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4. Genetically modified crops for industrial products and processes and their effects on human health

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4.1. Introduction

Industrial crops are grown as sources of chemicals or components that serve non-food uses. Products derived from plants for industrial applications include oils, fibres, fuels and pharmaceuticals. Some of these products are extracted from the plant and used directly by industry, while others require mechanical or chemical conversion to be suitable for application. The plant itself may be the conveyor of the product, for example vaccines produced in fruits and vegetables (Carter & Langridge, 2002).

The genetic modification of crops to improve industrial applications is relevant to human health because there are very few crops that are grown strictly for industrial use. Concern about quarantine of genetically modified (GM) industrial crops, to prevent intermingling with the food supply, has recently resulted in implementation of a more restrictive regulatory policy for such crops.[2] Since profitability in agriculture often relies on using as many parts of the crop as possible, for example selling the residual meal from oilseed processing for use in animal feed, industrial GM crops are required to undergo approval for such use. This chapter discusses GM crops that have important, commercial, non-food uses or are expected to have such uses in the future. Since many of the GM industrial crops proposed have not been commercially released, descriptions of their effects on human health are generic in nature. It is hoped that this chapter will at least identify fundamental benefits and risks associated with GM industrial crops and identify issues peculiar to this class of GM organisms.

Although a seemingly innumerable variety of GM crops has been proposed and developed, only a limited number have been introduced and achieved commercial success. Soybean, cotton, maize and canola are the major crops for which there are commercial GM varieties (Chapter 3) and, according to the International Service for the Acquisition of Agri-biotech Applications (ISAAA), they account for almost 100% of GM crops planted worldwide.[3] Currently, Laurate Canola and High-Oleate soybean are the only commercially available GM industrial crops that produce a product (oil) with a composition that has been altered to make it more useful for industry (Ohlrogge, 1999). Considerable biochemical research, combined with genomics and proteomics, is underway to elucidate biosynthetic pathways and identify relevant genes for the production of commercially valuable products (Mazur, Krebbers, & Tingey, 1999; Somerville & Bonetta, 2001; Timko &

[1] The author is a research chemist and is not responsible or consulted for developing official policy with regard to GM plant regulation. Any views or opinions expressed in this text are the author's own or a result of the consensus discussion. Official GM crop policies are under the purview of the US-EPA, US-FDA and USDA-APHIS.

[2] USDA (2002), Riverdale, MA, USA, available [December 2002] at http://www.aphis.usda.gov/ppq/biotech/pdf/pharm-2002.pdf.

[3] ETC Group (2002) *Ag Biotech Countdown: Vital Statistics and GM crops* (Update June, 2002), Winnipeg, Canada, Action Group on Erosion, Technology and Concentration, available [January 2003] at http://www.etcgroup.org.

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Cahoon, 1999; White & Benning, 2001). Many trial GM plants have been tested, at least in the greenhouse.[4] However, it is apparent that much of the promise of GM industrial crops is still in the first phase, that is plants engineered with single gene changes for agronomic improvement; the second phase, plants engineered for improved products resulting from more complex genetic alteration, is still in the research and development stage (Ohlrogge, 1999).

This chapter focuses on GM plants that are grown on a commercial scale and their industrial applications. Since a number of GM crops are under development and show some promise in terms of producing a desired product for industry, these are also considered. Finally, the effects of the industrial application of these crops on human health and any related effects are examined, with an emphasis on concerns that are unique to industrial GM crops.

4.2. Expected contribution of GM industrial crops

4.2.1. Vegetable oils

Oilseeds are an important source of chemicals for industry (Appleqvist, 1989; Broun & Somerville, 2001). Most temperate climate oilseeds produce oils containing the same five fatty acids (palmitate, stearate, oleate, linoleate and alpha-linolenate) in different proportions (Vles & Gottenbos, 1989). In addition to nutritive uses, these fatty acids are used to produce soaps and detergents, coatings, lubricants, cosmetics, plastics, plasticisers and numerous chemical derivatives (Hatje, 1989). For specific uses, certain fatty acids are more desirable. For example, the conjugated double bond system present in fatty acids of tung oil gives it excellent properties as a drying oil (Pryde & Rothfus, 1989); and the medium-chain fatty acid, lauric acid, which is supplied by coconut and GM canola (Somerville & Bonetta, 2001), is used as an anionic surfactant, whereas hydroformylation of petroleum is the alternative means for providing an equivalent surfactant (Porter, 1997). The possibility of replacing such petroleum products with plant-derived fatty acids has long been recognised and Laurate Canola was the first commercial product that proved the approach (Voelker *et al*., 1992). The ability to manipulate fatty acid composition in oilseed has resulted from a combination of three approaches. First, biochemical characterisation has identified most of the steps in fatty acid biosynthesis. Secondly, genetic identification and chemical characterisation of fatty acid biosynthetic mutants in mutated *Arabidopsis thaliana* has provided an extensive genetic map of fatty acid and lipid biosynthetic steps during plant growth and development (Browse & Somerville, 1991). Finally, the additional information needed to broaden the spectrum of fatty acids available from oilseeds has been provided by the identification, characterisation and cloning of unusual enzyme activities from plants that produce uncommon, industrially useful fatty acids (Broun, Shanklin, Whittle, & Sommerville, 1998). Hundreds of uncommon fatty acids, with unusual chemical functionalities, are produced by one or more oilseed plants (Hitchcock & Nichols, 1971). A considerable amount of research has gone into elucidating the biosynthetic process by which such fatty acids are made; much of the enzymology underlying the introduction of unsaturation, conjugated unsaturation, and hydroxyl, acetylenic and epoxy functionality is now understood (Broun *et al*., 1998). The enzymes that carry out each of these reactions are interrelated, can be interconverted by engineering appropriate amino acid residues, and to a limited extent can have their specificity for chain length and modification site altered (Broun *et al*., 1998). The specificity of the chemistry carried out on what is essentially a straight hydrocarbon chain is unprecedented for the bench chemist, and presents the possibility of 'green' chemistry carried out in green plants to produce desired chemicals for industrial applications.

It is the chemical functionality of a vegetable oil that can make it useful to industry; chemical functionality can alter physical properties or allow chemical precursors or useful derivatives to be made. For example, ricinoleate, the fatty acid from castor oil, has a mid-chain hydroxyl group that enhances its viscous properties for use as a grease and also enables production of an extensive range of chemical derivatives (Caupin, 1997; Pryde & Rothfus, 1989). Another industrially useful feature is high enrichment of a single component, such as oleate (from High Oleate soybean; Ohlrogge, 1999), as the expense of purifying the desired component can be eliminated. Some oils have fatty acids with unique uses, such as coconut oil or Laurate Canola oil, which both produce lauric acid. Lauric acid is especially suited for and widely used in detergent production (Knauf, 1995; Porter, 1997). Consistent composition is also important for industry, and this is usually closely tied to high content of a desired component. One goal of developing GM oilseeds for industrial use is to introduce the genetic changes necessary to meet one or more of these criteria. Laurate Canola and High-Oleate soybean are examples of two such crops. Laurate Canola produces an oil of greater than 50% lauric acid (dodecanoic acid), which is used to make soaps and surfactants (e.g. sodium dodecyl sulphate) that have desirable foaming properties, (Porter, 1997). High-Ole-

[4] The Animal and Plant Inspection Service (APHIS) of the US Department of Agriculture approves field tests of GM organisms in the USA (Information Systems for Biotechnology, *Field Test Releases in the U.S.,* Blacksburg, VA, USA, ISB, available [December 2002] at http://www.nbiap.vt.edu/cfdocs/fieldtests1.cfm).

Table 4.1. GM oilseeds with modified oil content

Crop	Modification	Stage	Use
Canola	High laurate content	Commercial	Detergent
Soybean	High oleate content	Commercial	Food, monomers
Canola	High stearate	Developed	Grease
Canola	Petroselenate	In development	Food, monomers
Soybean	Vernolate	In development	Plasticizer, coatings
Cotton	Low-saturates	In development	Food uses

Table 4.2. Industrial use vegetable oils

Oilseed	Application	Fatty acid
Flax	Coatings, plasticizer	α-linolenic acid
Castor	Lubricants, cosmetics, plastics	Ricinoleic acid
Coconut	Detergents	Lauric acid
Rapeseed	Slip agents	Erucic acid
Jojoba	Lubricants, cosmetics	Wax ester
Soybean	Inks, plasticizer, coatings	Linoleic acid

ate soybean oil contains greater than 80% oleic acid. Its high *cis*-monounsaturate content makes it beneficial for food use and also makes it a desirable chemical feedstock (Kinney, 2002). A high-oleate version of cottonseed oil has also been produced at the Commonwealth Scientific and Industrial Research Organisation (CSIRO; Anon., 2001a). A brief list of proposed GM oilseed crops is presented in Table 4.1.

Another application of transgenic technology is the development of oilseeds with improved agronomic characteristics. In fact, this has been the primary goal of agricultural chemical producers that have initiated programs to produce GM crops. Soybean, maize, cotton and canola are high volume oilseeds in the USA and other countries, and their major GM variants incorporate genes for herbicide resistance or insect resistance. In fact, according to the ISAAA, 77% of GM varieties have herbicide resistance, 15% have insect resistance and 8% have both (stacked traits).[5] As plant genomics and proteomics programs identify other agronomically useful genes, other transgenic traits will also be incorporated in GM crops (see Chapter 3). According to information from Canadian, US and Australian governmental regulatory agencies, upcoming traits include cold tolerance, drought tolerance and viral resistance.[6–8]

[5] ETC Group (2002) *Ag Biotech Countdown: Vital Statistics and GM crops* (Update June, 2002), Winnipeg, Canada, Action Group on Erosion, Technology and Concentration, available [January 2003] at http://www.etcgroup.org.

[6] *Canadian Food Inspection Agency*, available [December 2002] at http://www.inspection.gc.ca.

[7] *Animal and Plant Health Inspection Service*, available [December 2002] at http://www.aphis.usda.gov.

[8] *Office of the Gene Technology Regulator*, available [December 2002] at http://www.health.gov.au/ogtr/.

Vegetable oils have numerous industrial uses, depending on the fatty acid composition of the oil. A driving force behind development of GM oils is the perennial surplus of oils produced. The unused inventory of soybean oil may reach nearly 900 thousand tonnes (2 billion pounds) in any year (USDA, 2002). GM crops with altered oil composition hold the promise of reducing or preventing annual inventory carryover, thus stabilising or improving farm income. Table 4.2 presents oils derived from crops that are generally considered industrial crops. There are active research programs to modify the oil composition of several of these crops to expand their utility for industrial use or to produce an uncommon fatty acid in an agronomically preferred crop (Hildebrand, Rao, & Hatanaka, 2002; Kinney, 2002; Schultz & Ohlrogge, 2002); several are described below.

Soybean

Soybean oil from *Glycine max* represents the major oil crop of the USA and it also accounts for a considerable amount of world vegetable oil production, in spite of the fact that the bean produces approximately 20% oil (USDA, 2002). Efforts to expand its industrial uses by transgenic methods revolve around its fatty acid composition; soybean oil is normally high in linoleic acid and contains 10–15% saturated fatty acid. The High-Oleate soybean is primarily intended for food markets but it also is useful as a hydraulic oil, since it is liquid and stable to oxidation. It is also biodegradable, an important factor in reducing costs of oil spill clean up (Kinney, 2002). The high polyunsaturated fatty acid content of soybean oil makes it less desirable for food use because of the ease of rancidity, but lends itself to use in products where the drying quality of polyunsaturates is desirable, including printing inks and coatings (Erhan & Bagby, 1992; Pryde & Rothfus, 1989; Wilson, Burton, Pantalone, & Dewey, 2002). Efforts to develop GM soybeans that introduce additional unsaturation or epoxide groups into the oil support the expansion of industrial applications for soybean oil (Kinney, 2002; Hildebrand *et al*., 2002).

Flax

Flax oil (linseed oil) from *Linum usitatissimum* L. has a high content (55%) of alpha-linolenic acid, which makes it an excellent drying oil for coatings. As a food supplement it is also finding increasing use as a dietary source of the essential omega-3 fatty acids and lignans (Oomah, 2001). Although GM varieties with altered fatty acid composition have been developed, the only GM crop of flax that has been introduced commercially, a sulphonyl urea (herbicide) resistant plant, was withdrawn from the market as a result of the concern of Canadian flax growers that the European Union would not accept GM flaxseed for food uses (Kraweczyk, 1999).

Rapeseed and canola

Rapeseed oil, from *Brassica napa*, has a high content of erucic acid (13-docosaenoic acid); it is used as a slip agent to separate plastic sheets during manufacturing processes and also in fabric softeners (Ohlrogge, 1994). It can also be used to produce monomers for engineering plastics (Pryde & Rothfus, 1989) and efforts are underway to develop a GM rapeseed with higher erucic acid content (Brough *et al*., 1996). Since erucic acid is considered to be toxic, Canadian plant breeders developed canola, using traditional breeding to obtain a low erucate oil with other desirable characteristics. Because it has a greatly reduced capacity to produce fatty acid chains longer than 18 carbons, canola is a useful target crop for producing and accumulating novel fatty acids. Moreover, its close genetic heritage to Arabidopsis provides a wealth of relevant genetic information with which to manipulate canola gene expression. It was used to develop the first commercial GM oilseed with a modified oil composition (Knutzon *et al*., 1992) and it is considered the prime candidate for producing industrially useful oils (White & Benning, 2001).

4.2.2. Starch

Starch is a mixture of two types of alpha-D-glucopyranosyl polymers, the straight chain alpha-amylose, in which glucose units are linked by 1–4 linkages, and the branched chain amylopectin, in which glucose units are linked by both 1–4 and 1–6 linkages, the latter forming branchpoints. Like vegetable oil, starch is an energy reserve for seed germination and also serves a combination of food and non-food applications. Starch is used to produce adhesives, plastics, water absorbent polymers and, as a fermentable substrate, ethanol for fuel and lactic acid and other chemicals for polymer production. Starch gels are also important functional components of prepared foods, in addition to providing a dietary source of calories. There has been a considerable research effort to elucidate starch biosynthesis, and numerous genes that alter the products of starch biosynthesis have been identified (Kossman & Lloyd, 2000).

The principal sources of starch for industrial use are maize and potato, with starch derived from cassava, rice, and cereal grains increasing in market share. Each of these starch crops has GM derivatives in development or in the field, including some with modified starch content (Falk, Rassmussen, Schulman, & Jannson, 2001; Kossmann & Lloyd, 2000; Moffat, 1999). However, the genetic modification of starch structure is still in the discovery phase (Kossmann & Lloyd, 2000).

Properties of starch gels are dependent on interactions of the glucan chains with each other and with solute, most often water. Modifications of the starch structure can dramatically alter its properties. One example is starch produced by a GM potato in which antisense inhibition of three starch synthase genes results in production of short chain amylopectin free of amylose. The gelatinised starch is stable to freeze–thaw induced syneresis, the separation of the starch into a liquid and solid phase, through five freeze–thaw cycles. Low amylose starches (waxy starch) exist naturally, but they have stability to freeze–thaw syneresis for only one cycle; the food industry, therefore, usually uses chemical modification to produce a freeze-stable starch. This GM starch could thus replace the chemical processing currently required to produce starch gelatins that are stable to freezing. In some cases, modification to enhance the amylose content for improved plastic or adhesive applications could also result in a food with a reduced glycemic index (Jobling, Westcott, Tayal, Jeffcoat, & Schwall, 2002).

4.2.3. Fibre

Fibre crops represent another important class of industrial products. Cotton is the major textile fibre crop grown. Nutritive uses of cottonseed oil and cottonseed meal are as a food oil and animal feed, respectively (Anon., 2000). Cotton is the only GM fibre crop that has been commercialised, and it has been engineered for agronomic performance by the introduction of *Bt* toxin for insect resistance (see Chapter 3). Commercial GM cotton varieties have also been altered to express herbicide resistance genes for post-emergence weed control. Insect resistance provides a cost saving to growers and accounts for the high proportion of GM cotton grown in the USA and its rapid acceptance in other cotton producing nations (see Chapter 3). There are no commercial varieties using transgenic technology to modify fibre quality. However, there are reports of GM cotton plants that produce cotton with improved fibres, either longer, stronger or in higher yield (Wilkins, Rajasekaran, & Anderson, 2000). Since there are natural cotton varieties that produce coloured fibres and an array of floral colour pathways have been identified (Bourgaud, Gravot, Milesi, & Gontier, 2001; Kimmel *et al.*, 2002; Martin & Gerats, 1993), it is interesting to speculate that genes from these pathways might be used to expand the palette of colours available for natural cotton. The dye industry, which provided a major economic impetus for the development of organic chemistry and chemical manufacturing in the nineteenth century, may eventually convert to transgenic technology, one of the industries of the twenty-first century.

There are two types of flax, flax grown for oil, described above, and flax grown for fibre, which is used to produce linen, a premium textile. The volume of linen flax production is considerably less than that of cotton. There is no reported development of GM flax for linen production, although modifications used for GM oilseed flax varieties should be transferable. The fibre from the oilseed crop is strong, but too short for premium textile production. This fibre is used in specialty paper production, for example cigarette paper, and composite materials (Kraweczyk, 1999; USDA, 2002).

4.2.4. Forest products

Trees provide lumber, fuel and a source of fibre for paper production. Proposed improvements to trees for paper production envision large plantations of monoculture GM trees, modified to contain lower levels of lignin (Dinus, Payne, Sewell, Chiang, & Tuskan, 2001). The paper industry currently processes lumber to produce 185 million metric tons of pulp for paper, of which 70% is derived from chemical treatment, resulting in high environmental and economic costs (Pilate *et al.*, 2002). Reduction in lignin content could result in considerable cost savings and reduced water pollution. Although the knowledge to reduce lignin content by genetic modification is available and there are natural low-lignin varieties as well, the growth of such trees can be compromised if lignin content is too low (Pilate *et al.*, 2002). An aspen tree with modified lignin content is now undergoing field trials (Wu, Hu, & Han, 2000) and a recent evaluation of 4-year old poplars engineered to reduce lignin content resulted in a slight reduction of chemical input necessary for Kraft pulping (Pilate *et al.*, 2002). Since most GM trees grown for industrial production will be in plantations, the presence of these large monocultures will necessitate the incorporation of genes for insect resistance (Delledonne *et al.*, 2001; Harcourt *et al.*, 2000). Trees under consideration for plantation growth include GM versions of poplar (Pilate *et al.*, 2002), pine (Pena & Seguin, 2001), white spruce (Pena & Seguin, 2001) and eucalyptus (Harcourt *et al.*, 2000) species. In order to prevent out-crossing of the GM incorporated genes, genes that confer male sterility are likely to be used in GM tree plantations (Dinus *et al.*, 2001; Pena & Seguin, 2001). Varieties that do not fruit are also considered valuable when biomass is a consideration, as nutrients and energy are put into growth and not reproduction (Dinus *et al.*, 2001).

4.2.5. Protein

Protein from plants as a bulk industrial commodity is used in the production of adhesives and plastics. Genetic modification could be used to provide better quality adhesives or plastics, but the bulk nature of protein used suggests that such changes are not imminent. Numerous hydrolytic enzymes, lipases and proteases are used in industry, and these could also be produced in GM plants (Knauf, 1995). Transformation strategies specifically geared to production of high levels of specific proteins in plants have been developed (Bogorad, 2000). Laccase, an enzyme used in curing coatings, is the first commercialised industrial enzyme and is produced in GM corn; other proteins produced in GM corn by the same company include aprotinin and β-glucuronidase, proteins used for biochemical applications.[9]

Potato and tobacco have been modified to produce the proteins (spidroins) that make up spider silk (Scheller, Guhrs, Grosse, & Conrad, 2001). Spider silk is considered to be the strongest natural fibre, with a tensile strength comparable to that of steel but with high elasticity. It could be used to replace Kevlar as lightweight armour protective material, but it may also find use in woven textiles (Scheller *et al.*, 2001; Somerville & Bonetta, 2001). The spidroins have not yet been converted to fibre from these crops, so applications remain in the speculative realm.

4.2.6. Chemicals

Plant secondary metabolism has been an ongoing interest of chemists and biochemists for over a century. A broad range of chemicals can be obtained from plants, including pigments, flavour compounds, terpe-

[9] ProdiGene, *Product Development,* available [January 2003] at http://www.prodigene.com.

noids, polyketides and alkaloids (Bourgaud *et al*., 2001; Broun & Somerville, 2001). With the exception of secreted or vesicle-stored compounds, which can accumulate to a worthwhile volume in a harvested portion of a plant, only small compounds with high value, such as alkaloid drugs, would be worth producing in a GM plant, as small molecules tend not to accumulate to a high degree unless they are sequestered (Somerville & Bonetta, 2001). A poppy engineered for higher alkaloid production is currently in the testing stage.[10]

4.2.7. Natural polymers

In addition to starch, cellulose-fibre and protein, plants produce a variety of polymers. Natural rubber latex from *Hevea brasiliensis*, for example, is an important commercial polymer (USDA, 2002). There are currently initiatives in rubber-producing countries to use genetic modification to improve agronomic traits of *Hevea*, as well as yield and quality of yield of natural rubber latex from *Hevea*.[11]

Numerous bacteria make a biodegradeable plastic film consisting of a polymer of alpha-hydroxybutanoate (PHB) esters. In one of the early demonstrations of plant transformation involving the expression of multiple introduced genes, the model plant, *Arabidopsis thaliana* was altered to produce this plastic (Nawrath, Poirier, & Sommerville, 1994). Although chloroplast localisation of the product led to high accumulation in the plant, the plastic was not of suitable quality. Polyhydroxyalkanoate (PHA) copolymers have better properties, and a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate has been produced in *Arabidopsis* and *Brassicca* (Slater *et al*., 1999). Production of up to 15% (considered the profitable level) plant weight was achieved on a laboratory scale, but consistent high levels have not been realised.

A smaller market exists for carbohydrate polymers that are used as gums, for a variety of purposes, including thixotropic agents for paints (keeping mineral based pigments in suspension) and oil well drilling fluids, as well as numerous food uses to provide desirable textures and viscosities. Current research in genetics and proteomics is likely to elucidate the means to produce GM gums for such purposes (Chapple & Carpita, 1998).

4.2.8. Pharmaceuticals and nutraceuticals

A number of high value pharmaceutical proteins (i.e. various antibodies, vaccines, streptokinase, erythropoietin, human insulin, among others) are today isolated from humans or animals, or must be produced in bacteria or moulds by fermentation. These methods of production are costly and inefficient, and it is often necessary to purify the products extensively to ensure that they are safe for human or animal use (Giddings, Allison, Brooks, & Carter, 2000). Production of pharmaceutical proteins in plants, sometimes referred to as ‘Farmaceuticals’, is an attractive alternative. A number of food crop plants have been evaluated as model systems (i.e. potatoes, rice, lettuce, spinach, alfalfa, soybeans, barley, wheat, and canola). Tobacco, a non-food crop, could also be employed.

A novel approach to vaccination proposed the expression of vaccines in edible parts of plants, with vaccination resulting from the ingestion. A principal advantage of such an approach is vaccine delivery to poorly developed areas with limited access to proper medical care and storage conditions for manufactured vaccines. Effective edible vaccines have been developed in potato and lettuce (Walmsley & Arntzen, 2000; Yu & Langridge, 2001). Given concerns over possible commingling of farmed and BioPharmed crops,[12] it is less likely that edible vaccines will be widely adopted. However, plant production of vaccines retains certain advantages and the use of plants as bioreactors in which crude vaccines (to be further purified) are produced is likely to increase (Carter & Langridge, 2002).

If 5–10% of the soluble protein of corn could be turned into a desired protein, 15–25 kg protein per hectare could be produced. The first commercial plant-based pharmaceutical was hirudin, isolated from oilseed rape (Giddings *et al*., 2000). One company has recently announced commercial production of trypsin from corn plants as the world’s first large scale manufacture of a recombinant protein from a transgenic plant system.[13] The plant-based production of avidin has been reported by the same company (Hood, *et al*., 1997), which has also entered into a collaboration with the National Institutes of Health to produce an AIDS vaccine, based on its success with expression of a subunit vaccine against simian immune deficiency virus (SIV)[14] in corn. Anticoagulants and somatotropin[15] have also been pro-

[10] Office of the Gene Technology Regulator, *GMO Record*, Canberra, Australia, OGTR, available [January 2003] at http://www.health.gov/au/ogtr/.

[11] The International Rubber Research and Development Board, *IRRDB.NET*, available [January 2003] at http://www.irrdb.com.

[12] USDA (2002), Riverdale, MA, USA, available [December 2002] at http://www.aphis.usda.gov/ppq/biotech/pdf/pharm-2002.pdf.

[13] ProdiGene (2002) *ProdiGene launches first large scale-up manufacturing of recombinant protein from plant system,* College Station, Texas, ProdiGene, available [September 2002] at http://www.prodigene.com.

[14] ProdiGene (2002) *ProdiGene announces milestone in NIH collaboration for AIDS vaccine, College* Station, Texas, ProdiGene. Available [September 2002] at http://www.prodigene.com/0501.htm.

[15] SemBioSys (2001) *SemBioSys Genetic Inc. receives US patent for its plant-based somatotropin production technology,* Calgary, Canada, SemBioSys, available [September 2002] at http://www.Sembiosis.ca/pressreleases/SomatotropinPressRelease-Oct2001.pdf.

duced in corn. Field tests have been approved for corn producing cytokines, tobacco producing human urokinase, corn producing procollagen, rice producing human serum albumin, and barley producing antitrypsin and antithrombin (Giddings *et al*., 2000).

Tobacco is an attractive non-food crop for the production of pharmaceutical proteins. It produces high quantities of biomass and protein per hectare. It is also a closed pollinator with few diseases or pests. Tobacco can be easily transfected with a single-stranded RNA virus, which can be used to introduce desired proteins. Propagation of transfected cells for 24 h results in production of significant quantities of protein that can then be extracted from the plant matter. Tobacco mosaic virus (TMV) has been used as a vector to produce human and animal viral protein epitopes (Holtz, 2000). Production of proteins in tobacco provides an environment suitable for protein processing and folding while eliminating the presence of human or animal antigens and infections agents (e.g. viruses and prions).

GM plants can also be used to produce proteins that have medicinal or nutritional value, such as antibodies, recombinant enzymes, or human proteins (Daniell, Streatfield, & Wycoff, 2001). For example, infant formulae based on soybean or cow's milk could be supplemented with or eventually replaced by human milk proteins produced in plants. GM potatoes that produce human beta-casein have been reported (Chong *et al*., 1997). Expression levels of beta-casein reached 2% of the total plant protein, which is enough to produce about 7000 l of formula per hectare. Human alpha-lactalbumin (Takase & Hagiwara, 1998) has been expressed in tobacco, and lactoferrin, lysozyme and alpha-1-antitrypsin, have been expressed in rice (Rodriguez, Lonnerdal, Nandi, Wu, & Huang, 2000). It may, therefore, be possible to produce plant-derived human infant formulae or, alternatively, to introduce proteins ordinarily found only in human milk into commonly eaten crop plants.

The future use of plants to produce pharmaceuticals and human proteins will depend on a number of factors. It must be demonstrated that plants can economically produce significant quantities of high purity proteins that are safe to consume. Plants containing such proteins will need to be grown in isolation and the identity of the crops carefully preserved. Strict measures will need to be taken to ensure that they do not become commingled with conventional crops. It will need to be established that consumers will accept production using these new plants.

In addition to drugs, certain plants produce nutraceuticals. These are components present in some foods that have beneficial effects on health and are considered healthful enough to isolate and use to enrich foods deficient in the nutraceutical, or to take as supplements. Nutraceuticals proposed for enhanced expression in appropriate crops include gamma-linolenic acid, iso-flavones and plant sterols (Bourgaud *et al*., 2001; Kochian & Garvin, 2000; Napier & Michaelson, 2001).

4.2.9. Fuel

Ethanol from starch fermentation, wood, and biodiesel from vegetable oil represent the major fuel sources from plants. Research is underway to develop ways to generate fermentable carbohydrate from herbaceous plants and plant residues, in addition to increasing the yield of biomass (Vogel & Jung, 2001). Additional approaches to expand agricultural sources of fuel include GM tree crops, GM-enhanced starch content and utilisation to increase ethanol production from crops, such as maize. Increased vegetable oil content could lead to lower cost biodiesel fuel, and comparative genomics for gene discovery may lead to more productive GM oil crops (White & Benning, 2001).

4.2.10. Phytoremediation

Phytoremediation is the use of plants to decontaminate soil and water (Watanabe, 2001). Some plants naturally accumulate certain heavy metals and, with the discovery of phytochelatins, which bind a broad spectrum of heavy metal ions, GM plants, mainly *Brassica*, that grow in the presence of heavy metal ions and accumulate them in their tissues have been developed (Meagher, 2000). These can be used to clean up sites at mines and chemical dumps. A GM tobacco plant that expresses a bacterial nitroreductase will metabolise trinitrotoluene (TNT) to the less toxic aminodinitrotoluene (Hannink *et al*., 2001); other organic pollutants, including chloroaromatics and halogenated hydrocarbons, can be metabolised to inorganic residues (Meagher, 2000). Such plants point to the potential to use plants to treat soil and water polluted with heavy metal ions or organic chemical contaminants. Although the current experimental GM crops are small annuals, the expectation is that trees, such as the readily transformed poplar, will be used for phytoremediation, since their roots penetrate far into the soil, and they absorb much greater amounts of groundwater, therefore treating a greater volume than the annuals (Tzfira, Zuker, & Altman, 1998).

4.3. Outcomes and impacts of use of GM industrial crops

Section 4.2 describes a number of expected products arising from the introduction of GM crops. As noted, many of these are not commercially available. Some have been field tested, but most exist on a laboratory or greenhouse scale. Factual descriptions of the effects of GM industrial crops are limited to the few commercial types grown. The expected benefits from the next phase of GM crops include expanded use of renewable resources for fuel, fibre and chemical processes, decreased air and water pollution, and wider availability of food additives for enhancing food quality and nutrition.

4.3.1. Herbicide and insect resistance

Soybean, cotton, maize and canola are the major GM crops grown in the USA and elsewhere.[16] Although the manipulation of plant biochemical pathways is the key area in plant research that will increase industrial uses of GM crops, the major GM industrial use crops developed have been modified to include herbicide or insect resistance.[16] Based on conclusions from economic studies originating in the 1980s, which indicated that GM seed sales would be the key profitable area of GM plants, several of the major agricultural chemical manufacturers acquired seed companies and undertook research programs that would provide market niches for their chemical products. The driving forces behind this strategy were consumer concern about pesticide residues in crop plants, regulatory mandates against groundwater pollution, and directives requiring an expensive registration of crop chemicals for each specific crop.

The benefits derived from commercial GM crops include increased profitability for farmers, as a result of reduced chemical input (see Chapter 3). Reduced toxic exposure is an additional benefit; for example, farmers in China growing GM cotton experienced a 75% reduction in exposure to the toxic effects of agrochemicals (Huang, Rozelle, Pray, & Wang, 2002). In the USA, GM crops require less chemical input, leading to an increased profitability for the grower. The herbicide resistant GM crops require purchase of the agrochemical and thus guarantee sales for the chemical company. Insecticide resistant crops require less spraying and thus reduce environmental exposure. These reductions in chemical exposure are immediately beneficial to the farmer, farm workers and wildlife. They should also help alleviate consumer concern about chemical residues in food.

4.3.2. Crops entering food chain

The Starlink episode (see Chapter 8) provides the backdrop for concerns about industrial crops entering the food chain. Starlink was approved by the US Food and Drug Administration (FDA) for use only as animal feed, since the insecticidal protein CrY9c is not readily digested by pepsin and, as a result, the protein might pass intact into the human gut and sensitise the consumer (Kuiper, Kleter, Noteborn, & Kok, 2001). As yet, no human case of an allergic reaction to this protein has been identified. However, any food crop modified to produce a toxic, noxious or bioactive compound can present a potential health hazard. The crop and components of the crop must be isolated from the food supply, using a fail-safe identify preservation (IP) system (see Section 4.4).[17]

Cross-pollination with food crops is a particular concern, and several strategies for preventing it have been described (Daniell, 2002; Kuvshinov, Koivu, Kanerva, & Pehu, 2001). By their nature, pharmaceutical proteins produced in crops could have significant health effects if commingled with food crops. Some research groups propose to use male-sterile tobacco to prevent entry of such proteins into the food chain (Gruber *et al*., 2001; Menassa, Nguyen, Jevnikar, & Brandle, 2001). Such crops are expected to be grown in limited areas, and should be surrounded by a buffer crop to block cross-pollination or low probability revertants if male-sterile (Dale, Clarke, & Fonte, 2002; Daniell, 2002). An additional concern for non-sterile crops is seed drop during harvesting, which can result in germination and growth of the crop among the crop planted in the next growing season. A recent case highlights this problem. Some corn grown to produce a pharmaceutical protein in one growing season was left in a field and germinated among a crop of soybeans. As a result, the soybeans were destroyed.[18]

4.4. Standards of use for GM industrial crops

Industrial crops are considered beneficial because they are renewable resources, yield biodegradeable products and are environmentally benign. To the extent they provide materials that supplant products derived from petroleum, they are clearly 'green' alternatives to synthetic chemicals, and inherently benefit human health by reducing exposure to atmospheric and aqueous emissions from petroleum processing plants. However, the use of transgenic technology to expand applications of industrial crops for 'green' uses is seen in some quarters as 'anti-green' technology. Since much controversy about genetic modification is concerned with the safety of food derived from GM plants, genetic modification of industrial crops ought to be relatively free of controversy. Of course, this is not the case, for a number of reasons. Many by-products of industrial crop proces-

[16] ETC Group (2002) *Ag Biotech Countdown: Vital Statistics and GM crops* (Update June, 2002), Winnipeg, Canada, Action Group on Erosion, Technology and Concentration, available [January 2003] at http://www.etcgroup.org.

[17] USDA (2002), Riverdale, MA, USA, available [December 2002] at http://www.aphis.usda.gov/ppq/biotech/pdf/pharm-2002.pdf.

[18] Cohen (2002) *GM Crops Mishaps Unite Friend and Foe*. New Scientist Online News, 18 November, 17:10. Available [January 2003] at: http://www.newscientist.com/hottopics/gm/gm.jsp?id=ns99993073.

sing enter the food supply. For example, after oilseeds, such as industrial rapeseed, have been processed to remove the oil, the remaining meal is protein rich and processed for use as animal feed. In some cases, the meal may be used to produce foods for human consumption; for example, flax meal provides nutritional benefit in the form of lignans and omega-3 oil residue (Oomah, 2001). Cotton fibre is used by the textile industry and is limited to non-food use, but cottonseed meal and oil enter the food supply as animal feed and vegetable oil. Additionally, many crops have dual uses. Soybean is primarily a food crop, but soybean oil and soybean protein are also used for non-food applications, such as inks, coatings and adhesives. The Starlink maize incident in the recent past has made it clear that approving a food crop for strictly non-human use (animal feed) may not be sufficient to prevent it from entering the human food supply. As described by Kuiper and colleagues (Kuiper *et al.*, 2001), there were no genuinely harmful consequences of the Starlink corn to human health, only to positive perception of the GM crop industry. The more recent case described above (Section 4.3.2), involving commingling of GM corn carrying an unknown, presumed therapeutic protein, underlines the need for a failsafe IP system and appropriate quarantine (both space and time), if food crops are to be used to produce pharmaceuticals or potentially noxious products. In the case of Starlink, with animal feed being an inherently cheaper end-use than human food, there was no economic motivation to maintain it separately from other maize. While high economic value for the GM product is probably the safest assurance for IP of a GM industrial crop, it is evident that even the profit motive can fail.

For GM crop approval in the USA, the action and approval of three Federal Agencies is required (see Chapter 3). The FDA evaluates the crop in terms of direct and indirect food use, the Environmental Protection Agency (EPA) registers the crop in terms of potential environmental effects and the US Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) evaluates GM crops for their potential to disrupt the growth habit of domestic plants.

IP is the means by which a crop is maintained separately during storage, shipping and processing, and has been in practice for several specialty crops (Anon., 2001b) that have added value, for example, non-GM low-saturated soybean. Insofar as comingling a GM industrial crop with other crops could result in health problems, it is essential to segregate GM crops from other crops, in the field and post-harvest. The concept of IP and crop isolation is also important to market perception. Those opposed to eating food from GM crops require IP of non-GM crops, in addition to those non-GM crops that have enough added value that it is worth the extra cost of IP to maintain them as separate products. The cost of GM is in the range of 5–10% of the crop's value (Anon., 2001b). If a given GM crop delivers higher value to the end user, there is at least an economic incentive to keep it from contaminating a lower value crop.

4.5. Methods for evaluating outcomes and impacts of GM industrial crops

4.5.1. Risk assessment of industrial crops

The genetic modification of crops to make them better suited for industrial purposes necessitates an evaluation of their potential for hazard. The primary consideration is whether the modification to the crop makes it harmful. In every case, a designed crop, by definition, will produce the desired product. Based on existing toxicological data it is very likely that either the chemical product itself or related compounds will provide the means to develop a toxicological profile and determine any potential harm arising from production. A novel product would require an independent toxicological assessment. The crop residue remaining after extraction of the product will need to be evaluated in terms of remaining product, as well as changes in the crop residue resulting from the alterations required to make the product. Changes in metabolism brought about to enhance production of a single product can be, at least qualitatively, predicted, based on knowledge of biosynthetic pathways affected by the alteration. Potential detrimental effects would require testing for such changes, for example iso-flavone levels in glyphosate-resistant soybean. In short, aside from the additional product, the question to be answered is whether the crop retains 'substantial equivalence' to the non-GM crop (Kuiper *et al.*, 2001; see also Chapter 8). Finally, migration of the transgene(s) into other crops must be evaluated from the standpoint of potential for harm and the likelihood that it will actually occur. Knowledge of agronomic habit allows assessment of the latter and toxicological analysis provides the needed information for the former. Any potentially toxic or noxious product can be restricted to sterile strains. Although pollen release from GM crops to produce 'superweeds' is a common scenario for concern, a more significant problem may arise from commingling as a result of seed drop during harvest. It is likely that any such crop that is not controlled to prevent gene release will not be permitted (Smyth, Khachatourians, & Phillips, 2002). Transgenic technology holds out great promise for expanding the use of renewable resources in production of industrial products. Accordingly, it is essential that such a benefit be implemented to prevent any harmful effects.

4.5.2. Most likely beneficiaries

Aside from safety issues, the primary means to evaluate the success and the impact of a GM industrial crop is whether it is commercially successful. As stated above, there are many GM crops that have been successful in the greenhouse, and are ready to be or may have already been tested in the field, but are not yet commercially available. However, 75% of all cotton grown in the USA, 60% of soybean and 30–40% of maize are GM crops (Thayer, 2000). Clearly, these crops incorporate a genetic complement desired by US farmers, namely the expectation of lower costs for planting and maintaining the crop. The only GM crop to have a financial incentive built in for production is Laurate Canola. This crop was originally planted under contract, guaranteeing the farmer a higher price per pound, compared with conventional canola. Part of the financial incentive for developing Laurate Canola was the promise of a stable US crop for domestic producers of products that incorporate laurate. The prices of imported sources, coconut oil and palm kernel oil, fluctuate considerably, and in some years prices can reach levels over three times higher than that of canola oil (USDA, 2002). While economic incentive and the possibility of a stable domestic supply would seem attractive, Laurate Canola does not seem to have been successful as a commercial substitute for the tropical oils.

4.5.3. Time frame for anticipated benefits

In terms of providing a domestic source of laurate, Laurate Canola was a technical success. The levels of laurate were similar to those in imported oils (Voelker *et al.*, 1992), and the seed is still available commercially. Its long-term success remains an open question, although it is important as probably the first commercialised GM industrial crop. It should be noted that as part of a strategy to enhance the profitability of this crop, approval for food use of the oil was sought and received from US and Canadian regulators of GM crops. Even a well designed, well considered, commercially supported crop can prove to be less than successful as a result of unpredictable market forces that can affect demand for the product.

4.6. Knowledge gaps

4.6.1. Allergenicity

In some ways, transgenic technology for crops awaiting the call to commerce still has a 'black box' quality. Genes for given characteristics have been cloned and sequenced; perhaps quantities of the protein have been isolated after expression in bacteria or yeast. In many instances, the activity of the native protein has only been demonstrated by the change brought about *in planta*. Therefore, its potential for becoming a problem allergen remains unknown. In those cases where the primary product is free of the GM crop protein (oil, fibre, industrial chemicals) or the GM crop protein is the desired product (pharmaceuticals, industrial enzymes), the allergenicity of the protein is not a food health issue. If there is a possibility that the protein can be incorporated into human food, then protein allergenicity clearly becomes a consideration. In plants that have undergone 'metabolic engineering' the introduced gene(s) is (are) often over-expressed in order to redirect the flow of metabolites to the desired product leading to a relatively high level in the plant. Altered timing for expression may also be implemented. Promoter technology is still in a relatively primitive state. In oilseeds, it is common to use promoters that drive the synthesis of storage proteins, in order to limit expression of the gene introduced to the seed (Knutzon *et al.*, 1992) and because the storage proteins that are controlled by the promoters are produced at a high level (Hoglund, Rodin, Larsson, & Rask, 1992). As a result, high levels of an expressed protein can accumulate and, since storage proteins are expressed late into seed development, proteins produced to alter metabolism of the immature seed may be retained to a much higher degree than usual in the harvested seed, in which they would not normally be present. There are methods for demonstrating potential allergenicity, for example model pepsin digestion reactions (Kuiper *et al.*, 2001), and a 'decision-tree' for assessing allergenicity has been proposed (Metcalfe *et al.*, 1996). Animal models for allergenicity have also been proposed (Buchanan, 2001) to supplement the 'decision-tree'. In cases where the protein is not available in isolation, theoretical models to predict allergenicity from the protein sequence are essential to ensure the safety of associated by-products, such as seed meal.

4.6.2. Identity preservation

IP is an important facet of GM crop safety. Countries that have prohibitions against GM crop imports require certification of the import, and this requires IP. The exporter thus must maintain the harvested crop separately, from planting through storage and shipping. Strategies to do this have been developed, but they add expense (Anon., 2001b). If IP is required on a much broader scale, it is not clear how the costs to the growers and consumers will be affected. Furthermore, there are mechanisms that could lead to the breakdown of an IP system, including cross pollination from a GM crop and

GM seed carryover (Smyth *et al*., 2002). Ultimately, it is possible that, for full GM-free certification, it would be necessary to certify the nature of crops grown within a specific area and for the field to be certified as producing non-GM crops for a certain number of years. There may be other unknown mechanisms that result in IP breakdown as well.

4.6.3. Development of resistance

GM crops have been grown for a short time. One of the expected results of the GM crops that have been introduced was a diminution of pesticide and herbicide pollution. This appears to be the case in the short term, and seems likely to remain the case. Since resistance to applied pesticides has been commonly observed for cultivated non-GM plants, it seems possible that weeds and insects might develop resistance over time, as is thought to have occurred in the case of specific variants of the *Bt* toxin protein, and there are concerns about superweeds developing from GM crop pollen spreading to nearby, related weeds (Chapter 3; Smyth *et al*., 2002; Snow, 2002).

4.6.4. Pollen transfer

There is ecological concern about transmission of pollen from some types of plant, such as the *Brassica*s and tree crops, either into weedy relatives or into crops grown at some distance. This problem is not limited to GM plants. Canola, a rapeseed cultivar bred to produce low glucosinolates and low erucic acid, must be planted in isolation from industrial rapeseed, as each crop will result in seed with altered composition from the ideal: that is, excessive erucic acid in the canola, and less erucic acid in the industrial rapeseed. Because the products of industrial crops are not intended for consumption, and may even be noxious, risk management and containment, including the prevention of intercrop cross-pollination is essential. Approaches described by Daniell (2002) and Kuvshinov, Koivu, Kanerva, and Pehu (2001) can address the problem of out-crossing from GM crops.

4.6.5. Economics

A major argument for promoting transgenic technology has been the need to provide more food. Some industrial applications use surplus products, for example soybean oil, and provide a buffer against surplus crops causing a decline in farm profitability. However, it is not clear to what extent agriculture may be diverted away from food production, resulting in increased food costs, if industrial crops are grown in higher volume and have a higher value than food crops.

If the success of GM industrial crops is to be measured on the basis of their commercial success (see Hitz, 1999, for an economic analysis of GM industrial crop profitability), a corollary is that the success of such crops also can lead to economic disruption of an unpredictable order. If the application of transgenic technology in agriculture is as successful as has been heralded, significant volumes of products to replace petroleum and chemical manufacturing will be produced, affecting the prosperity of these industries. The overall benefit will be great, as 'cleaner' chemistry is introduced industry-wide, but industries and workers may be displaced.

In other cases, products that are currently obtained from crops grown in developing countries, such as castor oil, rubber and lauric acid, could be replaced by GM crops grown in temperate regions, causing economic displacement to the less wealthy countries. To the extent that GM products may be produced more cheaply in the developing countries, these countries will also benefit from the same new technologies. Where new uses, such as biodiesel fuel and fuel additives produced from castor oil and laurate (Choo, Ma, & Ong, 1997), will greatly expand demand, economic disruptions may be offset. Although economic disruption is not a direct human health effect, the two are inversely correlated. The impact of transgenic technologies in industrial agriculture on the world economy remains to be seen.

4.6.6. Public acceptability

At present little is known about how acceptable or otherwise the development of GM industrial crops might be to the general public; the topic is worthy of further empirical investigation, which will be carried out in the marketplace.

4.7. Conclusion

Crops can be genetically modified to produce oils, starch, fibre, protein or other chemicals useful for industrial processes. In particular, soybean oil, with high oleate content, and canola oil, rich in laurate, are both being produced by transgenic methods. Most such products are already available from agricultural or manufacturing sources that do not involve transgenic technologies.

A principal concern is how to use genetic modification technology in a way that gains the advantage of using renewable resources to replace products from petroleum and other non-renewable resources, while maintaining a safe and adequate human food supply. It is also crucial to ensure that GM crops designed to produce industrial products do not inadvertently enter the human food chain or contaminate food or other crops with their transgenes.

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