



**CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY**

Name(s) Nicolas Laqua; Annam Tran	Project Number 36339
Project Title Novel Transfection of Macroalgal Azolla and Lemna Mediated by Agrobacterium to Elevate Neutral Lipid Biofuel Production	
Objectives/Goals Abstract This affiliated research delves into genetic modification of the aquatic duckweeds <i>Azolla caroliniana</i> and <i>Lemna minor</i> in order to elevate triacylglycerol, diacylglycerol, wax ester, and other long chain lipid production for the purpose of biofuel production. These macroalgal duckweeds <i>Azolla</i> and <i>Lemna</i> were selected for a magnitude of reasons, including reduced difficulty of reliable harvest and rapid growth employed by both <i>Azolla</i> and duckweed. In order to catalyze potential biofuels, we transferred our gene of interest, bifunctional wax ester synthase/acyl-CoA diacylglycerol acyltransferase or <i>atfA1</i> , the key to biosynthesis of storage lipids in <i>Acinetobacter</i> sp. strain ADP1 located within the enzyme WS/DGAT, into <i>A. tumefaciens</i> strain pET21a-IfeR to allow catalyzation of triglycerides and WE from DAGs and fatty alcohols: Our research isolated and transformed <i>A. baylyi</i> ADP1's WS/DGAT into modified <i>Agrobacterium tumefaciens</i> pET21a-IfeR, acting as a vector for our <i>goi</i> , and isolated from <i>Acinetobacter baylyi</i> ADP1 via gel purification after electrophoresis and restriction digest. <i>A. tumefaciens</i> then transfected WS/DGAT into <i>Azolla</i> via novel spore propagation, and duckweed via calli induction. Upon utilizing <i>Agrobacterium tumefaciens</i> novelly for modification of <i>Azolla</i> , and performing modification of <i>Lemna</i> via calli induction and exposure to <i>A. tumefaciens</i> , both species' fatty acid production was statistically elevated. The product of processed duckweeds was utilized after solvent-lipid fsaa extraction; we converted our extracted lipid into functional biodiesel by transesterification. TAG production elevated an average 10% in the transfected <i>L. minor</i> samples; 7% in treated <i>Azolla</i> samples. Functional biodiesel production was obtained from the rapidly growing <i>azolla</i> and duckweed in the form of neutral lipids at a rate deemed "percent efficiency" of production efficiency: Dry weight percentage of long chain neutral lipids usable in biodiesel. The term "percent efficiency" (pe) models the elevation of fatty acids, increasing from 10.1 to 18.7 and 14.2 to 16.8, (<i>Lemna</i> / <i>Azolla</i> respectively). Based upon our experiment, <i>A. caroliniana</i> , and to a greater extent <i>L. minor</i> , are vectors for elevated fatty acid production, serving as a backbone for continued research into renewable, reliable, carbon negative biodiesel production from fast growing macroalgal sources.	
Summary Statement Through novel processes we transfected two species of macroalgal duckweed, <i>Azolla caroliniana</i> and <i>Lemna minor</i> , in order to elevate long chain lipid production for the purpose of biodiesel production.	
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