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Overexpression of the voltage-dependent anion channel 2 (*VDAC2*) gene induces drought resistance in *Arabidopsis thaliana*

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Abstract

Abiotic stresses, especially drought, trigger abscisic acid (ABA) signal conduction in plants. However, the molecular mechanisms are not well understood. We investigated the role of the voltage-dependent anion channel 2 gene (*AtVDAC2*) in drought resistance of *Arabidopsis* through ABA signalling. Some characteristics such as seed germination, root elongation, drought resistance, ABA related genes expressing, etc. were studied in plant transgenic lines. The *AtVDAC2* overexpressing plants displayed an ABA-hypersensitive phenotype with higher seed germination and root elongation, while plants with *AtVDAC2* knockdown (*AtVDAC2*-Dn) exhibited an ABA-insensitive phenotype. Additionally, *AtVDAC2*-OE plants showed enhanced resistance (about 40%) to drought than wide-type and *AtVDAC2*-Dn plants. In *AtVDAC2* transgenic plants, some downstream targets in the ABA and/or drought-signalling pathways were altered at various levels, suggesting the involvement of *AtVDAC2* in ABA-dependent drought resistance in *Arabidopsis*. Moreover, *AtVDAC2*-OE plants exhibited increased 9-cis-epoxycarotenoid dioxygenase 3 (*NECD3*) gene expression and generation of the reactive oxygen species hydrogen peroxide, suggesting that functional *AtVDAC2* may be involved in ABA and H_2O_2 production process. The overexpression of the *AtVDAC2* gene can confer drought resistance in *Arabidopsis*.

Keywords: ABA signalling, voltage dependent anion channel, drought resistance, *Arabidopsis*.
Abbreviations: ABA_abscisic acid, ABI_abscisic acid-insensitive, DAB_3,3-diaminobenzidine, Dn_knockdown, H_2O_2_hydrogen peroxide, MS_Murashige and Skoog, NECD_9-cis-epoxycarotenoid dioxygenase, OE_overexpression, PP2Cs_the protein phosphatase 2Cs, RCAR_regularoly component of ABA receptors, RD29B_regulated by drought 29B, ROS_reactive oxygen species, VDAC_voltage-dependent anion channel.

Introduction

Plants encounter various abiotic stresses such as drought, salinity, waterlogging, and low temperature during their life cycles. Therefore, they produce various biochemical and physiological responses to overcome these unfavourable conditions. For example, plants have to initiate adaptive mechanisms to survive under dehydration which leads to inhibition of physiological processes.

Phytohormone abscisic acid (ABA) regulates plant growth and developmental processes in response to changes in water status. In *Arabidopsis*, elevation of ABA levels can stimulate the activity of downstream targets to mediate drought stress adaption. The additional ABA-independent signal transduction pathway may also have a role in counteracting the effects of dehydration (Yamaguchi-Shinozaki and Shinozaki, 2006). Numerous studies have been conducted yet on ABA-regulated adaptation to drought stress such as RCAR (regulatory component of ABA receptors), ABI (abscisic acid-insensitive), RD29B (regulated by drought), NECD (9-cis-epoxycarotenoid dioxygenase) (Schroeder et al., 2001; Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006; Razem et al., 2008; Ma et al., 2009; Park et al., 2009). However, identification of novel genes involved in ABA signalling is required to improve our understanding of mechanism and action of this important phytohormone.

As it is known, the growth of plants is suppressed under drought stress, i.e., plants become smaller and production is reduced and ABA levels change as well（Westgate et al., 1985; Sharp et al., 1988; Bartels et al., 1988). Considering the change in matter accumulation and energy metabolism, voltage-dependent anion channel (VDAC) gene activates filtering of transport across the mitochondrial outer and inner membrane.

Recent research has demonstrated that abnormalities in *VDAC* gene lead to respiratory impairment in brewer's yeast (*Saccharomyces cerevisiae*) (Dihanich et al., 1987; Lee et al., 1998; Graham and Craigen, 2004). In mammals, abnormalities of the *VDAC* gene lead to impairment of the male reproductive system, central nervous system, and glucose homeostasis (Craigen and Graham, 2008). Recently, studies have also demonstrated a role for the *VDAC* gene in plants. For example, in *Arabidopsis thaliana*, the *VDAC3* gene (AtVDAC3) improved germination and growth under low temperature conditions (Yang et al., 2011). Under high salinity condition such with osmotic stress and drought, the VDAC expression is up-regulated in rice (Al Bitar et al., 2003). Moreover, salt exposure induces the *VDAC* gene expression in pearl millet (Desai et al., 2006). The *VDAC* expression is also upregulated by drought, freezing and salicylic acid treatment, but is not

upregulated after ABA treatment（Desai et al., 2006). Interestingly, VDAC expression has been shown to alter followed bacterial infections in tobacco plants (Tateda et al., 2009). Thus, these previous studies demonstrate that VDAC expression is always changed under biotic and abiotic stimuli, suggesting its importance in adaptive mechanisms to environmental changes.

Although we already know that plant growth is restrained under drought stress, and ABA levels is also changed, it is not clear whether VDAC is associated with growth inhibition under drought stress and plays an important role in ABA signalling.

A. thaliana contains 5 *VDAC* genes that exhibit high homology to one another (Clausen et al., 2004). In our lab, we have found some evidences that display *AtVDAC2* is involved in ABA-mediated early seedling development, such as germination (Yan et al., 2009). However, the functions of VDACs in *A. thaliana* have not been fully characterized and their role in the ABA-mediated drought stress response is still unclear.

In this study, we analysed ABA sensitivity under drought stress condition in *A. thaliana* using transgenic plant including overexpressing *AtVDAC2* (*AtVDAC2*-OE) or knockdown *AtVDAC2* (*AtVDAC2*-Dn) lines. It provided information on *AtVDAC2* and phenotypes associated with ABA-mediated drought stress response. This study will provide a good complement for studies of ABA and drought signal transduction networks.

Results and Discussion

AtVDAC2-OE plants displayed an ABA-hypersensitive phenotype

As a part of standardization, we first confirmed the up- or down-regulation of *VDAC2* in our transgenic lines. The expression of *VDAC2* was 140-fold higher in *AtVDAC2*-OE plants than in related wild-type plants, while the expression of *VDAC2* in *AtVDAC2*-Dn plants decreased to about 0.7-fold than wild-type (Fig. 1). Then, we selected 2 *AtVDAC2*-OE lines (OE2 and OE4) and 2 *AtVDAC2*-Dn lines (Dn2 and Dn8) for phenotypic analysis. Wild-type RLD plants were used as the control.

To confirm whether *VDAC2* played an essential role in ABA signalling in plants, we first analysed phenotypic differences between *AtVDAC2*-OE and *AtVDAC2*-Dn lines after treatment with ABA. The *AtVDAC2* transgenic plants were selected for their ability to germinate and root growth in MS medium with or without ABA. The findings from germination trail were similar to that reported by Yan et al. (2009). The *AtVDAC2*-Dn plants were capable of germinating in MS medium with 0.7 μM ABA. However, the germination rate of *AtVDAC2*-OE plants was relatively depressed (Fig. 2), while that of wide-type plants was between the 2 transgenic lines.

ABA was also observed to affect root growth in transgenic plants. As shown in Fig. 2, 0.5 μM ABA treatment resulted in reduced root lengths in the *AtVDAC2*-OE lines compared to wild-type plants. Conversely, ABA treatment in *AtVDAC2*-Dn lines led to increased root lengths compared to wild-type plants. Taken together, we speculated that the *AtVDAC2* gene strengthen the conduction of ABA signals. Excessively expressed *AtVDAC2* made plant sensitive to ABA and inhibited the seed germination and growth of root, while a low *AtVDAC2* gene expression led to ABA signal inhibition with less affected plant growth.

Response to drought stress in AtVDAC2 transgenic plants

Since *AtVDAC2* transgenic plants showed a marked change in ABA sensitivity, in terms of seed germination and root elongation, we next examined their responses to drought stress. Four-week-old plants were grown without water for 3 weeks until differences between the plants were visible and plants were then hydrated by watering for 1 week. After rehydration for 1 week, most of the *AtVDAC2*-OE plants, about 36% of the wild-type plants, and a few of the *AtVDAC2*-Dn plants were recovered (Fig. 3). These data suggested that *AtVDAC2*-OE plants had enhanced resistance to drought compared to wide-type and *AtVDAC2*-Dn plants.

AtVDAC2-OE plants produced more hydrogen peroxide as a result of ABA treatment

Drought stress triggers ABA-induced accumulation of reactive oxygen species (ROS). Thus, plants require ROS detoxification mechanisms to survive following drought stress (Smirnoff, 1993). The H_2O_2, an important superoxide free radical, is accumulated in plants after exposure to many types of stressors, such as drought, salinity, etc. Therefore, to characterize whether ROS production occurred in response to ABA treatment in *AtVDAC2* transgenic plants, we analysed H_2O_2 accumulation by DAB staining. The *AtVDAC2*-OE lines stained much darker and into a greater extent than wide-type plants. In turn, *AtVDAC2*-Dn lines were stained more lightly than wild-type plants (Fig. 4). These data showed that H_2O_2 accumulation was increased in *AtVDAC2*-OE lines, but decreased in *AtVDAC2*-Dn lines, suggesting the involvement of VDAC in mediating H_2O_2 accumulation, similar to previous studies of *VDAC* in *Nicotiana benthamiana* (Tateda et al., 2009). Thus, it is possible that H_2O_2 production was activated in response to ABA in *AtVDAC2*-OE lines but was blocked in *AtVDAC2*-Dn lines. The stain of DAB in the root of *AtVDAC2*-Dn lines was much deeper than that of *AtVDAC2*-OE lines (Fig. 4). We suggest that it might be due to migration ability of H_2O_2. The *AtVDAC2*-Dn lines produced H_2O_2 in root, and it could not be transferred to the upper parts of plant. This situation was opposite in *AtVDAC2*-OE lines. However, this needs more investigation to be sufficiently explained.

Transcriptional alterations in downstream targets in AtVDAC2 transgenic plants

To better understand the mechanisms mediating drought resistance in *AtVDAC2* transgenic plants, we analysed the expression of downstream targets of ABA signalling (Fig. 5). The expression of *RCAR1*, a foregone ABA receptor, was increased by 37- or 105-fold in *AtVDAC2*-OE plants compared to wild-type plants. However, it was decreased by about 0.2-fold in *AtVDAC2*-Dn plants. The *RCAR1* has been shown to act as a positive regulator of ABA signalling and ABA receptor and to interact with the protein phosphatase 2Cs (PP2Cs) *ABI1*and *ABI2* (Park et al., 2009; Ma et al., 2009; Szostkiewicz et al., 2010). The *RCAR1* acts as negative regulators of ABA response which is inactivated by the ABA receptor. Therefore, we analysed the expression of *ABI1*. Interestingly, this gene showed 4- or 58-fold up-regulation in *AtVDAC2*-OE plants when compared to wild-type plants. In contrast, *ABI1* expression in *AtVDAC2*-Dn plants was reduced to about 0.1-fold, compared to wild-type plants. Other ABA-inducible genes, such as *RD29B* and *NECD3* were also up-regulated in *AtVDAC2*-OE plants and down-regulated in *AtVDAC2*-Dn plants. Indeed, the expression of *RD29B*, which contains an

Table 1. Primers for quantitative real-time PCR.

Gene (access number)		Sequence (5′→3′)	Product (bp)
ACTIN (AT1G49240)	F	GATGAAGCTCAATCCAAACGA	228
	R	AGCAGGGGCATTGAAAGTCT	
VDAC2 (AT5G67500)	F	GCTGATGTTGCCACCCAATACAA	105
	R	TGGGAGGATCTCGGTAAGTGTGACT	
RD29B (AT5G52300)	F	TTCGGCCATATGTCATCGTTCTCTC	244
	R	ATGCTCCCTTCTCATGATGCTCTTC	
AtNECD3 (AT3G14440)	F	CAAGGTCGCAAGATTCGGGATT	257
	R	TGATCGGACGGCGAGTTGATT	
ABI1 (AT4G26080)	F	TGCTCTGCGATGGTGATACG	165
	R	CACCGCAGTTAGCGACGAAG	
RCAR1 (AT1G01360)	F	CTGTGCAGAGAAAACCAGTGTACC	173
	R	ACATTGACTTCTCTAAGACTGCCG	

ABA-responsive element in its promoter, was up-regulated by 60- or 35-fold in *AtVDAC2*-OE plants, compared to wild-type plants. However, it was down-regulated to about 0.4-fold in *AtVDAC2*-Dn plants. The expression of *NECD3*, which is involved in ABA biosynthesis, was increased by 14- or 90-fold in *AtVDAC2*-OE plants, but was decreased to about 0.2-fold in *AtVDAC2*-Dn plants, compared with wild-type plants. These findings suggested that overexpression of *VDAC2* enhanced the ABA biosynthesis and activated ABA signalling and ABA-related gene (*RCAR1*, *RCAR3*, *ABI1*, and *RD29B*) expression. So, the adaptation to drought stress in *AtVDAC2*-OE plants may be regulated by overexpression of *VDAC2* related to ABA signal transduction.

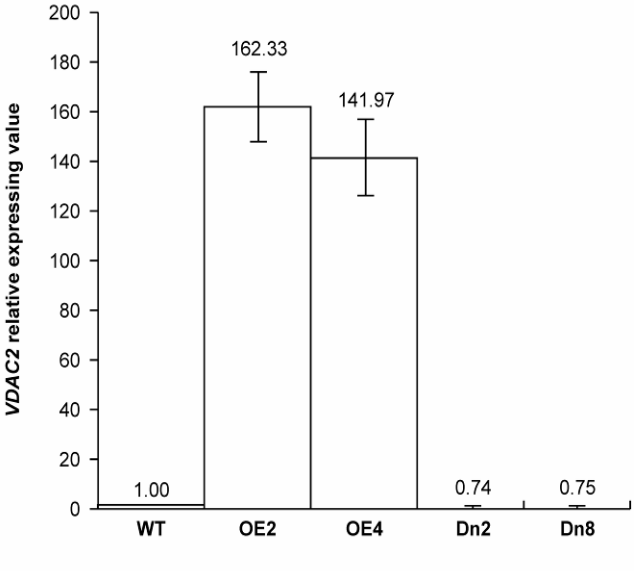

Fig 1. Relative expression of *VDAC2* in transgenic lines of *Arabidopsis* by qPCR. Relative expression of *VDAC2* was analysed by the ΔCT method after normalization of the CT of *VDAC* and related genes to CT of β-actin. The WT is *Arabidopsis thaliana* of the Reschiev (RLD) ecotype. The OE2 and OE4 are plants with *AtVDAC2* overexpression, while Dn2 and Dn8 are plants with *AtVDAC2* knockdown.

Discussion

VDAC is the most abundant protein of the outer mitochondrial membrane and is involved in transportation across the mitochondrial outer and inner membranes. Although studies have revealed the functions of VDACs in various physiological processes during the respiratory process (Graham and Craigen, 2004), glucose homeostasis and reproduction (Craigen and Graham, 2008), and seed germination under low temperature conditions (Yang et al., 2011), little is known about the involvement of VDAC in abiotic stress responses. The findings in the current study demonstrated that the overexpression of *AtVDAC2* enhanced ABA sensitivity during seed germination and root elongation and increased the accumulation of H_2O_2, promoting adaptive drought resistance in *Arabidopsis*.

Drought is one of the major abiotic stresses that can trigger severe damage to plants. Most plants generate ABA in response to drought. The ABA is a vital hormone that regulates plant growth, leaf abscission, seed and bud dormancy, and stress responses, and many drought- or ABA-induced genes, such as *RCAR1*, *ABI1*, *RD29B*, etc. (Park et al., 2009; Ma et al., 2009; Szostkiewicz et al., 2010). However, the interactions between ABA signalling and the drought stress response are still not clear, and it is imperative to identify novel genes that may be involved in plant adaptation to drought stress and the ABA response. In this study, we identified VDAC as an ABA-sensitive target of the drought stress response in *Arabidopsis*. In *Arabidopsis*, drought stress may initiate responses to either ABA-dependent or ABA-independent signal transduction (Yamaguchi-Shinozaki and Shinozaki, 2006). We found that overexpression of *AtVDAC2* enhanced ABA sensitivity, in terms of seed germination and root elongation and improved drought resistance. The opposite was the *AtVDAC2* knockdown. These data support that VDAC is involved in mediating the ABA signalling pathways in response to drought stress. Additionally, the expression levels of upstream components of the ABA-signalling pathway, such as *RCAR1* and *ABI1* and downstream components such as *RD29B* were all increased in *AtVDAC2*-OE plants and decreased in *AtVDAC2*-Dn plants. These expression patterns suggested that *AtVDAC2* most likely functions upstream of these components. Consistent with these results, RCAR proteins, of which RCAR1 was up-regulated in response to *VDAC2* overexpression, are essential for ABA signal transduction and are able to bind to the PP2Cs, upstream drought- and/or ABA-responsive targets that initiate downstream responses to drought stress (Park et al., 2009; Ma et al., 2009; Szostkiewicz et al., 2010).

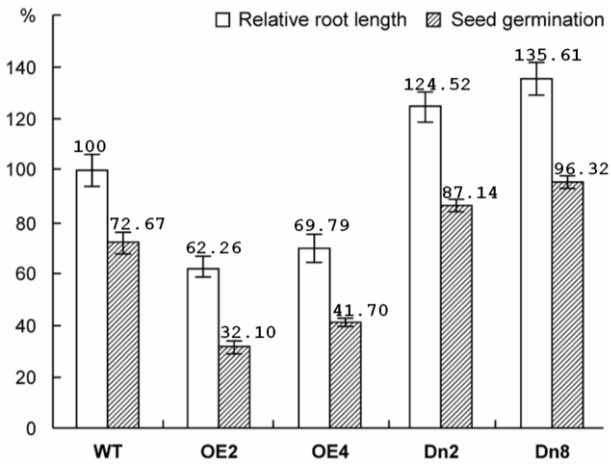

Fig 2. Seed germination rate and relative root length for *AtVDAC2* transgenic *Arabidopsis thaliana* (%). Seed germination was evaluated on the seventh day, and values are means ± SD (n ≥ 100). Relative root lengths compared with WT grown on MS medium with 0.5 μM ABA. Values are means ± SD (n ≥ 30). The WT is *Arabidopsis thaliana* of the Reschiev (RLD) ecotype and OE2 and OE4 are plants with *AtVDAC2* overexpression, while Dn2 and Dn8 are plants with *AtVDAC2* knockdown.

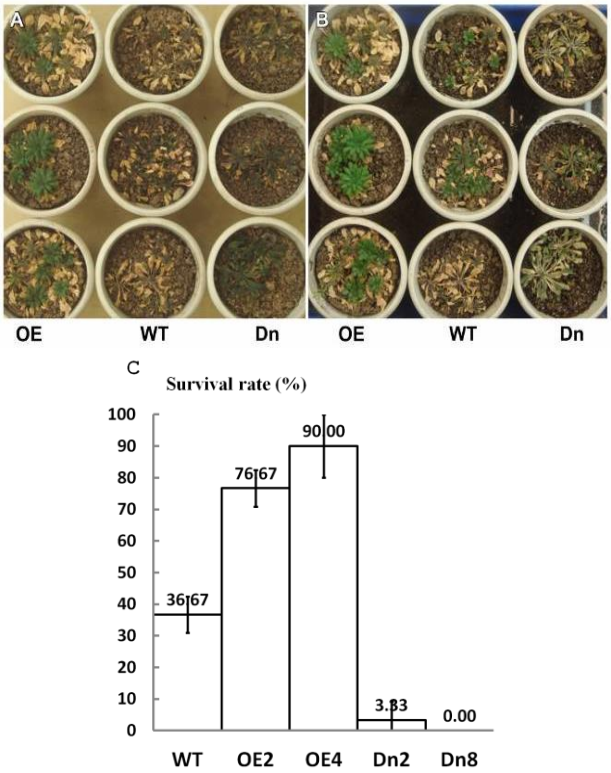

Fig 3. Growth of *AtVDAC2* transgenic *Arabidopsis thaliana* during drought conditions. (A) Drought conditions for 3 weeks. (B) Water recovered for 1 week. The WT is *Arabidopsis thaliana* of the Reschiev (RLD) ecotype, OE are plants with *AtVDAC2* overexpression, while Dn are plants with *AtVDAC2* knockdown. (C) Survival rate of the *AtVDAC2* transgenic *Arabidopsis thaliana* during drought conditions. Survival rate was analyzed when the plants were rewatered after a week. Values are means ± SD (n ≥ 30). The WT is *Arabidopsis thaliana* of the Reschiev (RLD) ecotype and OE2 and OE4 are plants with *AtVDAC2* overexpression, while Dn2 and Dn8 are plants with *AtVDAC2* knockdown.

Moreover, the elevated expression of *RCAR1* in *AtVDAC2*-OE plants may stimulate expression of downstream targets of ABA signalling. This was confirmed by the significantly increased expression of *RD29B* in *AtVDAC2*-OE plants (conversely, decreased expression in *AtVDAC2*-Dn plants). Thus, these results strongly suggested that *AtVDAC2* may confer drought resistance to plants via ABA signalling.

Drought stress causes endogenous ABA biosynthesis, and in turn, ABA triggers ROS production to mediate downstream responses (Pei et al., 2000; Zhang et al., 2001; Kwak et al., 2003). In this study, we observed that NECD3 expression increased, which may induce more endogenous ABA biosynthesis. The ROS production (i.e., H_2O_2) was promoted in plants after ABA treatment. These data indicated the involvement of *AtVDAC2* in ROS-mediated ABA signal transduction.

In summary, the findings of this study demonstrated the functions of *AtVDAC2* in the ABA-dependent drought stress response. Upregulation of the *AtVDAC2* gene induced *NECD3* gene expression, triggering ABA biosynthesis and activating the ABA signal transduction pathway. This promoted the expression of *RCAR1*, *ABI1*, and *RD29B*. Furthermore, *AtVDAC2* also activated ROS production (i.e., H_2O_2) by increasing ABA biosynthesis. Therefore, in *AtVDAC2*-OE plants, the ABA signals were conducted fast and smoothly, leading to the plants sensitive to ABA and tolerant to drought stress. However, in *AtVDAC2*-Dn plants, the ABA signalling path were blocked by the decreased expression of *AtVDAC2* and the plants remained sensitive to drought stress.

Through these mechanisms, plants were able to adapt to drought stress more effectively, revealing an important role for *VDAC2* in the drought stress response. We show that *AtVDAC2* improved the ABA synthesis related gene (*NECD3*) expression. Further research required to understand the mechanism and identify whether there are other signalling molecules involved.

Materials and Methods

Plant materials

Arabidopsis thaliana wild-type and transgenic plants used throughout this study were Reschiev (RLD) ecotype. *AtVDAC2* transgenic *Arabidopsis* plants were stored in our lab (Yan et al., 2009). We selected 2 *AtVDAC2*-OE lines (OE2 and OE4) and 2 *AtVDAC2*-Dn lines (Dn2 and Dn8) for phenotypic analysis. Wild-type RLD plants were used as the control. Plants were routinely grown in a growth chamber under 60% humidity at 23°C with a 16-h light/8-h dark photoperiod (250 μmol/m^2 sec^{-1}) in pots containing 1:1 vermiculite: soil mixture. For *in vitro* culture, seeds were surface sterilized in 75% alcohol solution for 15 s and 0.1% $HgCl_2$ for 5 min and were then washed 3 times in sterile distilled water. Stratification of the seeds was conducted during day 3 at 4°C, unless otherwise indicated. Afterwards, seeds were sown on Murashige and Skoog (MS) plates containing solid medium composed of MS basal salts and 3% Glc, solidified with 0.7% agar, and adjusted to pH 5.7 with KOH before autoclaving. Plates were sealed and incubated in a controlled-environment growth chamber.

Quantitative real-time PCR

Seeds from wild-type and transgenic plants were grown in pots. Plant material was collected and frozen in liquid nitrogen. Total RNA for quantitative reverse transcription (RT)-PCR was

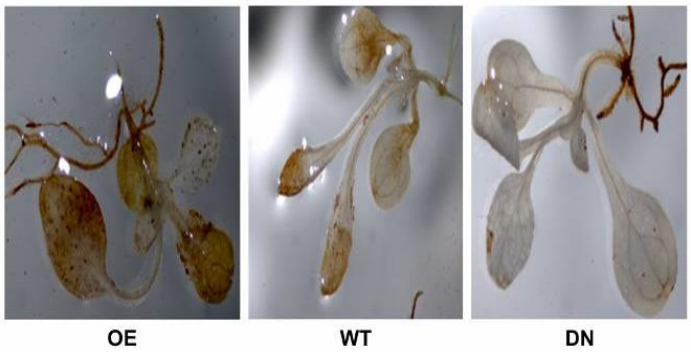

Fig 4. DAB Staining of VDAC transgenic *Arabidopsis* plants after ABA treatment. The WT is *Arabidopsis thaliana* of the Reschiev (RLD) ecotype. The OE are plants with *AtVDAC2* overexpression, while Dn are plants with *AtVDAC2* knockdown.

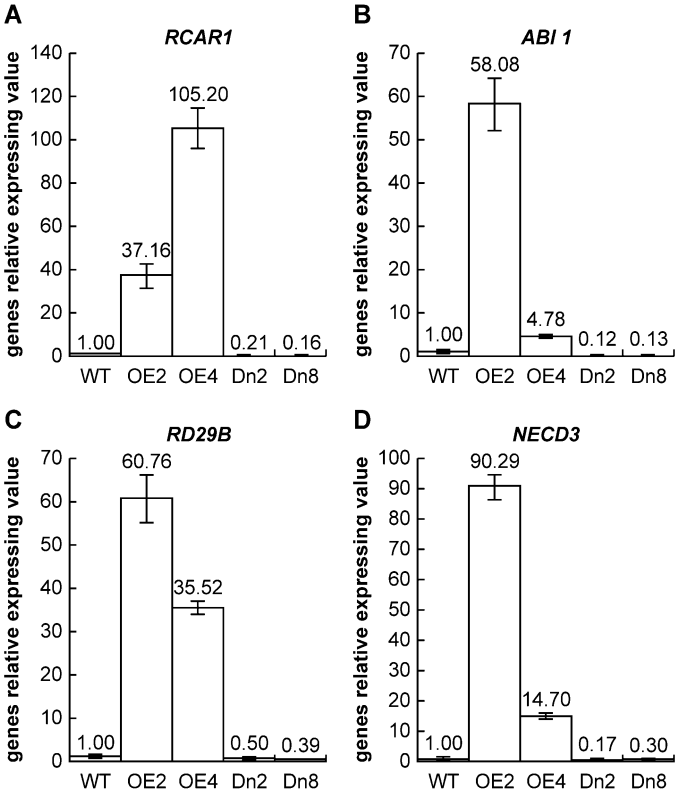

Fig 5. Relative expression of ABA signalling related genes in transgenic lines of *Arabidopsis* by qPCR. Relative expression of *RCAR1* (A), *ABI1* (B), *RD29B* (C), *NECD3* (D) was analysed by the ΔCT method after normalization of the CT of *VDAC* and related genes to that CT of β-actin. The WT is *Arabidopsis thaliana* of the Reschiev (RLD) ecotype, OE2 and OE4 are plants with *AtVDAC2* overexpression, while Dn2 and Dn8 are plants with *AtVDAC2* knockdown.

extracted from *Arabidopsis* 4-week-old seedlings using Trireagent (TIANGEN RNA Plant Kit, Tiangen Biotech, Beijing, China), according to the manufacturer's protocol. A 2-μg sample of total RNA from each sample was reverse transcribed to cDNA with ReverTra Ace qPCR RT Kit (TOYOBO, Osaka, Japan). The primer pair sequences for target genes are listed in Table 1.

Quantitative real-time PCR was conducted as follows: The cDNA reverse transcribed from RNA was used as a template for each real-time PCR reaction. Primer pairs were used at a final concentration of 0.3 μmol/L in a total reaction volume of 20 μL containing 10 μL 2× SYBR Green Realtime PCR Master Mix (TOYOBO). The DNA polymerase was activated by heat at 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 55°C for 15 s, and elongation at 72°C for 15 s. Data were collected on an iCycler instrument (Bio-Rad, Hercules, CA USA). All reactions were set up in triplicates so that comparable Ct (threshold) values could be obtained and also to calculate the standard deviations (S.Ds). The authenticity of the amplified product in each case was verified by performing melt curve analysis immediately after the quantitative PCR protocol. Quantitative analysis of the raw data was conducted in iQ5 software (Bio-Rad).

Germination and root growth assays

To measure ABA sensitivity, seeds were plated on MS medium with or without 0.7 μM ABA. Germination characteristics were observed every 24 h for 7 days. The experiments were repeated at least 3 times. The root growth assay for scoring ABA sensitivity was conducted by measuring root growth at 14 days after transferring of 3-day-old seedlings onto vertical MS plates containing 0.5 μM ABA.

3,3-diaminobenzidine (DAB) staining of tissues

H_2O_2 was visually detected in leaves using 3,3-diaminobenzidine (DAB) as a substrate (Thordal-Christensen et al., 1997). Briefly, seedlings were grown for 2 weeks on MS plates containing 0.5 μM ABA. Roots were then soaked in a 1 mg/mL solution of DAB, pH 3.8, for 8 h under light at 25°C. The seedlings were continually supplied with DAB solution until the experiments were terminated by immersion in boiling ethanol (96%) for 10 min. This treatment decolorized the leaves except for the deep brown polymerization product produced by the reaction of DAB with H_2O_2. After cooling, seedlings were extracted at room temperature with fresh ethanol for 4 h, preserved at room temperature in ethanol, and photographed.

Analysis of the effects of drought on Arabidopsis thaliana

Four-week-old *Arabidopsis* plants (wild-type and transgenic) were not watered for 3 weeks. After this time, images were taken to document morphological differences between plants. Plants were then rescued with irrigation for 1 week, and differences between plants were observed and recorded photographically. At the same time, counted the ssurvival plants and analyzed the ssurvival ration. The experiments were repeated 3 times.

Conclusion

Study of plant response to drought is becoming increasingly important, as drought is the most important environmental constraints to plant survival and to crop productivity. The understanding of drought stress signal transduction in plants is necessary to improve management practices in agriculture. Fortunately, many studies have been performed to build the net of drought stress signal transduction. But some details are still not clear. To supplement fundamental studies on drought stress

signal transduction in plants, we analysed ABA sensitivity of *A. thaliana* in drought stress condition using transgenic plant lines, including overexpressing *AtVDAC2* (*AtVDAC2*-OE) or with knockdown of *AtVDAC2* (*AtVDAC2*-Dn). We found that by changing the RNA expression of *AtVDAC2* in transgenic plant lines, the sensitivity of ABA and drought stress is changed. The overexpressing *AtVDAC2* plant lines showed inhibited seed germination and relative root length, while the *AtVDAC2*-Dn plant lines were quite the reverse. We also found that hydrogen peroxide and ABA related genes expression were changed as well. These findings indicate a novel functional link between changes in *AtVDAC2* and modulation of ABA and drought stress signal transduction. The excessive expression of *AtVDAC2* gene reinforces ABA and drought stress signalling, which inhibit the expression of *AtVDAC2* gene that suffocates these signals.

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