Development of Sustainable Transformation of *Miscanthus* × *giganteus* to Improve Photosynthesis

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Project Goals

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

The main objectives of this work are:

1) To improve the conversion of sunlight into plant biomass/metabolites through photosynthesis without the need for increased quantities of either water, or fertilizer.
2) To transfer ROGUE technologies from the lab bench to crops through an efficient pipeline.

Abstract

Improving photosynthetic efficiency of bioenergy crops, such as *Miscanthus* × *giganteus* and energycane, would provide sustainable sources of energy and bioproducts to achieve zero net greenhouse gas emission in future. These highly productive C_4 crops utilize the NADP-ME type C_4 photosynthesis. In this type of photosynthesis, the amount of pyruvate orthophosphate dikinase (PPDK) and rubisco limit the regeneration of phospho*enol*pyruvate (PEP) (Long *et al.*, 2013, Wang *et al.*, 2008, Naidu *et al.*, 2003). Research also demonstrated improvement in photosynthetic efficiency under fluctuating light when photoprotection response time is accelerated by overexpression of genes involved in non-photochemical quenching (NPQ): zeaxanthin epoxidase (ZEP), violaxanthin de-epoxidase (VDE) and Photosystem II subunit S (PsbS) (Kromdijk *et al.*, 2016). We propose that the overexpression of PPDK will alleviate rate limitation in C_4 photosynthesis, while increasing expression of NPQ-related genes can improve photosynthetic efficiency in *Miscanthus*. Although *M.* × *giganteus* is an ideal candidate of bioenergy crop with minimal invasive potential, the propagation of this feedstock is limited by the sterility of the plant due to its triploid genome (Boersma & Heaton, 2014). Biolistic transformation of *Miscanthus* is generally performed on calli induced from immature inflorescences collected during the summer (Sobańska *et al.*, 2019). However, this is a limitation to develop a continuous transformation system and has been a bottleneck of our effort to understand the effect of overexpressing the photosynthetic genes. In this study, we used calli induced from immature inflorescence and stem

meristemic tissue of *M.* × *giganteus* for biolistic transformation and performed a proof-of-concept experiment using fluorescent protein construct to check the efficiency of the systems. Preliminary data suggested that the transformed calli from stem meristemic tissue could be transformed and regenerated. These two embryogenic callus induction systems would provide a continuous source of calli for transformation to study the effect of photosynthetic genes, such as PPDK, VDE, ZEP and PsbS in *Miscanthus*.

References / Publications

Long SP, Spence AK (2013) Toward Cool C_4 Crops. *Annu Rev Plant Biol* 64(1):701–722.

Wang D, Naidu SL, Portis AR, Moose SP, Long SP (2008) Can the cold tolerance of C_4 photosynthesis in *Miscanthus* × *giganteus* relative to *Zea mays* be explained by differences in activities and thermal properties of Rubisco? *J Exp Bot* 59(7):1779–1787.

Naidu SL, Moose SP, Al-Shoaibi AK, Raines CA, Long SP (2003) Cold Tolerance of C_4 photosynthesis in *Miscanthus* × *giganteus*: Adaptation in Amounts and Sequence of C_4 Photosynthetic Enzymes. *Plant Physiol* 132(July):1688–1697.

Kromdijk J, et al. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* 354(6314):857–861.

Boersma NN, Heaton EA (2014) Propagation method affects *Miscanthus* × *giganteus* developmental morphology. *Ind Crops Prod* 57(M):59–68.

Sobańska K, et al. (2019) Optimised expression cassettes of hpt marker gene for biolistic transformation of *Miscanthus sacchariflorus*. *Biomass and Bioenergy* 127:105255.

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