Genetic engineering of commercially useful biosynthetic pathways in transgenic plants

Ganesh M. Kishore and Christopher R. Somerville

Monsanto Company, St Louis and Michigan State University, East Lansing, USA

In many economically important plant species, the chemical composition of one or more non-protein compounds determines the value of the plant and may have an important role in protecting the plant from environmental stress, including pests, drought, salt, temperature and light. A number of potential opportunities exist whereby the range or amount of such valuable compounds can be increased by genetic engineering.

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Introduction

Millions of tons of chemicals are produced annually from agricultural plants at prices below the cost of comparable petroleum or coal-derived commodities. The major commodity chemicals produced by plants can be grouped into carbohydrates, lipids and proteins. The highest volume commodities are starch, cellulose and sucrose, which are used for a wide variety of food and non-food purposes. Similarly, many plant lipids are utilized in both the food and non-food industries, whereas a few are used only for non-food benefits. Traditionally, plant proteins have had applications as food and feed, but industrial applications offer some exciting prospects for the forseeable future. The unit value of high-volume plant-derived non-proteinaceous chemicals is currently limited by the relatively narrow range of chemical structures produced in large quantities in the storage tissues of the major cultivated field crops. In some cases, the value is also limited by the fact that plant-derived chemicals are often mixtures of related structures which must be separated before being used or may not be present in high enough concentrations for cost-effective processing.

Historically, agriculture has been used for the production of food, feed, fiber and fuel, although during the last 100 years, petrochemicals have largely replaced crop products in the fuel industry. With the recognition that petrochemical resources are limited, non-renewable and, in some cases, hazardous to the environment, there is growing interest in exploring the possibility that the value of certain agricultural species may be enhanced by genetic modifications that alter the amount or composition of non-protein storage compounds which may replace petroleum in certain applications [1,2]. Replacement of petroleum-derived materials with renewable plant alternatives may also have a beneficial social effect by reducing the level of excess agricultural production in the developed world.

The value of many economically important plants is determined by the presence of compounds which may accumulate at concentrations of less than 1% of the dry weight of a particular tissue. For instance, the compounds used for flavorings, fragrances, colorings, pesticides or medicinal purposes, are typically present in low concentrations. As genes involved in the synthesis of these compounds become available, new opportunities to enhance production or modify the chemical composition of these compounds by plant genetic engineering have arisen. Furthermore, higher plants are thought to collectively accumulate as many as 100 000 secondary metabolites and polymeric derivatives [3]. Although many of these metabolic products could have important applications if they were available in significant quantities, in many cases they are produced in plant species that are not amenable to field production or are accumulated at low concentrations that are not commercially viable. The cloning of genes for enzymes which catalyze key steps in these pathways, or the transcription factors that regulate these pathways, may enhance the feasibility of producing these compounds in transgenic plants. Similarly, as the presumed role of many natural products is to provide protection against pathogens and predators [3,4], there will be many new opportunities to improve plant resistance to pathogens and pests.

In this brief overview, we discuss recent developments that bear directly on progress towards altering the chemical composition of crop plants by the introduction of cloned genes.

Abbreviations

ACP—acyl carrier protein; **CD**—cyclodextrin; **CoA**—coenzyme A; **GBSS**—granule-bound starch synthase; **GPP**—glucose pyrophosphorylase; **MCT**—medium-chain triacylglycerides; **PHA**—polyhydroxyalkanoate; **PHB**—polyhydroxybutyrate; **SPS**—sucrose phosphate synthase.

Production of carbohydrates

A substantial proportion of the photosynthetically fixed carbon in most plants is channelled into the biosynthesis of storage carbohydrates, primarily sucrose and starch. These two carbohydrates are by far the most widely used compounds in the food industry. Sucrose is the predominant sweetener in foods throughout the world. Starch, a polymer of glucose linked via α-1,4-glycosyl bonds, is complex and consists of the linear polymer of amylose as well as amylopectin, a polymer with 1,6-glycosyl-linked branches. Both molecules can be further distinguished by the number of glucose units present in the chain, by the degree of branching and by chemical modifications, such as phosphorylation. Typically, amylose starches contain 500–600 glucose units in a linear chain while amylopectins contain 25–30 glucose units per chain with branches on every sixth to tenth glucose residue. Starches are present as granules within the amyloplast (a differentiated plastid) and the granule size varies depending on the plant species from which the starch is isolated. The physicochemical, nutritional and textural properties of food are significantly influenced by the nature of the starch. Amylose starch is easily retrograded and sheared upon processing while amylopectin (waxy) is viscous and more susceptible to retrogradation. The caloric content of amylopectin is slightly higher than amylose (4.5 kcal g^{-1} compared to 4 kcal g^{-1}). Cooked amylose produces fiber-like structures that are resistant to digestion, serve as dietary fiber and require less insulin for metabolism. Thus, it is not surprising that specialized corn starches such as high-amylose starch and waxy starch are already employed by the food industry. Mutant corn lines producing these starches are cultivated to some extent but are not widely adapted because of lower yields associated with the mutations. As discussed later, genetic technology may be applied to produce a diverse range of specialized starches in a number of crops. In view of the differences between potato and corn starch, the functional properties of amylose or waxy potato starch are likely to be distinct from amylose or waxy starch produced in corn. In addition to starch and sucrose, a number of other carbohydrates produced by plants, such as cellulose, pectin, hemicelluloses and β-1,3-glucans, are used extensively in both the food and chemical industries and are also expected to become targets for alteration by genetic engineering.

Understanding the regulation of the biosynthesis of simple sugars such as mono- and disaccharides, as well as the more complex polysaccharides, is of paramount importance in view of the fundamental importance of carbohydrates in plant growth and development. Understanding the control points in carbon partitioning will also facilitate metabolic engineering to permit carbon flow into desirable biosynthetic pathways. Sucrose, synthesized in the leaves of most plants, is the carbon feedstock for the production of storage metabolites (carbohydrates, lipids, proteins, secondary metabolites) in sink tissues (e.g. roots, tubers and seeds). The enzyme sucrose phosphate synthase (SPS) catalyzes the first unique step of sucrose biosynthesis. Expression of maize SPS cDNA in transgenic tomato plants, under the control of the promoter for the small subunit of ribulose bisphosphate carboxylase, increased the level of sucrose up to 50% in leaf tissue with a corresponding reduction in starch [5•]. Thus, carbon partitioning within the photosynthetic tissue is regulated, at least in part, by SPS. A role for SPS in non-photosynthetic tissues has not yet been established. No unique physiological effects of this increased sucrose production were described, although a detailed study is clearly warranted. Sucrose is not only the exported form of photoassimilate in most plants but also regulates the expression of a number of genes [6••].

In addition to the use of SPS for influencing starch levels in plant tissues, a number of genes directly involved in the biosynthesis of starch have recently been scrutinized. The first unique step in starch biosynthesis in plants is catalyzed by the enzyme ADP-glucose pyrophosphorylase (ADPGPP). Expression of an *Escherichia coli* mutant *ADPGPP* gene in potato plants under the control of the patatin promoter enhanced the levels of starch and dry matter accumulation in potato tubers [7••] but had no effect on starch composition or granule size [8]. A higher dry matter content in the potato tuber increases the process yield and reduces the oil content of fried potato tuber, increases the process yield and reduces the oil content of fried potato products such as French fries and chips. Very low levels of expression of the *E. coli ADPGPP* gene resulted in the maximum accumulation of starch in the tuber, suggesting that some other step was limiting starch biosynthesis in these tubers. It is interesting that no significant increase in starch content was obtained by expression of the wild-type *E. coli ADPGPP* gene, suggesting that it is the allosteric regulation of this enzyme in the potato tuber that influences starch biosynthesis and not the absolute level of the enzyme.

Complementary experiments concerning the interaction between starch biosynthesis and plant development have exploited antisense inhibition of *ADPGPP* gene expression in transgenic plants [6••]. Unlike the bacterial ADPGPP, plant ADPGPP contains two subunits referred to as brittle (B) and shrunken (S). Both subunits are required for ADPGPP activity [9]. Transgenic potato plants expressing the antisense *ADPGPP-B* gene exhibited very low levels of ADPGPP activity (2–5% of wild type) and accumulated very little starch (2–5% of control). These plants had a dramatically higher number of tubers, although the tuber weight was reduced, possibly because of a reduction in sink strength. The low-starch potato tubers had higher levels of sugars, primarily sucrose (up to sixfold increase over control) and glucose (up to eightfold increase over control). Furthermore, there was a large reduction in the level of patatin and other storage proteins. The inter-relationship between ADPGPP activity and storage protein expression is complex and poorly understood but indicates that further research in this area may also lead to an understanding of the molecular techniques required to affect the levels of protein production in sink tissues.

Increases in plant starch production are important as a number of material applications of starch are now being developed. Perhaps the most interesting application is the production of biodegradable polymers based on combinations of starch and other polymers. These biodegradable polymers range from starch–polyethylene blends, where starch is a minor component, to the more complex plastics such as Novon™ and Fertec™ with a significantly high starch content. Some versions of Novon contain 80% or more starch while others contain ethylene–vinyl acetate or ethylene–acrylic acid copolymers. Fertec™ contains 50% starch and another unnamed chemical polymer. While low-starch polymers are primarily components of films, the high-starch polymers have a number of material applications from which films and rigid molecules can be manufactured [10].

A number of commercial properties of starch are based on a particular chemical composition which may be found only in selected genotypes of certain plant species. While breeding programs have been traditionally explored to generate specialty starches, these have not been entirely successful in view of the yield reduction observed in maize plants producing modified starches. Thus, there is interest in the possibility of producing modified starches by introducing genes that modify enzymes. Several genes involved in the biosynthesis of amylose and amylopectin have been cloned recently. Visser *et al.* [11] have demonstrated that potato plants expressing the antisense gene to the granule-bound starch synthase (GBSS) have little or no amylose compared to wild-type potato,which typically contains 21% amylose. However, there was no direct correlation between the extent of reduction in GBSS activity and amylose content. The effect of increased expression of the *GBSS* gene on the amylose content was not reported and may offer some prospects for increasing the amylose content. Recently, cDNA clones for a number of starch branching enzymes have also been isolated. However, our understanding of the nature of the branching reactions catalyzed by these enzymes is still in its infancy.

A number of modified starches occur in nature; of these, only the phosphorylation of potato starch is understood to some extent. It is generally believed that phosphate is incorporated into amylopectin following its biosynthesis, although the details of this process require further investigation. In the United States, the bulk of the starch used in industry is corn starch. As corn starch is not naturally phosphorylated, a chemical phosphorylation is required for the substitution of potato starch with corn starch. Similarly, hydroxyethyl starch, produced by the reaction of starch with ethylene oxide, is used extensively in the textile, paper, coating and printing industries. Hydroxypropyl starch can be a food-thickening agent. Cationic starches containing ammonium groups are employed in the paper industry and as flocculants in the purification of industrial water and ore benefaction. A more detailed understanding of the enzymology of starch modification should allow the production of a number of derivatives of starch in plants and thus eliminate the need for downstream processing.

Starch is also modified and converted to cyclodextrins by a number of microbial enzymes. Cyclodextrins (CDs) are cyclic oligosaccharides of six (α), seven (β) or eight (γ) α-1,4-linked glucopyranose units with an apolar cavity and a hydrophilic exterior. The cavity forms inclusion complexes with a wide range of molecules, such as colorants, flavorants and drugs. CDs are of interest as delivery systems in the pharmaceutical and pesticide industries. Although not yet approved in the United States for inclusion in foods, CDs are currently being used for the extraction of cholesterol from milk and eggs. Recently, Oakes *et al.* [12] have introduced a cyclodextrin glucosyl transferase gene into potato to produce α-cyclodextrins in the tubers. Potato plants containing the patatin promoter fused to the *Klebsiella pneumoniae* CD transferase gene were found to produce very low levels of CDs (0.001–0.01% of starch converted to CDs). The *K. pneumoniae* enzyme produces both α and β CDs at a ratio of 20:1. A similar ratio was detected in transgenic potato plants.

In addition to the importance of carbohydrates as biomaterials, there is recent evidence that carbohydrate metabolism may be manipulated to alter the environmental stress resistance of plants. It has been known for some time that in response to drought, low temperature or high salinity, many plants accumulate osmotically active, low molecular weight compounds such as sugar alcohols, proline or glycine-betaine. In order to directly test the role of sugar alcohol accumulation in providing salt tolerance, a bacterial gene encoding mannitol 1-phosphate dehydrogenase was constitutively expressed in transgenic tobacco [13]. This enzyme catalyzes the reversible interconversion of fructose-6-phosphate and mannitol 1-phosphate. The transgenic plants expressing this gene accumulated mannitol in tissues such as leaf and root (up to 6 $\mu mol\ g^{-1}$ fresh weight), whereas control tobacco plants did not contain any mannitol. The accumulation of mannitol was not predictable because the dehydrogenase functions to degrade mannitol in *E. coli*. It is possible that, in the plant, the mannitol 1-phosphate produced by this enzyme is dephosphorylated and the resulting mannitol is removed from the cytoplasmic pool either by vacuolar transport or phloem loading for export to the developing leaves. It is also possible that tobacco hexokinase does not recognize mannitol as a substrate and once mannitol 1-phosphate is dephosphorylated, it is not in equilibrium with other components of the hexose metabolic pathway.

Under normal growth conditions the transgenic and control plants had indistinguishable rates of growth. However, the transgenic plants exhibited significantly higher growth rates than control plants in media containing 250 mM NaCl [14••]. Therefore, it was considered likely that the relatively low amounts of mannitol in the tissues may have accumulated in a specific cellular compartment, such as the cytoplasm, which comprises only a small proportion of total cell volume. Although many questions remain concerning the

mechanistic basis of this effect, these results suggest a promising new approach to genetic manipulation of plant stress tolerance.

Production of novel oils

Many plants accumulate high levels of triacylglycerols as carbon storage reserves in seeds or mesocarp tissues. In the major field crops, the fatty acid composition of the triacylglycerols is limited to C_{16} to C_{22} acyl chains with up to three double bonds. Although these fatty acids can be instrumental in the production of a relatively wide variety of technical products, the value of the fatty acids is generally increased by the presence of certain functional groups that expand the applicability of the fatty acids as starting materials for industrial syntheses. For instance, the ubiquitous fatty acid oleic acid ($18:1^{\Delta 9}$) is converted to ricinoleic acid in developing castor seeds by the addition of a single hydroxyl group at the C_{12} position [15]. Ricinoleic acid is approximately twice as valuable as oleic acid and serves in a wide variety of non-food applications for which oleic acid is not suitable. Thus, there is substantial interest in the possibility of increasing the value of plant oils by altering the chemical structure of the oil. Higher plants produce at least 210 different kinds of fatty acids, many of which are likely to have industrial non-food uses of higher value than edible fatty acids [16].

A first step towards the genetic control of fatty acid composition has been to control the degree of fatty acid unsaturation. Progress toward this goal has been achieved by the recent cloning of the Δ6 [17], Δ9 [18] and Δ15 desaturases [19••]. In most plants, the Δ9 stearoyl acyl carrier protein (ACP) desaturase catalyzes the first desaturation step in seed-oil biosynthesis, converting stearoyl-ACP to oleoyl-ACP. Similarly, all higher plants are thought to contain several Δ15 desaturases which catalyze the conversion of linoleic acid to α-linolenic acid. By contrast the ability to synthesize petroselenic acid (*cis*-$18:1^{\Delta 6}$) is common in members of the Umbelliferae but not in other families. Petroselenic acid is of potential commercial interest because it can be chemically cleaved at the double bond to produce lauric acid (12:0), which is used in detergents, and adipic acid (6:0 dicarboxylic) which is the monomeric component of nylon 66. Expression of a cDNA for the Δ6 desaturase from coriander in transgenic tobacco resulted in moderate levels of accumulation of the desaturase protein but a low level of accumulation of petroselenic acid and $16:1^{\Delta 4}$ [17]. This suggests that this desaturase may require the presence of another factor for full activity. This is somewhat surprising as it shares approximately 70% amino acid sequence identity with the Δ9 desturase from castor, which has been shown to be fully active when expressed in *E. coli.*

In one of the first attempts at genetic engineering of oil composition, seed-specific antisense gene constructs of *Brassica rapa* stearoyl-ACP desaturase were used to reduce the protein concentration and enzyme activity of the desaturase in developing rapeseed embryos during storage lipid biosynthesis [20•]. As expected, the resulting transgenic plants had dramatically increased seed stearate levels. In conjunction with other oil quality parameters, high stearate content is a desirable result for certain applications of edible oils, such as margarine formulations and the production of confectionery fats. Preliminary experiments, in which the Δ15 desaturase from *Arabidopsis* was overexpressed in transgenic tissues, suggest that increased expression of this enzyme leads to increased synthesis of linolenic acid, a fatty acid that is used as a drying agent in paints and coatings. Thus, it appears to be likely that it will be possible to produce plants which produce oil containing much higher than normal proportions of linolenic acid. By extension of these promising results, the pending availability of other desaturase genes should facilitate the production of plant oils with virtually any desired degree of unsaturation.

Another goal of plant-oil engineering has been to produce plants that accumulate medium-chain triacylglycerides (MCT) with eight to 12 carbons, which are used for detergent synthesis and for specialized nutritional applications. Some species of higher plants accumulate MCT due to the presence of a medium-chain specific thioesterase, which cleaves elongating medium-chain fatty acids from ACP and thereby prevents the further elongation by the fatty acid synthase complex. The production of transgenic *Arabidopsis* and *B. napus* plants that accumulate high levels of 12-carbon fatty acids was accomplished by the introduction of a thioesterase gene from California bay (*Umbellularia californica*) [21••]. Even though these species do not normally accumulate MCT, the 12-carbon fatty acids appear to be specifically incorporated into storage triacylglycerols but not into membrane lipids. The mechanism by which such 'abnormal' fatty acids are excluded from membrane lipids is not known. However, this encouraging result suggests that it may also be possible to produce other unusual fatty acids in transgenic plants without a potentially harmful accumulation of unusual fatty acids in membrane lipids.

Several plant waxes such as carnauba and jojoba have a relatively high unit value because of the high costs of production from species which have a low yield or are not well adapted to mechanized agricultural practices. There is interest, therefore, in using genetic engineering to develop a field crop that accumulates high levels of wax esters. In most plants, waxes are produced as epidermal secretions that are harvested by boiling plant tissues or, in the case of carnauba, by threshing the wax from detached fronds. However, jojoba produces liquid wax esters, which accumulate as intracellular inclusions in much the same way as triacylglycerols in oilseeds. A first step towards a feasibility test of this concept was the recent report of the cloning of a fatty acid reductase gene from jojoba [22]. This enzyme catalyzes the first step in wax synthesis, the conversion of a fatty acyl coenzyme A (CoA) ester to a fatty alcohol. It is believed that only one additional enzyme, a fatty alcohol:acyl CoA transferase, is required to synthesize wax esters.

Production of novel polymers

Many species of bacteria accumulate excess carbon as small intracellular granules composed of aliphatic polyesters ($n \approx 8000$; where n is the number of monomers in the polyester), which are collectively designated polyhydroxyalkanoates (PHA). Depending on the species of the bacteria and the composition of the growth medium, the polymers may be composed of various monomers ranging from 3-hydroxypropionate to 3-hydroxylaurate. These compounds are thermoplastics with melting temperatures in the same range as petroleum-based thermoplastics such as polypropylene. However, because many species of soil bacteria secrete an enzyme called PHA depolymerase, plastic films produced from PHA are rapidly biodegraded when placed in soil. A copolymer of polyhydroxybutyrate and polyhydroxyvalerate is produced commercially by ICI under the trade name Biopol™ and is used to produce biodegradable containers. However, even though the bacterial cultures accumulate as much as 90% of their dry weight as PHA, the most optimistic projections indicate that it will not be possible to produce PHA by fermentation for less than about US$4 per kg compared to about $1 per kg for comparable petroleum-based plastics. By contrast, the production of starch in higher plants such as maize can be accomplished for about $0.1 per kg. Therefore, if the carbon that is normally present in starch or sucrose were quantitatively diverted to PHA or related compounds, it may be possible to produce these materials in large quantities and at low cost in higher plants.

The feasibility of producing small amounts of polyhydroxybutyrate (PHB) in plants was recently demonstrated by introducing genes for the enzymes acetoacetyl-CoA reductase and PHB-synthase from the bacterium *Alcaligenes eutrophus* into *Arabidopsis* [23••]. These two enzymes catalyze the reduction of acetoacetyl-CoA to 3-hydroxybutryl-CoA and the condensation of hydroxybutryl monomers into PHB. Transgenic plant lines that contained both genes accumulated PHB as electron-lucent granules in the cytoplasm, nucleus and vacuole. In size and appearance, these granules are similar to the PHB granules that accumulate in bacteria. Only about 0.1 mg of PHB was produced per gram of tissue. However, in these preliminary experiments, no effort was made to maximize production of the polymers, and further increases in production seem possible. The fact that granules accumulated in several cellular compartments raises the possibility that the granules are capable of penetrating membranes. As there is no precedent for this observation, it is not possible to predict whether this may be a significant hurdle in the large-scale production of the materials in plants.

If further studies demonstrate the feasibility of producing economically significant quantities of PHB in transgenic plants, there will be additional opportunities to produce novel polymers by expanding the range of hydroxyalkyl-CoA monomers produced by higher plants.

Terpenoids

Structures have been reported for more than 5600 plant terpenes [3], many of which have value as polymers (e.g. rubber), solvents, flavorings, fragrances or coloring agents. Certain terpenoids have also been implicated as antimicrobial agents and as regulators of insect behavior or development [3]. In spite of the prominence of these compounds in the plant kingdom, relatively little work has been undertaken, as yet, on the isolation of genes for terpene biosynthetic enzymes. However, the feasibility of altering the terpenoid pathway has now been demonstrated by the introduction of a cDNA for a sesquiterpene cyclase, trichodiene synthase, from *Fusarium sporotrichoides* into tobacco (*Nicotiana tabacum*) [24]. The enzyme is a member of a family of 100–200 enzymes which convert the ubiquitous precursor farnesyl pyrophosphate to cyclic sequiterpenoids. The fungal enzyme was active in the leaves of transgenic plants and trichodiene was detected at a level of 5–10 ng per gram fresh weight.

In the near term, such experiments provide a useful system for dissecting the mechanisms that regulate flux through terpenoid biosynthetic pathways. These experiments also suggest that it may be relatively straightforward to exploit the chemical diversity and biological activity of many terpenes as pest control agents.

Secondary metabolites

In many instances it is desirable to simply increase the level of accumulation of a compound in a plant that normally produces it at low levels. A notable example is the current necessity for processing about 370 000 kg of bark from *Taxus brevifolia* to produce about 25 kg of the anticancer drug, taxol [25]. The essential problem in these cases is that most compounds of interest are the product of a multienzyme pathway. Thus, it is probably necessary, in most instances, to increase the level of activity of many enzymes in order to effect an increased flux through the pathway. The results of several recent experiments suggest that in at least some multienzyme pathways, all the enzymes are under the transcriptional control of a small number of *trans*-acting factors. This raises the possibility of producing transgenic plants in which overexpression of a transcriptional factor causes a corresponding increase in the level of expression of the enzymes of a complex pathway.

This principle is exemplified by the recent production of high levels of anthocyanins in various tissues of maize by introducing a gene for the *Lc*-transcriptional factor placed under the control of a constitutive promoter [26]. The *Lc* gene is one of a family of related regulatory genes that control the pattern of accumulation of anthocyanin in various maize tissues. Constitutive expression of this transcriptional factor resulted in the accumulation of high levels of anthocyanins in tissues, such as roots, that do not normally accumulate

pigments. Similarly, expression of the closely related *C1* and *R* genes from maize in transgenic *Arabidopsis* plants also resulted in pigmentation of tissues that are not normally pigmented [27•]. Thus, by analogy, it seems likely that the isolation of *trans*-acting factors that regulate the accumulation of other classes of secondary metabolites may have a broad application in increasing the accumulation of related metabolites in heterologous species.

A related result, which promises a fresh approach to the production of valuable plant metabolites in tissue culture, involves the activation of a biosynthetic pathway by the introduction of a transcriptional enhancer adjacent to a regulatory locus. The feasibility of this approach was demonstrated by transforming tobacco protoplasts with T-DNA containing a transcriptional enhancer adjacent to the right border of the T-DNA. Selection for auxin-independent growth of the transformants resulted in the isolation of several tobacco cell lines which exhibited auxin-independent growth because of the activation of a previously unknown gene [28••]. It seems likely that if a suitable assay is available for the detection of calli that accumulate compounds of interest, this method may permit the isolation of cell lines that accumulate high levels of valuable metabolites.

There are now a number of examples of increases in the level of production of various metabolites, or the production of novel compounds, by the introduction of a single gene. A commercially promising example involves *Atropa belladonna*, which normally accumulates high levels of the anticholinergic tropane alkaloid hyoscyamine but has low levels of the more valuable alkaloid scopolamine. The enzyme hysocyamine 6β-hydroxylase, found almost exclusively in the pericycle of roots from *Hyoscyamus niger*, catalyzes two consecutive hydroxylation reactions which epoxidize hyoscyamine to scopolamine. Constitutive expression of the hydroxylase gene from *H. niger* in transgenic *A. belladonna* plants resulted in plants that accumulated scopolamine almost exclusively in the leaves [29].

Another recent example is the expression of stilbene synthase from groundnut (*Arachis hypogaea*) in transgenic tobacco calli [30]. Stilbene synthase catalyzes the condensation of *p*-coumaryl-CoA and three molecules of malonyl-CoA into 3,4,5-trihydroxystilbene (resveratrol). Transgenic tobacco calli containing the stilbene synthase gene, when treated with appropriate elicitors, produced resveratrol. Resveratrol has antifungal and antiplatelet activities. These examples highlight the novel possibilities for introducing new metabolic activities into crop plants to enhance pest resistance or to produce important pharmacological agents.

In many cases it seems likely that the overexpression of a single gene will not lead to significantly increased flux through a multistep pathway. For instance, in order to examine the regulation of the pathway for synthesis of the putrescine-derived alkaloid nicotine, root cultures of *Nicotiana rustica* were transformed with the *Saccharomyces cerevisiae* gene encoding ornithine decarboxylase under control of the cauliflower mosaic virus 35S promoter. The presence of the yeast gene enhanced ornithine decarboxylase activity in the plant tissue. However, the accumulation of putrescine and nicotine was only doubled, suggesting that other factors limit the metabolic flux into the nicotine biosynthetic pathway [31].

Conclusions

Although there is a relatively small number of examples, the available evidence suggests that it is generally feasible to use genetic engineering methods to enhance or permit production of economically or agronomically valuable compounds in transgenic plants. The main limitation at present is the relatively small number of structural and regulatory genes available for biosynthetic pathways.

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GM Kishore, Monsanto Agricultural Group, 700 Chesterfield Village Parkway, St Louis, MO 63198, USA.

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