

Accumulation of endogenous salicylic acid confers drought tolerance to *Arabidopsis*

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Abbreviations: SA, salicylic acid; ROS, reactive oxygen species; ; SUMO, small ubiquitin-related modifier; SHAM, salicylhydroxamic acid; DPI, diphenyleioidonium chloride

We investigated stomatal phenotype and drought tolerance of *Arabidopsis* salicylic acid-accumulating mutants, *acd6* and *cpr5*. In these mutants, the light-induced stomatal opening was impaired and the impairment of stomatal opening was restored by peroxidase inhibitors, salicylhydroxamic acid and azide. The *acd6* and *cpr5* mutant plants were more tolerant to drought stress than wild-type plants. Introduction of *nahG* gene into the *acd6* and *cpr5* mutants prevented the inhibition of stomatal opening and reduced the drought tolerance. Drought tolerance-related genes were more highly expressed in the *cpr5* and *acd6* mutant plants than in the wild-type plants. These results suggest that accumulation of salicylic acid improves drought tolerance through inhibition of light-induced stomatal opening in *Arabidopsis*.

Stomatal pores are formed by pairs of guard cells and mediate transpiration and carbon dioxide uptake. Stomatal movements modulate the rate of transpirational water loss, which is closely involved in drought tolerance of plants.¹

Arabidopsis siz1 mutant, which is impaired in the SIZ-type small ubiquitin-related modifier (SUMO) E3 ligase, accumulates more salicylic acid (SA) than *Arabidopsis* wild-type plants (Col-0)² and *SIZ1* is involved in regulation of abiotic stress responses.³⁻⁵ Reactive oxygen species (ROS) levels in *siz1* guard cells are higher than those in the wild-type guard cells and light-induced stomatal opening was inhibited by the *siz1* mutation, reducing water loss and enhancing drought tolerance.⁶ These phenotypes are suppressed by introduction of *nahG* gene, which encodes a salicylate hydroxylase that catalyzes the conversion of SA to catechol, into the *siz1* mutant. Peroxidase inhibitors, salicylhydroxamic acid (SHAM) and azide, suppressed the inhibition of light-induced stomatal opening in the *siz1* mutant, suggesting that ROS production mediated by peroxidases is involved in the inhibition of stomatal opening. In other SA-accumulating mutants, *cpr5* and *acd6*, ROS was accumulated in guard cells, light-induced stomatal opening was impaired, and drought tolerance was enhanced.⁶ These results suggest that SA accumulation induces ROS accumulation, resulting in inhibition of light-induced stomatal opening. However, it is to be clarified whether transpirational water loss is suppressed in the *cpr5* and *acd6* mutants and whether ROS production mediated by peroxidases is involved in the inhibition of light-induced stomatal opening in the *cpr5* and *acd6* mutants. In this study, the water loss from detached rosette leaves of the *cpr5* and *acd6* mutants and

the effects of SHAM and azide on the inhibition of light-induced stomatal opening in the *cpr5* and *acd6* mutants were examined.

In order to examine whether ROS production mediated by peroxidases is involved in the inhibition of light-induced stomatal opening of *cpr5* and *acd6* mutants, SHAM and azide were used as inhibitors for peroxidase. Stomatal opening assay was performed as described previously.⁶ Our previous result demonstrated that the inhibition of light-induced stomatal opening in *siz1* mutants was suppressed by SHAM and azide but not by an inhibitor of NADPH oxidase, diphenyleioidonium chloride (DPI). In agreement with our previous result, the inhibition of light-induced stomatal opening in the *cpr5* and *acd6* mutants was suppressed by SHAM and azide but not by DPI (Fig. 1). These results suggest that ROS production mediated by peroxidases is involved in the inhibition of light-induced stomatal opening of *cpr5* and *acd6* mutants. Together with the previous study,⁶ these results suggest that SA accumulation induces ROS production mediated by peroxidases, resulting in inhibition of light-induced stomatal opening. Water loss analysis of the detached leaves of *cpr5* and *acd6* mutants was performed as described previously.⁶ Water loss from rosette leaves of the *cpr5* and *acd6* mutants was slower than that of the wild-type plants (Fig. 2). Introduction of *nahG* gene into *cpr5* and *acd6* mutants increased the rate of water loss (Fig. 2), which is consistent with the stomatal phenotypes of *cpr5* and *acd6* mutants (Fig. 1). Moreover, the water-loss phenotype of *cpr5* and *acd6* mutants is in agreement with that of another SA-accumulating mutant, *siz1*.⁶

Transcription of drought-responsive genes, which encodes RD29A, β -glucosidase, RNA-binding, ribonuclease, dehydrin

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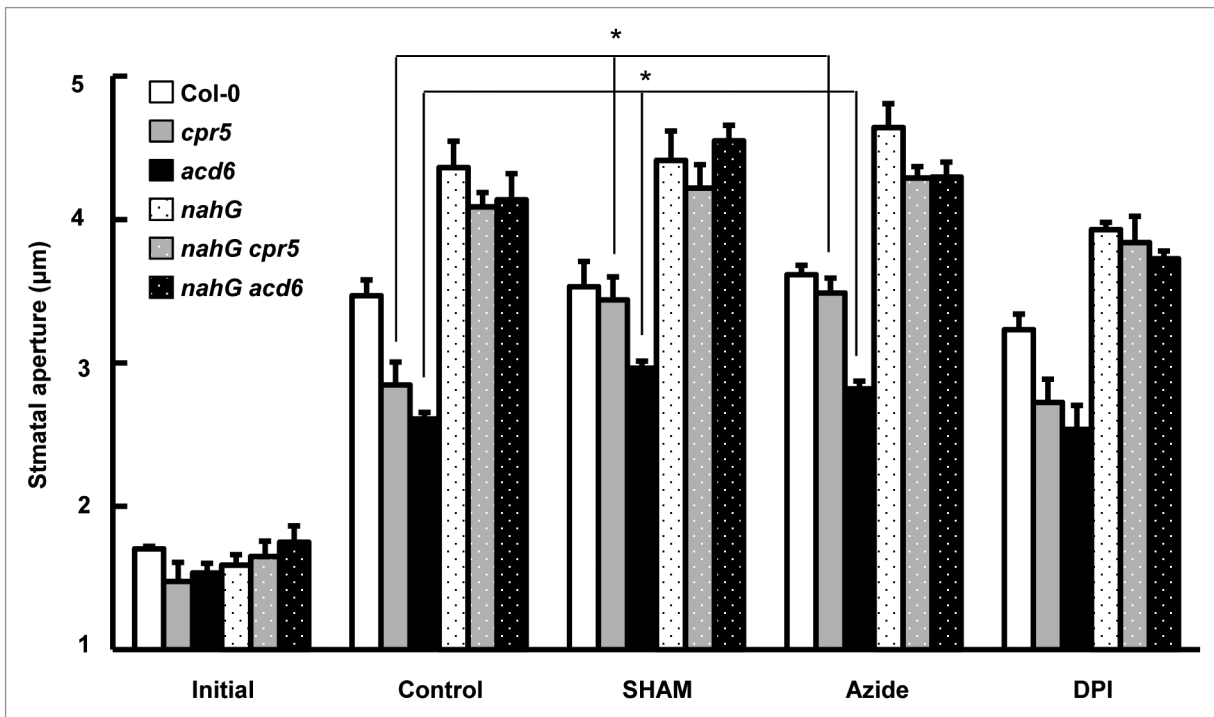


Figure 1. Peroxidases are involved in the inhibition of the stomatal opening of *cpr5* and *acd6* mutants. Four- to 6-week-old leaves were floated on buffer solution, containing 50 mM KCl, 50 μ M CaCl₂, 10 mM MES-Tris, pH 6.15. The stomatal aperture was measured after 2 h of incubation in the dark condition (Initial) and after 2 h of incubation in the dark condition and 3 h of incubation under light (Control). SHAM (2 mM), azide (1 μ M), or DPI (20 μ M) was added to the assay solution 30 min before light condition. Values are means \pm SE ($n \geq 3$). * indicates a significant difference from Control ($P < 0.05$), which is determined by the Student *t*-test.

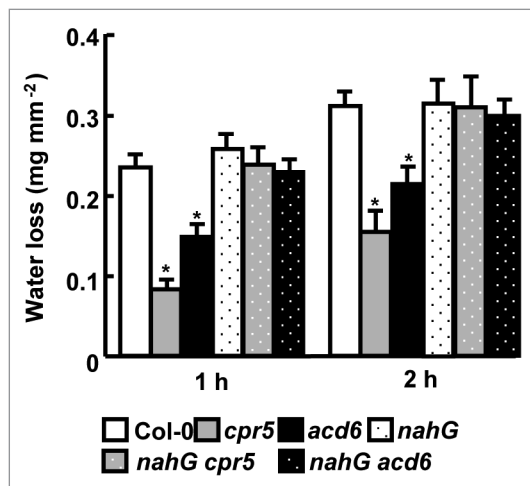


Figure 2. The *cpr5* and *acd6* mutants exhibited drought tolerance. The rosette leaves of 3-week-old plants were detached and were weighted at 0 h, 1 h, and 2 h. Water loss (mg mm⁻²) was calculated as fresh weight loss (mg) divided by leaf area (mm²). Leaf area was measured by ImageJ software. Data are means \pm SE ($n = 10$). * indicates a significant difference from Col-0 ($P < 0.05$), which is determined by the Student *t*-test.

LTI30, unknown protein (At5g61820), and alternative oxidase, was upregulated in the *cpr5* and *acd6* mutants under non-stress condition (Fig. 3). Transcription levels of these genes in *nahG cpr5* and *nahG acd6* mutants were lower than those in the *cpr5*

and *acd6* mutants (Fig. 3), suggesting that expression of these genes are upregulated by SA accumulation. Dehydrins play an important role in response to drought stresses.⁷ Therefore, these drought-response genes could be involved in the inhibition of light-induced stomatal opening by SA accumulation in the *cpr5* and *acd6* mutants.

This study demonstrates that endogenous SA accumulation improved drought tolerance accompanied by ROS production mediated by peroxidases and inhibited light-induced stomatal opening in *Arabidopsis* (Figs. 1 and 2). Moreover, accumulation of SA is induced by water deficiency in *Phillyrea angustifolia*⁸ and application of SA enhances drought tolerance of tomato and bean.⁹ *Arabidopsis adr1* and *myb96-1d* mutants, which accumulate SA, exhibit SA-dependent drought tolerance.¹⁰⁻¹³ These results indicate that SA accumulation plays a role in drought tolerance.¹⁴ Stomatal closure promoted by SA is also one of strategy for preventing bacterial entry.^{15,16} Because the *siz1*, *cpr5*, and *acd6* mutants highly accumulate SA,¹⁵⁻¹⁷ the inhibition of light-induced stomatal opening in these mutants is likely to contribute to blocking pathogen invasion as well as drought tolerance.

Together with our previous study,⁶ we concluded that SA accumulation induced ROS production mediated by peroxidases, inhibiting light-induced stomatal opening, which confers drought tolerance on *Arabidopsis*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

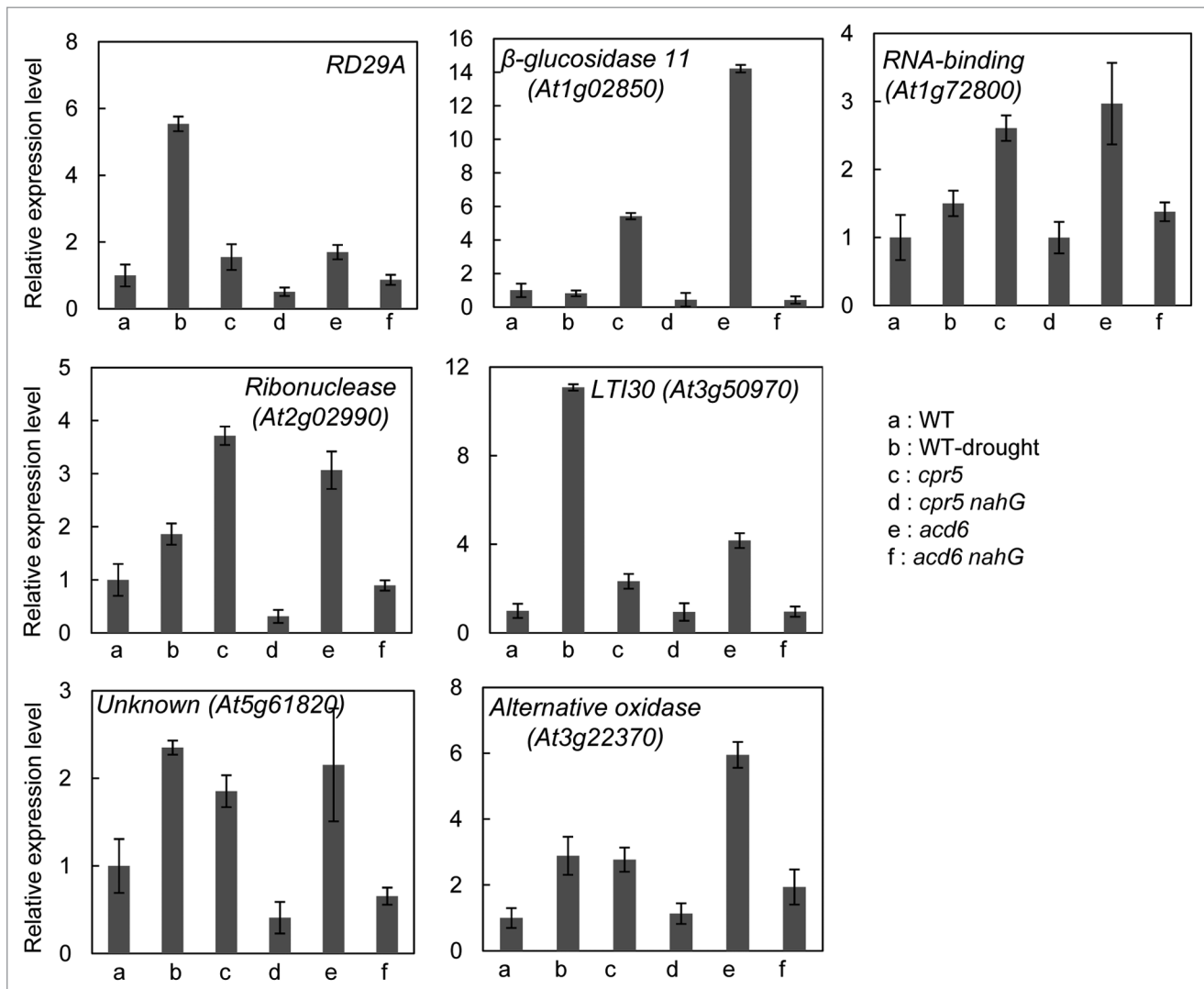


Figure 3. Drought-responsive genes are induced by the *cpr5* and *acd6* mutation. The relative expression level of these genes was measured by quantitative RT-PCR. *RD29A* expression was examined as a marker for drought stress. Three-week-old Col-0 (a), *cpr5* (c), *cpr5 nahG* (d), *acd6* (e), *acd6 nahG* (f) plants, grown under normal conditions, and Col-0 plants 1 h after detached (b), were harvested. Data are means \pm SD.

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