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AtNOA1 modulates nitric oxide accumulation and stomatal closure induced by salicylic acid in Arabidopsis

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Phytohormone salicylic acid (SA) has been documented to induce nitric oxide (NO) generation and stomatal closure in plants. However, the cellular components mediating these processes are limited. Here, we report that NO synthesis in guard cells and stomatal closure are markedly induced by SA in Arabidopsis wild type plants, whereas these effects caused by SA are suppressed significantly in *noa1* T-DNA mutant plants. These results suggest that AtNOA1 regulates SA-triggered NO accumulation and stomatal closure in Arabidopsis.

Key words: AtNOA1, nitric oxide, salicylic acid, stomatal closure, Arabidopsis

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Salicylic acid (SA) has been proposed to play important roles in many biological processes including pathogen defense, acclimation to abiotic stresses, growth and development in plants.[1-3] It has also been addressed to induce stomatal closure. Ca^{2+} and reactive oxygen species are involved in the process.[4-6]

It has been well documented that signal molecule nitric oxide (NO) mediates a great number of plant responses to phytohormone, biotic and abiotic stresses as well as developmental signals.[7] Besides, NO is also involved in ABA-, methyl jasmonate- or elicitor-induced stomatal closure in plants[8] although Lozano-Juste and León[9] recently reported that NO may not contribute to ABA-stimulated stomatal closure in Arabidopsis. NO synthase (NOS) and nitrate reductase (NR) are considered to be responsible for the NO generation and stomatal closure described above.[8] In addition, evidence indicated that NO is implicated in SA-promoted stomatal closure in *Vicia faba* and Arabidopsis.[10,11] NR and NOS are also required for the production of NO induced by SA in guard cells, and both genes *Nia1* and *Nia2* encoding NR in Arabidopsis are involved in this process.[10,11]

Up to now, no gene encoding NOS has been found in plants. Guo et al.[12] reported that AtNOS1 was involved in ABA-elicited NO accumulation and stomatal closure in Arabidopsis. AtNOS1 was initially considered as NOS contributing to NO production. However, subsequent investigations revealed that it has no NOS activity. The AtNOS1 was therefore suggested to be renamed as AtNOA1 for NO associated 1.[8,13] Recently, several lines of evidence indicated that AtNOA1 is a GTPase and mainly responsible for the posttranscriptional regulation of plastid-localized methylerythritol phosphate pathway enzyme.[14,15] Nevertheless, AtNOA1 indeed modulates NO accumulation in plants, possibly via an indirect way.[8,9] The detailed mechanisms need to be thoroughly investigated.

By using null mutant *noa1*, Zottini et al.[16] found that SA stimulates NO synthesis via activation of the AtNOA1 in roots and cultured cells in Arabidopsis. However, at present whether AtNOA1 modulates SA-induced NO accumulation and stomatal closure is unclear. We therefore investigated the action of AtNOA1 in stomatal closure triggered by SA.

AtNOA1 Regulates SA-Induced NO Production

Our previous results have shown that SA stimulated NO synthesis in guard cells, and the generated NO is required for

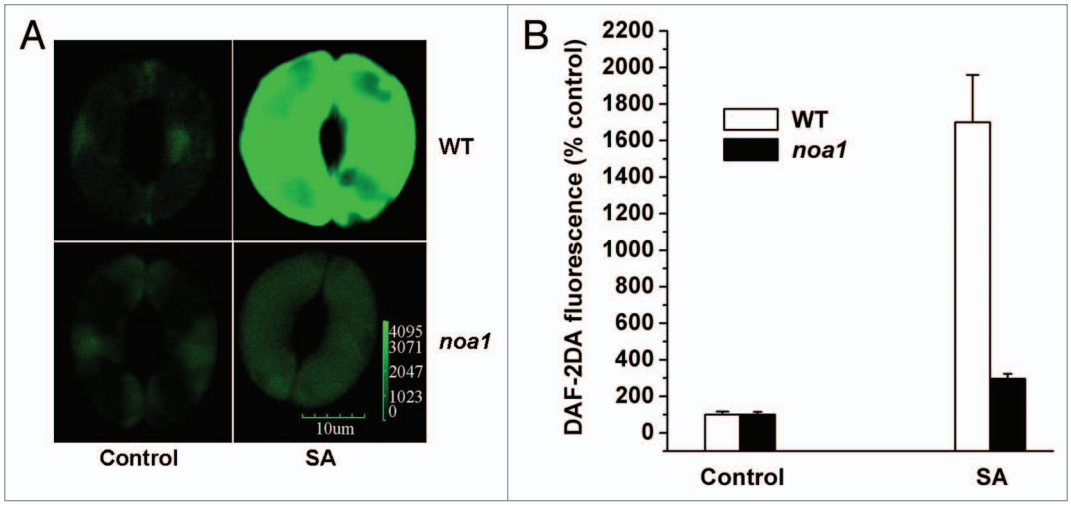

Figure 1. Suppression of SA-induced NO generation in *noa1* plants. (A) Confocal images showing NO synthesis in guard cells of WT and *noa1* plants treated by 100 μM SA for 15 min. (B) NO generation in guard cells from WT and *noa1* was monitored by DAF-2DA fluorescence in the absence or presence 100 μM SA. Fluorescence intensities were examined 15 min after treatments without or with SA.

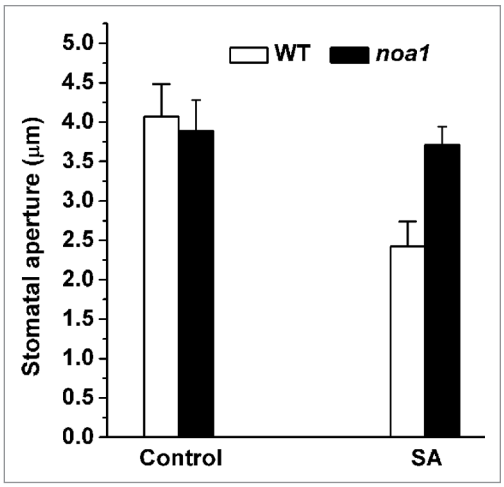

Figure 2. Effects of SA on stomatal aperture in WT and *noa1* plants. Abaxial epidermal peels were in buffer under light for 2.5 h to open the stomata, and then were treated with buffer (Control) or 100 μM SA (SA) for another 1 h. The stomatal aperture was determined. Values are the means of 150 measurements ± SE.

SA-promoted stomatal closure in wild type Arabidopis plants.[11] In this report, we observed that exogenous SA treatments markedly increased the intensity of the NO-specific fluorescent probe diaminofluorescein diacetate (DAF-2DA) in guard cells of WT plants. However, the effect caused by SA was significantly inhibited in null mutant *noa1* plants (CS6511, obtained from the Nottingham Arabidopsis Stock Centre, Nottingham, UK). The fluorescent intensity in WT is more than 5 folds of that in *noa1* (**Fig. 1**). The results imply that AtNOA1 regulates NO production in guard cells.

AtNOA1 Mediates SA-Induced Stomatal Closure

Responses of guard cells to SA were studied in WT and *noa1* plants. As shown in **Figure 2**, treatments with 100 μM SA resulted in pronounced decrease in the stomatal aperture of the epidermal peels in WT plants. However, the stomatal aperture from *noa1* plants had not significant change under the same treatment conditions. Moreover, the change pattern of stomatal aperture coincided with that of NO fluorescence intensity stimulated by SA in guard cells from WT and the mutant plants. These results suggest that NOA1 is involved in SA-induced NO accumulation and stomatal closure in Arabidopsis. These findings are also consistent with the results obtained from roots and cultured cells in Arabidopsis,[16] implying that NOA1 may play important roles in regulation of SA signaling in plants.

Acknowledgements

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