

**Running title:** ABA inhibits root cell elongation through repressing the cytokinin signaling

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## **Abstract**

Cell elongation, which plays an important role in root penetration into the soil, responds to a variety of environmental factors. A previous study demonstrated that abscisic acid, a phytohormone involved in stress responses, inhibits root growth by delaying the onset of cell elongation. In contrast, we recently reported that cytokinins promote elongation of root cells by enhancing actin bundling. However, the control of root cell elongation through the interaction between abscisic acid and cytokinin signaling has not yet been uncovered. Here, we show that abscisic acid-induced delay in cell elongation requires inhibition of cytokinin signaling; further, stress is signaled to cell elongation by the pathway mediated by B-type ARABIDOPSIS RESPONSE REGULATOR 2 (ARR2), which retards root growth.

## **Results**

The *Arabidopsis* root tip is divided into four distinct zones: the stem cell niche (SCN), the proximal meristem (PM), the transition zone (TZ), and the elongation/differentiation zone (EDZ)<sup>1, 2</sup>. Cells produced in the SCN actively divide in the PM and accumulate in a longitudinal direction to form the root meristem. At the boundary between the PM and the TZ, the cell cycle is modified from the mitotic cycle to the endocycle. In the endocycle, DNA replication is repeated without mitosis or cytokinesis, causing DNA polyploidization<sup>3</sup>. As a result, cell division ceases and rapid cell elongation occurs<sup>4</sup>. Then, cells gradually expand in the TZ and eventually more rapid cell elongation is initiated in the EDZ. Recently, we reported that dynamic actin reorganization triggers the transition from the TZ to the EDZ<sup>5</sup>. Therefore, *Arabidopsis* root cells undergo two modes of rapid cell elongation. The first mode is induced by the onset of endocycle at the border of the PM and the TZ, and the second mode results from actin reorganization at the border of the TZ and the EDZ<sup>5</sup>.

In addition, we observed that cytokinin (CK) signaling controls the number of TZ cells. The

transmembrane receptors ARABIDOPSIS HISTIDINE KINASE2/3/4 (AHK2/3/4) perceive CKs. Further, a phosphorelay to the B-type ARABIDOPSIS RESPONSE REGULATORS (ARRs), transcription factors controlling CK-responsive genes, transmits the signal<sup>6</sup>. At the boundary between the TZ and the EDZ, CK signaling that involves AHK3/AHK4 and ARR2 promotes actin bundling, which enables the second rapid cell elongation.<sup>5</sup> Indeed, *ahk3/4* and *arr2* mutants have more TZ cells than wild type owing to the delayed onset of the second rapid elongation.<sup>5</sup>

Abscisic acid (ABA) is a phytohormone known as the “stress hormone”<sup>7</sup>. ABA signaling mediates many types of abiotic stresses, such as drought, salinity, and heat and cold stress<sup>7</sup>. In roots, ABA plays an essential role in inhibiting cell division and elongation in response to soil conditions<sup>8,9</sup>. Previously, Zhang *et al.* (2010) reported that ABA has a positive effect on the TZ length in *Arabidopsis* roots, a phenocopy observed in *ahk3/4* and *arr2* mutants.<sup>8</sup> However, the crosstalk between ABA and CK signaling has not yet been analyzed, and it remains unknown whether abiotic stresses control root growth through modulation of CK signaling.

To examine the involvement of CK signaling in ABA-mediated control of the second rapid cell elongation, we first asked whether ABA controls the cell number in the TZ through the AHK3/AHK4-ARR2 pathway. Consistent with previous findings, 0.5  $\mu$ M or 10  $\mu$ M ABA treatment of *Arabidopsis* roots increased the TZ cell number, indicating an inhibition of the second rapid cell elongation (Fig. 1)<sup>8</sup>. As we recently described, *ahk3/4* and *arr2* mutants had more TZ cells than wild type in the absence of ABA. Interestingly, these mutants were tolerant of ABA treatment in terms of an increase in TZ cell number (Fig. 1)<sup>5</sup>. This result suggests that ABA-induced delay in the second rapid cell elongation requires inhibition of CK signaling through the AHK3/4-ARR2 pathway.

Nguyen *et al.* (2016) reported that ABA treatment reduced the transcript levels of B-type ARRs, *ARR1*, *ARR10*, and *ARR12*, in *Arabidopsis* roots<sup>10</sup>. However, whether ABA represses the expression of *ARR2*, which plays a major role in promoting the second rapid cell elongation, remains

unknown. Therefore, we investigated the expression pattern of ARR2 fused to b-glucuronidase (GUS), which was expressed under the 2-kb *ARR2* promoter, in the presence of ABA. A high GUS signal was observed in the area encompassing the TZ and the EDZ in the absence of ABA. However, the GUS signal was dramatically reduced by 0.5  $\mu$ M ABA treatment; further, only a weak signal was detected in the vasculature of 10  $\mu$ M ABA-treated roots (Fig. 2A).<sup>5</sup> As reported previously, ABA treatment reduced expression of *pARR1:ARR1-GUS* and *pARR12:ARR12-GUS* in a dose-dependent manner (Fig. 2B), implying that ABA downregulates not only ARR2 but also other B-type ARRs<sup>10</sup>. In contrast, neither 0.5  $\mu$ M or 10  $\mu$ M ABA treatment decreased the expression of *pAHK3:AHK3-GUS* or *pAHK4:GUS* (Fig. 2C), suggesting that ABA signaling does not affect the expression of CK receptors. *ARR1*, *ARR2*, and *ARR12* are known to promote the first rapid cell elongation by enhancing the transition from the mitotic cell cycle to the endocycle<sup>3</sup>. Therefore, downregulation of their expression by ABA may prevent mitotic cells from entering the endocycle, although mitosis is directly inhibited by ABA<sup>8</sup>. However, our data suggest that ABA-triggered repression of *ARR2* inhibits actin reorganization and delays the onset of the second rapid cell elongation, leading to inhibition of root growth. This result indicates that ABA controls cell elongation as well as cell division to effectively inhibit root growth under stressful conditions and that ARR2-mediated but cytokinin-independent pathway plays a crucial role in transmitting stress signals to actin-dependent cell elongation.

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## Figure legends

### Figure 1. ABA increases the cell number in the TZ through AHK3/4 and ARR2

(A) Cortical cells around the TZ of wild-type, *ahk3/4* and *arr2*. Three-day-old roots were treated with different concentrations of ABA for 4 days. The PM, TZ and EDZ cells are shown by white, red and blue outlines, respectively. The scale bars represent 50  $\mu\text{m}$ . (B) The TZ cell number in wild-type, *ahk3/4* and *arr2* treated with or without 0.5  $\mu\text{M}$  or 10  $\mu\text{M}$  ABA. Data are presented as mean  $\pm$  SD ( $n > 20$ ). Bars with different letters differ significantly from each other. Significant differences were determined by Tukey's test ( $P < 0.05$ ).

### Figure 2. Expression patterns of *ARR1*, *ARR2*, *ARR12*, *AHK3* and *AHK4* in the presence of ABA

Three-day-old roots were treated with or without different concentrations of ABA for 4 days, and subjected to GUS staining. (A) *pARR2:ARR2-GUS*, (B) *pARR1:ARR1-GUS* and *pARR12:ARR12-GUS*, and (C) *pAHK3:AHK3-GUS* and *pAHK4:GUS*. Yellow and red arrowheads indicate the boundaries between the PM and the TZ, and between the TZ and the EDZ, respectively. The scale bars represent 100  $\mu\text{m}$ .

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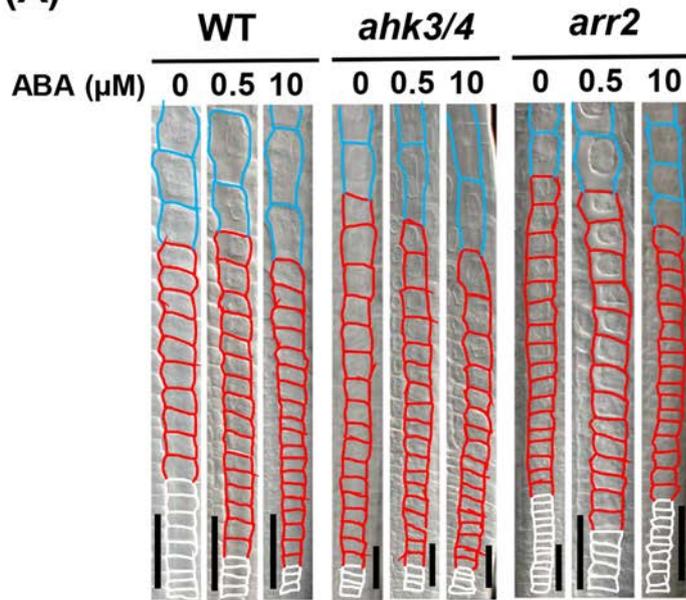
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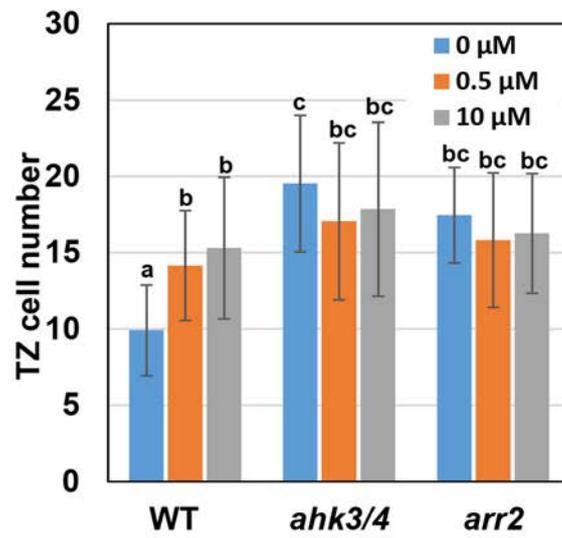
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Figure 1.

(A)



(B)



**Figure 2.**

