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ORIGINAL PAPER

Loss-of-Function Mutation of *ACTIN-RELATED PROTEIN 6* (*ARP6*) Impairs Root Growth in Response to Salinity Stress

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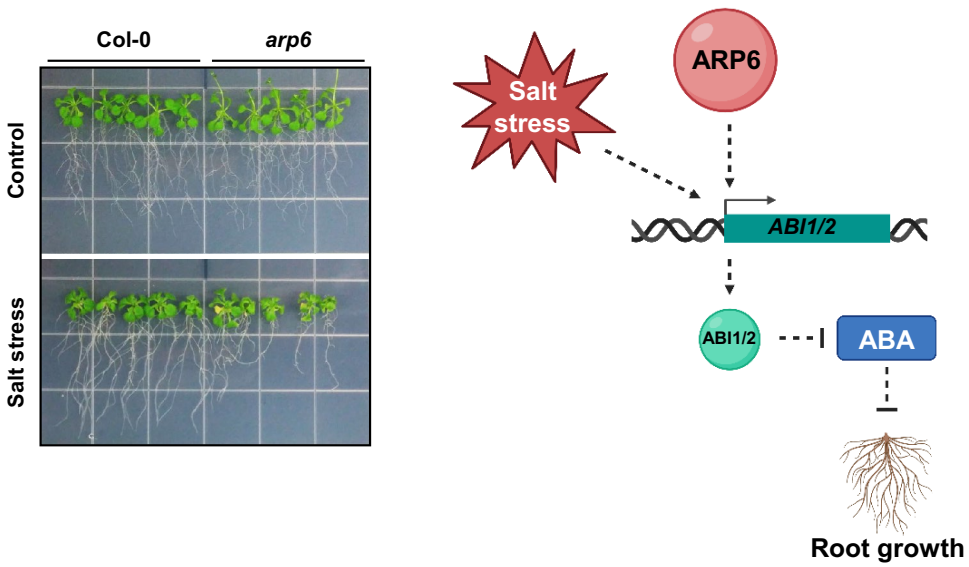
Abstract

H2A.Z-containing nucleosomes have been found to function in various developmental programs in Arabidopsis (e.g., floral transition, warm ambient temperature, and drought stress responses). The SWI2/SNF2-Related 1 Chromatin Remodeling (SWR1) complex is known to control the deposition of H2A.Z, and it has been unraveled that ACTIN-RELATED PROTEIN 6 (ARP6) is one component of this SWR1 complex. Previous studies showed that the *arp6* mutant exhibited some distinguished phenotypes such as early flowering, leaf serration, elongated hypocotyl, and reduced seed germination rate in response to osmotic stress. In this study, we aimed to investigate the changes of *arp6* mutant when the plants were grown in salt stress condition. The phenotypic observation showed that the *arp6* mutant was more sensitive to salt stress than the wild type. Upon salt stress condition, this mutant exhibited attenuated root phenotypes such as shorter primary root length and fewer lateral root numbers. The transcript levels of stress-responsive genes, *ABA INSENSITIVE 1* (*ABI1*) and *ABI2*, were found to be impaired in the *arp6* mutant in comparison with wild-type plants in response to salt stress. In addition, a meta-analysis of published data indicated a number of genes involved in auxin response were induced in *arp6* mutant grown in non-stress condition. These imply that the loss of H2A.Z balance (in *arp6* mutant) may lead to change stress and auxin responses resulting in alternative root morphogenesis upon both normal and salinity stress conditions.

Graphical Abstract



Keywords Abiotic stress · Abscisic acid (ABA) · ACTIN-RELATED PROTEIN 6 (ARP6) · Auxin · Chromatin modifications · H2A.Z

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Introduction

H2A.Z-containing nucleosomes have been suggested to play a dual role in the regulation of gene transcription [10, 25]. Hence, these H2A.Z-containing nucleosomes have been documented to define the transcription of various genes involved in development and environmental responses such as floral transition, anthocyanin biosynthesis, thermomorphogenesis, drought stress responses, and shade avoidance syndrome [3, 16, 4, 10, 25, 2, 19, 28]. It is known that the deposition and eviction of H2A.Z are, respectively, controlled by two major chromatin-remodeling complexes including SWR1 and INO80 [21, 1, 11]. ACTIN-RELATED PROTEIN 6 (ARP6) has been characterized as a component of the Arabidopsis SWR1 chromatin-remodeling complex [3, 16, 4]. The Arabidopsis *arp6* mutant has been investigated to exhibit several different phenotypes (e.g., early flowering, leaf serration, multiple phosphate-starvation-related phenotypes, high anthocyanins accumulation, and elongated hypocotyl) from wild-type plants [3, 4, 10, 23, 2].

In Arabidopsis, *ABA INSENSITIVE 1* (*ABI1*) and *ABI2* genes encode for two different clade A type 2C protein phosphatases (PP2Cs) [12]. These PP2Cs (ABI1 and ABI2) function as important factors in the abscisic acid (ABA) signaling pathway [8]. Previously, *abi1* and *abi2* mutants were shown to be more sensitive to ABA or abiotic stress (e.g., salt and heat stress conditions) [26, 9]. Auxin is one critical phytohormone that regulates root growth and development [8]. Besides, auxin was also found to contribute to control the root growth in response to salt stress [14]. In this study, the *arp6* mutant is found to show retarded root phenotypes such as shorter primary root length and fewer lateral root (LR) numbers in response to salt stress conditions. To support the phenotypic data, the transcript levels of *ABI1* and *ABI2* were also tested in wild-type and *arp6* plants grown under both normal and salt stress conditions. A meta-analysis of published data on *arp6* mutant indicated that a set of auxin-related genes was upregulated in this mutant compared with wild type. These imply that the loss of H2A.Z balance (in the *arp6* genome) can lead to changes in auxin signaling as well as salt stress response and eventually alterations in root morphogenesis in *Arabidopsis thaliana*.

Materials and Methods

Plant Materials and Growth Conditions

In this study, the used *arp6* mutant is originally named as *suppressor of FRIGIDA3* (*suf3*) which was isolated and characterized by Choi et al. [3]. Arabidopsis wild-type (Columbia, Col-0) and *arp6* mutant seeds were surface-sterilized and sown on half-strength Murashige and Skoog (1/2 × MS) (2% sucrose). The whole medium plates containing seeds were then stored at 4 ℃ for 3 days. Afterward, these plates were transferred to a growth chamber with the following conditions: long day (16 h light/8 h dark), light intensity (~ 100 µmol photons m^{-2} s^{-1}, white-light), and temperature (23 ± 1 ℃).

Salt Stress Treatments

Five-day-old seedlings were transferred to 1/2 × MS (2% sucrose) medium supplemented with 0 or 100 mM NaCl. Next, these seedlings were placed back to the same growth chamber and grown for phenotyping.

Quantitative Reverse Transcription PCR (RT-qPCR)

Two-week-old plants were transferred to 1/2 × MS (2% sucrose) medium supplemented with 0 (Control) or 250 mM NaCl (Salt stress) and were then placed back to the same growth chamber and grown for 6 h. Next, the plants were used for RNA extraction using TRIzol™ Reagent (Invitrogen™, Waltham, Massachusetts, USA). The RNA samples were subsequently subjected to cDNA synthesis and qPCR. The relative transcript levels of two genes, *ABI1* (F: 5′-GCC TACCCATTTCCTCCTTCTT-3′ and R: 5′-GGGTTTCCT GGATTGTGGGTA-3′) and *ABI2* (F: 5′-TTGCCCAGA ATCCAGGAAAC-3′ and R: 5′-AGACAACCTTAGCTA GCACATGA-3′), were calculated by the 2(-Delta Delta C(T)) method [15] and *ACTIN2* (F: 5′-GATCTCCAAGGC CGAGTATGAT-3′ and R: 5′-CCCATTCATAAAACCCCA GC-3′) was employed as the internal control.

Results

arp6 Mutant is Sensitive to Salt Stress

Salt stress was shown to increase H2A.Z-containing nucleosomes eviction resulting in activation of *AtMYB44* transcription [18]. This implies that histone variant H2A.Z also involves in the regulation of salt stress responses. In the present work, *arp6* mutant was used to test the growth performance upon salt stress condition. Five days after transferring to 1/2 × MS medium supplemented with NaCl (100 mM), both wild-type and *arp6* seedlings exhibited shorted primary roots than those on control conditions (Fig. 1A). Upon salt stress, the primary roots of *arp6* seedlings were significantly shorter than those of wild type (Fig. 1A). Besides, *arp6* mutant also showed fewer LR numbers than wild type

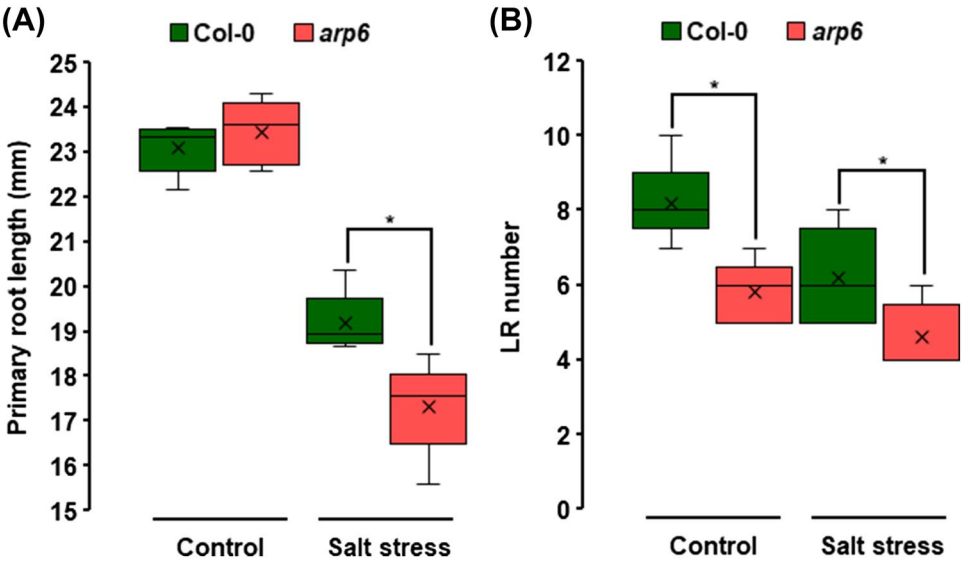

Fig. 1 Root phenotypes of *arp6* mutant in response to salt stress. Primary root length (**A**) and lateral root (LR) number (**B**) wild-type (Col-0) and *arp6* plants on the 5th day after exposure to salt stress condition (growth medium supplemented with 100 mM NaCl). Data were obtained from three independent experiments. Columns marked with an asterisk (*) indicate significant differences ($p < 0.05$)

on both control and salt stress conditions (Fig. 1B). When the plants (wild-type and *arp6*) were grown longer on the medium supplemented with NaCl (100 mM) (15 days), it was clearer to see that the *arp6* root system was much more sensitive to salt stress than wild type's one (Fig. 2A). The quantification of primary root length showed that salt stress condition dramatically reduced the root elongation in *arp6* in comparison with wild-type plants (Fig. 2B). These results indicate that loss-of-function mutation of *ARP6* increases the salt sensitivity, especially in root growth, in Arabidopsis.

ARP6 Positively Regulates Salt Stress-Responsive Genes

Next, we tested the transcript levels of stress-responsive genes in *arp6* mutant in response to salt stress. As shown in Fig. 3, salt stress dramatically induced the expression of *ABI1* and *ABI2* genes. However, the salt stress-mediated induction of *ABI1* and *ABI2* was significantly impaired in *arp6* mutant (Fig. 3). These molecular data support to explain why the *arp6* mutant is more sensitive to salt stress than wild-type plants. A prior study revealed that salt stress inhibited root growth via negative influences on auxin signaling and transport [14]. As indicated in Fig. 1B, *arp6* also exhibited fewer LR numbers even in normal growth condition. It implies that the lack of *ARP6* may also influence

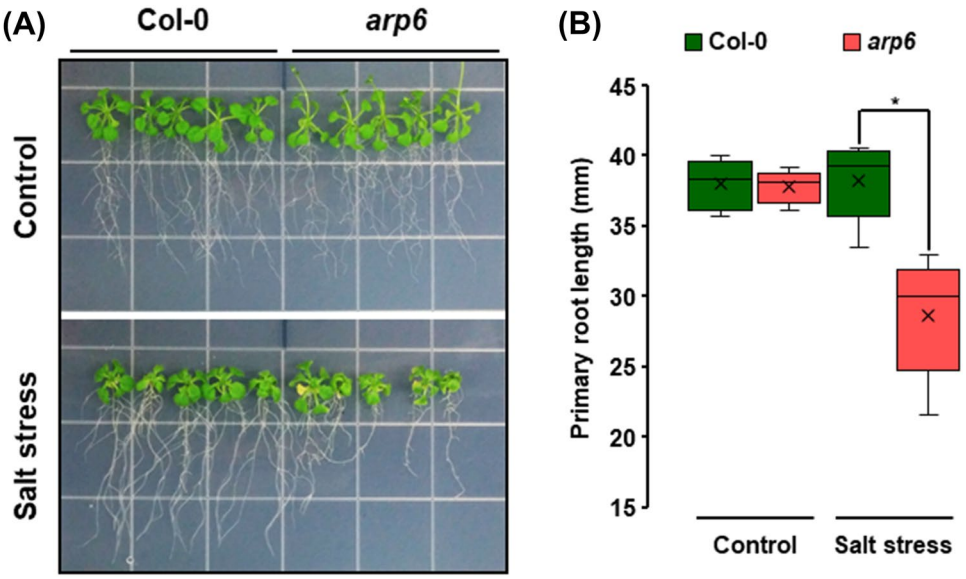

Fig. 2 Whole-plant pictures (**A**) and primary root length (**B**) of wild-type (Col-0) and *arp6* plants on the 15th day after exposure to salt stress condition (growth medium supplemented with 100 mM NaCl). Data were obtained from three independent experiments. Columns marked with an asterisk (*) indicate significant differences ($p < 0.05$)

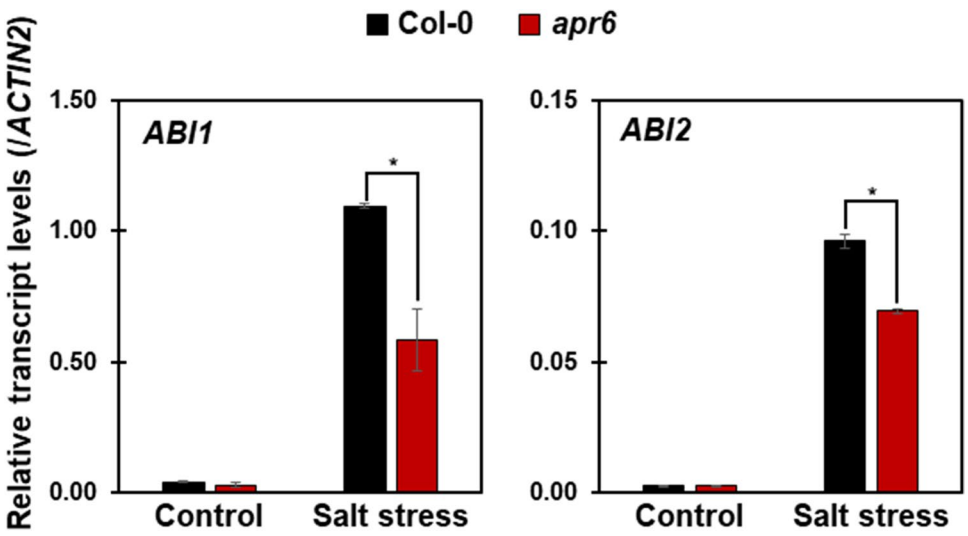

Fig. 3 Loss-of-function mutation of *ARP6* impaired the expression of stress-responsive genes upon salt stress condition. Two-week-old plants [wild-type (Col-0) and *arp6*] were either grown in normal condition (Control) or treated with NaCl (250 mM) for 6 h (Salt stress). Subsequently, the plants were subjected to RNA extraction and RT-qPCR to test the transcript levels of two stress-responsive genes, *ABI1* and *ABI2*. Data were obtained from three independent experiments. Columns marked with an asterisk (*) indicate significant differences ($p < 0.05$)

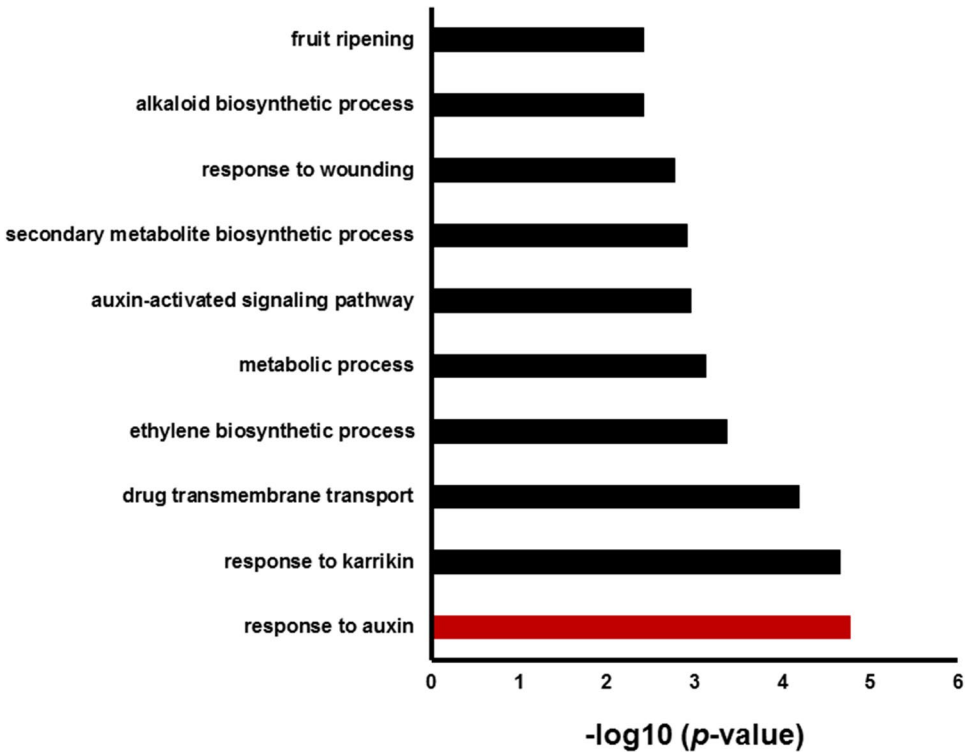

Fig. 4 Meta-analysis of upregulated genes in *arp6* plants grown in normal conditions. The data published by Sura et al. [25] were obtained and used for gene ontology (GO) analysis. The list of upregulated genes in *arp6* mutant when compared to wild-type plants grown in non-stress conditions was extracted from a prior published data [25]. Next, this list was used for GO analysis by DAVID Gene Functional Classification Tool [6]. Ten Biological Process (BP) categories which had highest [−log10 (*p-value*)] were shown

the auxin signaling or response in Arabidopsis. In fact, a meta-analysis of the published data from a previous study [25] showed that many genes involved in auxin response were upregulated in *arp6* mutant compared with wild-type when these plants were grown in normal condition (Fig. 4).

Discussion

A previous study reported that phosphate-starvation stress also strongly reduced the primary root length of *arp6* mutant when compared with wild type [23]. It is well known that different severe abiotic stress conditions (such as drought, salinity, and cold stresses) can significantly inhibit primary root elongation [29, 7]. A high concentration of NaCl (100 mM) in growth media was also found to reduce the lateral root numbers [30]. The

cytological analysis showed that salt stress conditions can cause a reduction in cell production and cell division as well as smaller mature cell length leading to unhealthy root morphogenesis [27, 13]. In the present work, we show that loss-of-function mutation of *ARP6* increased the salt sensitivity, and this was especially indicated as strongly attenuated root growth of *arp6* mutant upon salt stress (Fig. 1 and 2). To further explore the underlying mechanisms, expressions of stress-responsive genes were tested in wild-type and *arp6* plants grown under both normal and salt stress conditions. The salt stress significantly triggered the mRNA levels of *ABI1* and *ABI2* genes in both wild-type and *arp6* plants (Fig. 3). However, the mRNA levels of these genes were found to be lower in *arp6* than those in wild-type plants (Fig. 3). Previous studies did show that *abi1* and *abi2* mutants were more sensitive to ABA or abiotic stress (e.g., salt and heat stress conditions) in comparison with wild-type plants [26, 9]. These indicate that ARP6 may control the plant response to salinity stress via the regulation of different PP2C genes and these may consequently influence the ABA signaling pathway. ABA has been known to play as a negative regulator of root growth and branching [20, 24]. In the ABA signaling pathway, the PP2Cs (e.g., ABI1 and ABI2) proteins function as the brake which negatively influences the signal transduction [8]. As shown in Fig. 3, the loss-of-function mutation in *ARP6* resulted in the reduction of transcript levels of *ABI1* and *ABI2* genes upon salt stress treatment. Thus, it is possible that the ABA signaling transduction was highly activated in *arp6* mutant grown on salt-supplemented media leading to retarding root growth.

Auxin is known as a critical phytohormone in the regulation of root growth in both normal growth as well as salt stress conditions [5, 22, 14, 17]. Here, we found that the *arp6* mutant not only exhibited retarded root growth upon salt stress condition, but it also reduced the primary root growth under normal condition (Fig. 1 and 2). As shown in Fig. 4, the meta-analysis of published data on *arp6* mutant indicated that a set of auxin-related genes was upregulated in this mutant compared with wild type. Overall, these data suggest that the loss of balance in H2A.Z in the genome of *arp6* mutant may alter auxin signaling in plants resulting in inhibition of root growth upon both normal and salt stress conditions.

Conclusion

In this study, *arp6* mutant was found to exhibit impaired root growth such as shorter primary root length and fewer lateral root numbers in response to salt stress. The RT-qPCR analysis revealed that *ABI1* and *ABI2* transcript levels were significantly lower in *arp6* mutant in comparison with wild type upon salt stress treatment. Based on the phenotypic and gene expression analyses of *arp6* mutant, this study provides an additional role of H2A.Z histone variant in the regulation of plant response to salt stress.

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Author Contributions NHN designed and supervised the research. BHD and NHN performed the experiments. BHD and NHN wrote the manuscript. NTH and TDL read and commented on the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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Data Availability The data published by Sura et al. [25] were obtained and used for gene ontology (GO) analysis and are available at https://doi.org/10.1105/tpc.16.00573.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Approval This study was performed on the model plant (*Arabidopsis thaliana*) and was not involved in human participants and/or animals.

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