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• LETTER TO THE EDITOR •

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F-box gene *FOA2* regulates GA- and ABA- mediated seed germination in *Arabidopsis*

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Dear Editor,

GA and ABA antagonize each other in controlling seed germination, but the molecular mechanism is not fully understood. The F-box proteins act as the most important SCF (SKP1, cullin/CDC53, F-box protein) complex subunit of the ubiquitin (Ub)- 26S proteasome system, mediate diverse physiological processes ranging from hormonal signaling cascades to environmental stress responses (Santner and Estelle, 2010). We previously demonstrated that *FOA1* (F-box overexpressed/oppressed ABA signaling) is an ABA signaling-related gene, and plays a negative role in ABA signaling (Peng et al., 2012). Our preliminary results showed that F-box protein (AT3G16740), homologous to FOA1, named as FOA2, is highly expressed in silique (Duan et al., 2013), suggesting it may be involved in seed development.

In this study, we examined the expression patterns of *FOA2* during seed maturation and imbibition. Interestingly, with the maturity of the seeds the transcript level of *FOA2* increased, and reached plateau in the 27 DPA (days post-anthesis) silique (Figure 1A), whereas gradually decreased during seed imbibition (Figure 1B). Therefore, we postulated that *FOA2* might function in regulating seed dormancy and germination. To address this hypothesis, we identified T-DNA insertion mutant (*SALK_148443*) *foa2* and generated *FOA2* transgenic lines. The RT-PCR and qPCR results confirmed that the *foa2* is a null mutant, and the *FOA2* was overexpressed in transgenic lines (Figure S1A–D, Tables S1 and S2 in Supporting Information). Overexpression of *FOA2* delays seed germination (Figure S1F in Supporting Information), but the *foa2* mutant showed no difference compared to the wild type (Figure S1E in Supporting Information), which may be a result of functional redundancy with other homologous proteins. Interestingly, in the presence of 10^{-4} mol L^{-1} GA_3, the germination rate of *FOA2* transgenic lines restored to the wild type (Figure S2 in Supporting Information). Moreover, in the presence of 10^{-2} μmol L^{-1} GA biosynthesis inhibitor paclobutrazol (PAC), the seed germination rate of transgenic lines was reduced by about 75%, 70%, and 62.5%, respectively, whereas only 25% in the wild-type Col-4 (Figure 1C and D). However, the seed germination of *foa2* mutant reduced by 67%, while the wild-type Col-0 reduced by 80% (Figure 1C and E), indicating that overexpression of *FOA2* results in increased PAC sensitive phenotype, and *foa2* mutant is more resistant to PAC during seed germination. Moreover, the transcription of *FOA2* was up-regulated by GA treatment during seed imbibition (Figure S3 in Supporting Information). These findings suggesting a negative role for *FOA2* in GA mediated seed germination.

The balance between GA and ABA play central roles in

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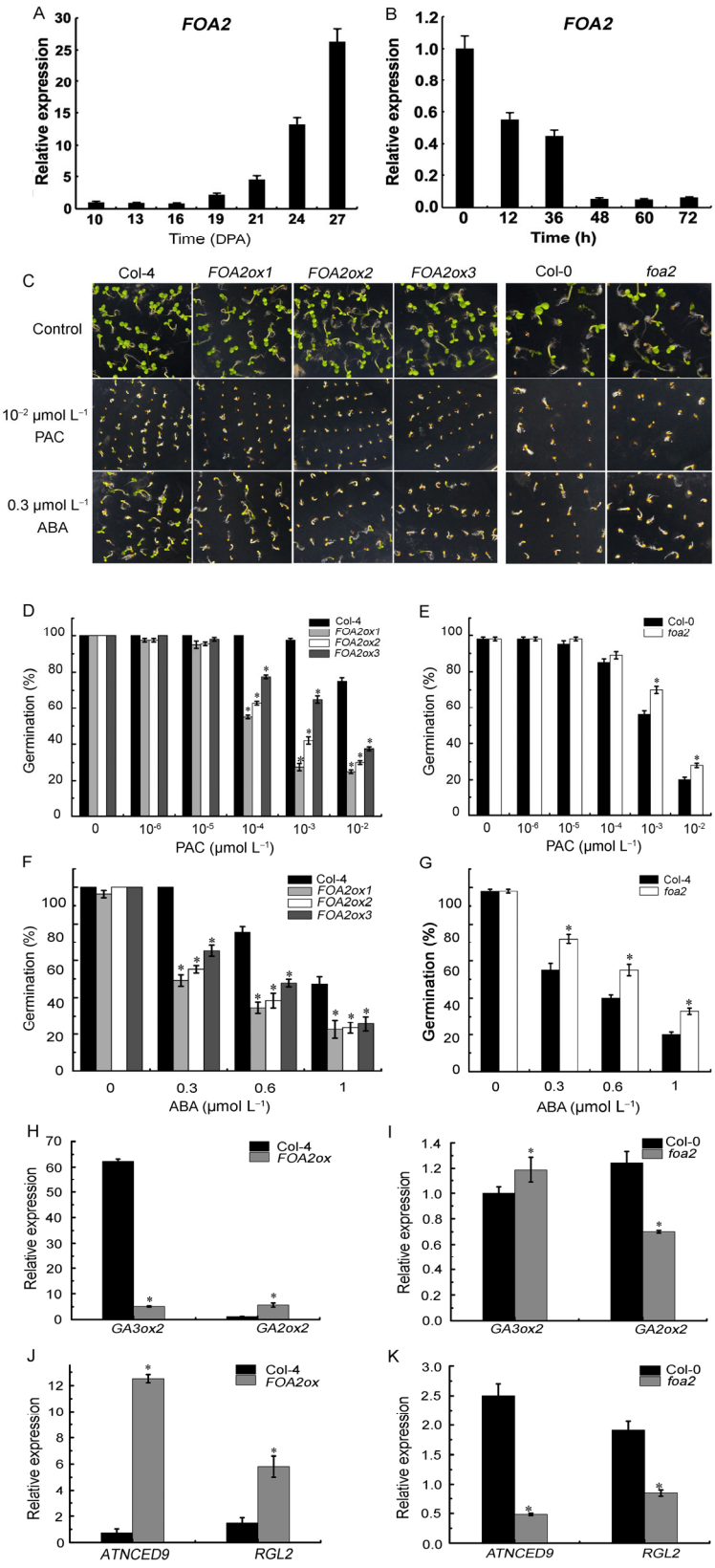

Figure 1 *FOA2* affects the response to paclobutrazol (PAC) and ABA on seed germination. A and B, Relative mRNA level of *FOA2* during seed maturation (A) and seed imbibition (B). C–G, Germination analysis of *FOA2* overexpression lines and *foa2* mutant in response to PAC (C, D, E), or ABA (C, F, G). H–K, The mRNA expression of GA anabolic gene *GA3ox2*, GA catabolic gene *GA2ox2*, ABA biosynthetic gene *NCED9* and a key DELLA gene *RGL2* during seed imbibition. Data were analyzed by t-tests and differences considered statistically significant with $P<0.05$. * denotes that a significant difference compared to the wild type.

the regulation of seed germination. ABA inhibits phase transitions from embryonic growth to germination. The expression profile of *FOA2* was similar to those obtained with ABI5, which is the key ABA signaling regulator (Lopez-Molina et al., 2001), increased during seed maturation and decreased during seed imibibition (Figure 1A and B). Moreover, the transcription of *FOA2* was down-regulated by ABA treatment during seed imbibition (Figure S3 in Supporting Information). Based on these results, we speculated that *FOA2* might associate with ABA during seed germination. To test this hypothesis, we examined the germination of *FOA2* overexpression lines and *foa2* mutant in response to ABA. As expected, the transgenic lines were more sensitive to ABA, and the seed germinations were decreased by 77.3%, 76.5% and 74.3%, respectively, whereas the wild-type Col-4 decreased by 52.8% with 1 μmol L^{-1} ABA treatment (Figure 1C and F). Nevertheless, the seed germination of *foa2* decreased by 67.3%, while the wild-type Col-0 decreased by 78% (Figure 1C and G). Taken together, these physiological data strongly suggest a positive role for *FOA2* in the ABA mediated inhibition of seed germination, while the down-regulation of *FOA2* expression by ABA might be a result of feedback regulation mechanism.

In *Arabidopsis*, *GA2ox2* is a key GA catabolic gene, loss of function of *GA2ox2* partly enhanced the seed germination ability (Yamaguchi et al., 1998). Consistent with the germination analysis results, *GA2ox2* were up-regulated in *FOA2* overexpression lines, and down-regulated in *foa2* mutant (Figure 1H and I). However, *GA3ox2*, which is a key GA biosynthesis gene (Seo et al., 2006), was down-regulated in *FOA2* overexpression lines and up-regulated in *foa2* mutant (Figure 1H and I). Additionally, the mRNA level of *RGL2* was increased in *FOA2* overexpression lines and reduced in *foa2* mutant during imbibition (Figure 1J and K). RGL2 is the gibberellic acid signaling repressor, has been shown to inhibit *Arabidopsis* seed germination by stimulating ABA synthesis (Lee et al., 2010). Interestingly, consistent with this notion, *NCED9*, which is essential for ABA biosynthesis (Lefebvre et al., 2006), was up-regulated in *FOA2* overexpression lines, and down-regulated in *foa2* mutant (Figure 1J and K). These findings therefore raised the possibility that *FOA2* functions to regulate GA metabolic and signaling and/or ABA biosynthesis, which in turn affect seed germination. It is interesting to clarify the relationship between *FOA2* and GA or ABA metabolic and signaling genes through additional genetic and biochemical data.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1	Characterization and germination assay of *foa2* mutant and *FOA2* transgenic plants.
Figure S2	Exogenous GA_3 recovered the delayed germinates in *FOA2* overexpression plant seeds.
Figure S3	The transcript level of *FOA2* in response to GA and ABA during seed imbibition.
Table S1	Primer sequences for semiquantitative reverse transcription (RT)-PCR and identifying homozygous lines of T-DNA insertion mutants
Table S2	Primer sequences for real-time fluorescence quantitative PCR (qPCR) used in this study

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