PLANT SIGNALING & BEHAVIOR
2021, VOL. 16, NO. 4, e1876379 (6 pages)
https://doi.org/10.1080/15592324.2021.1876379

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

RIC7 plays a negative role in ABA-induced stomatal closure by inhibiting H_2O_2 production

Zi-Dan Zhu, Hai-Jing Sun, Jiao Li, Ya-Xin Yuan, Jun-Feng Zhao, Chun-Guang Zhang, and Yu-Ling Chen

College of Life Sciences, Hebei Normal University, Shijiazhuang, China

ABSTRACT

When plants encounter environmental stresses, phytohormone abscisic acid (ABA) accumulates quickly and efficiently reduces water loss by inducing stomatal closure. Reactive oxygen species (ROS) is an important regulator in ABA-induced stomatal closure, and ROS generation is modulated by multiple components in guard-cell ABA signaling. ROP interactive CRIB-containing protein 7 (RIC7) has been found to negatively regulate ABA-induced stomatal closure. However, the molecular details of the RIC7 function in this process are unclear. Here, by using two *RIC7* overexpressing mutants, we confirmed the negative role of RIC7 in ABA-induced stomatal closure and found that guard cells of *RIC7* overexpressing mutants generated less H_2O_2 than the wild type with ABA treatment, which were consistent with the reduced expression levels of ROS generation related NADPH oxidase genes *AtRBOHD* and *AtRBOHF*, and cytosolic polyamine oxidase genes *PAO1* and *PAO5* in the *RIC7* overexpressing mutants. Furthermore, external applied H_2O_2 failed to rescue the defects of stomatal closure in *RIC7* overexpressing mutants. These results suggest that RIC7 affects H_2O_2 generation in guard cells, and the function of H_2O_2 is dependent on RIC7 in ABA-induced stomatal closure, indicative of interdependency between RIC7 and H_2O_2 in ABA guard-cell signaling.

ARTICLE HISTORY
Received 27 October 2020
Revised 11 January 2021
Accepted 11 January 2021

KEYWORDS
ABA; stomatal closure; RIC7; - H_2O_2

ABA is a phytohormone that regulates plant growth and development and plays a vital role in responses to multiple environmental stresses. Stomatal aperture variation regulates the gas exchange between plant and environment, which optimizing the water loss from transpiration and photosynthetic CO_2 assimilation. ABA accumulates quickly when the plants encounter abiotic stresses and enhances stomatal closure to reduce water loss. A series of cellular events happen during ABA regulation of stomatal closure, including cytosolic Ca^{2+} osscillation, activation of protein kinases and phosphatases, and reactive oxygen species (ROS) production.[1–4] However, the regulatory mechanism of ABA-induced stomatal closure has not been completely understood.

Plants have a distinct small G protein family ROPs, and Arabidopsis (*Arabidopsis thaliana*) genome encodes 11 ROP genes that act as molecular switches in multiple processes, including interdigitated growth of pavement cells, [5] polar growth of pollen tubes,[6] and polar auxin transport.[7] ROPs have also been found to be involved in the response to environmental stresses. For example, several lines of evidence indicate that ROP2, ROP6, ROP10, and ROP11 play roles in ABA-induced stomatal closure;[8–11] ROP2 and ROP7 negatively modulate red light-induced stomatal opening.[12,14] ROP-specific guanine nucleotide exchange factors RopGEFs convert the GDP-bound inactive forms of ROPs into the GTP-bound active forms. There are 14 RopGEF members in Arabidopsis, and a certain process needs the activation of distinct Rop-GEFs and ROPs: RopGEF1 and RopGEF4 act as specific regulators of ROP11 in ABA-induced stomatal closure;[10,15] ROP2 and ROP7 are activated by RopGEF2 in red light regulation of stomatal opening.[14] Furthermore, the ROP interactive CRIB-containing proteins (RICs) have been found to act downstream of ROPs. For example, RIC7 interacts with ROP2 and plays a role in the light-induced stomatal opening by inhibiting the exocyst subunit Exo70B1.[16] ROP2 and RIC7 negatively regulate ABA-induced stomatal closure.[8,16] However, the mechanism of RIC7 action in ABA-induced stomatal closure is unclear. Here, we provided convincing evidence to support the interdependency between RIC7 and ROS generation in ABA induction of stomatal closure.

RIC7 plays a negative role in ABA-induced stomatal closure and ABA inhibition of seed germination

To investigate the role of RIC7 in ABA-induced stomatal closure, we obtained two T-DNA insertion *ric7* mutants with the insertion sites in the promoter region of *the RIC7* gene (Figure 1a). The qRT-PCR result indicated that the two *ric7* mutants have higher *RIC7* expression levels than the wild type (Figure 1b). Since the *ric7-1* mutant is a *RIC7* null mutant in the previous report,[16] the two mutants with higher *RIC7* levels were designated as *ric7-2* and *ric7-3* in this research. Next, we checked the stomatal response of the two *ric7* mutants to light and ABA. The stomatal apertures of *ric7-2* and *ric7-3* were smaller than wild type after 2 h light illumination and were larger than wild type after 10 μM ABA treatment (Figure 2a), which are consistent with the stomatal response of *RIC7* overexpression lines in the previous report [16] confirming that *RIC7*

CONTACT Chun-Guang Zhang zhchg@hebtu.edu.cn; yulingchen@mail.hebtu.edu.cn Yu-Ling Chen College of Life Sciences, Hebei Normal University. Shijiazhuang, 050024, China.

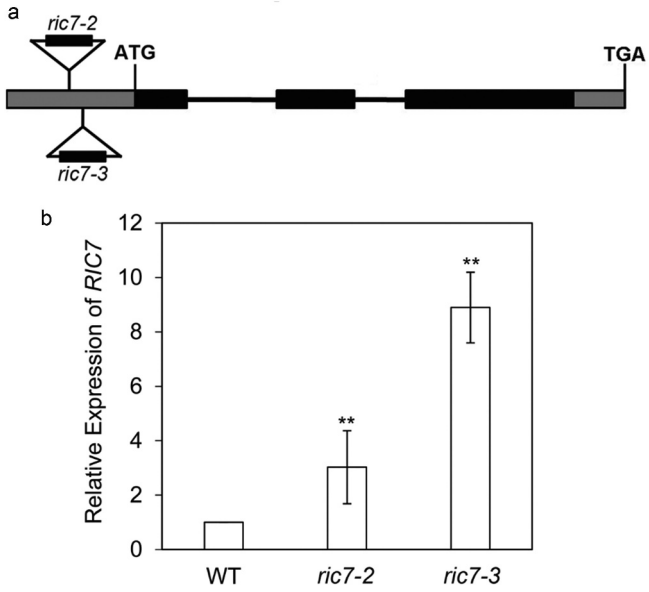

Figure 1. T-DNA insertions in promoter region of *RIC7* gene led to higher *RIC7* expression in *ric7-2* and *ric7-3* mutants. (a) The structure of *RIC7* gene and the insertion site of *ric7-2* and *ric7-3* mutants. (b) Expressions of *RIC7* in *ric7-2* and *ric7-3* mutants were higher than wild type (WT). (**, $p < .01$).

plays a negative role in ABA-induced stomatal closure. Furthermore, we examined the seed germination rate of *ric7* mutants with ABA treatment, and found that *ric7-2* and *ric7-3* mutants were also insensitive to ABA during seed germination: *ric7-2* and *ric7-3* mutants exhibited higher germination rates than the wild type with ABA treatment (Figure 2b), suggesting that RIC7 plays a negative role in ABA inhibition of seed germination.

Guard cells of *ric7* mutants generated less ROS with ABA treatment

ROS generation is an important cellular event in ABA-induced stomatal closure and accumulates to the highest level in guard cells after 30 min ABA treatment.[17] To investigate whether RIC7 affected H_2O_2 generation in ABA-induced stomatal closure, we checked the H_2O_2 levels in guard cells of the two *ric7* mutants without or with 30 min ABA treatments. The results showed that both *ric7-2* and *ric7-3* mutants have a relatively lower H_2O_2 level in guard cells without ABA treatment. Although H_2O_2 production increased in guard cells of both wild type and the two *ric7* mutants after ABA treatment, H_2O_2 accumulations in guard cells of *ric7-2* and *ric7-3* mutants were significantly lower than wild type (Figure 3 a and 3b), suggesting that RIC7 inhibits H_2O_2 generation in ABA-induced stomatal closure.

Expression levels of genes responsible for ROS generation in *ric7* mutants were lower than wild type in response to ABA

To investigate whether the lower ROS levels in *ric7* mutants were due to the lower generation capability or higher scavenging activities, we checked the expression levels of genes responsible for the ROS generation (*AtRBOHD/F* and *PAO1-5*) and scavenging (*CAT1-3* and *SOD1-2*) without or with ABA treatment, and showed here the expression levels of these genes with ABA treatment increased to at least twofold of the levels without ABA treatment in wild type or the expression levels of these genes exhibited a significant difference between *ric7* mutants and wild type. *AtRBOHD* and *AtRBOHF* are the guard cell-expressed NADPH oxidase genes, and the expression of *AtRBOHD* has been found to reach the highest induction by ABA at 30 min.[13] Therefore, the leaves were treated with ABA for 30 min, and the gene expression was detected by qRT-PCR in this research. The results showed that *AtRBOHD* and *AtRBOHF* expression levels increased upon ABA treatment, reaching about fourfold of the levels in untreated leaves of wild type; however, the expression levels of *AtRBOHD* or *AtRBOHF* with ABA treatment were significantly lower in *ric7* mutants than wild type. Furthermore, polyamine oxidase (PAO) catalyzes the oxidation of the higher PA spermidine and spermine, which contributes to the H_2O_2 accumulation. Silencing the Arabidopsis cytosolic *PAO1* and *PAO5* leads to the reduced H_2O_2 generation with salt stress.[18] Here, we showed that the expression levels of *PAO1* and *PAO5* increased upon ABA treatment, reaching twofold to fourfold of the levels in untreated leaves of wild type, whereas the expression levels

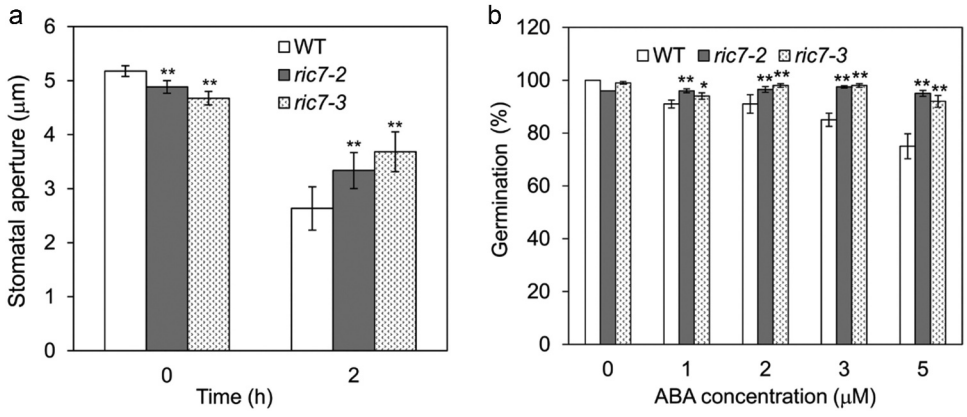

Figure 2. RIC7 played a negative role in ABA-induced stomatal closure and ABA inhibition of seed germination. (a) Stomatal apertures of *ric7-2* and *ric7-3* mutants were larger than wild type after ABA treatment. (b) Seed germination rates of *ric7-2* and *ric7-3* mutants were higher than wild type (WT) in the MS medium containing different concentrations of ABA. (*, $p < .05$; **, $p < .01$).

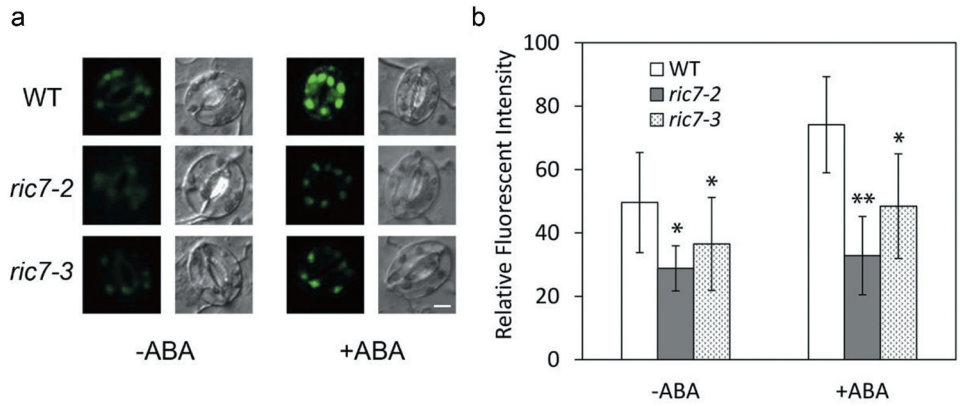

Figure 3. H_2O_2 levels in guard cells of *ric7-2* and *ric7-3* mutants were lower than wild type without and with ABA treatment. The representative pictures (a) and statistical results (b) showing the H_2O_2 levels in guard cells of wild type (WT), *ric7-2* and *ric7-3* mutants. (bar = 5 μm; *, $p < .05$; **, $p < .01$).

of *PAO1* and *PAO5* with ABA treatment in *ric7* mutants were greatly lower than the levels in wild type. The dynamic changes of cellular H_2O_2 level are determined by the balance between the generation and scavenging rates. We next checked the expression levels of catalases (CAT), the efficient scavengers of H_2O_2, upon ABA treatment, and found that *CAT2* and *CAT3* expression levels increased upon ABA treatment, reaching to over twofold higher than the levels in untreated leaves of wild type whereas the expression levels of *CAT2* and *CAT3* with ABA treatment in *ric7* mutants were greatly lower than wild type (Figure 4). These results suggest that the lower H_2O_2 accumulation in guard cells of *ric7* mutants is likely due to the reduced expression levels of the H_2O_2 generation related *to AtRBOHD/F* and *PAO1/5* genes, not due to the higher expression of scavenging-related genes. Unexpectedly, the expression levels of *AtRBOHD/F* and *PAO1/5* were higher in *ric7-2* and *ric7-3* mutants without ABA treatment. It has been shown that AtRBOHF were phosphorylated by OST1, an important kinase in ABA signaling,[19] indicating that the activities of these enzymes were also post-translational regulated. These combined results suggest that the lower H_2O_2 accumulations in *ric7* mutants with ABA treatment attribute to the lower expression of the genes responsible for H_2O_2 generation.

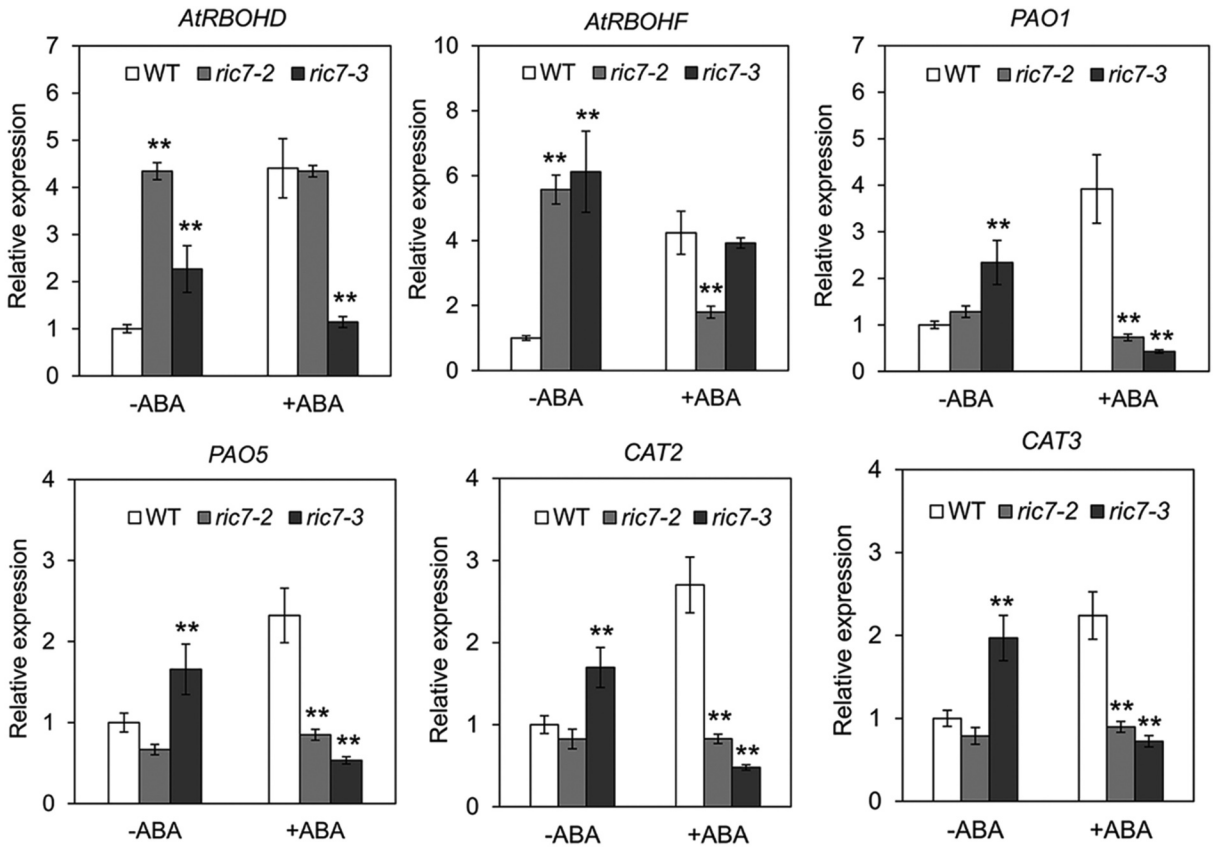

Figure 4. The expression levels of H_2O_2 generation related genes *AtRBOHD/F* and *PAO1/5*, and the scavenging related genes *CAT2/3* in *ric7* mutants before and after ABA treatment. (**, $p < .01$).

H_2O_2 failed to induce stomatal closure in *ric7* mutants as in wild type

To explore whether the reduced H_2O_2 productions in guard cells of *ric7-2* and *ric7-3* mutants were responsible for the reduced stomatal closure in response to ABA, we examined the effect of external applied H_2O_2 on stomatal closure of *ric7-2* and *ric7-3* mutants. Unexpectedly, H_2O_2 failed to induce stomatal closure of *ric7-2* and *ric7-3* mutants as in wild type: *ric7-2* and *ric7-3* mutants exhibited larger stomatal apertures than the wild type with H_2O_2 treatment (Figure 5), suggesting that RIC7 is required for the induction of stomatal closure by H_2O_2.

ROS plays an essential role in multiple stimuli-induced stomatal closures,[15,20,21] and ROS generation was regulated by GPA1, OST1, BAK1, or ABI1.[22–25] Here, we provided convincing evidence to support that RIC7 inhibits guard-cell H_2O_2 generation induced by ABA (Figure 3), which is likely due to the lower expression levels of ROS generation related genes (Figure 4). At the same time, the external applied H_2O_2 failed to induce stomatal closure in *ric7-2* and *ric7-3* as in wild type (Figure 5), suggesting that the function of H_2O_2 is dependent on the existence of RIC7 in guard-cell ABA signaling. It has been shown that H_2O_2 forms a positive feedback loop with pepper *CaWRKY27* expression in the response to heat stress.[26] H_2O_2 also forms a feed-back regulation with anthocyanin, a plant water-soluble antioxidant: H_2O_2 induces accumulation of anthocyanin while anthocyanin affects ROS levels and the sensitivity of plants to ROS stress.[27] Therefore, the conclusion in this research supports the interdependency of H_2O_2 and RIC7 in ABA-induced stomatal closure, which providing a new clue in the regulatory network of guard-cell signaling.

Materials and methods

Plant materials and growth conditions

The Arabidopsis used in this study was the Col-0 background, and *ric7-2* (SALK_136344) and *ric7-3* (SALK_150242) were T-DNA insertion mutants. Seedlings were grown in a greenhouse under long-day conditions (16-h-light/8-h-dark cycle), with a photon flux density of 150 μmol m^{-2} s^{-1} and a temperature of 18°C to 22°C. To check the expression levels of *RIC7* in the *ric7-2* and *ric7-3* mutants, total RNA from leaves of three- to four-week-old plants was isolated using TRIzol (Invitrogen), and cDNA was prepared using the PrimeScript RT reagent kit (Takara). The relative expression of the *RIC7* in the corresponding mutants was performed using SYBR Premix ExTaq (Takara). The primers used for qRT-PCR are F: 5'-GGACCGTCTGATAATGCCACTG-3' and R: 5'-TCTAGTCCGACCACCAAACTCT-3'. The qRT-PCR was conducted in a Real-Time PCR System (ABI PRISM 7500; Applied Biosystems). Each experiment was repeated three times. For determination of seed germination with ABA treatment, seeds were sterilized with 75% (v/v) ethanol for 5 min and then rinsed with 95% (v/v) ethanol for 30 s. Seeds on the one-half MS medium supplemented with 0, 1, 2, 3, or 5 μM ABA were kept at 4°C for 48 h in darkness, and then incubated in the plant growth chamber (Percival). After 5 days, the seed germination rates of wild type and *ric7* mutants were calculated.

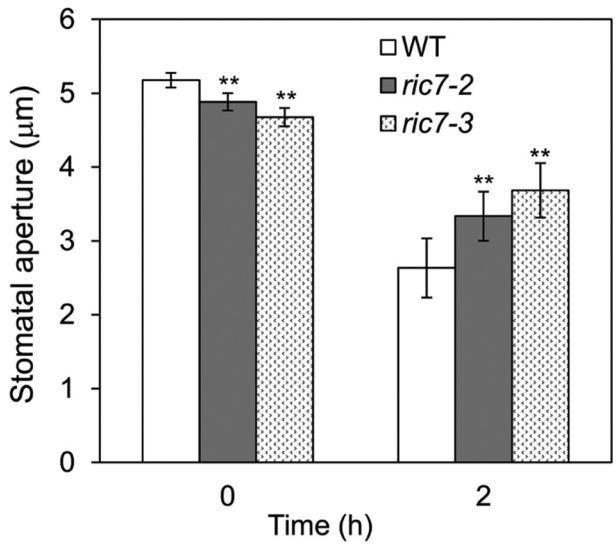

Figure 5. External applied H_2O_2 failed to induce stomatal closure in *ric7-2* and *ric7-3* mutants as in wild type (WT). (**, $p < .01$).

Stomatal aperture assays

Stomatal aperture assays were performed essentially as described in 14. In brief, the fully expanded rosette leaves of three- to four-week-old plants were collected and illuminated with white light (150 μmol m^{-2} s^{-1}) for 2 h, and then the epidermis was peeled and incubated in MES buffer (10 mM MES, 30 mM KCl, 0.1 mM $CaCl_2$, pH 6.1) containing 10 μM ABA or 10 μM H_2O_2 for 2 h. The stomatal apertures before and after ABA or H_2O_2 treatments were determined under a microscope. Fifty stomata were randomly selected for three independent replicates. The data are presented as the means ± SE (n = 150).

Detection of the H_2O_2 level in guard cells

H_2O_2 detection in guard cells was performed as described previously.[17] Abaxial epidermal strips were peeled from the leaves with open stomata and then were incubated in MES buffer containing 50 μM H_2DCF-DA (Molecular Probes) in the dark for 15 min, washed for three times. The epidermal strips were then transferred to MES buffer containing 10 μM ABA for 30 min. H_2O_2 levels in guard cells were detected by CLSM with a setting of 488 nm excitation and 525 nm emission. The experiments were repeated at least three times with 30 cells for each treatment.

Checking the expression level of ROS generation and scavenging related genes

To check the expression levels of ROS generation and scavenging-related genes without or with ABA treatment, fully expanded rosette leaves of three- to four-week-old plants of wild type, *ric7-2*, and *ric7-3* mutants were sprayed with 50 μM ABA. Half an hour later, the leaves were harvested and total RNA was isolated, and then cDNA was prepared using the PrimeScript RT reagent kit (Takara). Expression levels of the ROS generation and scavenging-related genes were detected

using SYBR Premix ExTaq (Takara). The primers used for qRT-PCR are *AtRBOHD*, F, 5'- AGCTTCACAATTATTGCACGAG-3', R, 5'- TCTCCAGTTAGGTTTAGCGAAG-3'; *AtRBOHF*, F, 5'-TATTGGAGACCATCTTGCTTGT-3', R, 5'-CGTTAAAACCGGTTAGTCGATC-3'; *PAO1*, F, 5'-GTGTCGGTGGTAAAGAGTCTAA-3', R, 5'- CTTTAACTTGAGAATCGCCGAG-3'; *PAO5*, F, 5'- TGCTGAATTGTTTAGTCCTCCT-3', R, 5'- TTCTTTGAGACATCTCGACGAA-3'; *CAT2*, F, 5'- TGGGGCCTTCCTTTTAAGTTAT-3', R, 5'-TGGGGCCTTCCTTTTAAGTTAT-3'; *CAT3*, F, 5'-CTTGTGGTTCCTGGAATCTACT-3', R, 5'-AGGATCAAACTTTGAGGGGTAG-3'.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the National Science Foundation of China (grant no. 32070191 to Y.-L.C.), and Natural Science Foundation of Hebei Province (grant no. C2019205168 to C.-G.Z.), and Scientific Research Foundation of Hebei Province for the Returned Overseas Chinese Scholars (to C.-G.Z.).

References

1. Geiger D, Scherzer S, Mumm P, Marten I, Ache P, Matschi S, Liese, A, Wellmann C, Al-Rasheid KAS, Grill E, et al. Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca $^{2+}$ affinities. Proc Natl Acad Sci USA. 2010;107(17):8023–8028. doi:10.1073/pnas.0912030107.
2. Hauser F, Waadt R, Schroeder JI. Evolution of abscisic acid synthesis and signaling mechanisms. Curr Biol. 2011;21(9):R346–355. doi:10.1016/j.cub.2011.03.015.
3. Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI. Mechanisms of abscisic acid-mediated control of stomatal aperture. Curr Opin Plant Biol. 2015;28:154–162. doi:10.1016/j.pbi.2015.10.010.
4. Wang P, Song CP. Guard-cell signalling for hydrogen peroxide and abscisic acid. New Phytol. 2008;178(4):703–718. doi:10.1111/j.1469-8137.2008.02431.x.
5. Lin D, Ren H, Fu Y. ROP GTPase-mediated auxin signaling regulates pavement cell interdigitation in Arabidopsis thaliana. J Integr Plant Biol. 2015;57(1):31–39. doi:10.1111/jipb.12281.
6. Gu Y, Vernoud V, Fu Y, Yang Z. ROP GTPase regulation of pollen tube growth through the dynamics of tip-localized F-actin. J Exp Bot. 2003;54(380):93–101. doi:10.1093/jxb/erg035.
7. Huang J-B, Liu H, Chen M, Li X, Wang M, Yang Y, Wang C, Huang J, Liu G, Liu Y, et al. ROP3 GTPase contributes to polar auxin transport and auxin responses and is important for embryogenesis and seedling growth in Arabidopsis. Plant Cell. 2014;26(9):3501–3518. doi:10.1105/tpc.114.127902.
8. Hwang J-U, Jeon BW, Hong D, Lee Y. Active ROP_2 GTPase inhibits ABA- and CO_2-induced stomatal closure. Plant Cell Environ. 2011;34(12):2172–2182. doi:10.1111/j.1365-3040.2011.02413.x.
9. Lemichez E, Wu Y, Sanchez JP, Mettouchi A, Mathur J, Chua NH. Inactivation of AtRac1 by abscisic acid is essential for stomatal closure. Genes Dev. 2001;15(14):1808–1816. doi:10.1101/gad.900401.
10. Li Z, Liu D. ROPGEF1 and ROPGEF4 are functional regulators of ROP11 GTPase in ABA-mediated stomatal closure in Arabidopsis. FEBS Lett. 2012;586(9):125–1253. doi:10.1016/j.febslet.2012.03.040.
11. Zheng Z-L, Nafisi M, Tam A, Li H, Crowell DN, Chary SN, Schroeder JI, Shen J, Yang Z. Plasma Membrane–associated ROP10 small GTPase is a specific negative regulator of abscisic acid responses in arabidopsis. Plant Cell. 2002;14(11):2787–2797. doi:10.1105/tpc.005611.
12. Jeon BW, Hwang J-U, Hwang Y, Song W-Y, Fu Y, Gu Y, Bao F, Cho D, Kwak JM, Yang Z, et al. The arabidopsis small G protein ROP2 is activated by light in guard cells and inhibits light-induced stomatal opening. Plant Cell. 2008;20(1):75–87. doi:10.1105/tpc.107.054544.
13. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI. NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in arabidopsis. Embo J. 2003;22(11):2623–2633. doi:doi:10.1093/emboj/cdg277
14. Wang W, Liu Z, Bao L-J, Zhang -S-S, Zhang C-G, Li X, Li H-X, Zhang X-L, Bones AM, Yang Z-B, et al. The RopGEF2-ROP7/ROP2 pathway activated by phyB suppresses red light-induced stomatal opening. Plant Physiol. 2017;174(2):717–731. doi:10.1104/pp.16.01727.
15. Li Y, Xu SS, Gao J, Pan S, Wang GX. Glucose- and mannose-induced stomatal closure is mediated by ROS production, Ca $^{2+}$ and water channel in Vicia faba. Physiologia Plantarum. 2016;156(3):252–261. doi:10.1111/ppl.12353.
16. Hong D, Jeon BW, Kim SY, Hwang J-U, Lee Y. The ROP2-RIC7 pathway negatively regulates light-induced stomatal opening by inhibiting exocyst subunit Exo70B1 in Arabidopsis. New Phytol. 2016;209(2):624–635. doi:10.1111/nph.13625.
17. Li X, Li J-H, Wang W, Chen N-Z, Ma T-S, Xi Y-N, Zhang X-L, Lin H-F, Bai Y, Huang S-J, et al. ARP2/3 complex-mediated actin dynamics is required for hydrogen peroxide-induced stomatal closure in Arabidopsis. Plant Cell Environ. 2014;37(7):1548–1560. doi:10.1111/pce.12259.
18. Sagor GHM, Zhang S, Kojima S, Simm S, Berberich T, Kusano T. Reducing cytoplasmic polyamine oxidase activity in arabidopsis increases salt and drought tolerance by reducing reactive oxygen species production and increasing defense gene expression. Front Plant Sci. 2016;7:214. doi:10.3389/fpls.2016.00214.
19. Sirichandra C, Gu D, Hu H, Davanture M, Lee S, Djaoui M, Valot B, Zivy M, Leung J, Merlot S, et al. Phosphorylation of the Arabidopsis AtrbohF NADPH oxidase by OST1 protein kinase. FEBS Lett. 2009;583(18):2982–2986. doi:10.1016/j.febslet.2009.08.033.
20. Wang H-Q, Sun L-P, Wang L-X, Fang X-W, Li Z-Q, Zhang -F-F, Hu X, Qi C, He JM. Ethylene mediates salicylic-acid-induced stomatal closure by controlling reactive oxygen species and nitric oxide production in Arabidopsis. Plant Sci. 2020;294:110464. doi:10.1016/j.plantsci.2020.110464.
21. Wang Z, Wang FX, Hong YC, Huang JR, Shi HZ, Zhu JK. Two chloroplast proteins suppress drought resistance by affecting ROS production in guard cells. Plant Physiol. 2016;172(4):2491–2503. doi:10.1104/pp.16.00889.
22. Murata Y, Pei Z-M, Mori IC, Schroeder J. Abscisic acid activation of plasma membrane Ca $^{2+}$ channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in abi1-1 and abi2-1 protein phosphatase 2C mutants. Plant Cell. 2001;13(11):2513–2523. doi:10.1105/tpc.010210.
23. Mustilli A-C, Merlot S, Vavasseur A, Fenzi F, Giraudat J. Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell. 2002;14(12):3089–3099. doi:10.1105/tpc.007906.
24. Shang Y, Dai C, Lee MM, Kwak JM, Nam KH. BRI1-associated receptor kinase 1 regulates guard cell ABA signaling mediated by Open Stomata 1 in Arabidopsis. Mol Plant. 2016;9(3):447–460. doi:10.1016/j.molp.2015.12.014.

25. Zhang W, Jeon BW, Assmann SM. Heterotrimeric G-protein regulation of ROS signalling and calcium currents in Arabidopsis guard cells. J Exp Bot. 2011;62(7):2371–2379. doi:10.1093/jxb/erq424.
26. Dang F, Lin J, Xue, B, Chen Y, Guan D, Wang Y, He S. *CaWRKY27* negatively regulates H_2O_2-mediated thermotolerance in pepper (*Capsicum annuum*). Front Plant Sci. 2018;9:1633. doi:10.3389/fpls.2018.01633.
27. Xu ZH, Mahmood K, Rothstein SJ. ROS induces anthocyanin production via late biosynthetic genes and anthocyanin deficiency confers the hypersensitivity to ROS-generating stresses in Arabidopsis. Plant and Cell Physiology. 2017;58(8):1364–1377. doi:10.1093/pcp/pcx073.