Enhancing energy density in crop biomass by redirecting photosynthate into triacylglycerols in vegetative tissues

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Abstract: The GLRBC plant oil project aims at increasing the content of high energy compounds in vegetative tissues of plants. The concept of enhancement of energy density in plant biomass is synergistic with other GLBRC efforts to develop lignocellulosic feed stocks for biofuel. Enhancing the energy yield of plant biomass can be achieved by accumulating energy-dense compounds such as triacylglycerol (TAGs).

The plant oils group of GLBRC is testing if carbon partitioning can be redirected from starch to oil in plant biomass. We have engineered *Arabidopsis* plants to overproduce the transcription factor WRI1 that controls oil accumulation in seeds, and we have reduced starch biosynthesis by RNA interference (RNAi) of ADP-glucose pyrophosphorylase (AGPase) expression. The resulting transgenic lines accumulated less carbohydrate and produced up to 1% oil per DW in the vegetative tissues. The relative contribution of TAG compared to starch to the overall energy density increased in the AGPRNAi-WRI1 double transgenic line. In addition, these transgenic Arabidopsis lines resulted in the accumulation of 10% oil per DW when grown on a medium supplemented with 3% sugar. Heterologous expressions of a type 2 diacylglycerol acyltransferase (DGTT2) from *Chlamydomonas reinhardtii* in Arabidopsis transgenic lines also led to the accumulation of TAG in seedlings and mature leaves. The abundance of oil droplets and very long chain fatty acid (VLCFA) in the mature plants was confirmed by ESI-MS. Gene stacking of 35S-DGTT2 and AGPRNAI-WRI1 increased oil content close to 2% per DW in the Arabidopsis seedlings. These transgenic lines accumulated up to 14% oil per DW when medium was supplemented with 3% sugar. Transgenic rutabaga lines expressing the AGPRNAI-WRI1 (double gene) construct (T_1 transgenic rutabaga plants) accumulated up to 5% oil per DW in soil grown leaves as measured by ESI-MS.

In addition, we used biodiversity and EST sequencing to discover a novel acyltransferase gene EaDAcT (*Euonymys alatus d*iacyl*glycerol a*cetyl*t*ransferase) from burning bush that produces acetyl-glycerols. These novel oils are low viscosity and therefore can be used directly in some diesel engines. Expression of EaDAcT in *Arabidopsis* seeds resulted in the accumulation of acTAGs, up to 65 mol % of total TAG in the seed oil. The development of novel strategies to address compartmentalization of oil metabolism by metabolic flux analysis is in progress.