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ABA INSENSITIVE4 promotes rather than represses PHYA-dependent seed germination in Arabidopsis thaliana

Light quality plays vital roles in the life cycle of plants. For example, in seeds of many species, light quality determines the levels of the gibberelic acid (GA) and abscisic acid (ABA) phytohormones, which promote and repress seed germination respectively (Seo et al., 2006). In Arabidopsis thaliana, the photoreceptors phytochrome A (PHYA) and phytochrome B (PHYB) distinguish between full light (rich in red wavelength; R) and shade light (rich in far-red wavelength; FR) to regulate seed germination (Lymperopoulos et al., 2018). PHYB is reversibly activated and deactivated by R and FR light, respectively. Unlike the effect upon PHYB, both R and FR light irreversibly activate PHYA and once active, PHYA is more resistant to proteasome-mediated degradation (Shinomura et al., 1994, 1996 Debraux & Fankhauser, 2010). Both PHYA and PHYB promote germination by targeting the transcription factor PHYTOCHROME INTERACTING FACTOR 1 (PIF1) for protein degradation (Shen et al., 2005; Oh et al., 2006). When PHYA and PHYB are inactive PIF1 accumulates and regulates expression of genes leading to low GA/ABA ratios, which in turn repress germination (Oh et al., 2004, 2007; Kim et al., 2016). Conversely, upon PHYA and PHYB activation, and the subsequent PIF1 degradation, GA/ABA ratios increase to promote germination.

In addition to their different light-quality dependent activation, PHYA and PHYB have distinct patterns of accumulation: while PHYB accumulates from the beginning of seed imbibition; PHYA is only detectable later by a 60 min long FR light exposure (FR/FR; Fig. 1a), or a light at later stages of imbibition activates PHYA, but still deactivates PHYB. Thus, an initial short FR light pulse followed later by a 60 min long FR light exposure (FR/FR; Fig. 1a), or a continuous 48 h FR light treatment (FR48; Fig. 1a), results in activation of PHYA and deactivation of PHYB.

ABA acts through the signalling factors ABA INSENSITIVE3 (ABI3), ABI4 and ABI5, which are B3-, AP2- and bZIP-type transcription factors, respectively (Finkelstein et al., 1998; Finkelstein & Lynch, 2000; Clerkx et al., 2003). These three factors were originally identified as mutations that resulted in seeds that were insensitive to ABA treatments (Koornneef et al., 1984; Finkelstein, 1994). The corresponding genes were later found to also be involved in ABA signalling in other biological processes distinct from seed germination (Rohde et al., 2000; Signora et al., 2001; Rush et al., 2012). The roles of ABI3 and ABI5 in light-dependent seed germination have been described previously: ABI3 gene expression is induced under PHYB deactivating light conditions and, in turn, ABI3 controls expression of ABA-response related genes including ABI5 (Piskurewicz et al., 2009). ABI5 plays a more of a secondary role with a relatively modest effect under light conditions leading to PHYA activation and PHYB deactivation (Lee et al., 2012). This is probably due to the fact that the jasmonic acid precursor oxylipin cis-12-oxo-phytodienoic acid (OPDA) also plays a critical role in light-quality dependent repression of seed germination in an ABI5 independent manner (Barros-Gálvao et al., 2019).

Regarding ABI4, it has been shown to control lipid metabolism in seeds, sugar-directed growth arrest, lateral root development, and plastid-to-nucleus retrograde signalling (Wind et al., 2013). Intriguingly, previously published transcriptomic analysis revealed that, opposite to expectation, ABI4 gene expression is repressed by FR light conditions, which are known to increase ABA levels (Oh et al., 2009; Vaistij et al., 2018). These observations prompted us to investigate the role of ABI4 on the light-quality dependent germination pathway. We first compared ABI3, ABI4 and ABI5 gene expression upon FR/FR light treatment in phy-a-211 mutant seeds, which do not germinate under these conditions (Lee et al., 2012). Twelve hours after the end of the second FR light treatment (61 h after imbibition, hai; Fig. 1a) ABI3 and ABI5 expression was, as expected, increased in mutant seeds compared to wild-type control seeds (Fig. 1b). By contrast, ABI4 expression was repressed in phy-a-211 seeds (Fig. 1b). This shows that PHYA, an inducer of germination, promotes ABI4 expression. We then assessed germination of lack-of-function abi4-1 mutant seeds (120 hai; Fig. 1a). We also analysed seeds of an abi4-1 complemented line (abi4-1comp). As expected, under FR/R conditions, seeds of all genetic backgrounds analysed germinated at similar high rates (Fig. 1c,d). Under FR light conditions, germination of all seeds was severely repressed (Fig. 1c,d). By contrast, under FR/FR and FR48 light conditions, while wild-type and abi4-1comp seeds germinated at relatively high and similar levels, abi4-1 mutant seeds germinated at lower rates (Fig. 1c,d). These observations demonstrate that, in contrast to what has been reported for ABI5 (Lee et al., 2012), ABI4 promotes PHYA-dependent germination.

Previous studies showed that ABI4 and ABI5 are not only involved in ABA signalling, but they also positively feedback to regulate expression of genes leading to reduced GA/ABA ratios...
(Lee et al., 2012; Shu et al. 2013, 2016). This led us to assess whether expression of key genes involved in GA-biosynthesis (GA3ox1 and GA3ox3), ABA-biosynthesis (NCED6 and NCED9), and ABA-breakdown (CYP701A1, CYP701A2 and CYP701A3), are regulated by ABI4 under FR48 light conditions (52 hai; Fig. 1a). We found that, while expression of GA-biosynthesis and ABA-breakdown related genes were unchanged, ABA-biosynthesis genes were up regulated in abi4-1 seeds (Fig. 1e). This prompted us to measure ABA levels in FR48 light treated seeds (52 hai; Fig. 1a). We found that, in accordance with the increased NCED6 and NCED9 expression, ABA was present at higher levels in abi4-1 compared to wild-type seeds (Fig. 1f). These results show that, in contrast to its role in promoting ABA accumulation in post-germination developmental stages (Shu et al., 2016), ABI4 represses ABA accumulation during PHYA-dependent promotion of seed germination.

Fig. 1 ABSCISIC ACID INSENSITIVE 4 (ABI4) promotes phytochrome A (PHYA)-dependent germination and represses both abscisic acid (ABA) accumulation and MOTHER-OF-FT-AND-TFL1 (MFT) gene expression in Arabidopsis thaliana. (a) Schematic of the experimental set up. Seeds were imbibed on water-agar plates for 4 h under low light and then treated with: (1) two successive 5 min far-red (FR) and red (R) light pulses to activate PHYB (PHYB_on) during the period when PHYA does not accumulate; (2) only one 5 min FR light pulse to deactivate PHYB (PHYB_off) during the period when PHYA does not accumulate; (3) an initial 5 min FR light pulse followed 44 h later (48 h after imbibition, hai) by a second 60 min long FR light exposure to activate PHYA and deactivate PHYB (PHYA_on, PHYB_off); or (4) a continuous FR exposure for 48 h (FR48) to activate PHYA and deactivate PHYB (PHYA_on, PHYB_off). Seeds were kept in the dark between and after light treatments. (b) Relative expression of ABI3, ABI5 and ABI4 in FR/FR treated wild-type (WT) and phy-a-211 seeds 12 h after the end of the second FR treatment (61 hai). (c, d) Germination (120 hai) of FR/R, FR and FR/FR (c) and FR/R, FR and FR48 (d) treated WT, abi4-1 and abi4-1comp seeds. (e) Relative expression of GA3ox1, GA3ox2, NCED6, NCED9, CYP701A1, CYP701A2 and CYP701A3 in FR48 treated WT and abi4-1 seeds (52 hai). ABA (f) and 12-oxo-phytodienoic acid (OPDA) (g) levels in FR48 treated WT and abi4-1 seeds (52 hai). Data are means ± SD of three (for gene expression) and four (for germination and phytohormones levels) biological replicates. Asterisks indicate statistically significant difference according to two-tailed Student’s t-test (**, P < 0.01; *** P < 0.001). (h) Model of the role of PHYA and ABI4 in the promotion of seed germination: FR light (FR/FR and FR48 treatments in our experimental set up) activates PHYA (PHYA_on) (i) and deactivates PHYB (PHYB_off) (ii). PHYA_on promotes ABI4 gene expression (iii). ABI4 represses accumulation of ABA (iv) by inhibiting expression of the ABA-biosynthesis NCDE6 and NCDE9 genes (not depicted in the model). ABI4 acts, at least partially, through MFT (v) to repress seed germination (vi).
We reported previously that OPDA is a key repressor of germination (Dave et al., 2011, 2016). More recently we also showed that, under FR light conditions, and to a lesser extent under FR48 light conditions, OPDA acts in parallel to the action of ABA (Barros-Galvão et al., 2019) in repressing seed germination. Hence, we also measured OPDA and found that levels were decreased in abi4-1 seeds (Fig. 1g). In our previous study, we showed that the abnormally high germination of ABA and OPDA deficient mutant seeds is repressed by either ABA or OPDA treatments (Barros-Galvão et al., 2019). Presumably, in the present study the high ABA levels that lead to germination inhibition compensate for the low OPDA levels in abi4-1 seeds.

In two previous studies from our laboratory, we demonstrated that MOTHER-OF-FT-AND-TFL1 (MFT) is a repressor of seed germination, that it is a key factor of the ABA-signalling pathway, and that MFT gene expression is promoted by shade light (Vaistij et al., 2013, 2018). This prompted us to assess whether MFT expression is regulated by ABI4. We found that, under FR48 light conditions (52 hai; Fig. 1a), MFT expression was increased in abi4-1 seeds (Fig. 1e). This observation shows that, as for NCDE6 and NCDE9, ABI4 inhibits MFT expression. Whether this repression is due to direct or indirect ABI4–MFT/NCDEs interactions remains to be determined.

In conclusion, previous studies have established that ABI3, ABI4 and ABI5 are ABA-signalling effectors repressing germination (Koornneef et al., 1984; Finkelstein, 1994). It has also been demonstrated that ABI3 and ABI5 act under shade light conditions to inhibit germination (Piskurewicz et al., 2009; Lee et al., 2012). In the current study, we reveal an unexpected role for ABI4 in promoting, rather than repressing, PHYA-induced germination (Fig. 1h): upon light activation, PHYA promotes ABI4 gene expression (probably through the action of PIF1). In turn, ABI4 represses expression of NCDE6 and NCDE9, which leads to a decrease in ABA levels in the seed. ABI4 also reduces MFT gene expression either directly or indirectly as its effect on ABA, which itself promotes MFT expression (Xi et al., 2010). Interestingly, it has been reported that, as under PHYA activating light conditions (Fig. 1b), the pattern of expression of ABI4 is opposite to that of ABI3 and ABI5 in both Arabidopsis seed dormancy cycling (Footitt et al., 2011, 2014) and in Aethionema arabicum light-dependent seed germination (Méraï et al., 2019). This suggests that the germination-promoting role of ABI4 is a more general phenomenon. The evolutionary processes resulting in ABI4 function switching from a repressor to a promoter of seed germination depending on the environmental conditions remain to be elucidated.

Methods

The mutant abi4-1 and phyA-211 lines were described previously (Finkelstein, 1994; Reed et al., 1994). The abi4-1comp line was obtained by transformation of the mutant line with a pGREEN derived binary vector (pGTI0242AGR) carrying the ABI4 coding sequence under the control of the CaMV 35S promoter. All Arabidopsis lines used in this work are of the Columbia ecotype. Plant growth conditions, seed collection, germination assays, RNA extraction, primer sequences, quantitative polymerase chain reactions (qPCRs) conditions and phytohormones extractions were described previously (Dave et al., 2016; Vaistij et al., 2018; Barros-Galvão et al., 2019). For gene expression analysis, transcript levels as determined by qPCR were normalized to UBQ11 expression and expressed relative to the lower of the expression levels in each of the wild-type vs mutant comparisons.

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Author contributions

TB-G, FEV and IAG planned and designed the research. TB-G, AD, ADG, DH and FEV performed experiments and analysed data. TB-G, FEV and IAG wrote the manuscript.

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