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Arabidopsis Tóxicos en Levadura 78 (*AtATL78*) mediates ABA-dependent ROS signaling in response to drought stress☆

Ji Yeon Suh [a], Soo Jin Kim [a, 1], Tae Rin Oh [a], Seok Keun Cho [a], Seong Wook Yang [a, b, *], Woo Taek Kim [a, **]

[a] Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea
[b] Section of Plant Biochemistry, Department of Plant and Environmental Sciences, Faculty of Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark

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ABSTRACT

Plants have developed a variety of complicated responses to cope with drought, one of the most challenging environmental stresses. As a quick response, plants rapidly inhibit stomatal opening under the control of abscisic acid (ABA) signaling pathway, in order to preserve water. Here, we report that *Arabidopsis Tóxicos en Levadura* (*ATL*), a RING-type E3 ubiquitin ligase, mediates the ABA-dependent stomatal closure. In contrast to wild-type plants, the stomatal closure was fully impaired in *atatl78* mutant plants even in the presence of exogenous ABA and reactive oxygen species (ROS). Besides, under high concentrations of Ca^{2+}, a down-stream signaling molecule of ABA signaling pathway, *atatl78* mutant plants successfully closed the pores. Furthermore, AtATL78 protein indirectly associated with catalases and the deficiency of *AtATL78* led the reduction of catalase activity and H_2O_2, implying the function of AtATL78 in the modulation of ROS activity. Based on these results, we suggest that *AtATL78* possibly plays a role in promoting ROS-mediated ABA signaling pathway during drought stress.

1. Introduction

Ubiquitin (Ub)-mediated post-translational modifications of proteins occur in diverse cellular processes including environmental stress responses [1–3]. Three enzymes, E1 activating, E2 Ub-conjugating and E3 ligase, coordinately facilitate the tethering of poly-ubiquitins to a target protein for its subsequent degradation. In the process, known as the Ubiquitin-Proteasome System (UPS), E3 ligases majorly play roles in target recognition and tagging poly-ubiquitins [4]. Among single-subunit E3 ligases, the RING-type E3 ligases cover a large proportion - around 477 RING E3 ligase proteins - of defined E3 ligases, such as the HECT-type, RING-type, and U-box-type E3 ligases in *Arabidopsis thaliana* [5]. Many studies recently have reported that several RING-type E3 ligases are involved in abiotic and biotic stress responses of plants [4]. *Arabidopsis Tóxicos en Levadura* (*ATL*) gene family is composed of at least 80 gene members that belong to the RING-type E3 ligases [6]. Recently, we reported that *AtATL78* is a negative regulator of the cold stress response and inversely, functions as a positive regulator of drought stress response in *Arabidopsis* [7]. We further demonstrated that the deficiency of *AtATL78* results in a dramatic reduction of hydrogen peroxide (H_2O_2). As a consequence of H_2O_2 reduction, the expressions of abscisic acid (ABA)-dependent genes, such as *RD22*, *RD29*, and *RAB18*, were notably decreased in *atatl78* mutant plants [7]. These results implied that *AtATL78* may be a functional E3 ligase for the immediate transmission of ABA signals to down-stream genes in response to water deficit.

ABA is an essential hormone that directly regulates the stomatal closure in concert with other hormones such as jasmonates, ethylene, auxins and cytokinins and thus prevents water loss [8]. In

Abbreviations: ATL, Arabidopsis Tóxicos en Levadura; UPS, Ubiquitin-Proteasome System; ROS, Reactive oxygen species; ABA, Abscisic acids.

☆ The nucleotide sequence data reported here has been deposited in the GenBank database under accession number NM_103813 (*AtATL78*).

* Corresponding author. Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea.
** Corresponding author. Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea.
E-mail addresses: swyang@plen.ku.dk (S.W. Yang), wtkim@yonsei.ac.kr (W.T. Kim).
[1] Current address: Department of Radiation Biology, Korea Atomic Energy Research Institute, Daejon 305-600, Republic of Korea.

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the stomatal closure process, inositol-1,4,5-triphosphate (IP_3) seems to mediate ABA signals to activate Ca^{2+}-permeable channel in plasma membrane (PM) that triggers an influx Ca^{2+} into cytoplasm of guard cells and subsequently depolarizes PM [8,9]. In addition to the IP_3-mediated signaling, recent studies reported that H_2O_2, a type of reactive oxygen species, seems to transiently relay the ABA-dependent activation of Ca^{2+}- permeable channel as a second messenger [10]. Within the ABA signaling pathway, NAD(P)H oxidase encoded by two genes – *A. thaliana respiratory burst oxidase homolog D and F* (*AtrbohD and F*) – generates H_2O_2 in the guard cells. Mutant plants of the genes are impaired in stomatal closure in response to ABA [11,12]. Moreover, a recent study reported that *GUARD CELL HYDROGEN PEROXIDE-RESISTANT1* (*GHR1*), which encodes a receptor-like kinase, modulates H_2O_2-mediated ABA signaling for stomatal closure, suggesting the important role of H_2O_2 in the control of stomatal aperture [13]. However, the detailed processes of H_2O_2-signaling in Ca^{2+} mediated stomatal closure are not yet fully understood.

In this study, in order to elucidate the H_2O_2 –mediated signaling, we further investigated *AtATL78* gene whether it is a new components for H_2O_2-mediated ABA signaling under drought stress.

2. Materials and methods

2.1. Plant materials and growth conditions

A. thaliana ecotype Columbia (Col-0) plants and *atatl78* (SALK_058308) T-DNA insertion mutant allele were used for all the experiments. *35S:RNAi-AtATL78* knock-down plants used in this paper were generated by Kim and Kim [7]. The plants were grown in a growth chamber at 22 °C under long-day conditions (16 h light/8 h dark) for 4 weeks before stress treatments.

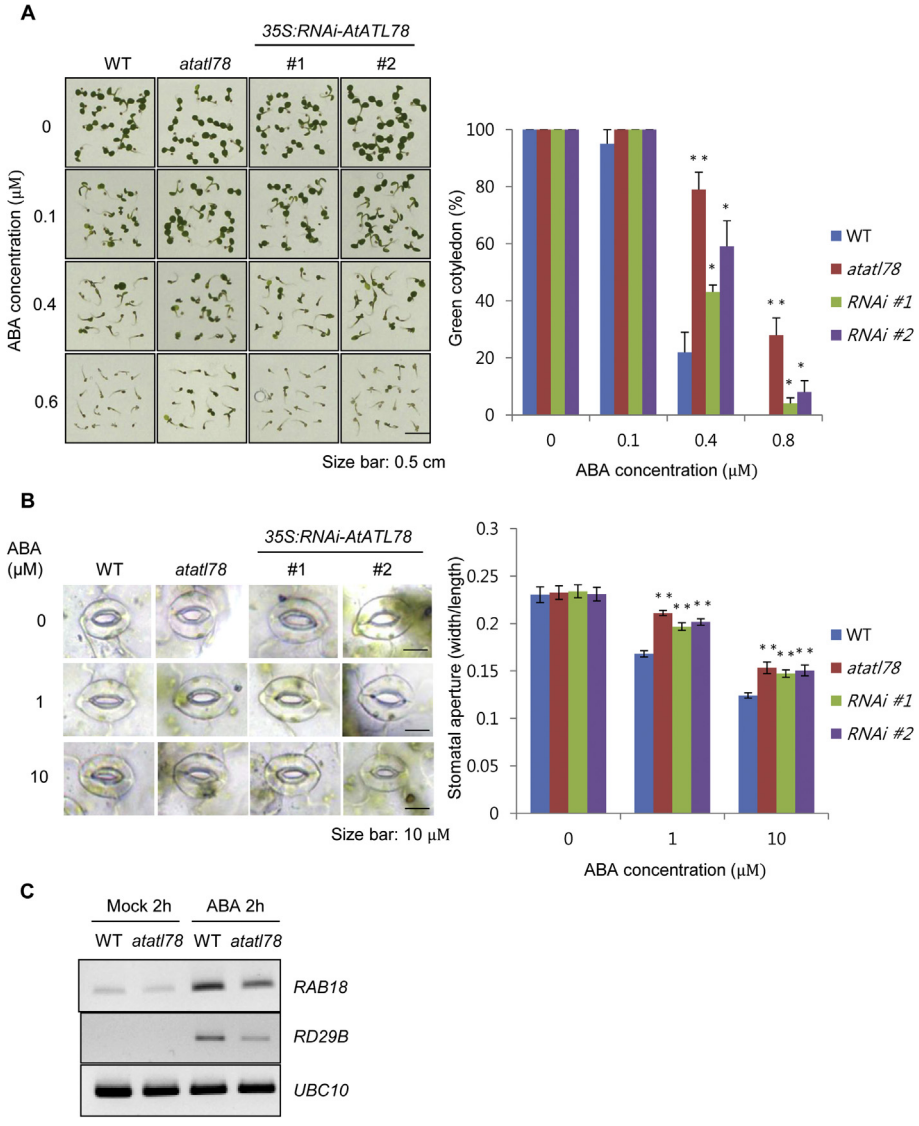

Fig. 1. Phenotypic analysis of wild-type (WT), *atatl78* mutant and *35S:RNAi-AtATL78* plants in response to ABA. (A) ABA sensitivity of wild-type, *atatl78* and *35S:RNAi-AtATL78* plants in the germination stage. Average and SD values were determined from three biological replicates (n = 25). Bar = 0.5 cm. Error bars represent SD (n > 70; *P < 0.05, **P < 0.005, Student's t test). (B) Stomatal movement profiles of wild-type, *atatl78* mutant and *35S:RNAi-AtATL78* plants. Rosette leaves were incubated with ABA (0, 1.0, and 10 μM) for 2 h and 30 stomatal apertures in epidermal peels were measured per replicate. Three replicates were performed for each experiment. Bars = 10 μm. Error bars represent SD (n > 90; *P < 0.05, **P < 0.005, Student's t test). (C) RT-PCR analysis of ABA-induced marker genes (*RAB18* and *RD29B*) in wild-type and *atatl78* plants. Ubiquitin Conjugating Enzyme10 (UBC10) was used as a loading control.