IMPROVING THE NUTRITIONAL AND FUNCTIONAL QUALITIES OF PLANT FOOD THROUGH GENETIC ENGINEERING

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Summary

Over the past 10 years, some 40 different species of food and fibre plants have been successfully modified by genetic engineering (GE) technology. Most of the early developments in plant genetic engineering involved the introduction of genes from viruses or bacteria into plants in order to generate varieties which were resistant to viral diseases or herbivorous insects, or which were tolerant to herbicides. In recent years, plant genetic engineers have turned their attention towards the elimination or reduction of undesirable characteristics in plants in order to improve the processing or nutritional characteristics of food plant products. Genetic engineering has provided tomato varieties whose fruit is more robust and flavoursome, or which ripens more slowly, than normal tomatoes. These transgenic tomatoes offer considerable advantages to growers and producers in terms of reduced losses during transport and handling. GE technology is also being used to develop potatoes which are less likely to brown as a result of bruising or cutting thereby affording savings to the potato chip industry. This anti-browning technology also offers potential benefits in other food plants such as apples, pears, beans, lettuce, grapes and sugarcane. Modification of the fatty acid composition of seed oils is also being achieved by manipulating genes from oil-seed plants such as flax (linseed). The length and degree of fatty acid unsaturation in storage oil triglycerides are determined by specific plant enzymes. A number of genes encoding some of these enzymes have been isolated from various sources, manipulated in vitro and introduced into oil crop plants with the net effect of significantly altering the fatty acid composition, thereby leading to seed oils with improved nutritional or functional properties.

I. INTRODUCTION

The advent of recombinant DNA technology some 20 years ago heralded a new and exciting era in the biological sciences. Our ability to cut, join and precisely manipulate DNA, the stuff genes are made of, has led to enormous advances in our understanding of the structure, function and regulation of genes which dictate the characteristics and activities of living organisms and their constituent cells. These technologies for remodelling the genetic material have come to be known as genetic engineering, a powerful new branch of biotechnology. Genetic engineering technology allows us to endow plants and animals with novel traits by introducing genes from other unrelated organisms thereby circumventing the constraints of conventional breeding. The introduced novel gene (transgene) is stably incorporated (integrated) into the genetic material of the recipient to generate a transgenic organism. These are sometimes referred to as genetically manipulated organisms or GMOs. The range of organisms into which novel genes can be introduced (transformed) by genetic engineering technologies is currently limited to those species for which gene transfer and regeneration procedures have been developed. The most common transgenic organisms under development at present are plants, where a large range of food and fibre varieties have been engineered with improved agronomic, nutritional or processing traits.

In genetically engineering higher organisms such as plants, two novel genes are added to the complement of 50,000 or more genes normally present in the plant cell nucleus. One of these two

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novel genes determines the desirable characteristic while the other gene provides resistance to an agent such as an antibiotic or herbicide which is used to select those cells which have been transformed, and hence, which have the potential to give rise to a transgenic plant (Figure 1). The magnitude of the resultant genetic change is extremely small (analogous to the splicing of one-tenth of a second of extraneous noise into a ninety-minute audio tape) and the introduced segment of DNA, corresponding to the transgenes, is not hazardous per se. The focus of concern is generally on the nature of the proteins which are determined by the coded information in the novel genes. The concerns about such proteins relate to their potential pathogenicity, toxicity or allergenicity to humans and to other organisms.

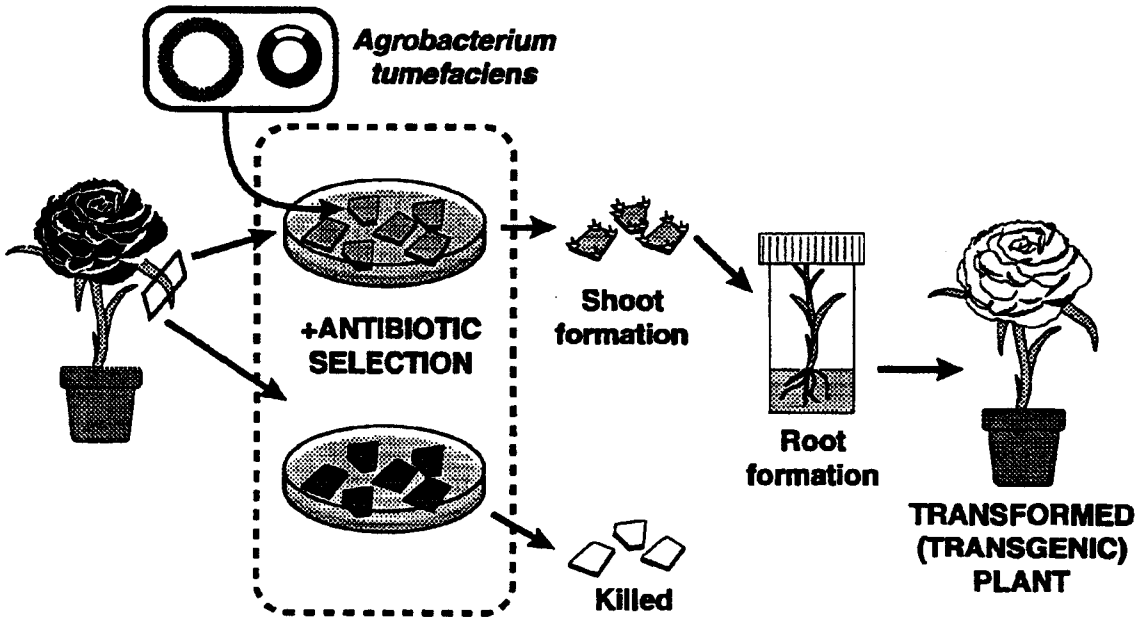

Figure 1. Transformation of novel genes into plants. The soil bacterium *Agrobacterium tumefaciens* is able to induce tumours on dicotyledenous plants by inserting part of its own DNA into a plant chromosome. Mutants of *A. tumefaciens,* which are unable to induce plant tumours, are now used to insert novel genes into a wide range of food plants. This is achieved by mixing the bacteria with segments of plant tissue (such as leaf) and then cultivating the tissue on a synthetic medium containing an antibiotic which kills untransformed cells. Plant cells which contain the novel gene, and an associated antibiotic resistance gene, survive in the presence of the antibiotic and can be induced, by hormone treatment, to develop into a fully-functional transformed (transgenic) plant.

Plant genetic engineering objectives can be divided into two main categories: (1) adding a desirable characteristic, and (2) removing an undesirable characteristic. Adding a desirable characteristic involves the introduction of a gene from a taxonomically-unrelated organism and results in the production of a new protein whose effect is to provide the plant with a new trait. Removing an undesirable characteristic requires the identification and isolation (cloning) of a gene in the target plant which controls the undesirable characteristic. This gene is manipulated in the laboratory (by literally turning it upside down) to generate a so-called antisense gene (Figure 2). This manipulated gene is then inserted into the original plant species with the net effect of reducing or eliminating the activity of the original gene, and thereby, manifestation of the undesirable characteristic. Examples of how these strategies have been employed to genetically engineer plant foods with improved nutritional or processing qualities are described below.

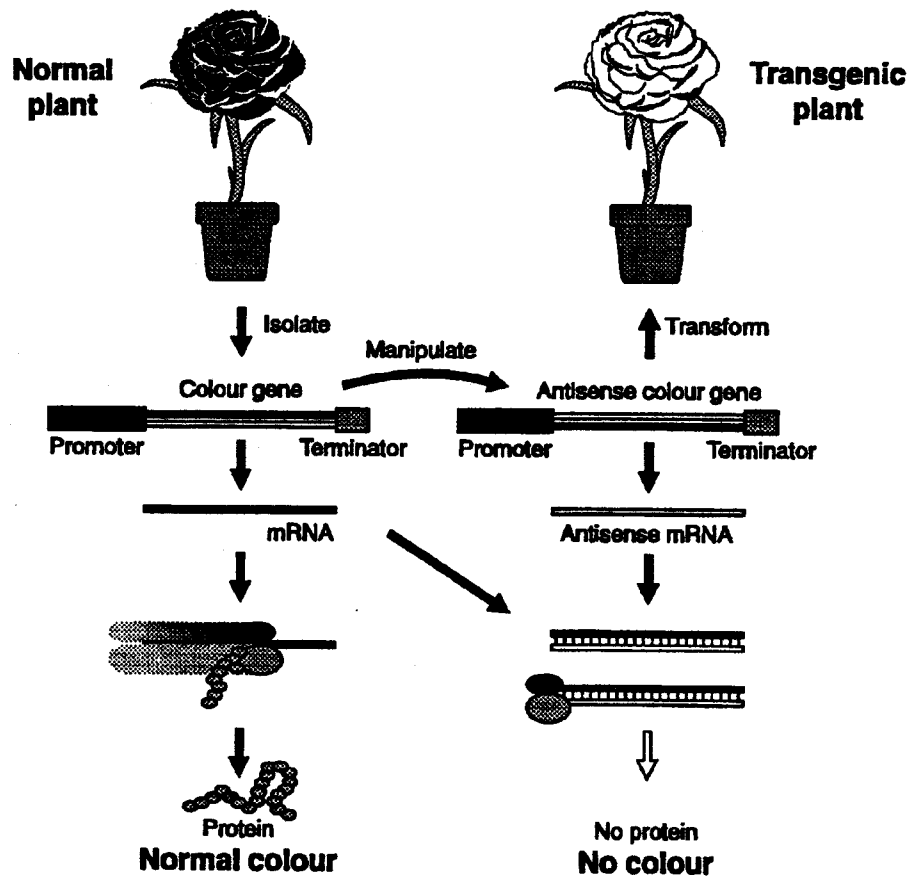

Figure 2. Removing an undesirable characteristic by antisense gene technology, using flower colouration as an example. A gene, which encodes a key protein (eg an enzyme) involved in colour formation, is isolated by cloning. This gene consists of three functional parts, namely, promoter and terminator regions which turn the gene on and off, respectively, and an intervening coding region which specifies the structure of the protein product of the gene. To construct an antisense gene, the coding region in inverted relative to the promoter and terminator regions. When this antisense gene is transformed into the original plant, it produces an antisense messenger RNA (mRNA) which is capable of hydrogen bonding (pairing) to the normal mRNA thereby preventing protein synthesis and colour development

II. IMPROVING THE OUALITY OF FOOD PLANT PRODUCTS

(a) Tougher and tastier tomatoes

Most fresh plant products such as tomatoes are handled, transported and stored between harvest and consumption. The physiological state of tomatoes at the time of picking will determine how long they can be stored, and their quality when consumed. Quality loss can result from a number of factors such as overmaturation, fungal or bacterial infection, and water or temperature stress. Avoiding or delaying any of these factors would obviously increase the market value of tomatoes and result in a better quality product for the consumer. In general, tomatoes are harvested when firm and green which allows about two weeks for transport and storage and a further week on display shelves before their quality declines. Picking tomatoes early in order to prevent damage

in transit to the point of sale can, however, prevent the full accumulation of sugars (fructose and glucose) and organic acids (citric and malic) which are important contributors to tomato flavour.

The softening of fruit such as tomatoes during the ripening process is largely due to changes in structure of the celluloses, hemicelluloses and pectins which are the major components of the fruit cell wall (Brady 1989). During the ripening process, there is an increase in the activity of hydrolytic enzymes such as cellulases and polygalacturonase (PG) which break down the cell wall polysaccharides leading to fruit softening. In tomatoes, PG was originally thought to be the major hydrolytic enzyme involved in softening (Kuhlemeyer et al. 1987). The first application of antisense gene technology in plants involved manipulating PG gene expression in tomatoes (Smith et al.1988). When an antisense PG gene was transformed into the original tomato variety (from which the PG gene was isolated) a number of transgenic plants were obtained whose fruit exhibited reduced PG activity down to 1% of that of the parent plant. Despite this significant reduction in PG activity, there was no measurable change in fruit softening, however, pectin degradation (catalysed by PG) in the cell walls was retarded (Smith et al. 1990). This observation lead to the conclusion that, although PG is important for pectin degradation, it is not the primary determinant of tomato fruit softening. Although these antisense PG tomatoes appear to soften normally, they do have other characteristics which add to their commercial value. For example, they are more resistant to cracking and mechanical damage, and hence to secondary fungal infection, than normal tomatoes which represents a considerable advantage in handling and processing. The fact that the antisense PG tomatoes are more robust than normal means that they can be picked somewhat later than normal, thus allowing further development of their flavour.

(b) Slow-ripening tomatoes

The normal ripening process of tomatoes involves concomitant changes in colour, sweetness, texture and flavour. The onset of ripening is accompanied by an increase in respiration which is known to be stimulated by the phytohormone ethylene (Lyons and Pratt 1964). The addition of exogenous ethylene stimulates mature fruit to ripen earlier than normal (Lyons and Pratt 1964) while the removal of ethylene from the vicinity of picked fruit delays the onset of ripening (Jeffery et al. 1984). In plants, ethylene is synthesised from the precursor s-adenosylmethionine (SAM) which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase. ACC is then converted to ethylene by the enzyme ACC oxidase which is also known as ethylene forming enzyme (EFE) (Yang 1985).

Several different approaches have been used to genetically engineer tomatoes which are delayed in their ripening as a result of the perturbation of ethylene biosynthesis. Oeller et al. (1991) transformed an antisense ACC synthase gene into tomato and isolated a line in which ACC synthase was severely reduced and ethylene production was less than 0.5% of that of the parent plant. The tomatoes from the antisense ACC synthase plant were defective in all the normal responses associated with ripening. When exogenous ethylene was added, these fruit ripened normally and were indistinguishable from the parental tomatoes. The results of this work clearly established that ethylene is a key biochemical signal required to initiate the various processes leading to tomato ripening.

In a slightly different approach, Hamilton et al. (1991) genetically engineered tomatoes by introducing an antisense EFE gene. Fruit from the resultant transgenic tomato lines displayed reduced ethylene production and delayed ripening, although not to the same extent as the antisense ACC synthase fruit. Rather than manipulating the production of ethylene, Klee et al. (1991) transformed tomatoes with a bacterial gene (from a species of *Pseudomonas)* which encodes the enzyme ACC deaminase. In transgenic tomatoes expressing this gene, ACC is broken down to α-ketobutyric acid thereby severely reducing ethylene accumulation and retarding normal fruit ripening. This approach of using a novel gene encoding a degradative enzyme is considerably more versatile in scope than that of using an antisense gene since the latter requires a high degree of nucleotide sequence similarity between the antisense and target genes.

(c) Seed oils for food and function

Over 70% of the world's vegetable oil supplies are derived from four crop plants: soybean, sunflower, rape and palm (Scowcroft 1990). There is an increasing demand for high-quality oilseeds, designed for both edible and non-edible market applications. This demand has coincided with the emergence of plant genetic engineering technologies for the modification of target crop species. The length and degree of fatty acid unsaturation in storage oil triglycerides determine their suitability for various applications. In general, oils which contain a higher proportion of mono- and polyunsaturated fatty acids are more desirable from a health and dietary viewpoint than those with a predominance of saturated fatty acids. The latter types of oils are more suited to industrial applications such as cooking, lubrication, or in paint and polymer production. It has been estimated that the volume of vegetable oil used for chemical production would double if full use was made of them, rather than petrochemicals (Pryde and Rothfus 1989).

Biochemical and genetic studies over the past 10 years have increased our understanding of lipid biosynthesis and the enzymes most likely to determine the composition of a storage oil (Somerville and Browse 1991; Slabas and Fawcett 1992). Some of the genes encoding these enzymes have been cloned, manipulated and introduced into oilseed plants with the objective of specifically altering the fatty acid composition, and thereby the functional or nutritional properties, of the resultant seed oil. Most seed oils are composed of triacylglycerols whose synthesis occurs in three stages. The first of these involves synthesis of the fatty acids palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acid in the plastid. Depending on the plant species, oleic acid can then be metabolized to other fatty acids by desaturation, elongation, hydroxsylation etc. to generate modified fatty acids. In the final step of oil synthesis, the various fatty acids form an acyl-CoA pool in the endoplasmic reticulum where they are acted upon by acyltransferases to form triacylglycerols (see Hills and Murphy 1991; Somerville and Browse 1991; Topfer et al. 1995).

Using a conventional mutation breeding approach, Green (1986) was able to demonstrate that major modification of seed oil composition can be achieved. Linseed oil, which is derived from flax, contains 50% linolenic acid (C18:3), a polyunsaturated fatty acid which renders the oil highly oxidizable. This property makes linseed oil unsuitable for eating or cooking but ideal as a drying agent in paints. Green (1986) was able to isolate flax mutants whose seed contained reduced amounts of linolenic acid. Two such mutants were crossed to generate a flax variety (Linola) which yielded oil containing only 2% linolenic acid. Because of its low linolenic acid content, Linola oil can be used for cooking or in margarine. Biochemical analysis revealed that Linola is defective in linoleic acid desaturase activity which convertslinoleic (C18:2) to linolenic (C18:3) acid. Genetic engineering approaches are currently being employed to develop flax and other oil plant varieties, containing appropriate antisense genes, for the commercial production of specific types of oils.

Lauric acid (C12:0) is the principal fatty acid found in coconut and palm kernel oils. These vegetable oils are commonly known as lauric oils which are mainly used in detergent, soap, lubricant and cosmetic manufacture. In 1994, the American biotechnology company Calgene announced the development of a rape variety whose seed oil contains about 40% lauric oil (normal rapeseed oil does not contain this fatty acid). This was achieved by identifying an enzyme that controls lauric acid synthesis in the California bay laurel tree. The purified enzyme was used to isolate its corresponding gene from the bay laurel which was then genetically engineered into rape plants to generate the high lauric acid variety (Baum 1994).

(d) Reduced browning of fruit and vegetables

Much of the browning which occurs in fruits and vegetables following bruising, injury or during processing is caused by the enzyme polyphenol oxidase (PPO) which occurs in most plants. Such browning results in an unsightly appearance of plant produce and often spoils its flavour. The browning reaction results from the oxidation of phenolic compounds in fruit and vegetables by PPO which is released when plant tissues are damaged by wounding or bruising. Food companies currently use chemicals such as sulphur dioxide to prevent browning. Sulphur

dioxide, however, may pose health risks, particularly to asthmatics and is being phased out as a food preservative.

A natural solution to the problem of fruit and vegetable browning has been developed at the CSIRO Division of Horticulture in Adelaide by a team lead by Dr Simon Robinson. In 1992, Robinson cloned the gene for PPO from grapevines. Using the grapevine gene, he has since cloned similar PPO genes from potatoes, apples, beans, sugarcane and lettuce. The browning reaction is a major contributor to production losses during the processing of food products derived from these plants. For example, the potato chip industry rejects 20-30% of tubers because of browning caused by bruising. In collaboration with Dr Peter Waterhouse (CSIRO Division of Plant Industry, Canberra), Robinson has introduced an antisense PPO gene into potato cultivar Atlantic which is used for crisp production. A large number of transgenic potato lines containing the antisense PPO gene have been generated and these exhibit low levels of PPO activity ranging from 1 to 10% of normal levels. When tubers from some of these low PPO transgenic potatoes are cut or bruised, they fail to show any obvious browning even after 16 hours. In contrast, normal tubers exhibit obvious browning within two or three hours after injury. While this work has initially focussed on potatoes, the antisense PPO strategy may also find application in reducing the browning of grapes, apples, lettuces, peaches, strawberries, bananas, pineapples, sugarcane, wheat and barley.

(e) Modifying the amino acid content of lupins

Feeding non-ruminant animals a diet containing sufficient total protein, but lacking one or more of ten essential amino acids, will result in sub-optimal growth rate and meat quality (Boorman 1980). The amino acid which limits the growth rate of an animal varies with dietary intake, composition of the diet, and the animal species (Boorman 1980). Pigs and chickens are often fed a diet consisting mainly of wheat which is low in the amino acid lysine. For this reason, non-ruminant diets are supplemented with other sources of protein such as peas and lupins. While these legume grains contain adequate amounts of lysine, they contain only low amounts of the sulphur amino acids (SAA) methionine and cysteine. Animal feed formulas are therefore supplemented with purified SAA at a significant cost to the pig and poultry industries.

A genetic engineering approach to improving the SAA content of peas and lupins is being pursued at the CSIRO Division of Plant Industry in Canberra by a team lead by Dr T.J. Higgins. This approach has involved the introduction of a sunflower gene (SFA8) into peas and lupins (Tabe et al. 1993) . The sunflower gene encodes a seed storage protein which contains 23% methionine plus cysteine. Seeds from transgenic lines of pea and lupin, containing the SFA8 gene, have been shown to contain significantly higher SAA contents than those of the parental plants. The seed protein from a transgenic lupin line, for example, contained 44% more SAA than normal lupins. This SFA8containing transgenic lupin variety is currently being bulked up in order to produce enough seed for animal feeding trials.

III. CONCLUSION

The above examples are representative of the types of genetic engineering strategies which are currently being applied to a variety of over 40 different food and fibre plants. A number of other novel applications of this technology to the improvement or modification of food plants have been reviewed by Beck and Ulrich (1993) and Comai (1993). Genetic engineering is a valuable adjunct to conventional breeding practice. The transformation of novel genes into plants involves the introduction of a relatively insignificant amount of genetic material (DNA), compared with conventionally-bred hybrids which initially contain equal contributions of DNA from both parental lines. As a consequence, genetically-engineered varieties may require only a single selfed cross to establish a pure-breeding (homozygous) line compared with the three or more back-crosses needed to establish conventionallybred varieties.

Undoubtedly, genetic engineering will continue to provide many other novel strategies for the improvement of plant productivity and the nutritional or functional qualities of plant foods. This technology can provide solutions to agricultural problems which could not be achieved by traditional plant breeding. In contrast to the more empirical approaches which have been employed in traditional biotechnology over the past few thousand years, the techniques of genetic engineering are precise and rapid. We now have the ability to isolate genes from virtually any living organism and to determine the biological message coded within such genes. With this information we can then devise ways of introducing a particular gene into another living organism with the objective of improving human health, plant productivity or quality, and environmental sustainability.

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