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Genetically Engineered Plants for Quality Improvement

D. S. BRAR[1], T. OHTANI[2] AND H. UCHIMIYA[3]

[1] International Rice Research Institute, P. O. Box 933, Manila, Philippines; [2] Kirin Brewery Co.,LTD, Research and Development Division, Jingumae 6–26–1, Shibuya-ku, Tokyo 150, Japan and [3] Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

Introduction

Recent advances in plant molecular biology have opened new avenues for the production of genetically engineered plants and in the precise transfer of novel genes into crop plants from diverse sources, not feasible before. The development of efficient procedures for the culture of somatic cells, pollen and protoplasts and for plant regeneration from a large number of plant species and improved DNA vector systems based on Ti and Ri plasmids of *Agrobacterium*, direct DNA transfer methods, transposable elements, series of promoters, marker genes and a large number of cloned genes, have made genetic manipulations more precise and directed (Uchimiya, Handa and Brar, 1989). As a result of these developments, genetically engineered plants have been produced in more than 90 plant species. A series of genes has been transferred through various transformation techniques including genes for several agronomically important traits such as herbicide resistance, disease and insect resistance (*Table 1*). These developments have also resulted in precise understanding of the genome organization and regulation of gene expression in higher plants (Weising, Schell and Kahl,1988; Willmitzer, 1988; Uchimiya, Patena and Brar, 1993; Shi *et al.*, 1994). Although transformation has become a routine and straightforward process in several plant species, isolation of genes governing economic traits is a continuing challenge. In this review, we will focus on the advances made in genetic engineering of plants leading to the production of transgenic plants with specifically improved quality characteristics.

Modification of storage proteins and amino acid compositions

Seeds of higher plants contain large quantities of storage proteins. These proteins have been classified on the basis of their solubility in various solvents. Albumins (soluble

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Table 1. Foreign genes inserted (expressed) into genetically engineered plants (Brar and Uchimiya, 1990; Uchimiya, Patena and Brar, 1993).

Classification	Foreign genes
Marker genes	
Drug resistance	Neomycin phosphotransferase, chloramphenical acetyltransferase, dehydrofolate reductase, hygromycin phosphotransferase, streptomycin phosphotransferase
Opine synthesis	Octopine synthase, nopaline synthase
Visible coloration	β-glucuronidase, luciferase, β-galactosidase
Herbicide tolerance	*aro*A and EPSP (glyphosate), *bar* (phosphinothricin), *bxn* (bromoxynil), ALS (sulfonylurea), *tfd*A (2, 4-D)
Insect tolerance	*Bt* toxin, proteiase inhibitor
Virus resistance	Tobacco mosaic virus coat protein, alfalfa mosaic virus coat protein, potato virus coat protein, cucumber mosaic virus coat protein, cucumber mosaic virus antisense coat protein, rice stripe virus coat protein, papaya ring spot virus coat protein, maize dwarf mosaic virus coat protein, plumpox virus coat protein, satellite RNA
Fungal resistance	Chitinase, ribosome-inactivating protein (RIP)
Cold tolerance	Glycerol-3 phosphate acyl transferase
Salinity tolerance	*mtl-D* (mannitol-1-phosphate dehydrogenase)
Seed storage protein	Phaseolin, phytohemagglutinin, conglycinin, patatin, zein, glutenin
Male sterility	Ribonuclease gene (barnase)
Fertility restoration	Ribonuclease inhibitor (barstar)
Photosynthesis	Chlorophyll a/b-binding protein ribulose-1, 5-biphophate carboxylase small subunit phosphoenolpyruvate carboxylase
Pigmentation	Dehydroquercetin reductase, chalcone synthase
Nodulation	Lectin, leghemoglobin
Transposon	Ac, Ds, Tam, En-I
Others	Polygalacturonase, heat shock protein, tubulin, ATP synthase, isopentenyl transferase, metallothionein, pathogenesis-related protein, phytochrome

in water) and globulin (soluble in salt solutions) are major storage proteins found in dicotyledonous plants, whereas prolamins (alcohol soluble) and glutelins (soluble in acid or basic solutions) are those found in monocotyledons. The high levels of accumulation of storage proteins and the resultant ease of gene cloning as well as their economic value make storage proteins an important material for genetic manipulations. Enhanced levels of seed storage proteins would improve the nutritional quality of seeds of crop plants. The most important objective is to alter the amino acid compositions to improve the nutritional properties of seeds. De Lumen (1990) has discussed some of the molecular approaches to improve the nutritional and functional properties of seeds for food purposes. Other objectives include reducing the level of anti-nutritional factors, modifying starch contents and modifying oil quality and content.

The storage proteins of major crops are insufficient in one or more of the essential amino acids. For example, wheat, barley, maize and sorghum accumulate major storage proteins which are low in lysine, while storage proteins of legumes are insufficient in sulphur-containing amino acids. Barley and sorghum are also low in threonine, and maize in tryptophan. Therefore, manipulation of the nutritional quality of proteins involves an alteration of the amino acid compositions in the seeds to provide nutritionally balanced products for human and livestocks.

Genetic engineering can be used to improve protein quality by way of increasing the proportion of a specific amino acid within a protein. This approach would require the isolation and cloning of the gene encoding a specific protein, and modifying its primary structure by altering codons for the limiting amino acids. The modified gene,

along with its regulatory sequences, would then be reintroduced into the host plant species. The genes of a number of plant storage proteins have been cloned and expressed in transgenic plants, for example, the zein genes of maize, the B1 hordein gene of barley, the glutenin genes of wheat and the glycinin and conglycinin genes of soybean.

Three molecular approaches are being used in altering the amino acid compositions of seeds: (a) Identification of naturally occurring seed storage proteins with high levels of the desired amino acids, followed by cloning the corresponding gene and expressing this gene at high levels in the species distinctly differ from the sources of genes, (b) modification by recombinant DNA technologies so that they encode proteins similar to wild type proteins but possess higher levels of the desired amino acids and (c) modification in the pool size of the desired amino acids for the synthesis of seed storage proteins by an alternative metabolic pathway.

Expression of modified storage protein genes

A number of reports are available on tissue specific and developmentally regulated expression of modified seed storage protein genes in higher plants. Storage protein genes of monocotyledons have been expressed and synthesized in dicotyledonous plants and vice versa. For instance, a wheat high-molecular-weight glutenin gene was expressed in tobacco (Robert, Thompson and Flavell, 1989). Hoffman *et al.* (1987) found that unmodified 15-kD zein in tobacco seeds using the phaseolin promoter was accumulated up to 1.6% of total protein. However, when Ohtani *et al.* (1991) attempted to express the modified 19-kD zeins in tobacco seeds previously tested in the *Xenopus* system in tobacco seeds using the same promoter, they found that these were degraded, as was the unmodified version of the protein. The different results obtained with the 19-kD and 15-kD zeins may be due either to structural differences or to the inability of the 19-kD zeins to form protein storage vacuoles on their own.

Saalbach *et al.* (1988) have reported the construction of a modified legumin (11S globulin) gene from *Vicia faba* in which, through a frameshift, the final exon was modified to contain four methionine residues. This exon was then substituted for the corresponding exon in the 11S globulin from soybean, resulting in the production of a hybrid glycinin-modified legumin gene. The modified gene was then transferred into both yeast and tobacco plants. It accumulated in the vacuoles of the yeast, but no modified protein could be detected in transgenic tobacco seeds (Saalbach *et al.*, 1990). Since the mRNA was detectable, the defect appears to be at the translational or post-translational level.

Analysis showed that translated protein was (relatively slowly) degraded during the intracellular transport process. Zein genes can be expressed in dicotyledons if a suitable dicot promoter is available (Hoffman *et al.*, 1987). Both chimeric and modified storage proteins have been expressed in *Brassica napus* (De Cleraq *et al.*, 1990). Expression at mRNA level is correctly regulated in most cases, but, protein levels are not always as expected. Altenbach *et al.* (1989) reported an increase in the methionine content of tobacco seeds from 3.60% to 4.74% of the total protein by achieving the expression of at least 5 copies of a chimeric methionine rich gene. It is important that such traits behave as a single locus, and this can be made possible by passing multiple copies of genes into a single T-DNA. Expression levels on the order

of 1%–5% of total protein with 10%–20% of the desirable amino acids would be desired, assuming no shift in the expression of other proteins. Obtaining significant expression levels of chimeric seed storage protein genes on a consistent basis remains a major challenge. Yang *et al.* (1989) constructed a synthetic gene coding for a protein high in essential amino acids. This synthetic gene fragment (HEAAE-DNA) of 292 base pairs in length, codes for a protein composed of about 80% essential amino acids, and was introduced into potato. Transgenic potato plants contain the synthetic gene insert. The gene was transcribed and translated in the transformed plants. Plant seeds generally contain low levels of free threonine and only trace amounts of free lysine. To examine the effects of these free amino acid levels on seed proteins, Karchi, Shaul and Galili (1994) introduced feed back-insensitive bacterial enzyme genes, dihydrodipicolinate synthase and aspartate kinase, in transgenic tobacco plants. They found that co-expression of both bacterial genes in the same plant, which was generated by cross pollinating transgenic plants expressing either one of the two genes, resulted in a significant increase in the proportions of lysine and threonine in seed albumins. Protein transport and compartmentalization further affect the expression of seed storage proteins. The genetically engineered plants thus should not disrupt any stage of the complex process of protein biosynthesis.

Expression of modified storage proteins in transgenic plants is still in its infancy. Information on the effect that overexpressing proteins rich in a particular amino acid will have on amino acid pools or other physiological factors in transgenic plants is lacking. Protein quality is not the only parameter of importance, and the interaction of changes in protein quality or quantity with changes in oil or starch needs to be examined to provide a useful product.

Production of novel carbohydrates

The reserve starch stored in tissues such as fruits, seeds and tubers, used for animal or human food, or processed for industrial use could be modified through genetic engineering techniques. It is also possible to produce altered starches with unique rheological properties or other novel carbohydrates. A novel source of cyclodextrins (CDs) has been by production in the tubers of transgenic potato plants (Oakes, Shewmaker and Stalker, 1991). CDs have potential applications in stabilization of flavors, odors and other compounds in food plants. It has been also suggested that CDs could be used for the removal of undesirable compounds from food, such as caffeine and cholesterol (Shewmaker and Stalker, 1992). The cyclodextrin glycosyltransferases (CGT), which produce CD from starch, are found only in bacteria and are used in batch fermentors with hydrolyzed starch to produce CDs commercially. Shewmaker and Stalker (1992) introduced the CGT gene from *Klebsiella pneumoniae* into potato. A chimeric gene consisting of the patatin promoter for tuber specific expression, a small subunit of ribulose bisphosphate carboxylase (SSU) transit peptide for plastid targeting, the CGT structural gene from *Klebsiella* and the nopaline synthase 3' region were all introduced into potato. CDs were produced into transgenic potatoes at levels corresponding to 0.001%–0.01% of total starch being converted to CDs. The level of CGT expression and thus production of CDs might be further increased by synthesizing a CGT gene that lacked high AT regions.

Starch biosynthesis occurs in the plastids of plant cells. ADP-glucose

pyrophosphorylase (*ADPase)* has been suggested to play a critical role in plant starch synthesis, as it is in the bacterial pathway for glycogen biosynthesis (Stark *et al.*, 1992). Plants ADPGPPs are tetramers that contain two distinct subunits and are regulated by 3-phosphoglyceric acid and inorganic phosphate as positive and negative effectors, respectively. Activity of ADPGPPs is thus regulated in plants in a complex manner.

Stark *et al.* (1992) introduced an *E. coli*-derived mutant glgC16 gene, which corresponds to plant ADPGGP but is less dependent on fructose 1,6-bisphosphate, an activator, and is less sensitive to inhibitor AMP in *E. coli*. The chimeric glgC16gene was constructed with 35S or patatin promoter and chloroplast transit peptides derived from an *Arabidopsis* SSU gene. In transgenic tubers expressing glgC16gene under the control of patatin promoter, on average, 35 % more starch was found than in control tubers.

The expression of a gene can sometimes be inhibited by a transcript of the corresponding antisense gene. An antisense is a gene in which the opposite strand serves as the template during transcription. The basic concept of antisense gene technology is the down regulation of gene expression by interrupting the flow of information from DNA to protein through the biological inactivation of mRNA. This is accomplished by introducing a gene that, when expressed, encodes an antisense RNA molecule that is complementary to the target mRNA sequence. Subsequent base-pairing results in a non-functional RNA duplex. The efficiency of antisense technology can be enhanced further through the use of organ or tissue specific promoters. Muller-Rober, Sonnewald and Willmitzer (1992) produced transgenic potato plants in which the expression of ADP-glucose pyrophosphorylase (*AGPase*) was inhibited by introducing a chimeric gene containing the coding region of the subunits of the *AGPase* linked in an antisense orientation to the CaMV 35 S promoter. Partial inhibition of the *AGPase* enzyme was achieved in leaves and almost complete inhibition in tubers. As a result, starch formation was abolished in tubers, instead up to 30% of the dry weight of the transgenic potato tubers was represented by sucrose and up to 70% by glucose.

Inhibition of granule-bound starch synthase (GBSS) in potato plants transformed with antisense constructs have been observed (Visser *et al.*, 1991). In those cases, total suppression of GBSS activity tubers containing amylose free starch was obtained. The *waxy* gene in rice encodes GBSS. A 1.0 Kb portion of the sequence of rice *waxy* gene was inserted in an antisense orientation (Shimada *et al.*, 1993). The resulting plasmid was introduced into japonica rice protoplasts. Seeds from transgenic rice plants showed a significant reduction in the amylose contents of grain starch. Manipulation of such genes could be used to reduce the amylose contents of the grain starch in cereals.

Engineering plant genes for modification of fatty acid composition

Modifying the fatty acid composition is an important way to produce improved vegetable oils for food and manufacturing industries. The properties of fats and oils are determined by their fatty acid composition, which affect nutritional quality and oxidative stability. The fatty acid composition of plant membranes and of the triacylglycerols of seed storage lipids is governed by complicated biosynthetic

pathways. In the past, conventional plant breeding approaches have successfully been used to produce desirable alterations in the fatty acid compositions of seed oil. Notable examples include development of rapeseed varieties with low erucic acid contents and reduction in the level of polyunsaturated fatty acids in the soybean, sunflower and linseed.

Manipulation of biosynthetic pathways in transgenic plants also offers new opportunities of modifying fatty acid compositions. However, only limited information is available on the biochemistry and regulation of lipid metabolism. Due to the fact that many key enzymes of lipid metabolism are membrane-bound, attempts to solubilize and purify them from plant sources have been constrained. In many crop species, fatty acid compositions are not suitable for their intended use. Fatty acids are being used extensively for industrial purposes such as lubricants, plasticizers and surfactants. The two-thirds of vegetable oils used for food purposes can be improved in their nutritional quality. Genetic engineering can be applied to modify fatty acid compositions using approaches such as : (a) adding new enzymes, (b) over-expressing existing enzymes and (c) antisense RNA to reduce expression of endogenous enzymes.

The predominant plant fatty acids consist of chain lengths of 16 or 18 carbons and 1–3 double bonds. These fatty acids are synthesized from acetyl-CoA by a series of reactions that are localized in plastids. Modification of fatty acids is primarily by membrane-bound enzymes in the endoplasmic reticulum. These reactions include desaturation to introduce additional double bonds and assembly via acyl transferase reactions to yield triacylglycerol, the storage form of fatty acids in seeds.

Isolating genes for lipid metabolism

Most of the cDNAs and genes encoding enzymes involved in *de novo* fatty acid biosynthesis have been cloned. Some examples include acetyl-CoA carboxylases, components of type II fatty acid synthase, acyl carrier protein (ACP) desaturases and ACP thioesterases (Töpfer and Martini, 1994; Ohlrogge, 1994). *Arabidopsis* is an amenable material for such studies because of its small genome size, availability of comprehensive RFLP maps and gene tagging methods. Identification of markers close to *fad E* gene will facilitate cloning of the wild-type allele by chromosome walking using a contiguous cosmid or yeast artificial chromosome library. A transgenic line containing the DS transposable element closely linked to *fad E* would provide an opportunity to produce a transposon induced mutants. Genes for lipid metabolism can be cloned for which mutants (*fad E, fad F, fad I*) are available in *Arabidopsis*. Analysis of these mutants shows that over expression of cloned *fad E* and *fad F* in transgenic plants could result in highly unsaturated oil. Similarly, increased levels of long chain fatty acids should be possible by over-expression of *fad I*.

Increasing saturated fatty acids

Vegetable oils are an important source of lipids in human diets and they now constitute 15%–20% of the total calorie intake in industrialized countries. The vegetable fats are either used in the form of hydrogenated oil, which increases the saturated fat content of the oil. An alternative to hydrogenation of vegetable oil would be desirable. One of the genetic engineering approaches involves antisense RNA to alter the activity of the

enzyme stearoyl-ACP desaturase. Knutzon *et al.* (1992) used seed-specific antisense gene constructs in *B. napus* and *B. rapa*. The antisense expression of stearoyl-ACP desaturase mRNA reduced the enzyme activity, and thus the contents of stearic acid in the seed oil was increased up to 20-fold (from 2%–40%). Seeds of *B. napus* with 39% stearic acid have normal germination and oil whereas in *B. rapa* seed germination and oil content were reduced. Arondel *et al.* (1992) cloned a gene from *A. thaliana* that encodes an omega-3 desaturase based on the genetic map position of a mutation affecting membrane and storage lipid fatty acid composition. A complementary cDNA clone for the desaturase was identified and introduced into the wild type and mutant plants. Transgenic tissues showed increased amounts of the fatty acid produced by desaturase.

Reducing saturated fatty acids in dietary oils

Vegetable oils generally contain far less saturated fatty acids than the 40%–50% found in animal fats. However, most vegetable oils still contain 10%–20% saturated fatty acids. The major saturated acid in most plant oils is palmitic acid (16:0). Transformation of soybean with an additional acyl-ACP thioesterase led to reduction of thioesterase activity and approximately 2-fold reduction in saturated fatty acids in somatic embryos (Yadav *et al.*, 1993).

Transformation of plants with additional membrane-bound desaturases can thus potentially convert saturated fatty acids to unsaturated ones. Introduction of rat and yeast genes coding for the enzyme stearoyl-CoA desaturase into tobacco plants resulted in reduced levels of saturated fatty acids and increased level of (16:1) palmitoleic acid. Grayburn, Collins and Hildebrand (1992) produced transgenic tobacco by incorporating a stearyl-CoA desaturase gene from rat. The transformed calli and leaves showed an increase in the monounsaturated 16 and 18 carbon fatty acids, and these results show that desaturase from an organism from a different kingdom can function in plants. Polashock, Chin and Martin (1992) introduced a Δ-9 fatty acid desaturase gene from yeast (*Saccharomyces cerevisiae*) into tobacco. The transformed plants expressing the yeast gene at the mRNA level exhibited an approximately 10-fold increase in the level of palmitoleic acid (16:1) in leaf tissues. Other plant organs had increased 16:1 levels ranging from 2 to 20-fold. This fatty acid is found in very low levels (less than 2%) in untransformed plants. The results show that cytoplasmic yeast enzyme functions in plants and alters fatty acyl and membrane lipid compositions. Thus it may be feasible to produce broad changes in lipid compositions of plants through the introduction of synthetic or heterologous lipid biosynthetic genes.

Engineering fatty acids for industrial use

Fatty acids such as lauric acid (12:0) from coconut and palm kernel, erucic acid (22:1) from rapeseed, and linolenic acid (18:3) from flax are used for industrial purposes. For example, erucic acid, a major constituent of some *B. napus* varieties, when converted to erucamide is used extensively in plastic film manufacture. If the erucic acid can be increased from its current level of 50% to 90% through genetic engineering, it would improve its availability for industrial use.

The seeds of the California bay tree (*Umbellularia californica*)which produce 70% medium-chain fatty acids in its seed oils, contains a special acyl-ACP, thioesterase that is specific for lauroyl-ACP. Voelker *et al.* (1992) isolated a cDNA clone by encoding 12:0-acyl-carrier protein thioesterase (BTE).

As is evident from the examples of high lauric acid and high stearic acid production, addition or modification of a single enzyme can result in changed fatty acid composition. A fatty acid modification that might simultaneously increase unsaturation in diets and reduce the need for hydrogenation is the production of petroselinic acid rich vegetable oil. Cahoon, Shanklin and Ohlrogge (1992) isolated cDNA encoding the 36kD peptide from a coriander endosperm cDNA library that codes for an acyl-ACP desaturase involved in petroselinic acid biosyntheses. Expression of this cDNA under the control of CaMV 35S promoter in tobacco callus was accompanied by accumulation of petroselinic acid and 4- hexadecenoic acid, both of which were absent from control callus. However, the levels of petroselinic acid in transgenic tobacco callus were quite low, 1% to 4% of the total fatty acid. The synthesis of petroselinic acid and 4-hexadecenoic acid in transgenic tobacco demonstrates the potential to produce new unsaturated fatty acids through genetic engineering techniques.

Engineering plant genes for longer shelf life of fruits

Almost half of fruits and vegetables are lost due to spoilage. The ripening of tomato fruits is characterized by a series of biochemical processes that result in the change of color, flavor, and texture of ripe tomato fruit. Polygalacturonase (PG) is the major enzyme involved in pectin metabolism during fruit ripening and has been associated with cell wall breakdown, fruit softening, and loss of tissue integrity. Large amounts of PG enzyme activity accumulate specifically during fruit ripening, resulting from the de novo synthesis of PG mRNA and protein. Ethylene, which acts as a hormone in plants to cause fruit ripening, triggers this spoilage. Controled ethylene reduction can extend the ripening period to 20 days in tomato as compared to the normal 10 days of ripening. Two approaches involving (a) reduction in PG activity by antisense gene expression and (b) engineering genes controlling ethylene production could be used to develop plants with longer shelf life of fruits.

Antisense RNA has been used to regulate gene expression in bacteria, *Drosophila*, *Xenopus* and mammalian cells, and the technology has been used to reduce gene expression in transformed plants (Rothstein *et al.*, 1987). The constitutive expression of an antisense chalcone synthase gene results in alteration of flower pigmentation in *Petunia* and tobacco (Van der Krol *et al.*, 1988). The antisense RNA technique has been used to inhibit gene expression during fruit development. Antisense constructs based on a gene for polygalacturonase (Sheehy, Kramer and Hiatt, 1988; Smith *et al.*, 1988) and 1-aminocyclopropane-1 carboxylic acid (ACC) synthase (Oeller *et al.*, 1991) have been used to reduce softening and ripening of fruits. Several groups of workers have isolated cDNA and genomic PG clones and have used these sequences to specifically manipulate PG gene expression. A gene construct containing CaMV35S promoter and full-length of PG cDNA in reverse orientation was used to produce transgenic tomato cv UC 82B (Sheehy, Kramer and Hiatt, 1988). The constitutive synthesis of PG antisense RNA in transgenic plants resulted in a substantial reduction in the level of PG mRNA and enzymatic activity

Table 2. Some examples on genetically engineered plants for quality improvement

Modification	Engineered enzyme (gene)	Source of gene	Engineered plant	Reference
Lauric acid production	Acyl-ACP thioesterase	California bay tree (*Umbellularia californica*)	*Arabidopsis*	Voelker *et al.* 1992
Increased stearic acid	Antisense of stearoyl-ACP desaturase	*Brassica napus*	*Brassica napus*	Knutzon *et al.*, 1992
Increased palmitoleic acid	Stearyl-CoA desaturase	Rat	Tobacco	Grayburn, Collins and Hildebrand, 1992
Increased palmitoleic and oleic acids	Stearyl-CoA desaturase and oleic acids	Yeast	Tobacco	Polashock, Chin and Martin, 1992
Reduced saturated fatty acid	Acyl-ACP thioesterase	Soybean	Soybean	Yadav *et al.*, 1993
Petroselinic acid production	Acyl-ACP desaturase	Coriander	Tobacco	Cahoon, Shanklin and Ohlrogge, 1992
Increased alpha-linolenic acid	w3 desaturase	Arabidopsis	Arabidopsis	Arondel *et al.*, 1992
Reduced ethylene synthesis and extended shelf of tomato fruit	1-aminocyclopropane-1-carboxylic acid (ACC) deaminase	*Pseudomonas* sp.	Tomato	Klee *et al.*, 1991
Reduced polygalacturose activity	Polygalacturonase (PG)	Tomato	Tomato	Sheey, Kramen and Hiatt, 1988, Smith *et al.*, 1988
Reduced synthesis of ethylene forming enzyme	ethylene forming enzyme (EFE)	Tomato	Tomato	Grierson 1992, Picton *et al.*, 1993
Production of novel carbohydrate	cyclodextrin glycosyltransferase (CGT)	*Klebsiella pneumoniae*	Potato	Oakes, Shewmaker and Stalker, 1991

in ripening fruits. The steady-state levels of PG antisense RNA in green fruits of transgenic plants were lower than the levels of PG mRNA normally attained during ripening. The PG activities of ripe fruits analyzed in 1416 transformants showed over tenfold reduction when compared with the control fruits. Interestingly, the lycopene content responsible for red pigmentation of fruits did not differ in transgenic and control plants.

Smith *et al.* (1988) also reported a striking inhibition of expression of the endogenous developmentally regulated gene for PG in transgenic tomatoes expressing antisense RNA. The low level of PG mRNA in ripe fruits may be due to some process occurring in the nucleus, interference with transcription, processing or transport rather than inhibition of translation by the formation of RNA/RNA hybrids in the cytoplasm. Antisense gene technology has also been used to reduce ethylene biosynthesis (Hamilton, Bouzayen and Grierson, 1990). Reduction in ethylene production has been successfully used to prolong shelf life of fruits. Ethylene is synthesized from 5-adenosylmethionine by way of the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC). The cDNAs encoding ACC synthase and ACC oxidase have been cloned. Klee *et al.* (1991) cloned a gene encoding ACC deaminase, which was then introduced into tomato UC 82B. Transgenic plants exhibited reduced ethylene synthesis and did not show any abnormality. The fruit from transgenic plants showed delay in ripening and the mature fruits remained firm for at least 6 weeks longer than the non transformed control fruits. Picton *et al.* (1993) analyzed fruit ripening in transgenic tomatoes expressing an antisense ethylene-forming enzyme (EFE). The rate of over-ripening and fruit spoilage was reduced in EFE-antisense fruits. Oeller *et al.* (1991) described the ACC-synthase antisense transformants in which the synthesis of the ethylene precursor ACC was almost completely inhibited. Ripening of these fruits was inhibited but could be restored by adding ethylene. Hamilton, Lycette and Grierson (1991) also showed that the synthesis of ethylene during fruit ripening was inhibited by EFE-antisense transgene and over-ripening of fruit was delayed. Inability to inhibit tomato fruit ripening with antisense-EFE compared with antisense-ACC synthase reflects a difference in the stability of polypeptides encoded by targets of respective antisense genes.

Prospective

During the last decade, major advances have been made in the production of transgenic plants and in the regulation of gene expression in these transgenic plants. Transgenic plants have been produced in more than 90 plant species and a series of marker genes, including several agronomically important genes for herbicide resistance, insect and disease resistance have been incorporated through various transformation procedures. Most importantly, genetic engineering techniques for improving the quality characteristics are being focused upon modification of storage proteins, improving the amino acid compositions, production of novel carbohydrates, improving fatty acid compositions, reducing anti-nutritional factors and developing plants with longer shelf life of fruits (*Table 2*). However, majority of the quality characteristics are quite complex involving several biosynthetic pathways. More basic information is needed on biochemical aspects to apply genetic engineering technology for the quality improvement of crop plants.

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