

Vegetable Research and Information Center

Vegetable Biotechnology

Applications of Biotechnology in Vegetable Breeding, Production, Marketing, and Consumption

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Bioengineered Products Reach The Marketplace

Revolutionary discoveries in biology in the 1970's and 1980's fueled predictions of dramatic changes in agriculture and stimulated entrepreneurial excitement and investment. Driven by continuing advances in knowledge, technology, and commercial experience, these predictions are now being realized in the marketplace. Beginning in 1994, the first wave of products from biotechnological applications to vegetables were introduced in pilot test markets. Vine-ripe tomatoes with extended shelf life, processing tomatoes with superior quality and deep red color, squash with novel virus resistance, and potatoes genetically modified to produce an insect-killing protein are examples of the traits introduced into commercial vegetable varieties with the tools of biotechnology

These first products, such as the Flavr Savr® tomato which promised superior vine-ripened flavor, received both public visibility and regulatory scrutiny. Less visible to the public is the astounding behind-the-scenes impact of biotechnology in vegetable breeding, production, processing, and marketing. While not replacing traditional crop breeding and horticultural expertise, biotechnology has dramatically expanded the tools available for the genetic improvement and production of vegetables. The revolutionary (evolutionary in terms of biological sciences as a whole) advances that have resulted from public and private investments in basic and applied research are now entering commercial application, to the benefit of both producers and consumers.

Advances in biotechnology also raise important questions.

What are these tools? How are they used in vegetable breeding? What new vegetable products are on the market and can be anticipated for the future? How is this technology accessed for a new product concept? What are the benefits and limitations of these new technologies? How will they affect the vegetable industry and the consumer? Are these foods safe? Who regulates the biotechnology industry? How will they be marketed?

The answers to these questions require an understanding of the methods by which vegetable varieties have traditionally been developed and how the new techniques are being used to complement this process. This brochure provides an overview and introduction to biotechnology as it is being applied for the improvement of vegetables and serves as an introduction and background for additional informational pamphlets on specific commodities or issues in vegetable biotechnology available through the **Agricultural Biotechnology in California (ABC) Series**. It also addresses important food safety, environmental and regulatory issues that have been raised in conjunction with agricultural biotechnology. A glossary at the end of this brochure defines the terms highlighted in the text.

What is Biotechnology?

Biotechnology, in the simplest and broadest sense, is the utilization of living organisms or their components to provide useful products or processes. This definition encompasses essentially all of agriculture, since agriculture is based on the production of plants and animals to provide food, fiber and other products for human use. It also includes familiar uses of microorganisms, such as the yeast used in brewing or baking or the symbiosis of legume plants with nitrogen-fixing bacteria (such as commercial *Rhizobium* inoculants for peas). Thus, humans have been employing biotechnology for at least 10,000 years since the origin of agriculture. Recently, however, the term 'biotechnology' has become synonymous with '**genetic engineering**.' The **cloning** and movement of **genes** among life forms as diverse as bacteria, tomatoes or sheep is now possible. In this context, biotechnology refers to a wide range of enabling technologies that allow the alteration of heritable traits outside of the living organism and the subsequent re-introduction of the new trait into an organism for specific purposes. These new varieties are called **transgenic** because they require the **recombination** of genetic materials from different organisms to cause the desired change.

Some of these enabling techniques are applied without resulting in a transgenic plant. These advanced technologies are sophisticated enhancements of methods that have long been common practice. For example, potatoes are propagated primarily by planting the buds, or "eyes," present on the tubers. This vegetative reproduction ensures that the new plants are identical to their parents. As one tuber can give only a few new plants, one for each "eye," several rounds of seed increase are needed to produce commercial quantities of potato "seed." Unfortunately, this also allows the transmission of disease-causing bacteria or viruses that may have infected the parent plant. **Micropropagation**, the mass production of identical plants from tiny buds of the parent plant, is a biotechnique that can eliminate these pathogens from the progeny plants while retaining the advantages of vegetative reproduction. Similarly, the individual cells of a plant can be separated, multiplied, and **regenerated** into whole plants through a process known as **tissue culture**. In this way, thousands of copies of a single plant can be made and certified pest-free. These techniques are valuable for propagating plants and are an essential component of more advanced methods of genetic modification.

Genetic Basis of Crop Improvement

While these tissue culture techniques are valuable, they do not, in principle, modify the characteristics that are encoded in the plant's genes. **Genes** are the hereditary units, composed of **DNA** (deoxyribonucleic acid), that are strung together to form the **chromosomes** within the nucleus of every cell. All the genetic instructions that determine an organism's characteristics are "packaged" in the chromosomes. The biological systems within an organism convert these instructions into proteins (termed **expression**), one for each gene. The individual or combined activities of these proteins execute all the functions that determine individual traits. Sexually reproducing organisms receive half of their chromosomes from each parent. Since one chromosome of each pair is transmitted randomly to the next generation, the offspring contain a mixture of the traits possessed by both parents. This results in variation in the characteristics of the progeny in each generation. In addition, random changes (or **mutations**) sometimes naturally occur in the DNA itself, potentially altering the characteristic encoded by the gene in which the mutation occurs.

Early agriculturists, either consciously or unconsciously, selected plants that had improved characteristics, and these characteristics were passed on via their genes to subsequent generations, resulting in our domesticated crops. The majority of the changes in crops that are associated with domestication (seed retention on the plant, easier harvesting, greater size of the harvested organs, changes in plant form, loss of bitter and toxic substances, etc.) were already accomplished by the time of historic agricultural civilizations such as the Egyptian, Chinese, or Mayan. With the development of the science of genetics early in this century, breeders had much greater understanding of how to recombine genes via sexual crosses to improve crop yields or modify plant characteristics. In combination with improved agronomic practices, genetic improvement of crop varieties has led to dramatic increases in crop yield and quality, particularly in the past 50 years.

Although most of the 'wild' traits have been eliminated from our domesticated crops, continuous genetic improvement efforts are essential. In addition to further improvements in product quality, yield, and regional performance, there are always new strains of diseases and pests appearing that require new resistant varieties. Thus, the genetic improvement of crops is not something that can be done once and for all. Rather, it requires a deliberate search for novel genetic combinations that can be the source of new consumer or production characteristics to maintain the quality and yield of vegetable crops. Modern biotechnology has created new tools to augment selection strategies and novel sources of genetic combinations for breeders to achieve these advancements.

New Tools for Plant Breeding

For many crops, breeders have relied heavily on the introduction of genes from related wild, but **interfertile**, species to provide new characteristics. In tomatoes and potatoes, for example, resistance to diseases such as *Fusarium* and *Verticillium* and pests such as Root Knot Nematode have been introduced into commercial varieties from related wild relatives discovered in South America. Virtually all of our domesticated crops, including vegetables, are dependent upon genes derived from a wide array of **germplasm**, or genetically distinct variants, of each species. Following a sexual cross between the wild and domesticated types, the parent genes will

segregate, or randomly distribute in subsequent generations according to the laws of genetics. Breeders want to follow these genes and know which progeny have inherited specific genes. This is generally done by visual assessment, disease or pest screens, chemical analysis or other criteria. Often, no visual trait (**phenotype**) is apparent or is only measurable in a mature plant.

Molecular markers are 'tags' that can be used to identify specific genes and locate them on the chromosomes. There are a number of different kinds of molecular markers that can be used to confirm the presence of a gene or even locate its relative position on the chromosome. Geneticists are able to use the markers to develop **genetic maps** of the arrangement of genes in the chromosomes and to identify individual plants containing specific advantageous combinations of genes. Enzymes and proteins (the products of the genes) for a few important traits have been used for many years to monitor or reveal inheritance in the same way. Molecular markers for DNA allow the geneticist to "see" the genes and their arrangement on the chromosomes directly, without having to rely on the expression of the trait.

An example of the use of biochemical markers in vegetable breeding is the introduction of the Root Knot Nematode resistance (*Mi*) gene into commercial tomato varieties. This gene was originally discovered in a wild relative of tomato and was transferred into cultivated tomatoes by conventional sexual crosses. However, the wild tomato parent contributed many additional traits that were not desirable, such as small fruit, poor color, and bitter or toxic compounds. After many generations (more than 10 years) of selection, most of the wild type chromosomes had been eliminated except for a short section containing the *Mi* gene. However, this section also contained a distinct form (or **isozyme**) of a common enzyme, acid phosphatase, that was directly adjacent (**linked**) to the *Mi* gene. Once this association, or **linkage**, was discovered, breeders could determine whether an individual plant had inherited the *Mi* gene simply by testing which form of the acid phosphatase enzyme was present. This made further breeding and selection of nematode resistant plants much more convenient, as the isozyme tests can be conducted on very small seedlings, while tests for nematode resistance require inoculating test soil with the pest and then growing plants to full size. Unfortunately, since the acid phosphatase gene is not identical with the *Mi* gene, plants were eventually found which contained the isozyme marker but did not have nematode resistance, so a linked marker such as this is not a guarantee that the desired gene has been inherited.

This limitation can be minimized or eliminated using DNA-based markers, one of the new tools of biotechnology. In the case of the *Mi* gene, DNA sequences have been isolated that overlap with parts of the desired gene and therefore are more certain to be inherited with it. Researchers are also using these sequences to isolate the *Mi* gene itself to facilitate breeding this valued trait into new varieties and even other species. In other cases, the gene of interest has been isolated, or **cloned**, and copies are available to use as the marker. An advantage of these types of markers is that they identify the DNA of the gene itself, rather than the **expression** of enzymes or proteins. The protein may be present in only small amounts and therefore be difficult to detect, or it may be affected by other genes or by the environment in which the plant is growing. In addition, in many vegetables, including lettuce or tomato, relatively little variation is found among enzymes or proteins to use as markers to identify different lines, while a virtually inexhaustible number of DNA-based markers can be generated to distinguish among even closely related varieties.

A simple way to appreciate this powerful tool for vegetable selection is to visualize the UPC bar-code found on most products in the supermarket. Each set of bars in the code specifies a

product with both a unique set of characteristics and shared characteristics with items of a similar type. The unique product code, organized as a series of bars, gives the item a clear and differentiated identity when the scanner translates the code. This traceable "fingerprint" of the product can be used for a diversity of applications in source-tracking, pricing, inventory management and marketing. In a similar manner, a modern vegetable breeder can create a bar-code-like "**fingerprint**" that allows the physical identification and tracking of desirable or undesirable traits in a breeding and selection program. The unique ladder-like pattern is the result of differences in the genetic blueprint of the breeding line or hybrid. With specialized techniques, detection can be as sensitive as a single **nucleotide base** change in the millions of component units comprising a DNA molecule.

Having multiple markers, or codes, is particularly useful if the breeder wishes to simultaneously follow the inheritance of several genes. For example, there are at least 15 genes that can result in resistance to various strains of Lettuce Downy Mildew. In order to develop cultivars which possess several of these genes to give broad spectrum resistance, the rare individuals who inherit all of the desired genes from both parents must be identified. Rather than screening each seedling against all of the strains of the pathogen, which is difficult to accomplish, the seedlings can be screened using molecular markers specific for each gene, and only those possessing all of the desired genes are grown for the next generation. Similarly, for traits that are dependent upon a number of genes, such as soluble solids content of tomatoes, multiple markers closely associated with desirable genes can be screened and specific individuals can be selected. This technology has the advantage of being suitable for automation to process thousands of samples.

Marker-assisted breeding is also valuable for incorporating, or **introgressing**, specific desirable genes from wild relatives into domesticated varieties. Since the desired gene is present at only one or a few locations in the **genome**, markers identifying other regions of the wild type chromosomes can be selected against, eliminating those (generally undesirable) genes from the progeny. This markedly reduces the time required to develop horticulturally acceptable cultivars containing genes from their distant relatives. One can think of the desired gene as the 'needle in the [genetic] haystack.' Traditional methods essentially require sorting through the entire stack to find the needle, while molecular markers can be thought of as providing a metal detector to easily find the specific part of the haystack where the needle is located. These tools allow breeders to narrow their search for desirable traits to much smaller plant populations, greatly streamlining the development and evaluation of new cultivars.

Genetic Engineering in Plants

Until recently, introduction of new genes into a crop could only be accomplished by sexual crossing, and was therefore limited only to close relatives of the crop species. Plants have mechanisms that prevent fertilization by pollen other than that of their own or closely related species, so a desirable trait of cabbage, for example, could not be transferred to lettuce because such crosses are incompatible and no progeny could be produced.

Over the past 25 years, methods have been developed to selectively alter the genetic instructions in the DNA that direct the growth and development of living organisms. Collectively known as "**genetic engineering**" or "**recombinant DNA**" (**rDNA**) technology, these methods allow scientists to identify, cut out, and then reconnect specific genes (or DNA segments) into a carrier

DNA (or **vector**). This DNA segment can then be introduced into the same or a different organism, and when it executes its instructions (or is “**expressed**”), it will transfer the characteristic coded by the gene to the receiving organism. Because DNA is chemically identical among all organisms, the instructions on these **cloned** pieces of DNA can be readily exchanged and “understood” between organisms as dissimilar as bacteria and humans.

To illustrate the basic concept of rDNA, think of DNA as the biological equivalent of videotape. All organisms carry the instructions they need to grow and function encoded on linear pieces of DNA, just as images are encoded magnetically in the linear pieces of videotape. When the videotape is played, the encoded information is converted electronically into the images displayed on the TV screen. Similarly, organisms convert the genetic information in the DNA into proteins, which then carry out the functions necessary for life. Just as technicians can copy and splice sections of videotape to create new scenes, scientists can now copy and exchange genes among organisms to introduce new characteristics.

Because of their simple genetic makeup, transferring DNA to or among bacteria is relatively easy. Thus, the first applications of rDNA technology were to introduce useful genes into bacteria in order to produce large amounts of specific products. Insulin, for example, is now produced by expressing the human insulin gene in bacteria. Approximately 70% of all cheese produced is now processed using a recombinant enzyme called chymosin produced in bacteria, rather than the very similar enzyme (rennet) isolated from the stomach lining of calves. Transferring DNA into higher organisms is somewhat more complex, but has been achieved for most important agricultural plants and animals. Thus, in theory, any gene from any organism is potentially transferable to other organisms by rDNA techniques.

In plants, transfer of genes, or **transformation**, can be accomplished by several methods. One fascinating approach uses a bacterial pathogen, *Agrobacterium tumefaciens*, to transfer the desired DNA into the plant. The bacterium naturally transfers part of its DNA into the plant's chromosomes, where it then causes the production of compounds that the bacterium consumes. Scientists have learned how to ‘disarm’ the pathogen so that it can no longer impose its own changes, but it retains the ability to transfer DNA into the host plant. Desired genes can be spliced into the bacterial DNA and then *Agrobacterium*, like a video editor, will transfer them into the plant without causing disease.

Unfortunately, *Agrobacterium* does not efficiently infect all types of plants, most importantly the cereals (wheat, maize, rice). Using alternative methods, DNA can be injected directly into cells via two techniques, **electroporation** and particle acceleration or **biolistics**. In electroporation, the plant cell walls are removed using enzymes, and the resulting naked cells (**protoplasts**) are mixed with the desired DNA and subjected to an electric field. The field makes the cell membranes more permeable and allows the cells to take up the DNA. The biolistic or particle gun actually blasts the DNA into the cells. The DNA is coated on tiny tungsten or gold beads, then fired at high speed into plant tissues. Some of the cells will be penetrated by the beads and will incorporate the DNA carried by the beads into their chromosomes. The **transgenic** cells resulting from any of these processes can be selected and regenerated into whole plants by tissue culture, and will then possess the characteristic encoded by the transferred gene.

Genetic engineering provides an alternative to sexual crosses for transferring desirable traits into crop plants. Since only a single (or a few) specific genes are transferred, the host plant retains all of the desirable agronomic traits already fixed into the plant by many rounds of selection. Genetic engineering allows the breeder to introduce a novel gene or make a highly

specific change only to the gene of interest without bringing along other genetic “baggage” that has to be eliminated to restore the original qualities of the variety.

The most significant advantage, however, is that genes can be transferred among organisms that are not sexually compatible. For example, some of the genes used for controlling tomato ripening and for conferring insect or herbicide resistance in genetically engineered crops currently on the market have originally come from bacteria or from plant species unrelated to the crop. A single gene conferring resistance to Mosaic Virus was recently transferred from tobacco to tomato using rDNA techniques. However, genetic engineering does not eliminate the need for continued breeding and selection. Transformation, by any method, has the potential to simultaneously alter other genetic traits in some individuals. After introduction of a novel trait, the variety still must be tested for yield and quality under field conditions and perhaps be incorporated into a **hybrid** cultivar before it will receive commercial acceptance.

Genetic engineering, or **molecular accelerated breeding (MAB)**, is differentiated from conventional breeding by the unique ability to introduce a single or multiple combination of traits to an established cultivar or elite breeding line. Successful commercial lines that growers want and buyers request can be rapidly re-created to provide enhanced agronomic or consumer market traits with a minimal re-investment in field evaluations. Value-added traits are being incorporated into existing cultivars in development times of 2-3 years using MAB, rather than the 8-10 years typical with conventional breeding, while preserving the features that made them profitable to grow.

Direct and Indirect Benefits of Agricultural Biotechnology

The long-term beneficial impact of biotechnology on vegetable production will be realized both directly and indirectly. Many of the products will benefit producers and processors by improving the economic efficiency of production. California vegetable production faces many challenges in maintaining productivity while protecting the environment. Biotechnology will contribute to environmental quality protection by reducing the frequency of agricultural pesticide applications and by allowing more environmentally compatible materials and alternative methods to be employed. For example, plant-based pest resistance in both transgenic and conventionally selected varieties will reduce dependence on broad spectrum pesticides. To enjoy continuing benefits from this approach, producers will need to carefully manage their use of such varieties to prevent or delay the development of resistance in the target organisms (see ABC Series: *Pest Resistance Management - Bt Potatoes – DANR #72XX*). Alternative strategies for reducing herbicide use include introducing color or leaf morphology modifications into seedlings to enhance the use of precision, robotic cultivation.

Biotechnology is also being applied to develop microorganisms for biological control of pests. For example, an insect-attacking virus (baculovirus) has been modified using rDNA techniques to produce a protein toxin from a gene originally obtained from scorpions. Field trials in California and other states on several vegetable crops have been proposed. These types of products are much slower to be commercialized as they provoke far more public concern than plant-based pest resistance. On the other hand, DNA-based tools allow the detailed analysis of microbial interactions with plants, with other microbes and with the environment in ways never before possible.

Another major issue facing all of California agriculture is expanding urban development. Many of the major vegetable producing regions are also highly desirable for residential and recreational uses. Biotechnology can help develop balanced solutions to improve the compatibility of agriculture with increasing urban pressures.

The value of traits with predicted environmental benefits must be evaluated in relation to broader social issues. It has been argued, for example, that developing plants that can grow successfully on marginal crop land (by incorporating salt tolerance) will only accelerate the irreversible loss of prime farm land to urbanization and poor management practices. The reality of world population growth, however, demands that all tools and solutions to improving food production capacity be explored. The responsible application of these tools will further the sustainability of agricultural resources and systems.

What types of direct consumer benefits are expected?

Diversity has been the hallmark of California vegetable production. California leads the nation in production of over 35 major vegetable crops and is the dominant or sole producer of many more specialty vegetable items which enrich our quality of life. A recent report by the FDA has revised the food pyramid to emphasize the importance of vegetables in human nutrition. California will play a key role in the application of biotechnology to deliver consistent, year-round quality of major produce items and novel vegetable products to consumers. Two major categories of direct consumer benefit from biotechnology are sensory and nutritional quality and food safety.

Sensory and Nutritional Quality

Biotechnology has been applied to improving the sensory properties and shelf life of vegetables. Some of the innovations, particularly in texture or flavor enhancement, have not been highly publicized as originating from plants modified by methods included in a broad definition of biotechnology. These include carrot, potato, celery, pepper, and melon varieties improved through applications of tissue culture. Public awareness of developments in agricultural biotechnology is primarily associated with genetic engineering, particularly of tomato.

In 1994, the first genetically engineered food product reached consumer markets. Calgene's Flavr Savr[®] tomato received widespread publicity during development and commercial introduction. The targeted benefit, shared by several companies developing new tomato varieties, was to deliver to the market tomatoes with improved flavor and reduced rates of softening or decay. Flavr Savr[®] technology utilizes the inhibition of the enzyme polygalacturonase (PG) to achieve this goal while DNA Plant Technology, Corp. (Endless Summer[™]) and Agritope, Inc. (SAMase[®]) have focused on blocking the production of ethylene, a plant hormone produced in ripening fruit and other tissues (for details see ABC Series: *Tomato—DANR #72XX*). In each case, improved flavor quality was expected from allowing the fruit to remain on the vine until the full, natural sensory potential for acids, sugars, and aromatics was attained. These "vine-ripe" traits, often absent from mass-marketed tomatoes that are picked green, are then realized as ripening continues during distribution. Although production issues have slowed large scale commercialization, these enhanced sensory quality tomatoes were generally well received by consumers in test markets and limited retail distribution.

Future products entering the commercial pipeline seek to specifically modify the accumulation and stability of sensory traits by increasing the sweetness and by maintaining the

acid balance during maturation and ripening of tomatoes. Both plant and non-plant genes have been introduced into test tomato varieties to increase sucrose accumulation, increase the conversion of sucrose to fructose in the fruit, and sustain organic acids during ripening of tomatoes.

One interesting outcome of the focus on consumer traits by the biotechnology industry has been a resurgence of effort to improve tomato sensory quality by conventional breeding. Capitalizing on the general trend for increased consumption of fruits and vegetables and the value-added traits demonstrated by Flavr Savr® and Endless Summer™, an immediate impact at the marketplace has been enhanced interest and demand among wholesale buyers for higher sensory quality tomatoes. This created an opportunity for seed companies and growers to focus more attention on developing conventionally-bred varieties and handling practices that deliver these value-added traits. The consumer now has greater access to higher quality tomatoes from both field and greenhouse production. Sustained improvements in tomato quality will result from continued expansion of sources of diversity, the precision of gene identification, and the control of trait expression that will be available to breeders.

Food Safety

Biotechnology is playing a key role in the development of rapid and sensitive diagnostic tools for food borne pathogens, microbial toxins, and other contaminants. Recent outbreaks of *Salmonella* and *Escherichia coli* (*E. coli*) 0157:H7 in fresh vegetables and processed fruit have heightened awareness of the need for proper sanitation and handling. Biotechnology is contributing new detection methods and information about the sources and persistence of food-borne pathogens in production, processing and distribution to the consumer.

Who Regulates Biotechnology Products?

Are Biotech Foods Safe?

The federal agencies responsible for food, human, and environmental safety apply the same basic requirements to the products of biotechnology as to conventional vegetable production and consumption. A coordinated system of review and regulation has evolved and continues to be refined among the United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). The review and notification system includes many other federal, state, and local agencies in the process depending on the specific application and intent of the product or food.

Various resources are available which detail the review process, permitting, and product registration requirements for safety testing and approvals from research through commercialization (see Additional Sources of Information). In general for products related to vegetable production or consumption, the EPA is the lead agency if the product is a microbe or plant with genetically-engineered pesticidal traits. As with any new pesticide, a program of toxicity assessment for target and non-target organisms is conducted early in development. The review of experimental field testing and registration of biotechnology products is focused on product traits, without assuming that biotechnological processes are inherently risky. Current regulations, nonetheless, require special conditions or considerations unique to biotechnology products. A non-transgenic product with the identical product trait will likely have reduced regulatory requirements for approval. As experience has been gained in regulating biotechnology over the

past 10 years, all of the agencies involved have streamlined their procedures and review or notification requirements to reflect the growing database of environmental and food safety information.

The FDA is the primary agency regulating food safety and the same procedures apply to genetically engineered vegetables that are used to safeguard all foods in the marketplace. Guidelines are in place that provide criteria for determining whether a product of biotechnology results in a “new ingredient” and requires pre-market review and approval, as with any food additive.

FDA experts regularly engage in informal consultations with commercializing companies to assist them in determining the status of a particular product. Several genetically-modified vegetable products, including tomatoes, potatoes, and squash have been the subject of formal or informal FDA review.

Compliance with a FDA policy statement on food safety testing for new plant cultivars, first released in 1992, has been thorough. Companies have provided data that details the required genetic background, nutrient levels, and toxicant composition of the products for FDA review. Critics argue, however, that the safety assessment is an internal process that follows loose guidelines to reach a determination of “No Concern”. Regulators and the food industry counter that the system works well for all food ingredients or additives and no new regulations are needed. Product liability concerns have been a strong motivator for the agricultural biotechnology industry to proceed with prudence.

The USDA is the lead agency that provides oversight for research and testing of non-pesticidal products and importation or creation of potential pests. The process of development and testing of major genetically-modified vegetable crops, such as potato and tomato, has become routine based on the nature of the crop and experience with several hundred field trials throughout the world. More rapid product development and field testing is now possible as a result of a “delisting” procedure available for those crops with sufficient science-based data for the absence of pest or weediness traits in the test product. Many other factors, such as outcrossing potential (breeding with other related plant species), are equally important in removing a plant from permit or notification requirements. For example, tomato is delisted from regulatory review by the USDA, but the high outcrossing rates for sunflower, a native to North America, make it a less likely plant to be delisted in the immediate future. Much of the notification procedure can now be completed quickly using simple electronic, on-line forms. Significantly, the USDA has ruled that all current and future Flavr Savr® tomato varieties are exempted from further regulatory review. Thus, once a genetically modified trait has received regulatory exemption, that trait can be bred into other varieties without repeating the formal review process.

Shouldn't Biotech Foods Be Labeled ?

Blanket requirements for labeling of produce developed by biotechnological processes are not likely to be implemented. This controversial subject has been debated vigorously for several years among regulators, consumer advocates, the research community, and the agricultural biotechnology industry. The consensus, at this time, is that blanket, mandated labeling is not in the best interest of the consumer.

A special label that merely identifies a tomato or potato as being harvested from a genetically engineered variety may falsely imply to some that it is better and to others that it is inherently dangerous. Labels that carry enough information to describe the full complexities of the

process would be burdensome to the retailer, increase packaging waste, and not be readily understood by the majority of consumers. The label information for each element of the process or area of food safety concern would be either overwhelming or grossly oversimplified, and therefore uninformative. An informed decision by the consumer to use or avoid biotechnologically derived produce, whether fresh or processed, would not be attainable in practice.

Special circumstance labeling for kosher or vegetarian diet requirements has been suggested by special interest advocates for produce developed with the use of genes derived from animals or shellfish. Rabbinical organizations have taken the position that a single gene or even several genes transferred from an animal or shellfish source does not embody the essence of the being and creates no automatic conflict with a kosher diet.

At this time, marketing companies are voluntarily placing stickers on fresh market tomatoes and tomato paste that use the phrase "Grown from genetically-modified seed." In the future, consumers may have the option to access FDA-maintained electronic databases for coded produce and processed foods. It is doubtful, however, that this solution will address broader consumer concerns that deal with the degree of confidence in the food safety assessment and approval process.

Shouldn't labels identify produce that might cause allergies?

Allergens are substances that produce an allergic reaction. The introduction of new allergies from conventional or molecular breeding is possible, but of very low probability. The common example used to argue that genetically engineered foods should be labeled involves reactions to peanut allergens. Genes transferred from a peanut plant to a snap pea, it is argued, may cause allergic reactions in individuals sensitive to peanut. The food product, therefore, should be "hazard labeled" to warn consumers. As with kosher or vegetarian issues, one or several genes are not the essence of a living organism. All peanut-gene products won't cause allergic reactions. Nonetheless, recent studies have shown the concerns to have a valid basis. A specific gene from the Brazil nut, known to code for a protein allergen, was transferred to other plant species. This protein, produced in the cells of the test crop, caused an allergic reaction in previously non-allergenic test foods.

Inability to test effectively for potential allergenic reactions remains a significant barrier to regulatory approval of genetically engineered foods. While predicting the allergenic potential of a substance is not a well established science, known protein allergens have common structural components that are good indicators for caution. These properties are being catalogued at the University of Geneva, Switzerland, which maintains a large database for computer-based comparisons to introduced proteins. Products of cloned genes with potentially allergenic traits are likely to be avoided as the commercial development costs to ensure food safety are presumed to be very large and therefore financially risky. As a greater understanding of the specific features and identity of allergens becomes known, the tools of biotechnology will be utilized to eliminate the allergenic components and increase food safety and quality.

Strategies For Commercialization

The steps between concept, discovery, reduction to practice and commercialization for biotechnology-based products are very complex. Several branch points in the path are reached at each key phase that affect both technical and business strategic decisions. Access to a broad

array of enabling licenses and technologies must be obtained to have “freedom to operate” in a commercial application. Very broad patents cover the primary tools of biotechnology and are the ground floor access points. A novel application may result in a new patent, but sale of the resultant product requires negotiation of license rights for those technologies utilized in the construction and introduction of any new gene to a plant or microbial product.

Future Prospects

The future strategic application of biotechnology is expected to follow a trend of increasing technological sophistication. During the pre-commercial phase, extensive research was required to develop the basic technologies for gene identification, manipulation of desired traits, understanding of plant developmental regulation of genes and the stable re-introduction into competitive varieties. The progression of products will extend applications from single-gene modifications to multiple-gene introductions and developmentally or environmentally regulated gene expression. The level of participation of growers in the economic consequences of marketing of biotechnology products will be specific to the application. As in the mainstream of the produce industry, strategic alliances between agricultural biotechnology companies, large grower/shippers-processors, and seed companies is a rapidly developing trend. Vertical integration to control all components of the production and marketing system is viewed as the best approach to ensure quality, safety, proprietary protection, and profitability for both conventional and biotechnology-derived products.

Additional Sources of Information:

Biotechnology Information Center
National Agricultural Library
10301 Baltimore Blvd.
Beltsville, Md 20705-2351
Tel: (301) 504-5947 Fax : (301) 504-7098
E-mail :biotech@nalusda.gov

Biotechnology Industry Organization (BIO)
1625 K Street, NW Suite 1100
Washington, DC 20006-1604
Tel: (202) 857-0244
Fax: (202) 857-0237
E-mail: bio@iia.org

International Food Information Council Foundation
1100 Connecticut Ave. N.W., Suite 430
Washington, D.C. 20036
<http://ificinfo.health.org>

Other On-line Biotechnology Access Sites:

APHIS Biotechnology and Scientific Services
<http://www.aphis.usda.gov//bbep/bp/index.html>
National Biotechnology Impact Assessment Program
<http://nbiap.biochem.vt.edu>
Union of Concerned Scientists
<http://www.ucsusa.org>

Glossary

- Agarose gel** - a firm gel made from materials purified from seaweed. Used in the electric-field separation of pieces of isolated DNA.
- Agrobacterium tumefaciens** - a soil-borne bacterium that can naturally transfer genetic information into plant cells, thereby causing Crown Gall disease (see Ti plasmid.) This ability has been harnessed to transform (introduce desired sections of DNA into) many plant species.
- Allele** - one of several naturally existing forms of a gene or DNA sequence occurring at the same position (locus) on chromosome pairs.
- Allergen** - a protein that is capable of triggering an allergic reaction
- Amino acid** - the biochemical units from which all proteins are made. There are twenty different amino acids that occur most commonly in the proteins of all life forms.
- Antibiotic** - a substance that kills or prevents the growth of microorganisms; often produced by other microbes (bacteria or fungi).
- Antigen** - a substance (often a protein) which triggers the production of antibodies.
- Antisense (RNA)** - a molecule of mRNA which has been transcribed from the noncoding (antisense) strand of a DNA molecule. The antisense mRNA binds with the functional (sense) mRNA, effectively 'silencing' the gene and thus turning off the trait.
- Attenuated (relating to a pathogen or plasmid)** - a debilitated form of a disease-causing microorganism or its genetic machinery. Rendered unable to cause disease, the microbe or genetic element can be used for model studies, immunization, or transformation (see Ti-plasmid).
- Bacteriophage (or phage)** - a virus that infects only bacteria.
- Bacterium (plural bacteria)** - a simple organism consisting of one cell or short chains of cells, in which there is no nucleus. The chromosomal DNA is free within the cytoplasm as a single circular strand.
- Baculovirus** - a virus that infects only insects. Baculovirus tend to be highly specific and are limited in host range. These have been modified to inject protein toxins into their natural host insect.
- Base (as in nucleotide base)** - one of the chemical sub-units found in nucleic acid molecules which (in groups of three, or triplets) carry the code for particular amino acids. In DNA, the bases are adenine (A), thymine (T), cytosine (C) and guanine (G). Uracil (U) replaces thymine in RNA molecules.
- Base pair** - two bases on different strands of a nucleic acid which join together reversibly. In DNA, cytosine always pairs with guanine and adenine always links to thymine. (In RNA molecules, adenine joins to uracil.)
- Biolistics (in plant transformation)** - a patented process and apparatus that accelerates a particle (tungsten or gold microbeads) coated with purified DNA . The particles bombard a section of target plant tissue and the DNA is incorporated into the plant cells, either transiently to allow gene identification or function studies or in a stable form in the chromosome. Typically, biolistic transformation occurs at a low but economically satisfactory efficiency.
- Biotechnology** - In the broadest sense, the utilization of living organisms or their vital processes or components to provide useful products. This definition can include activities as diverse as wine, beer, or bread making, composting, release of parasitic wasps to control insect pests, and conventional plant or animal breeding. In the current modern usage, biotechnology is identified with techniques that collectively allow the precise identification, isolation, alteration, and re-introduction of heritable traits to living organisms for specific purposes.
- cDNA (complementary DNA)** - a DNA strand synthesized on an RNA template by the action of the enzyme Reverse Transcriptase. This reverses the usual process of RNA synthesis on a DNA template. Application of this technology allows the isolation an unknown gene using cDNA probes synthesized from a known amino acid sequence of the target protein.
- Chromosome** - the organized structure containing DNA that carries genetic information. Humans have 23 pairs of chromosomes in their body (somatic) cells, one of each pair from each parent. Corn (maize) has 20 pairs while tomato has 12.
- Clone (of plants)** - a plant propagated vegetatively, as by the use of buds or "eyes" of potatoes or shoot or root cuttings. The resulting plants are genetically identical to the parent plants.

Clone (of cells) - group of genetically identical cells arising from mitotic division.

Clone (of DNA, as to clone a gene) - to propagate and purify identical copies of a particular piece of DNA by enzymatic and biochemical techniques.

Clonal (propagation of plants) - to make identical copies of a particular plant by vegetative methods which include techniques known as tissue culture.

Codon - a group of three bases in a nucleic acid sequence which encode a particular amino acid. The three-base group may also act as a signal to stop or start gene translation (protein synthesis) or perform some other function of gene regulation.

Complementary (pairing of bases) - the pairing of specific bases in nucleic acid molecules between adenine and thymine (or uracil in RNA) and cytosine and guanine.

Crossing-over - a natural process occurring during meiosis (formation of pollen or sperm and egg cells) in sexually-reproducing organisms during which sections of similar (homologous) chromosome pairs are exchanged, often resulting in new traits.

DNA (deoxyribonucleic acid) - in most organisms, DNA carries the primary genetic information. DNA is a polymer consisting of long chains of nucleotides. Each nucleotide consists of a base linked to a sugar (deoxyribose) and a phosphate molecule.

DNA ligase - an enzyme that acts as a biochemical glue to join nucleotides in a DNA strand.

Diploid - a cell or organism with two complete sets of chromosomes; e.g. most human cells (except for the sex cells) have two sets - one from each parent.

Dominant - one of a pair of alleles that determines the phenotype of an individual, regardless of the nature of the other allele (see Recessive).

Double helix - the three-dimensional structure of DNA, in which the two opposing strands are arranged as a right-handed helix. The two strands are formed by complimentary pairing of nucleotide bases; there are normally about 10 base-pairs per turn of the helix.

Electrophoresis - a technique for separating different-sized fragments of DNA in an agarose gel based on their rate of movement in an electric field. DNA fragments generally carry a negative electrical charge and move towards the positive electrode when a current is applied. Large fragments move relatively slowly, whereas small pieces move more rapidly. The differing size classes are visualized in a gel with stains to reveal the banding pattern.

Electroporation (in plant transformation) - introduction of purified DNA into protoplasts by application of an electric field. The DNA is incorporated into the protoplasts, either transiently for gene identification or function studies or in a stable form in the chromosome.

Endonuclease - enzymes that are utilized as "molecular scissors" to cut nucleic acid molecules at specific sites along their length. Such enzymes are naturally produced by microorganisms as a defense against 'foreign' nucleic acids from invading viruses or bacteriophages.

Enzyme - a protein catalyst which speeds up a specific chemical reaction.

Eukaryote - an organism which has most of its genetic material in the form of chromosomes contained within the nucleus of a cell.

Exon - A functional genetic sequence that is expressed by a cell. Genes often contain additional sequences (introns) that are spliced out of the mRNA before the mRNA is used as the template for protein synthesis.

Expression - the manifestation of a particular characteristic specified by a gene (not all genetically expressed traits are manifested by the organism, being masked by other dominant genes). 'Expression' also refers to the production of proteins by a genetically-modified organism.

Fingerprint (of DNA) - a reproducible banding pattern created by a number of biochemical techniques of DNA isolation and/or amplification that clearly differentiate a genetic component in an individual, group, or population.

Gene - the basic unit of informational inheritance consisting of a sequence of DNA and generally occupying a specific position within the genome. Genes may be structural, which encode for particular proteins; regulatory, which control the expression of the other genes; or genes for transfer or ribosomal RNA.

Gene probe - a specific single-stranded DNA sequence used to detect a complimentary DNA or RNA sequence.

Genetic code - The groups of three nucleotide bases (codons) which specify a particular amino acid.

- Genetic engineering** -The production of new combinations of genetic material by the modification of DNA outside of a cell and the subsequent transfer of the DNA into an organism in which the specific sequence of nucleotides does not naturally occur.
- Genetic fingerprinting** - a set of techniques which enable genetic relationships of similarity, uniqueness, and evolutionary relatedness to be established.
- Genetic map (relating to chromosomes)** - an experimentally determined diagram which shows the relative positions of genes on chromosomes.
- Genome** - the complete (haploid) set of chromosomes carried by a sex cell.
- Genotype** - the full genetic composition of an organism.
- Germplasm** - genetically distinct variants of a species that can represent a valuable natural resource of plant diversity.
- Haploid** - half the usual number of chromosomes; e.g. sperm (or pollen) and egg cells are haploid.
- Heredity** - the transfer of genetic information from parents to their offspring.
- Heterozygote (n.); Heterozygous (adj.)** - a diploid organism or cell containing two different forms (alleles) of a particular gene or chromosome.
- Homologous (of chromosomes or DNA)** - chromosomes that are highly similar due to relationship by descent but not necessarily exact duplicates.
- Homozygote (n.); Homozygous (adj.)** - a diploid organism or cell with two identical forms (alleles) of a gene.
- Interfertile (for plants in nature or an agronomic breeding population)** - plants that are sexually compatible and capable of producing viable seed.
- Introgression (in plant breeding)** - the introduction of desirable traits from wild species by the lengthy process of repeated backcrossing and selection.
- Intron** - A stretch of DNA in a gene which is processed out of a mature mRNA and not expressed as a protein (see Exon).
- Isozyme** - differentially charged proteins that can be reproducibly separated and identified, generally using starch gel electrophoretic procedures. Commercial labs routinely perform this procedure for genetic purity testing on a number of crops.
- Ligase** - an enzyme used to biochemically “glue” double-stranded DNA molecules together.
- Linkage (as in linked genes)** - the tendency of pairs or groups of genes to be inherited together because they are physically close within a single chromosome.
- Locus** - a genetically defined site of a specific gene or DNA sequence on a chromosome.
- MAB** - see molecular accelerated breeding
- Marker (genetic)** - a distinguishing feature that can be used to identify a particular gene location on a chromosome. Markers may be morphological (e.g., a physical trait such as determinant growth habit, leaf form, color, flower shape) or biochemical (e.g., isozymes, enzymes or other proteins, DNA fragment size).
- Marker-assisted selection (in plant breeding)** - increased breeding selection efficiency achieved using DNA markers that allow earlier selection of positive traits and elimination of negative traits in a plant population. The breeder can potentially reduce the population size more rapidly in selecting for qualitative or quantitative traits.
- Meiosis** - the process of division of the nucleus of a cell during which the diploid chromosome number is reduced to the haploid and genetic segregation occurs.
- Micropropagation** - the mass production of clonal copies of a donor or parent plant by tissue culture techniques. The initial steps in micropropagation take place in synthetic solid or liquid growth media. Some processes can be conducted on a mass scale in culture tanks.
- Mitosis** - the process of division of the nucleus of a cell in which the chromosomes duplicate and divide to yield two identical nuclei. Nuclear division is usually followed by cell duplication.
- Molecular accelerated breeding (MAB)** - the use of genetic engineering techniques to speed the process of developing new varieties containing beneficial traits
- mRNA** - messenger RNA. A section of RNA transcribed from a DNA molecule that carries the code for the amino acid sequence of a protein.
- Mutation** - a random or directed change in the structure of DNA or the chromosomes. Often results in a visible or detectable trait alteration.

Nucleic acid - large biological polymers (DNA or RNA) composed of nucleotides.

Nucleotide - the 'building blocks' from which nucleic acids are composed. Each nucleotide consists of a base, a sugar and a phosphate group.

Nucleus - the membrane-bound structure within eukaryotic cells that contains the chromosomes.

PCR - see Polymerase chain reaction

Phenotype - an individual or combined set of characteristics of an organism that can be determined without genetic tests; generally the visible characteristics which result from interaction of both genetic and environmental effects, although it may also refer to a trait determined only by biochemical analysis or other analytical assay.

Plasmid - a small ring of DNA found in many bacteria and some yeast. Plasmids are able to replicate independently of the chromosome, and may pass from one cell to another. They are the principal agents used in genetic engineering for cloning and transformation.

Protoplasts (for tissue culture and transformation) - "naked cells" released following enzymatic treatment to remove the rigid walls of plant cells. These cells can be caused to fuse together, potentially resulting in recombination, or more typically made permeable in an electric field to facilitate DNA uptake for transformation.

Polygenic - controlled by or associated with more than one gene; generally confer complex traits such as flavor or solids content in tomatoes or most forms of pest resistance.

Polymerase (DNA or RNA) - an enzyme which catalyses the synthesis of nucleic acid molecules.

Polymerase chain reaction (PCR) - a laboratory process by which a specific DNA sequence is copied many millions of times in only a few hours using highly specialized (thermostable) polymerase enzymes.

Polymorphism - the presence of several forms of a genetic characteristic in an individual or population.

Primer - a short piece of single-stranded DNA that acts as the starting template for the synthesis of a complementary strand of DNA.

Protein - a molecule composed of a chain of many amino acids that acquires a particular folded shape due to the amino acid sequence. Both the sequence of the amino acids and the pattern of folding are involved in the specific function of the protein.

QTL (Quantitative Trait Loci; in plant breeding) - Multiple loci or genetic regions that affect the phenotype of an individual.

rDNA - see recombinant DNA

Recessive - a trait expressed in organisms that are homozygous for a particular gene, but not in those who are heterozygous for the gene.

Recombinant DNA (rDNA) - DNA formed external to a living cell by joining DNA from two or more different sources (a laboratory process not requiring reproducing organisms).

Regenerate (in plant tissue culture) - to vegetatively reproduce (clone) a whole plant from single cells or isolated plant tissues.

Replication (of DNA) - the synthesis of new a double-stranded DNA molecule complementary to an existing single strand.

Restriction enzyme - an enzyme that will cut DNA molecules only at sites where particular sequences of base pairs occur.

RNA (ribonucleic acid) - a biological polymer similar to DNA, but with the sugar ribose instead of deoxyribose in its structure, and with the base uracil in place of thymine. Various forms of RNA are found: mRNA (messenger RNA); tRNA (transfer RNA); and rRNA (ribosomal RNA). Most RNA molecules are single-stranded, although they can form double-stranded hydrogen-bonded segments.

Segregation (in plant genetics and breeding) - the distribution of inherited genetic traits from a breeding pair among the progeny of subsequent generations, according to the laws of genetics.

Sequence (relating to DNA or protein) - the precise order of bases in a nucleic acid or of amino acids in a protein polypeptide.

Somatic cells - "body" cells, or cells other than cells responsible for sexual inheritance.

Structural gene - a gene that codes for a protein, such as an enzyme.

Ti-plasmid (relating to plant transformation) - a plasmid, carried by the bacterium *Agrobacterium tumefaciens*, that naturally integrates into plant chromosomes where it triggers the growth of tumorous galls (Crown Gall disease of plants). 'Disarmed' versions of this plasmid, with its tumor-inducing ability removed, are used as agents of transformation to genetically modify many plant species.

Tissue culture (in plant biotechnology) - the process of regeneration of a plant from single cells, isolated embryos, or small bits of plant tissue on liquid or solid media. The media is supplemented with a customized balance of nutrients and plant hormones known to induce the formation of roots, shoots or both from disorganized plant tissue, called callus.

Trait - a phenotypic characteristic associated with the expression of a single gene.

Transcription - the synthesis of a strand of RNA by cellular enzymes using the sequence of bases present in a single strand of the DNA molecule as a template.

Transformation - a change in the genetic composition of a cell or organism brought about by the integration and expression of purified DNA.

Transgenic (adj.) - an organism containing genetic material from other species introduced via the process of transformation.

Translation - synthesis of protein directed by the DNA sequence information encoded in a mRNA molecule.

Transposon - a mobile piece of DNA that can insert at random into plasmid or chromosomal DNA (so-called "jumping gene").

Vector - a piece of DNA containing an introduced gene that is used to deliver the gene to another organism

Virus - a submicroscopic infectious agent that contains genetic material but must invade a cell in order to replicate itself.