***Physcomitrella patens* mutant lines with reduced sensitivity to abscisic acid are hypersensitive to freezing and osmotic stress.**

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Protonema cells of *Physcomitrella patens* acquire tolerance to freezing stress when they are treated with abscisic acid (ABA). Freezing tolerance (LT_{50}) of the cells cultured under the optimal growth condition is around -2˚C but treatment with 10^{-5} M abscisic acid dramatically alters LT_{50} to -10˚C after one day and to over -15˚C after two days. We previously showed that ABA induced accumulation of various stress-related transcripts, heat-stable LEA-like proteins, and soluble sugars such as sucrose and theanderose (Minami et al., 2003; Nagao et al., 2006). Increase in freezing tolerance and induction of stress-related gene expression and sugar accumulation were also observed when the cells with subjected to hyper-osmotic stress treatment, suggesting that the treatment provoked the same response as did ABA. To better understand intracellular signaling events leading to freezing tolerance, we isolated mutant lines AR1 to 7 with reduced sensitivity to ABA by screening of protonema cells derived from UV-mutagenized protoplasts. Of these, protonemata of the line AR7 showed growth similar to that of wild type and were able to form gametophore and sporophyte. Unlike wild type, protonemata of AR7 did not stop their growth even when they were transferred onto a medium containing 10^{-5} M and were not able to acquire freezing tolerance. Amounts of ABA-induced transcripts were remarkably reduced while those of ABA-suppressed transcripts were increased in the AR7 cells. Furthermore, the AR7 cells were sensitive to short- and long-term treatment of hyper-osmotic stress. These results indicate that a signaling device necessary for transducing both ABA and osmotic stress signals might be impaired in the mutant cells.

Minami et al., *J. Plant Physiol.* 160: 475-483 (2003)
Nagao et al., *Phytochemistry* 67: 702-709 (2006)