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SHORT COMMUNICATION

Leaf temperature responses to ABA and dead bacteria in wheat and Arabidopsis

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ABSTRACT

Stomatal densities, aperture openness and their responsiveness to environmental change determine plant water loss and regulate entry of pathogens. Stomatal responsiveness is usually assessed on restricted areas of leaves or isolated epidermal peels floated in solution. Analyzing these responses in the whole plant context could give valuable additional information, for example on the role of mesophyll in stomatal responses. We analyzed stomatal responses to the phytohormone abscisic acid (ABA) and pathogenic elicitors in intact plants by dynamic measurement of leaf temperature. We tested whether ABA-induced stomatal closure in wheat requires external nitrate and whether bacterial elicitor-induced stomatal closure can be detected by dynamic thermal imaging in intact Arabidopsis. We found that wheat was hypersensitive to all applied treatments, as even mock-treated leaves showed a strong increase in leaf temperature. Nevertheless, ABA activated stomatal closure in wheat independent of exogenous nitrate. Pathogenic elicitors triggered a fast and transient increase in leaf temperature in intact Arabidopsis, indicating short-term stomatal closure. The data suggest that the dynamics of pathogen-induced stomatal closure is different in whole plants compared to epidermal peels, where elicitor-induced stomatal closure persists longer. We propose that dynamic thermal imaging could be applied to address the effect of pathogenic elicitors on stomatal behavior in whole plants to complement detached sample assays and gain a better understanding of stomatal immunity.

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Stomata are microscopic pores in leaves that, when open, enable CO_2 uptake for photosynthesis but at the same time allow water loss via transpiration and provide sites of entry for pathogens. Plants perceive changes in abiotic and biotic environmental conditions and manage CO_2 uptake, water loss and pathogen stress by adjustment of stomatal aperture, or in the long term, development. The phytohormone abscisic acid (ABA) is a key messenger in environmental stimuli-induced stomatal closure,[1] whereas pathogens trigger stomatal closure[2] and suppress stomatal density in developing leaves.[3]

Stomatal responses to ABA and pathogenic elicitors, such as flagellin, lipopolysaccharides (LPS) or chitin, have mostly been studied in isolated epidermal peels or detached leaves.[2,4–6] While this approach is well established and widely used, stomata in detached samples do not always behave as in attached leaves. For example, by using dynamic thermal imaging of leaf temperature we recently showed that ABA does not trigger fast systemic stomatal closure in distal leaves in intact Arabidopsis,[7] unlike previously reported in isolated epidermal peels.[8] We also found that exogenous nitrate was not required for quick and strong ABA-induced stomatal closure in intact barley,[7] in contrast to other experiments with detached leaves.[9] Here, we tested whether stomata in another grass, wheat, also respond to ABA independent of exogenous nitrate. We further analyzed whether dynamic thermal imaging could be applied to study pathogenic elicitor-induced stomatal closure in Arabidopsis.

To study the effect of ABA and nitrate on wheat stomata, we carried out dynamic thermal imaging experiments as reported previously.[7] Briefly, we acclimated young wheat plants (11–17 d old) in pots placed horizontally on their side under ~150 µmol m^{-2} s^{-1} light at ~50% relative humidity (RH) for ~2 h and applied 5 µM ABA (in aqueous solution with 0.05% ethanol and 0.012% Silwet L-77, mock treatment was 0.05% ethanol with 0.012% Silwet L-77 in water) on wheat leaves by paintbrush. We measured leaf temperature with 1-min intervals before and 1.5 h after application of ABA. Temperature in hormone-treated leaves increased by ~2.2°C both in the absence and presence of 5 mM potassium nitrate indicating a reduction in stomatal water loss (Figure 1a, b). Thus, similar to barley,[7] exogenous nitrate was not required for efficient stomatal closure in wheat. However, wheat leaves were more sensitive than barley to any treatment: mock and nitrate treatments increased leaf temperature by ~0.5°C in barley,[7] but ~1.5°C in wheat leaves (Figure 1a, b). Thus, the difference between mock- and ABA-treatment at the end of the experiment was not statistically significant (Figure 1b). Higher sensitivity of wheat stomata compared to those of barley has been observed before in experiments addressing darkness-induced stomatal closure and stomatal responses to simultaneously applied darkness and low CO_2 levels.[10] However, the difference between increases in leaf temperature in response to ABA and mock treatments was similar between barley[7] and wheat (Figure 1b), suggesting a similar ABA-specific response in the two grasses.

We also asked whether dynamic thermal imaging could be applied to analyze stomatal responses to other stimuli than ABA. We tested the effect of pathogenic elicitors on leaf temperature in

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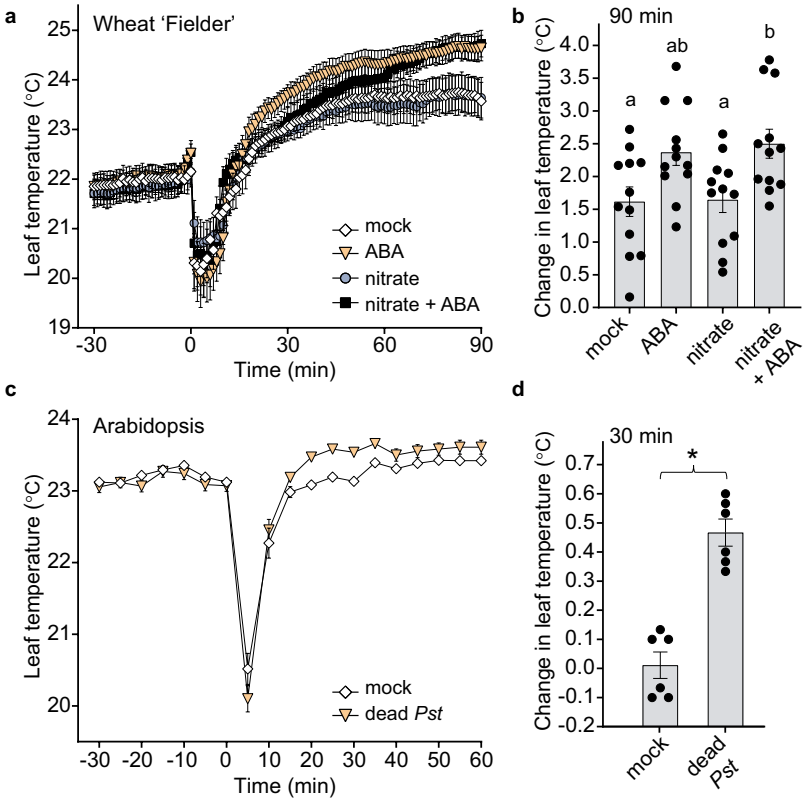

Figure 1. Leaf temperature responses to ABA and dead bacteria. (A) Response of leaf temperature to 5 µM ABA with or without 5 mM nitrate in wheat and (B) respective temperature change by 1.5 h after treatment. (C) Response of leaf temperature to dead *Pseudomonas syringae* (*Pst*) in Arabidopsis and (D) respective temperature change by 30 min after treatment. Mean ± SEM is shown in all panels, $n = 12$ plants in (A) and (B), $n = 6$ plants in (C) and (D), letters or star denote statistically significant differences between groups (one-way ANOVA with Tukey *post hoc* test in (B) and Student's *t*-test in (D)) and dots show individual data points (plants) in (B) and (D).

Arabidopsis, because an approach to study pathogen-induced stomatal closure in the context of an intact plant would be valuable for the field of stomatal immunity. We acclimated Arabidopsis plants under ~150 µmol m^{-2} s^{-1} light at ~50% RH for ~2 h. Then we resuspended the debris from autoclaved stationary phase culture of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) in water to an $OD_{600} = 0.2$, added Silwet L-77 to 0.012% and sprayed the acclimated plants with the suspension or with a mock solution of water with 0.012% Silwet L-77. We monitored leaf temperature before and after treatment with 5-min intervals. Dead bacteria caused a fast increase of ~0.5°C in leaf temperature 20–30 min after treatment (Figure 1c, d), indicating pathogenic elicitor-induced stomatal closure. Leaf temperature increase triggered by dead pathogens was weaker than that caused by ABA.[7] Stomata also appeared to reopen rapidly, as 1 h after treatment there was only a minor difference in leaf temperature between *Pst*- and mock-treated leaves (Figure 1c), whereas ABA-induced stomatal closure persisted at least for an hour.[7]

In nearly all studies, the effect of pathogenic elicitors on stomatal aperture is studied in isolated leaves or epidermal peels floated in solution with either bacteria or elicitors such as flagellin, LPS or chitin. In these assays, pathogen- and elicitor-induced stomatal closure is usually measured 1–2 h after treatment, whereas coronatine-producing strains lead to stomatal reopening 3–4 h after treatment.[2,11,12] In our experiments with intact plants, bacteria were killed by autoclaving before application to the plant surface and hence the fast reopening of stomata starting ~30-40 min after treatment could not be caused by coronatine. Instead, it may have been due to attenuation of elicitor-induced signaling. For example, upon perception of its ligand, the flagellin receptor FLAGELLIN-SENSITIVE 2 (FLS2) is internalized and sent for degradation within 20–40 min,[13] likely to avoid over-stimulation of immune responses in the continued presence of the signal. Similar mechanisms may underlie the transient increase in leaf temperature caused by pathogenic elicitors present in the suspension of dead bacteria (Figure 1c).

Our results point to important differences between the behavior of stomata depending on whether pathogenic elicitors are perceived from adaxial leaf surface of attached leaves or abaxial or inner surfaces in detached leaves or epidermal peels floated in solution. Longer persistence of stomatal closure in detached samples may be due to constitutive presence of large amounts of signal that reaches stomata, whereas surface-applied bacterial debris may be less effective in reaching receptors in guard cells. Possibly, pathogens on leaf surface are not sufficient to trigger efficient stomatal closure, whereas microbes that have entered the plant via stomata or wounds can be better perceived by plant immune system. Our experiments suggest that pathogenic elicitors-induced stomatal closure can be recorded in real time in intact plants by following leaf temperature. In the future, a similar approach could potentially be used to study the effects of pure elicitors, such as flagellin, LPS and chitin, on stomatal closure in a whole plant context.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Merilo E, Laanemets K, Hu H, Xue S, Jakobson L, Tulva I, Gonzalez-Guzman M, Rodriguez PL, Schroeder JI, Broschè M, et al. PYR/RCAR receptors contribute to ozone-, reduced air humidity-, darkness-, and CO_2-induced stomatal regulation. Plant Physiol. 2013;162:1652–1668. doi:10.1104/pp.113.220608.
2. Melotto M, Underwood W, Koczan J, Nomura K, He SY. Plant stomata function in innate immunity against bacterial invasion. Cell. 2006;126:969–980. doi:10.1016/j.cell.2006.06.054.
3. Dutton C, Hõrak H, Hepworth C, Mitchell A, Ton J, Hunt L, Gray JE. Bacterial infection systemically suppresses stomatal density. Plant Cell Environ. 2019;42:2411–2421. doi:10.1111/pce.13570.
4. McAinsh MR, Brownlee C, Hetherington AM, Mansfield TA. Partial inhibition of ABA-induced stomatal closure by calcium-channel blockers. Proc R Soc Lond B Biol Sci. 1991;243:195–201.
5. Kuhn JM, Boisson-Dernier A, Dizon MB, Maktabi MH, Schroeder JI. The protein phosphatase *AtPP2CA* negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of *abh1* on *AtPP2CA* mRNA. Plant Physiol. 2006;140:127–139. doi:10.1104/pp.105.070318.
6. Liu Y, Maierhofer T, Rybak K, Sklenar J, Breakspear A, Johnston MG, Fliegmann J, Huang S, Roelfsema MRG, Felix G, et al. Anion channel SLAH3 is a regulatory target of chitin receptor-associated kinase PBL27 in microbial stomatal closure. eLife [Internet]. 2019;8. cited 2020 Jan 24. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6776436/
7. Hõrak H, Fountain L, Dunn JA, Landymore J, Gray JE. Dynamic thermal imaging confirms local but not fast systemic ABA responses. Plant Cell Environ. 2021;44:885–899. doi:10.1111/pce.13973.
8. Devireddy AR, Zandalinas SI, Gómez-Cadenas A, Blumwald E, Mittler R. Coordinating the overall stomatal response of plants: rapid leaf-to-leaf communication during light stress. Sci Signal. 2018;11:eaam9514. doi:10.1126/scisignal.aam9514.
9. Schäfer N, Maierhofer T, Herrmann J, Jørgensen ME, Lind C, Von Meyer K, Lautner S, Fromm J, Felder M, Hetherington AM, et al. A tandem amino acid residue motif in guard cell SLAC1 anion channel of grasses allows for the control of stomatal aperture by nitrate. Curr Biol. 2018;28:1370–1379.e5. doi:10.1016/j.cub.2018.03.027.
10. Merilo E, Jõesaar I, Brosché M, Kollist H. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. New Phytol. 2014;202:499–508. doi:10.1111/nph.12667.
11. Lim CW, Luan S, Lee SC, Prominent A. Role for RCAR3-mediated ABA signaling in response to *Pseudomonas syringae* pv. *tomato* DC3000 Infection in Arabidopsis. Plant Cell Physiol. 2014;55:1691–1703. doi:10.1093/pcp/pcu100.
12. Du M, Zhai Q, Deng L, Li S, Li H, Yan L, Huang Z, Wang B, Jiang H, Huang T, et al. Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack. Plant Cell. 2014;26:3167–3184. doi:10.1105/tpc.114.128272.
13. Robatzek S, Chinchilla D, Boller T. Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. Genes Dev. 2006;20:537–542. doi:10.1101/gad.366506.