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Overlapping and distinct roles of AKIN10 and FUSCA3 in ABA and sugar signaling during seed germination

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Abbreviations: ABA, abscisic acid; GA, gibberellic acid; FUS3, FUSCA3; SNF1, sucrose non-fermenting 1; AKIN10, arabidopsis Snf1-related kinase homolog 10; SnRK1, Snf1-related kinase 1; ML1:FUS3, FUS3 overexpression; 35S:AKIN10, AKIN10 overexpression; WT, wild type; FLU, fluridone

The B3-domain transcription factor FUSCA3 (FUS3) is a master regulator of seed maturation and also a central modulator of hormonal responses during late embryogenesis and germination in *Arabidopsis thaliana* (Arabidopsis). Recently, we have identified AKIN10, the Arabidopsis ortholog of Snf1 (sucrose non-fermenting-1)-related kinase1 (SnRK1), as a FUS3-interacting protein. We demonstrated that AKIN10 physically interacts with and phosphorylates FUS3 at its N-terminal region, and genetically interacts with FUS3 to regulate developmental phase transition and lateral organ growth. Snf1/AMPK/SnRK1 kinases are important sensors of the cellular energy level, and they are activated in response to starvation and cellular stress. Here we present findings that indicate FUS3 and AKIN10 functionally overlap in ABA signaling, but play different roles in sugar responses during germination. Seeds overexpressing *FUS3* and *AKIN10* both display ABA hypersensitivity and delayed germination. The latter is partly dependent on de novo ABA synthesis in both genotypes, as delayed germination can be partially rescued by the ABA biosynthesis inhibitor, fluridone. However, seeds and seedlings overexpressing *FUS3* and *AKIN10* show different sugar responses. *AKIN10*-overexpressing seeds and seedlings are hypersensitive to glucose, while those overexpressing *FUS3* display overall defects in osmotic stress, primarily during seedling growth, as they show increased sensitivity toward sorbitol and glucose. Hypersensitivity to sugar and/or osmotic stress during germination are partly dependent on de novo ABA synthesis for both genotypes, although are likely to act through distinct pathways. This data suggests that AKIN10 and FUS3 both act as positive regulators of seed responses to ABA, and that AKIN10 regulates sugar signaling while FUS3 mediates osmotic stress responses.

Introduction

Seed formation is a critical adaptation in the plant life cycle, as it allows embryos to temporarily cease growth in adverse conditions until the environment becomes favorable. During the maturation phase of embryogenesis, the embryo accumulates nutrient reserves, acquires desiccation tolerance and enters a stage of dormancy. The transition from embryonic to vegetative development (germination) is tightly regulated by the hormones abscisic acid (ABA), which promotes dormancy and inhibits germination, and gibberellic acid (GA), which has the opposite effect of breaking dormancy and stimulating germination.[1,2] In Arabidopsis, B3-domain transcription factors of the AFL (ABSCISIC ACID INSENSITIVE3, FUSCA3, LEAFY COTYLEDON2) family act as master regulators of late embryogenesis, as loss- and gain-of-function mutations in these genes greatly affect seed maturation.[3]

Genetic and molecular analyses indicate FUSCA3 (FUS3) inhibits the transition from the embryonic to the vegetative phase of development by promoting ABA accumulation while inhibiting GA biosynthesis.[4] The loss-of-function mutant, *fus3-3*, bypasses dormancy and enters postembryonic development prematurely due to a lower ABA/GA ratio.[4-7] Conversely, ectopic expression of *FUS3* post-embryonically (*ML1:FUS3*) delays seed germination and plant development by increasing ABA level while repressing GA biosynthesis.[4,8] The stability of the FUS3 protein also appears to be tightly regulated by ABA, GA and the 26S proteasome.[4,9]

In our recent study, AKIN10, was identified as an interactor of FUS3 from yeast two-hybrid screens.[10] AKIN10 belongs to the sucrose-non-fermenting 1 (Snf1)-related kinase1 (SnRK1) family and acts as a central regulator of cellular energetics in plants.[11] Our results indicate AKIN10 physically interacts with and phosphorylates FUS3 at its N-terminal region, and delays its degradation in a cell-free system. Overexpression of *AKIN10* (*35S:AKIN10*) causes delays in developmental phase transitions (germination and flowering) and defects in lateral organ formation. These phenotypes can be partially rescued by the *fus3-3* mutation, suggesting *FUS3*

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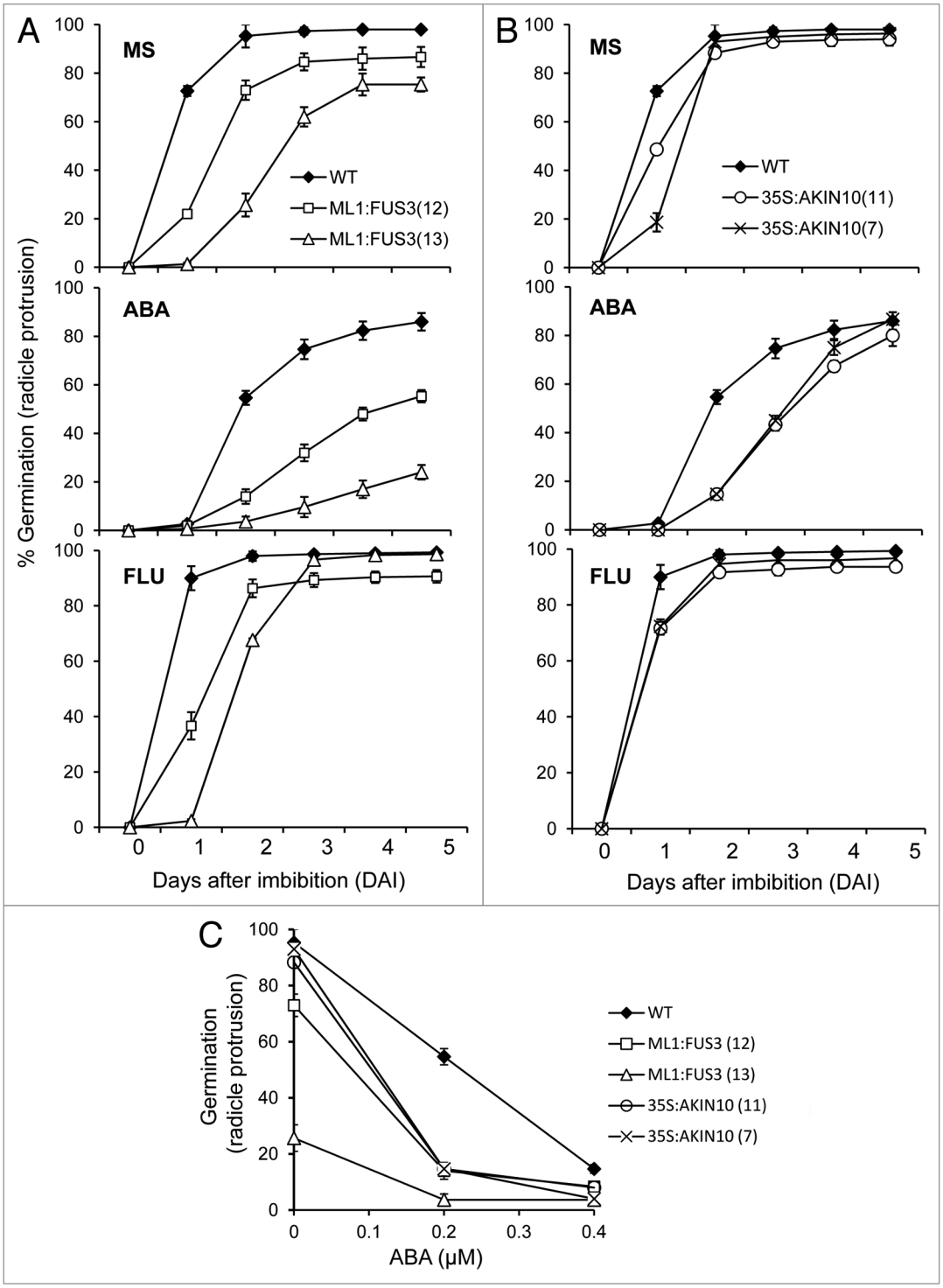

Figure 1. Overexpression of *FUS3* or *AKIN10* leads to ABA hypersensitivity and delayed germination, which is partly dependent on de novo ABA synthesis. Germination (radicle protrusion) kinetics of seeds from WT and two independent lines of *ML1:FUS3* (A) or *35S:AKIN10* (B) on MS media or MS supplemented with 0.2 μM ABA or 10 μM fluridone (FLU). WT germination is significantly higher ($p < 0.01$) than *35S:AKIN10* on 10 μM FLU at 1 d after imbibition (DAI). (C) ABA dose-response curves for WT, *ML1:FUS3* and *35S:AKIN10* seed germination (radicle protrusion) 2 d after imbibition. WT germination is higher ($p < 0.01$) than *ML1:FUS3* and *35S:AKIN10* at 0.4 μM ABA. Averages from 3 experiments ± SD are shown. 100–150 seeds were used in each experiment.

and *AKIN10* act in overlapping pathways to regulate developmental phase transitions and lateral organ development.[10] Interestingly, SnRK kinases also regulate stress responses, hormonal and sugar signaling pathways by global transcriptional modulations.[12-16] This prompted our investigation of the role of AKIN10 and FUS3 in ABA and sugar responses during germination.

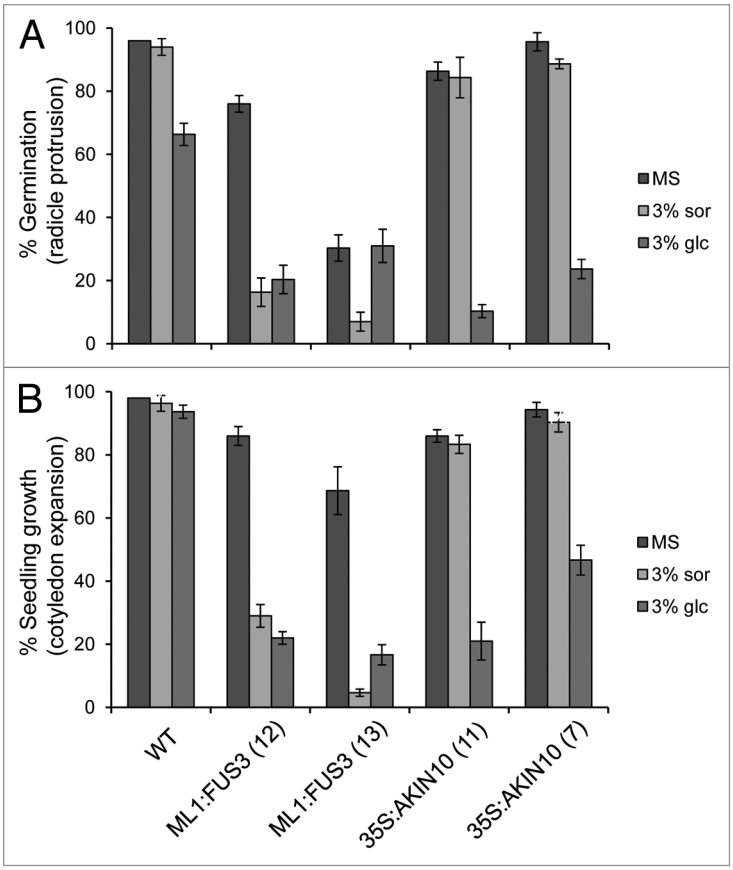

Figure 2. Overexpression of *FUS3* or *AKIN10* leads to different responses to sugar during seed germination and seedling growth. (A) Germination (radicle protrusion) of WT, *ML1:FUS3* and *35S:AKIN10* seeds 2 d after imbibition on MS ± 3% sorbitol (sor) or 3% glucose (glc). (B) Seedling growth (cotyledon expansion) of WT, *ML1:FUS3* and *35S:AKIN10* seeds 4 d after imbibition on MS ± 3% sor or 3% glc. Averages from 3 experiments ± SD are shown. 100–150 seeds were used in each experiment.

Results

We first tested germination rates (radicle protrusion) of two independent lines overexpressing *AKIN10* (*35S:AKIN10*) and *FUS3* (*ML1:FUS3*) on ABA (see Material and Methods). Both *35S:AKIN10* and *ML1:FUS3* transgenic plants were previously shown to delay seed germination on minimal MS medium,[8,12] with *ML1:FUS3* showing a greater germination delay than *35S:AKIN10* (**Fig. 1**). The delayed germination has been attributed to the heightened sensitivity to and level of ABA in *ML1:FUS3* seeds,[4,8] but the cause of delay remains unknown for *35S:AKIN10* seeds. To test whether altered ABA sensitivity contributes to the *35S:AKIN10* germination delay, wild type (WT), *ML1:FUS3* and *35S:AKIN10* seeds were germinated on a low concentration of ABA (0.2 μM). ABA delayed WT germination, though germination rates recovered to approximately 80% in 5 d (**Fig. 1**). *ML1:FUS3* germination was more sensitive to ABA compared with WT, and reached only 20–50% after 5 d of treatment (**Fig. 1A**). *35S:AKIN10* germination was also hypersensitive to ABA, but recovered to approximately 80% within 5 d of treatment (**Fig. 1B**). Germination rates tested on 0.4 μM ABA show that both genotypes are slightly hypersensitive to this concentration of ABA compare with WT (**Fig. 1C**). This suggests that both AKIN10 and FUS3 positively regulate ABA sensitivity during germination.

In order to dissect the role of ABA in the delayed germination of *FUS3*- and *AKIN10*-overexpressing seeds, WT, *ML1:FUS3* and *35S:AKIN10* germination rates were assayed in media supplemented with 10 μM fluridone, an inhibitor of phytoene desaturase which reduces ABA synthesis.[17,18] If delayed germination of the transgenic lines is due to increased ABA synthesis, then fluridone should rescue this delay. Germination rates of WT, *ML1:FUS3* and *35S:AKIN10* seeds were higher in the presence of fluridone compared with untreated seeds (**Fig. 1A and B**), suggesting de novo ABA synthesis negatively regulates germination in all genotypes. However, *ML1:FUS3* and *35S:AKIN10* seeds still germinated later than WT on fluridone (**Fig. 1A and B**), and their rates did not increase even at a higher concentration of fluridone (50 μM; data not shown). This suggests de novo ABA synthesis alone cannot explain the delayed germination phenotype of *ML1:FUS3* and *35S:AKIN10* seeds.

ABA and sugar signaling pathways are intricately related and share common downstream signaling components.[19-21] Since *AKIN10* is known to partake in sugar signaling[11,22] and since *FUS3* expression is regulated by sugar,[23] we investigated the role of *FUS3* and *AKIN10* in sugar signaling during germination. WT, *ML1:FUS3* and *35S:AKIN10* germination rates were assayed two days after imbibition on 3% glucose or 3% sorbitol as an osmotic control. At the concentration tested glucose, but not sorbitol, significantly reduced WT seed germination, as previously described (**Fig. 2A**).[21,24] *35S:AKIN10* germination was similar to WT on sorbitol, but showed hypersensitivity on glucose (**Fig. 2A**). Surprisingly, *ML1:FUS3* germination was reduced by sorbitol, while the effect of glucose varied between the two transgenic lines (**Fig. 2A**). To better understand the effect of exogenous glucose application, seedlings growth rates (cotyledon expansion) were assayed 4 d after imbibition in the presence of the sugars. In this case, cotyledon expansion of both *ML1:FUS3* transgenic lines was hypersensitive to both sorbitol and glucose, whereas *35S:AKIN10* cotyledon expansion was inhibited specifically by glucose (**Fig. 2B**). These results indicate that overexpression of *FUS3* during germination causes hypersensitivity toward osmotic stress, whereas overexpression of *AKIN10* leads to hypersensitivity specifically toward glucose. We next tested whether the osmotic hypersensitivity of *ML1:FUS3* and glucose hypersensitivity of *35S:AKIN10* seeds during germination are dependent on increased ABA synthesis. Germination rates were assayed 2 d after imbibition on 10 μM fluridone in the presence and absence of 3% glucose or sorbitol. In both cases, fluridone was able to partially restore the germination delay imposed by sorbitol and glucose on *ML1:FUS3* and glucose on *35S:AKIN10* (**Fig. 3**). These results indicate the hypersensitivity of *ML1:FUS3* seeds to osmotic stress and glucose hypersensitivity of *35S:AKIN10* seeds are both partially dependent on de novo ABA synthesis.

Discussion

Collectively, the data shown here demonstrate a positive role of *AKIN10* and *FUS3* in ABA responses during germination, as well as distinct roles in sugar and osmotic stress responses during seed germination and seedling growth. Indeed, FUS3 plays an inhibitory role under osmotic stress, while the inhibitory role of AKIN10 is specific for glucose. These data complement previous findings showing that plants overexpressing *AKIN10* display defects in post-embryonic development and root elongation on exogenous ABA and glucose.[12,14] Notably, the germination of seeds overexpressing *AKIN10* was previously shown to be unaffected by 3 μM ABA.[14] This is likely due to the high concentration of ABA used by Jossier et al.,[14] and that we recorded germination rates over multiple time points after imbibition.

Although fluridone was able to elevate the germination kinetics in seeds of both genotypes on MS media, it was not sufficient to fully recover the delayed germination of *ML1:FUS3* and *35S:AKIN10* seeds to the WT level. This suggests de novo ABA synthesis is only partially responsible for *ML1:FUS3* and *35S:AKIN10* delayed germination. It is possible that overexpression of *FUS3* or *AKIN10* already increases ABA level during embryogenesis, thus increasing seed dormancy. This is likely the case for FUS3, considering that a short activation of FUS3 indeed increases ABA level in *ML1:FUS3-GR* seedlings, while loss-of-function *fus3-3* embryos contain less ABA.[4,5] The germination delay of *35S:AKIN10* seeds was more closely, but not completely, rescued by fluridone. Although no differences in ABA level were previously found between WT and AKIN10-overexpressing seedlings,[14] this does not exclude the possibility of higher ABA accumulation in *35S:AKIN10* embryos compared with WT. In addition, ABA accumulation prior to germination may also explain why fluridone was unable to fully restore *ML1:FUS3* and *35S:AKIN10* reduced germination in the presence of glucose. Alternatively, ABA-independent pathways may be activated by FUS3 and AKIN10 during germination.

In conclusion, the data presented here indicate both FUS3 and AKIN10 act as positive regulators of ABA signaling during germination, although showing different sensitivity to the hormone, but they play different roles in sugar and osmotic stress signaling. *AKIN10* is involved in glucose-specific pathway(s), while *FUS3* modulates osmotic stress responses. Both sugar and osmotic responses regulated by AKIN10 and FUS3 are partly dependent on de novo ABA synthesis, likely through distinct pathways. It remains to determine if phosphorylation of FUS3 by AKIN10 is required to modulate seed sensitivity to ABA.

Material and Methods

Arabidopsis seeds of WT (Col-0), *FUS3*-overespressing (*ML1:FUS3-GFP*;[4,9]) and *AKIN10*-overexpressing (*35S:AKIN10-HA*;[10]) lines were vernalized and germinated as previously described.[9] ABA, fluridone, sorbitol and glucose were supplemented in the media at the concentrations specified in each experiment. Germination was considered positive when the radicle had emerged from the seed, while seedling growth was scored positive when the cotyledons were expanded. The *ML1:FUS3* construct was previously shown to rescue the *fus3-3* embryonic phenotypes, including desiccation intolerance of the seeds, and to ectopically express *FUS3* post-embryonically.[4,9] In contrast, *35S:FUS3* does not rescue the *fus3-3* mutant and causes co-suppression when transformed in a WT background (data not shown). Therefore, the 35S promoter could not be used to overexpress *FUS3*. Conversely, *35S:AKIN10* has been shown to overexpress *AKIN10* post-embryonically.[10] Since *ML1* and *AKIN10* expression levels are very similar throughout development, as measured in several microarrays (eNorthern; http://bar.utoronto.ca), we did not attempt to overexpress *AKIN10* using the *ML1* promoter.

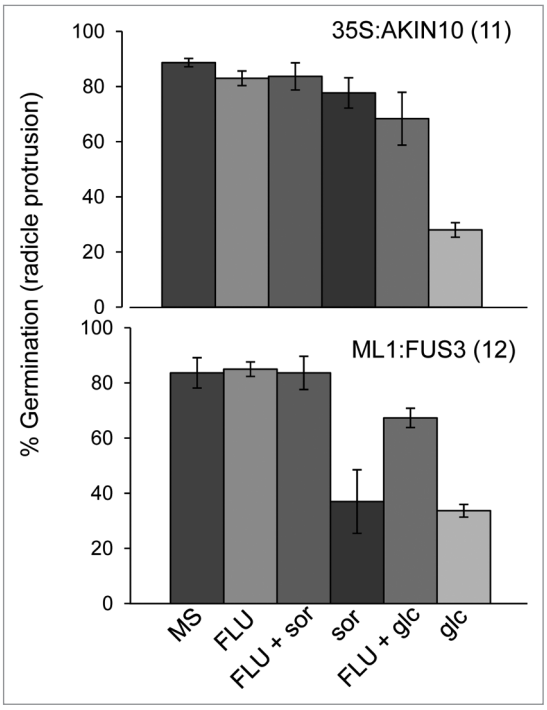

Figure 3. Osmotic stress hypersensitivity of *ML1:FUS3* seeds and glucose hypersensitivity of *35S:AKIN10* seeds are both partially dependent on de novo ABA synthesis. Germination (radicle protrusion) of *35S:AKIN10* and *ML1:FUS3* seeds 2 d after imbibition on MS, 10 μM fluridone (FLU), 3% sorbitol (sor) ± 10 μM FLU, and 3% glucose (glc) ± 10 μM FLU. Averages from 3 experiments ± SD are shown. 100–150 seeds were used in each experiment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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