Plants as Factories for Technical Materials[1]

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Until the latter part of the 19th century, humans were largely dependent upon contemporaneous biological sources for the production of all organic materials. Plants and animals provided the only sources of fibers, coatings, lubricants, solvents, dyes, waxes, fillers, and insulation, fragrances, detergents, sizing, leather, wood, paper, rubber, and many other types of materials. As recently as 1930, 30% of industrial organic chemicals were derived from plants (13). The discovery of extensive petroleum reserves and advances in chemistry and petroleum engineering resulted in a major shift to reliance on fossil sources of organic feedstocks and the development of materials such as inexpensive plastics, with properties that could not be duplicated by abundantly available natural materials. Nevertheless, many important materials are still derived from plants and animals. Wood, cork, paper, and leather remain ubiquitous. Cotton, ramie, hemp, flax, sisal, wool, and silk are also important sources of fiber for many applications. Rubber from natural latex is still the only material that can be used to produce tires that will reliably withstand the forces associated with airplane landings. Linseed oil is still used to make paint, although linoleum is no longer produced. Thus for many applications, biological sources can still be used to produce materials on the scale necessary to meet the needs of populous industrialized nations.

The traditional strategy for using plants has been to modify, by breeding and selection, a species that produces something useful so that it is suited to our needs. Many of the important food species such as maize or the many varieties of *Brassica oleracea* no longer bear much resemblance to wild progenitors because of strong selection for useful traits. Attempts have been made to improve jojoba for production of wax esters and guayule for latex production and species such as crambe, meadowfoam, *Euphorbia lagascae*, *Lesquerella fendleri*, and *Cuphea* sp. for technically useful oils (5). However, these initiatives have met with limited success because of an inability to develop lines with acceptable production, quality, and agronomic properties. Except for relatively small-scale production of jojoba and guayule, there does not appear to have been a newly created field or plantation crop for production of technical materials during the last century. Several species that produced useful materials such as kapok, which was used for applications such as waterproof fiber filling in life vests, have declined because of the labor costs associated with harvesting the materials.

Interest in the possibility of using genetically engineered plants as factories seems to have several motivations (19); in the short term, it would be desirable to diversify crop production by producing high value technical materials in crop plants and create potentially large new markets for excess agricultural production. In the longer term, the concept addresses a widely held social goal of developing more sustainable and environmentally benign methods of meeting our needs for materials that are currently produced by chemical synthesis from declining petroleum or coal feedstocks. In addition, it is possible to envision the production in plants of novel biologically inspired materials, with properties not easily simulated through chemical synthesis.

Lingering concern about the consequences of the 1973 oil embargo appear to have been the motivation for the first article that clearly described the concept of using genetic engineering to produce an industrial product in plants. Even before the first paper describing the production of a transgenic plant had appeared (24), Melvin Calvin (1) explicitly outlined the basic steps by which *Euphorbia lathyrus* could be engineered to produce industrial quantities of sesquiterpenes. This paper and related work from Calvin and colleagues established the concept of engineering plants as factories, but because of economic considerations, the work on *E. lathyrus* was discontinued. In retrospect it was unrealistic to expect that the first applications of genetic engineering would be directed toward creation of a new crop for a nonexistent market. Calvin's ideas remain interesting, but until the price of petroleum increases substantially there will not be sufficient economic incentive to attempt the engineering of *E. lathyrus*. Perhaps the major lesson from Calvin's work was the necessity of minimizing the threshold for the introduction of a new crop. In practice this has focused attention on using genetic engineering to make incremental changes in plants that are already grown on a large scale.

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MODIFYING STARCH AND OIL

To utilize plants as a source of organic molecules, the proposed products must accumulate to levels that justify the costs associated with growing and processing the plant material (6). For low M_r water-soluble compounds of industrial utility, the osmotic effects of high level solute accumulation place practical limits on how much can be accumulated. In general, microorganisms are the preferred vehicles for production of water-soluble small molecules such as organic acids and amino acids because they frequently secrete the compounds into the media. By contrast, high M_r soluble compounds such as proteins or water insoluble compounds such as starch, oil, terpenoids, polyalkanoates, and cell wall polysaccharides are not significant osmolytes and can, in principle, be produced at very high concentrations in some types of plant cells.

Because starch and oil are already produced from plants in very large amounts and have a diverse range of industrial applications, some of the first applications of genetic engineering were directed toward modifying starch structure and oil fatty acid composition for food and non-food uses. There is a vast literature concerning chemical modification of starch and the many non-food uses for the resulting polymers (23). The use of starch as a plastic and as a component of plastics began in the 1970s and a number of products of this type are commercially produced. Although the ideal starch properties for polymer applications do not seem to have been explicitly defined as yet, it is apparent that factors such as the amylose/amylopectin ratio and the size of granules has a significant influence on the properties of plastic made from starch (25). Amylose ethers have mechanical properties similar to those of high-density polyethylene. If amylose was available in large quantities at prices similar to starch, amylose-ethers could replace polyethylene and polystyrene in applications where biodegradability was desirable (25). Because of progress in cloning and understanding the genes involved in starch biosynthesis, it is just a matter of time before a type of starch that has been optimized for polymer applications is available from transgenic plants (4, 17). Because of the magnitude of demand for polymers, this will have a major effect on the economics of agriculture.

The idea of creating new oil crops by domestication of plants that accumulate unusual fatty acids seems to have been most vigorously pursued by a group of chemists at the U.S. Department of Agriculture northern Regional Research Center in Peoria Illinois (5). Beginning in the 1960s, they conducted an analysis of the fatty acid composition of the seed oil of approximately 8,000 species. These studies showed that higher plants collectively produce more than 500 kinds of fatty acids, which differ by chain length and the presence of double bonds, hydroxyls, epoxy groups, acetylenes, conjugated double bonds, cyclopropanes and cyclopropenes, furan groups, and cyclopentenes, to name a few (13, 22; Fig. 1). As thinking about the uses of recombinant DNA methods spread in the plant biology community in the early 1980s, the idea of using genetic engineering to transfer the biosynthetic capability to produce novel fatty acids into crop plants spread very quickly among the small community of people interested in plant lipids (9). The basic assumption was, and is, that if industrially useful fatty acids could be produced in plants in large amounts and reasonably high purity, they would be useful starting materials for the synthesis of a wide variety of materials, including polymers, elastomers, plasticizers, paints and coatings, detergents, and hydraulic and lubricating fluids (but see 6). By way of illustration of how fatty acids are used as synthons, the steps required to convert a plant lipid to nylon is shown in Figure 2.

At the time that the idea of producing industrially useful fatty acids in transgenic plants emerged in the mid-1980s, no gene involved in plant lipid metabolism had been cloned and no enzyme purified to homogeneity. It was not until the early 1990s that genes for key enzymes began to become available (14). As these genes have been used to create plants with novel oils, a new problem has emerged; the expression of a new biosynthetic capability frequently does not lead to more than 5% to 20% of the total oil being composed of the desired compound (13). Thus there is now heightened interest in understanding what factors control the amount of particular fatty acids. In view of the fact that some plants such as *Ricinus communis* (Castor) produce oil that is >80% composed of a single fatty acid, there is a biological precedent for achieving the quality of oil that would be acceptable to the chemical industry.

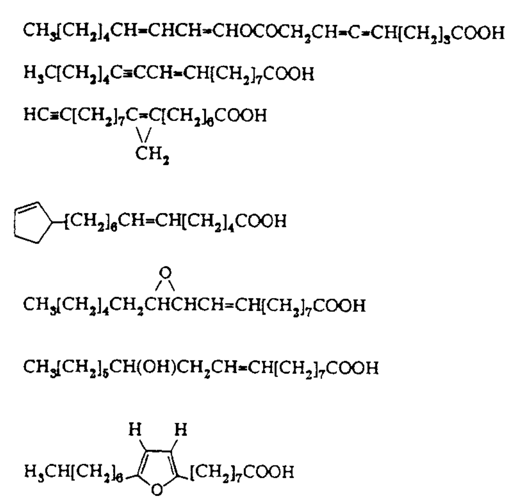

Figure 1. Some examples of the chemical diversity of plant seed fatty acids (20). 1, A component of stillingia oil from *Sapium sebiferum*; 2, crepenynic acid from *Crepis foetida*; 3, sterculynic acid from *Sterculia alata*; 4, gorlic acid, from the family Flacourtiaceae; 5, vernolic acid from *Vernonia galamensis*; 6, ricinoleic acid from *Ricinus communis*; and 7, a furan-containing fatty acid from *Exocarpus cupressiformis*.

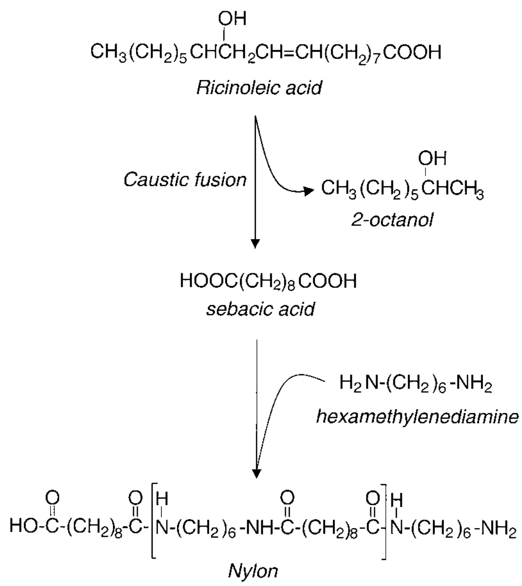

Figure 2. Production of nylon 6,10 from ricinoleic acid, the principal component of castor bean oil. By continuously removing water produced in the condensation reaction, polymerization progresses at a high conversion rate due to Le Chatelier's principle. Details of the synthesis are available at www.psrc.usm.edu/macrog/nysyn.htm.

However, to date the only example of a transgenic producing more than about 50% of a novel fatty acid is the high lauric acid canola developed by Calgene (10).

In addition to the problem of increasing the purity of desirable fatty acids, the field crops used for oil production accumulate oil in seeds along with relatively large amounts of protein that is used for food and feed. It would be useful to uncouple the production of oil and protein so that the value of the oil was not dependent on the value of the protein. This may be particularly important for production of useful nonedible fatty acids, which may prove to be unacceptable as post-processing carryover even at low levels in seed proteins used for food and feed. More importantly, field crops such as potato, cassava, and sugar beet, which produce large amounts of starch, but relatively small amounts of protein, are much more productive than plants that produce oil. Therefore, it would be useful to learn how to modify these plants so that they accumulate oil rather than starch. Since these plants are used for production of roots and tubers, but can be propagated by seed, it should be possible to produce materials in the roots and tubers of these species that would otherwise interfere with the germination of seeds. This is obviously an ambitious goal. However, the recent identification of a mutant of Arabidopsis in which part of the root becomes filled with oil suggests that it may eventually be possible to accomplish this goal by altering the cellular identity of root or tuber cells so that they take on the identity of oil producing cell types (12).

INTRODUCTION OF NOVEL CAPABILITIES

Nature is the palette of genetic engineering. Thus the evolution of ideas about what might be produced in plants is rooted in knowledge of what is already produced by various organisms. One of the earliest examples of producing a new material in plants was the production of polyhydroxybutyrate (PHB) in Arabidopsis (16). At the time this work began, a copolymer of PHB and polyhydroxyvalerate was commercially produced by Imperial Chemical Industries via bacterial fermentation and sold under the trade name BioPol. It is unfortunate that BioPol is expensive to produce compared with plastics derived from petroleum and is, therefore, not in widespread use. The idea behind the plant work was that since starch and sugar could be produced in plants at costs well below the cost of commodity plastics, it might be possible to produce PHB and related polyhydroxyalkanoates at similarly low costs. The first evidence for production of PHB in plants was obtained by modifying two genes from the bacterium *Alcaligenes eutrophus* so that the polypeptides were produced in the plant cytoplasm (16). The resulting plants were tiny and produced only traces of PHB. However, when the genes were restructured so that the polypeptides were targeted to the chloroplasts, the plants exhibited normal growth and development and plants with as much as 14% of dry weight of PHB were obtained (11). The plastic produced was brittle and not of suitable quality for most applications. More recently, scientists at Monsanto have produced transgenics that produce a copolymer of hydroxybutyrate and hydroxyvalerate, raising the possibility that it will eventually be possible to produce a useful material (18). However, because of the possibility that very high levels of production in chloroplasts will disrupt their function, it may be necessary to learn how to increase the number of chloroplasts to obtain higher levels of production.

The variety of fibers produced in nature greatly exceeds the relatively small number of synthetic polymers that are used for fibers. Before synthetic polymers such as nylon and polyesters became widely available, some efforts to produce man-made alternatives to silk involved the production of silk-like fibers from seed proteins such as zein from corn, arachin from peanuts, and casein from soybean (8). For example, the production of a fiber based on zein (Vicara) reached an output of 10 million pounds by the mid-1950s. Preceding the production of Vicara, the Ford Motor Company had developed a soybean casein fiber for use in automobile upholstery in the late 1930s. However, these natural protein fibers suffered from a number of deficiencies and were quickly replaced by synthetic fibers. More recently, silk-like proteins have been produced in bacteria through genetic engineering, and consequently, interest in protein based materials is resurgent. This interest is attributable to the technical advantages that certain protein polymers have over synthetic ones. Unlike the random monomer arrangements, which typify synthetic polymers, the ordered blocks of amino acids found in certain structural proteins impart a greater degree of crystallinity to the polymer. Thus collagen and keratin have more in common with the new highly oriented superfibers such as Kevlar and thermotropic polyesters than with conventional plastics.

In the early 1980s it was discovered that the structural properties of most fibrous proteins could by mimicked by polypeptides consisting of short amino acid repeats and that structural blocks derived from natural proteins could be used to design artificial genes (2). Since this time, polymer compositions that mimic silk, laminin, elastin, collagen, and keratin have been produced in *E. coli*. It has also become apparent that the structural blocks from different natural proteins can be combined to have properties that are not inherent in the synthetic block or the natural protein. A potentially large number of unique polymers can be produced using this principle and more than 20 patents are based on this idea. Despite high expression of some polymers in bacteria, commercial production of protein polymers has yet to be achieved. The expression of an elastin-like polymer in plants (3), albeit at low levels, represents a first step toward the development of a low cost production system that may bring this thread of ideas to fruition. It has generally been difficult to produce transgenic plants that accumulate more than a few percent of total protein from an introduced gene (15). However, the successful commercial production of avidin in maize (7) provides an example of progress toward realizing the goal of producing industrial proteins in plants.

CONCLUSIONS

The genetic engineering of plants for production of industrial materials is still in the conceptual stage. A limited number of specific goals have been defined and there have been some promising preliminary results. The results to date suggest that as in any engineering project, broad theoretical knowledge of the entire system is required to ensure progress. It seems likely that progress will be dependent on concurrent initiatives to understand the basis of cellular differentiation, morphogenesis, metabolism, and many other aspects of growth and development that have little direct connection with metabolic engineering. Progress toward understanding the function of all genes in Arabidopsis and other angiosperms (21) will greatly facilitate progress toward the development of a true engineering discipline in which it is possible to envision a goal and predict the outcomes of making specific changes in the genetic composition of a plant.

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