“Building on success in systems design of high yielding low-input energycanes for marginal lands” OR “Renewable Oil Generated with Ultra-productive Energycanes (ROGUE)”

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Project goals

Combining functional genomics and metabolic modeling, through DOE ARPA-E support, we identified and up-regulated key genes for TAG synthesis and transport and down regulated those involved in TAG catabolism to force accumulation of TAG in sugarcane within a modified oleosin coating in a Florida cultivar of sugarcane. Because oil contains far more combustion energy per unit mass than both structural and non-structural carbohydrates, we similarly identified and engineered increased photosynthetic capacity to provide the additional energy required to support lipid synthesis without loss of total biomass. In sugarcane we achieved 10% increases in total productivity and up to 8% tri-acyl glycerides (TAGs) in the leaves. This project now aims to further advance these technologies in Energycane and *Miscanthus x giganteus,* which is also something termed an energycane. Why have we chosen these two crops? They are: 1) Technologically farm- and factory-ready. That is, technologies for planting, growing, harvesting, transporting and crushing the juice, including extraction of oil, from their stems are well established (we have patented an efficient method for extracting this lipid from grass stems). 2) The most productive crops known, for the sub-tropical and temperate zones of the USA, respectively, in terms of their ability to convert sunlight energy into the chemical energy stored in the plant. 3) Energycane is highly productive on marginal soils in the Gulf States; Miscanthus can be grown on such soils from the north of the Gulf States into eastern Canada, so avoiding competition with food crop production and opening up many thousands of acres to under-utilized land to profitable use. 4) Both energycane and Miscanthus are closely related and share much of their genetic code with sorghum, allowing reference to this now detailed structural and functional genomic database. Having this template greatly speeds the pace at which genetic changes will be engineered. 5) Neither crop requires good soil or much fertilizer and they use water more efficiently than grain crops. 6) Both are easily contained under field conditions because they do not produce viable seeds under continental US field conditions, making them biosafe for transgenic modification. 7) As non-food crops, biolistically transformed, deregulation should not be required, facilitating quick commercial uptake. 8) Both crops are clonally propagated and since we are transforming elite US cultivars, the transformed plants will provide commercially viable material without the requirement of any further breeding.

At their exceptional levels of productivity, engineering energycanes and Miscanthus to accumulate vegetable oils (tri-acyl glycerides) to 20% of dry weight will provide ≥17x the amount of oil per acre than can currently be obtained from soybean, and 50x that of Camelina. With ~2,400,000 acres of marginal, under-utilized or abandoned cropland in the SE and E of the USA (Figure 1) this could provide sufficient diesel and jet fuel to satisfy >66% of the nation’s current use.

These crops will be biolistically engineered with multi-gene constructs for both oil accumulation in their vegetative tissues and increased photosynthetic capacity to provide the energy for oil synthesis. This will be achieved by: 1) Continued development of our mathematical models allowing *in silico* optimization and engineering of photosynthesis and of oil synthesis/accumulation to guide *in vivo* genetic transformation. 2) Further development of our energycane and Miscanthus biolistic transformation systems to deliver higher efficiencies and speeds, including DNA editing. 3) Expansion of our Golden Gate libraries for the development of multi-gene constructs, which will be used in combination with re-transformation strategies. 4) Increased activity of tri-acyl glyceride (TAG) synthesis through up-regulation and addition of genes involved in synthesis, including up-regulation of transcription factors and editing, RNAi knock-downs of TAG catabolic pathways, and accumulation of modified protein coats to protect the oil globules. 5) Redirection of sucrose within the parenchyma storage cells, via modification of transporters (up- and down-regulation) to fuel TAG synthesis in addition to targeting of TAG accumulation to storage parenchyma. 6) Computationally guided engineering improvement of photosynthetic efficiency, including genetic modification of the crop canopy, leaf structure and metabolism to increase efficiency of light, water and nitrogen use. 7) Further development of our transient expression systems for testing and selecting best constructs for stable transformation. 8) Field testing in FL, MS and IL of resulting germplasm. Overall goal to increase photosynthetic efficiency by 50% and accumulate oil to 20% of shoot biomass, and provide farm-ready germplasm.

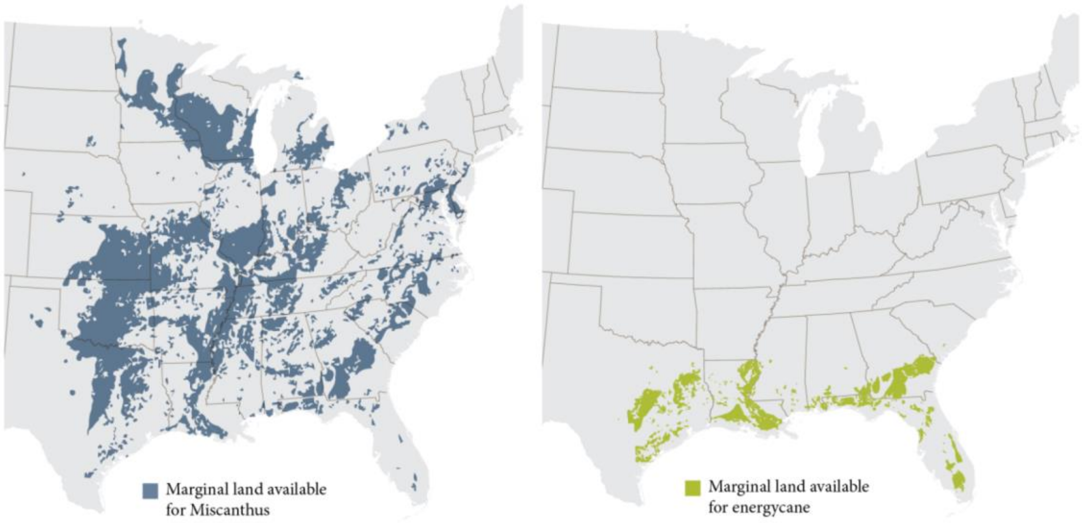

Figure 1. Marginal land availability for energycane and Miscanthus