 (Col-0 background) and Ler (Landsberg *erecta*) were used). **c**, Temporal changes in expression profiles of glycolysis and acetate fermentation pathway genes during drought treatment. Boxes indicate relative expression changes in genes encoding enzymes (Supplementary Table 2). **d**, Drought sensitivity of WT plants (ecotype Nossen) for acetate biosynthesis genes, *pdc1* and *aldh2b7* mutants. **e**, Survival rate of *pdc1* and *aldh2b7* mutants during the drought stress test. Asterisks indicate significant differences between WT and mutants. All error bars denote the s.d., $n = 3$. *$P < 0.01$ (Student's *t*-test).

during drought conditions in wild-type plants (Supplementary Fig. 4). In *hda6*, transcripts of both genes accumulated during the early stages of the drought response, suggesting that HDA6 acts as a negative regulator of their expressions under non-stressed conditions. We also analysed the changes in transcript and metabolite levels of glycolysis and its connective pathways (Fig. 1c and Supplementary Fig. 5). Remarkably, the transcription of glycolytic genes was downregulated by drought in wild-type and *hda6* plants (Fig. 1c and Supplementary Table 2). From the results of metabolomic analysis, the accumulation of glucose was detected in both wild-type and *hda6* mutant plants under drought condition. In contrast, fructose-6-phosphate level was decreased at day 14 of soil drying in *hda6* mutant compared with wild-type plants. On the TCA cycle, similarly 2-oxoglutarate and isocitrate levels were reduced in *hda6* mutant at day 14 of soil drying (Supplementary Fig. 5). The downregulation of glycolysis and the activation of acetate synthesis under drought indicated that *PDC1* and *ALDH2B7* genes function not only in anoxia but also in response to drought. To verify the role of *PDC1* and *ALDH2B7* in the drought response, we tested the sensitivity of their mutants to drought. Indeed, both *pdc1* and *aldh2b7* (ref. 18) mutants showed severe sensitivity to drought stress (Fig. 1d,e and Supplementary Fig. 6). In addition, *PDC1* and *ALDH2B7* double overexpression plants exhibited enhanced drought tolerance (Supplementary Fig. 7).

To determine how HDA6 regulates *PDC1* and *ALDH2B7* expression, we examined the HDA6 binding activity and the corresponding histone modification status on these loci using a chromatin immunoprecipitation assay (Fig. 2a,b). The results showed that HDA6 binds to the *PDC1* and *ALDH2B7* loci in wild-type plants, predominantly at the transcribed gene body regions. Notably, the HDA6 binding levels dramatically decreased after drought treatment. This decrease in HDA6 binding mirrored histone H4 acetylation (H4Ac) changes (Fig. 2c,d): H4Ac levels were low in wild-type plants under normal conditions but were greatly increased during the drought response. These changes indicated that the dissociation of HDA6 triggers increased H4Ac and transcriptional upregulation of these loci. In *hda6*, the H4Ac enrichment occurred earlier and at higher levels than in wild-type plants. Altogether, these results demonstrated that HDA6 represses the acetate biosynthetic pathway directly under normal conditions and that HDA6 dissociates from these regions to trigger the epigenetic and transcriptional changes that activate this pathway under drought stress.

We next asked whether the activation of *PDC1* and *ALDH2B7* regulated by HDA6 increases the endogenous acetate levels during drought conditions (Fig. 3a). Mass spectrometry analysis showed that the acetate content increased substantially during the drought response in wild-type plants. In contrast, *pdc1* and *aldh2b7* did not show any significant increase in acetate levels. Therefore, the endogenous acetate in wild-type plants actually increases in response to drought, and that both *PDC1* and *ALDH2B7* are required for acetate production. In *hda6*, acetate accumulated compared with wild-type plants. Acetate abundance correlated with increased plant drought tolerance: in *hda6*, the acetic acid levels were higher than in wild-type plants and survival was enhanced, but the acetate biosynthesis-deficient *pdc1* and *aldh2b7* were more sensitive to drought. This is the first indication that acetate biosynthesis is essential for plant drought tolerance. To confirm this conclusion, the drought tolerance of plants pretreated with exogenous acetic acid was assessed. Plants grown in the presence of 10, 20 and 30 mM acetic acid exhibited strikingly increased drought tolerance (Supplementary Figs 8 and 9a), in contrast to other organic acids (Fig. 3b, Supplementary Figs 10 and 11); supporting the specific role of acetic acid in this process.

The drought tolerance promoted by acetic acid was studied further by comparing transcriptional regulation during drought stress between acetic acid-pretreated and water-pretreated wild-type plants. Microarray analyses showed that 357 genes were induced under drought, specifically in the acetic acid-pretreated plants (Fig. 3c and Supplementary Table 3). Gene ontology enrichment analysis showed that the categories of JA and ethylene responses with defence/immune processes were enriched among these genes (Supplementary Table 4), in agreement with the partial, constitutive JA phenotype of *hda6* (ref. 19). Previously, extensive crosstalk was reported between the JA, ethylene and defence response pathways[20,21]. To determine the functional downstream regulators of the acetic acid-dependent drought tolerance, the drought sensitivity of mutants for the JA receptor COI1 (ref. 22), the JA biosynthetic enzyme AOS (ref. 23) and the essential positive regulator of ethylene

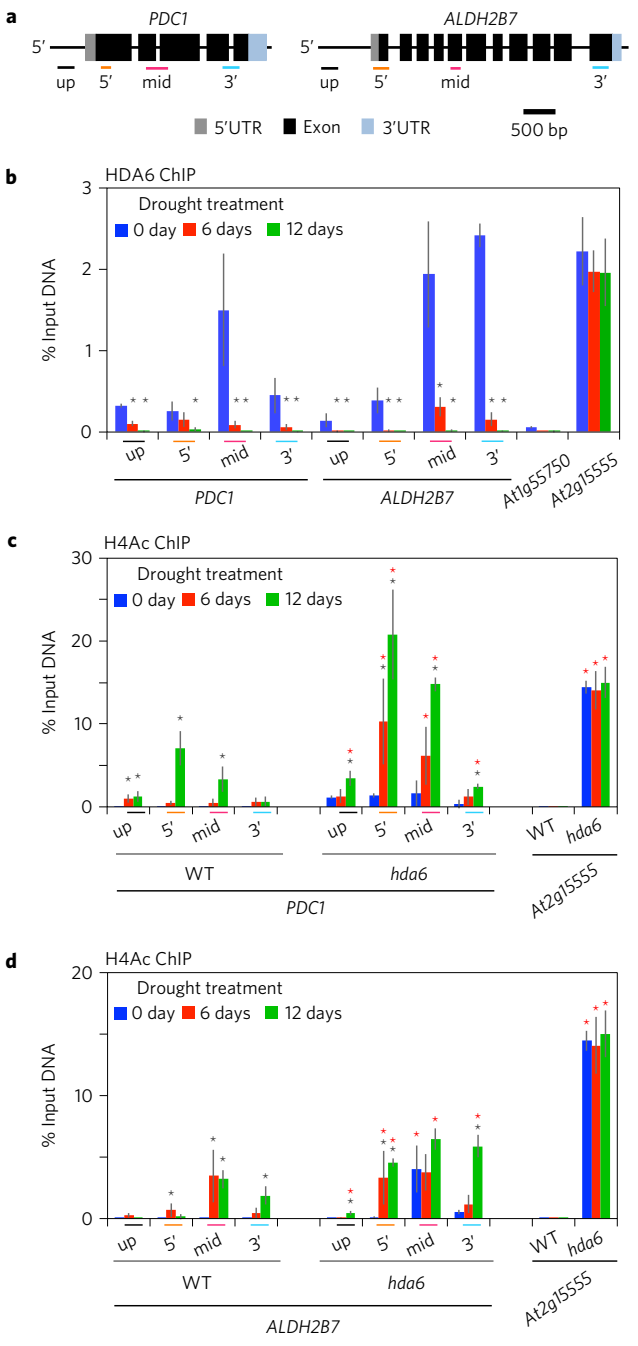

Figure 2 | HDA6 directly regulates acetate biosynthetic pathway genes, *PDC1* and *ALDH2B7*. a, Graphical representation of changes in HDA6 binding/histone H4 acetylation (H4Ac) sites (underlined) on the *PDC1* and *ALDH2B7* genes, obtained by a chromatin immunoprecipitation assay (ChIP) assay. **b**, Enrichment of HDA6 binding determined on the *PDC1* and *ALDH2B7* gene regions by ChIP assays. Drought stress treatment caused a decrease in HDA6 binding to *PDC1* and *ALDH2B7* in the WT. Asterisks indicate significant differences between non-stressed (0 day) and drought stress conditions (6 and 12 days). **c**,**d**, Enrichment of H4Ac on the *PDC1* and *ALDH2B7* genes under drought stress conditions in WT and *hda6* plants. *At2g15555* and *At1g55750* genes are positive and negative control sites for HDA6 binding and histone H4 acetylation enrichments, respectively[13]. Grey asterisks indicate significant differences between non-stressed and drought stress conditions. Red asterisks indicate significant differences between WT and *hda6*. All error bars denote the s.d. The results of independent replicates were averaged ($n = 3$). *$P < 0.01$ (Student's *t*-test).

responses EIN2 (refs 24,25) were tested. The *aos* mutant[26] showed drought sensitivity (Supplementary Fig. 12). The *coi1-16B* mutant[27] showed drought sensitivity, and acetic acid pretreatment did not ameliorate this phenotype (Fig. 3d and Supplementary Fig. 13). In contrast, the *ein2-5* mutant[24] did not show altered sensitivity (Supplementary Fig. 9b). Thus, *COI1* is essential to mediate acetic acid-dependent drought tolerance. A physical interaction between HDA6 and COI1 proteins using a yeast two-hybrid system was reported[19]. It suggests that HDA6 regulates JA signalling pathways cooperatively with COI1 and the chromatin status of COI1 target gene regions might be influenced. We next measured the JA levels in wild-type plants treated with acetic acid. Transient biosynthesis of JA and jasmonoyl–isoleucine (JA-Ile) was greatly induced by acetic acid treatment at 24 h, but abscisic acid (ABA) levels remained unchanged (Fig. 3e). The expression of the JA biosynthetic enzyme AOC3 (ref. 28) was induced by acetic acid (Supplementary Fig. 9c). Despite the transient JA increase during acetic acid treatment, the downstream genes of JA signalling, such as the key regulator MYC2 (ref. 29), were not transcriptionally activated (Supplementary Fig. 9d and Supplementary Table 3). However, these genes, involving *MYC2* showed higher induction after the drought stimulus in the acetic acid-pretreated plants than in plants pretreated with water. Thus, it suggested that acetic acid primes the activation of a COI1-mediated signalling pathway and the transient production of JA might define the downstream target genes. The other acids, formic acid and lactic acid, also affected *AOC3* gene induction; however, these acids could not enhance drought tolerance (Supplementary Figs 10 and 14).

To understand how acetic acid functions in priming gene activation, we monitored the behaviour of acetic acid in wild-type plants. In human cells, exogenous acetic acid treatment promotes intracellular pH increase and elevated histone acetylation associated with cell proliferation[30]. We detected an analogous pH upshift in *Arabidopsis* (Supplementary Fig. 15): by adding 10 mM acetic acid to the soil, the pH level of the xylem sap solution increased, which agreed with the observed pH increase of the xylem sap in plants responding to drought[31]. When ^{14}C-labelled acetic acid (^{14}C-AA) was absorbed through the roots, ^{14}C-AA became evident in the shoots after 1 h (Supplementary Fig. 15). The total amount of absorbed ^{14}C-AA was 500 nmol per shoot, which was approximately fivefold higher than in *hda6* under drought conditions (Fig. 3a and Supplementary Fig. 15b,c). From the results of metabolomics analysis using plants treated with 10 mM acetic acid for five days, there was no difference of accumulation in 88 kinds of metabolites compared with plants treated with water for five days (Supplementary Table 5). It suggested that even though acetic acid with a high concentration of 10 mM is given, the amount absorbed by plants is not so high (Supplementary Fig. 15). It seems that the acetic acid absorption by plants correlates to soil pH (Supplementary Fig. 16). Up to 20 mM acetic acid concentration, the soil pH was kept in pH 7. However, the soil pH was shifted down to lower pH by addition of excessed 50 mM acetic acid. In connection with these changes in pH, plant growth seems to be inhibited by the treatment with 50 mM acetic acid (Supplementary Fig. 8 and 16). Interestingly, ^{14}C-AA was incorporated into proteins with a molecular size corresponding to histone H4, detected by western blotting analysis after 3 h (Fig. 3f). Our results suggested that the acetic acid is converted to acetyl-CoA and is used as a substrate for histone acetylation. In addition, from the results of ChIP-seq analysis, histone H4 acetylation (H4ac) of the gene body region was enriched genome-wide by addition of 10 mM acetic acid for nine days (Supplementary Fig. 17 and Supplementary Table 6). The 106 genes, including *MYC2*, considered to be downstream target genes of the acetic acid–JA pathway to confer plant drought tolerance were involved within the H4ac-enriched genes (Supplementary Table 6). Herein,

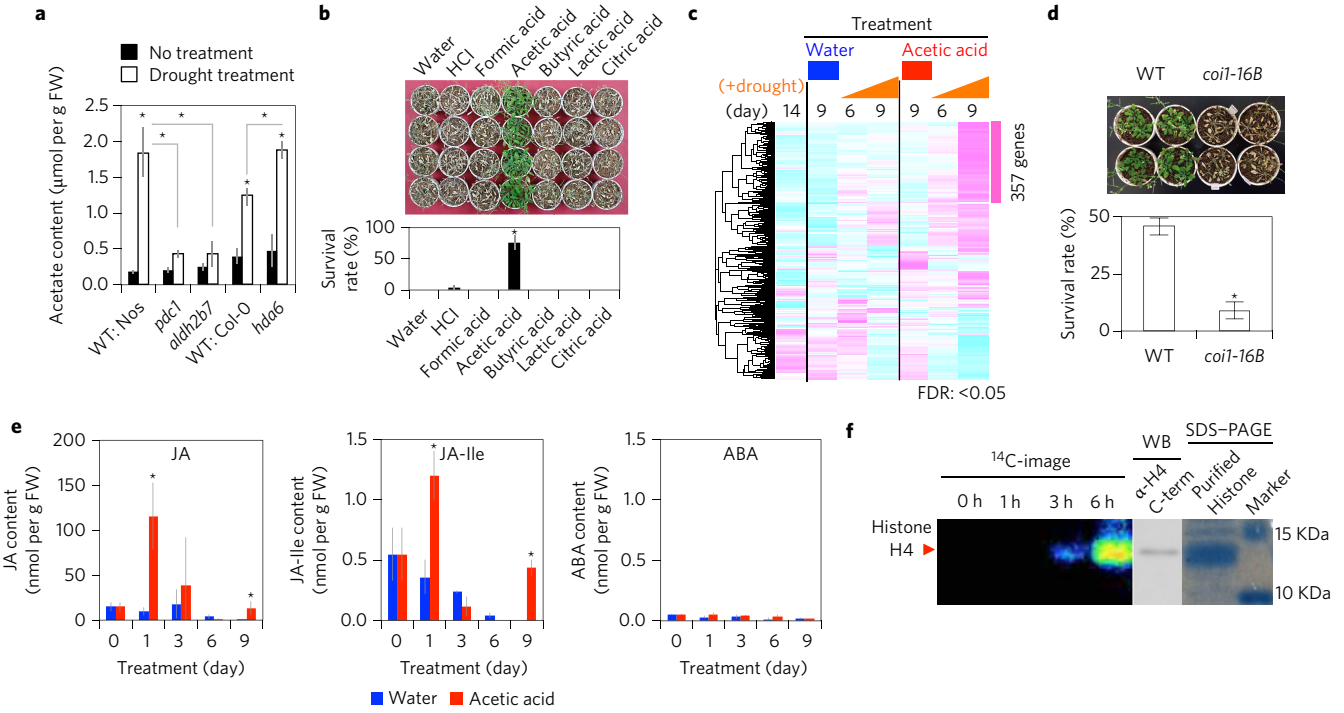

Figure 3 | Acetic acid promotes jasmonate signalling and histone acetylation. a, Measurement of endogenous acetic acid contents in WT and mutant plants under drought stress conditions after 14 days. **b**, The effect of several organic acids on plant drought tolerance after 14 days. The lower panel shows the survival rate of WT plants during the drought stress test with several organic acids. **c**, Clustering display of genes regulated differentially by water or acetic acid and drought-continuous treatments in the WT. Three hundred and fifty-seven genes were highly induced by drought treatment in the WT pretreated with acetic acid. **d**, Drought sensitivity of the *coi1-16B* mutant. The lower panel shows the survival rate of the WT and *coi1-16B* mutant during the drought stress test. **e**, Measurement of plant hormones, JA, JA-Ile and ABA in WT plants treated with acetic acid. Transient synthesis of JA and JA-Ile by acetic acid treatment was detected on the first day. **f**, Incorporation of exogenous ^{14}C-AA into the histone H4 protein. Semi-purified histone proteins (50 ng) were loaded in each lane. The incorporation of a substrate including a ^{14}C radiotracer derived from ^{14}C-AA is shown as a ^{14}C image over a time course (0, 1, 3 and 6 h). WB, western blotting analysis using anti-histone H4 antibody; SDS–PAGE, semi-purified histones stained with Coomassie brilliant blue. The error bars denote s.d., $n = 6$ in **a**, $n = 3$ in **b**–**d** and $n = 4$ in **e**. *$P < 0.01$ (Student's t-test).

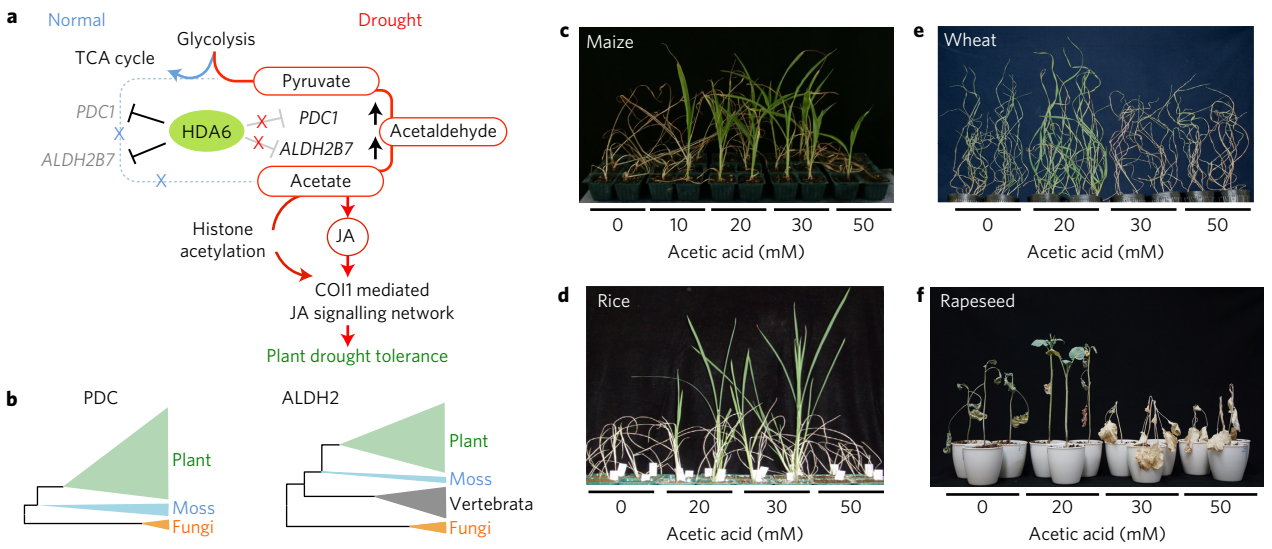

Figure 4 | Proposed mechanism for acetic acid-induced drought tolerance and its evolutionary conservation. a, HDA6 acts as a switch during drought stress to redirect the metabolic flux, leading to the accumulation of acetic acid through the activation of specific biosynthetic regulatory genes and the JA signalling pathway in *Arabidopsis*. The histone acetylation derived from acetate might function in the priming effect of the COI1-mediated JA signalling pathway. **b**, Schematic representation of the conservation of *PDC* and *ALDH2* homologous genes in eukaryotes (see also Supplementary Fig. 18). **c**–**f**, Acetic acid confers drought tolerance on maize, rice, wheat and rapeseed plants.

we propose that in *Arabidopsis*, the activation of the acetate biosynthetic pathway and the resulting acetate increase are indispensable for acquiring drought tolerance. We considered that HDA6 acts as an ON/OFF switch that controls this pathway directly. The alteration of metabolic flux in response to drought is consistent with the results of transcriptome analyses (Fig. 4a). Our phylogenetic

analyses showed that ALDH2B7 is conserved widely from fungi to humans (Fig. 4b and Supplementary Fig. 18). In contrast, PDC1 is only conserved in moss, fungi and plants. In yeast, the *acetaldehyde dehydrogenase4* mutant shows oxidative stress sensitivity under glucose starvation, and this sensitivity is recovered by supplying exogenous acetic acid[32]. As drought also induces oxidative stress in plants[3,4], taken together, these results indicated that this novel stress-responsive mechanism is conserved in autotrophic eukaryotes. To test whether acetic acid could enhance the drought tolerance of plants other than *Arabidopsis*, we assessed its effect when supplied exogenously. We found that concentrations between 20 and 30 mM enhanced drought tolerance in both monocots and dicots, such as rice, wheat, maize and rapeseed plants (Fig. 4c–f and Supplementary Fig. 18). In conclusion, acetate, a basic and simple biochemical compound, has a significant role as an initial factor that orchestrates plants' survival capability, which connects fundamental metabolism, epigenetic regulation and hormone signalling, ultimately conferring plant drought tolerance. Integrative studies of transcription factors and histone modifications will reveal the undiscovered players in the acetic acid-mediated network for environmental adaptation.

Methods

Plant materials and growth conditions. *A. thaliana* (L.) Heynh plants were used in this study. The *hda6* mutants *axe1-5* and *sil1*, and their respective wild types, DR5 (Col-0 background) and Ler (Landsberg *erecta*)[9,10] were used for the drought tolerance tests. The *axe1-5* (*hda6*) mutant and its parental DR5 plants (wild type) were used for other experiments. The *pdc1* (No. 13-1034-1) and *aldh2b7* (No. 15-2168-1) mutants, and their *DS* donor line (ecotype: Nossen)[18], were used for the drought sensitivity tests. In addition, *coi1-16B*, *aos* and *ein2-5* mutants[22–24] were also used for drought sensitivity tests. Seeds were sown in soil (Professional soil No. 2, Dio Chemicals) in 9-cm-diameter pots, stratified for two to four days at 4 °C in the dark, and then grown under a 16 h light/8 h dark regimen at 22 °C.

Wild-type wheat plants (*Triticum aestivum* cv. Chinese Spring) were used. Seeds were germinated in Petri dishes on filter paper saturated with water for three days at 4 °C in the dark and then grown for two days at 21 °C. Seedlings were transplanted into soil (Golden planting mix, IRIS Ohyama) in 9-cm-diameter pots under a 16 h light/8 h dark regimen at 21 °C.

Wild-type rice plants (*Oryza sativa* L. cv. Nipponbare) were used. Seeds were germinated on soil (Bon Sol No.2, Sumitomo Chemical) in pots (6 cm × 6 cm × 4.5 cm) and grown under a 14 h light/10 h dark regimen at 30 °C.

Seeds of maize plants (*Zea mays* L. cv. Canberra) were germinated on soil (Hanazanmai (Sakata Seed)/Gardening soil No. 1 (Nihon Hiryo): 3:1) in pots (6 cm × 6 cm × 4.5 cm) and the plants were grown for nine days under a 14 h light/10 h dark regimen at 30 °C.

Seeds of rapeseed (*Brassica napus* cv. Wester) were sown on soil (Professional soil No. 2, Dio Chemicals) in 9-cm-diameter pots, stratified for two to four days at 4 °C in the dark, and then grown under a 16 h light/8 h dark regimen at 22 °C.

The mutant lines of histone modifiers used for initial genetic screen are listed in Supplementary Table 7.

Drought tolerance tests. Watering of three-week-old *Arabidopsis* plants was withheld for approximately two weeks under a 16 h light/8 h dark regimen at 22 °C, and 40% humidity in the greenhouse. The plants were then rewatered, and the number of surviving plants was counted after five days. The average survival rate was calculated for three independent experiments ($n = 40$). Pretreatment of *Arabidopsis* plants with acids or salts was performed as follows: when plants were two weeks old, a solution of organic acids (10 mM of hydrochloric acid, formic acid, acetic acid, butyric acid, lactic acid or citric acid) or 0, 1, 10, 20, 30 and 50 mM acetic acid, was supplied to the soil and the plants were grown for nine days. After removing the treatment solutions (administered from the bottom of the pot by capillarity using a paper towel), plants were subjected to the drought stress test. The average survival rate was calculated for three independent experiments ($n = 48$).

Four-week-old wheat plants were pretreated with 0, 20 and 50 mM acetic acid (supplied to the soil) and grown for nine days under a 16 h light/8 h dark regimen at 22 °C, and 40% humidity in the greenhouse. After removing the treatment solutions as above, plants were subjected to the drought stress test. The plants were then rewatered for five days to determine plant survival. The average survival rate was calculated for three independent experiments ($n = 15$).

Two-week-old rice plants were pretreated with 0, 20, 30 and 50 mM acetic acid supplied to the soil and plants were grown on soil for 4 days under a 14 h light/10 h dark regimen at 30 °C in a growth chamber. Removal of treatment solutions and drought treatment (four days) was performed as described above. The plants were then rewatered for ten days to determine plant survival. The average survival rate was calculated for three independent experiments ($n = 8$).

Ten-day-old maize plants were used for the drought test. Water in the soil was removed from the bottom of the pot by capillarity using a paper towel and the plants were then treated with 0, 10, 20, 30 and 50 mM acetic acid for four days. The acetic acid solution was removed using a paper towel and the plants were incubated under drought conditions for six days. After rewatering for five days, their survival was checked. The average survival rate was calculated for three independent experiments ($n = 4$).

One-month-old rapeseed plants were pretreated with 0, 20, 30 and 50 mM acetic acid supplied to the soil and the plants were grown on soil for nine days under a 16 h light/8 h dark regimen at 22 °C, and 40% humidity in the greenhouse. After removing the treatment solutions, plants were subjected to the drought stress test. The plants were then rewatered for five days to determine plant survival. The average survival rate was calculated for three independent experiments ($n = 12$).

RNA preparation. Total RNA was extracted from the aerial parts of *Arabidopsis* plants, either before or after drought stress, using the Plant RNA Purification Reagent (Invitrogen) according to the manufacturer's instructions. For quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR) and reverse transcription polymerase chain reaction (RT–PCR), cDNA was synthesized using the QuantiTect Reverse Transcription synthesis kit (Qiagen). The primers are listed in Supplementary Table 8.

Microarray analysis. Three-week-old *Arabidopsis* plants were subjected to drought stress treatment, and the aerial parts were collected after 0, 6, 9, 12 and 15 days. Three biological replicates were performed. Microarray analysis was performed using an Agilent Arabidopsis microarray platform, as described previously[13]. The microarray data are available on the GEO website (GEO ID: GSE46365). Drought stress-responsive genes were selected using the following criteria: expression level change >1.5 fold and an unpaired *t*-test false discovery rate (FDR) of <0.05. At each time point, upregulated genes in *hda6* were compared with their levels in wild-type plants. Two-week-old wild-type plants (Col-0) were treated with 10 mM acetic acid solutions for nine days, and then subjected to drought stress treatment. The aerial parts were collected after 0, 6, 9, 12 and 15 days of drought stress and used for microarray analysis. Three biological replicates ($n = 12$) were taken. The microarray data are available on the GEO website (GEO ID: GSE46524). Acetic acid-responsive genes were selected using the following criteria: the genes showed an FDR less than 0.05 at more than one time point, when water-drought treated wild-type plants series and acetic acid-drought treated wild-type plants series were compared at each time point, using an unpaired t-test and the FDR method[33] in the R 2.12.1 software. The acetic acid responsive genes were hierarchically clustered for heatmap display.

Clustering of expression patterns in the microarray data. The gene expression heatmap was obtained using heatmap.2 in the gplots package of R ver. 2.1.12 (R Core Team). Hierarchical gene clusters were built using Ward's minimum variance method clustering with Euclidean distance, after which the $\log_2$ normalized values were transformed into *Z*-scores. The heatmap colouring reflects the rank of the Z-scores.

Chromatin immunoprecipitation. *Arabidopsis* chromatin immunoprecipitation (ChIP) assays were performed essentially as described previously[34], with three biological replicates. The antibodies used in this study were an anti-HDA6 antibody[13] and an anti-H4 tetra-acetylation (06-866) from Millipore. Precipitated DNA was analysed using quantitative PCR (Power SYBR real time reagent and StepOnePlus; Applied Biosystems). We estimated the absolute fraction of DNA recovered from the INPUT (% input DNA) by comparing the reaction threshold cycle of the ChIP sample to a dilution of its own INPUT. Statistical significance was determined using Student's *t*-test. *At1g55750* was used as a negative control for HDA6 binding, and *At2g15555* was used as a positive control for HDA6 binding and enrichment of histone H4 acetylation[13]. The primers used are listed in Supplementary Table 8.

ChIP-seq analysis. ChIPed DNA (250 ng) was used to make sequencing library for a SOLiD 5500 sequencer (Thermo Fisher). Library preparation and sequencing were performed as described in instruction manuals. Sequences were mapped on the TAIR10 genome using bowtie (version 0.12.8) with default options. Since the software had problems with our SOLiD outputs, paired-end sequences were aligned as individual files and aggregated after alignment of either file. Peaks were identified using MACS2 (version 2.1.0) with nomodel option. Whole-genome histone acetylation was evaluated using the normalized read count of all peaks. Mapped reads were aggregated for every 25 bp bin of a peak and statistically evaluated between treated and untreated organisms with the Wilcoxon test. Peaks with *q*-values <0.05 and a logarithm of fold change (logFC) >0.25 were identified as significant peaks. Associated genes with the peaks of acetylated regions and differentially acetylated peaks were enumerated using our in-house program and gene annotation file provided by the TAIR database. Since the majority of acetylated histones are located downstream of TSS, we identified acetylated genes responding to acetic acid as genes having peaks from 500 bp upstream of TSS to 1.5 kb downstream of TSS. All raw files and processed files were deposited at the NCBI GEO (ID: GSE95817). The Integrative Genome Browser 9.0.0 was used to visualize the

processed data (http://bioviz.org/igb/index.html). Sequencing was done using each ChIPed DNA ($n = 1$) and result of H4ac enrichments was validated by ChIP-qPCR ($n = 3$) on three target gene regions (*NHL3, ZAT10* and *At5g42050*), a negative gene region (*At2g15555*) and a positive gene region (*UBQ10*).

Measurement of acetate. Three-week-old *Arabidopsis* plants were subjected to a drought stress treatment for 12 (Nos background plants) or 14 days (Col-0 background plants), and rosette leaves were harvested. Well-watered plants of the same age were used as controls. Harvested leaves were immediately weighed and frozen in a plastic tube. Frozen tissues were homogenized in five volumes (*w*/*v*) of cold (4 °C) water using a mixer mill (Shake Master Neo; Bio Medical Science) with a zirconia bead for 1 min at 20 Hz. Following centrifugation at 15,000*g* for 1 min at 4 °C, the supernatants were diluted with an equal amount of acetone. The sample extracts (1 µl) were analysed using a gas chromatography–mass spectrometry (GC–MS) system (GCMS-QP2010 Plus) equipped with a nitroterephthalic acid modified polyethylene glycol (DB-FFAP) column (60 m, 0.25 mm internal diameter, 0.5 µm film thickness; Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The oven temperature was held at 100 °C for 5 min and then heated at 10 °C min^{-1} to 230 °C. The injector temperature was maintained at 250 °C, and the ion source and interface temperatures were 230 and 250 °C, respectively. The sample was injected in split injection mode with a 100:1 split ratio. Acetate was detected using the selected ion monitoring mode at *m*/*z* 60.

Metabolite profiling. Metabolite profiling was performed as described previously[35], with slight modifications. All chemicals and reagents used were of spectrometric grade. We extracted 25 mg fresh weight (FW) of aerial plant parts per millilitre of extraction medium containing ten stable isotope reference compounds. After centrifugation, a 200 µl aliquot of the supernatant was drawn and evaporated to dryness for derivatization, as described previously[35]. The equivalent of 56 µg FW of extract was analysed by gas chromatography with time-of-flight mass spectrometry (GC–TOF–MS). (LECO, St Joseph, MI, USA). Data were normalized using the cross-contribution compensating multiple standard normalization (CCMN) algorithm[36].

Statistical data analysis of metabolomics data. Statistical data analyses were performed by R statistical software (http://cran.r-project.org). Statistical comparison of the metabolome data with $\log_2$ transformation between acetate treatment and control was conducted using a Welch's *t*-test with correction by false discovery rate (FDR)[33].

Measurement of xylem sap pH. Xylem sap was collected from six-week-old *Arabidopsis* plants grown in soil. Before and after acetic acid treatment, shoots corresponding to the hypocotyl of 15–20 plants were cut off. Exudates from the cut ends were collected for 30 min. Approximately 10 µl of the exudate was subjected to pH determination using a pH meter (B-712).

Measurement of soil pH and water content. Ten grams of soil (Professional No. 2) were treated for nine days with 25 ml of an acetic acid–deionized water solution in a 50 ml plastic tube and vigorously shaken for 30 min at 200 rpm with a Shaker NR-30 (Taitec), and then kept at room temperature for 30 min. The pH of the supernatant was measured with a pH meter PH-6011A (Custom). Soil water contents were measured with a soil water content meter DM-18 (Takemura Denki).

Measurement of phytohormones. Approximately 100 mg (fresh weight) of the aerial parts of *Arabidopsis* plants were subjected to quantification of JA, jasmonoyl–isoleucine (JA-Ile) and abscisic acid (ABA). Extraction and fractionation were performed using the method described previously[38]. [$^2H_2$]JA-Ile was purchased from OlChemim Ltd. The hormones were determined using an ultra high-performance liquid chromatography-Q-Exactive™ system (Thermo Fisher) using an ODS column (Aquity UPLC BEH C18, 1.7 µm, 2.1 × 100 mm; Waters) as previously described[36].

^{14}C-Labelled acetic acid incorporation. Acetic acid transport to shoots was examined using ^{14}C-labelled acetic acid. Ten millilitres of 10 mM acetic acid containing 12MBq of [1-^{14}C]-acetic acid was supplied from the bottom of the pot containing 18-day-old seedlings. A shoot was excised at each treatment time and placed on cardboard at 4 °C. After being covered with a thin plastic film, the shoots were exposed to an imaging plate (BAS–IP-MS, GE Healthcare) for one day, and then the ^{14}C image was acquired by an FLA-5000 image analyser (FujiFilm). The amount of acetic acid in the aerial part was calculated from the signal intensity, using imaging analysis software (Image Gauge ver. 4.0, FujiFilm).

Western blotting. Three *Arabidopsis* plants for each sample were fixed with liquid nitrogen and disrupted by shaking for 1 min using an SK mill (Tokken). Histone proteins were purified from the crude protein fraction using a Histone purification kit (Active motif). Protein concentrations were measured using a Qubit protein assay kit and Qubit 2.0 Fluorometer (Thermo Fisher). Histone proteins were separated by SDS–PAGE on a 15% gel (SuperSep Ace, 15%, Wako Pure Chemical Industries). Histone proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using an iBlot Dry Blotting System (Thermo Fisher). To detect the histone H4 proteins, an antibody specific for the histone H4 C-terminus (ab10158, Abcam) was used as the primary antibody.

Development and characterization of transgenic lines expressing *PDC1* and *ALDH2B7* under control of the *TSPO* promoter. *Arabidopsis TSPO* (*At2g47770*) is a small intron less gene and its expression is up regulated by drought stress[39]. A 1.4 kb promoter region upstream of *TSPO* gene was cloned with forward and reverse primers as 5′-ATAGTAGACTGCGGCCGCACCATGGCA-3′ and 5′-TGTCCTGAGATCTAGATACAAACGAAACGTCCAAAAC-3′, respectively. A *Not*I site was introduced into the forward primer, while a *Xba*I site was introduced into the reverse primer and a 1.4 kb region of the *Arabidopsis TSPO* promoter was cloned into the pGreenII 0029 and pGreenII 0229 vectors. Then, the *ALDH2B7* and *PDC1* genes were cloned downstream of the *TSPO* promoter in pGreenII 0229 and pGreenII 0029, respectively. The primers used for the cloning of *PDC1* were 5′-CTCGAATTCCCCGGGTTAATTAAATTAATC-3′ and 5′-ATCAGGCCGTCGACGCCGGATC-3′ containing *Sma*I and *Sal*I sites, respectively. The primers used for the cloning of *ALDH2B7* were 5′-AATCGGCCCCCGGGTTAATTAAATTAATC -3′ and 5′-TGGAGCTAGTCGACTTATGGCCGA-3′ containing *Sma*I and *Sal*I sites, respectively. Transgenic *Arabidopsis* plants expressing both of these genes were developed through *Agrobacterium tumefaciens* (GV3101)-mediated transformation of plants by the floral dip method[40]. The both plasmids containing *PDC1* or *ALDH2B7* were transformed into *Arabidopsis* wild-type (Col-0) plants at one time. *Neomycin phosphotransferase II* (*NPT II*), and the *bialaphos resistance* (*bar*) genes were used to screen transgenic plants using kanamycin (km) and bialaphos for *PDC1* and *ALDH2B7*, respectively.

Homozygous lines for plants expressing *ALDH2B7*, *PDC1* or both were developed, and the drought-inducible expression of these genes was confirmed through RT–qPCR analysis. cDNA for each sample was synthesized from 200 ng RNA using the QuantiTect Reverse transcription kit according to the manufacturer's instructions (Qiagen). For *PDC1*, the forward and reverse primers were 5′-AGAGCACCATCCTCATTTCATT-3′ and 5′-TGGCTTTTTCTTTCTTGAGGAG-3′, respectively. Forward and reverse primers used for *ALDH2B7* were 5′-CATGCTCATAGCAACAGACGA-3′ and 5′-CAGCAGCTAAACCGTACCTTG-3′, respectively. *Actin 2*, used as an internal control, was amplified using the forward and reverse primers 5′-TGAAGTGTGATGTGGATATCAGG-3′ and 5′-GATTTCTTTGCTCATACGGTCAG-3′, respectively.

For the drought treatment, seeds were sown in Dio propagation mix no. 2 professional soil (Dio Chemicals) at 22 °C (16 h light/8 h dark cycle, 60 µmol m^{-2} s^{-1} photon flux density). Two weeks after germination, plants were subjected to the drought treatment by removing excess water from the trays and ceasing any subsequent watering. The survival ratio of the wild-type and the transgenic plants was calculated by counting the number of survived plants at 48 h after rewatering the plants. For measurement of biomass, plants were grown in soil for two weeks and shoots were subsequently harvested and fresh weight was measured. The samples were then subjected to heat drying at 55 °C for five days and sample dry weights were measured. The weight of pots containing two-week-old plants was recorded and changes in the pot weight was expressed as percentage change in soil water content.

Data availability. The transcriptome and epigenome data are available at the GEO website under the accession numbers GSE46365, GSE46524 and GSE95817. The data that support the findings of this study are available from the corresponding authors upon request.

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Author contributions

J.M.K., T.K.T., and M.S. conceived the project. J.M.K., and T.K.T. designed the experiments. J.M.K. and T.K.T. carried out all drought stress and growth tests in *Arabidopsis*. J.M.K. performed the ChIP assay. T.K.T. and J.I. performed the qRT–PCR and RT–PCR expression analyses. J.M.K., K.T. and N.I.K. performed the radioactive incorporation assay. F.M. measured the acetic acid concentration by GC–MS. M.K., A.F. and K.S. carried out the metabolomic analyses. Y.T. and H.S. measured the phytohormone levels. J.I., M.T. and T.M. supported the microarray analyses. A.M. analysed the microarray data. S.M. and T.S. measured the xylem sap pH. J.M.K., D.O. and Y.H. carried out the drought stress test in rice and maize. J.M.K., M.A., H.T., K.K. and Y.O. carried out the drought stress test in wheat and rapeseed. T.A.E carried out data analysis for ChIP-seq. C.T. supported the management of plants and seeds. J.M.K., T.K.T. and A.D. identified the link with JA and conceived the experiments using mutants of the jasmonate signalling pathway genes. J.M.K., M.A., S.R. and K.B. analysed the transgenic plants expressing *PDC1* and *ALDH2B7*. T.K., K.S. and A.D. supplied the *pdc1*, *aldh2b7* and *coi1-16B* mutant seeds, respectively. J.M.K., T.K.T., A.D. and M.S. wrote, reviewed and edited the manuscript.

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Correspondence and requests for materials should be addressed to J.M.K. and M.S.

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Competing interests

The authors declare no competing financial interests.