Sustainable Improvement of C4 Photosynthesis in Bioenergy Grasses

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Project Goals

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

1. Increasing oil accumulation and targeting this to the mature stem
2. Increasing photosynthetic efficiency to power oil synthesis
3. Multi-gene construct transformation of energycane and Miscanthus
4. Field testing, processing and techno-economic analysis

Abstract

Bioenergy feedstock, such as energycane (*Saccharum* spp.) and miscanthus (*Miscanthus* × *giganteus*), are some of the NADP-ME type C4 perennial grasses adapted to grow in marginal croplands which could be engineered to improve their photosynthetic efficiency to increase yield (Mitchell et al. 2016). These grasses can then be grown in marginal croplands to avoid the need to utilize more land for agriculture (Wang et al. 2021, Mitchell et al. 2016). Regeneration of phospho*enol*pyruvate (PEP) in the NADP-ME type of C4 photosynthesis is limited by the activity of pyruvate orthophosphate dikinase (PPDK) and rubisco (Long et al., 2013, Wang et al., 2008, Naidu et al., 2003). In order reduce the bottleneck effect of PPDK, we hypothesized that increased expression of PPDK in bioenergy crops could improve C4 photosynthesis. Research also shows that photosynthetic efficiency could be improved under fluctuating light when a faster photoprotection response time is observed by overexpressing violaxanthin de-opoxidase (VDE), photosystem II subunit S (PsbS) and zeaxanthin epoxidase (ZEP) (note as VPZ hereafter) involved in non-photochemical quenching (NPQ) (Kromdijk et al. 2016).

Using the particle bombardment-mediated transformation approach, synthetic constructs of *MxgPPDK* and *SbPPDK* were transformed into energycane at the University of Florida and into miscanthus at the University of Illinois at Urbana-Champaign. A total of 3 transgenic energycane lines of overexpressed *MxgPPDK* gene

and its corresponding wild type were grown in the greenhouse and photosynthetic measurements of 8- to 12-week-old plants showed increased V_{pmax} (the rate of PEP carboxylation). Field trial data of MxgPPDK transgenic lines are currently being analyzed. Preliminary measurements of the photosynthetic efficiency of 29 SbPPDK transgenic lines are being carried out in the greenhouse.

Calli induced from immature inflorescence of *Miscanthus* × *giganteus*, were transformed with synthetic constructs bearing *VPZ* or *PPDK* and several putatively transformed plants obtained. PCR genotyping of plants transformed with VPZ constructs using selection marker-specific primer pairs to detect the presence of BAR, NPTII or oHPT selection markers yielded 18, 9 and 29 positive lines out of 62, 114 and 46 putative transformants, respectively. Over 100 plants putatively transformed SbPPDK constructs in 2020 are currently being genotyped. Photosynthetic measurements of the miscanthus transgenic lines will be made in summer 2022.

References/ Publications

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