

Characterization and Functional Analysis of *ABSCISIC ACID- INSENSITIVE3*-like Proteins from *Physcomitrella patens*

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The transcriptional regulator VIVIPAROUS1 (VP1)/ABSCISIC ACID INSENSITIVE 3 (ABI3) is involved in ABA signaling during seed development and is composed of four, highly conserved functional domains, which have been characterized only in seed plants. We isolated three (A,B,C) *ABI3*-like genes (*PpABI3*) from the moss *Physcomitrella patens* and undertook a comparative approach to understand the role of these functional domains in the ABA response. We showed that PpABI3A enhances the expression of both *Em-GUS* and *PpLEA1-GUS* in protonemal cells and recently demonstrated that the triple deletion lines ($\Delta abi3ABC$) are impaired in the expression of *PpLEA1-GUS*. This result clearly shows that the link between the ABA response and the transcriptional regulator ABI3 was present before the seed plants and suggests that this link maybe related to drought/desiccation tolerance.

Although PpABI3A can also activate the wheat *Em* promoter in barley aleurone cells, it was unable to fully complement the *abi3-6* mutant phenotypes in Arabidopsis. We showed that this is most likely due to a weak interaction between the B1 domain of PpABI3A and the bZIP transcription factor ABI5. We isolated two *ABI5*-like genes from *P. patens* and show that PpABI5A fully interacts with VP1 in protonema and aleurone. However, PpABI5A interacts with PpABI3A only in protonema and not aleurone cells, indicating that the lack of interaction in seed plants appears not to be with the PpABI5, but rather with the PpABI3.

To further identify the defect in PpABI3 that prevents its full interaction with ABI5 proteins, we showed that the conserved leucine of the *abi3-8* mutation is required for the interaction between VP1/ABI3 and ABI5. The PpABI3 proteins lack a leucine at this site in the B1 domain. However, altering PpABI3A to have a leucine at this site was not sufficient to improve the interaction with HvABI5. Replacing the full B1 domain from VP1 into PpABI3A results in an intermediate level of interaction with HvABI5, suggesting that other parts of VP1 are needed.