Novel food products from genetically modified crop plants: methods and future prospects

Jim M. Dunwell

School of Plant Sciences, The University of Reading, Reading, UK

Summary Using a variety of *in vitro* techniques, it is now possible to isolate a selected gene sequence from any source and introduce it into any major crop plant. Millions of hectares of such genetically modified (GM) or transgenic plants are already being grown commercially, mostly in North America. To date, the most widely grown GM crops (soybean and maize) are those with modified agronomic traits (herbicide or insect tolerance); the products from these commodity crops are now included in a wide range of processed foods. This review describes the methods used to generate these GM crops and then discusses the range of modified food products that can be generated using this new technology. Such products include those with altered protein, starch or oil quality, as well as examples of improved micronutrient or vitamin content. Much of this work, particularly that aiming to develop food with specific health benefits, is still at the experimental stage, but there is no doubt that many GM foodstuffs, with an increasing variety of qualitative changes, will reach the market in the coming years. The rate at which such products are developed commercially depends to a large extent on the public reaction to a technology still poorly understood by most consumers.

Keywords Allergen, oil, protein, starch, transgenic, vitamin.

Introduction

Over the last few years, genetically modified (transgenic) plants have moved from being laboratory curiosities to major commercial products. The first such genetically modified (GM) products that reached the consumer were tomatoes with modified ripening and processing characteristics, and these have been quickly followed by several field crops (corn and soybean) with improved herbicide (Nida *et al*., 1996; Padgette *et al*., 1996) and/or insect tolerance (Carozzi & Koziel, 1997; Perferon, 1997). The most recent estimates suggest that the market for transgenic seed has already reached several hundred million dollars per year, that more than 15 million hectares are being grown in the USA in 1998 and that the exported material from these crops (principally from the USA) is now included in many processed food products in Europe. In any consideration of GM food it is important to distinguish those food products that have been deliberately designed to have an altered food quality (e.g. tomato) from those that have been derived from transgenic crops (e.g. corn and soybean) with altered agronomic traits.

Because of space constraints, this review will not cover the whole subject of food biotechnology (Dörnenburg & Lang-Hinrichs, 1994) or indeed of the use of transgenic plants to produce new products for industrial and pharmaceutical purposes (Owen & Pen, 1996); instead it will consider first the methods used to generate transgenic plants and will then consider some of the types of modification that can be produced using these methods. It will then describe issues that determine the speed of commercialization, and some of the regulatory and public perception aspects relating to this new class of food product.

Correspondent: Fax: +44 (0)118 931 6577.
e-mail: J.Dunwell@reading.ac.uk

Transformation methods

Although the first attempts to produce transgenic plants were made in the 1960s, it was not until the early 1980s that success was achieved. Since that time, the methods have become commonplace for many crop plants and there are few, if any, important crops from which transgenics have not been produced (Dunwell, 1995). Initially, most success was reported with techniques that used the soil bacterium *Agrobacterium tumefaciens* as a vector for introduced genes, and at that time it was assumed by many that cereal crops were not susceptible to this method. There was much interest therefore in the discovery of direct DNA transfer using small metal particles (usually gold) propelled at high speed into the target tissue, which is usually the scutellum of an immature zygotic embryo (Takumi & Shimada, 1997). The general subject of particle bombardment or 'biolistics' has been reviewed by Daniell (1997).

Within the last few years, *Agrobacterium*-based protocols have been amended to allow uptake by cereal tissue (Ishida *et al*., 1996), and therefore there is no longer any need to rely on direct DNA methods for species such as rice, wheat and maize. In addition to the two principal methods described above, several other techniques have been developed (for a review see Potrykus & Spangenberg, 1995). These include the uptake of plasmid DNA directly into protoplasts (cells from which the cell wall has been removed), and the perforation of cell walls with electrical or physical methods such as by vortexing with needle-shaped crystals of silicon carbide – an industrial abrasive (Wang *et al*., 1996).

In addition to major advances in methods for gene transfer, the last decade has witnessed great improvements in the understanding of the particular DNA sequences required for high and stable levels of transgene expression. It is now possible for the molecular biologist to include in the 'construct' to be transferred, specific 'promoters' that control the site of expression of the gene of interest. For example, there is a range of well-characterized promoters that permit high-level expression in the seed (Vincentz *et al*., 1997), especially in the endosperm – the preferred site of activity for many of the transgenes used in cereals (Russell & Fromm, 1997).

When designing a DNA construct and selecting a transformation method to use for a particular species, a choice can be made on the basis of either technical efficiency or, where necessary, commercial freedom; many of the available methods and relevant gene sequences are the subject of granted patents.

Targets for modification

Protein

Among the first transgenic experiments were many in which efforts were made to amend the storage protein composition, or express a storage protein gene in parts of the plant other than the seed. As these methods have now been refined and the sequences of many storage proteins are known in detail (Lawrence *et al*., 1994), it is possible to design specific alterations to protein quality, either to modify the content of a particular amino acid (Saalbach *et al*., 1994; De Clercq *et al*., 1996; Marcellino *et al*., 1996; Coleman *et al*., 1997; Molvig *et al*., 1997; Falco *et al*., 1998) or to induce functional changes (Gidamis *et al*., 1995; Utsumi *et al*., 1997) or to introduce a novel protein such as the high-molecular-weight glutenins implicated in bread-making quality (Shewry *et al*., 1995; Blechl & Anderson, 1996; Barro *et al*., 1997; Shimoni *et al*., 1997). This last method of wheat improvement has recently been granted a US patent (Blechl & Anderson, 1997).

Another recent opportunity provided by transgenic methods is the possibility of reducing the content of those proteins with specific allergenic properties. Such an approach has been demonstrated successfully in rice (Nakamura & Matsuda, 1996; Tada *et al*., 1996) and could be adopted in peanut, for which the allergenic protein(s) have been isolated (Burks *et al*., 1997; Stanley *et al*., 1997; Shin *et al*., 1998) and for which the relevant transgenic methods have now been developed (Mansur *et al*., 1995; Singsit *et al*., 1997). The general issue of the allergenic status of transgenic food (Nordlee *et al*., 1996) is most important and will be considered in more detail below in the section covering regulatory aspects.

An interesting recent publication related to the issue of human allergenicity is the report that the human milk β-casein protein has been produced in transgenic potato tubers (Chong *et al*., 1997) and the human alphaA-lactalbumin in tobacco leaves (Takase & Hagiwara, 1998). These findings open the way for the reconstitution of human milk from edible plants as a replacement for bovine milk in baby foods, and the consequent prevention of gastric and intestinal diseases in children. Of course, the public acceptability of this type of product is a particularly sensitive issue (Hoban, 1996) (see below).

Carbohydrates

There are two main approaches to the modification of the carbohydrate content of crops and thereby of foodstuffs. The first comprises a qualitative or quantitative change to an existing compound, usually sucrose (Klann *et al*., 1996; Secor *et al*., 1997) or starch (Bruinenberg *et al*., 1995; Sivak & Preiss, 1995), whereas the second involves the introduction of a novel product or products. This second approach is exemplified by the introduction into chicory of fructan-encoding genes from onion (Vijn *et al*., 1997). Fructans (Ebskamp *et al*., 1994; Hellwege *et al*., 1997) are of increasing significance to many food companies interested in relatively high-value, non-calorific carbohydrates, and it is likely that this type of product will soon be available with any desired chain length and degree of branching. Other examples of high-value carbohydrates produced in plants include bacterial cyclodextrins that have been produced in transgenic potato tubers (Oakes *et al*., 1991).

Oils/fats

The oil quality, and therefore nutritional value, of a food is determined by the combination of its chain length and degree of saturation, with the combination being a characteristic of a particular species (Cahoon *et al*., 1997). In recent years, many of the genes that control these two features have been cloned, and transgenics with altered oil quality are widespread (Topfer *et al*., 1995; Zou *et al*., 1997; Eccleston & Ohlrogge, 1998). For example, Sayanova *et al*. (1997) produced a transgenic tobacco that contained a delta6-desaturase gene from borage and was thus able to accumulate gamma-linolenic acid, a component of evening primrose oil widely used as a dietary supplement.

Vitamins, micronutrients and secondary products

Until relatively recently it was not possible to modify selectively the vitamin content of plants by genetic modification. However, there is now sufficient biochemical understanding of some of the critical pathways, and modified plants have been produced. For example, phytoene synthase, the enzyme that condenses two molecules of geranyl geranyl diphosphate, is necessary for beta-carotene (provitamin A) synthesis in plants. Recently, the gene encoding this enzyme in daffodil has been transferred into rice and expressed in the endosperm (Burkhardt *et al*., 1997), a staple food for two billion people, many of whom suffer vitamin A deficiency. Similar studies are under way on various fruit and vegetables, including tomato, in order to improve vitamin/antioxidant content (Hauptman *et al*., 1997), an objective likely to be aided by the recent analysis of the pathway for vitamin C synthesis in higher plants (Wheeler *et al*., 1998).

In the area of flavours and fragrances, aroma compounds such as vanillin, benzaldehyde and 4-(R)-decanolide constitute a market of several thousand tons per year (Krings & Berger, 1998). The biosynthetic pathway for each of these compounds is now relatively well understood, and it can be assumed that directed synthesis in transgenic plants will soon be accomplished. General aspects of secondary metabolism, including isoprenoid synthesis, are covered in a recent review by McCaskill & Croteau (1997).

Pharmaceuticals/antibodies

One of the most active areas of research at present is the production of medically active compounds in plants. These vary from the oils and vitamins as described above to more general aspects of dietary composition (Knauf & Facciotti, 1995) and particularly to therapeutic antibodies (Ma & Hein, 1995; Fiedler *et al*., 1997) and vaccines (Arntzen, 1998). Perhaps the best example of food-related antibody production in plants is the use of the antibody raised to the adhesion protein of *Streptococcus mutans*, one of the bacteria associated with dental caries. In the most recent study (Ma *et al*., 1998), a functional comparison was made between a monoclonal secretory antibody raised in plants and its parent murine IgG antibody. It was found that the former plant-derived antibody afforded specific protection in humans against oral streptococcal colonization for at least 4 months.

A most interesting example of the use of antigen production within plants (Tacket *et al*., 1998) is provided by the recent results of Ma *et al.* (1997), who showed that a transgenic plant expression system was capable of synthesizing an immunogenic form of the diabetes-associated autoantigen, glutamic acid decarboxylase. When provided as a dietary supplement, the transgenic plant inhibited the development of diabetes in a non-obese diabetic mouse.

The proposed advantage of vaccine production in plants (Mason & Arntzen, 1995; Lam & Arntzen, 1996, 1997; Arntzen, 1997; Arakawa *et al*., 1997, 1998) lies in the fact that new vaccines need to be inexpensive, easily administered, and capable of being stored and transported without refrigeration (banana or potato seem to be the favoured targets); without these features, developing countries may find it difficult to adopt vaccination effectively. In addition to a role in combatting disease, plant-derived vaccines have been suggested recently to have potential as a means of contraception (Smith *et al*., 1997). The public acceptability of plant and other GM vaccines has been considered by Danner (1997); see also below.

Other

There are many other specialized examples of new products being produced in transgenic plants. These range from the expression of synthetic (Wallis *et al*., 1997) or natural antifreeze proteins from the Arctic flounder (Hightower *et al*., 1991) or beetle (Duman, 1997) – a method designed to protect foodstuffs from the deleterious effects of freezing – to novel non-calorific sweeteners such as thaumatin (Zemanek & Wasserman, 1995). The use of this latter type of protein to sweeten food is the subject of a recently granted US patent (Fischer *et al*., 1998). In contrast to the addition of novel compounds to improve food quality, some companies are developing methods to remove unwanted compounds. For example, it might be possible to generate transgenic coffee (Adams & Zarowitz, 1994), in which caffeine synthesis is inhibited (a 'naturally decaffeinated' product).

Regulatory considerations

A major consideration for companies involved in the development of transgenic foodstuffs is the cost and time required for full regulatory assessment of the new product (Day, 1996). One of the most important aspects of this process is the confirmation of essential equivalence, that is that the novel product is not significantly different from the conventional one in terms of its chemical composition (Shinmoto *et al*., 1995; Anan *et al*., 1996; Klein *et al*., 1998) and especially its allergenicity (Astwood & Fuchs, 1996; Lehrer *et al*., 1996; Lehrer & Reese, 1997; Metcalfe *et al*., 1996; Gendel, 1998a,b). This latter aspect is vital in light of the increasing incidence of food-related (Moneret-Vautrin, 1998) and other (Caballero & Martin-Esteban, 1998) allergenic responses among the general public. The content of known dietary inhibitors such as lectins (Peumans & van Damme, 1996) and alpha-amylase inhibitors (Pusztai *et al*., 1995) also need to be considered.

Timescales for commercialization

This issue has been addressed by Dunwell (1996), who examined the relative rate of product development of transgenic and non-transgenic varieties. Much of the difference, where it exists, is due to the extensive backcrossing programme often required to transfer the introduced gene into the required background and to any field trials (Tieman *et al*., 1995) and regulatory testing demanded (Bright *et al*., 1996; Miele, 1997).

Public acceptance

There has been much recent discussion about the public response to GM food (Burke, 1998; Wymer, 1998), whether it be in the form of a uniform product comprising entirely material from a modified plant (eg tomato puree), or more particularly where the processed product of a modified plant (eg soya flour) is included as a minor constituent in a large number of products. In the latter case there is no evidence that the 'GM product' is any different in terms of its chemical composition to the 'normal product' (Padgette *et al*., 1996), yet there is a vociferous lobby arguing against such products on the grounds either of safety (see Dunwell, 1997) or of a general moral opposition to 'tampering' with nature. These issues have been considered in several studies, either in relation to specific types of product (Hoban, 1996; Danner, 1997) or as part of a general survey of public reactions to risk (Frewer *et al*., 1998).

Future prospects

It is difficult in this area of science to predict how quickly new products will become available and be accepted by the consumer (see above). In principle, it is now possible to introduce any gene from any organism into any plant, and to express that new product in any part of the plant, be it seed, leaf, root or tuber. Increasingly, food is being considered not merely as a source of basic nutrition but rather as a product with specific medicinal properties (Goodman *et al*., 1997), so-called 'functional food'. For example, changes in the basic composition of the sort discussed above will be followed by more radical modifications; powerful human growth factors have already been produced in fruit (Kobayashi *et al*., 1996) and leaves (Lee *et al*., 1997), and it would be relatively simple to reduce the levels of toxic compounds such as oxalate (Ito *et al*., 1996), a compound that should be avoided by those suffering from urolithiasis – the deposition of kidney and bladder stones. As we move towards food with more specific health benefits (Knauf & Facciotti, 1995), and the opportunities created by such products, the difficulty of prediction lies not so much in the world of science but rather in estimating commercial success.

Conclusion

We are moving rapidly into a new age of food design in which the value of high-quality food products will be enhanced further by the application of transgenic techniques. In the final analysis, the extent of these changes, and their eventual value, will be determined by the most important arbiter, the consumer (Thompson, 1997).

Acknowledgements

I would like to thank Zeneca and the Biotechnology and Biological Sciences Research Council for financial support during the preparation of this review.

References

Adams, T.L. & Zarowitz, M.A. (1994). Stably transformed coffee plant cells and plantlets. US Patent 5334529.

Anan, T., Ito, H. & Monma, S. (1996). Chemical contents in fruits of transgenic tomato carrying the TMV coat protein gene, nontransgenic tomato, and other *Lycopersicon* species. *Journal of the Japanese Society of Horticultural Science*, **65**, 635–644.

Arakawa, T., Chong, D.K., Merritt, J.L. & Langridge, W.H. (1997). Expression of cholera toxinB subunit oligomers in transgenic potato plants. *Transgenic Research*, **6**, 403–413.

Arakawa, T., Chong, D.K. & Langridge, W.H. (1998). Efficacy of a food plant-based oral cholera toxin B subunit vaccine. *Nature Bio/Technology*, **16**, 292–297.

Arntzen, C.J. (1997). Edible vaccines. *Public Health Reports*, **112**, 190–197.

Arntzen, C.J. (1998). Pharmaceutical foodstuffs – oral immunization with transgenic plants. *Nature Medicine*, **4**, 502–503.

Astwood, J.D. & Fuchs, R.L. (1996). Allergenicity of foods from transgenic plants. *Monographs on Allergy*, **32**, 105–120.

Barro, F., Rooke, L., Bekes, F., *et al.* (1997). Transformation of wheat with high molecular weight subunit genes results in improved functional properties. *Nature Bio/Technology*, **15**, 1295–1299.

Blechl, A.E. & Anderson, O.D. (1996). Expression of a novel high-molecular-weight glutenin subunit gene in transgenic wheat. *Nature Bio/Technology*, **14**, 875–879.

Blechl, A.E. & Anderson, O.D. (1997). Glutenin genes and their uses. US Patent 5650558.

Bright, S.W.J., Greenland, A.J., Halpin, C.M., Schuch, W. & Dunwell, J.M. (1996). Environmental impact from plant biotechnology. *Annals of the New York Academy of Sciences*, **792**, 99–105.

Bruinenberg, P.M., Jacobsen, E.J. & Visser, R.G.F. (1995). Starch from genetically engineered crops. *Chemistry & Industry*, **6**, 881–884.

Burke, D. (1998). Why all the fuss about genetically modified food? Much depends on who benefits. *British Medical Journal*, **316**, 1845–1846.

Burkhardt, P.K., Beyer, P., Wunn, J., *et al.* (1997). Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *The Plant Journal*, **11**, 1071–1078.

Burks, A.W., Shin, D., Cockrell, G., Stanley, J.S., Helm, R.M. & Bannon, G.A. (1997). Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *European Journal of Biochemistry*, **245**, 334–339.

Caballero, T. & Martin-Esteban, M. (1998). Association between pollen hypersensitivity and edible vegetable allergy: a review. *Journal of Investigative Allergology and Clinical Immunology*, **8**, 6–16.

Cahoon, E.B., Lindqvist, Y., Schneider, G. & Shanklin, J. (1997). Redesign of soluble fatty acid desaturases from plants for altered substrate specificity and double bond position. *Proceedings of the National Academy of Sciences of the USA*, **94**, 4872–4877.

Carozzi, N. & Koziel, M. (1997). *Advances in Insect Control: The Role of Transgenic Plants*. London: Taylor & Francis.

Chong, D.K., Roberts, W., Arakawa, T., *et al.* (1997). Expression of human milk protein β-casein in transgenic potato plants. *Transgenic Research*, **6**, 289–296.

Coleman, C.E., Clore, A.M., Ranch, J.P., Higgins, R., Lopes, M.A. & Larkins, B.A. (1997). Expression of a mutant alpha-zein creates the floury2 phenotype in transgenic maize. *Proceedings of the National Academy of Sciences of the USA*, **94**, 7094–7097.

Daniell, H. (1997). Transformation and foreign gene expression in plants by microprojectile bombardment. *Methods in Molecular Biology*, **62**, 463–489.

Danner, K. (1997). Acceptability of bio-engineered vaccines. *Comparative Immunology and Microbiology of Infective Diseases.*, **20**, 3–12.

Day, P.R. (1996). Genetic modifications of plants: significant issues and hurdles to success. *American Journal of Clinical Nutrition*, **63**, 651–656.

De Clercq, A., Krebbers, E., Vandekerckhove, J., Barreto De Castro, L., Gander, E. & Van Montagu, M. (1996). Process for the production of transgenic plants with increased nutritional value via the expression of modified 2S storage albumins. US Patent 5589615.

Dörnenburg, H. & Lang-Hinrichs, C. (1994). Genetic engineering in food biotechnology. *Chemistry & Industry*, **4,** 506–510.

Duman, J.G. (1997). Transgenic plants having a nucleic acid sequence encoding a dendroides antifreeze protein. US Patent 5633451.

Dunwell, J.M. (1995). Transgenic cereal crops. *Chemistry & Industry*, **18,** 730–733.

Dunwell, J.M. (1996). Time-scale for transgenic product development. *Field Crops Research*, **45**, 135–142.

Dunwell, J.M. (1997). Is transgenic food dangerous? A personal view. *Focus on Biopesticides*^{Plus}, November, 1–2.

Ebskamp, M.J., van der Meer, I.M., Spronk, B.A., Weisbeek, P.J. & Smeekens, S.C. (1994). Accumulation of fructose polymers in transgenic tobacco. *Bio/Technology*, **12**, 272–275.

Eccleston, V.S. & Ohlrogge, J.B. (1998). Expression of lauroyl-acyl carrier protein thioesterase in *Brassica napus* seeds induces pathways for both fatty acids oxidation and biosynthesis and implies a set point for triacylglycerol accumulation. *Plant Cell*, **10**, 613–622.

Falco, S.C., Keeler, S.J. & Rice, J.A. (1998). Chimeric genes and methods for increasing the lysine and threonine content of the seeds of plants. US Patent 5773691.

Fiedler, U., Phillips, K.J., Artsaenko, O. & Conrad, U. (1997). Optimization of scFv antibody production in transgenic plants. *Immunotechnology*, **3**, 205–216.

Fischer, R., Kim, S.-H., Cho, J.M., Penarrubia, L., Giovannoni, J. & Kim, R. (1998). Endogenously sweetened transgenic plant products. US Patent 5739409.

Frewer, L.J., Howard, C., Hedderley, D. & Shepherd, R. (1998). Methodological approaches to assessing risk perceptions associated with food-related hazards. *Risk Analysis*, **18**, 95–102.

Gendel, S.M. (1998a). The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Advances in Food and Nutrition Research*, **42**, 45–62.

Gendel, S.M. (1998b). Sequence databases for assessing the potential allergenicity of proteins used in genetically modified foods. *Advances in Food and Nutrition Research*, **42**, 63–92.

Gidamis, A.B., Wright, P., Haque, Z.U., Katsube, T., Kito, M. & Utsumi, S. (1995). Modification tolerability of soybean proglycinin. *Bioscience, Biotechnology and Biochemistry*, **59**, 1593–1595.

Goodman, R.M., Knauf, V.C., Houck, C.M. & Comai, L. (1997). Molecular farming. US Patent 5629175.

Hauptman, R., Eschenfeldt, W.H., English, J. & Brinkhaus, F.L. (1997). Enhanced carotenoid accumulation in storage organs of genetically engineered plants. US Patent 5618988.

Hellwege, E.M., Gritscher, D., Willmitzer, L. & Heyer, A.G. (1997). Transgenic potato tubers accumulate high levels of 1- ketose and nystose: functional identification of a sucrose sucrose 1-fructosyltransferase of artichoke (*Cyanara scolymus*) blossum discs. *The Plant Journal*, **12**, 1057–1065.

Hightower, R., Baden, C., Penzes, E., Lund, P. & Dunsmuir, P. (1991). Expression of antifreeze proteins in transgenic plants. *Plant Molecular Biology*, **17**, 1013–1021.

Hoban, T.J. (1996). Anticipating public reaction to the use of genetic engineering in infant nutrition. *American Journal of Clinical Nutrition*, **63**, 657S–662S.

Ishida, Y., Saito, H., Ohta, S., Hiei, Y., Komari, T. & Kumashiro, T. (1996). High efficiency of maize (*Zea mays* L.) transformation mediated by *Agrobacterium tumefaciens*. *Nature Bio/Technology*, **14**, 745–750.

Ito, H., Miura, N., Masai, M., Yamamoto, K. & Hara, T. (1996). Reduction of oxalate content of foods by the oxalate degrading bacterium, *Eubacterium lentum* WYH-1. *International Journal of Urology*, **3**, 31–34.

Klann, E.M., Hall, B. & Bennett, A.B. (1996). Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiology*, **112**, 1321–1330.

Klein, J., Altenbuchner, J. & Mattes, R. (1998). Nucleic acid and protein elimination during the sugar manufacturing process of conventional and transgenic sugar beet. *Journal of Biotechnology*, **60**, 145–153.

Knauf, V.C. & Facciotti, D. (1995). Genetic engineering of foods to reduce the risk of heart disease and cancer. *Advances in Experimental Medicine and Biology*, **369**, 221–228.

Kobayashi, S., Nakamura, Y., Kaneyoshi, J., Higo, H. & Higo, K.-I. (1996). Transformation of kiwifruit (*Actinidia chinensis*) and trifoliate orange (*Poncirus trifoliata*) with a synthetic gene encoding the human epidermal growth factor (hEGF). *Journal of the Japanese Society of Horticultural Science*, **64**, 763–769.

Krings, U. & Berger, R.G. (1998). Biotechnological production of flavours and fragrances. *Applied Microbiology and Biotechnology*, **49**, 1–8.

Lam, D.M. & Arntzen, C.J. (1996). Vaccines produced and administered through edible plants. US Patent 5484719.

Lam, D.M. & Arntzen, C.J. (1997). Anti-viral vaccines expressed in plants. US Patent 5612487.

Lawrence, M.C., Izard, T., Beuchat, M., Blagrove, R.J. & Colman, P.M. (1994). Structure of phaseolin at 2.2 Å resolution. Implications for a common vicilin/legumin structure and the genetic engineering of seed storage proteins. *Journal of Molecular Biology*, **238**, 748–776.

Lee, J.S., Choi, S.J., Kang, H.S., *et al.* (1997). Establishment of transgenic tobacco cell suspension culture system for producing murine granulocyte-macrophage colony stimulating factor. *Molecules and Cells*, **7**, 783–787.

Lehrer, S.B., Horner, W.E. & Reese, G. (1996). Why are some proteins allergenic? Implications for biotechnology. *Critical Reviews in Food Science and Nutrition*, **36**, 553–564.

Lehrer, S.B. & Reese, G. (1997). Recombinant proteins in newly developed foods: identification of allergenic activity. *International Archives of Allergy and Immunology*, **113**, 122–124.

Ma, J.K. & Hein, M.B. (1995). Immunotherapeutic potential of antibodies produced in plants. *Trends in Biotechnology*, **13**, 522–527.

Ma, S.W., Zhao, D.L., Yin, Z.Q., *et al.* (1997). Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. *Nature Medicine*, **3**, 793–796.

Ma, J.K., Hikmat, B.Y., Wycoff, K., *et al.* (1998). Characterization of a recombinant plant monoclonal secretory antibody and preventative immunotherapy in humans. *Nature Medicine*, **4**, 601–606.

McCaskill, D. & Croteau, R. (1997). Prospects for the bioengineering of isoprenoid biosynthesis. *Advances in Biochemical Engineering and Biotechnology*, **55**, 107–146.

Mansur, E., Lacorte, C. & Krul, W.R. (1995). Peanut transformation. *Methods in Molecular Biology*, **44**, 87–100.

Marcellino, L.H., de Neshich, G.S.a., Grossi, M.F., Krebbers, E. & Gander, E.S. (1996). Modified 2S albumins with improved tryptophan content are correctly expressed in transgenic tobacco plants. *FEBS Letters*, **385**, 154–158.

Mason, H.S. & Arntzen, C.J. (1995). Transgenic plants as vaccine production systems. *Trends in Biotechnology*, **13**, 388–392.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. & Fuchs, R.L. (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Critical Reviews in Food Science and Nutrition*, **36**, 165–186.

Miele, L. (1997). Plants as bioreactors for biopharmaceuticals: regulatory considerations. *Trends in Biotechnology*, **15**, 45–50.

Molvig, L., Tabe, L.M., Eggum, B.O., Moore, A.E., Craig, S., Spencer, D. & Higgins, T.J. (1997). Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. *Proceedings of the National Academy of Sciences of the USA*, **94**, 8393–8398.

Moneret-Vautrin, D.A. (1998). Modifications of allergenicity linked to food technologies. *Allergy and Immunology*, **30**, 9–13.

Nakamura, R. & Matsuda, T. (1996). Rice allergenic protein and molecular-genetic approach for hypoallergenic rice. *Bioscience, Biotechnology and Biochemistry*, **60**, 1215–1221.

Nida, D.L., Kolacz, K.H., Buehler, R.E., *et al.* (1996). Glyphosate-tolerant cotton: genetic characterization and protein expression. *Journal of the Science of Food and Agriculture*, **44**, 1960–1966.

Nordlee, J.A., Taylor, S.L., Townsend, J.A., Thomas, L.A. & Bush, R.K. (1996). Identification of a Brazil-nut allergen in transgenic soybeans. *New England Journal of Medicine*, **334**, 688–692.

Oakes, J.V., Shewmaker, C.K. & Stalker, D.M. (1991). Production of cyclodextrins, a novel carbohydrate, in the tubers of transgenic potato plants. *Biotechnology*, **9**, 982–986.

Owen, M.R.L. & Pen, J. (eds) (1996). *Transgenic Plants: a Production System for Industrial and Pharmaceutical Proteins*. Chichester: Wiley.

Padgette, S.R., Taylor, N.B., Nida, D.L., *et al.* (1996). The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *Journal of Nutrition*, **126**, 702–716.

Perferon, M. (1997). Progress and prospects for field use of *Bt* genes in crops. *Trends in Biotechnology*, **15**, 173–177.

Peumans, W.J. & van Damme, E.J.M. (1996). Prevalence, biological activity and genetic manipulation of lectins in foods. *Trends in Food Science and Technology*, **7**, 132–138.

Potrykus, I. & Spangenberg, G. (eds) (1995). *Gene Transfer to Plants*. Berlin: Springer-Verlag.

Pusztai, A., Grant, G., Duguid, T., *et al.* (1995). Inhibition of starch digestion by alpha-amylase inhibitor reduces the efficiency of utilisation of dietary proteins and lipids and retards the growth of rats. *Journal of Nutrition*, **125**, 1554–1562.

Russell, D.A. & Fromm, M.E. (1997). Tissue-specific expression in transgenic maize of four endosperm promoters from maize and rice. *Transgenic Research*, **6**, 157–168.

Saalbach, I., Pickardt, T., Machemehl, F., Saalbach, G., Schieder, O. & Muntz, K. (1994). A chimeric gene encoding the methionine-rich 2S albumin of the Brazil nut (*Bertholletia excelsa* H.B.K.) is stably expressed and inherited in transgenic legumes. *Molecular and General Genetics*, **242**, 226–236.

Sayanova, O., Smith, M.A., Lapinskas, P., *et al.* (1997). Expression of a borage desaturase cDNA containing an N-terminal cytochrome b5 domain results in the accumulation of high levels of delta6-desaturated fatty acids in transgenic tobacco. *Proceedings of the National Academy of Sciences of the USA*, **94**, 4211–4216.

Secor, G.A., Borokov, A.Y., McClean, P.E. & Sowokinos, J.R. (1997). Modulation of sugar content in plants. US Patent 5646023.

Shewry, P.R., Tatham, A.S., Barro, F., Barcelo, P. & Lazzeri, P. (1995). Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex. *Bio/Technology*, **13**, 1185–1190.

Shimoni, Y., Blechl, A.E., Anderson, O.D. & Galili, G. (1997). A recombinant protein of two high molecular weight glutenins alters gluten polymer formation in transgenic wheat. *Journal of Biological Chemistry*, **272**, 15488–15495.

Shin, D.S., Compadre, C.M., Maleki, S.J., *et al.* (1998). Biochemical and structural analysis of the IgE binding sites on Ara h1, an abundant and highly allergenic peanut protein. *Journal of Biological Chemistry*, **273**, 13753–13759.

Shinmoto, H., Tomizawa, A., Kobori, M., Tsushida, T. & Shinohara, K. (1995). Assessment of the mutagenicity of extracts of TMV-coat-protein-gene induced transgenic tomato by the umu-test. *Bioscience, Biotechnology and Biochemistry*, **59**, 2151–2152.

Singsit, C., Adang, M.J., Lynch, R.E., *et al.* (1997). Expression of a *Bacillus thuringiensis* cryIA (c) gene in transgenic peanut plants and its efficiency against lesser cornstalk borer. *Transgenic Research*, **6**, 169–176.

Sivak, M.N. & Preiss, J. (1995). Progress in the genetic manipulation of crops aimed at changing starch structure and increasing starch accumulation. *Journal of Environmental Polymer Degradation*, **3**, 145–152.

Smith, G., Walmsley, A. & Polkinghorne, I. (1997). Plant- derived immunocontraceptive vaccines. *Reproduction, Fertilisation and Development*, **9**, 85–89.

Stanley, J.S., King, N., Burks, A.W., *et al.* (1997). Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Archives of Biochemistry and Biophysics*, **342**, 244–253.

Tacket, C.O., Mason, H.S., Losonsky, G., Clements, J.D., Levine, M.M. & Arntzen, C.J. (1998). Immunogenicity in humans of a recombinant bacterial antigen in a transgenic potato. *Nature Medicine*, **4**, 607–609.

Tada, Y., Nakase, M., Adachi, T., *et al.* (1996). Reduction of 14–16 kDa allergenic proteins in transgenic rice plants by antisense gene. *FEBS Letters*, **391**, 341–345.

Takase, K. & Hagiwara, K. (1998). Expression of human alpha- lactalbumin in transgenic tobacco. *Journal of Biochemistry,* **123**, 440–444.

Takumi, S. & Shimada, T. (1997). Variation in transformation frequencies among six common wheat cultivars through particle bombardment of scutellar tissues. *Genes and Genetic Systems*, **72**, 63–69.

Thompson, P.B. (1997). Food biotechnology's challenge to cultural integrity and individual consent. *Hastings Center Report*, **27**, 34–38.

Tieman, D.M., Kausch, K.D., Serra, D.M. & Handa, A.K. (1995). Field performance of transgenic tomato with reduced pectin methylesterase activity. *Journal of the American Society of Horticultural Science*, **120**, 765–770.

Topfer, R., Martini, N. & Schell, J. (1995). Modification of plant lipid synthesis. *Science*, **268**, 681–686.

Utsumi, S., Katsube, T., Ishige, T. & Takaiwa, F. (1997). Molecular design of soybean glycinin with enhanced food qualities and development of crops producing such glycinins. *Advances in Experimental Medicine and Biology*, **415**, 1–15.

Vijn, I., van Dijken, A., Sprenger, N., *et al.* (1997). Fructan of the inulin neoseries is synthesised in transgenic chicory plants (*Cichorium intybus* L.) harbouring onion (*Allium cepa* L.) fructan:fructan 6G-fructosyltransferase. *The Plant Journal*, **11**, 387–398.

Vincentz, M., Leite, A., Neshich, G., *et al.* (1997). ACGT and vicilin core sequences in a promoter domain required for seed-specific expression of a 2S storage protein gene are recognised by the opaque-2 regulatory protein. *Plant Molecular Biology*, **34**, 879–889.

Wallis, J.G., Wang, H. & Guerra, D.J. (1997). Expression of a synthetic antifreeze protein in potato reduces electrolyte release at freezing temperatures. *Plant Molecular Biology*, **35**, 323–330.

Wang, K., Drayton, P., Frame, B., Dunwell, J.M. & Thompson, J. (1996). Whisker-mediated plant transformation: an alternative technology. *In Vitro Cell and Developmental Biology, Plants*, **31**, 101–104.

Wheeler, G.L., Jones, M.A. & Smirnoff, N. (1998). The biosynthetic pathway of vitamin C in higher plants. *Nature*, **393**, 365–369.

Wymer, P. (1998). Genetically modified food: ambrosia or anathema. *Chemistry & Industry*, 1 June, 422–426.

Zemanek, E.C. & Wasserman, B.P. (1995). Issues and advances in the use of transgenic organisms for the production of thaumatin, the intensely sweet protein from *Thaumacoccus danielli*. *Critical Reviews of Food Science and Nutrition*, **35**, 455–466.

Zou, J., Katavic, V., Giblin, E.M., *et al.* (1997). Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. *Plant Cell*, **9**, 909–923.

