# Genetic Engineering of Oil Crops for Oil Quality

*Cheah Suan Choo**

## INTRODUCTION

"As we move through the next millenium, biotechnology will be as important as the computer" - so predicts John Naisbitt and Patricia Aburdene in Megatrends 2000. This promising technology, in fact, has humble beginnings in the basic biological sciences such as microbiology, biochemistry and molecular biology. Through the 20 years of its development, biotechnology has acquired many definitions. In simple terms, it is a set of techniques that uses organisms or parts of organisms to enhance existing industrial processes or innovate new ones. As such, biotechnology is not an industry, but serves industry.

Biotechnology in the plant agricultural sector has focussed on the application of the techniques of cell culture and genetic engineering to crop improvement. These new techniques should therefore be looked upon as additional tools in plant breeding. Estimates of its economic potential are often optimistic. Figures ranging from US$12.6 billion to US$67 billion have been quoted as probable values of the world agricultural biotechnology market for the year 2000 (Busch *et al.*, 1991). Product sales of goods derived from biotechnology in the United States (US) reached US$5.9 billion last year, mainly in the pharmaceutical and health care sector (Burrill *et al.*, 1993). However, experts predict that agricultural biotechnology will soon move into profitability with genetically engineered tomatoes and biopesticides about to be marketed (Orr, 1993). Products derived from recombinant plants could have a market value of US$300 million by the year 2002 (Lelen, 1992).

Vegetable oils and fats are important agricultural commodities in the world market with a total value estimated to be as high as US$35 billion per year (Anon., 1987). The current annual world demand of less than 60 million tonnes is expected to double by the year 2003-2007 (Mielke, 1993). It is envisaged that biotechnology can help fulfill this increasing demand as well as add value to the commodity. The market value of modified oils derived from rapeseed, soya bean, sunflower and oil palm has been estimated to be nearly US$3 billion (Anon., 1987). This is probably a modest estimate as another study gave a value of US$1.4 billion for modified soya bean oil alone (Anon., 1990a).

Interest in the application of biotechnology to oil crops has been fueled by several factors. Firstly, the technology presents an opportunity for the creation of novel oils not possible by conventional breeding as it enables the transfer of genes across species, genera and kingdom. Secondly, recent innovations derived from studies of the plant genome using molecular probes have proven useful in accelerating plant breeding. These new techniques have important implications in the selection of quantitative traits such as oil yield and quality in oil crop breeding. Thirdly, there is much interest in developing oil crops for industrial uses. This interest stems mainly from two issues – increasing public awareness in preserving the environment and the phenomenon of idle land in Europe. Vegetable oils are being viewed favourably as a renewable, environment friendly resource as the manufacture of natural oleochemcials is less hazardous to the environment than the processing of fossil oils for petrochemicals. In Europe, the cultivation of industrial oil crops on arable land being laid idle due to over-production of food crops has been considered to be an attractive alternative (van Soest *et al.*, 1993).

## TARGETS FOR MANIPULATING OIL QUALITY

As the main economic product of oil crops is oil, it is not surprising that improving oil yield and quality are the most profitable targets of genetic engineering in these crops. Manipulating oil yield is not an easy task as this trait is controlled by many genes. The improvement of oil quality is, however, an achievable target. *Table 1* lists some of these targets. The desired modifications fall into two generalized categories: (i) changes in fatty acid chain length, and (ii) alterations in the saturated level of the oil.

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** Biology Division, PORIM*

### Chain Length

Currently, much effort is directed towards genetic engineering temperate oil crops for the production of medium chain triglycerides (MCT). Oils containing medium chain fatty acids (C12 and C14) are produced mainly in the tropics. In order to secure a local supply of these oils, which incidentally account for 44% of the consumption of the oleochemical industry (Hirsinger, 1986), several concerns in North America and Europe have resorted to using genetic engineering for the production of MCT in transgenic rapeseed. Recently, a successful attempt at producing MCT in rape was reported. A thioesterase enzyme that terminates fatty acid synthesis at laurate (C12:0) was identified in the California bay plant. The gene of this enzyme was isolated and introduced into oilseed rape, resulting in an accumulation of laurate at levels of between 10% and 24% in the seed oil of the transgenic plants (Voelker *et al.*, 1992). Laurate is not normally detectable in rapeseed.

### Saturate Level

The targets of manipulation for oil quality include both increases and decreases in saturate levels of the oil. An increase in unsaturation is desirable in some oils due to consumer demands for monounsaturated and polyunsaturated dietary oils and fats. On the other hand, a lower level of the polyunsaturated linolenic acid (C12:3) content will enhance the stability of other oils. Decreased unsaturation in some oils is also desirable in order to reduce the need for hydrogenation in margarine production.

There is interest in increasing the stearate content of temperate oil crops. This is motivated by the substantial price differential between cocoa butter and the commodity oils. The high stearate oils could presumably be sold as cocoa butter equivalents. Most plant oils have low stearate contents. The challenge to increase stearate in rapeseed oil using *in vitro* technology was taken up by a company in the United States. The gene of the enzyme which desaturates stearate to oleate was blocked thereby causing stearate to accumulate in the oil produced (Knutzon *et al.*, 1992). Indeed, the new oil contained 20-fold higher amounts of stearate than normal rapeseed oil.

## INDUSTRIAL OILS THROUGH GENETIC ENGINEERING

Of the global supply of vegetable oils, almost 90% is used in the food sector (Schmid, 1987). The volume going into non-edible industrial uses is therefore very small. The opportunities for expanding this application are ample given the myriad of chemical reactions which can produce industrial feedstocks from plant oils. Some interesting possibilities are listed in *Table 2*. Application of genetic engineering to the production of industrial oils in plants has the advantage that marketing of the products will not have to face the problem of consumer acceptance as is currently occuring with engineered foods.

The oleochemical industry has requirement for oils with high lauric (C12:0), oleic (C18:2) and erucic (C22:1) contents (Hirsinger, 1986). The availability of an additional functional group in the normal monofunctional oleochemical could lead to new uses in the polymer industry (Zoebelein, 1992). Long chain waxes, currently used in skin care and pharmaceutical formulations, could have important high volume applications as specialty lubricants and in low-calorie foods if produced in sufficient quantities (Gillis, 1988).

Several plant species are known to produce oils enriched for chemicals useful to the oleochemical industry (*Table 3*). It is possible to harness these plants for oil production by domestication of the uncultivated species. The theoretical time scale for such a process has been estimated to be about 24 years (Knowles, 1989). However, the experience with Cuphea, a potential source of short and medium chain fatty acids, shows that it could take longer (Arndt, 1985). Moreover, agronomic problems could further delay large-scale production (Graham, 1989). Producing a novel oil through gene manipulation of an existing crop, on the other hand, has the advantage that production can occur using well-established cultivation and processing practices.

*In vitro* genetic techniques are being applied in some ingenious ways to overcome the need to domesticate wild species. Current efforts in the attempt to transfer a desaturase gene from coriander to oilseed crops for the production of petroselinic acid is a good example. Petroselinic acid can be used as a precursor of lauric and adipic acid by chemically cleaving the molecule at its double

**TABLE 1. TARGETS OF GENE MANIPULATION FOR OIL QUALITY**

| Crop | Desired FAC | Potential Use |
|---|---|---|
| Rape | Increased C12:0<br>Increased C16:0<br>Increased C18:0<br>Increased C18:1<br>High C22:1 (trierucoylglycerol) | Detergents<br>Margarine<br>Cocoa Butter Equivalent<br>Frying Oil<br>Oleochemical Feedstock |
| Soya Bean | Reduced C16:0<br>Increased C18:0<br>Reduced C18:3 | Improve Nutritional Properties<br>Margarine<br>Oil Stability |
| Sunflower | Increased C18:1 | Olive Oil Substitute/ Oleochemical Feedstock |
| Flax | Reduced C18:3 | Oil Stability |
| Cacao | Decreased Unsaturation | More Solid Chocolate |
| Oil Palm | Increased C18:0<br>Increased C18:1 | Cocoa Butter Equivalent<br>Olive Oil Substitute/ Oleochemical Feedstock |

**TABLE 2. PLANT FATTY ACIDS USEFUL AS INDUSTRIAL FEEDSTOCKS**

| Plants | Fatty Acid | Chemical Reaction | Products | Use |
|---|---|---|---|---|
| Castor | Ricinoleic Acid | Caustic Fusion | 2-octanol<br>+<br>Sebacic acid | Perfumes, Soaps<br>Polymers, plasticizers |
| | | Pyrolysis | Heptanal<br>+<br>Undecylenic acid | Flavouring<br>Nylon II, fungistat |
| Olive | Oleic Acid | Oxidative Ozonolysis | Pelargonic acid<br>+<br>Azelaic acid | Lubricants<br>Plasticizer |
| | | Reductive ozonolysis | Pelargonadehyde<br>+<br>Azelaaldehyde ester | Nylon 9 |
| Rape | Erucic acid | Oxidative ozonolysis | Pelargonic acid | Lubricants |
| | | | Brassylic acid | Nylon IIII, Nylon 1313 |
| Coriander | Petroselinic acid | Oxidative ozonolysis | Lauric acid | Detergents |
| | | | Adipic acid | Nylon 66 |

bond at carbon 6. This unusual fatty acid is found in abundance in the seeds of the *Umbelliferae* family to which the spice crop coriander belongs. The coriander desaturase gene which adds a double bond to stearic acid in the delta-6 position has recently been cloned and transferred to tobacco, causing this unusual fatty acid to accumulate in the transgenic tissues (Cahoon *et al.*, 1992).

Ricinoleic acid is another unusual fatty acid of industrial importance which is a target of manipulation. This fatty acid is derived from oleic acid by the action of a hydroxylase enzyme that inserts the hydroxyl group in the delta-12 position (Moreau and Stumpf, 1981). Currently, castor seeds are the main source of this fatty acid, and there is a shortage in supply due to poor yields and the

TABLE 3. POTENTIAL PLANT SOURCES OF INDUSTRIALLY USEFULL FATTY ACIDS

| Fatty Acid | Plant |
|---|---|
| Long Chain Fatty Acids | Cramble<br>Limnanthes<br>Lunaria<br>Sapindaceae |
| Medium Chain Fatty Acids | Cuphea<br>Babassu<br>Lauraceae |
| Hydroxy Fatty Acids | Lesquerella<br>Dimorphotheca |
| Conjugated Fatty Acids | Calendula<br>Impatiens |
| Epoxy Fatty Acids | Vernonia<br>Stokesia<br>Euphobia Lagascae |
| Dicarboxylic Acids | Umbelliferae |
| Acetylenic Acids | Sterculia<br>Crepis |
| Allenic Acid | Labiatae |
| Wax Esters | Jojoba |

toxicity of its meal. It is conceivable that transfer of the hydroxylase gene from castor to a more productive oil crop that has the capacity to overproduce oleic acid could help ease the situation.

Another objective of genetic engineering plants for industrial oils is the production of fatty alcohols and long chain waxes. The seeds of the jojoba plant produce a liquid wax consisting of a mixture of esters formed from linear long chain (mainly C20 and C22) alcohols and unsaturated fatty acids. Although jojoba cultivation has been successfully implemented in some regions of the United States, the production cost is still high (Gillis, 1988). For this reason, a California-based genetic engineering company has taken interest in producing the waxes in rapeseed (Kridl *et al.*, 1993).

It has been argued that although growing oil crops for industrial feedstocks to replace petrochemicals is an attractive alternative, the volume required is too enormous for agriculture to handle alone (Taylor, 1991). The situation could improve if a high yielding crop like the oil palm was to be grown. The commercial *guineensis* palm has an oil yield which is more than ten times that of the annual oil crops. Being a perennial crop with an economic life of more than 20 years has its advantages as well. The stability of inheritance of the transferred genes from generation to generation is not as great a problem as in annual plants. Studies directed towards making *in vitro* gene technology amenable to oil palm will therefore be imperative in the effort to produce chemical feedstocks in the field rather than in factories.

## TOWARDS COMMERCIALIZATION

No oil crop developed by *in vitro* gene technology has been commercialized to date. Given the rapid pace of developments and the intensification of research efforts, it is anticipated that this will happen in the foreseeable future. It is thus pertinent that some of the issues currently faced in the commercialization of genetically modified plants be examined.

### Regulatory Approval

Gaining regulatory approval appears to be one of the major impediments to commercialization of transgenic crops and their products. In the industrialized countries, approval has to be sought at two levels. In the first place, permits are required for testing the plants in the field. Subsequently, marketing of products derived from genetically modified plants is subject to approval of the relevant authorities.

Gaining regulatory approval for field testing is getting easier in the developed countries as much has been learnt from previous experiences. However, obtaining approval for marketing products derived from manipulated plants is still being put to test. In the United States, the marketing of the world's first genetically modified food, a tomato engineered for delayed ripening, has provided the FDA with the opportunity to evaluate and formulate policies for the introduction of transgenic products. The much awaited statement came on 26 May 1992 when the FDA released its policy statement that no pre-market examination was required if "the food constituents of new plant varieties are the same or substantially similar to substances currently found in other foods, such as proteins, fats, oils and carbohydrates" (Anon., 1992). As the United States is the policy leader in biotechnology regulatory procedures, this statement should help allay fears of impending problems associated with the marketing of oils and fats derived from transgenic plants.

## Intellectual Property Rights

Intellectual property rights (IPR) in the form of utility patents has been said to be an integral part of biotechnology commercialization. The controversy that surrounds it has done much to hinder the bringing of some products to market, and technical developments appear to have outpaced patent legislation. Much of the controversy is derived from the widespread belief that life forms are not patentable, but in 1988, this belief was challenged when a patent was granted by the US Patent and Trademark Office (PTO) to a transgenic mouse (often called the Harvard mouse) that carries a human cancer gene. Besides the United States, living creatures including plants and animals are now patentable in Europe and Japan.

In the evaluation of whether IPR is going to be an issue in future commercial exploitation of plant biotechnology, one needs to examine the developments that are occurring in several international flora. The International Union for the Protection of New Varieties of Plants (UPOV) is the international organization that provides the framework for Plant Breeders' Rights (PBR), under which new plant varieties are protected. The 1991 amendment to the UPOV Convention introduced patentability of plant varieties.

During the Uruguay Round of trade negotiations under the auspices of the General Agreement on Tariffs and Trade (GATT), the issue of "Trade Related Intellectual Property Rights" (TRIPs) was introduced. Included in this proposal was the implementation of IPR on biotechnological inventions and plant varieties either in the form of either patents or PBR, or both. This has caused much concern among developing countries who see it as a ruse of the developed nations to impose IPR laws on them (Anon, 1990b). The industrialized countries, on the other hand, argue that it will provide a more conducive environment for international technology transfer and foreign investment (Norichika, 1990).

The third and most recent international forum to touch on IPR in biotechnology is the Biodiversity Treaty introduced during the United Nations (UN) Conference on Environment and Development held at Rio de Janiero in June 1992. The treaty primarily addressed the need for preservation of the earth's biological diversity. Several articles in the treaty however eluded to issues pertaining to biotechnology and IPR of genetic resources. Taken as a whole, the treaty recognized that each nation has ownership rights over the genetic resources within its boundaries and that a nation should be rewarded for use of its genetic resources (Rhein, 1992). The implications of this treaty on the commercialization of biotechnology are far-reaching as genetic resources form the cornerstone of this technology. If ratified, the terms of the treaty will allow genetic resource-rich nations to play a bigger role in the global commercialization of biotechnology.

## Socio-economic Implications

The extent of the success of plant biotechnology will undoubtedly be determined by its socio-economic impact. The economic benefits are much touted, but its potential to change agricultural structure is seldom publicized. The studies of Hill and Florkowski (1991) used models to show that high yielding genetically modified crops will result in a decrease in total acreage of planted land in the US. Transgenic disease and pest resistant crops will cause relocation of crop cultivation. For soya bean, they predicted that while improved resistant varieties offer an opportunity for production to expand into marginal areas, overall it will be likely that increased production will cause land to be withdrawn from utilization (Florkowski and Purcell, 1988). They indicated that future demand for nutritionally desirable oils and industrial oleochemicals can be met by applying gene technology to oil crops.

For the marketing of any biotechnological innovation to succeed, the product must be acceptable to the producers and the consumers. In the case of agricultural biotechnology, the producers are the farmers. In a survey conducted in the United States, farmers identified improved plant and animal strains which have the capability of increasing yield as the major benefit to be derived from biotechnology (Hayenga, 1988). Ironically, when asked to identify their greatest fear, the response was that increased yield will lead to lower farm prices, less agricultural acreage and reduced number of farm jobs.

Perhaps of all the factors to be considered in marketing a genetically modified product, public perception of the technology is the most important. In a survey conducted by the United States office of Technology Assessment (OTA), 62% of the public thought that benefits to be derived from biotechnology will outweigh the perceived risks (Hayenga, 1988). However, 52% believed that genetically engineered products are likely to represent a serious threat to the environment. In Denmark, public acceptance of biotechnology is low. Surveys showed that only 20% of those interviewed support biotechnology. However, a large proportion (65%) were neutral. It has been said that fear or nonchalance towards biotechnology is often rooted in ignorance (Miflin, 1992). To remedy this situation, several public and private organizations are embarking on educational programmes to demystify biotechnology .

## CONCLUSION

Agricultural biotechnology is today at a crossroad. Technical progress is fast overtaking the ability to handle the legal and socio-economic issues. The common occurrence of "first"s in this area is testament to this. In the oils and fats industry, it is now technically feasible to modify plants to produce novel lipids. Where and how this technology is developed in future could determine the fate of some nations (Stumpf, 1988).

Globalization of the biotechnology trade is an inevitable phenomenon most as major players here are multinational companies. It is rather alarming to note that the present rate of consolidation of the seed industry will eventually lead to only 10 to 12 multinational companies dominating the seed trade (Kidd, 1987). Current international events appear to indicate that this trend will lead to a head-on collision between the technology-rich industralized countries and the resource-rich developing nations (Shand, 1993). How the powerful tools of biotechnology developed by the former are used to systematically exploit the genetic wonderland of the latter is a challenge the world faces today. To quote from Calestous Juma's book, The Gene Hunters : "Biotechnology has empowered humanity to create as well as destroy life using genetic information ....... Never has mankind lived through such moments of prospect and fear before. Biotechnology and genetic resources have now moved to the agenda of national and regional security ..... innovate or perish".

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