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Arabidopsis SMO2 regulates seed germination and ABA response

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Arabidopsis *SMALL ORGAN2* (*SMO2*) encodes a functional homologue of yeast TRM112, and disruption of *SMO2* results in a defect in progression of cell division and organ growth. Here, we show that SMO2 mediates the abscisic acid (ABA) response during seed germination. *smo2* exhibits an obvious delay of seed germination and is hypersensitive to abscisic acid (ABA), while transgenic seeds overexpressing *SMO2* are hyposensitive to ABA. Given that SMO2 is required for proper cell division in root apex, our observation suggests that SMO2-regulated progression of cell division is involved in ABA response during seed germination.

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Seed germination is a process that initiates post-embryonic development in higher plants, during which a series of processes, including transcription, translation and DNA repair followed by directional cell expansion and/or division, are resumed and eventually the radicle penetrates seed coat.[1,2] As a fundamentally biological process, cell division is generally considered to occur only at the completion of germination,[2] because the cabbage (*Brassica oleracea* L.) radicle has been found to be able to grow and protrude from the seed coat in the absence of cell cycles.[3] However, recent study in Arabidopsis demonstrates that D-type cyclins activate cell cycle reentry in root meristem and promote seed germination,[4] suggesting that cell division is also important for seed germination.

Abscisic acid (ABA) signaling has been defined to negatively regulate seed germination, and many identified molecules involved in the regulation of seed germination so far are associated with ABA and its signaling.[5,6] Recent studies also reveal that progression of cell division is inhibited by ABA during seeds germination of maize (*Zea mays* L.) and coffee (*Coffea arabica* 'Rubi').[7,8] Our previous work demonstrates that Arabidopsis SMO2 modulates progression of cell division during organ growth. In this report, we further describe that SMO2 affects ABA response during seed germination. Our finding suggests that the role of ABA in seed germination may, at least in part, involve the progression of cell division.

Seed Germination is Delayed in *smo2*

In addition to its smaller organs, Arabidopsis *smo2* knockout mutant was found to exhibit obviously delayed seed germination compared to WT. To investigate the effect of *SMO2* on seed germination, we compared germinating time between wild type (WT) and *smo2* seeds, by examining the percentage of germinated seeds after stratification. As shown in **Figure 1**, WT seeds began to germinate at about 16 h and all the seeds completed germination at 24 h after stratification, however, *smo2* seeds began to germinate at 20 h and almost all the seeds germinated at 48 h after stratification. This finding demonstrates that disruption of *SMO2* delays seed germination. Previous studies revealed that disturbance of some cell cycle regulators, such as CYCD4;1 or CYCD1;1, leads to a delay of seed germination due to the retardation of the onset of cell proliferation.[4] Given that mutation of *SMO2* impedes progression of cell division in root apical meristem, our observation suggests that the delay of seed

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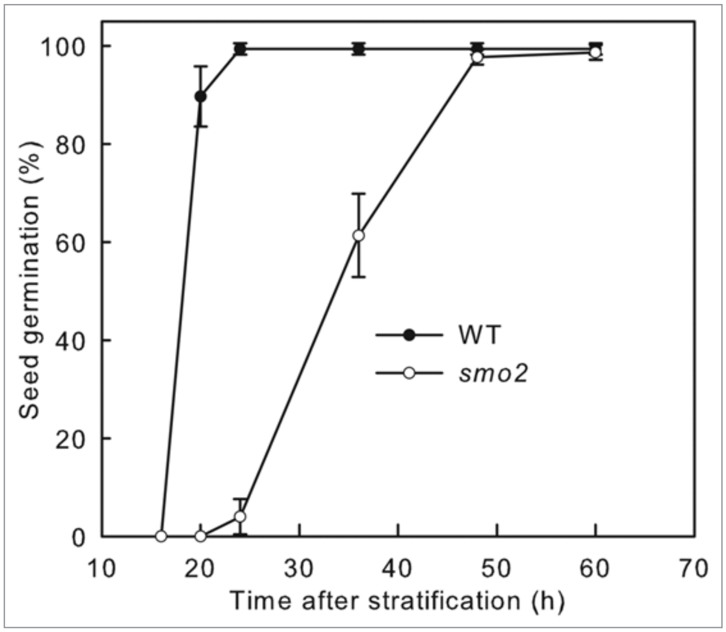

Figure 1. The delayed germination of *smo2* seeds. WT and *smo2* seeds were germinated on plates containing 1/2 MS medium (0.5 MS salts, 1% sucrose, and 0.6% agar) and stratified at 4°C in the dark for 2 d. The plates were transferred to a culture room with 22 ± 1°C under an illumination of 80–90 μmol m^{-2} s^{-1} and a 16-h light/8-h dark photoperiod. The germination was defined as obvious emergence of the radicle from seed coat. The percentage of germinated seeds were examined at indicated times and data were collected from three biological replicates.

germination in *smo2* is related to the defect in cell division progression.

Mutation or Overexpresssion of *SMO2* Alters ABA Sensitivity

ABA is a classical plant hormone that has been defined to negatively regulate seed germination.[5,6] Because seed germination was delayed in *smo2*, we therefore speculated whether the role of *SMO2* in seed germination is related to ABA response. To test this, the seeds of WT, *smo2* and two independent *35S-SMO2* transgenic lines (*SMO2-OE 4-4* and *SMO2-OE 13-3*) were germinated on the media supplemented with different concentrations of ABA. As shown in **Figure 2A–C**, compared to WT, *smo2* showed hypersensitive to ABA, and conversely the *SMO2-OEs* exhibited apparently less sensitive to ABA. Cotyledon greening assay showed that, when seeds were germinated on the medium with 0.2 μM ABA, 64% of WT seedlings became greening, whereas greening seedlings in *smo2* and *SMO2-OE* were 27% and 100% (**Fig. 2D**), respectively, indicating that knockout or ectopic expression of *SMO2* affects ABA sensitivity during seeds germination.

Conclusions

Our previous work revealed that SMO2 is required for proper cell cycle progression. Here, we further show that SMO2 affects seed germination and ABA response. These observations strongly suggest that the ABA-regulated seed germination is associated with progression of cell division. Similar cell cycle progression defects and altered

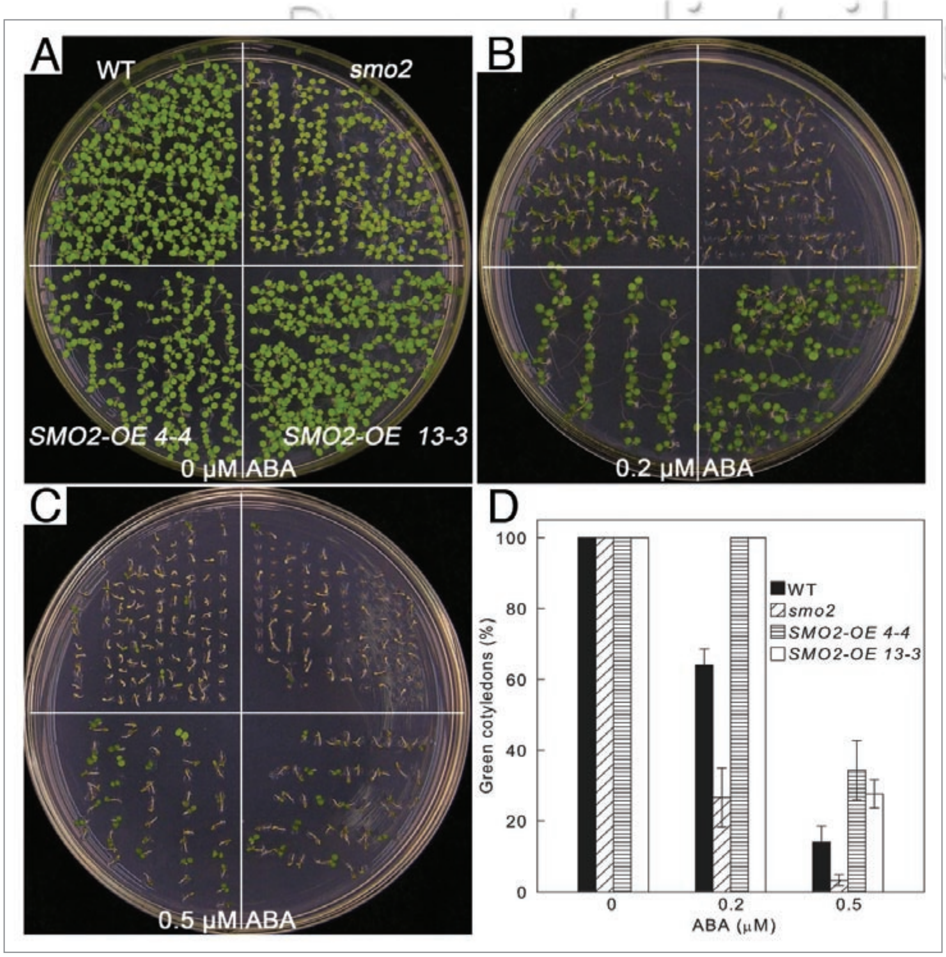

Figure 2. The ABA sensitivity of WT, *smo2* and *35S-SMO2* transgenic seedlings. (A–C) 6-day-old seedlings grown on 1/2 MS medium containing 0 μM ABA (A), 0.2 μM ABA (B) and 0.5 μM ABA (C). Seeds of WT, *smo2* and two independent *35S-SMO2* lines (*SMO2-OE 4-4* and *SMO2-OE 13-3*) were germinated on media with/without ABA after stratified at 4°C for 2 d. The panel of genotypes in (B and C) was same as in (A). (D) Percentage of seedlings with greening cotyledons. At least 50 seeds from each genotype were examined, and the data were from three biological replicates and shown as average values ± SDs.

ABA sensitivity have been reported in Arabidopsis *abo4-1* mutant, which contains a mutation in the DNA polymerase ε.[9] In addition, ABA have been found to have inhibitory role in G_1-S progression in cell cycle,[7,10] and *smo2* has the defect in G_2-M phase and possible G_1-S progression. Therefore, it is likely that proper cell division progression is, at least in part, required for ABA-regulated seed germination.

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