Modification of plant components
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With respect to plant biotechnology, 1995 and 1996 will be marked by the commercialization of the first genetically engineered plant oil and a number of ground-breaking publications. The modification of plant components using transgenic technology is not just 'switching' phenotypes from one host to another, rather, it is a means for producing valuable novel products that are normally not found (or are difficult to find) in plants. Active research is being carried out with similar schemes in both academic laboratories and biotechnology companies. As a result, the traditional line that separates the 'basic' research of universities and the 'practical' work of industry is becoming fuzzy. Although many roadblocks remain, judging from the progress made in the past two years, the genetic engineering of plant components is heading towards a bright and exciting future.

Introduction
When this subject was reviewed two years ago, the author concluded that the dream of producing novel components in plants was approaching reality [1]. In the past year or so, we have seen the realization of the commercialization of the first genetically engineered plant oil, laurate canola, as well as a few other products reaching commercial stage. It is a giant step forward in plant biotechnology, because it shows that at least in several areas the transformation has been made from discovery research (or 'proof of concept') to products in the market. On the literature front, a large number of papers in related fields has been published during 1995 and 1996, although only a few can claim to describe breakthroughs. This review covers a broad area, because almost any phenotype resulting from a genetic manipulation, including both classical breeding and molecular engineering, should be associated with modified plant components. We will therefore try to focus this review on novel components made possible only by transgenic manipulation, that is, those unlikely to be obtained by conventional breeding.

It comes as no surprise that most published papers describe work on economically and commercially important crop plants, such as maize, soybean, cotton, tobacco and canola. Molecular farming, the use of plants to produce industrial or pharmaceutical polypeptides and biopolymers, continues to show great promise. Antibodies and vaccines produced in plants bring well-publicized excitement into the field. Polysaccharide modifications in plants are a major focus of research in both academia and biotechnology companies. As reviewed below, novel vegetable oils remain one of the most successful examples of what plant biotechnologists have long promised to deliver. Furthermore, we are equally excited to see some new technical breakthroughs that have potential for developing new products.

New developments
Several recent studies have brought manipulating metabolic partitioning in transgenic plants to our attention. These manipulations were achieved by either overexpressing heterologous genes or suppressing endogenous ones [2*] to alter the metabolic sinks. By introducing into potato a gene encoding the tryptophan decarboxylase (TDC) from Catharanthus roseus, Yao et al. [3] were able to create an artificial pool of tryptamine through the redirection of tryptophan. Although the resulting transgenic plants became more susceptible to pathogenic infection, the work presents an elegant example of how metabolic sinks can be modified. In another interesting work [4], with the hope of increasing terpenoid indole alkaloid production, the same TDC gene overexpressed in C. roseus crown gall calli also resulted in the accumulation of tryptamine but failed to achieve the goal. Nonetheless, it is encouraging to observe the continuous efforts using transgenic plants to increase the production of alkaloids and other pharmaceutically important secondary metabolites. On the positive side, at least a 1000-fold increase in 4-hydroxybenzoate glucosides was obtained in transgenic tobacco expressing the bacterial ubiC gene [5]. Also, the production of carotenoids in transgenic tomato plants by overexpressing a fruit phytoene synthase resulted in a reduction of gibberellin and a dwarf phenotype [6].

Many important enzymes require biotin. The Arabidopsis biotin auxotroph bio1 fails to grow on biotin− basal media, accumulates anthoceanin in sepal and lacks epidermal wax. The bio1 mutant is apparently defec-ive in the conversion of 7-keto-8-aminopelargonic acid
to 7,8-diaminopelargonic acid. The gene encoding the *Escherichia coli* enzyme catalyzing this conversion was introduced into the *bio1* mutant, and rescued the auxotroph phenotype [7]. This study demonstrates that the plants share a similar biotin synthetic pathway with bacteria, a significant advance in possible metabolic engineering.

The characteristic flavors of fruit and vegetables are largely determined by the contents of certain volatile aldehydes and their alcohol derivatives, some of which are antimicrobial. Interestingly, these compounds are derived from polyunsaturated fatty acids by the fatty acid hydroperoxide lyase, a member of the cytochrome P450 superfamily [8]. Although the commercial significance of this enzyme remains to be determined, it is fascinating to see another cytochrome that shares common substrates from multiple metabolic pathways.

On the technology front, there were recently several eye-catching breakthroughs in crops that serve as novel biomaterials. 1996 saw the high-efficiency transformation of maize using *Agrobacterium*-mediated processes [9]. Another milestone is the development of transgenic cassava plants by bombardment [10] or *Agrobacterium* [11] procedures. The success achieved in cassava was largely attributed to the development of friable embryogenic callus and embryogenic suspension culture systems [12]. Considering that both maize and cassava are the major sources of food for millions of people in the world, these developments should be viewed as landmark accomplishments in plant biotechnology. Also worthy of mention is the strategy of utilizing the site-specific yeast recombinase for gene manipulation in plants [13], which may have broad application.

Two other important crops have also achieved excellent results with high-efficiency gene transformation. After disappointing attempts on sugar beets using *Agrobacterium*, Hall *et al.* [14] developed a polyethylene glycol-mediated transformation protocol. The breakthrough came after the authors discovered that stomatal guard cells are the preferred cell types for transformation and regeneration. In addition, the direct exposure of calli of the grapevine *Vitis vinifera*, a major cultivar for wine and table grapes, to *Agrobacterium* has been known to cause necrosis and subsequent cell death. Perl and co-workers [15] discovered that the addition of antioxidants to the media reduced cell death and resulted in an efficient transformation procedure.

**Cotton**

Imagine cotton fields covered with bolls in various shades of blue, brown, red and black. That is what a recent US patent [P1] suggests will be possible, based on the interesting concept of transferring genes controlling pigment biosynthesis from prokaryotic or eukaryotic systems to cotton, regulated by fiber-specific promoters. If successful, not only will there be color fabrics that will not fade after washing, there may also be the possibility of eliminating color dyes, major pollutants from textile mills. To date, large-scale commercial production of the naturally occurring colored cotton is unfeasible because of problems associated with low yield, poor fiber quality and weak colors. Genetic engineering technology may provide the means to solve these problems.

Recent studies of plant cellulose have revealed additional insights into the mechanism of cotton fiber biosynthesis; progress in this field was highlighted in two review papers [16,17]. A significant advance is the identification of novel plant cellulose synthase cDNAs, the cotton CelA genes, as a result of collaboration between Calgene (Davis, CA, USA) and Delmer's group (see [18,19]). The gene shares significant sequence homology with bacterial cellulose synthase genes such as BecA from *Aerobacter xylinum* [20]. Earlier, Delmer and colleagues also reported the identification of a membrane-associated sucrose synthase, and suggested that this enzyme may form a complex with the cellulose synthase as a supplier of UDP-glucose [21]. Several cotton fiber specific mRNAs and their gene promoters have been isolated by researchers at Agracetus (Middleton, WI, USA). The function of one, E6, was analyzed in transgenic cotton plants by antisense expression [22]. Although no apparent change of phenotype was observed, their approach of using fiber-specific promoters to drive antisense gene expression opens the door to similar experiments in the future. Most recently [23], the E6 promoter was used in cotton to express one of the genes encoding polyhydroxybutyrate (PHB). When combined with another PHB synthesis gene behind the cauliflower mosaic virus 35S promoter, the resulting fibers contain PHB and have better insulating properties than unmodified fibers (see Table 1).

**Polysaccharides**

Polysaccharides other than cellulose are also of commercial importance. The biosynthesis of starch was reviewed recently by Martin and Smith [24]. For example, antisense transgenic plants were studied to determine whether or not the enzyme acid invertase determines the amount of reducing sugars in cold-stored potato tubers [25]. In another example, transgenic plants expressing a bacterial mannito-1-phosphate dehydrogenase produced significant levels of polyols (sugar alcohols), which are not usually produced in plants. This led to a recent patent being issued to the University of Arizona [P2]. Plants producing polyols apparently exhibit enhanced growth rates, vigor and stress tolerance. The commercial value of such products remains to be seen.

**Proteins and biopolymers**

One of the most pleasant surprises of the year is the formation of artificial Z-membranes in transgenic plants [26]. These ‘zipped’ (Z-)membranes, formed by the fusion proteins of the avian infectious bronchitis virus (IBV) M protein and β-glucuronidase (GUS), allow the
transgene products to be concentrated within the membrane structures and therefore escape degradation. This system might be particularly useful for overexpressing integral membrane proteins that tend to be problematic in many other expression systems. The strategy of organ- or organelle-specific expression in molecular farming is continuing to be explored (see, for example, [27,28]), after a significant breakthrough reported in 1994, in which high-level transgene expression is controlled by a nuclear DNA-encoded and plastid-targeted T7 RNA polymerase [29].

Several laboratories are expanding the concept of engineering value-added crop plants. The new approaches include producing high-molecular-weight glutenin in transgenic wheat to improve baking quality [30,31], and making the engineered Brazil nut 2S albumins [39]. Expression of the vicilin deletion mutant proteins provides some insights into factors influencing the accumulation and targeting of recombinant components in seed and nonseed tissues [40].

The major hurdles for the commercial tobacco-based production of pharmaceutical proteins include overall low expression levels, uncharacterized post-translational modifications and regulatory issues involved in human drug use [41]. Overcoming these issues is no trivial task, and most of them have to be determined on a case-by-case basis. Companies that are in advanced positions in both technologies and patents will have the upper hand in success.

### Table 1

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<th>Cotton fiber engineered for enhanced thermal properties.</th>
<th>Control</th>
<th>Transgenic</th>
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<tbody>
<tr>
<td>Total heat uptake (J g⁻¹)</td>
<td>619</td>
<td>691</td>
</tr>
<tr>
<td>Fiber</td>
<td>618</td>
<td>695</td>
</tr>
<tr>
<td>Thermal conductivity (W m⁻¹ K⁻¹)</td>
<td>0.283</td>
<td>0.264*</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.263</td>
<td>0.242*</td>
</tr>
<tr>
<td>Heat capacity (J g⁻¹°C⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cp 36°C</td>
<td>1.862</td>
<td>2.022*</td>
</tr>
<tr>
<td>Cp 60°C</td>
<td>2.692</td>
<td>3.889*</td>
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Data abstracted from [23*]; see reference for explanation of units and measurements. *Lower thermal conductivity and increased ability to hold heat demonstrate improved thermal characteristics of transgenic cotton fiber. Cp, specific heat value as measured by differential scanning calorimetry.

Fatty acids

This continues to be a rapidly developing area, evidenced by the significant progress that has been made since three recent review papers were published [42–44]. High-laurate canola is the first commercial genetically engineered plant oil to hit the market [45*]. One unique feature of the triglycerides (or triacylglycerols [TAGs]) in this oil is the predominant presence of 18:1 (18 carbon; 1 double bond; olate) at the sn-2 position (i.e. TAG structure is laurate-oleate-laurate). In food applications, it is suggested that such structural arrangements have health benefits compared with the tropical laurate oils, which have a random distribution of saturates in their TAGs. In industrial applications, however, the exclusion of the sn-2 position sets a theoretical 67% limit for laurate in TAGs. Knutzon et al. [46] tackled this issue by cloning a gene from coconut encoding a 1-acyl-sn-glycerol-3-phosphate acyltransferase (or lysophosphatidic acid acyltransferase [LPAAT]; see Fig. 1). The LPAAT is specific for medium chain-length substrates, and is apparently capable of incorporating laurate into the sn-2 position of TAGs. A homolog of the coconut LPAAT gene has also been isolated from meadowfoam [47–49]. This LPAAT has specificity towards erucic acid (22:1), and could be a key enzyme for the development of a high-erucic acid rapeseed (HEAR) oil. HEAR oils are of industrial interest for being the precursors of a number of specialty industrial chemicals. Although these very long chain fatty acids are found in HEARs, they are essentially absent from edible canola due to a mutation in the fatty acid elongation (FAE) system. Metz and colleagues [50*] purified a ketoacyl-CoA synthase from jojoba, cloned a cDNA, and restored long chain fatty acid biosynthesis to canola transgenically. This result suggested that the lesion affecting erucic acid synthesis in canola is the condensing enzyme. Taking a parallel genetic approach, James et al. [51] cloned the Arabidopsis FAE1 locus; its DNA sequence reveals significant homology to that of condensing enzymes in general and strong homology to that of the jojoba enzyme in particular. Currently, approximately 50,000 tons of HEAR oils are produced annually in Europe and North America. Transgene technology can not only increase the weight contents of the very long chain fatty acids, it can also ‘custom-make’ oils with different long-chain fatty acids.

It is debatable whether or not tobacco is a good host for the production of novel components; however, because it is an excellent host for genetic modification, tremendous efforts aimed at generating transgenic tobacco plants have been made in both biotechnology companies and academic laboratories. Although it is not an ideal choice as a carrier for recombinant oral vaccines, much progress has been made in producing transgene products that may or may not require further purification. After successfully producing phytase in transgenic plant seeds, Verwoerd et al. [57] achieved high-level accumulation of the same enzyme in tobacco leaves. Transgenic tobacco cells apparently allow the correct processing of transgene products, at least in the cases of Robinia bark lectin [38] and the engineered Brazil nut 2S albumins [39]. Expression of the vicilin deletion mutant proteins provides some insights into factors influencing the accumulation and targeting of recombinant components in seed and nonseed tissues [40].
lysophosphatidic acid. (b) Limnanthes has high activity on shorter-chain saturated fatty acids and selective for unsaturated 18-carbon acyl-CoA, whereas the other enzymes in this pathway are typically nonselective. However, coconut LPAAT group to create triacylglycerol. Reactions (a), (b) and (d) each draw on acyl-CoA pools available in the cytoplasm. In most plants, LPAAT is very phosphatase activity removes the phosphate group to generate diacylglycerol. (d) Finally, diacylglycerol acyltransferase attaches the third acyl group to create triacylglycerol. Assembly of triacylglycerols in oilseeds. (a) Glycerol-3-phosphate is acylated to form 1-acyl-sn-glycerol-3-phosphate, also known as lysophosphatidic acid. (b) 1-acyl-sn-glycerol-3-phosphate is acylated to form phosphatidic acid by an enzyme known as LPAAT. (c) A phosphatase activity removes the phosphate group to generate diacylglycerol. (d) Finally, diacylglycerol acyltransferase attaches the third acyl group to create triacylglycerol. Reactions (a), (b) and (d) each draw on acyl-CoA pools available in the cytoplasm. In most plants, LPAAT is very selective for unsaturated 18-carbon acyl-CoA, whereas the other enzymes in this pathway are typically nonselective. However, coconut LPAAT has high activity on shorter-chain saturated fatty acids [46] and Limnanthes contains an LPAAT enzyme with appreciable levels of activity on erucic acid [47-49].

Acids (e.g. 20:1, 22:1 and 24:1) by introducing elongases with different substrate specificities.

Ever since the successful production of laurate in rapeseeds, it followed as a logical step to produce other ‘exotic’ fatty acids in oilseeds. Normal canola seeds do not accumulate significant amounts of medium-chain fatty acids (i.e. 8:0, 10:0, 12:0 and 14:0); however, when a 8:0/10:0-specific thioesterase from Cuphea hookeriana was expressed in rapeseeds, high levels of 8:0 and 10:0 fatty acids were accumulated [52**]. The product oils enriched in 8:0 and 10:0 but lacking 12:0 and 14:0 is a significant achievement because there are no economically viable natural sources of such oils. Structural TAGs with 10-carbon chains or shorter have broad applications for nutritional and industrial uses, including infant formulae, intravenous feeding, athletic supplements, and biodegradable lubricants and biodiesel (Table 2). Currently, these types of TAGs can only be made using expensive transesterification procedures. The ratio of different medium-chain fatty acids in the oils can also be regulated by additional thioesterase activities from other sources (see, for example, [53]).

Table 2

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<th>Possible benefits of vegetable oils with eight- and ten-carbon fatty acids.</th>
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<td>More readily absorbed</td>
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<td>More easily digested</td>
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<tr>
<td>Enhanced ‘delivery’ of essential unsaturated fatty acids in sn2 position</td>
</tr>
<tr>
<td>Catabolism more likely than direct deposition as fat, compared to longer chains</td>
</tr>
<tr>
<td>Less calories on a molar basis</td>
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<tr>
<td>Higher glycerin content</td>
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Abstracted from a wide range of nutrition literature. In addition, C8 and C10 fatty acids can be used as feedstocks for synthetic motor lubricants.

Striking results can be achieved in oilseeds by either the overexpression of a heterologous thioesterase (see above) or the antisense inhibition of an endogenous thioesterase [54]; however, it is not trivial to identify a thioesterase with the desired substrate specificity, and often the enzyme kinetics are too poor to result in dramatic changes in the lipid. Protein engineering may be the answer to some of the problems. Initial attempts to modify the substrate specificities of enzymes and to understand the catalytic mechanisms of the thioesterases have demonstrated the feasibility of designing desirable specificity and activity [55,56]. Ideally, detailed three-dimensional structures should be available to guide more rational mutagenesis. The recently solved crystal structure of a Δ9-stearoyl-ACP desaturase may allow researchers to do just that [57*].

Another ambitious but less well-publicized goal is to use plants as a delivery vehicle for long-chain polyunsaturated fatty acids (PUFAs; oils that typically contain 18–22 carbons with two or more double bonds). Many PUFAs, including arachidonic acid (ARA; 20:4), eicosapentaenoic acid (EPA; 20:5), docosahexaenoic acid (DHA; 22:6) and precursors such as γ-linolenic acid (GLA; 18:3), aid the prevention and treatment of heart disease, asthma, arthritis, and some cancers. The PUFA synthetic pathways have been identified in certain marine unicellular algae and some fungi. The largest commercial production of EPA and DHA is from fish oil, but the products are relatively expensive because of the need for fractionation, antioxidation, and deodorization. Therefore, the production of PUFAs in edible plant oils (e.g. canola and soybean oil) becomes the most attractive and cost-effective alternative. Despite the apparent rationale behind the production of PUFAs in plants, it is by no means straight-forward: the PUFA synthetic pathways in algal and fungal sources are poorly defined and the enzymes and substrates involved are uncharacterized. Also, even with the mechanism understood and the gene cloned, the compatibility of such a multigene system with that of plants remains unknown. It is difficult to predict how to engineer a plant to produce ARA or DHA without years of hard work and enforceable technical breakthroughs. A landmark accomplishment in this area was GLA accumulation in transgenic plants as a result.
of the expression of the Δ6-desaturase gene [58**]. More recently, the same group isolated another Δ6-desaturase from the plant Borage, and successfully used it for GLA production in Arabidopsis seeds (T Thomas, personal communication).

Conclusions

Engineering plant components through transgenic technology is no longer simply a proposed concept, and modified plants will continue to deliver benefits to consumers and growers. Many problems remain unsolved, however, and as research progresses, more problems will surface. Major hurdles include the stability of high levels of transgene expression and physiological effects of the transgene on the host plants (especially on germination and yield). Furthermore, there are gaps in communication between industries and research laboratories as to what is needed and what can be done. To overcome all of these problems, a collaboration of chemists, molecular biologists, plant breeders, business people, and even educators is required. Finally, we encourage those who would like a possible preview of new products for the near future to check out the list of the field trial permits issued by the US Department of Agriculture (available on the Internet at http://www.aphis.usda.gov/BBEP/).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

** of outstanding interest


This paper reviews the strategies that have been applied to this field. It illustrates well the approaches for increasing additional cellular activities to the reduction of endogenous enzymatic activities in transgenic plants.


18. Carpita N, McCann M, Giffing LR: The plant extracellular matrix: news from the cell's frontier. Plant Cell 1996, 8:1451-1463. This is a comprehensive meeting report that also reviews the most recent progress. First-time unpublished data are reported. It paints a clear picture, showing that cellulose research has moved from ultracellular structures and chemical analysis to molecular pinpointing of the biological mechanisms at gene levels.

19. Pear JR, Kawagoe Y, Schreckengost WE, Delmer DP, Stalker DM: ** Higher plants contain homologues of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. Proc Natl Acad Sci USA 1996, 93:12637-12642. Years after the bacterial CelA gene was cloned, its siblings in higher plants were finally identified. This opens the door to the understanding and engineering of cellulose biosynthesis in higher plants, the ultimate sources of cellulose.


22. John ME: Structural characterization of genes corresponding to cotton fiber mRNA, E6: reduced E6 protein in transgenic plants. Proc Natl Acad Sci USA 1996, 93:12769-12773. This paper demonstrates the technical feasibility of altering, in a tissue-specific manner, a basic structural property of cotton fiber. Although the
modification of the thermal properties of the fiber was a stated objective, one wonders if this result could have been predicted. Nonetheless, because cotton is a major world crop of central economic value to certain developing countries, the precedent of using the technology to increase the functional value of cotton fiber is an exciting one.


25. Zrenner R, Schüler K, Sonnewald U: Soluble acid invertase • determines the hexose-to-sucrose ratio in cold-stored potato tubers. Planta 1996, 198:246–252. The accumulation of hexoses (mainly glucose and fructose) in cold-stored potato is a major problem in food processes. It was believed that hexoses are the breakdown products of sucrose by invertases. This study found that there is a strong correlation between the hexose:sucrose ratio and the soluble invertase activities. Antisense inhibition of the soluble acid invertase reduced the accumulation of hexoses but increased the sucrose content during cold storage, and therefore the amounts of total soluble sugars remained unchanged. It thus suggests that the invertases regulate the ratio of hexose and sucrose, but do not control the total amount of soluble sugars in cold-stored potato tubers.

26. Gong FC, Gidding TH, Meeli JB, Staehelin LA, Galbraith DW: • Z-membranes: artificial organelles for overexpressing recombinant integral membrane proteins. Proc Natl Acad Sci USA 1996, 93:2229–2233. When the avian IBV M protein was expressed in tobacco cells as a fusion protein with GUS, a formed a novel membrane organelle. The fusion proteins are trapped within specialized, zippered endoplasmic reticulum membranes, allowing them to accumulate in a stable and presumably active form while sequestered away from the rest of the endoplasmic reticulum components. They apparently do not affect plant growth. It is unclear if the same type of structures can be formed in plant cells other than tobacco, although similar membranes have been produced in yeast. The foreign proteins have to be expressed as chimeric fusions in this system; it might affect the enzyme activity in some cases. This is still, however, an interesting discovery and deserves further exploration.

27. Parmenter DL, Boehle JG, Van Roojen GJH, Yeung EC, • Moloney MM: Production of biologically active hirudin in plants seeds using oleosin partitioning. Plant Mol Biol 1995, 29:1167–1170. The seed-ambient oil body targeted oleosin was used as a carrier for the production of hirudin, a pharmacologically valuable protein. The oleosin-hirudin fusion protein accumulated to about 1% of the total seed proteins in transgenic Brassica napus (oilseed rape). The authors further dissected the amino acid sequence requirements of the oleosin domains for the oil body targeting, revealing that both the amino-terminal and central oleosin domain are important for the process (28).


35. Poiger T, Nawrath C, Sonnewald C: Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants. Bio-Technology 1995, 13:142–150. The authors propose the use of bacteria and transgenic plants to produce biodegradable polymers such as polyhydroxyalkanoates, including PHB. Currently, most PHAs are commercially produced by bacterial fermentation; however, plants may turn out to be the most cost-effective system for large-scale production.

36. Jensen LG, Olsen O, Kops O, Wolf N, Thomsen KK, Von Wettstein O: Transgenic barley expressing a protein-engineered, thermostable (1,3-1,4)-β-glucanase during germination. Proc Natl Acad Sci USA 1996, 93:3487–3491. This paper shows an elegant example of expression in plants of a bacterial enzyme with improved functions through protein engineering. The Bacillus glucanases have been previously engineered to improve thermostability, and have been shown to be more thermostable than their barley counterparts. Computer modeling provided the possible structural basis for the increased stability. The bacterial gene encoding the novel enzyme was reconstituted to optimize the codon usage for expression in barley. The transgenic plants produced the thermostable enzyme during germination. The scheme illustrated in this paper is being applied in many other areas. There is no doubt that more work that resembles the experiment discussed will be described in the coming years.


45. Del Vecchio AJ: High-laurate canola. International News on Fats, Oils and Related Materials 1996, 7:230–243. This is a clear portrait of the development and the characteristics of the novel oil. It reminds us that the laurate canola is more than just a simple substitute for the natural tropical laurate oils. Doors are now open for fine-tuning the product to custom-fit the consumer requirements.


50. Lassner MW, Lardizabal K, Metz JG: A jojoba β-ketoacyl-CoA synthase cDNA complements the canola fatty acid elongation mutation in transgenic plants. Plant Cell 1996, 8:281–292. This paper demonstrates the key role of the β-ketoacyl-CoA synthase in determining the chain lengths of fatty acids in oil seeds, and reveals that the low-erucic acid canola is indeed caused by the mutation in this gene. This work also opens up, together with the meadowfoam LPAAT [46], the possibility of producing a transgenic rapeseed oil containing high levels of very long chain fatty acids.


52. Dehesh K, Jones A, Knutson DS, Voelker TA: Production of high levels of 8:0 and 10:0 fatty acids in transgenic canola by overexpression of Ch FatB2, a thioesterase cDNA from Cuphea hookeriana. Plant J 1996, 9:167–172. Yet another success story of the plant thioesterases. This paper re-emphasizes a proven path of redirecting fatty acid synthesis in oilseeds: identifying organisms producing the target oil, isolating genes encoding the thioesterase, and characterizing the gene product in vitro followed by transgenic expression.


55. Yuan L, Voelker TA, Hawkins DJ: Modification of the substrate specificity of an acyl-acyl carrier protein thioesterase by protein engineering. Proc Natl Acad Sci USA 1995, 92:10639–10643. Taking advantage of conserved amino acid sequences in the thioesterases, domain-swapping and site-directed mutagenesis led to the identification of regions and residues that might be critical for substrate recognition. This paper demonstrates that subtle changes of amino acid could result in altered substrate specificity. The active-site residues of the plant thioesterase were also identified by site-directed mutagenesis and enzyme assays [56].


57. Lindqvist Y, Huang W, Schneider G, Shanklin J: Crystal structure of ∆9 stearoyl-acyl carrier protein desaturase from castor seed and its relationship to other di-iron proteins. EMBO J 1996, 15:4081–4092. The first structure of a fatty acid desaturase has been determined! The aim is to understand the structural basis of the enzyme mechanism and to guide the redesign of novel specificity.

58. Reddy AS, Thomas TL: Expression of a cyanobacterial ∆6-

** Patents of special interest
