#28: Analyzing Somatic Embryogenesis Gene Expression in Response to Tissue Culture Enhancer PLA1 Protein

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Advances in molecular biology and bioinformatics are now unraveling the basis for why 'conditioned media' aids tissue culture regeneration of plants. Extracellular Phytocyanin-Like-Arabinogalactan-1 or PLA1 protein has been shown to improve somatic embryogenesis (SE) in cotton (*Gossypium hirsutum*); Poon et al. (2012) applied GhPLA1 proteins to tissue culture media for a two-fold increase in embryogenic calli production. Our work focuses on production of orthologous PLA proteins and investigation of their respective efficacies as a media additive towards enhanced regeneration of recalcitrant plants through SE. Specifically, we are evaluating PLA1 protein based on 1) observed effects on embryo production in *Theobroma cacao* SE tissue culture and 2) the effects of protein treatment on SE marker genes such as BBM, LEC1, LEC2, AGL15, and FUS3. Understanding how PLA1 interacts with SE genes can give insight into the complex orchestration of gene expression during plant embryo development. The generality of many elements surrounding the SE tissue culture process should allow improvement across all plants. GhPla1 protein was applied to *T. cacao* by dripping protein solution as a way to use less protein than media addition, while achieving the same enhancement effects. This is consistent with our goal to implement exposure in temporary immersion bioreactor propagation systems. Preliminary results showed embryos were produced earlier in protein treated tissue and induced expression of SE transcription factor: BBM compared to non-treated tissue. Gene expression of BBM and other SE genes were analyzed via qPCR. In addition to GhPla1, we are developing PLA1 proteins from *Theobroma cacao*, *Oryza sativa*, and *Dioscorea rotundata* using recombinant *E. coli* protein expression. PLA proteins may prove to be a valuable addition to the in vitro toolbox (beyond plant hormones) for plant species that are difficult to transform or propagate.