Expanding the contribution of plant oils as biofuels

The seeds of green energy

John Ohlrogge (Michigan State University, USA) and Kent Chapman (University of North Texas, USA)

Plant oils represent one of the most energy-rich sources of renewable fuels available in Nature. Most of these oils occur in the form of triacylglycerols (TAGs) that can be transformed into biodiesel by conversion of their acyl chains into fatty acid methyl esters. In 2009, 14 billion litres of biodiesel were produced worldwide from plant oils (largely in the EU). This compares with 70 billion litres of ethanol (largely from Brazil and the USA). Both of these fuels now depend on land and crops (e.g. oil seeds, palm trees, maize and sugar cane) that are also used for foods. To meet growing demand and avoid competition with food, major expansion of biofuel production and development of new sources of biofuel are required. In this article, we outline how plants synthesize oils and describe some ways in which supplies of oils from plants could be increased to provide a larger contribution to renewable energy supplies.

The major biofuels available today, ethanol and biodiesel currently contribute only a small fraction of the world transportation fuel consumption of approximately 3000 billion litres. The annual world production of vegetable oils was 144 billion litres in 2009, most of which is used for food. From this supply, production of biodiesel from plant oils provides ~5% of diesel fuel in Europe. For comparison, in the USA, ethanol (largely from maize) contributes ~6% of the US petrol (gasoline) volume. For the USA, production of renewable fuels is targeted to increase 3-fold and reach 137 billion litres (36 billion gallons) by 2022 and similar increases are targeted for the EU. To meet this growing demand and avoid competition with food, major expansion of biofuel production in combination with development of new sources of biofuel will be required.

Because cellulose and hemicellulose and lignin from plant cell walls are the most abundant forms of terrestrial biomass, most research to increase biofuel supplies has focused on conversion of these polymers into liquid fuels that can substitute for petroleum. However, plant oils have several advantages over ‘lignocellulosics’ as feedstocks for liquid fuels. First, these oils have twice the energy content per kg compared with carbohydrates and 25% more energy than ethanol. Secondly, they do not require fermentation for conversion into liquid fuels, but rather can be extracted and converted into biodiesel fuel with lower energy inputs and capital costs and with substantially higher net energy output and greenhouse gas reduction. Thirdly, their highly reduced carbon chains are compatible with diesel engines that are 30% more efficient than petrol engines. Despite these advantages, plant oils have received far less consideration than lignocellulosics as a future source of bioenergy. A major reason is the currently limited supply of suitable plant oils, most of which is now used for food. Can plant oil production be expanded for use as biofuel? Below we briefly describe how plants synthesize oils and we outline strategies that could increase their production in the future.

Plant lipid biosynthesis

Plants and algae synthesize and store fatty acids mostly as TAGs through co-operation between two cellular compartments: plastids and the endoplasmic reticulum (ER). Fatty acids are synthesized de novo in the plastids and then are exported and assembled into TAGs in the ER. The enzymatic machinery for TAG synthesis overlaps that of membrane glycerolipids, and the basic steps of this pathway are similar in plants and other eukaryotes. The initial step that commits carbon to fatty acid synthesis begins with acetyl-CoA carboxylase, an enzyme that forms malonyl-CoA from acetyl-CoA and bicarbonate. Fatty acids are then assembled in two-carbon increments by the fatty acid synthase (FAS) complex where acetyl units donated from malonyl-CoA are added sequentially to an elongating acyl chain tethered to an acyl carrier protein (ACP).

After assembly by FAS, C₁₈ fatty acids may be released from the ACP by fatty acyl-ACP thioesterases (FAT). This can occur before or after introduction of a double bond thereby yielding saturated or mono-unsaturated C₁₈ fatty acids, respectively. Chain elongation to acyl-ACP may also be terminated earlier in the FAS cycle by FAT yielding saturated C₁₆ fatty acids. Some plants which store medium and short-chain fatty acids have specific FATB isoforms...
selective for shorter acyl-ACPs, and thus FATB specificity is a major regulatory point for determining acyl chain length in plant lipid synthesis. Furthermore, the FATA isoform generally has preference for mono-unsaturated fatty acids, whereas saturated acyl-ACPs are hydrolysed by FATB-type isoforms. Since polyunsaturated fatty acid synthesis that occurs in the ER utilizes only the mono-unsaturated fatty acids supplied from the plastid, the relative activities of FATA and FATB in the plastid indirectly regulate the composition of polyunsaturated fatty acids in TAGs as well. Additionally, the β-ketoacyl ACP synthase II (KASII) isoform specifically catalyses the elongation step from C_{16:0}-ACP to C_{18:0}-ACP, and stearoyl-ACP desaturase (SAD) then converts stearoyl-ACP into oleoyl-ACP. Thus the relative activities of KASII and SAD together with FATB and FATA determine the chain length and proportion of saturated and C_{18:1} fatty acids leaving the plastid for TAG assembly in the ER. Because the chain length and level of unsaturation of fatty acids are the main determinants of the viscosity, volatility and oxidative stability of plant oils, the plastid-localized enzymes described above play a key role in determining the suitability of plant oils as liquid fuels. Genes for these enzymes or specific variants have been utilized widely in the successful engineering of designer oils in crop plants.

The fatty acids produced by the plastid reactions described above are next transported to the ER as acyl-CoA esters. The assembly of three fatty acids into TAG first involves two sequential acyl-CoA-utilizing acyltransferases that esterify the sn-1 and sn-2 positions of glycerol 3-phosphate to synthesize phosphatidic acid (PA). PA is hydrolysed by PA phosphatase to form diacylglycerol (DAG) which is converted into TAG by a DAG acyltransferase (DGAT) or by transfer of an acyl group from phosphatidylcholine (PC). Polyunsaturated fatty acid synthesis in the ER occurs almost exclusively on PC. Considerable acyl exchange also occurs between the phospholipid and DAG pools leading to TAG synthesis. The acyl composition of the DAG intermediate and the TAG that is stored are determined by the available pool of exported acyl-CoAs from the plastid, the substrate specificities of the acyltransferases, and by acyl desaturation and editing of PC in the ER. The regulation of flux between the PC, acyl-CoA, DAG, PA and TAG pools is incompletely understood and represents an important area for future research.

In oilseeds, TAGs are rapidly synthesized during seed maturation, and the biosynthetic enzymes are under genetic regulation in combination with pathways, leading to accumulation of other seed storage reserves (i.e. protein and carbohydrate). A few key transcriptional regulators of TAG accumulation have been identified including WRINKLED1 (WRI1) and these work with seed maturation pathways that also prepare the seed for desiccation. After synthesis in seeds, TAGs are packaged into oilbodies of relatively uniform size that have a phospholipid monolayer and major structural proteins called oleosins that coat the lipid droplet surface.

The prevailing landscape for world vegetable oil production

Of the oil-producing crops currently grown worldwide, oil palm, soya bean, oilseed rape and sunflower...
represent the four largest contributors. Most oilseeds accumulate 20–50% oil by weight, but the mesocarp surrounding seeds of oil palm can reach 90% oil by weight. Oil palm is now the most productive world oil crop, contributing over 35% of world plant oil while occupying only 5% of area devoted to oil crops. In the last 20 years, palm oil production has more than quadrupled largely because of its high yield (up to 10 metric tons/ha), low cost of production, and growing demand. Currently about three-quarters of the palm oil enters food markets and the rest is used for industrial or biofuel purposes. With current world production at more than 50 billion litres of oil, palm represents a formidable potential source of bioenergy. Indonesia, in particular, has invested recently in new plantations, but Africa, India, and South America represent areas where palm oil production may also increase substantially. However, concerns have been expressed over expansion due to ecological damage from deforestation and conversion of tropical forests into agricultural plantations.

Furthermore, as is the case with all vegetable oil crops, palm oil demand is increasing, driven by population growth and increased consumption as food by emerging economies. Consequently, major increases in plant oil production must be made in a rational sustainable fashion, to support increasingly competitive needs of the world for both food and fuels.

**Methods to increase plant oil production**

While expanded acreage of oil palm in the tropics may be one realistic means to influence future plant oil supplies, several other opportunities are also under development. These include genetic engineering to enhance seed/fruit oil production, dedicated alternative oilseed crops for bioenergy uses, production of oils in vegetative tissues of crops, and utilization of algal or microbial processes that would not compete with arable land use. It is likely that solutions will not be found with an individual avenue, but rather will require a portfolio of combined strategies over various time horizons that, along with improved efficiencies in energy usage, will facilitate a reduced dependence on fossil-based carbon sources. Although it is not possible to cover all strategies under development, a few examples are provided to emphasize the potential for increasing plant oils.

**Efforts to boost oils from seeds and fruits**

Genetic engineering of oil palm has proven difficult and not yet reached commercial success. In contrast, most other major oil crops have been engineered. Strategies to increase TAG accumulation in their seeds have capitalized on the inherent capacity of plant reproductive tissues to produce large amounts of storage reserves for progeny utilization. Although increasing oil in seeds of crops or model plants has been reported using many strategies, we mention two approaches that have been documented to be successful in multiple field trials and without compromise in crop yields.

An early and key success in increasing seed oil content in soya beans was obtained by overexpression of the last acyltransferase enzyme in TAG biosynthesis (DGAT). This approach increased yields of oil per ha by 7.5%, which, at current world production levels, would represent over 3 billion litres of additional soya bean oil with an added value of approximately US$4 billion. In addition to this example of targeting a specific enzyme in the TAG pathway, seed oil has also been increased by manipulating expression of the WRI1 transcription factor that regulates multiple enzymes in glycolysis and fatty acid synthesis. Overexpression of WRI1, first shown to enhance *Arabidopsis* seed TAG levels, has now increased oil in maize by more than 25%. If applied to all world maize crops, plant oil production could be increased by approximately 6 billion litres. Whereas the above examples prove that very profitable increases in seed oil yields can be obtained, it is clear that new strategies will be needed if agriculture is to provide oils that can contribute more than a minor fraction of world fuel needs.

**Alternative sources of plant oils**

One new strategy for the production of oils by agricultural production may be to elevate oil content in tissues where oil does not normally accumulate, for example leaves and stems. If this can be accomplished with very-high-yielding dedicated energy crops such as *Miscanthus* or switchgrass (Figure 3), impressive increases in oil production could occur. Most plant cells have the capacity to synthesize TAGs, and, to a limited extent, TAGs are found in all plant tissues. However, TAGs normally do not accumulate to significant percentages in vegetative tissues, despite this metabolic capacity. There are several notable exceptions to this generalization (such as fruit mesocarp of olive, avocado and palm, the tuber tissues of nutsedge and the stem tissues of Mongolian oilwood), and this suggests that the production of oil in vegetative tissues may be a realistic strategy. Understanding more about the regulation of TAG synthesis and accumulation in non-seed tissues could provide important new strategies to alter the carbon-partitioning balance in
biomass to favour the accumulation of oil in addition to or in place of carbohydrate. Producing biomass with 10% oil on a dry weight basis could have a major positive impact on the recovery of energy from dedicated biomass crops. For example, average yields of Miscanthus of 22 metric tonnes dry weight per ha are achieved in Illinois and would yield 150 GJ of energy/ha if converted into ethanol (at 320 litres of ethanol/tonne of biomass). If this harvestable biomass included 10% TAG (or other hydrocarbons), the energy yield from the crop would be increased by 30–40% and oil yields per ha would exceed oilseed rape at least 2-fold. If the ~12 million ha of maize now grown in the US for ethanol were instead planted with such a crop, 24 billion litres of biodiesel could be produced from a non-food crop. Whether such a crop is processed for diesel fuels before fermentation of the remaining lignocellulose, or burned directly to produce bioelectricity, a significant increase in energy yield can occur from producing oil in vegetative tissues of biomass crops even if only 10% of dry weight.

Is such engineering of energy crops feasible? It should be noted that most plant leaves already contain 5% fatty acid by weight, in the form of less easily recovered polar membrane lipids. Recent research suggests that overexpressing seed transcription factors (LEC2, WRI1) or up-regulating the synthesis of TAG (via DGAT) or blocking the turnover of TAG can all increase the amount of oil accumulating in vegetative tissues. To date, the oil contents achieved have been low, and it is likely that a combination of strategies will be necessary to result in the accumulation of 10% or more TAG in dedicated biomass crops. An additional biodiesel source under intense development is algae which could produce lipids for biofuel without competition for arable land for food production. In theory, this is an attractive proposition because yields on a per-area basis could outcompete conventional cropland. Many strategies have been proposed and considerable efforts have intensified to exploit algal (and cyanobacterial) systems for large-scale oil production. Growing algae in open pond or raceway systems, although the most economical means, has the caveat of developing mixed species populations that may be difficult to manage for reproducible yields of defined oil products. Alternatively, large-scale photobioreactors of high-yielding single-species cultures may offer more predictable outputs, but are less favourable from an economic standpoint for large volumes of liquid transportation fuels. Perhaps bioreactors may be most important for higher value, speciality chemical feedstock production (for example, for lubricants). In any case, algal systems for the production of renewable carbon feedstocks represent a future approach from a technical standpoint, but with economic challenges. However, major ongoing genomics and other efforts are likely to identify spe-

Figure 3. Miscanthus sinensis
cific species, growth conditions or metabolic targets that can be manipulated to improve biofuel production in aquatic environments.

**Future oils designed for wider uses**

The conversion of TAG into fatty acid methyl esters to produce biodiesel is required to reduce the viscosity and melting point of the oil. This process adds at least 10% to the cost of biodiesel. One strategy that achieves lower viscosity through metabolic engineering may be production of acetyl-TAG. These structures possess a two-carbon (rather than a long-chain) acyl group at the sn-3 position of TAG which results in a viscosity similar to diesel #4 grade without the need for conversion into fatty acid methyl esters. Levels of 40% of acetyl-TAG content in Arabidopsis seeds have recently been achieved. Plants have also been engineered to produce medium-chain fatty acids with 8–12-carbon chain lengths. These saturated fatty acids are more stable than the usual unsaturated plant oils and the shorter chain length also reduces viscosity. Eight-carbon chain lengths would be highly compatible with petrol, and of particular interest for jet fuel. Another recent opportunity has emerged from the discovery of two genes from cyanobacteria that together produce alkanes and alkenes from intermediates of fatty acid synthesis. These very stable and energy-rich structures with 13–17-carbon chain length have been produced in fermentations of E. coli expressing the cyanobacterial genes. If also successfully introduced into plants or high-oil-producing algae, the potential for a less costly non-fermentative route to highly desirable alkanes may become feasible.

In summary, together with fermentation of lignocellulosics, plant oils should be included as an important component of a sustainable vision for bioenergy. But, as with most other bio-based fuel sources, new developments are required to provide abundant supplies that are balanced with growing worldwide food requirements.

Biofuel research in the J.O. laboratory is supported by US Department of Energy–Great Lakes Bioenergy Research Center Agreement DE-FC02-07ER64494, and in the K.C. laboratory by Department of Energy agreement DE-SC0000797.

John Ohlrogge is Professor of Plant Biology at Michigan State University. For most of his career, he has studied plant lipid metabolism, with a focus on regulation of fatty acid biosynthesis. Research has been carried out in oilseeds and also in leaves and other tissues. Approaches have included biochemistry, in vivo labelling, microarrays, and expressed sequence tag (EST) sequencing. Together with Mike Pollard, John has also studied the biosynthesis of the extracellular lipid polymers, cutin and suberin.

Kent Chapman is Professor of Biochemistry at the University of North Texas. Research in the Chapman laboratory is focused mostly in the areas of lipid signalling and lipid compartmentation in plants. Experimental emphasis ranges from enzymology and lipid identification to cellular and molecular studies of lipid pathway function. New ideas about the conservation and divergence of lipid metabolism and function in eukaryotes have emerged from these efforts.

**References**