Overcoming Recalcitrance of Genetic Transformation in Energycane for Improving Cold Tolerance and Biomass Yield

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Project Goals:

The Renewable Oil Generated with Ultra-productive Energycane (ROGUE) project aims to engineer the two most productive American biofuel crops, energycane and *Miscanthus*, to produce a sustainable supply of biodiesel, biojet fuel and bioproducts.

1) **Overcoming recalcitrance in tissue culture of energycane to establish an efficient genetic transformation protocol.**
2) **Over expression of pyruvate Pi dikinase in energycane to enhance biomass yield and cold tolerance.**

Abstract:

Energycane, an interspecific hybrid within the genus saccharum, has a superior photosynthetic efficiency, biomass accumulation and persistence in tropical and subtropical regions. In contrast to sugarcane, energycane has a high proportion of the ancestral species *Saccharum spontaneum* in its genome which contributes to higher tiller number, biomass yield and persistence in addition to a reduced stem diameter and sugar content. Pyruvate orthophosphate dikinase (PPDK) has been proposed as rate limiting enzyme in C4 photosynthesis. It regenerates the substrate phosphoenol pyruvate (PEP) for the initial carbon-fixation step. C4 plants are also severely limited by low temperature, possibly because PPDK is highly cold-labile and partially dissociates below 14 ºC. A previous study suggested that *Miscanthus x giganteus* achieves cold tolerance by increasing the amount of the enzyme rather than its intrinsic properties. Therefore, we decided to explore the over-expression of *Miscanthus x giganteus* PPDK in energycane under its native regulatory sequences. However, energycane is far more recalcitrant in tissue culture than sugarcane. Visual browning of the newly excised explants is a major hurdle that needs to be overcome to establish an efficient genetic transformation protocol for this target species.

Experiment I: We investigated effects of several supplements (e.g. anti-oxidants/anti-browning agents) in the tissue culture medium on visual tissue browning in energycane. Reduction in visual tissue browning, callus induction and regeneration response were evaluated to determine the optimal concentration and combination of these anti-browning agents when added to culture media. The combination of 2 to 3 anti-browning agents significantly reduced visual tissue browning while increasing the number of regenerating plantlets from energycane callus.

Experiment II: Calli were generated from five different genotypes of energycane in order to evaluate the genotype response to callus induction and regeneration. Two energycane genotypes with the highest callus induction and regeneration frequency were selected for biolistic transformation. Transgenic calli were regenerated on the media containing selection agent and regenerated plantlets were transferred to the soil. Independent transgenic events were confirmed by PCR.

Experiment III: In the present study, Miscanthus PPDK gene (*M-PPDK*) was introduced under its native regulatory sequences into energycane callus by biolistic gene transfer. The transgenic calli were regenerated on the media containing selection agent. The regenerated plants will be evaluated for the effect of *PPDK* overexpression on photosynthetic efficiency, cold tolerance and biomass accumulation.

References

Matsuoka S., Kennedy A.J., dos Santos E.G.D., Tomazela A.L., and Rubio L.C.S. 2014. Energy cane: Its concept, development, characteristics, and prospects, Adv. Bot., Article ID 597275.

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