Opportunities for biotechnology in the development of new edible vegetable oil products

(The following was presented by Dr. W.R. Sharp of DNA Plant Technology Corp. as a keynote talk at the 76th annual meeting of the American Oil Chemists' Society held May 1985 in Philadelphia.)

Abstract

New plant cell culture technologies are poised to have significant impact on the edible oil market. The biotechnology techniques of somaclonal and gametoclonal variation, protoplast fusion and clonal propagation can be selectively leveraged against particular oil crop species to develop improved genetic traits for processing and/or the consumer quality of the specific edible oil. The application of these technologies for development of improved genetic traits requires continued development of tissue culture procedures for some oil crops. Plant tissue culture biotechnology can lead to improvements in the health and nutritional characteristics of vegetable oils and can improve oil yield and other agronomic characteristics in a shorter period of time than conventional breeding. Biotechnology can also be leveraged against underexploited oilseed crops such as cuphea and castor bean for development of edible oil products.

Current edible oil crops

The world edible vegetable oil production in 1984-1985 was 41 million metric tons (1). Soybean oil is the largest source of edible oil (33% of total), followed by oil palm (17%), sunflower (15%), rapeseed (14%) and cottonseed (10%).

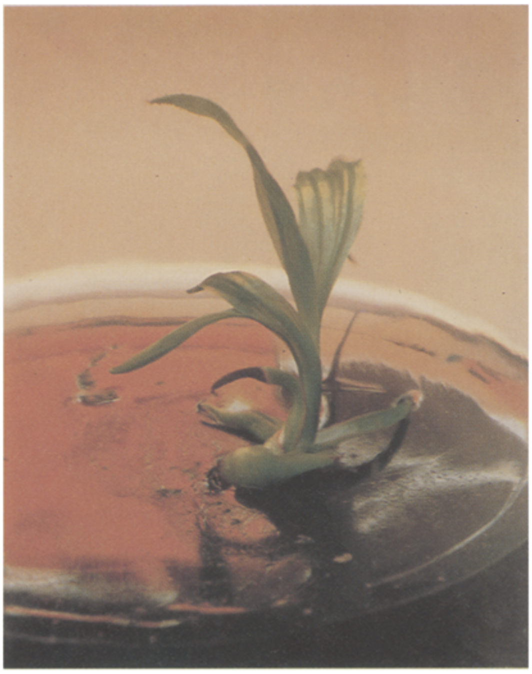
Eight countries are responsible for 86% of total world edible vegetable oil production: U.S.A. (28%), China (20%), India (9%), Brazil (8%), U.S.S.R. (7%), Argentina (6.6%), Malaysia (5%), and Canada (3%). The U.S.S.R. and India were the world's leading importers of fats and oils in 1984-1985 with 1.6 and 1.5 million tons, respectively. The two most populous nations, China and India, showed consumption increases of 11.4% and 4.3% respectively from the 1983-1984 to the 1984-1985 season (1).

The relative importance of soybean and cottonseed for the edible oil market is obvious. However in both crops, oil is not the only economic motivation for production. In the case of soybean, the meal frequently is the more valuable commodity, and in cotton, the fiber is more important than oil. Oil production is the principal market motivation for oil palm, sunflower, coconut, olive and rapeseed.

Another interesting aspect related to sources of edible oils is their relative efficiencies, based on units of land, to produce 1.0 metric ton of oil (Table 1). This relative efficiency may significantly affect future decisions that relate to land usage and crop selection to produce specific edible oils (2).

The ultimate goal for improvement among edible oil crops is to build consumer and processing benefits into target species that already have high oil content or high yields. Sunflower, safflower, peanut, sesame and rapeseed could be listed as possible candidates among annual species and palms among the perennials. However, taking into account that the world increase in edible oil since the late 1960s has been from acreage increases of soybean, rapeseed, sunflower and oil palm (3), one could suggest that the main targets for biotechnology improvement programs for oil crops would be the following: (a) developed countries—soybean, sunflower and rapeseed; (b) developing countries—palm oil and soybean.

In developed countries there will be shifts in acreage according to economic factors such as price of land or consumer or processing benefits versus crop efficiency. It is possible that sunflower and/or rapeseed could displace soybean acreage



W.R. Sharp, R.J. Whitaker, M.R. Sondahl, D.A. Evans, J.E. Bravo, J.F. Marsden and R.J. Orton
DNA Plant Technology Corp., 2611 Branch Pike, Cinnaminson, NJ 08077

L.C.S. Ramos
Institute of Agronomy, Campinas, SP, Brazil

Selected elite oil palms can be clonally propagated using tissue culture to produce genetically uniform, high yielding palms. Photo courtesy of DNA Plant Technology Corp.

if protein meal is in over-supply, or other added-value characteristics are incorporated into these two oilseed crops. Sunflower and rapeseed presently are more amenable to biotechnology techniques for genetic modification than soybean, indicating that these two crops will be more reasonable targets for improvement in the near term. This situation could change because of recent success in the tissue culture regeneration of soybean which makes soybean more amenable to genetic improvement through the biotechnology tools used for other crops.

Palm oil should continue to be an important oil source in developing countries because: (a) it is adapted to tropical rain forest areas where western-style crop agriculture is difficult; (b) land is available at relatively low prices: (c) it is characterized by an efficient conversion rate, i.e., tons of oil per unit land; (d) labor costs are relatively low, and (e) it is amenable to clonal propagation techniques. Soybean will continue to be grown in developing countries as long as good agricultural land is available for acreage expansion and improved varieties are available. Soybean probably will not displace other food crops in competing for good agricultural land. New soybean varieties are presently available for cultivation at lower latitudes, (e.g., 12°S, Brazil).

The availability of new oil crop varieties (technological input) will be important in determining the relative importance of individual oil crop species. Access to new varieties of soybean, rapeseed or sunflower for lower latitudes without compromising yield, quality and disease resistance could have significant impact in developing areas. Likewise, new varieties with added consumer or processing benefits, associated with high oil yields, would change the relative importance of some edible oil species in developed countries. The development of high oleic acid varieties of sunflower and the use of conventional breeding to develop canola, i.e., rapeseed with oleic acid in place of erucic acid, demonstrate that fatty acid composition can be significantly modified. Several laboratories have reported modified fatty acid composition in germplasm collections (4), induced mutations (5) and somaclonal variants of oil palm (6). These developments all suggest that it is feasible to develop new oil crops using biotechnology approaches.

Technologies

Agricultural biotechnology focuses on the applied aspects of biology and exploits the use of here-and-now genetic modification procedures for the production of products for the processor or consumer. This is consistent with the subject matter of this paper which focuses on test-tube technologies suited to the modification of commercial edible oil crops with the goal being to develop modified edible oil products.

The test-tube technologies that can be immediately leveraged against development of new edible oil plant varieties with modified oil chemistry consist of an arsenal of cellular biotechnology tools for genetic improvement. These include somaclonal variation, gametoclonal variation, somatic cell hybridization and clonal propagation (7). Somaclonal variation and gametoclonal variation exploit the occurrence of genetically modified cells that occur naturally in certain plant tissues. These are aseptically excised from the plant and grown on a simple nutrient formulation contained in a test tube to produce new improved plant breeding lines. Somaclonal variation makes use of somatic cells or the "body cells" of the plant, e.g., leaf cells, stem cells, etc. while gametoclonal variation pertains to the use of gametes, e.g., the pollen or egg cells (8).

Regeneration of plants from

TABLE 1

Efficiency of Oil Production in Oil Crop Plants, Data Based on Optimum Yield Values (FAO 1980)

Oil crop	Area needed for production of 1.0 ton of oil	Relative efficiency[b]	Seed oil %	Optimum seed yield (ton/ha)
Cottonseed	1.6	0.9	22	2,800
Soybean	1.4	1.0	18	4,000
Rapeseed[a]	0.9	1.5	38	3,000
Sesame	0.8	1.7	48	2,500
Peanut	0.8	1.7	32	4,000
Safflower	0.7	2.0	35	4,000
Sunflower	0.7	2.0	40	3,500
Olive	0.4	3.5	25	10,000
Coconut	0.4	3.5	62	4,000
Oil palm	0.14	10.0	50	35,000

[a] Based on Canadian Spring rape.

[b] Based on soybean at 4,000 ton per hectare as 1.0.

somatic cells, or plant protoplasts (wall-less somatic cells) using tissue culture can result in the recovery of genetically modified plants called somaclonal variants. Likewise, genetic variability occurs following the regeneration of plants from pollen or the male gametes grown in tissue culture. This allows the researcher to develop genetically modified plants from cells sourced from commercial plant varieties. These are used to develop new breeding lines for use in the development of improved varieties. Somaclonal variation has been characterized in several oil-producing crops including oil palm and rapeseed. In several food crops, somaclonal variants have been used for developing new breeding lines that have been important to plant breeding programs in developing varieties with new agronomic and processing benefits. The principle benefit of somaclonal and gametoclonal variation is that it cuts the time in half for new variety development to three or four years, from seven or eight years, for annual crops, e.g., rapeseed, soybean, sunflower and cotton, and to seven to eight years, from 30 to 40 years, for perennial crops, e.g., oil palm.

Protoplast fusion is a new technology that permits the development of unique hydrid plants impossible to achieve via conventional sexual hybridization (9). These new hybrids, when integrated into a breeding program, will permit development of plant varieties that are otherwise not possible. The unique formation of genetic combinations by protoplast fusion technology results from combining the best genes of plant A with plant B by fusing the cells of two plants together in the test tube using certain chemical fusion agents. Interspecies somatic hybrid plants have been produced in several crops including rapeseed. The hybrid genetic material can then be incorporated into a conventional breeding program. Somatic hybrids from closely related species will permit the rapid development of new gene combinations of value to the plant breeder.

Attention recently has been directed toward use of fusion products in crop improvement. The primary limitation is certainly the inability to regenerate plants from protoplasts for a number of species (Table 2). A number of hybrid constructions could be proposed to complement cereal and legume breeding programs, but very little success has been reported in plant regeneration from protoplasts of these important crops. Hence, the limitation of plant regeneration from protoplasts precludes short-term application of this technology to crops such as soybean, coconut and sunflower.

Clonal propagation, on the other hand, allows for the scale-up of certain improved genetic combinations without going through the seed process. This technology is especially applicable to perennial crops. Trees selected for higher yields or markedly different oils can be produced using clonal propagation technology. It requires decades for the development and introduction of new tree varieties using conventional breeding practices which focus on development of new seed varieties. Clonal propagation technology is currently in place for the production of genetic carbon copied of oil palm trees.

The genetically modified plants resulting from somaclonal variation, gametoclonal variation, somatic hybridization and clonal propagation technologies are grown on a simple growth medium in a test tube environment. The cells grow and develop into small plantlets in response to certain growth hormones. The plantlets are subsequently transferred to greenhouse conditions to allow for maturation and seed set. Plants with good vigor are thereafter tested in breeding trials. Here selections are made by a plant breeder for value-added traits. The benefits of this approach to crop improvement are two-fold: (a) development of new commercial varieties within half the time required by using conventional breeding alone, and (b) the fine tuning of existing commercial plant varieties with the addition of genes for certain value added characteristics, i.e., modification of oil chemistry.

The technologies discussed so far are currently in place and can be used to bring forward improved varieties of edible oil plants. It is apparent that the field is moving rapidly and certain new technologies for genetic modification are moving downstream.

The next group of genetic modification tools to be used in variety improvement will most likely consist of subcellular genetic modification and molecular biology. The former pertains to the organelles (e.g., mitochondria, chloroplasts) which are small membrane bound structures which contain ca. 5% of the total genetic information found in the cell, including important genes for photosynthesis efficiency, male sterility, herbicide resistance and certain kinds of disease resistance. The remaining 95% of the DNA is found in the nucleus. Progress has been made during the past few years in regard to the genetic modification of the organelles as well as to the transfer of organelles from cell A to cell B using protoplast fusion technology.

One promising near-term molecular technology is pollen transformation (10). Here isolated pollen is incubated in crude DNA extracts for uptake and insertion of genes. The treated pollen is then used in pollination for production of seed with new genetic attributes.

TABLE 2

Technical Feasibility of Genetic Modification of Major Oilseed Crops

	Classical genetics	Tissue culture cloning	Somaclonal or gametoclonal variation	Protoplast	Hybrid seed technology
Soybean	+[b]	−[a]	+	−	−
Palm	−	++[c]	++	−	+
Sunflower	++	+	+	−	+++[d]
Rapeseed	++	++	+++	++	++
Cotton	++	+	+	+	
Coconut	−	−	−	−	−

[a] = virtually no progress.

[b] = limited progress on technology development.

[c] = success reported in several laboratories, evidence that it will eventually be suitable for application.

[d] = technology in place; suitable for application.

Wish list

To precisely define the opportunities for leveraging biotechnology genetic modification tools against the improvement of oil crops, one must consider the wish list of benefits and then balance the list against technical limitations. In general, perennial crops, such as oil palm and coconut, require a longer time frame for genetic improvement than do annual crops such as soybean and rapeseed.

For perennial crops, the most important technology for short-term improvement is tissue culture propagation. Selections of superior oil crop individuals can be identified in the plantation and introduced into cell culture for clonal propagation. Utilizing this technology, oil crops with unique oil composition or specific hybrid combinations can be produced in large numbers. Several companies, such as Unilever (UK) and Sime Darby (Malaysia) and the DNA Plant Technology Corp./United Fruit Co. joint venture (U.S.-Costa Rica) have begun to apply this technology to oil palm. It is anticipated that additional companies will use this technology to scale up production of proprietary palms. A longer term approach for perennials could include somaclonal and gametoclonal variation. Somaclonal variation already has been documented in oil palm, resulting in clones with unique fatty acid composition (6). Once somaclones are identified and verified under field conditions, selected palms could again be clonally propagated to produce sufficient plant material of clonal origin. In the longer term, gametoclonal variation could be used to develop new parents for production of unique hybrids which could then be cloned.

For annual crops such as soybean, cottonseed, sunflower and rapeseed, which have short generation times and rapid turnover of plant material, seed propagation rather than clonal propagation would be more desirable. Therefore, it is more economical to produce genetic modifications that are transmitted through seed. The major annual oilseed crops can be grouped based on availability of technology. Rapeseed is most amenable to these technologies, followed by cotton and sunflower, while soybean has only recently been regenerated from cell culture. This would suggest that different short-term technologies would be used for each crop.

For example, the development of a soybean with high oleic acid could be approached using mutation and conventional breeding approaches, somaclonal variation, or by protoplast fusion. The first two approaches would represent short- or intermediate-term approaches. Mutation breeding has already been used to produce soybean lines that are high in oleic acid (5). While soybean regeneration has been traditionally viewed as difficult, several recent publications state that whole plants can be obtained from culture of immature embryos. This would suggest that somaclones could be generated in a short period of time and progeny of regenerated plants screened for altered fatty acid composition. Soybean has one undesirable feature; it is not amenable to hybrid seed production. Consequently, any new lines developed would have to be protected by legal rather than by genetic means.

Genetic modification of rapeseed should prove more feasible than the other annual crops. Rapeseed is very easy to regenerate for either somaclonal or gametoclonal variation. In addition, plant regeneration from fused protoplasts of rapeseed has been documented (11). Rapeseed might be the best candidate to approach for the development of new plant varieties that contain ω-3 fatty acids described by some researchers as having positive health benefits. Somaclones and wild species germplasm would first be screened for presence of ω-3 fatty acids. Once uncovered, these lines could be transferred into cultivated rapeseed using conventional breeding or protoplast fusion. Rapeseed also holds promise for hybrid seed production as male sterility has been identified. Once in the appropriate backgrounds, this trait could facilitate hybrid seed production for the genetic protection of new developments. The ω-3 fatty acids will be subject to oxidative degradation which would greatly reduce shelf-life of oils containing these compounds. However, the canola varieties of rapeseed already contain high oleic acid, which gives improved shelf-life. In rapeseed, polyunsaturated fatty acids are located preferentially in the β position of triglycerides. These oils are significantly more resistant to oxidation than if the polyunsaturated fatty acids are in the α or α-prime position. If this acyl configuration were maintained in new rapeseed varieties, this could produce a high concentration of triglycerides that are stable and offer unique health benefits.

With the advent of new technologies, additional opportunities exist to improve other crops for oil production. Two crops, cuphea and castor bean, will be viewed as case studies.

Coconut and palm kernel oils are the main sources of lauric acids for the manufacture of soaps, detergents, lubricants and other products in the food and health areas. The lauric acid groups are comprised of caprylic, capric, and lauric acids which have 8, 10 and 12 carbon chains respectively. This group accounts for 60% of the total fatty acids of coconut and palm kernel oil. Cuphea is an annual species that may contain over 90% of these lauric acids. The remarkable variability in the fatty acid composition present among cuphea species (4,12-15) makes them potential candidates for specific industrial, health or food use.

Cuphea species have not yet been domesticated, and so cuphea still needs to be tailored to fit agronomic requirements. Elimination of seed shattering, seed dormancy, indeterminate flowering habit, inadequate plant architecture for mechanical harvesting, and sticky hairs would all be necessary to make cuphea an agronomic reality. Indeed, some mutants have already been selected that do not possess sticky hairs (16). It is possible to combine traditional breeding and new biotechnology techniques for the improvement of cuphea. Somaclonal variation and micropropagation could be successfully used in cuphea to quickly improve agronomic characteristics. High seed yield and high seed oil content also could be obtained. Biotechnology could play a crucial role in the process of domestication of cuphea, speeding up the time required by conventional breeding, by providing altered genotypes with direct agronomic or industrial applications. Development of cuphea into an annual temperate crop would provide the temperate region with an alternative for production of medium chain triglycerides.

The medium carbon length of the lauric acids resembles those found in diesel fuel. Although the level of saturation needs to be modified, cuphea represents a potential source for renewable energy.

Castor bean is a tropical and subtropical oil crop principally used for industrial applications, including some minor pharmaceutical use. The striking feature of castor bean is the presence of a unique fatty acid, ricinoleic acid. This fatty acid accounts for about 90% of castor bean oil with virtually no chemical variation (1). This feature entails exciting industrial applications for castor bean oil (17).

The biosynthesis of fatty acids in castor bean has been extensively studied (18-20). Oleic acid is the precursor of ricinoleic acid, and a single hydroxyl group replaces one hydrogen from oleic acid near the double bond. Considering these characteristics of castor bean oil, one could speculate that the biosynthesis of ricinoleic acid could be under genetic control of one or a few genes. This could be assessed through genetic studies, using a ricinoleic acid-free mutant. A very high oleic acid variety of castor bean could then be developed by blocking ricinoleic acid synthesis. This would be an extremely useful novelty crop for tropical and subtropical regions considering that castor bean is an annual crop, producing high yields (1.5-2.0 tons oil per ha), and is amenable to mechanical harvesting.

References

1. *J. Am. Chem. Soc. 62*:1166 (1985).
2. Ferwerda, J.O., in *CRC Handbook of Tropical Food Crops*, edited by F.W. Martin, 1984, pp. 59-108.
3. Wiedermann, L.H., *J. Am. Oil Chem. Soc. 60*:448 (1983).
4. Hirsinger, F., *Ibid. 62*:76 (1985).
5. Hammond, E.G., and W.R. Fehr, in *Biotechnology for the Oil and Fats Industry*, edited by C. Ratledge, P. Dawson and J. Rattray, AOCS, Champaign, IL, 1984, pp. 89-96.
6. Jones, L.H., *J. Am. Oil Chem. Soc. 61*:1717 (1984).
7. Sharp, W.R., D.A. Evans and P.V. Ammirato, *Europ. Chem. News (Biotechnology Supplement) 42*:35 (1984).
8. Evans, D.A., W.R. Sharp and H.P. Medina-Filho, *Amer. Jour. Bot. 71*:759 (1984).
9. Evans, D.A., *Bio/Technology 1*:856 (1983).
10. Ohta, Y., *Proc. Nat. Acad. Sci. 83*:715 (1986).
11. Pelletier, G., C. Primard, F. Vedel and P. Chetrit, *Molec. Gen. Genet. 191*:244 (1983).
12. Graham, S.A., and R. Kleiman, *J. Am. Oil Chem. Soc. 62*:81 (1985).
13. Graham, S.A., F. Hirsinger and G. Robbelen, *Amer. Jour. Bot. 68*:908 (1981).
14. Hirsinger, F., and G. Robbelen, *Z. Pflanzenzucht. 85*:275 (1980).
15. Wolf, R.B., S.A. Graham and R. Kleiman, *J. Am. Oil Chem. Soc. 60*:27 (1983).
16. *Ibid. 62*:6 (1985).
17. Ramos, L.C.S., J.S. Tango, A. Savi and N.R. Leal, *Ibid. 61*:1841 (1984).
18. Naughton, F.C., *Ibid. 51*:65 (1974).
19. Moreau, R.A., and P.K. Stumpf, *Plant Physiol. 67*:672 (1981).
20. Vick, B., and H. Beevers, *Ibid. 62*:173 (1978).