

## **Biolistic Transformation of *Miscanthus* Species. Preliminary Studies.**

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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.**

**The aim of this work is to engineer energycane and *Miscanthus* to produce an abundance of natural oil that can be converted into biodiesel, biojet fuel, and bioproducts.**

### **Abstract:**

The *Miscanthus x giganteus* has proved a highly productive and cold tolerant C4 biomass feedstock. As a sterile interspecific hybrid, it lacks genetic variation. Bioengineering offers the opportunity to add desirable traits to this productive chassis, such as genes for triacylglyceride accumulation and improved photosynthetic efficiency. Since the crop is clonally propagated, once successfully transformed, the issues that face seed propagated plants in moving to farm-ready propagules, such as copy number, segregation, silencing and breeding into elite cultivars are by-passed.

Three plasmid vectors were used for transformation. The vector pCAHGA (optimized pCAMBIA 1201) with the *ohpt* marker gene with codon from monocots under the Ubi gene promoter from *Zea mays* (GenBank JX947345.1), and the reporter gene *uid* (GUS) under the Act1 promoter from *Oryza sativa* (GenBank S44221.1). Two other transformation vectors, engineered to encode genes known to modulate the photoprotection pathway in plants were also used [1, 2].

The *Miscanthus* transformation was carried out using the microparticle bombardment (biolistic method) established by Bioengineering Team from Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland.

Callus from *Miscanthus sinensis* and *M. x giganteus* was induced from immature inflorescences on the C-17 medium (with 5 mg/l 2,4-D, 0.5 mg/l BAP and 90 g/l maltose). After 8-10 weeks callus was transferred on the osmotic medium (MIC with 36.4 g/l sorbitol and mannitol each) 24 hours before transformation. Biolistic® PDS-1000/He gene gun and 1.0 µm gold particles were used for transformation. The distance between the stopping screen and target cells was 9 cm. Plant regeneration were conducted on 190-2 medium with 0.5 mg/l KIN and 0.5 mg/l NAA and 200 mg/l of kanamycin or 5 mg/l hygromycin B [3].

Currently, molecular analyses are carried out to check the presence of the transgene in the obtained plants.

### References

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- [2] Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., and Long, S. P. 2016. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science (New York, N.Y.)* 354, 6314, 857–861.
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