Genetic Engineering of Flavor and Shelf Life in Fruits and Vegetables

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Twenty years ago, consumers only ate fruit that was "in season." Fruit was not shipped long distances and supply was dependent on local production areas. Demand for year-round, inexpensive produce has led to consolidation of production where temperatures are warm, sunshine is plentiful, and labor is cheap. In most cases, produce is now shipped long distances before being consumed. Tomatoes grown in Mexico are trucked 3,500 miles, bananas grown in Ecuador travel 6,000 miles on a boat, bell peppers from Holland greenhouses are flown 5,000 miles to market. Unfortunately, fruit was not designed to be shipped long distances. In order to make it to market, fruit is picked before it is ripe (and before it has any taste). Despite harvesting immature fruits, post-harvest losses of fruits and vegetables still exceed 25 percent of crop production and the fruit that makes it to market has often been described as tasteless.

Strategies for Flavor and Shelf-Life Improvement

Flavor can be addressed by increasing intensity (concentration) of an important chemical component of flavor. This can be accomplished by increasing production of a specific compound (e.g., sucrose), by shifting the balance of compounds (glucose versus sucrose), or by blocking production of undesirable compounds (such as starch). Genes have been isolated to explore increased sweetness. These include sucrose phosphate synthase (increased sucrose), invertase (interconversion of sugars), and ADPG-pyrophosphorylase (sugar to starch conversion).

Alternatively, flavor can be improved by delaying harvest until the fruit is ripe. Greater firmness or reduction in ethylene production can permit fruit to be retained on the vine until ripe, when it has achieved full flavor potential. Increased firmness through regulation to decrease fruit softening enzymes has met with limited success to date. Control of ethylene content appears to be more promising. Ethylene controls ripening and rotting in many fruits and vegetables. By reducing ethylene concentration in fruit, it is possible to arrest development of the fruit and permit the fruit to remain on the vine until ripe.

Case Study: Ethylene Regulated Tomatoes

Technology

Tomato fruits produce ethylene in high concentrations during ripening. There is a burst of ethylene production, called the climacteric, that occurs when the tomato fruit begins to turn from green to red (Grierson and Covey, 1988). The increased concentration of ethylene in tomato fruit is associated with color development, fruit softening, increased respiration, and sugar accumulation. Ethylene is a simple chemical. Its biosynthesis is understood well and the genes that are required for biosynthesis have been identified. In addition, genes have been identified that degrade intermediates in ethylene biosynthesis.

Ethylene formation is the result of a three-step biosynthetic pathway (Imaseki, 1991): Methionine is converted to S-adenosyl-L-methionine (SAM) by the enzyme methionine adenosyltransferase. SAM is converted to 1-amino-cyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACC-S). ACC is converted to ethylene by the enzyme ACC oxidase. Genes encoding the enzymes involved in biosynthesis (ACC synthase and ACC oxidase) have been identified, isolated, and cloned. It is possible to use techniques to block expression of those genes, thereby blocking or reducing biosynthesis of ethylene. Alternatively, enzymes have been identified in microorganisms or viruses that metabolize the intermediates in ethylene biosynthesis. These include ACC deaminase, an enzyme capable of degrading ACC, and SAM hydrolase, an enzyme capable of degrading SAM.

The process for developing genetically engineered plants with reduced concentrations of ethylene in the fruit is complicated and requires several technologies that have been developed over the past twenty years. These technologies include gene introduction into plants, selection of transformed cells, regeneration of plants from genetically transformed cells, and gene expression.

Commercial Opportunity

The principle commercial benefit of ethylene-regulated tomatoes is long shelf-life. Tomatoes blocked for ACC-synthase can survive in laboratory conditions for 90 days. At DNA Plant Technology Corp., we completed commercial tests on a hybrid tomato variety that was blocked for ACC-S using Transwitch™ technology. In commercial tests, these ethylene-regulated tomatoes have a 40 day shelf-life. This includes harvest, five to seven days ethylene treatment to complete color development, five to seven days to ship to market, five to seven days storage in the local market at a repacker, seven days in the supermarket, and 10 days with the consumer prior to consumption. This contrasts with the maximum of 20 days for current commercial tomato varieties.

The current commercial system works as follows (How, 1991):

1. Growers harvest a tomato field two or three times during a season. All tomatoes (unless red ripe) are harvested.
2. Tomatoes are brought from the field to a packing shed where they are sorted by size and color. Tomatoes with some red color are usually sold at a discount price in the local market because they are already beginning to soften and will not survive trucking over long distances. The 90+ percent green tomatoes are placed in 25 pound boxes and treated for one to three days with 100-150 ppm ethylene, then trucked to a repacker at a local market.
3. Upon receipt, the repacker sorts tomatoes for color. Tomatoes that developed color in transit are shipped to supermarkets (or food service outlets) immediately. The remaining tomatoes receive an additional two to seven days of ethylene treatment and then are shipped to market.

Extended shelf life provides a distinct advantage throughout the chain of commercialization.

Grower: The ACC-S regulated tomato reaches full size and flavor potential, begins to degrade chlorophyll, but does not turn red in the field. At this stage, the tomato can remain in the field for several weeks without rotting or softening. This attribute affords the grower two benefits. First, all the tomatoes are harvested, i.e., no over ripe tomatoes are left in the field. This increases field yield per acre. Second, harvest costs per pound are lower because fewer total harvests are required.

Packer: Over-ripe tomatoes are sold at a discount in local markets (Florida, California, and Mexico). Because all the ACC-S regulated tomatoes that are harvested can be shipped to remote markets, revenue per pound is increased. In addition, tomatoes left on the vine generally increase in size. Large tomatoes command an average premium price of four to six cents per pound.

Repacker: When tomatoes arrive at a repacker they are sorted based on stage of ripeness. Losses at the repacker range from six to ten percent of tomatoes shipped from production areas. These are discarded tomatoes that have rotted in route or are showing signs of impending deterioration. Ethylene-regulated tomatoes are superior, with decreased losses.

Retailer: Because current tomatoes rot in two to three days at retail, orders and shipments are made daily by most large supermarkets. Loss due to rotting can reach 20 percent of product at the retail store level. Both issues are addressed by a long shelf-life tomato. Moreover, better tasting tomatoes can be sold at a premium price, resulting in increased revenue and profit per square foot in the produce section of the supermarket.

Consumers: Because ethylene-regulated tomatoes are left on the vine until they reach full maturity, the consumer purchases a better tasting tomato. Moreover, there should be a significant reduction in the number of rotten tomatoes in consumer homes.

The commercial potential of a better tasting, longer shelf-life tomato that is sold at a premium price can be quantified as follows:

per 100 lbs. harvested	standard	ethylene controlled
Revenue	$20.00	$60.00
Costs	$16.00	$38.00
Margin	$ 4.00	$22.00
Margin %	20%	37%

Intellectual Property

The technology that is required to make an ethylene-regulated tomato is the subject of several patents and represents a mine field that must be successfully navigated prior to introducing a product to market. The present intellectual property situation is as follows:

Germplasm: Nearly all commercial acreage of fresh market tomatoes is grown using proprietary seed developed by a seed company. The open pollinated, publicly available varieties (e.g., Rutgers, Floradade) cannot compete with current hybrid varieties in yield and disease resistance, hence genetically engineered open pollinated varieties are not commercially viable. As proprietary seed is controlled by seed companies, relationships must be negotiated with seed companies to obtain access to parental seed for genetic engineering. The largest tomato seed companies are Seminis, Ferry Morse, and Sun Seeds. Other companies with breeding capability have developed proprietary tomato germplasm using breeding or cellular genetics (Morrison and Evans, 1996). In addition to hybrids and the protection inherent in segregation of hybrid seeds, most companies also seek Plant Variety Protection Certificates on one or both of the hybrid's parents. Those layers of protection, along with seed production capability, assure seed company involvement in commercialization of genetically engineered tomatoes.

Gene Introduction: There are two methods available for gene introduction in tomatoes. *Agrobacterium tumefaciens*-mediated gene transfer is the preferred method. *Agrobacterium* plasmids are modified to incorporate desired promoters and genes and the natural infection behavior of the bacterium is used to deliver the DNA into plant cells. Broad claims have not been issued on this technology. However, several components of this technology, such as the use of binary vectors, are the subject of patents. A secondary gene introduction method is ballistics. Ballistics uses some method to shoot DNA into plant cells. Variations on the type of microprojectile and the acceleration device have been optimized during experimentation for different crop plants and likely would need to be optimized for tomatoes. DuPont Corp. has claims in a U.S. patent that are directed to the ballistics technology. Other patents are issued or pending on various modifications of the ballistics approach for specific plant species.

Plant regeneration: Regeneration using standard tissue culture methods is routine in tomato. The method using leaf pieces of tomatoes as explant material has been used in several laboratories since the early 1980's. No broad patents cover the commonly used techniques for tomato regeneration.

Promoters: A wide range of promoters have been identified that will express DNA in plants. Some of these, including 35S, derived from cauliflower mosaic virus, are the subject of issued or pending patents (Fraley, et al., 1994). Much effort has been directed to isolation of tissue-specific promoters. Fruit-specific promoters in tomato would be ideal to ensure that ethylene regulation is directed to the tomato fruit and to ensure that ethylene production (involved in disease resistance) is not blocked in leaf and other tissue.

Selectable markers: Markers are foreign genes that are inserted into target plant cells at the same time as a segment of DNA that can modify ethylene. Because ethylene regulation cannot be selected in a test tube, the selectable marker permits transformed cells to proliferate while non-transformed cells die. Resistance to kanamycin (conferred by the nptII gene) is a preferred selectable marker that has received both EPA and FDA approval for use in tomatoes and other crop plants. Kanamycin resistance is the subject of an issued patent in the United States and a pending patent in Europe (Rogers and Fraley, 1991).

Genes: Segments of DNA can be patented in the United States. For ethylene, patents are issued or pending on each gene that has been identified in ethylene biosynthesis or degradation. For example, the USDA has a pending patent on the ACC-S gene that we have used. The gene isolation work was completed at the USDA in Albany, California (Oeller, et al., 1991). The USDA has licensed the gene to multiple companies within different fields to ensure broad commercialization of the government funded technology. Other genes have been isolated including ACC-oxidase (Hamilton, et al. 1990), ACC-deaminase (Klee, 1994), and SAM hydrolase (Good, et al., 1993).

Gene expression: In order to block ethylene biosynthesis using ACC-synthase or ACC-oxidase, the endogenous genes must be regulated to decrease their expression. Two methods have been used in tomatoes: Transwitch® and antisense. Transwitch® is a unique phenomenon in which some plants resulting from transformation with a homologous gene are suppressed for the target gene (Jorgensen, 1995). Antisense achieves a similar result, i.e., gene suppression, by inserting a gene sequence that is complementary to the target gene (Shewmaker, et al., 1992). Each technique is the subject of issued patents in the United States and Europe.

Commercialization Implications

Despite the demonstrated value of ethylene-regulated tomatoes, no one is presently in the market with such a product. This product has extremely high barriers to entry due to the intellectual property situation. The companies closest to market are DNA Plant Technology, Monsanto/Calgene, and Zeneca Plant Sciences. It is likely that each party will need licenses to intellectual property from other companies in order to successfully commercialize an ethylene-regulated tomato. While the current patent situation presents an additional commercialization hurdle to the three companies, it also tends to eliminate the prospect of future new entrants developing and marketing an ethylene-regulated tomato.

Research and Planning Implications

Several general lessons have been learned from attempts to commercialize ethylene-regulated tomatoes that apply to all commercial projects using plant genetic engineering.

1. Many patents are still pending. Work initiated today using technology developed by others may never be commercialized in the absence of licenses. Many of the basic agricultural biotechnology patents have not been resolved, with broad patents still possible or likely for gene introduction and gene regulation technology.
2. In commercial or academic genetic engineering research, genes need to be inserted into the best existing germplasm. This affords an appropriate benchmark to value the technology and eases adoption of new varieties. Seed company and technology-provider alliances involving universities and research laboratories are more likely in the future.
3. The earliest product developed is not always the first to be commercialized. The complex business system of fresh market tomatoes and the complex patent situation have delayed commercialization. If a new genetically engineered variety requires modification of growing or handling practices, adoption is slow.

4. In a complex business system a novel trait may have multiple benefits. The ethylene-regulated tomato provides benefits to growers, packers, repackers, retailers, and consumers while originally targeted to increase shelf-life.

SUMMARY

The initial hurdles for commercialization of products of plant genetic engineering included technical, regulatory, and consumer acceptance. As the first products are now ready for market introduction, it is obvious that an additional major hurdle exists: intellectual property. Patents have been granted that cover a number of basic biotechnology methods and the use of specific genes for genetic engineering. For some technologies and genes, patents already have been issued and represent barriers to commercialization. For other technologies, the patents are still pending and represent an uncertainty. Indications from the first products being commercialized in cotton, corn, and soybean suggest that patents are being aggressively enforced and are being used to establish competitive advantage in the marketplace. Clearly, if broad patents are upheld by the patent office and the courts, alliances and cross licenses will be necessary for commercialization.

The modification of consumer-preferences traits could have implications for several, perhaps unintended, steps in the chain of commercialization. For example, ethylene regulation in tomato will primarily result in a tomato with longer shelf-life. However, the tomato will not be red when it reaches full maturity, can stay in the field for a longer period of time prior to harvest, requires post-harvest ethylene treatment, and results in delivery of a better tasting tomato to consumers. These features of the ethylene-regulated tomato require modification of harvest practices and modification of handling and packing procedures. Moreover, production trials and operation tests may suggest modification of pesticide practices, staking, and handling in the field; sorting, ethylene treatment, and shipping from the field; as well as packaging and pricing for the retailer. Ultimately, successful commercialization of new consumer-preference traits in fruits and vegetables requires more than simple insertion of a gene into a plant.

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