

## Genetic Modification of Proteins in Food

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### I. INTRODUCTION

All cultivated plants grown for food have been improved by conscious selection and breeding. Yield, quality, and resistance to biotic and abiotic stresses have all been enhanced to improve agricultural productivity and satisfy mankind's growing needs for food and feed. Plant breeding is an exceptionally successful enterprise that has fashioned the raw material of unimproved germ plasm into the modern high-yielding crop and pasture varieties on which we now depend. Until recently, plant breeders had to depend on empirical methods to reach their goals. They intercross varieties with different characters expecting to recover progeny, in later segregating generations, that combine all of the desired features. After extensive testing these are introduced as new varieties. However, the discovery of plant transformation is changing the way that many breeders approach the challenge of creating new varieties to fulfill specific needs. Directed genetic change provides an important new tool and introduces the possibility of using genetic information from almost any life form, because the genetic code is universal.

Deliberate modification of the protein makeup of food through plant breeding had to await the discovery of the role of proteins and how they affect either the quality of the food material or their suitability for human consumption. Several examples of this in wheat, rice, and soybean are discussed below. Genetic theory implies that each time genetic changes are made in crop plants through plant breeding, there are associated changes in the proteins involved in the production of the new phenotypes.

The genetic controls of inheritance involve the transcription of DNA to messenger RNA (mRNA), which is then translated into the amino acid sequences of polypeptide chains and proteins. Many of the protein products of gene expression are enzymes that direct the synthesis of various cell components that collectively determine the characteristics of the cells, which in turn make up the tissues, organs, and the organism as a whole. The timing and coordination of the expression of these genes are governed by other genetic components called regulatory elements. The effect of this orchestration is the expression of cascades of different genes that, acting together, bring about the changes recognized as growth, development, and differentiation. These are observed as a plant passes from a seedling to a mature flowering structure, to produce a harvestable product that is consumed as food.

When breeding introduces changes in an organism's genetic constitution, usually subtle, but sometimes profound, variation is expected in its proteins. New proteins may be formed, or the balance of existing ones may be changed. A long history of producing new varieties of crop plants by introducing genetic information from a range of related wild species has very rarely resulted in forms that have had to be withdrawn from the market because of health concerns. Plant breeders have introduced thousands of new crop varieties that have had little, if any, effect on food safety.

The concern over genetic engineering stems from the fact that this new technology provides plant breeders access to genetic information from any living organism or indeed to synthetic DNA sequences. The developers of new plant varieties using genetic engineering have the responsibility

of establishing that the newly introduced varieties, and the food products developed from these varieties, are as safe and nutritious as their traditional counterparts. This article compares the types of new plant varieties being developed by new technologies based on plant molecular biology with conventional plant breeding, and summarizes the directions that are being taken in crop plant development. This article focuses particularly on how genetic engineering could alter the allergenic potential of foods derived from major crops.

## II. EARLY PLANT BREEDING

The practice of selecting superior parent plants from which to save seed had its origins during the transition that primitive humans made from hunter-gatherers to cultivators and keepers of livestock. There was a conscious selection of seed lots that gave good yields of easily harvested fruits, seeds, and other edible plant parts.

The first plants were chosen by hunter-gatherers through trial and error. We can assume they quickly learned to avoid plants that were poisonous, bitter tasting, made them sick, or that failed to satisfy their hunger. Seeds collected in times of plenty that were kept for the winter were probably planted in the spring or early summer. In this way we may suppose that the value of husbandry was learned and cultivation became a way of life for groups who were not content to depend entirely on chance in their foraging for food. As early agriculture developed, seeds and plants would have been exchanged and the better forms would have been prized for such qualities as yield, high rate of seed germination, and good flavor. The better farmers practiced selection, recorded their observations, and began to take the first steps in plant breeding by keeping seed lots from different sources separate. Such farmer's lines in Britain were given names that are recorded in early farming journals and diaries.

Modern plant breeding had its origins in the rediscovery of Mendel's observations from genetic experiments with peas. Mendel's factors, later called genes, showed that inheritance was particulate and did not result from the blending of characters, like mixing ink and water.

Biffen,<sup>1</sup> working in Cambridge, England, showed that resistance to yellow rust in wheat was inherited as a Mendelian character. Those who followed him began the process of deliberate selection of forms resistant to the diseases of our major food crops. If sufficient variation was not found among the varieties grown by farmers, then related unimproved and wild varieties and species were collected and screened to find the characters that were needed. Plant explorers and collectors sent and brought back seeds and live plants to be stored in germ plasm banks and botanical gardens. These stores of genetic variation came to be treasured as sources of genetic variation that could be found nowhere else short of returning to the original, often remote, collection sites. The best of modern germ plasm banks maintain detailed catalogs of their materials with computerized data banks showing the origins and characteristics of all the accessions. Because seeds deteriorate over time they must be periodically sown to regenerate fresh seeds for storage to maintain a supply that is available to plant breeders and others.<sup>2</sup>

Many other characters were included in the hybridization and selection programs as plant breeding gathered momentum in the early twentieth century. These programs all had the common objectives of improving the yield and quality of the harvested product and selecting for resistance to environmental stresses to maximize adaptation to the regions where the crops would be grown. The first consumers to test the products of plant breeding programs were the breeders themselves. By discarding forms with poor yield and low quality, the better ones were saved and compared with the best available materials. Improvements were kept and used for further hybridization. Ultimately, the market decides what has value. The crop plant varieties grown today are the result of the accumulation of many incremental steps in plant breeding. However, the early successes in breeding for disease resistance were followed by the discovery that the pathogens could also change.<sup>3</sup> Just as human disease organisms have evolved forms that are resistant to antibiotics, plant pathogens developed mutant forms that attacked the formerly resistant varieties. The Canadian plant pathologist Johnson,<sup>4</sup> who worked with the stem rust disease of wheat, described this process as human-guided evolution.

Most of the first modern plant breeding programs were carried out with public funds in universities and specialized institutions. Private plant breeders also existed, but at first there were no laws to protect the varieties that they developed. Hybrid maize, first introduced in the 1930s, began to change this dramatically.<sup>5</sup> Because the parentage of each hybrid was a trade secret, the knowledge of how to make it remained with the owner of the maize inbred lines used to prepare the F1 hybrid seed sown by farmers. Fresh seed had to be purchased each year for planting. If the farmer planted seed saved from his own crop it gave rise to a mixture of genetic forms caused by gene and chromosome segregation.

The introduction of plant breeder's rights in the 1950s in North America and Europe allowed the rapid growth of the private sector plant breeding industry by granting plant breeders rights and placing restrictions on the ability of farmers to sell seeds of nonhybrid crops to other farmers for planting.<sup>6</sup> The royalty income from new cultivars of crop plants financed the production of further improvements.

Improvements in agronomic traits such as yield and disease resistance continue to be driving forces behind today's seed industry, but, increasingly, attention is also focused on specialty traits, including high oilseed grains, low saturated fat oilseeds, and modified corn starches. Such traits command premium values in the marketplace and may offer unique opportunities for the application of new technologies.

### III. PLANT BREEDING AND BIOTECHNOLOGY COMPARED

In conventional plant breeding, sexual crosses are made between different parents that may each have different characteristics that the breeder seeks to combine. The principal limit to the number and kinds of characters that can be introduced is the sexual compatibility between the plants that are being hybridized. If they are genetically distant, or unrelated, the likelihood of obtaining seeds from crosses is very low. Even when seeds are obtained they may fail to germinate or the plants they give rise to may be sterile. For example, in breeding tomatoes the principal sources of new

genetic variation are other varieties of the same cultivated species *Lycopersicon esculentum*, or other species of *Lycopersicon* that are sexually compatible.<sup>7</sup> Table 1 shows a list of disease resistance traits that were introduced by traditional breeding. Although species in the genus *Solanum*, which includes the cultivated potato, are related they are not sufficiently closely related to produce seeds when crossed with tomato. If fertilization does take place following pollination, the development of the fertilized egg is often aborted and no viable seeds are formed. Sometimes hybrid embryos that would otherwise abort can be rescued by dissection and placing them on a nutrient medium for further development into seedlings.<sup>8</sup>

When crosses between parents produce fertile plants in the following generations, the progeny are selected for their conformation to the breeder's objectives. If the parents differ in many genes the precise combination of characters will occur rarely among the many different segregating forms. As a consequence, breeders must work with large segregating progeny families. These families are screened to select plants that approach the desired form, quality, yield, and resistance to pests and pathogens. These selection programs are rigorous and to be effective must be carried out over successive years and seasons in order to verify that the end products stably inherit the desired characteristics. For example, in winter wheat, a large breeding program may make up to 1000 crosses each year, but not produce new and proven finished varieties until some 8 to 10 years later.<sup>9</sup>

As a result of plant breeding, hundreds of new varieties of crop plants are introduced each year. There are accurate statistics on the numbers of new cultivars of each crop species introduced each year in countries that require variety registration and national trials. These trials test the distinctiveness, uniformity, stability, and the yield and quality of harvested product in order for new introductions to qualify for breeders royalties on seed sales (Table 2). The commercial lifetime of new plant varieties is often short, considering that it may take from 6 to 10 years to produce a new product. In the U.K., varieties of small grain cereals may have a commercial lifespan of 5 to 7 years before being replaced by other superior

**TABLE 1**  
**Resistance in Wild Species of *Lycopersicon* and *Solanum* of Some Economically Important Diseases of Tomato**

| Disease                         | Responsible organism                                   | Source of resistance                                                                           |
|---------------------------------|--------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Bacteria                        |                                                        |                                                                                                |
| Bacterial canker <sup>a</sup>   | <i>Clavibacter michiganese</i>                         | <i>L. hirsutum</i> , <i>peruvianum</i> ,<br><i>pimpinellifolium</i>                            |
| Bacterial speck <sup>a</sup>    | <i>Pseudomonas tomato</i>                              | <i>L. pimpinellifolium</i>                                                                     |
| Bacterial spot                  | <i>Xanthomonas vesicatoria</i>                         | <i>L. esculentum</i> var.<br><i>cerasiforme</i>                                                |
| Bacterial wilt                  | <i>Pseudomonas solanacearum</i>                        | <i>L. pimpinellifolium</i>                                                                     |
| Fungi                           |                                                        |                                                                                                |
| Collar rot                      | <i>Alternaria solani</i>                               | <i>L. hirsutum</i> , <i>peruvianum</i> ,<br><i>pimpinellifolium</i>                            |
| Leaf mold <sup>a</sup>          | <i>Cladosporium fulvum</i>                             | <i>L. esculentum</i> var.<br><i>cerasiforme</i>                                                |
| Fruit anthracnose <sup>a</sup>  | <i>Colletotrichum coccodes</i>                         | <i>L. esculentum</i> var.<br><i>cerasiforme</i>                                                |
| Target leaf spot                | <i>Corynespora cassiicola</i>                          | <i>L. pimpinellifolium</i>                                                                     |
| Didymella canker                | <i>Didymella lycopersici</i>                           | <i>L. hirsutum</i>                                                                             |
| Fusarium wilt <sup>a</sup>      | <i>Fusarium oxysporum</i><br>f. sp. <i>lycopersici</i> | <i>L. pimpinellifolium</i>                                                                     |
| Phoma blight                    | <i>Phoma andina</i>                                    | <i>L. hirsutum</i>                                                                             |
| Late blight <sup>a</sup>        | <i>Phytophthora infestans</i>                          | <i>L. pimpinellifolium</i>                                                                     |
| Phytophthora fruit rot          | <i>Phytophthora parasitica</i>                         | <i>L. pimpinellifolium</i>                                                                     |
| Phytophthora root rot           | <i>Phytophthora parasitica</i>                         | <i>L. esculentum</i> var.<br><i>cerasiforme</i>                                                |
| Corky root <sup>a</sup>         | <i>Pyrenochaeta lycopersici</i>                        | <i>L. peruvianum</i>                                                                           |
| Septoria leaf spot <sup>a</sup> | <i>Septoria lycopersici</i>                            | <i>L. esculentum</i> var.<br><i>cerasiforme</i> , <i>hirsutum</i> ,<br><i>pimpinellifolium</i> |
| Grey leaf <sup>a</sup>          | <i>Stemphylium</i>                                     | <i>L. pimpinellifolium</i>                                                                     |
| Verticillium wilt <sup>a</sup>  | <i>Verticillium albo-atrum</i>                         | <i>L. esculentum</i> var.<br><i>cerasiforme</i>                                                |
| Dahlia wilt                     | <i>Verticillium dahliae</i>                            | <i>L. peruvianum</i>                                                                           |
| Nematodes                       |                                                        |                                                                                                |
| Potato cyst                     | <i>Globodera pallida</i>                               | <i>L. hirsutum</i>                                                                             |
| Sugarbeet                       | <i>Heterodera schactii</i>                             | <i>L. pimpinellifolium</i>                                                                     |
| Root-knot <sup>a</sup>          | <i>Meloidogyne incognita</i>                           | <i>L. peruvianum</i>                                                                           |
| Viruses                         |                                                        |                                                                                                |
| Spotted wilt <sup>a</sup>       | TSWV                                                   | <i>L. pimpinellifolium</i>                                                                     |
| Tobacco mosaic <sup>a</sup>     | TMV                                                    | <i>L. peruvianum</i>                                                                           |
| Tomato yellow<br>leaf curl      | TYLCV                                                  | <i>L. cheesmanii</i><br><i>L. hirsutum</i> , <i>peruvianum</i> ,<br><i>pimpinellifolium</i>    |
| Cucumber mosaic                 | CMV                                                    | <i>L. peruvianum</i> ,<br><i>S. lycopersicoides</i>                                            |
| Curly top <sup>a</sup>          | CTV                                                    | <i>L. peruvianum</i>                                                                           |
| Potato Y <sup>a</sup>           | PYV                                                    | <i>L. esculentum</i> var.<br><i>cerasiforme</i>                                                |

<sup>a</sup> Resistance has been incorporated into cultivars.

Adapted from Rick et al.<sup>75</sup>

**TABLE 2**  
**Number of New Varieties of Major**  
**Agricultural Crops Registered in 1995 in the**  
**European Catalog**

| Crop                                 | Number of new varieties |
|--------------------------------------|-------------------------|
| Alfalfa                              | 26                      |
| Cotton                               | 9                       |
| Flax                                 | 12                      |
| Maize                                | 233                     |
| Pea                                  | 28                      |
| Rapeseed                             | 43                      |
| Sorghum                              | 25                      |
| Soybean                              | 24                      |
| Sunflower                            | 89                      |
| Wheat (soft and durum)               | 114                     |
| Vegetables (46 species) <sup>a</sup> | 1760                    |
| Total                                | 2363                    |

<sup>a</sup> As of December 1, 1994.

varieties. Maize hybrids in the U.S. have a similar short commercial lifespan.

Most of the variation used by plant breeders has come from germ plasm collections that include cultivars and land races produced by earlier generations of farmers and breeders, related wild species collected from the crop's geographic center of origin, and material developed by today's successful seed companies. Much of the variation held in germ plasm banks arose from spontaneous mutation during evolution. Mutations are inherited changes in the DNA in which one or more nucleotide bases are deleted or are replaced with other different bases. If a mutation confers a selective advantage it survives and, in some areas, can replace its progenitor form. In most crops, breeders see spontaneous mutations arise and sometimes select them for propagation. For example, in fruit trees so-called bud sports are known in which the fruit has a different color or form. Pink-fleshed grapefruit and variants of dessert apple varieties have arisen in this way.<sup>10</sup>

Not surprisingly, there has been interest in increasing the normally low frequency of spontaneous mutation. One method uses the property of certain chemicals, called mutagens, and ionizing radiation (X-rays, gamma rays, neutrons) to cause changes in the DNA.<sup>11</sup> The majority of such induced mutations are deleterious. In crop plants where large numbers of seedlings resulting from

mutagen treatments can be easily screened to detect new forms, there has been limited success in recovering disease- and herbicide-resistant forms. Another method makes use of the spontaneous variation observed when unorganized plant callus tissue, cultured on artificial media, is allowed to regenerate meristems and shoots (Figure 1). The plants that are recovered often show considerable variation in morphology and other characters. Although much of this somaclonal variation is of limited practical interest, there are claims that useful forms have been recovered which have given rise to new and superior varieties.

Tissue culture and mutagenesis have been combined, as in the case of chemically mutagenized *Brassica* pollen microspores that were cultured on medium containing herbicide from which fertile double-haploid herbicide-resistant plants were obtained.<sup>12</sup> Since 1964, at least 14 new plant varieties derived through induced mutation have been registered in Canada. These include Empress barley, Redwood 65 flax, Shamrock apple, Early Blenheim apricot, Sunburst sweet cherry, and Stellar canola.<sup>11</sup>

#### IV. PLANT TRANSFORMATION

Recombinant DNA technology overcomes the barriers imposed by sexual compatibility. Because

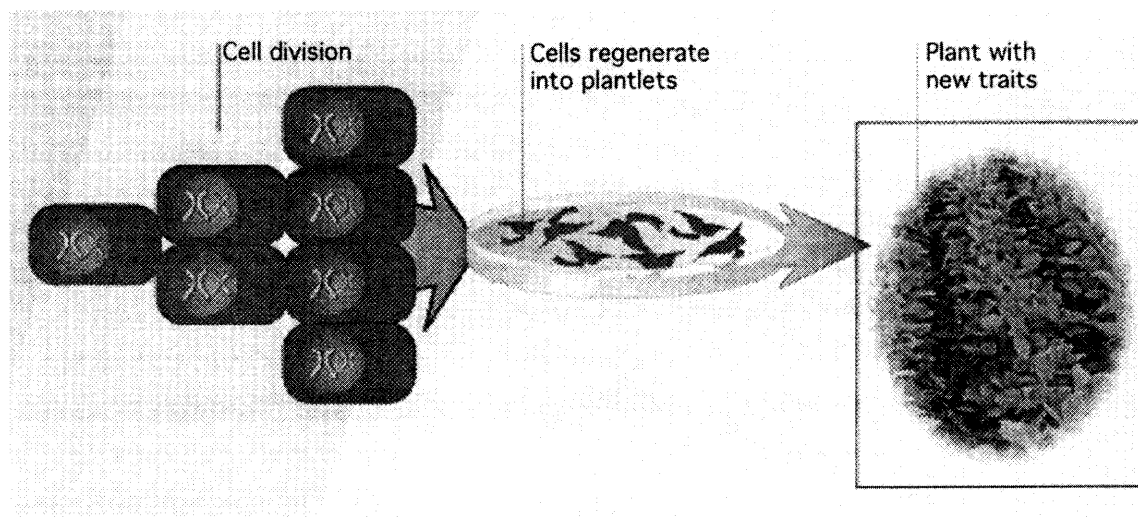


**FIGURE 1.** Plant regeneration. (Figure courtesy of Monsanto Company.)

of such technology, it is now technically possible to introduce into the tomato genetic information not only from the tomato, but from any other organism. Genes from viruses, bacteria, fungi, other green plants and other organisms can be introduced, although genes from nonplant sources may need to be substantially altered to allow them to be efficiently processed by plant cells. The process of introducing foreign DNA into plants is called plant transformation. The following is a brief description of the principal methods used in crop plant transformation intended to guide the unfamiliar reader. The earlier publication by the International Food Biotechnology Council<sup>13</sup> covers in more detail this and other aspects of genetically engineered foods and provides many more references to the literature.

The technology for carrying out genetic transformation was developed about 15 years ago.<sup>14</sup> Several different methods may be employed, but all depend on tissue culture on suitable media. Tissue cultures usually consist of unorganized cells (callus) that do not form recognizable organs like leaves, stems, or roots. However, under special culture conditions some are able to form small organized meristematic regions that develop shoots from which whole fertile plants can be regenerated.

A method of transformation commonly used for dicotyledonous plants (these generally have

broad leaves and not the long narrow strap-shaped leaves of the grasses) depends on the crown gall bacterium, *Agrobacterium tumefaciens*.<sup>15</sup> This is a plant pathogen that invades wounds in the tissues of dicotyledonous plants. The bacterium introduces a small segment of its own DNA into some of the nuclei of the cells of its host where it becomes an integral part of one or more of the host chromosomes. In nature, this results in the formation of a tumor, or gall, at the site of infection which provides a refuge for the bacterium. In the laboratory, the tumor-inducing (Ti) DNA of the crown gall bacterium was disarmed so that it no longer causes plant disease. This was done by deleting the genes carried by the Ti plasmid that are responsible for tumor cell formation. After deletion of these genes all that is left are the left and right ends, or "borders", of the piece of DNA that is inserted in the host nucleus together with a few bases of DNA between them into which foreign DNA can be spliced. The disarmed Ti DNA, carrying the foreign DNA between the borders, is still inserted in the host nucleus, but no tumors are formed. The cells with introduced DNA are normal. In this way a desired gene can be spliced into the disarmed Ti DNA and, when carried into the host cell nucleus, becomes an integral part of the host chromosome (Figure 2). Provided the introduced genes have appropriate controlling elements, or promoters, attached to them, they will be ex-

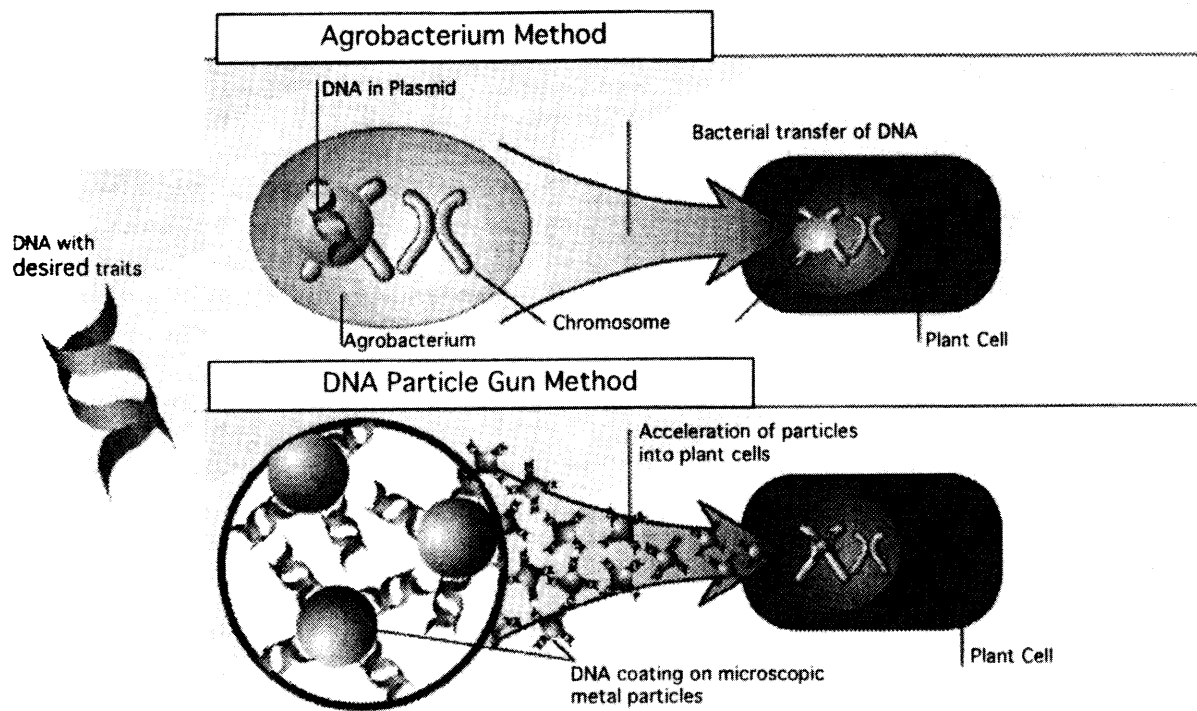


FIGURE 2. Plant transformation. (Figure courtesy of Monsanto Company.)

pressed at the correct stage of host plant development and in the appropriate tissues (e.g., leaves, flowers, fruits, or seeds).

The efficiency of transformation by the crown gall Ti DNA is low. Only 1 in 10,000 to 1 in 100,000 of the host cells exposed to the transforming bacteria are transformed.<sup>16</sup> If the untransformed cells are allowed to grow, they will crowd out the transformed cells. Therefore, a method of selecting host cells that have been transformed is needed.<sup>17</sup> The cells of many dicotyledonous plants in culture are sensitive to the antibiotic kanamycin. If the Ti DNA also carries a gene for kanamycin resistance (a gene that is commonly present in soil bacteria) with a constitutive plant promoter that causes the gene to be expressed all the time, only transformed cells that carry this gene will grow on tissue culture medium containing the antibiotic. The untransformed cells are killed. Antibiotic-resistant plants have no agronomic value. However, when a useful gene is linked to the kanamycin resistance gene, there is a high probability that the transformed tissue that expresses kanamycin resistance will also carry and express the other gene.

The "biolistic" method of transformation is an alternative to the *Agrobacterium* method and is often used for monocotyledonous plants that are not susceptible to the pathogen. The method uses microscopic gold or tungsten particles coated with transforming DNA that are accelerated, by explosive charge, high-pressure helium, or by electric discharge, toward tissue culture cells held in a partial vacuum<sup>18</sup> (Figure 2). A small proportion of the cells, penetrated by DNA-coated projectiles, become transformed. Provided they carry a suitable selectable marker, such as resistance to a herbicide that would normally kill the plant, they can be selected from the background of untransformed tissue and allowed to regenerate to recover whole plants. Variants of this method use carborundum particles or ground glass and a vortex mixer to carry DNA into host cells.

Yet another method uses protoplasts of cultured cells, from which the rigid cell walls are removed by enzyme treatment. DNA enters the cells, either by natural uptake of DNA or by electroporation when the protoplasts, suspended in a suitable osmoticum, are pulsed with an electric field.<sup>19</sup> The short current flow opens small

holes in the outer protoplast membrane greatly increasing the permeability of the cells to DNA molecules.

## V. PLANT TRANSFORMATION AND CROP IMPROVEMENT

At first, the principal limitation to the use of genetic engineering in crop plant improvement was the difficulty in carrying out transformation at a sufficiently high frequency to recover useful numbers of plants. The development of reliable methods for culturing regenerable cell lines required lengthy experimentation. The methods often needed adjustment to suit the idiosyncracies of

individual cultivars within a crop species. The cereals proved to be particularly difficult. The methods for rice<sup>20</sup> and wheat<sup>21</sup> have been developed only recently, aided by the use of herbicide resistance markers, and are not yet routine. Table 3 lists the plants that have been transformed successfully to date.

Another limitation lay in isolating and characterizing the genes to be used in transformation programs. Until the development of plant molecular biology, there was little progress toward understanding the biochemistry and genetic controls of the characters of most interest to plant breeders. Only with this detailed knowledge is it possible to plan and design changes that are likely to result in plant improvements.

**TABLE 3**  
**Plants That Have Been Modified Using Genetic Engineering Techniques**

|                                   |                                  |                             |
|-----------------------------------|----------------------------------|-----------------------------|
| Alfalfa                           | Fennel                           | Pea                         |
| <i>Allocasuarina verticillata</i> | Flax                             | Peach                       |
| <i>Anagallis arvensis</i>         | Foxglove                         | Petunia                     |
| Apple                             | Geranium                         | Plum                        |
| Apricot                           | Grape                            | Poplar                      |
| <i>Arabidopsis thaliana</i>       | Horseradish                      | Poppy                       |
| Asparagus                         | Kiwi                             | Potato                      |
| Aspen                             | Lettuce                          | Raspberry                   |
| <i>Atropa belladonna</i>          | Lemon                            | Rice                        |
| Barley                            | Licorice                         | Rose                        |
| Black currant                     | Lily                             | Rye                         |
| <i>Brassica carinata</i>          | Lotus                            | Snap Dragon                 |
| <i>Brassica juncea</i>            | Maize                            | <i>Solanum dulcamara</i>    |
| Broccoli                          | <i>Medicago truncatula</i>       | <i>Solanum muricatum</i>    |
| Brown Sarson                      | Morning Glory                    | Sorghum                     |
| Buckwheat                         | Muskmelon                        | Soybean                     |
| Cabbage                           | <i>Nicotiana bigelovii</i>       | Spruce                      |
| Canola                            | <i>Nicotiana clevelandii</i>     | Strawberry                  |
| Carnation                         | <i>Nicotiana glauca</i>          | <i>Stylosanthes humilis</i> |
| Carrot                            | <i>Nicotiana hesperis</i>        | Sugarbeet                   |
| Cauliflower                       | <i>Nicotiana plumbaginifolia</i> | Sugar cane                  |
| Celery                            | <i>Nicotiana rustica</i>         | Sunflower                   |
| Chicory                           | Nightshade                       | Sweet potato                |
| Chrysanthemum                     | Oats                             | Tobacco                     |
| Cotton                            | Oil Seed Rape                    | Tomato                      |
| Cranberry                         | <i>Onobrychis viciifolia</i>     | Tulip                       |
| Cucumber Orchid                   | Orange                           | Walnut                      |
| Eggplant                          | Orchardgrass                     | Wheat                       |
| European larch                    | Papaya                           | White clover                |
|                                   |                                  | Yam                         |

Adapted from Fisk and Dandekar.<sup>76</sup>

## VI. GENE CLONING

Much of modern plant molecular biology involves the identification and isolation of genes involved in plant development and metabolism. Most of this is fundamental research to establish the nature and controls of the many pathways involved. Many of the characters of principal interest to breeders (e.g., yield, drought, and stress tolerance) are controlled by many genes. The analysis of how and where to make changes that would increase food production has barely begun. Some of the initial genes that have been isolated and reintroduced into plants are shown in Table 4. Some of these products are already commercially available, whereas others will likely reach the marketplace in the next few years. The following sections describe some of the characters introduced by transformation that are currently in use in plant breeding programs. Plants that have been modified by transformation are said to be transgenic or genetically engineered. The term *genetically modified* is sometimes used, although this could

equally well apply to any organism that has been improved by breeding.

## VII. GENE SUPPRESSION TECHNOLOGY

DNA transcription occurs in only one direction along the molecule, from the 5' to the 3' end. If a coding sequence is inserted in the DNA in an inverted order, transcription occurs from the antisense strand of the DNA molecule instead of from the sense strand. As a result, an antisense form of mRNA is produced, which may bind to the sense form of RNA produced by the normal gene in the cell. Binding is highly specific because it relies on accurate base pairing. If the two mRNA forms interfere with each other, the interference can prevent normal levels of translation into an amino acid sequence. As a consequence, the level of gene product may be greatly reduced, resulting in an altered phenotype.

Following review by the U.S. Department of Agriculture (USDA) and the Food and Drug

**TABLE 4**  
**Near-Term Products of Plant Biotechnology**

| Crop     | Trait                   | Primary gene(s)        | Organization               |
|----------|-------------------------|------------------------|----------------------------|
| Tomato   | Virus resistance        | Viral coat protein     | China                      |
| Tomato   | Delayed softening       | Anti-polygalacturonase | Calgene                    |
| Tobacco  | Bromoxynil tolerance    | Nitrilase              | Rhône Poulenc              |
| Tomato   | Delayed ripening        | Anti-ACC synthase      | DNA Plant Technology       |
| Tomato   | Delayed ripening        | ACC deaminase          | Monsanto                   |
| Tomato   | Delayed softening       | Anti-polygalacturonase | Zeneca                     |
| Squash   | Virus resistance        | Viral coat proteins    | Asgrow                     |
| Cotton   | Bromoxynil tolerance    | Nitrilase              | Calgene                    |
| Potato   | Insect resistance       | <i>cryIIIA</i>         | Monsanto                   |
| Maize    | Basta resistance        | <i>bar</i>             | AgrEvo                     |
| Maize    | Insect resistance       | <i>cryIA(b)</i>        | Ciba Seeds                 |
| Flax     | Sulfonylurea tolerance  | Acetolactate synthase  | University of Saskatchewan |
| Rapeseed | High laurate            | Anti-12:0 thioesterase | Calgene                    |
| Rapeseed | Basta tolerance         | <i>bar</i>             | AgrEvo                     |
| Soybean  | Glyphosate tolerance    | EPSPS                  | Monsanto                   |
| Canola   | Glyphosate tolerance    | EPSPS/GOX              | Monsanto                   |
| Rapeseed | Male sterility          | Barnase/Barstar        | PGS                        |
| Cotton   | Insect resistance       | <i>cryIA(c)</i>        | Monsanto                   |
| Cotton   | Glyphosate tolerance    | EPSPS                  | Monsanto                   |
| Maize    | Insect resistance       | <i>cryIA(b)</i>        | Monsanto                   |
| Maize    | Glyphosate tolerance    | EPSPS/GOX              | Monsanto                   |
| Potato   | Virus resistance (PVY)  | Viral coat protein     | Monsanto                   |
| Potato   | Virus resistance (PLRV) | Viral replicase        | Monsanto                   |

*Note:* ACC, 1-amino-1-cyclopropane-carboxylic acid; EPSPS, 5-enolpyruvylshikimate-3-phosphate; GOX, glyphosate oxidoreductase; *cryIIIA*, the insecticidal gene from *B. thuringiensis* subsp. *tenebrionis*; *cryIA(b)*, the insecticidal gene from *B. thuringiensis* subsp. *kurstaki* strain HD-1; *bar*, phosphinothricin acetyltransferase; Barnase is the male sterility gene and barstar is the restorer gene.

Administration (FDA), a tomato carrying such an antisense construct was introduced in the U.S. in 1994. This tomato has an antisense form of the gene for the enzyme polygalacturonase (PG), in addition to the normal sense form of the gene.<sup>22</sup> PG is produced during the natural ripening of the fruit and is partly responsible for the softening that occurs once full ripeness is reached. The enzyme disrupts the materials that hold the cells of the fruit tissue together. Some tomato plants that carry the antisense PG gene had markedly reduced levels of PG during fruit ripening. Fruits borne by these plants show some delay in the softening process that normally accompanies fruit ripening. The antisense gene adds up to 5 d to the life of the fruit at the stage of full red ripeness before softening spoils it, allowing fruit to ripen on the vine before harvest.

These tomatoes were produced by transformation using a disarmed Ti plasmid vector that also carried the selectable marker gene for resistance to the antibiotic kanamycin. Detailed evidence was provided to the FDA to show that the levels of known toxic compounds normally found in the tomato, such as the alkaloid tomatine, as well as important nutrients, such as vitamins A and C, were no different than the cultivar from which it was derived. The engineered variety employed a selectable marker that causes the tomato to produce the enzyme neomycin phosphotransferase II in all tissues, including the fruit. This enzyme degrades kanamycin, rendering it inactive. Neomycin phosphotransferase is not known to be toxic to mammals, but attention was focused instead on its potential to interfere with the clinical use of the antibiotic even though kanamycin is only infrequently used in human medicine. It was shown that the enzyme was rapidly destroyed in the gut so destroying its activity. There is no evidence of transfer of genes from plant food materials in the gut to microorganisms that are part of the gut microflora.<sup>23</sup> The FDA concluded that there was no hazard presented by the tomato and imposed no restrictions on its sale or requirements that it be labeled as a product of genetic engineering. This tomato was marketed in 1994 as the first genetically engineered food in the U.S.

Since the discovery of antisense technology, it has been found that gene suppression may often

occur in genetically engineered plants that carry sense constructs. Called trans- or co-suppression, the technique offers an alternative, and sometimes simpler, method of turning off the activities of particular genes.<sup>24</sup> Vine-ripened tomatoes that use co-suppression to reduce ethylene production have recently gone on sale in the U.S. after FDA review. In the context of allergenicity, because little or no foreign genetic information encoding protein is introduced by gene suppression technology, the only risk would appear to come from unspecific effects due to inadvertent disruption of other genes (see below) or from the products of marker genes used to select the transformants.

## VIII. HERBICIDE TOLERANCE

In developed world agriculture, weed control is almost entirely carried out by using chemicals that do not harm the crop, but kill its principal weeds. Although some of the early herbicides had undesirable environmental effects, many have been replaced by newer chemicals that are used at very low doses and that are rapidly degraded by soil microorganisms and therefore do not persist in the environment. Most of these chemicals can only be used on the crops for which they were developed. They are not selective on other crops. For example, the widely used herbicide, glyphosate, cannot be used directly on any crops because it is a general herbicide designed to kill all unwanted vegetation. The analysis of the mode of action of herbicides has led to the discovery of genes for tolerance to them. These may, for example, involve the substitution of an altered form of a key enzyme that is no longer inactivated by the herbicide or the use of a gene that degrades the herbicide.<sup>25</sup> Because the mechanisms of resistance are known the nature of the determinative protein changes is also known, and with far greater precision than is possible with conventional plant breeding. An advantage of herbicide tolerance is that it can be selected for directly by incorporating the herbicide in the culture medium used after transformation to introduce the gene for tolerance. Herbicide-tolerant varieties will encourage more selective use of herbicides and will promote soil conservation practices such as reduced tillage.

## IX. DISEASE RESISTANCE

In crop plant development, much effort is expended on breeding varieties that are innately resistant to diseases and pests. The growing disenchantment with chemical pesticides, their effects on the environment, and the perception that some may pose risks to human health has greatly enhanced interest in genetic resistance. There is much active research on the nature and mechanisms of plant disease resistance that will increasingly influence the ways in which we develop resistant crop plant varieties. The traditional method, which is still the most widely practiced, is to survey germ plasm to look for other cultivars, and related and wild species, to find individual plants or families that show high or useful levels of resistance when inoculated with the disease organism or when they are exposed to conditions that support heavy levels of disease infection. The most promising resistant accessions are crossed with one or more modern cultivars to begin a backcrossing program to isolate the genes for resistance while retaining a genetic background adapted for commercial use. Linkage to adverse traits, such as low yield, may make this a long and tedious process that may take up to 10 or more years. It is also a process that favors the selection of resistance genes that are dominant with major effects. Unfortunately, the usefulness of such genes has very often proven short-lived in agriculture, because they strongly select pathogen races that are not affected by them. Some authors advocate the selection of minor gene resistance on the grounds that it is more stable and long lasting.<sup>26</sup>

A variety of mechanisms are responsible for disease resistance. All plants are capable of defense-response reactions. The responses begin within minutes of wounding or injury, or invasion by an organism that is detected as a pathogen. One of the first responses is the appearance of a group of proteins, called pathogenesis-response (PR) proteins.<sup>27</sup> The role and function of the PR proteins are not fully understood. At later stages of response, a variety of compounds that inhibit the growth of invading microorganisms are formed, including products of phenol-propanoid metabolism. There is also strong evidence of the production of compounds that signal systemic responses. These reactions have effects at a dis-

tance from the original invasion site, not only elsewhere on the same leaf, but on other leaves and plant parts. This phenomenon is called systemic-acquired resistance, and appears to play an important role in protecting plants against disease organisms.<sup>28-30</sup>

Not surprisingly, an early goal of plant molecular biology was to isolate and clone genes for disease resistance. By the summer of 1994, a number of reports of the successful cloning of disease resistance genes in maize, *Arabidopsis*, tomato, flax, and tobacco had appeared.<sup>31-34</sup> These genes appear to be responsible for such components of the disease resistance reactions as a maize enzyme that inactivates a fungal pathotoxin,<sup>35</sup> and putative recognition proteins that detect the products of avirulence alleles carried by fungal or bacterial pathogens.

The extent to which naturally occurring genes for resistance will be isolated for use in genetically engineered plants as a means of speeding up breeding for disease resistance remains to be seen. However, because this approach directly parallels the very successful and, so far, very safe conventional breeding approach, the risk of allergenicity seems low. Even if a gene for resistance were obtained from a plant known to be allergenic, e.g., a legume, and was introduced into food plants belonging to other genera and families, the gene product could be evaluated for potential allergenicity in premarket testing (for details see example with soybean protein below and "Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants" by Dean D. Metcalfe et al. in this issue).

In conventional plant breeding, disease and pest-resistant cultivars have not been singled out for special testing. The reason for this is that there has been little evidence that hazards have been introduced in this way. The products of conventional plant breeding programs are thus covered by the safety standard of not requiring premarket approval if the product is generally recognized as safe (GRAS). In the debates over the dangers from pesticide residues in food crops, some have argued that the levels of naturally occurring toxicants in our daily diet are very much higher than the trace amounts of pesticides that are the cause of so much concern. It has also been suggested that naturally occurring antioxidants in the same

plant foods, such as ascorbic acid (vitamin C) and riboflavin, may protect humans from the effects of these toxicants.

## **X. NOVEL MECHANISMS FOR DISEASE AND PEST RESISTANCE**

### **A. Virus Resistance**

A number of important and very damaging plant diseases are caused by viruses. Many of these are transmitted by insects, such as aphids, and plant hoppers that feed by sucking plant sap. In 1986, Powell-Abel et al.<sup>37</sup> discovered that if the coat protein gene for the virus causing tobacco mosaic (TMV) was introduced into the host plant, its expression interfered with the replication and systemic spread of the virus. This made the plant resistant to virus infection. Others soon showed that this was a general phenomenon that applied to many other plant viruses and their hosts.<sup>38,39</sup> Because effective virus resistance was uncommon up to that time, the discovery was seen as the harbinger of a new way to control virus diseases. One of the first potential commercial applications has been the introduction of viral coat protein genes of the cucumber mosaic virus, watermelon mosaic virus, and zucchini yellow mosaic virus into squash.<sup>40</sup> The engineered squash is resistant to all three viruses but contains viral coat proteins that constitute less than 0.1% of the total protein of the squash fruit. This compares favorably with the somewhat greater virus content of fruit from plants naturally infected with the viruses that are commonly consumed because these viruses are widespread (see Table 1). These products do not pose a new allergenic risk.

Another approach to virus resistance involves cloning the viral gene that encodes a replicase. This is an enzyme that the virus needs in order to replicate its own genomic RNA. Somewhat surprisingly, plants that express viral replicase show resistance to the virus concerned and it is assumed that this is because the excess replicase interferes with orderly replication of the viral genome.<sup>41</sup> Viruses also produce certain proteins that appear to be responsible for enlarging the lumen of the plasmodesmata that connect adja-

cent host cells. This enlargement is necessary to allow the virus particle to move from cell to cell to establish systemic infection. Genes for viral movement proteins have been cloned and expressed in host plants and can interfere with viral movement, thereby conferring resistance.<sup>42</sup>

### **B. Fungal Disease Resistance**

The observation that the cell walls of many fungal pathogens are made of chitin, and that some host plants respond to fungal infection by producing chitinase, has encouraged the production of novel transgenic plants that express this enzyme constitutively. Various sources of chitinase genes have been employed including other plants (bean), and bacteria (*Serratia marcescens*). Although interesting, and potentially promising, results have been reported,<sup>43</sup> there are not yet any cultivars in use that employ this as a primary defense against fungal infection.

### **C. Insect Resistance**

Bt is a successful commercial biological insecticide based on the crystal protein endotoxin produced by some strains of the soil bacterium *Bacillus thuringiensis*. When ingested by larvae of sensitive insects, the insecticidal protein is cleaved and an active part of the molecule attaches to the brush border membrane of the larval intestine.<sup>44</sup> As a result, the intestinal wall loses its property of semipermeability and the insect leaks hemolymph into the intestinal cavity, becoming dehydrated and eventually dies. There are a number of different *B. thuringiensis* endotoxin proteins identified that are specifically active against different groups of insects.<sup>45</sup> For example, the cryI proteins are active against many lepidopteran larvae, whereas the cryIII proteins are active against coleopteran larvae. Extensive use of Bt insecticides containing the cryI proteins has not shown any evidence of human toxicity or allergenicity.<sup>46,47</sup> Consequently, biotechnologists were anxious to use *B. thuringiensis* genes encoding various insecticidal proteins, coupled to constitutive promoters, to protect transgenic plants

from insect attack. This strategy is currently in use to protect cotton plants from attack by larvae of the boll worm, potato plants from attack by the Colorado potato beetle,<sup>48</sup> and maize plants from attack by the European corn borer.<sup>49</sup>

The use of Bt toxins in the tissues of crop plants used for food raises issues that must be addressed. Clearly in each case there must be satisfactory evidence that the form of the protein used poses no risk to humans or livestock, and that the protein is not an allergen. Several Bt-based transgenic plant products, including potato, maize, and cotton, are nearing market introduction (see Table 4). Of these Bt-based products, the insect-resistant potato product has completed USDA review, FDA consultation, and has been approved by the U.S. Environmental Protection Agency (EPA).<sup>50</sup> The EPA specifically assessed the safety of the insecticidal Bt protein, from *B. thuringiensis* subsp. *tenebrionis*, including its allergenic potential (based on a history of safe use and its susceptibility to digestion), and identified no significant concerns. The Bt-based insect-resistant cotton and maize products also completed the USDA, FDA, and EPA review processes in 1995. In some cases, the expression of insecticidal proteins can be restricted to certain tissues that are not consumed through the use of tissue-specific promoters. Bt could be expressed in the leaves and stems of potatoes, but it would not be produced in tubers. It is also possible to design the expression of the insecticidal gene so that it only occurs in response to injury such as that caused by a chewing insect. Any such system must provide economically useful control.

An important issue associated with the use of any pesticide is that its effectiveness can be compromised if pests develop resistance. For example, large acreages of transgenic crops expressing Bt will likely exert a strong selection pressure for any insects that become less sensitive to the insecticidal protein. Resistance to Bt has been observed in the diamondback moth from extensive use of Bt insecticide.<sup>51</sup> This is in spite of the fact that selection is potentially less intense because the insecticide is relatively short-lived in nature; it is degraded by ultraviolet (UV) light and washed off the foliage by rain. In a transgenic plant the expression of Bt, under the control of a constitu-

tive promoter, will be constant throughout the life of the plant. Developers of Bt crops are aware of the risk of resistance and have recommended special management practices that may need to be implemented to conserve Bt as an efficient biological insecticide.

Another strategy employed against insects is the use of a trypsin inhibitor gene isolated from an exotic collection of cowpea germ plasm, the seeds of which are unusually resistant to the larvae of moths that attack the grain in storage.<sup>52</sup> By interfering with the digestion of the plant proteins ingested by feeding larvae, transgenic plants that express this gene show moderately high levels of resistance. Trypsin and other protease inhibitors are common components of many of our major food and feed crops. However, any transgenic food crop containing a new trypsin inhibitor would need to be tested to assure safety, especially because the Kunitz trypsin inhibitor is known to be an allergen in soybeans. Appropriate resistance management strategies would need to be developed for any transgenic crop.

Both Bt and trypsin inhibitors are examples of biological pesticides that the EPA proposed to regulate as "plant-pesticides" under the provisions of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act.<sup>53</sup>

## **XI. QUALITY TRAITS: ENHANCED PROTEIN CONTENT AND ALTERED PROTEIN EXPRESSION**

### **A. Maize and Soybean**

Some deficiencies are identified when the amino acid composition of plant proteins is compared with the ideal composition for human and animal nutrition. For example, legume proteins have a less than ideal content of methionine and cysteine, both of which are important sulfur-containing amino acids. Livestock feed based on soybean meal must be supplemented with methionine to provide a nutritionally adequate diet. Maize meal fed to livestock has limiting amounts of the amino acid lysine. Many research groups have addressed these problems. For example, in maize

grain, the lysine content is increased in a mutant called opaque-2.<sup>54</sup> Much effort has been expended in using this gene in breeding programs to produce feed with a higher lysine content. Because of other effects on grain storage and agronomic characters that appear to be closely linked to the high lysine gene(s), these efforts have not been very successful in competition with manufactured lysine feed supplements of microbial origin. Messing and his colleagues<sup>55</sup> used another approach to improve the grain methionine content by selectively increasing the amount of specific methionine-rich proteins in maize endosperm.

In soybean, Townsend et al.<sup>56</sup> used a cloned gene from a Brazil nut that encodes a 2S storage protein found in the nut meat and introduced this gene by transformation. The Brazil nut 2S protein has a methionine content of 18%. The protein was expressed in the seeds of transgenic soybean, resulting in a significant improvement in nutritional quality. The same gene has also been expressed in canola (oilseed rape) where it increases the methionine content of seed meal by more than 30%.<sup>57</sup> However, because Brazil nuts are allergenic for some sensitive individuals, the developers investigated the allergenic potential of the 2S protein. The expressed 2S protein from the soybean was tested against immunoglobulin E (IgE) from the sera of eight Brazil nut-sensitive individuals using the RAST inhibition test and immunoblotting.<sup>58</sup> Seven of the eight sera detected the 2S protein, suggesting that it is likely to be a major allergen in Brazil nuts and would therefore need to be labeled if used in fresh or processed foods in compliance with the 1992 FDA policy<sup>59</sup> on foods derived from new plant varieties. In view of this restriction, development of this product has been discontinued.

## B. Rice Proteins

Some Japanese children suffer from an atopic dermatitis (AD) that is caused by a 16-kDa globulin protein in the rice they consume. This protein is heat stable and resistant to proteolytic enzymes in the gut. Although the allergen can be destroyed by enzyme treatment, the cost of producing hypoallergenic rice in this way is excessive. Japa-

nese scientists set out to produce mutants in which the amount of allergenic protein was reduced. Several such mutants were isolated by Nishio and Iida<sup>60</sup> following treatment of rice seeds with a chemical mutagen. In one mutant, with good agronomic qualities, the amount of the allergenic protein was reduced by about 50%. However, two others, each with only trace amounts of the allergen, were almost sterile and therefore of little use in agriculture. Izumi et al.<sup>61</sup> characterized the allergen and sequenced the gene that encodes it. Matsuda et al.<sup>62</sup> reported that they have succeeded in significantly reducing the amount of the 16-kDa allergen protein in rice by introducing an antisense RNA. Further studies are underway to try to further reduce or eliminate this major allergen in rice. Similar antisense approaches could be used to decrease the levels of other allergenic proteins in food crops, for example, peanuts, soybeans, and Brazil nuts.

## C. Wheat Endosperm Proteins

Wheat breeders have for some time been aware of the incidence of celiac disease, a gluten-sensitive enteropathy that affects between 1 and 16 people per 8000 in Europe. People with celiac disease have to avoid consuming wheat and all products made with wheat flour. Although not an IgE-mediated allergic response, celiac disease could, in theory, be alleviated by breeding wheat varieties that lack the offending gliadin proteins responsible. These are relatively small molecules that range from 10 to 50 kDa in size. Recent information<sup>63</sup> shows that several molecules are reactive and carry and share repeated sequences of nucleotides. The genetic control of the rice allergen is relatively simple and lends itself to the mutational or genetic engineering approaches described. In wheat, the situation is more complex. Unlike rice, which has a single genome, wheat is an allohexaploid with three ancestral genomes. This means that there is likely to be redundancy in the genetic controls with up to three sets of genes for each developmental and metabolic pathway. Also, the offending molecules carry shared repeated amino acid sequences. If these are responsible for the enteropathy, the task

of changing them to reduce significantly their impact on sensitive individuals will be difficult, if not impossible. However, there is a considerable amount of research on the composition, structure, and molecular biology of wheat endosperm proteins in general because the gliadins, together with larger molecular weight glutenins, determine the baking quality of wheat flours.<sup>64</sup>

#### D. Modified Oil and Starch

Vegetable oils derived from the seeds of crops such as maize, soybean, and sunflower represent an important component of the human diet in many countries, and have achieved new prominence as consumers have sought to reduce dietary intake of animal fats. Oil composition can be affected by climatic conditions, as well as by the genetics of each species. Oil profiles have been significantly changed through traditional breeding and selection. The level of erucic acid in wild *Brassica napus* has been reduced from more than 50% to less than 5% to yield low erucic acid rapeseed oil, otherwise known as canola oil.<sup>65</sup> Canola oil has established a reputation as an especially healthy oil because it has the lowest levels of saturated fatty acids of all oilseed crops. Sunflower varieties have been selected that have a high concentration of the polyunsaturated fatty acid, linoleic acid, that makes the oil particularly suitable for dressings and salad oil. Other sunflower varieties have been bred to have a high concentration of oleic acid that improves the oil quality for frying.<sup>66</sup> Hot-processed vegetable oils contain very little, if any, protein and are unlikely to elicit allergenic reactions. Studies with a limited number of subjects have shown that individuals who are allergic to soybeans, peanuts, or sunflowers can consume soybean, peanut, or sunflower oil without ill effects.<sup>67-69</sup>

More recently the 12:0 ACP thioesterase gene from the California bay (*Umbellularia californica*) was introduced into canola to generate a line that produces a laurate-rich oil.<sup>70</sup> High laurate canola oil can replace tropical oils in nondairy coffee whiteners and whipped toppings, as well as in confectionery products. After consultation with the FDA, the first crop of laurate canola was

grown and harvested in 1995.<sup>71</sup> Other oil profiles (e.g., high oleic canola and high stearate, low polystearate soybean) altered through genetic engineering will likely reach the market in the late 1990s.<sup>71</sup>

There are numerous other examples of enhancing or modifying the quality aspects of crop plants. Considerable research and success have been reported in modifying the starch content and/or structure of starch in several food crops. The overall amount of starch has been increased significantly in potato and other crops by expressing a mutant of the *Escherichia coli* ADP glucose pyrophosphorylase protein selectively in the potato tuber.<sup>72</sup> Starch is typically composed of a combination of a linear polymer of glucose called amylose and a branched glucose polymer called amylopectin. Amylose-free starch was produced in transgenic potatoes by inhibiting the amount of granule-bound starch synthase.<sup>73</sup> The degree of branching may also be altered by modifying the levels or activities of other enzymes in starch metabolism. The ability to specifically modify and design new starches will enhance the use of starches as thickeners, bulking agents, sources of calories, and stabilizers in foods. Because starches are not involved in allergenic responses, and the enzymes being engineered are already present in food, these applications are unlikely to raise significant allergenic concerns.

## XII. COINCIDENTAL GENETIC CHANGES

Although the predicted or intended changes that result from plant transformation can be described and confirmed, there is concern that other random changes may have unpredictable effects. There is no control over the site of DNA integration, or of the number of copies of foreign DNA introduced into the recipient genome. Therefore, it is usual to make a number of different primary transformants to select those that are most vigorous and best express the new characters in a manner similar to the selection approach used in traditional breeding. If the integrated DNA disrupts an important coding sequence of an existing gene, this could have undesirable effects on the fitness of the resulting plant after regeneration,

which will cause the material to be rejected during field screening. The site of integration of the foreign DNA may also affect the expression of neighboring genes, and may turn them off or even turn on genes that are normally silent. Again, this may have effects on plant fitness that will lead to those lines being discarded.

It is possible that either kind of effect may alter the protein balance or constitution of the transgenic food plant and raises questions about its safety. However, there are natural processes leading to protein variation that occur in conventional plant breeding. These include genome mixing, transposition due to chromosome breakage and reunion, and loss or gain of whole chromosomes as well as mutations caused by the insertion of transposons; the so-called jumping genes.<sup>13</sup> Despite these significant genomic disruptions, traditional plant breeding has a long history of safe product development. New plant varieties that have been developed by sexual crossing or transformation undergo several generations of further breeding and evaluation to test the products for field performance. This is to establish that they have value to the end user or processor and do not carry deleterious traits. In addition to extensive scientific investigation conducted by industry into the safety of transgenic varieties of plants, such plants are also subject to consideration by FDA under the agency's "consultation process."<sup>58</sup> Under this mechanism, industry has the opportunity to provide science-based assurance that transgenic varieties of plants pose no safety concern to the health of consumers.

A considerable data base has accumulated since the first products of plant biotechnology were engineered (Table 4). The data show that the composition of the food and feed products derived from transgenic plants is equivalent to that of their traditionally bred counterparts. At a recent World Health Organization workshop, Fuchs et al.<sup>74</sup> presented compositional data on 20 independently transformed lines including five crops and six products. No meaningful differences were observed after analyzing over 450 components of these plants. These results establish that the introduction of new traits by genetic engineering, coupled with the traditional plant breeding meth-

ods used to select specific lines for marketing, produce food products that are equivalent to those derived from traditional breeding.

### **XIII. SUMMARY**

Plant breeders have been extremely successful in improving the quality and yield of the major crops, while maintaining the safety of the food supply. This success has been achieved with very little understanding of the biochemical mechanisms that determine the selected traits. Each time a cross is made, tens of thousands of genes are mixed and reassorted, largely at random. The skill of the breeder lies in selecting the lines to be crossed and recognizing the preferred progeny, discarding those that lack the desirable trait or exhibit undesirable properties.

With the advent of recombinant DNA technology, breeders have not only extended the range of biological materials from which genes can be accessed, but have also gained new insights into genome organization and gene structure as well as the nature and function of the proteins that those genes encode. Such knowledge affords exquisite specificity in altering the genetic makeup of new crop varieties. For example, resistance to insect pests can now be achieved through the addition of a single well-characterized gene, instead of introducing thousands of unwanted genes from a wild relative that code for uncharacterized and possibly toxic proteins that must be eliminated by generations of backcrossing and screening to recover a commercially acceptable insect-resistant line. The technology also affords unique opportunities to identify the individual components of foods that may cause allergies, and to remove them from food, or change them, so that the food can be consumed safely.

A number of commercial products derived through genetic engineering have been approved through regulatory processes that address environmental and food safety concerns. These products are available, or will shortly be available, to growers, producers, and consumers. They will provide foods and feeds that are produced with fewer chemical inputs and have improved nutritional composition and quality.

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