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Transgenic strategy to improve drought tolerance in plants using a Rab gene from Sporobolus stapfianus

Crops such as maize are highly susceptible to dehydration creating yield loss for farmers during periods of drought. One approach to improve drought tolerance and sustain productivity during dry episodes is to introduce genes that control dehydration tolerance from plants that are naturally adapted to arid climates. An African grass, Sporobulus stapfianus, is a desiccation tolerant plant that can recover from the complete loss of water from its vegetative tissues. Earlier work has suggested that a specific gene, which encodes a Rab protein involved in membrane biogenesis, is active during both dehydration and in the recovery process following rehydration of Sporobulus stapfianus. Assuming that improved capacity for membrane biogenesis is an integral aspect of drought tolerance, we hypothesize that overexpression of the Rab gene in drought sensitive plants will improve tolerance to dehydration. Thus, the objective is to overexpress the Rab protein in two drought sensitive models, Physcometrella patens and Arabidopsis, to determine if a correlation can be drawn between Rab protein expression levels and dehydration tolerance. The Rab protein coding sequence was moved into a Gateway™ entry vector using a PCR based cloning procedure. From the entry vector, the Rab coding sequence was transferred into three destination vectors using the recombination properties of bacteriophage lambda. The vectors facilitated the formation of three different Rab genes; one driven by an ubiqitin promoter for high-level expression, one by a heat shock promoter for inducible expression, and another by the 35s promoter for moderate expression. The engineered Rab genes will be introduced into Physcomitrella patens and Arabidopsis via protoplast transformation and Agrobacterium directed gene transfer respectively. Correlations between RNA and protein expression levels and toleration of dehydration will be assessed at the cellular level with Physcomitrella patens and at the whole plant level with Arabidopsis.