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Engineering Oilseeds for Industry

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Over the last century the world has become dependant on fossil fuels as the primary source of hydrocarbons for energy and industry. These resources are finite and non renewable and their exploitation releases massive amounts of carbon dioxide into the atmosphere. The identification of sustainable replacements has been an ongoing area of research for many years. Plant triacylglycerol (TAG) oils have shown considerable potential to replace petrochemicals in a wide variety of applications ranging from fuel and lubricants to the manufacture of polymers, surfactants, and surface coatings. Their utilization by these industries has however been limited by a number of factors, the most important being the inability of vegetable oil production to compete with the low price of mineral oil. The vegetable oil industry has therefore focused on producing oil for food and food processing uses. In 2005 for example, world oilseed production exceeded 390 million metric tonnes (MMT) yielding over 115 MMT of oil (Gunstone 2006). Less than 20% of this is estimated to have entered the industrial market.

With the recent rapid increase in crude oil prices and the focus of political attention on renewable energy and bio-sustainability, the market for vegetable oils is beginning to undergo some fundamental changes. Currently this is lead by the demand for vegetable oil for the manufacture of biodiesel, as illustrated by the more than 3 fold increase in biodiesel production in the US from 25 million gallons in 2004 to 78 million gallons in 2005 (www.emerging-markets.com). It is important to realize that although biodiesel production has clear environmental benefits (Hill et al. 2006; Schubert 2006) it is not a viable replacement for fossil fuels in transportation and energy usage. The reason for this is that it is simply not possible to produce enough oil to substitute for more than a fraction of our current petroleum consumption. An area where vegetable oils show long term potential, either as TAG or as a source of fatty acids, is in higher value applications such as lubricant manufacture and as industrial raw materials. There are, however, considerable challenges that must be overcome for plant oils to achieve their full potential for many industrial processes. Some of these will be discussed below.

CHALLENGES AND OPPORTUNITIES

Fatty Acid Diversity

The chemistry of plant oils offers both advantages and disadvantages to industrial processes. The fatty acid composition of the oil determines its properties and its utility. Most plants only produce a small number of fatty acids, either 16 or 18 carbons in length with zero to three double bonds at specific positions. These are often referred to as the "common" fatty acids and they are the predominant fatty acids of commercial vegetable oils (Table 1), a market dominated by only 4 species; soybean (*Glycine max* L., Fabaceae), oil palm (*Elaeis guineensis* Jacq., Arecaceae), rapeseed (*Brassica napus* L., Brassicaceae), and sunflower (*Helianthus annuus* L., Asteraceae). Within the plant kingdom, however, a diverse array of "unusual" fatty acids are synthesized, many of which would be potentially valuable industrial raw materials if available in sufficient quantity. These fatty acids differ from the common fatty acids in aspects such as chain length, degree of unsaturation and double bond position, or by the presence of functionalities such as hydroxyl, epoxyl, and acetylenic groups, and even covalently bound halogens (Badami and Patil 1981). Although these fatty acids can be present at high levels in seed oils they are usually only found in a small number of plant species, the majority of which do not have agronomic characteristics suitable for their development as commercial oil crops.

Since the development of molecular biology techniques, considerable effort has been directed at identifying the genes encoding enzymes responsible for synthesis of unusual fatty acids. The goal was to transfer these to existing oilseed crop plants to produce novel oil for industrial uses. This topic has been covered extensively by a number of recent reviews (Thelen and Ohlrogge 2002; Murphy 2002; Drexler et al. 2003; Jaworski and Cahoon 2003). In most instances results have been disappointing, with only low levels of unusual fatty acids being produced in the engineered crops. The target fatty acids were also found both in the TAG and the membrane lipids of the transformants whereas in the native species they were only found at significant levels in TAG (Thomaeus et al. 2001; Cahoon et al. 2006). Incorporation of certain unusual fatty acids into membrane lipids is thought to be detrimental to membrane function and the native plants have developed mechanisms for their exclusion (Millar et al. 2000). The challenge is now to understand and overcome the factors that are limiting the accumulation of these fatty acids in transformed plants. Part of the solution will be to gain a better understanding of the processes of TAG assembly, particularly in plants that make unusual fatty acids, but also in existing oilseed species. It is likely that multiple factors are responsible for high level production of unusual fatty acids and there is no single gene that will allow this in a transgenic oilseed. Furthermore, when higher levels are achieved, it may also be necessary to modify the breakdown of unusual fatty acids during seed germination so that the engineered crops are able to efficiently utilize the novel fatty acids that they are synthesizing.

Current strategies to increase unusual fatty acid content are now focusing on “pathway engineering,” the cloning of biosynthetic enzymes and enzymes involved in TAG assembly from species of interest and their co-transformation into the target oilseed. There is increasing evidence that enzymes of TAG assembly from plants that make unusual fatty acids have high specificity for these products (Millar et al. 2000) whereas homologous enzymes in the target crops show a preference for the common fatty acids.

An alternative to the transfer of fatty acid biosynthetic genes to existing crops is to apply molecular biology techniques to aid in the development of new crops, or to improve the characteristics of existing minor oil crops that produce the target fatty acids. Techniques such as TILLING (Henikoff et al. 2004) or RNA interference (Wang and Waterhouse 2002) can be applied to silence specific genes in a plant and the development of molecular markers can greatly speed up breeding programs. The major obstacle to this approach is that agronomic traits are generally complex and often not well understood. Application of these techniques to improve specific characteristics such as fatty acid composition, or to reduce the content of undesirable seed components, such as ricin in castor bean (*Ricinus communis* L., Euphorbiaceae) may however have potential.

Increasing the portfolio of fatty acids available to industry may be more successful in the short term for fatty acids that have value even if they are not the major component of the oil. The presence of relatively low levels (10% to 15%) of ricinoleic acid (12-hydroxy oleic acid) in a vegetable oil high in oleic acid, for example, has been shown to greatly improve the characteristic of the oil in lubricant applications (Grushcow and Smith 2006). It has already been shown that these levels can be achieved by transformation of oilseeds with the oleate-12 hydroxylase gene from castor bean (Broun et al. 1998; Smith et al. 2003; Grushcow and Smith 2006). Programs are currently underway to develop a commercial crop that produces a seed oil containing 15% ricinoleic acid (Linnaeus Plant Sciences Inc., unpubl.).

Homogeneity of Fatty Acid Content

Vegetable oils are generally heterogeneous in composition with each TAG molecule containing a number of different fatty acids. As an industrial feedstock this mixture is not ideal. In processes that involve the hy-

Table 1. Typical percentage fatty acid compositions of the major commercial vegetable oils.

		Oil palm		Rapeseed		
Fatty acid[z]	Soybean	Mesocarp	Kernel[y]	Canola	High erucic[x]	Sunflower
Lauric (12:0)			45			
Myristic (14:0)			18			
Palmitic (16:0)	9	48	9	4	4	7
Stearic (18:0)	4	4	3	2	1	5
Oleic ($18{:}1^{\Delta 9}$)	24	36	15	60	24	19
Linoleic ($18{:}2^{\Delta 9,12}$)	54	10	8	20	16	68
Linolenic ($18{:}3^{\Delta 9,12,15}$)	8			11	11	1
Erucic ($22{:}1^{\Delta 13}$)					43	

[z]Common name and numerical abbreviation ($x{:}y^{\Delta z}$) where x is chain length, y is number of double bonds and z is double bond position.
[y]Accounts for less than 10% of total palm oil production (Gunstone 2006).
[x]Grown for non food uses.

drolysis of the oil, fractionation steps will be required to isolate the fatty acids of interest and this adds to the processing costs of the material. Although there may be value in each of the fatty acids, the most suitable oil would be homogeneous containing a very high percentage of a single fatty acid. Considerable progress has been made in recent years in increasing the homogeneity of fatty acids that naturally exist in the major crop species. A good example is the increase in oleic acid (18:1) content achieved through conventional and mutational breeding of canola (*Brassica napus*), soybean, sunflower, safflower (*Carthamus tinctorius* L., Asteraceae), and peanut (*Arachis hypogaea* L., Fabaceae) where cultivars containing this fatty acid in the range of 80% to 90% have been generated (Drexler et al. 2003) Similar levels have been achieved in canola, soybean, and cotton by genetic engineering (Kinney 1996; Stoutjesdijk et al. 2000; Liu et al. 2002). Although developed for food uses, the availability of a relatively pure source of oleic acid at commodity prices has stimulated interest in the industrial uses of these oils.

Breeding strategies have also been applied to increase the content of linolenic acid (18:3) from around 45% to over 65% in flax (*Linum usitatissimum* L., Linaceae) and erucic acid (22:1) to similar levels in rapeseed, making these oils more suitable for their role as industrial feedstocks. Biological constraints however appear to exist which may limit the accumulation even of naturally occurring fatty acids in some seed oils. Erucic acid for example in rapeseed is not efficiently esterified to the center (*sn-2*) position of the TAG molecule. The theoretical maximum limit of erucic acid in this species would therefore be only 67% of total seed fatty acids. Efforts to overcome this barrier by overexpressing the condensing enzyme responsible for erucic acid synthesis in conjunction with an acyltransferase enzyme capable of catalyzing the incorporation of erucic acid into the *sn-2* position, have been successful in altering the distribution of the fatty acid in TAG, but have not significantly increased the total amount in the seed (Han et al. 2001; Katavic et al. 2001). This fatty acid, and 18:3 in flax, are the end products of a pathway of fatty acid modification in the developing seed. High level accumulation would therefore be expected to require efficient conversion of substrate in addition to efficient incorporation of the product into TAG.

TECHNICAL CONSIDERATIONS

In addition to the scientific challenges associated with developing vegetable oils with new fatty acid profiles there are a number of technical issues that must be considered. The choice of fatty acids will be determined by their utility and also by the size of the market for the oil. This is complicated by the fact that the markets may not yet exist as the product is essentially a new chemical feedstock. Rather than trying to introduce too many novel fatty acids it would be more logical to restrict the number to a few key substrates that can be further converted to the desired feedstock. In this respect monounsaturated fatty acids may be of particular value as the single area of reactivity (the double bond) will allow more control over chemical modification. The presence of the double bond also allows the application of olefin metathesis chemistry to produce dicarboxylic acids and alkenes.

Identity preservation will be a key issue and seeds destined for industrial uses will require segregation from those grown for food use. Mechanisms must be available to control all stages of production and also to ensure that traits are not accidentally transferred to other varieties or species. Possible approaches to simplify this include the engineering of existing major oilseed crops, but with geographic separation during production, or the establishment of platform crops that are not widely grown, but which have the potential to be developed into industrial oilseeds. For temperate zones, candidates include members of the Brassicaeae such as *Brassica juncea* L., *Brassica carinata* A. Braun, *Camelina sativa* L., and crambe (*Crambe abyssinica* Hochst. Ex. R.E. Fries). The advantage of a species such as *B. carinata* is that it can grow well on marginal land that is less suitable for the production of food crops such as *B. napus* (Cardone et al. 2003; Warwick et al. 2006).

For oilseed engineering to be successful it is important to consider all aspects of the seed, not just the oil. A co-product in vegetable oil production is the meal left over after oil extraction. Uses will have to be found for the meal and this may be influenced by the nature of any residual fatty acids and the presence of anti-nutritionals such as glucosinolates. The ability of seeds to germinate and establish should not be affected by the novel fatty acids and any impact on oil content would need to be offset by an increase in value. For some countries it may also be important to breed in agronomic characteristics such as herbicide resistance. The new crops should provide valuable raw materials for industry but also bring value to the producers, and this means the farmers who are growing the crops. Perhaps most importantly, large scale development of industrial crops must not compromise the ability to grow food.

Vegetable oils have a bright future as a source of renewable raw materials for industry. Considerable effort is still required, however, to complete our understanding of how plants synthesize oils of particular fatty acid compositions and how these processes can be modified to optimize vegetable oils for specific purposes.

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