The coordination of guard-cell autonomous ABA synthesis and DES1 function in situ regulates plant water deficit responses

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**Graphical Abstract**

**Abstract**

**Introduction:** Drought stress triggers the synthesis and accumulation of the phytohormone abscisic acid (ABA), which regulates stomatal aperture and hence reducing plant water loss. Hydrogen sulfide (H\textsubscript{2}S), which is produced by the enzyme L-cysteine desulfhydrase 1 (DES1) that catalyzes the desulfuration of L-cysteine in Arabidopsis, also plays a critical role in the regulation of drought-induced stomatal closure. However, little is known about the regulation of DES1 or the crosstalk between H\textsubscript{2}S and ABA signaling in response to dehydration.

**Objectives:** To demonstrate the potential crosstalk between DES1-dependent H\textsubscript{2}S and ABA signaling in response to dehydration and its regulation mechanism.

**Methods:** Firstly, by introducing guard cell-specific MYB60 promoter, to produce complementary lines of DES1 or ABA3 into guard cell of des1 or aba3 mutant. And the related genes expression and water loss under ABA, NaHS, or dehydration treatment in these mutant or transgenics lines were determinate.

**Results:** We found that dehydration-induced expression of DES1 is abolished in the abscisic acid deficient 3 (aba3) mutants that are deficient in ABA synthesis. Both the complementation of ABA3 expression in guard cells of the aba3 mutants and ABA treatment rescue the dehydration-induced expression of DES1, as well as the wilting phenotype observed in these mutants. While the drought-induced expression of ABA synthesis genes was suppressed in des1 mutants. While the addition of ABA or the
expression of either ABA3 or DES1 in the guard cells of the aba3/des1 double mutant did not alter the wilting phenotype of these mutants, the wild type phenotype was fully restored by the expression of both ABA3 and DES1, or by the application of NaHS.

Conclusion: These results demonstrate that the coordinated synthesis of ABA and DES1 expression is required for drought-induced stomatal closure in Arabidopsis.

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Determination of endogenous H2S content

Endogenous H2S content was measured by the formation of methylene blue, according to the method previously published [25]. Briefly, Arabidopsis leaf samples (0.2 g) were extracted in 1 ml of phosphate buffer solution (pH 6.8, 50 mM) containing 0.1 M EDTA and 0.2 M AsA. The supernatant was mixed with 1 ml of phosphate buffer solution (pH 6.8, 50 mM) containing 1 M HCl in a test tube to release H2S, following by absorbance measurement. Solutions with different concentrations of NaHS were treated in the same way as the assay samples, and used for the quantification of H2S.

Statistical analysis

Error bars represent ± SD three biological replicates, analyzed using SPSS version 23.0. The comparison of two groups was performed by Student’s t-test. * and ** represent P < 0.05 and P < 0.01, respectively. Different letters indicate significantly different at P < 0.05 according to Duncan’s multiple range test (P < 0.05).

Results and discussion

De-novo ABA synthesis is critical for dehydration-induced DES1 expression and responses to dehydration stress

We have recently demonstrated that the abundances of DES1 transcript is induced by ABA treatment of wild-type Arabidopsis thaliana leaves [23]. To investigate the potential role of ABA in dehydration-induced DES1 expression, we first analyzed the expression of DES1 gene in response to dehydration stress in the wild-type and aba3 mutants, which are defective in the last step of ABA synthesis in plants [7,8]. As predicted, DES1 was induced in wild-type rosette leaves within 6 h after detachment. However, the induction of DES1 expression was not observed in the detached aba3 mutant (Fig. 1A). Treatment with ABA, but not NaHS, stimulated DES1 expression in both wild-type and aba3 mutant leaves (Fig. 1B and C). This observation suggests ABA3-mediated ABA synthesis is required for the induction of DES1 expression in response to dehydration stress.

Although ABA can be transferred from the vascular tissues to the guard cells [4,26], guard cells house the complete ABA synthesis pathway [11]. Moreover, ABA3 mediated-guard cell ABA synthesis is required for stomatal closure. To test whether de novo ABA synthesis in the guard cells is required for DES1 expression and the related dehydration responses, we further use pMYB60:ABA3 plants in which ABA synthesis was rescued in the mature guard cells of the aba3 mutants [12]. DES1 expression was significantly increased in pMYB60:ABA3 aba3 lines after 6 h dehydration, similar to wild-type (Fig. 1D). This result suggests that ABA3-mediated guard-cell ABA synthesis is sufficient to trigger dehydration induced-DES1 expression. DES1 is involved in stomatal closure and related drought tolerance responses [15,18,20]. To further evaluate the possible link between the functions of ABA3 and DES1 in drought stress responses, DES1 was specifically expressed in the guard cell of aba3 mutant under control of guard cell-specific MYB60 promoter, to produce pMYB60:DES1aba3 lines. In agreement with previous results [11], the complementation of ABA3 in guard cells rescued the wilting phenotype of the aba3 mutants. Moreover, the pMYB60:ABA3 aba3 plants showed a similar response to dehydration stress as the wild-type (Fig. 1E) However, there were no significant differences between the pMYB60:DES1aba3 plants and the aba3 mutants, indicating that ABA3-mediated guard-cell ABA synthesis is sufficient for dehydration-induced DES1 expression and related dehydration tolerance.

Table 1

| Primer            | Sequence (5’-3’)       | Description                     |
|-------------------|------------------------|---------------------------------|
| des1-1P           | GCCGGCTTTGTCTCTCTCTTC  | Identification for des1 mutant  |
| des1-1R           | AGTAAACCCTCCACACCTCC   | Identification for des1 mutant  |
| LBD1.3            | ATTTCGCGAGATTGACGAC    | for promoter pMYB60-pCAMBIA1305-GFP |
| pMYB60-SacE-F     | cagattgaattcgagctcTGTTGCACTAAGTGGTTTAC | for des1-pCAMBIA1305-GFP |
| pMYB60-SpeE-R     | agtccgagcttaagctgATGTAACAAATAACGCCGTAGG | Identification for pMYB60:ABA3-pCAMBIA1305-GFP |
| DES1-BamHI-R      | gctccgtcattagctgTCAACTGGCAATAACCTCAGTT | Identification for pMYB60:DES1-pCAMBIA1305-GFP lines |
| pMYB-DES-F        | ACTATGGGTGATTTAGCC     | for RT-PCR                       |
| pMYB-DES-R1       | CCAGGCTATCTTCTTG       |                                 |
| pMYB-ABA3-R       | TAAACAGGGCAACAGGA      |                                 |
| pMYB-ABA3-R       | CCAAGGCCACTAGGATAA     |                                 |
| qDES1-F           | TCGACTCAGCATGATAGTACGCT |                                 |
| qDES1-R           | TGTACACTTGGATACCACTCTCT |                                 |
| qABA3-F           | TTCCGCTGACCAACGAGAAC   |                                 |
| qABA3-R           | ATGGACCTCCAGGGGAAAG    |                                 |
| qNED3-F           | TTCAGGCGGCTATTACGTTCT  |                                 |
| qNED3-R           | GTGTGACACGACTGGCATAA   |                                 |
| qZEP-F            | TTCGGATTTGCTGTTGGTT    |                                 |
| qZEP-R            | GATAGAATCCCCCGAGGAC    |                                 |
| qAAO3-F           | TAGCAAAGCTCCAGGATGT    |                                 |
| qAAO3-R           | CTCGCCCACTCATATCCCC    |                                 |
| qACTIN7-F         | CCGCTCGACTCATGACTCT    |                                 |
| qACTIN7-R         | TACGCTCCTGATCGGAGG     |                                 |
| qUBQ10-F          | GCCCTCTTATATACCTCATGAAATAG | Identification for pMYB60:ABA3-pCAMBIA1305-GFP lines |
| qUBQ10-R          | AAAGAGATAACGGAACGGAACATAGT | Identification for pMYB60:ABA3-pCAMBIA1305-GFP lines |

Cysteine and/or the products of cysteine metabolism might trigger ABA synthesis and contribute to the regulation of stomatal closure in response to drought [27]. Cysteine is also the substrate of
the Moco-sulfurylase ABA DEFICIENT3 required for activation of ALDEHYDE OXIDASE 3 (AAO3). To investigate the possible role of DES1, which degrades cysteine to produce H2S, we first checked the expression profiles of genes encoding proteins responsible for ABA synthesis in the leaves wild-type plants and des1 mutants, including ZEAXANTHIN EPOXIDASE (ZEP), 9-cis-EPOXYCAROTENOID DIOXYGENASE (NCED3), ALDEHYDE OXIDASE 3 (AAO3), and ABA DEFICIENT3 (ABA3). Exposure to dehydration increased the accumulation of all of these transcripts in the wild-type but not in the des1 mutants (Fig. 2). These results indicate that DES1 is required for dehydration-induced ABA synthesis. Combined with the results shown in Fig. 2, these data suggest that the downstream products of DES1-mediated cysteine metabolism, trigger drought-induced ABA synthesis rather than cysteine per se. In support of

![Fig. 1. ABA denovo synthesis is critical for drought induced DES1 expression and plant drought tolerance. The induction of DES1 expression in aba3 mutant, DES1 expression in 1-month-old wild type and aba3 mutant leaves after detachment (A), detachment with ABA (10 μM) (B) or NaHS (100 μM) (C) pretreatment in 6 hr were detected. (D) The induction of DES1 in aba3 mutant and aba3 mutant carrying ABA3 in the guard cells. Relative transcript levels are shown using actin7 and UBQ10 as internal control. (E) Relative dehydration stress phenotype of wild type, aba3, and aba3 mutant seedlings carrying ABA3 or DES1 in the guard cells. The excised leaves of 1-month-old Col-0, aba3, and aba3 mutant seedlings carrying ABA3 (pMYB60:ABA3) or DES1 (pMYB60:DES1) in the guard cells were put into the eppendorf tubes immediately, with photo (the upper panel) taken as 0 hr. The lower panel photo was taken 1 hr later. Bar = 0.5 cm. Error bars represent ± SD three biological replicates. * p < 0.05; ** p < 0.01; *** p < 0.001, Student’s t test.](image-url)
this conclusion, it has been reported that ABA synthesis contributes to H2S-induced drought tolerance in wheat [17]. To confirm the function of DES1-produced H2S in ABA-mediated dehydration responses, we crossed the aba3 and des1 mutants to obtain aba3 des1 double mutants. Functional characterization revealed that aba3 des1 plants possessed much lower endogenous H2S levels than either parental lines or wild-type (Fig. 3A; [25]). We also noticed that the H2S level was relatively lower in aba3 mutant than wild-type, suggesting that DES1-produced H2S is regulated by ABA synthesis. In this study, drought-induced ABA biosynthesis was impaired in des1 mutant, and vice versa (Figs. 1, 2). Importantly, the levels of cysteine accumulation were about 20–25% higher in the des1 mutant leaves than the wild-type regardless of the growth stage of the plant [21]. These results implied DES1-H2S signals might be divergent from cysteine assimilation [27], both of which may fine-tune ABA synthesis and signal in plants.

Subsequently, we observed that the rate of water loss was greater in the aba3 des1 double mutant than those of either the aba3 or des1 single mutants, or the wild-type leaves following detachment (Fig. 3B). This result suggests that DES1 is involved in ABA3-mediated ABA synthesis during plant responses to dehydration. Taken together, these results increase our understanding of the intrinsic crosstalk between the DES1/H2S and ABA signaling pathways and shed more light onto the regulatory role of H2S in plant cells.

The coordination of guard-cell autonomous ABA synthesis and in situ DES1 function in controlling plant dehydration responses.

DES1 is expressed in the cytosol of mesophyll and epidermal cells, including the guard cells at all stages of development [23].

Fig. 2. DES1 is involved in drought-induced ABA synthesis. The induction of genes involved in ABA synthesis were abolished in des1 mutant. Expression profile of ABA-synthesis genes in 1-month-old wild type and des1 mutant leaves after detachment were detected within 6 hr. Relative transcript levels are shown using actin7 and UBQ10 as internal control.

Fig. 3. Endogenous H2S production and relative water content in ABA3 and DES1 related mutant and complementation lines. (A) Endogenous H2S production in 1-month-old leaves of wild type, aba3, des1 mutant and aba3 des1 double mutants. (B) Relative water loss of wild type, aba3, des1 mutant and aba3 des1 double mutant leaves within 50 min. (C) Relative water loss of aba3 des1, pMYB60:DES1 aba3 des1, pMYB60:ABA3 aba3 des1, and pMYB60:ABA3 pMYB60:DES1 aba3 des1 leaves within 50 min. The 1-month-old leaves were excised, and fresh weight was measured every 5 min within 50 min. The fresh weight was measured immediately, setting as 0% water loss. Error bars represent ± SD three biological replicates. Different letters indicate significantly different at P < 0.05 according to Duncan’s multiple range test (P < 0.05). * p < 0.05; ** p < 0.01; Student’s t test.
We have recently reported that DES1 is required for in situ \( \text{H}_2\text{S} \) production in guard cells and the related control of stomatal closure [22]. To evaluate the contribution of guard cell specific-DES1 expression in the ABA3-mediated dehydration response, DES1 or ABA3 were introduced into the \( \text{aba}3 \text{ des1} \) double mutants under the control of the \( \text{pMYB60} \) promoter, to produce \( \text{pMYB60:ABA3 aba3 des1} \) and \( \text{pMYB60:DES1 aba3 des1} \) lines respectively, as well as \( \text{pMYB60:ABA3 pMYB60:DES1 aba3 des1} \) plants. The \( \text{pMYB60:ABA3 aba3 des1} \) and \( \text{pMYB60:DES1 aba3 des1} \) plants exhibited similar rates of dehydration-induced water loss to the \( \text{aba3 des1} \) double mutants. Hence, complementation with either DES1 or ABA3 in guard cell could not prevent the dehydration-induced water loss in the \( \text{aba3 des1} \) double mutants. The \( \text{pMYB60:ABA3 pMYB60:DES1 aba3 des1} \) leaves displayed a significant decrease in dehydration-induced water loss compared to the \( \text{aba3 des1} \) mutants (Fig. 3C).

These results suggest that DES1 and ABA3 in the guard cells interact synergistically to control water loss in response to dehydration. The leaves of the \( \text{aba3 des1} \) double mutants wilt after detachment. The expression of either \( \text{ABA3} \) or \( \text{DES1} \) in the guard cells of the \( \text{aba3 des1} \) mutants did not prevent wilting. However, the leaves of the \( \text{pMYB60:ABA3 pMYB60:DES1 aba3 des1} \) plants exhibited a similar wilting behavior to the wild-type following detachment (Fig. 4). It is worthy to note that the guard cell specific overexpression of \( \text{DES1} \) delayed the wilting phenotype and reduced water loss following exposure to dehydration stress [23]. Both the \( \text{pMYB60:DES1 aba3 des1} \) and \( \text{pMYB60:DES1 aba3 des1} \) plants exhibit greater wilting in response to dehydration than the wild-type (Figs. 1 and 4). These finding suggest that ABA synthesis in guard cells is sufficient to support DES1 functions in the dehydration stress response. Most importantly, the wilting phenotype of \( \text{pMYB60:DES1 aba3 des1} \) plants can be fully restored by ABA pretreatment. This was not observed in the \( \text{aba3 des1} \) and \( \text{pMYB60:ABA3 aba3 des1} \) mutant line. In contrast, NaHS pretreatment rescued the wilting phenotype in all of the mutant lines, a finding that indicates that DES1 is required in guard cells for ABA-triggered dehydration responses.

Taken together, these results suggest that the coordination of guard-cell autonomous ABA synthesis and DES1 functions control the dehydration response in \textit{Arabidopsis}.

Conclusions

In summary, the findings presented here demonstrate that ABA3-mediated guard-cell autonomous ABA synthesis is required (and is sufficient) for \( \text{DES1} \) expression and related dehydration responses, DES1 is involved in dehydration-induced ABA synthesis. Moreover, the \textit{in situ} \( \text{DES1} \) function of guard cell are required for ABA-triggered dehydration responses. Using transgenic lines that specifically express ABA3 and DES1 in the guard cells, we found that the expression of either ABA3 or \( \text{DES1} \) in the guard cells of the \( \text{aba3 des1} \) mutants failed to alter wilting, the wild-type phenotype was fully restored by simultaneous expression of ABA3 and \( \text{DES1} \), or following treatment with NaHS but not ABA. Taken together, these results suggest that the coordination of guard-cell autonomous ABA synthesis and DES1 functions control the dehydration stress response in \textit{Arabidopsis}. These findings not only extend our current knowledge of \( \text{H}_2\text{S} \) function in guard cells and ABA signaling, but they also provide new targets for plant improvement strategies, seeking to produce crops that are better able to withstand changing environmental conditions.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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References

[1] Blatt M. Cellular signaling and volume control in stomatal movements in plants. Annu. Rev. Cell Dev. Biol. 2010;16:221–41. doi: https://doi.org/10.1146/annurev.cellbio.16.122109.133417

[2] Kim TH, Böhmer M, Hu H, Nishimura N, Schroeder JI. Guard cell signal transduction network: advances in understanding abscisic acid, CO2, and Ca2+ signaling. Annu. Rev. Plant Biol. 2010;61:561–91. doi: https://doi.org/10.1146/annurev-arplant-042809-112226

[3] Zhu Jian-Kang. Abiotic Stress Signaling and Responses in Plants. Cell 2016;167:313–24. doi: https://doi.org/10.1016/j.cell.2016.08.029

[4] Boursiac Y, Lézan S, Corrátge-Faille C, Gojon A, Krouk G, Lacombe B. ABA transport and transporters. Trends Plant Sci. 2013;18:325–33. doi: https://doi.org/10.1016/j.tplants.2013.01.007

[5] Seo M, Koshiba T. Transport of ABA from the site of biosynthesis to the site of action. Plant Res. 2011;124:501–7. doi: https://doi.org/10.1016/j.pt.2011.04.041

[6] Hauser F, Li Z, Waadt R, Schroder J. Snapshot: abscisic acid signaling. Cell 2017;171:1708–1708.e6. doi: https://doi.org/10.1016/j.cell.2017.11.045

[7] Bottner F, Oreb M, Mendel RR. ABA3 is a molybdenum cofactor sulfurase required for activation of aldehyde oxidase and xanthine dehydrogenase in Arabidopsis thaliana. J. Biol. Chem. 2001;276:40381–4. doi: https://doi.org/10.1074/jbc.M708549200

[8] Xiong L, Bhatia M, Lee H, Zhu JK. The Arabidopsis LO5/ABA3 locus encodes a molybdenum cofactor sulfuration J. Biol. Chem. 2008;283:9642–50. doi: https://doi.org/10.1074/jbc.M708549200

[9] Xiong L, Bhatia M, Lee H, Zhu JK. The Arabidopsis LO5/ABA3 locus encodes a molybdenum cofactor sulfuration. J. Biol. Chem. 2008;283:9642–50. doi: https://doi.org/10.1074/jbc.M708549200

[10] Nambara E, Marion-Poll A. Abscisic acid biosynthesis and catabolism. Annu. Rev. Plant Biol. 2017;68:1708–1708.e0. doi: https://doi.org/10.1146/annurev-arplant.56.032604.144046

[11] Gotor C, Laureano-Marín AM, Moreno I, Aroca A, García I, Romero LC. Signaling by hydrogen sulfide and cyanide through post-translational modification, J. Exp. Bot. 2015;66:1285–92. doi: https://doi.org/10.1093/jxb/eru225.

[12] Ma D, Ding H, Wang C, Qin H, Han Q, Hou J, et al. Alleviation of drought stress by hydrogen sulfide is partially related to the abscisic acid signaling pathway in wheat. PLoS ONE 2016;11.: doi: https://doi.org/10.1371/journal.pone.0163082.

[13] Gotor C, García A, Aroca, A.M. Lauroeano-Marín, I. Arenas-Alfonseca, Jurado-Flores, et al. Signaling by hydrogen sulfide and cyanide through post-translational modification, J. Exp. Bot. 2020;71:423–426. doi: 10.1093/jxb/erz225.

[14] Guo H, Zhou H, Zhang J, Guan W, Xu S, Shen W, et al. L-cysteine desulfhydrase-related H2S production is involved in OsSE5-promoted ammonium tolerance in roots of Oryza sativa. Plant Cell Environ. 2017;40:177–90. doi: https://doi.org/10.1111/pce.12983.

[15] Du XZ, Jin ZP, Liu X, Yang GD, Pei YX. H2S is involved in ABA-mediated stomatal movement through MPK4 to alleviate drought stress in Arabidopsis thaliana. Plant Soil. 2019;435:295–307. doi: https://doi.org/10.1007/s11104-018-3954-0.

[16] C. Gotor, I. García, A. Aroca, A.M. Lauraeano-Marín, I. Arenas-Alfonseca, Jurado-Flores, et al. Signaling by hydrogen sulfide and cyanide through post-translational modification, J. Exp. Bot. 2020;71:423–426. doi: 10.1093/jxb/erz225.

[17] Gotor C, Laureano-Marín AM, Moreno I, Aroca A, García I, Romero LC. Signaling by hydrogen sulfide and cyanide through post-translational modification, J. Exp. Bot. 2015;66:1285–92. doi: https://doi.org/10.1093/jxb/eru225.