Towards Oil Cane: Engineering Energycane for Hyperaccumulation of Lipids and Improved Agronomic Performance

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Project Goals:

Renewable Oil Generated with Ultra-productive Energycanes—or ROGUE—is engineering the two most productive American crops—energycane and Miscanthus—to produce a sustainable supply of biodiesel, biojet fuel, and bioproducts.

Project goals are to:
1) Overcoming recalcitrance in tissue culture and genetic transformation of energycane.
2) Engineer energycane to produce an abundance of lipids in the form of triacylglycerol which can be converted into biodiesel, biojet fuel, and bioproducts.
3) Altered expression of flowering genes, pyruvate Pi dikinase and proteins involved in chloroplast division in energycane to enhance biomass yield and cold tolerance.

Abstract

Metabolic engineering towards hyper-accumulation of lipids [e.g. triacylglycerol (TAG)] in the vegetative tissues of high biomass yielding crops is a new strategy to improve lipid yields for biofuel production. Energycane is an ideal feedstock for this approach due to its superior biomass production and persistence. However, energycane is among the most recalcitrant crops in tissue culture, impeding its genetic transformation.

Visual browning of the newly excised energycane explants is a major hurdle that needs to be overcome to establish an efficient genetic transformation protocol for this target species. We investigated effects of several culture media supplements (e.g. anti-oxidants/anti-browning agents) in the tissue culture medium on visual tissue browning in energycane. The combination of 2 to 3 anti-browning agents significantly reduced visual tissue browning while increasing the number of regenerating plantlets from energycane callus.

Plant lipid is one of the most energy-rich and abundant forms of reduced carbon available from nature. A multigene expression construct was used to elevate the production of free fatty acids, catalyze their conversion into TAG and prevent TAG hydrolysis. This construct was transferred into energycane callus, using the biolistic particle delivery system. Presence of transgenes in the regenerated plants were confirmed by PCR.

Lipid yield per land area from high biomass crops like energycane is determined by the lipid concentration in the biomass, the total biomass yield and the extractability of the lipids from the biomass. Flowering of energycane is expected to affect oil yield and the extractability of oil. Upon flower induction vegetative growth ceases and sucrose/oil that has accumulated in the stalks is re-mobilized for use in reproductive development. Often flowering also leads to dehydration of the stalk tissues, which negatively affects stalk density, and also compromises sugar extraction in conventional sugarcane or lipid extraction in metabolically engineered lipid cane. Therefore, we recently generated transgenic energycane plants harboring a construct for RNAi mediated suppression of multiple flowering genes. Since energycane is vegetatively propagated for establishment of plantings, suppression of flowering will not require an altered agronomic practice while improving the biosafety of the engineered crop.

Genetic improvement of photosynthetic efficiency could potentially be achieved by developing a photosynthetically more effective canopy. To evaluate the effect of chloroplast size on light penetration into the canopy and biomass production, we intend to modify the expression of the cytoskeletal Filamenting temperature-sensitive Z (FtsZ) protein, which is critical for chloroplast division. Overexpression and RNAi constructs of FtsZ were introduced into energycane callus and regenerated through somatic embryogenesis. 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. Therefore, we decided to explore the over-expression of Miscanthus x giganteus PPDK in energycane. MxgPPDK with its native regulatory sequences were introduced into energycane callus by biolistic gene transfer. The regenerated plants will be evaluated for the effect of PPDK overexpression on photosynthetic efficiency, cold tolerance and biomass accumulation.

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