Genetic engineering and functional foods
Engenharia genética e alimentos funcionais

RALF GREINER
Federal Research Centre for Nutrition and Food, Centre for Molecular Biology
Address correspondence to:
Ralf Greiner
Haid-und-Neu-Strasse 9, D-761341 Karlsruhe, Germany,
e-mail: ralf.greiner@bfel.de

ABSTRACT

GREINER, R. Genetic engineering and functional foods. *Nutrire:* rev. Soc. Bras. Alim. Nutr. = J. Brazilian Soc. Food Nutr., São Paulo, SP, v. 32, n. 3, p. 123-141, dez. 2007.

Functional foods are the food-industry response to the continuously increasing request of consumers for foods that are both attractive and healthy. The main targets of functional foods are intestinal health, immune system activity, mental performance, caries, menopause symptoms, cancer, cardiovascular disease, diabetes, osteoporosis and child skeletal development. Most of the functional foods designed so far are derived from traditional foods by adding so-called functional ingredients, by modifying the technological process during industrial food preparation or by modifying the composition of the raw material used for food production. However, gene technology is thought to be a powerful technique to improve the nutritional quality of food raw materials. The modification of product quality characteristics using gene technology depends on a well-established understanding of the pathways for biosynthesis of plant products, a rapidly expanding knowledge about the genetic control of these pathways, and an increasing availability of cloned genes for key enzymatic steps. Quality-improved crops derived from genetic engineering are expected to reach the market in the near future. Crops with an improved protein quality, with an improved nutritional quality of the plant oil, crops rich in vitamins, minerals, antioxidants or low in undesired compounds as well as crops with an altered secondary metabolite production or altered carbohydrate composition have been developed by genetic engineering. These examples give an idea of the genetic engineering potential to produce health-promoting foods.

Keywords: Genetic. Engineering. Nutrition. Negative effect. Diet.

RESUMEN

Los alimentos funcionales son la respuesta de la industria de alimentos a la creciente demanda de los consumidores por alimentos que sean al mismo tiempo atrayentes y saludables. Los principales objetivos de los alimentos funcionales son la salud intestinal, del sistema inmunológico, el desempeño mental, las caries, los síntomas de la menopausia, cáncer, enfermedades cardiovasculares, diabetes, osteoporosis y desenvolvimiento óseo en niños. La mayoría de los alimentos funcionales desarrollados hasta el momento son derivados de los alimentos tradicionales a los cuales se les adicionan los ingredientes funcionales, se les modifica el proceso tecnológico de industrialización o se les altera la composición de materias primas utilizadas en su producción. Sin embargo, se tiene por cierto que la tecnología genética es un instrumento poderoso para mejorar la calidad nutricional de las materias primas alimenticias. La modificación de las características de calidad del producto utilizando tecnología genética depende de un conocimiento asentado de las vías metabólicas de síntesis de productos vegetales, un conocimiento en rápida expansión sobre el control genético de tales vías y una creciente disponibilidad de genes clonados para la expresión de enzimas claves de algunos pasos de esas vías. Se espera que cultivos con calidad mejorada originarios de ingeniería genética lleguen al mercado en un futuro próximo. Cultivos con mejor calidad de proteínas y lípidos, con mayor concentración de vitaminas, minerales y antioxidantes, con bajos tenores de compuestos indeseables y también cultivos con metabolitos secundarios modificados o composición alterada de carbohidratos son ejemplos de logros ya alcanzados por la ingeniería genética. Los ejemplos mencionados permiten visualizar el potencial de la ingeniería genética para la producción de alimentos promotores de la salud.

Palabras clave: Ingeniería genética. Nutrición. Efecto negativo. Dieta.

RESUMO

Os alimentos funcionais são a resposta da indústria alimentícia à sempre crescente demanda dos consumidores por alimentos ao mesmo tempo atraentes e saudáveis. Os principais alvos dos alimentos funcionais são a saúde intestinal, a atividade do sistema imune, o desempenho mental, cáries, sintomas da menopausa, câncer, doenças cardiovasculares, diabetes, osteoporose e desenvolvimento ósseo de crianças. A maioria dos alimentos funcionais desenvolvidos, até o momento, são derivados de alimentos tradicionais pela adição dos ditos ingredientes funcionais, modificação dos processos tecnológicos durante o preparo industrial dos alimentos ou alteração da composição das matérias-primas usadas na produção dos alimentos. Contudo, acredita-se que a tecnologia genética seja um poderoso instrumento para melhorar a qualidade nutricional das matérias-primas alimentícias. A modificação das características de qualidade do produto usando a tecnologia genética depende de um conhecimento bem embasado sobre as rotas metabólicas de síntese de produtos vegetais, um conhecimento em rápida expansão sobre o controle genético de tais rotas metabólicas, e uma crescente disponibilidade de genes clonados para expressão de enzimas-chave de alguns passos destas rotas. Espera-se que culturas com qualidade melhorada derivadas da engenharia genética cheguem ao mercado num futuro próximo. Culturas com qualidade protéica melhorada, com melhor qualidade nutricional do óleo vegetal derivado, culturas ricas em vitaminas, minerais, antioxidantes ou com baixos teores de compostos indesejáveis, bem como culturas com produção de metabólitos secundários alterados ou composição alterada de carboidratos já foram desenvolvidas pela engenharia genética. Estes exemplos dão uma idéia do potencial da engenharia genética para produzir alimentos promotores de saúde.

Palavras-chave: Engenharia genética. Nutrição. Efeito negativo. Dieta.

INTRODUCTION

Consumers' demand for foods that are both attractive and healthy is steadily increasing. Food marketing is following this demand and functional foods are the food industry's direct response to the increasing request for healthy foods. Therefore functional foods are branded foods, which claim, explicitly or implicitly, to improve human health or well being. Today the discussion about functional foods is mainly focused on definitions and on legislative issues related to the presence of this kind of products on the market. Especially the definition of functional foods is a contentious issue. Even in Japan, where functional foods originated, the term itself was not adopted because it was agreed that all foods are already functional (BAILY, 1999). "Foods that provide health benefits beyond basic nutrition" is a widely used definition for functional foods. However, this definition does not provide a clear demarcation between functional and other foods, because almost any food can have some beneficial effects on some bodily function (KATAN; DE ROOS, 2004). By this definition even tap water could be called a functional food, because a liberal intake of water prevents cystitis, kidney and bladder stones, and possibly bladder cancer. A more concrete definition was provided by the Institute of Medicine of the US National Academy of Sciences, which defined functional foods as, "those foods in which the concentration of one or more ingredients have been manipulated or modified to enhance their contribution to a healthful diet" (COMMITTEE ON OPPORTUNITIES IN THE NUTRITION AND FOOD SCIENCES, 1994). A more market-oriented definition was given by Nestlé (2002), who described functional foods as, "products created just so that they can be marketed using health claims".

Functional foods can be classified into two main categories according to the expected effects: those aiming to improve physiological functions and those aiming to reduce the risk of specific pathologies (FOGLIANO; VITAGLIONE, 2005). The main targets of functional foods are gut health, immune system activity, mental performance, caries, menopause symptoms, cancer, cardiovascular disease, diabetes, osteoporosis and child skeletal development (FOGLIANO; VITAGLIONE, 2005; KATAN; DE ROOS, 2004).

FUNCTIONAL FOOD DESIGN

Most of the functional foods designed so far are derived from traditional foods by adding so-called functional ingredients, by modifying the technological process during industrial food preparation or by modifying the composition of the raw material used for food production. Gene technology is thought to be a powerful technique to improve the nutritional quality of food raw material. Characteristics of genetically modified crops, such as herbicide tolerance and insect resistance, have become known as "input traits", and constitute the first wave of GM products to reach the market. The benefits from input traits are confined mainly to crop production systems and are therefore captured principally by growers and agribusiness. The modification of product quality characteristics using gene technology depends on a well-established understanding of the pathways for biosynthesis of plant products, a rapidly expanding knowledge of the genetic control of these pathways, and an increasing availability of cloned genes for key enzymatic steps. Quality improved crops are expected to reach the market in the near future. Golden Rice, a genetically engineered rice cultivar that is high in provitamin A is the most famous example of a quality improved transgenic product and it gives an impression of the potential of genetic engineering to produce health-promoting foods. In the following more examples will be given where genetic engineering is expected to result in crop quality modifications with potential benefits on human health.

REDUCTION OF MICRONUTRIENT MALNUTRITION

Deficiencies of micronutrients such as iron, zinc, iodine, and vitamin A in human populations are widespread, affecting over three billion people and the numbers are increasing (WELCH; GRAHAM, 2004). The development of micronutrient-enriched staple crops via molecular biological techniques was suggested as a powerful complementary intervention to already existing strategies for combating micronutrient malnutrition (BOUIS, 2000). The use of these biofortified crops is expected to be a more sustainable and less expensive approach to reduce the enormous global problem of 'hidden hunger'. In addition, biofortified crops could reach a larger number of people including the most vulnerable ones such as resource-poor women, infants, and children than nutrient supplements or fortified foods. A further advantage of biofortified crops is the missing need of changing habits both for producers and consumers. In recent years 'proof of concept' studies have been published using transgenic approaches to biofortified crops. For example, the development of provitamin A-rich 'Golden Rice' and iron-rich rice at the ETH Zurich was a milestone in the application of gene technology to enrich a staple crop with micronutrients (BEYER et al., 2002; LUCCA; HURRELL; POTRYKUS, 2002).

Provitamin A-rich plants

Vitamin A is needed for vision, bone growth, reproduction, immune function, gene expression, embryonic development, cell division and cell differentiation as well as healthy respiratory and gastrointestinal systems. Vitamin A deficiency is a serious public health problem in parts of the developing world. Night blindness is one of the first signs for vitamin A deficiency, but it can also result in permanent blindness. Furthermore, vitamin A deficiency diminishes the ability to fight infections resulting in an increase of the incidence and severity of infectious diseases (WEST; DARNTON-HILL, 2001). In Asia, vitamin A deficiency is associated with the poverty-related predominant consumption of rice, which lacks carotenoids exhibiting provitamin A-activity in the edible part of the grain. Therefore, introduction of the complete provitamin A biosynthetic pathway into the rice endosperm could be a simple and effective complement to supplementation programs. Through farming and local trade, those rice cultivars are expected to reach the target populations, namely the urban poor and rural populations, particularly those living in remote areas. Because there is no rice germplasm available, which is capable of synthesising carotenoids in the endosperm, genetic engineering was the only way to enable the accumulation of provitamin A in the rice endosperm. The transgenic approach has become feasible because of the rapid progress in the development of rice transformation technology and the identification of the entire carotenoid biosynthetic pathway on molecular level in numerous bacteria and plants. In a proof-of-concept study it was shown that it is possible to establish a biosynthetic pathway *de novo* in rice endosperm, which results in the aspired accumulation of provitamin A (BEYER et al., 2002). To enable the rice endosperm to produce β-carotene, the most effective precursor of vitamin A, three genes have been introduced in a single, combined transformation effort into rice (BEYER et al., 2002). The genes coding for phytoene synthase and lycopene β-cylase were both derived from daffodil (*Narcissus pseudonarcissus*) and placed under the control of the endosperm-specific glutelin promoter. The third gene introduced codes for phytoene desaturase. It originates from the bacterium *Erwinia uredovora* and was placed under the control of the constitutive CaMV 35S promoter. The expression of these three genes in the rice endosperm covers the requirements for β-carotene synthesis, because it enables the endosperm to convert geranylgeranyl diphosphate via phytoene and lycopene to β-carotene. Geranylgeranyl diphosphate, an early intermediate in provitamin A biosynthesis, was already shown to be synthesised by immature rice endosperm (BEYER et al., 2002). Because of the characteristic yellow colour of the polished grains, the name 'Golden Rice' was given to the genetically modified rice that produces β-carotene in the endosperm. A maximum level of 1.6μg/g total carotenoids was reported for the transgenic lines (BEYER et al., 2002). This level has not been surpassed in subsequent experiments using alternative rice varieties (PAINE et al., 2005). While cooking for 10 to 15 minutes in water a reduction in the total carotenoid content by about 10% in the cooked rice grains compared with the non-cooked rice was observed (DATTA et al., 2003). The limited production of provitamin A in 'Golden Rice' is cited in the media as the major hurdle to the success of this particular approach to combat vitamin A deficiency. A promising strategy to increase provitamin A content in the rice endosperm is by identifying the metabolic rate-limiting bottlenecks in 'Golden Rice'. It was shown that the presence of the daffodil gene encoding lycopene β-cyclase was not necessary for β-carotene production in the rice endosperm (BEYER et al., 2002). Phytoene synthase and phytoene saturase alone were capable of driving β-carotene synthesis. Furthermore, it was hypothesised that the second daffodil originated enzyme (phytoene synthase) used to develop 'Golden Rice', was at the rate-limiting step in provitamin A accumulation (PAINE et al., 2005). Through systematic testing of other plant phytoene synthases in a model plant system, the maize enzyme was shown to substantially increased carotenoid accumulation. Thus 'Golden Rice 2' was developed by introducing the maize gene encoding phytoene synthase in combination with the *Erwinia uredovora* phytoene desaturase gene used to generate the original 'Golden Rice' (PAINE et al., 2005). A maximum content of 37μg/g total carotenoids with a preferential accumulation of β-carotene was reported. This represents an increase of 23-fold in total carotenoids compared to the original 'Golden Rice'.

Since it is know that dietary fat facilitates the utilization of carotenoids (RIBAYA-MERCADO, 2002), an increase in β-carotene levels in oilseeds might be a suitable approach to improve availability of dietary provitamin A. As β-carotene is lipid-soluble, the majority of it will be extracted in the seed oil. Research has shown that carotenoide levels in canola (*Brassica napus*) seed can be raised up to 50-fold, α- and β-carotenes being the predominant ones, by the introduction of just one gene (bacterial phytoene synthase gene) targeted to the plastid (VERPORTE; MEMELINK, 2002). In 2000, the Tata Energy Research Institute (TERI), a not-for-profit research institute in India, started a project in partnership the Michigan State University and the Monsanto Company with the overall goal to develop locally acceptable varieties of high β-carotene mustard ('Golden mustard') (MONSANTO, 2000). Once developed and if adopted by Indian farmers these enhanced mustard varieties will yield cooking oil high in β-carotene. This cooking oil has the potential of helping hundreds of thousands of children suffering from vitamin A deficiencies, particularly in northern and eastern India, where mustard oil is commonly used for food preparation and cooking.

High iron rice

Iron deficiency is estimated to affect about 30% of the world population (WHO, 1992), making iron by far the most deficient nutrient world wide. The major consequences are reduced mental and motor development in infants, reduced body growth, decreased immune function, tiredness and poor work performance, and poor pregnancy outcome, including increased mortality of mother and children. The poor absorption of iron from unrefined cereals and/or pulses, the major dietary component in populations of developing countries is considered a major factor in the aetiology of iron deficiency anaemia. Unrefined cereals and/or pulses are high in phytate, which is a potent inhibitor of iron absorption (KONIETZNY; GREINER, 2003). In addition, the intake of foods that enhance iron absorption such as fruits, vegetables or meat is often limited in these populations.

Since the amount of bioavailable iron depends on both iron intake and iron absorption, an approach to reduce the prevalence of iron deficiency anaemia in developing countries would be to increase the total level of iron in the edible parts of stable crops while at the same time increasing the concentration of compounds which promote its uptake and/or decreasing the amount of compounds which inhibit its absorption either by plant breeding or by genetic engineering. To improve white or polished rice as a source of iron, three proteins were expressed in its central endosperm; a *Phaseolus* phytoferritin, an endogenous cysteine-rich metallothionein-like protein, and an *Aspergillus fumigatus* phytase (LUCCA et al., 2002). Expression of the phytoferritin approximately doubled the endosperm iron content and the cysteine-rich peptides have been shown to improve iron absorption in the gut. The *Aspergillus fumigatus* phytase was selected because of its reported high thermal stability (WYSS et al., 1999) and the hope of retaining activity following cooking. However, the enzyme was completely inactivated during cooking (LUCCA; HURRELL; POTRYKUS, 2001). Identification and/or development of phytases that retain thermotolerance following expression in plant tissues and cooking remain a target of active research.

IMPROVEMENT OF MINERAL BIOAVAILABILITY FROM PLANT-BASED FOODS

Regarding micronutrient malnutrition, iron is not the only mineral of concern. Zinc deficiency is suggested to be as widespread as iron deficiency. Zinc deficiency in children is associated with poor growth, reduced motor and cognitive development, impaired immune response and increased infectious diseases. Furthermore, evidence is accumulating that zinc deficiency is associated with complications of pregnancy and childbirth, lower birth weight, and other fatal effects lasting through childhood. Two further mineral that are of interest in respect to a better supply of the population are calcium and magnesium. Calcium helps to maintain bone density and lower the incidence of osteoporosis. Magnesium is needed to prevent magnesium tetany (severe muscle spasms). Transgenic approaches to increase mineral content in the edible parts of plants have been carried out in the past by expression of components of the mineral acquisition system or mineral storage proteins, but the changes were minor (AL-BABILI; BEYER, 2005). A more promising approach to improve mineral bioavailability seems to be the reduction of mineral absorption inhibitors such as phytate or polyphenolic compounds in the crop plants. The introduction and expression of microbial phytase encoding genes into several different crop plants including sesame, soybean, canola, potato, rice, wheat, maize and sugarcane have been reported (reviewed in GREINER; KONIETZNY, 2006). If properly targeted, overexpression of phytase during seed development can result in reduced phytate levels in the mature seed. Furthermore, mineral bioavailability can be improved by using crop plants high in phytase activity through phytate hydrolysis during digestion in the stomach (SANDBERG; ROSSANDER HULTHEN; TÜRK, 1996) or during food processing (GREINER; KONIETZNY, 2006). Introduction or increase of phytate-degrading capability in microorganisms used for food fermentation such as *Saccharomyces cerevisiae*, *Lactobacillus sanfranciscensis* or *Lactobacillus plantarum* is a further approach to improve phytate hydrolysis during food processing. Recently, a genetically modified phytase-secreting *Lactobacillus plantarum* strain was reported (KEROVUO; TYNKKYNEN, 2000), but the secretion levels were far too low for an industrial application. Furthermore, a *Saccharomyces cerevisiae* strain producing high levels of extracellular phytase activity was constructed (HARALDSSON et al., 2005), but its capability to contribute significantly to phytate hydrolysis during fermentation needs to be studied first. Last but not least, low phytate mutants in maize, barley, rice and soybeans were isolated recently (RABOY, 2002) and their potential for improving the absorption of iron, zinc and calcium has been shown (MENDOZA, 2002).

FURTHER POTENTIAL APPLICATIONS OF PHYTASES IN FUNCTIONAL FOOD DEVELOPMENT

Much scientific information has been reported in the last few years linking diet, specific foods, or individual food components with the maintenance of human health and the prevention of chronic diseases. Individual *myo*-inositol phosphate esters, the dephosphorylation products of phytate, have been shown to have important physiological functions in man. D-*myo*-inositol(1,2,6)trisphosphate, for example, has been studied in respect to prevention of diabetes complications and treatment of chronic inflammations as well as cardiovascular diseases (CARRINGTON et al., 1993; CLAXON et al., 1990) and due to its antiangiogenic and antitumour effects *myo*-inositol(1,3,4,5,6)pentakisphosphate was suggested as a promising compound for anticancer therapeutic strategies (MAFFUCCI et al., 2005). In addition, dietary phytate was reported to prevent kidney stone formation (GRASES et al., 2000), protect against diabetes mellitus (YOON; THOMPSON; JENKINS, 1983), atherosclerosis, coronary heart disease (JARIWALLA et al., 1990), caries (KAUFMAN; KLEINBERG, 1971), and against a variety of cancers (VUCENIK; SHAMSUDDIN, 2003). However, it is not at all established that phytate itself is the active compound. Several *myo*-inositol phosphates were linked with different physiological effects (SHEARS, 1998). Thus, phytate dephosphorylation products generated during food processing or food digestion may act as the functional compounds. Because the number and distribution of the phosphate residues on the *myo*-inositol ring determines the metabolic effects triggered by the individual *myo*-inositol phosphate isomer, a controlled dephosphorylation of phytate may result in individual food components maintaining human health and preventing chronic diseases. So far enzymatic phytate dephosphorylation is the most promising approach to get access to an individual *myo*-inositol phosphate isomer. Different phytases may exhibit different phytate degradation pathways and therefore lead to the generation and accumulation of different *myo*-inositol phosphate intermediates (reviewed by KONIETZNY; GREINER, 2002). If individual phytate degradation products are established to be metabolically active, phytases may find application in food processing to produce foods with improved nutritional value, health benefits and maintained sensory properties (functional foods) (GREINER et al., 2002).

TRANSGENIC PLANTS WITH AN ELEVATED ANTIOXIDANT LEVEL

It is common knowledge that antioxidants protect from dangerous substances called free radicals. Free radicals are highly reactive compounds that are created in the body during normal metabolic functions or introduced from the environment. They are inherently unstable, since they contain “extra” energy. To reduce their energy load, free radicals react with certain compounds in the body, and in the process, interfere with the cells’ ability to function normally. Free radicals are believed to play a role in more than sixty different health conditions, including the aging process, cancer and atherosclerosis. Reducing exposure to free radicals and increasing intake of antioxidants has the potential to reduce the risk of free radical-related health problems. Antioxidants work in several ways: they may reduce the energy of the free radical, stop the free radical from forming in the first place, or interrupt an oxidising chain reaction to minimize the damage caused by free radicals. The major classes of dietary compounds with antioxidant activity are carbohydrates such as vitamin C, caroteinoids, such as lutein and lycopene, N or S containing compounds, such as indols or allicin, lipids, such as ω-3-polyunsaturated fatty acids, and polyphenolic compounds, such as flavonoids, isoflavons and tocopherols (TSAO; AKHTAR, 2005). Production of various antioxidants in transgenic plants could provide additional health benefits by reducing the risk of cancer, cardiovascular disease and age-related macular degeneration.

Recent research has focused upon manipulation of carotenoid content and composition in crop plants to improve their nutritional value for human consumption (BOTELLA-PAVÍA; RODRÍGUEZ-CONCEPCIÓN, 2006; NAIK et al., 2003). Especially the enormous progress in cloning of carotenogenic genes has opened up the possibility of genetic manipulation of carotenoid biosynthetic pathway in plants. Although the major value of carotenoids in human nutrition is their role as provitamin A, dietary carotenoids also provide health benefits based on their antioxidant properties. Some dietary carotenoids may have more protective roles than others, and certainly they have different antioxidant capacities. Besides the above mentioned examples of plants in which carotenoid biosynthesis has been manipulated through genetic engineering, tomatoes, carrots, and potatoes have been targets to develop foods enriched in carotenoids (BOTELLA-PAVÍA; RODRÍGUEZ-CONCEPCIÓN, 2006; NAIK et al., 2003). Through introduction of the phytoene desaturase gene of *Erwinia uredovora* into tomato under the control of the CaMV 35S promoter, β-carotene levels of the tomato fruit increased 3-fold, up to 45% of the total carotenoid content (RÖMER et al., 2000). However, total carotenoid levels were not elevated in these transgenic tomatoes. The level of lycopene, a carotenoid with strong antioxidant properties, was increased up to 2.1-fold in the ripe tomato fruit by introduction of a bacterial gene encoding phytoene synthase fused to a chromoplast-targeting sequence and overexpression in a fruit-specific fashion (FRASER et al., 2002).

The carotenoid content of potatoes has been significantly increased by overexpression of a bacterial phytoene desaturase gene in the plastids of developing tubers of *Solanum tuberosum*, a species with low carotenoid levels, and *Solanum phureja*, a carotenoid-accumulating species (DUCREUX et al., 2005). The transgenic *S. tuberosum* and *S. phureja* lines accumulated around 7-fold and 3-fold higher carotenoid levels, respectively than the untransformed lines. In addition, the carotenoid profile changed dramatically in the transgenic tubers. They were highly enriched in lutein and β-carotene.

The levels of antioxidants in seed oils may also be enhanced, once genes in the tocopherol and tocotrienol pathways are expressed transgenically. So far, it has been shown that the relative proportions of α-tocopherol (vitamin E) and ß-tocopherol, which differ in their effectiveness as antioxidants, can be modified by gene technology (YAN; KERR, 2002). There has been one successful attempt to increase plant *L*-ascorbic acid content through genetic engineering (HANCOCK; VIOLA, 2002). The knowledge and availability of genes involved in the biosynthesis of different flavonoids make it feasible to genetically up-regulate the overall flavonoid biosynthesis or to engineer the pathway towards new flavonoid species in crop plants. For example, introduction and overexpression of a chalcone isomerase encoding gene from Petunia in tomatoes led to a 70-fold increase of the amount of the flavonol quercetin glycoside in the tomato peel (MUIR et al., 2001). A further approach to increase antioxidant levels in crop plants was recently reported by Niggeweg, Michael and Martin (2004). They developed genetically modified tomatoes with elevated levels of the antioxidant chlorogenic acid by overexpression of hydroxycinnamoyl transferase.

REDUCTION IN UNDESIRED COMPOUNDS AND SECONDARY METABOLITE PRODUCTION

Phytate is not the only antinutrient or undesired compound that is reduced in content or eliminated in food products by using gene technology. Reduction in protease inhibitors (WELHAM; DOMONEY, 2000) and elimination of allergens (SHEWRY; TATHAM; HALFORD, 2001) are two examples were genetic engineering has found application so far. Food allergy can be a serious nutritional problem in children and adults and avoidance of the food is the only treatment available. Current technology allows gene expression to be down-regulated using antisense or co-suppression and future developments may allow targeted gene mutation or gene replacement. Transgene-induced gene silencing was for example used to prevent the accumulation of a major allegen in soybean (HERMAN et al., 2003) and peanut (DODO; KONAN; VIQUEZ, 2005).

The biosynthetic pathways for the synthesis of secondary metabolites are rapidly being elucidated using biochemistry, gene technology and genomics in model systems such as *Arabidopsis*. The increasing availability of key genes controlling these pathways is now enabling the content of these valuable micronutrients to be enhanced in plant foods. It is possible to use gene technology to increase greatly the naturally low levels of phytosterols in oils up to levels that have a nutritional benefit (MURPHY, 2003). A well-established and large body of scientific evidence has proven that plant sterol enriched foods significantly lower LDL-cholesterol (JUDD et al., 2002) and high phytosterol vegetable oil from transgenic plants will provide a excellent source of cholesterol-lowering plant sterols.

Isoflavones are naturally occurring plant compounds that are being studied for their substantial health benefits. They are found almost exclusively in soybeans and other leguminous plants. The reported health benefits include relief of menopausal symptoms, reduction of osteoporosis, improvement in blood cholesterol levels, and lowering risk of certain hormone related cancers and coronary heart disease (SETCHELL; CASSIDY, 1999). Identification of two soybean genes encoding isoflavone synthase paved the way for manipulating the expression of isoflavones in legumes in order to produce valuable plant products or nutritionally enhanced foods (JUNG et al., 2000). It was already demonstrated that introduction of the soybean isoflavon synthase encoding gene produced a functional enzyme even in a plant species that does not naturally produce isoflavones. Since the plant produced genistein in levels up to 2ng/mg of fresh weight, the potential for producing isoflavones in nonisoflavone-producing crop species was demonstrated (JUNG et al., 2000).

IMPROVED PROTEIN QUALITY

Eight amino acids are generally regarded as essential for humans: tryptophan, lysine, methionine, phenylalanine, threonine, valine, leucine, and isoleucine. Legume seeds and oilseeds are deficient in both cysteine and methionine. Consequently, there is an interest in increasing the methionine content of legume seeds. The main interest in improving the sulphur amino acid content of legume seeds has focused on the methionine-rich 2S albumins from Brazil nut and sunflower. Expression of 2S albumin genes has resulted in significant increases in the methionine content of oilseed rape, narbon bean and lupine (MÜNTZ et al., 1998). However, the Brazil nut protein can be allergenic to some humans and the commercial development of seeds expressing this protein has therefore been suspended. It is clear that, in some species, amino acid reserves may limit the plant's ability to respond to the added demand imposed by the transgene and the limited sulphur reserves may simply be re-allocated from endogenous proteins to the new sulphur sink (DEMIDOV et al., 2003). Attempts have been made also, using *in vitro* gene mutation, to alter appropriate amino acid codons into methionine and/or lysine codons, or to insert stretches of additional codons for these amino acids, into individual genes for legume and oilseed storage proteins (MÜNTZ et al., 1998). In addition, amino acid metabolism within the seed has been modified in order to increase the free amount of the respective essential amino acid by engineering regulatory key enzymes in the biosynthetic pathway (GALILI; HÖFGEN, 2002). High-lysine soybean and rapeseed have been produced by this approach (FALCO et al., 1995). It has been shown recently that expression of both a methionine-rich albumin and a feed-back insensitive bacterial aspartate kinase, to stimulate methionine biosynthesis, can result in narbon bean protein with 2-2.4 times higher methionine content than the wild-type (DEMIDOV et al., 2003). A similar approach was used to increase lysine content in potatoes (SÉVENIER et al., 2002). The introduction of a gene encoding a feedback-insensitive bacterial dihydrodipicolinate synthase resulted in a 6-fold increase of lysine compared to the non-transgenic potatoes and the introduction of a mutated version of the potato gene which give rise to a feedback-insensitive dihydrodipicolinate synthase led to a 15-fold increase in lysine content. Milled rice is low in protein concentration and lysine is limiting in rice. Thus, high-quality protein rice was developed to combat protein-energy malnutrition (POTRYKUS, 2001). High-quality protein rice contains a balanced mix of all essential amino acids. The transgenic concept is based on the expression of one single synthetic gene (*Asp1*) encoding a storage protein with a balanced mixture of all essential amino acids under endosperm-specific regulation.

Human milk proteins are believed to have a multitude of biological activities benefiting the newborn infant. Such functions include antibacterial and antiviral activities, enhancement of the immune system and increased nutrient absorption. By using genetic engineering human milk proteins can be produced in microorganisms, animals and plants and therefore they will available not only to breast-fed infants. Recombinant human lactoferrin from *Aspergillus oryzae* is already commercially available (WARD et al., 1992). Due to the extensive purification needed the cost is most likely to high for its use as a food additive. Furthermore, transgenic cows have been used as a production system for human lactoferrin (KRIMPENFORT, 1993), but cow's milk containing human lactoferrin has not yet found a market. Expression of human milk proteins in potatoes is attractive, because potatoes are a normal part of the diet of many people, but expression levels of human lactoferrin in potatoes are so far low (CHONG; LANGRIDGE, 2000). Because rice is one of the first non-milk foods introduced to infants and because very high expression levels can be achieved, rice is now being used for the expression of several human milk proteins, such as lactoferrin, lysozyme and α_1-antitrypsin (CHOWANADISAI et al., 2003; HUANG et al., 2002; NANDI et al., 2002).

IMPROVED NUTRITIONAL QUALITY OF PLANT OILS

The manipulation of seed oil content *via* transgene insertion was an early goal of the application of modern biotechnology to agriculture and has led to the production of crops with modified oils that are under trial, principally in the USA and Canada. There are three obvious targets for the modification of edible oils in seeds. First, to increase the total 18:0 and 18:1 content of the plant oil so that it has an acceptable solid fat functionality for use in margarine and other confectionery applications but without the deleterious health effects of partially hydrogenated oils rich in *trans*-fatty acids (THELEN; OHLROGGE, 2002). Using transgenic approaches, the oleic acid levels have been raised to 88% in soybean oil, to 89% and 75% in canola oil from *Brassica napus* and *Brassica juncea* respectively, and to 77% in cottonseed oil (LIU; SINGH; GREEN, 2002; Thelen and Ohlrogge, 2002). In addition, transgenic soybeans, sunflower, cotton and canola produce an oil enriched in stearic acid (35-40%) (THELEN; OHLROGGE, 2002). By inter-crossing a high-oleic and a high-stearic genotype of cotton, it has been possible to obtain a cottonseed oil with intermediate levels of oleic and stearic acid (LIU; SINGH; GREEN, 2002). Secondly, work is ongoing to increase monounsaturates (18:1) with a concomitant decrease in polyunsaturates (18:2, 18:3), combined with a reduction in total saturates (16:0, 18:0) (COUGHLAN; KINNEY, 2002). This would yield oil that is more chemically stable and having a reduced total saturated fat content. High-oleic/low-linolenic and high-lauric oilseed rape is being grown commercially in Canada. Furthermore, a low-saturated (3%)/low-linolenic (3%) soybean oil is on the market in the USA. Thirdly, there is increasing interest in producing very long-chain polyunsaturates, such as linolenic acid (18:3ω6, GLA), docosahexenoic acid (22:6ω3, DHA) and eicosapentenoic acid (20:5ω3, EPA), which are nutritionally beneficial as precursors for certain prostaglandins and as cholesterol-lowering agents (HUANG et al., 2002). The accumulation of up to 68% linolenic and up to 17% stearidonic acid (18:4ω3, SDA) in oilseed rape has been reported (URSIN, 2003). It seems likely, therefore, that genetically modified oilseeds having nutritionally effective levels of long-chain polyunsaturates will become a reality but there is still much progress needed, in particular for some fatty acids (e.g. fish oil-types).

ALTERED CARBOHYDRATE COMPOSITION

Carbohydrates provide dietary fibre as starch and β-glucans, the basis of most beverages ranging from fruit juice and soft drinks to cognac, and dietary nutrients. So far, the main efforts in carbohydrate engineering in plants have been directed to altering starch yield and type, and to changing the degree of branching in amylopectin (SCHULMAN, 2002). Applications of starch and other plant carbohydrates range from fat substitutes to fibre as resistant starch in novel foods. Additional objectives include the production of fructans, as potential pro- or prebiotic antitumorigenic components of the human diet (SÉVENIER et al., 2002). Through introduction of the first enzyme involved in fructan synthesis, the sucrose sucrose:fructosyltransferase, from Jerusalem artichoke into sugarbeet a dramatic change in the nature of the accumulated sugar was observed. The amount of sucrose left in the transgenic tap root was less than 10% of the amount in the non-transgenic control, indicating that over 90% of the sucrose was channelled into fructan synthesis.

EDIBLE VACCINES

The production of vaccines in transgenic plants was first proposed in 1990 however no product has yet reached commercialization. Recent work suggests that plants provide economic bio-reactors for large-scale production of pharmaceutical proteins. Therefore, plants offer an attractive alternative for the production and delivery of subunit vaccines (DANIELL; STREATFIELD; WYCOFF, 2001). In the past 15 years, a range of different plant and vector systems have been used for the production of a long list of antigens that includes viral, bacterial, enteric and nonenteric pathogen antigens as well ass autoimmune antigens (DANIELL; STREATFIELD; WYCOFF, 2001; RIGANO; WALMSLEY, 2005; STREATFIELD; HOWARD, 2003). An increasing body of evidence demonstrates that these plant-produced antigens can induce immunogenic responses and confer protection when delivered orally. Plant-based vaccines are relatively inexpensive to produce and production can be rapidly scaled up. There is also the potential for oral delivery of these vaccines, which can dramatically reduce distribution and delivery costs. Plants being studied for oral vaccine production include potato, banana, papaya, tomato, lettuce, carrot, rice, wheat, maize, soybean, apple and lupine. The transgenic plants were developed to protect for example from hepatitis B, *Vibrio cholera*, enterotoxigen *Escherichia coli*, Respiratory Syncytial Virus, and Norwalk Virus.

FUTURE ASPECTS AND PERSPECTIVE

The continued development of quality improved crops remains a great challenge. Most transgenic varieties developed initially carry only a single modified trait, but genetic engineering is now moving to the introduction of multigenic or stacked traits. Such lines can be developed either by multiple or sequential transformation, or by conventional crossing of single trait lines. Plant biotechnology is now at the threshold of an exciting new area in which emphasis is on the manipulation of metabolic pathways. Over-expression of single biosynthetic genes in plants may yield enhanced amounts of the desired metabolites within a pathway or even novel metabolites not normally produced by the plant. Inhibition of the activity of genes for biosynthetic enzymes may be utilised to knock out pathway side-branches or catabolism of a particular secondary metabolite, thus enhancing product yield.

Over-expression of regulatory genes of biosynthetic pathways represents an elegant way to manipulate several pathway enzymes more easily. Regulatory genes are also implicated in the responses of a plant to environmental challenges, the control of flowering, seed development, fibre development, partitioning of photo-assimilate into different storage compounds and a multitude of other important metabolic pathways. In order to manipulate plant productivity and product quality effectively, it is critical, therefore, to understand these metabolic networks and their regulation. The value of transgenic technologies as laboratory tools cannot be over-emphasised. In the future, these technologies will become more refined and more predictable, with respect to promoter behaviour, genome integration sites for transgenes, control of downstream pleiotropic effects of novel genes and regulation of transgenes by nuclear matrix attachment regions.

In the longer term, commercial uptake and exploitation of many transgenic lines being developed currently will not take place for many reasons. The majority will serve as laboratory tools to unravel aspects of the function of genes that may be exploited later for crop improvement using conventional breeding. Although transgenesis may represent the only means of achieving some desirable traits, it must be viewed alongside traditional breeding methods as part of a suite of techniques available to modern plant breeding. Besides fundamental considerations of public acceptance and gene product safety, there are other issues that may prevent particular crops or crop products from reaching the marketplace. These include the consequences of gene flow, problems associated with traceability and segregation of food and non-food plants, and methods for maintaining strict control over access to plants producing, for example, pharmaceutical compounds. These are all issues that are being considered and addressed currently in various nations by appropriate regulatory authorities; further discussion of these issues falls outside the scope of this paper. Additionally, commercial viability will weigh against the further development of some transgenic products when extraction/production costs and competitiveness against 'natural' products are considered. Limitations to achieving commercial concentrations of products exist in many cases. The reasons for particular limitations, for example a lack of specific acyltransferases in the case of some fatty acids (LARSON et al., 2002), are beginning to be understood as solutions to these and many other problems continue to be sought.

REFERÊNCIAS/REFERENCES

AL-BABILI, S.; BEYER, P. Golden Rice – five years on the road – five years to go? *Trends in Plant Science,* v. 10, n. 12, p. 565-573, 2005.

BAILY, R. Foods for specified health use (FOSHU) as functional foods in Japan. *Canadian Chemical News*, p. 18-19, May 1999.

BEYER, P.; AL-BABILI, S.; YE, X.; LUCCA, P.; SCHAUB, P.; WELSCH, R.; POTRYKUS, I. Golden rice: Introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *Journal of Nutrition,* v. 132, n. 3, p. 506S-510S, 2002.

BOTELLA-PAVÍA, P.; RODRÍGUEZ-CONCEPCIÓN, M. Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum,* v. 126, n. 3, p. 369-381, 2006.

BOUIS, H. E. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition,* v. 16, n. 7/8, p. 701-704, 2000.

CARRINGTON, A. L.; CALCUTT, N. A.; ETTLINGER, C. B.; GUSTAFSSON, T.; TOMLINSON, D. R. Effects of treatment with *myo*-inositol or its 1,2,6-trisphosphate (PP56) on nerve conduction in streptozotocin-diabetis. *European Journal of Pharmacology,* v. 237, n. 2-3, p. 257-263, 1993.

CHONG, D. K.; LANGRIDGE, W. H. Expression of full-length bioactive antimicrobial human lactoferrin in potato plants. *Transgenic Research,* v. 9, n. 1, p. 71-78, 2000.

CHOWANADISAI, W.; HUANG, J.; HUANG, N.; LÖNNERDAL, B. Stability of recombinant human alpha-1-antitrypsin produced in rice in infant formula. *Journal of Nutritional Biochemistry* v. 14, n. 7, p. 386-393, 2003.

CLAXON, A.; MORRIS, C.; BLAKE, D.; SIREN, M.; HALLIWELL, B.; GUSTAFSSON, T.; LÖFKVIST, B.; BERGELIN, I. The anti-inflammatory effects of D-*myo*-inositol-1.2,6-trisphosphate (PP56) on animal models of inflammation. *Agents Actions,* v. 29, n. 1-2, p. 68-70, 1990.

COMMITTEE ON OPPORTUNITIES IN THE NUTRITION AND FOOD SCIENCES FANBIOM *Opportunities in the Nutrition and Food Sciences:* research challenges for the next generation of investigators. Washington DC: National Academy Press, 1994.

COUGHLAN, S. J.; KINNEY, A. J. Transgenic plants as sources of modified oil. In: OKSMAN-CALDENTEY, K. M.; BARZ, W. H. (Ed.). *Plant biotechnology and transgenic plants.* New York: Marcel Dekker, 2002. p. 305-321.

DANIELL, H.; STREATFIELD, S. J.; WYCOFF, K. Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends in Plant Science,* v. 6, n. 5, p. 219-226, 2001.

DATTA, K.; BAISAKH, N.; OLIVA, N.; TORRIZO, L.; ABRIGO, E.; TAN, J.; RAI, M.; REHANA, S.; AL-BABILI, S.; BEYER, P.; POTRYKUS, I.; DATTA, S. K. Bioengineered 'golden' indica rice cultivars with β-carotene metabolism in the endosperm with hygromycin and mannose selection systems. *Plant Biotechnology Journal,* v. 1, n. 2, p. 81-90, 2003.

DEMIDOV, D.; HORSTMANN, C.; MEIXNER, M.; PICKARDT, T.; SAALBACH, I.; GALILI, G.; MÜNTZ, K. Additive effects of the feed-back insensitive bacterial aspartate kinase and the Brazil nut 2S albumin on the methionine content of transgenic narbon bean (*Vicia narbonensis* L.). *Molecular Breeding,* v. 11, n. 3, p. 187-201, 2003.

DODO, H.; KONAN, K.; VIQUEZ, O. A genetic engineering strategy to eliminate peanut allergy. *Current Allergy and Asthma Reports,* v. 5, n. 1, p. 67-73, 2005.

DUCREUX, L. J. M.; MORRIS, W. L.; HEDLEY, P. E.; SHEPARD, T.; DAVIES, H. V.; MILLAM, S.; TAYLOR, M. A. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. *Journal of Experimental Botany,* v. 56, n. 409, p. 81-89, 2005.

FALCO, S. C.; GUIDA, T.; LOCKE, M.; MAUVAIS, J.; SANDERS, C.; WARD, R. T.; WEBBER, P. Transgenic canola and soybean seeds with increased lysine. *Biotechnology,* v. 13, n. 6, p. 577-582, 1995.

FOGLIANO, V.; VITAGLIONE, P.Functional foods: Planning and development. *Molecular Nutrition & Food Research,* v. 49, n. 3, p. 256-262, 2005.

FRASER, P. D.; RÖMER, S.; SHIPTON, C. A.; MILLS, P. B.; KIANO, J. W.; MISAWA, N.; DRAKE, R. G.; SCHUH, W.; BRAMLEY, P. M. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proceedings of the National Academy of Science* USA, v. 99, n. 2, p. 1092-1097, 2002.

GALILI, G.; HÖFGEN, R. Metabolic engineering of amino acids and storage proteins in plants. *Metabolic Engineering,* v. 4, n. 4, p. 3-11, 2002.

GRASES, F.; MARCH, J. G.; PRIETO, R. M.; SIMONET, B. M.; COSTA-BAUZÁ, A.; GARCÍA-RAJA, A.; CONTE, A. Urinary phytate in calcium oxalate stones formers and healthy people. *Scandinavian Journal of Urology and Nephrology,* v. 34, n. 3, p. 162-164, 2000.

GREINER, R.; KONIETZNY, U. Phytase for food application. *Food Technology and Biotechnology,* v. 44, n. 2, p. 125-140, 2006.

GREINER, R.; LARSSON ALMINGER, M.; CARLSSON, N. G.; MUZQUIZ, M.; BURBANO, C.; CUADRADO, C.; PEDROSA, M. M.; GOYOAGA, C. Enzymatic phytate degradation – a possibility to design functional foods? *Polish Journal of Food and Nutrition Sciences,* v. 11, p. 50-54, 2002.

HANCOCK, R. D.; VIOLA, R. Biotechnological approaches for L-ascorbic acid production. *Trends in Biotechnology,* v. 20, n. 7, p. 299-305, 2002.

HARALDSSON, A. K.; VEIDE, J.; ANDLID, T.; LARSSON ALMINGER, M.; SANDBERG, A. S. Degradation of phytate by high-phytase *Saccharomyces cerevisiae* strains during simulated gastrointestinal digestion. *Journal of Agriculture & Food Chemistry,* v. 53, n. 13, p. 5438-5444, 2005.

HERMAN, E. M.; HELM, R. M.; JUNG, R.; KINNEY, A. J.; Genetic modification removes an immunodominant allergen from soybean. *Plant Physiology,* v. 132, n. 1, p. 36-43, 2003.

HUANG, J.; NANDI, S.; WU, L.; YALDA, D.; BARTLEY, G.; RODRIGUEZ, R.; LÖNNERDAL, B.; HUANG, N. Expression of natural antimicrobial human lysozyme in rice grains. *Molecular Breeding,* v. 10, n. 1-2, p. 83-94, 2002.

JARIWALLA, R. J.; SABIN, R.; LAWSON, S.; HERMAN, Z. S. Lowering of serum cholesterol and triglycerides and modulation of divalent cations by dietary phytate. *Journal of Applied Nutrition,* v. 42, n. 1, p. 18-28, 1990.

JUDD, J. T.; BAER, D. J.; CHEN, S. C.; CLEVIDENCE, B. A.; MUESING, R. A.; KRAMER, M.; MEIJER, G. W. Plant sterol esters lower plasma lipids and most carotenoids in mildly hypercholesterolemic adults. *Lipids,* v. 37, n. 1, p. 33-42, 2002.

JUNG, W.; YU, O.; LAU, S. M.; O'KEEFE, D. P.; ODELL, J.; FADER, G.; MCGONIGLE, B. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. *Nature Biotechnology,* v. 18, n. 2, p. 208-212, 2000.

KATAN, M. B.; DE ROOS, N. M. Promises and problems of functional foods. *Critical Reviews in Food Science and Nutrition,* v. 44, n. 5, p. 369-377, 2004.

KAUFMAN, H. W.; KLEINBERG, I. Effect of pH on calcium binding by phytic acid and its inositol phosphoric acid derivatives and on the solubility of their calcium salts. *Archives of Oral Biology,* v. 16, n. 4, p. 445-460, 1971.

KEROVUO, J.; TYNKKYNEN, S. Expression of *Bacillus subtilis* phytase in *Lactobacillus plantarum* 755. *Letters in Applied Microbiology,* v. 30, n. 4, p. 325-329, 2000.

KONIETZNY, U.; GREINER, R. Molecular and catalytic properties of phytate-degrading enzymes (phytases). *International Journal of Food Science & Technology,* v. 37, n. 7, p. 791-812, 2002.

KONIETZNY, U.; GREINER, R. Phytic Acid: Nutritional Impact. In: CABALLERO, B.; TRUGO, L.; FINGLAS, P. (Ed.). *Encyclopedia of food science and nutrition.* London: Elsevier, 2003. p. 4555-4563.

KRIMPENFORT, P. The production of human lactoferrin in the milk of transgenic animals. *Cancer Detection and Prevention,* v. 17, n. 2, p. 301-305, 1993.

LARSON, T. R.; EDGELL, T.; BYRNE, J.; DEHESH, K.; GRAHAM, I. A. Acyl CoA profiles of transgenic plants that accumulate medium-chain fatty acids indicate inefficient storage lipid synthesis in developing oilseeds. *The Plant Journal,* v. 32, n. 4, p. 519-527, 2002.

LIU, Q.; SINGH, S. P.; GREEN, A. G. High-stearic and high-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. *Plant Physiology,* v. 129, n. 4, p. 1732-1743, 2002.

LUCCA, P.; HURRELL, R.; POTRYKUS, I. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theoretical and Applied Genetics,* v. 102, n. 2-3, p. 392-397, 2001.

LUCCA, P.; HURRELL, R.; POTRYKUS, I. Fighting iron deficiency anemia with iron-rich rice. *Journal of the American College of Nutrition,* v. 21, n. 3, p. 184S-190S, 2002. Supplement.

MAFFUCCI, T.; PICCOLO, E.; CUMASHI, A.; IEZZI, M.; RILEY, A. M.; SAIARDI, A.; GODAGE, H. Y.; ROSSI, C.; BROGGINI, M.; IACOBELLI, S.; POTTER, B. V. L.; INNOCENTI, P.; FALASCA, M. Inhibition of the phosphatidylinositol 3-Kinase/Akt pathway by inositol pentakisphosphate results in antiangiogenic and antitumor effects. *Cancer Research,* v. 65, n. 18, p. 8339-8349, 2005.

MENDOZA, C. Effect of genetically modified low phytic acid plants on mineral absorption. *International Journal of Food Science & Technolology,* v. 37, n. 7, p. 759-767, 2002.

MONSANTO. *Leading Indian Research Institute Partners with Monsanto and Michigan State University to develop "Golden Mustard",* 2000. Disponível em: <www.monsanto.com>.

MUIR, S. R.; COLLINS, G. J.; ROBINSON, S.; HUGHES, S. G.; BOVY, A. G.; DE VOS, C. H.; VAN TUNEN, A. J.; VERHOEVEN, M. E. Overexpression of petunia chlacone isomerise in tomato results in fruits containing dramatically increased levels of flavonols. *Nature Biotechnology,* v. 19, n. 5, p. 470-474, 2001.

MÜNTZ, K.; CHRISTOV, V.; SAALBACH, G.; SAALBACH, I.; WADDELL, D.; PICKARDT, T.; SCHIEDER, O.; WÜSTENHAGEN, T. Genetic engineering for high methionine grain legumes. *Nahrung,* v. 42, n. 314, p. 125-127, 1998.

MURPHY, D. J. Agricultural biotechnology and oil crops: current uncertainties and future potential. *Applied Biotechnology, Food Science and Policy,* v. 1, n. 1, p. 25-38, 2003.

NAIK, P. S.; CHANEMOUGASOUNDHARAM, A.; PAUL KHURANA, S. M.; KALLOO, G. Genetic manipulation of carotenoid pathway in higher plants. *Current Science,* v. 85, n. 10, p. 1423-1430, 2003.

NANDI, S.; SUZUKI, Y. A.; HUANG, J.; YALDA, D.; PHAM, P.; WU, L.; BARTLEY, G.; HUANG, N.; LONNERDAL, B. Expression of human lactoferrin in transgenic rice grains for the application in infant formula. *Plant Science,* v. 163, n. 4, p. 713-722, 2002.

NESTLÉ. *Food politics*: how the food industry influences nutrition and health. London: University of California, 2002.

NIGGEWEG, R.; MICHAEL, A. J.; MARTIN, C. Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology,* v. 22, n. 6, p. 746-754, 2004.

PAINE, J. A.; SHIPTON, C. A.; CHAGGAR, S.; HOWELLS, R. M.; KENNEDY, M. J.; VERNON, G.; WRIGHT, S. Y.; HINCHLIFFE, E.; ADAMS, J. L.; SILVERSTONE, A. L.; DRAKE, R. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology,* v. 23, n. 4, p. 482-487, 2005.

POTRYKUS, I. *Nutritionally enhanced rice to compete malnutrition disorders of the poor*, 2001. Disponível em: <http://nutrition.tufts.edu/ppt>.

RABOY, V. Progress in breeding low phytate crops. *Journal of Nutrition,* v. 132, n. 3, p. 503S-505S, 2002.

RIBAYA-MERCADO, J. D. Influence of dietary fat on β-carotene absorption and bioconversion into vitamin A. *Nutrition Reviews,* v. 60, n. 4, p. 104-110, 2002.

RIGANO, M. M.; WALMSLEY, A. M. Expression systems and developments in plant-made vaccines. *Immunology and Cell Biology,* v. 83, n. 3, p. 271-277, 2005.

RÖMER, S.; FRASER, P. D.; KIANO, J. W.; SHIPTON, C. A.; MISAWA, N.; SCHUCH, W.; BRAMLEY, P. M. Elevation of the provitamin A content of transgenic tomato plants. *Nature Biotechnology,* v. 18, n. 6, p. 666-669, 2000.

SANDBERG, A. S.; ROSSANDER HULTHEN, L.; TÜRK, M. Dietary *Aspergillus niger* phytase increases iron absorption in humans. *Journal of Nutrition,* v. 126, n. 2, p. 476-480, 1996.

SCHULMAN, A. Transgenic plants as producers of modified starch and other carbohydrates. In: OKSMAN-CALDENTEY, K. M.; BARZ, W. H. (Ed.). *Plant biotechnology and transgenic plants.* New York: Marcel Dekker, 2002. p. 255-282.

SETCHELL, K. D.; CASSIDY, A. Dietary isoflavones: biological effects and relevance to human health. *Journal of Nutrition,* v. 129, n. 3, p. 758S-767S, 1999.

SÉVENIER, R.; VAN DER MEER, I. M.; BINO, R.; KOOPS, A. J. Increased production of nutriments by genetically engineered crops. *Journal of the American College of Nutrition,* v. 21, n. 3, p. 199S-204S, 2002. Supplement.

SHEARS, S. B. The versatility of inositol phosphates as cellular signals. *Biochimica & Biophysica* Acta, v. 1436, n. 1-2, p. 49-67, 1998.

SHEWRY, P. R.; TATHAM, A. S.; HALFORD, N. G. Genetic modification and plant food allergens: risks and benefits. *Journal of Chromatography B:* Biomedical Sciences and Applications, v. 756, n. 1-2, p. 327-335, 2001.

STREATFIELD, S. J.; HOWARD J. A. Plant production systems for vaccines. *Expert Review of Vaccines,* v. 2, n. 6, p. 763-775, 2003.

TSAO, R.; HUMAYOUN AKHTAE, M. Nutraceuticals and functional foods: I. Current trends in phytochemical antioxidant research. *Journal of Food, Agriculture & Environment,* v. 3, n. 1, p. 10-17, 2005.

THELEN, J. J.; OHLROGGE, J. B. Metabolic engineering of fatty acid biosynthesis in plants. *Metabolic Engineering,* v. 4, n. 1, p. 12-21, 2002.

URSIN, V. Modification of plant lipids for human health: Development of functional land-based omega-3-fatty acids. *Journal of Nutrition,* v. 133, n. 12, p. 4271-4274, 2003.

VERPOORTE, R.; MEMELINK, J. Engineering secondary metabolite production in plants. *Current Opinion in Biotechnology,* v. 13, n. 2, p. 181-187, 2002.

VUCENIK, I.; SHAMSUDDIN, A. M. Cancer inhibition by inositol hexaphosphate (IP6) and inositol: From laboratory to clinic. *Journal of Nutrition,* v. 133, n. 11S-I, p. 3778S-3784S, 2003.

WARD, P. P.; LO, J. Y.; DUKE, M.; MAY, G. S.; HEADON, D. R.; CONNEELY, O. M. Production of biologically active recombinant human lactoferrin in *Aspergillus oryzae. Bio/Technology,* v. 10, n. 7, p. 784-789, 1992.

WELCH, R. M.; GRAHAM, R. D. Breeding for micronutrients in staple food crops from a human nutritive perspective. *Journal of Experimental Botany,* v. 55, n. 346, p. 353-364, 2004.

WELHAM, T.; DOMONEY, C. Temporal and spatial activity of a promoter from a pea enzyme inhibitor gene and its exploitation for seed quality improvement. *Plant Science,* v. 159, n. 2, p. 289-299, 2000.

WEST, K. P. JR.; DARNTON-HILL, I. Vitamin A deficiency. In: SEMBY, R. D.; BLOEM, M. W. (Ed.). *Nutrition and health in developing countries.* Totowa, NJ: Humana Press, 2001. p. 267-306.

WHO. *National strategies for overcoming micronutrient malnutrition*. Geneva: World Health Organisation, 1992. (Document A4513).

WYSS, M.; BRUGGER, R.; KRONENBERGER, A.; REMY, R.; FIMBEL, R.; OESTERHELT, G.; LEHMANN, M.; VAN LOON, A. P. G. M. Biochemical characterization of fungal phytases (*myo*-inositol hexakisphosphate phosphohydrolase): Catalytic properties. *Applied and Environmental Microbiology,* v. 65, n. 2, p. 367-373, 1999.

YAN, L.; KERR, P. S. Genetically engineered crops: their potential use for improvement of human nutrition. *Nutrition Reviews,* v. 60, n. 5 Pt1, p. 135-141, 2002.

YOON, J. H.; THOMPSON, L. U.; JENKINS, D. J. A. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *American Journal of Clinical Nutrition,* v. 38, n. 6, p. 835-842, 1983.

Recebido para publicação em 17/05/07.
Aprovado em 21/10/07.