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Commercialized Biotechnology, Food for Thought: Introduction to the Colloquium

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In the 1970s, the study of molecular biology charted a course leading to genetic engineering of plants in 1983 with the Ti plasmid of the soil bacterium *Agrobacterium tumefaciens*. This bacterium allowed the transfer of foreign DNA into the plant genome, using an antibiotic resistance marker for selection of transformed cells. After many technological breakthroughs, scientists were able to engineer resistance to plant viruses and insects due to expression of a variety of factors, including viral coat proteins and *Bacillus thuringiensis* (Bt) protein, tolerance to herbicides, control of fruit ripening and softening in tomatoes due to downregulation of cell wall enzymes or ethylene pathway enzymes, engineered male sterility and its restoration, modified carbohydrate composition, and altered oil composition. Plants resulting from this research have been commercialized to some extent (Wilkinson, 1997).

Some interesting statistics indicate that the United States leads the world in approved biotech products, followed by Canada and Japan. Biotech acreage reached 50 million in the United States, including 40% of cotton, 30% of soybeans, and 20% of corn (Buckner, 1999). Worldwide, 73 million acres are producing biotech crops as of 1999. This is important because biotechnology becomes a factor in the

projected global population increase and subsequent food shortage by virtue of anticipated increased yields. It could reduce the need to expand agriculture into marginal agricultural and environmentally sensitive areas, such as wetlands, forests, or rain forests, to produce crops, since the best agricultural lands around the world are already in production (Buckner, 1999).

Unfortunately, despite a tripling of food production since 1950, much of it accomplished with the aid of numerous scientific advances, 800 million people are still undernourished in the world, and billions are malnourished. Most of this poor nutrition is in Asia and Africa, yet by 2050 it is predicted that we will need three times the current food supply for high-quality diets, or one-third as much just to stave off starvation. Curiously, in the global community it is the well fed who are rejecting biotechnology (Avery, 2000). Europeans and, to a lesser extent, Americans, view GMO foods as menacing or dangerous based on scientifically inaccurate assumptions, or object on moral or ethical grounds because they perceive biotechnology to be challenging the natural order (Anonymous, 1999).

However, this is not the first time that a scientific advance relating to the food supply has been met with suspicion by consumers. Canned food, pasteurized milk, artificial insemination of farm animals, hormones in animal feed, microwave cooking, and irradiation of foods are some examples. The anticipated benefits of recombinant DNA tech-

nology by the food industry and much of the scientific community include a more abundant and economical food supply, improvements in nutritional quality, improved shelf life for fresh fruits and vegetables, foods with reduced allergenicity, development of functional foods and vaccines, conversion of nonproductive toxic soils to productive arable land, and more environmentally friendly agricultural practices [Institute of Food Technologists (IFT), 2000a].

Some examples of the above include a biotech rice carrying a gene from corn that imparts a higher rate of photosynthesis, resulting in a 35% yield increase; transgenic acid-tolerant crops developed in Mexico and predicted to increase crop yields by 50% in the tropics, thus saving some rain forests; and a golden rice that was engineered to contain beta-carotene (precursor to vitamin A), which is predicted to alleviate vitamin A deficiency and related blindness in Asia (Avery, 2000).

In 1992, the FDA provided a general outline for the safety assessment of recombinant DNA biotechnology-derived food products based on risk analysis related to the characteristics of the products. In fact, the safety assessment of foods derived from biotechnology has been more stringent than for conventionally derived products, yet concerns still exist (IFT, 2000b). One concern is allergenicity. Naturally occurring proteins found in commonly allergenic foods such as peanuts, milk, and seafood can cause gastrointestinal or cutaneous symptoms or even more serious anaphylactic shock reactions. All food allergens are proteins and since genetic modifications involve the introduction of new genes into plants, hence the production of new proteins, potential new allergenicity is possible. Thus, recombinant DNA-derived foods are assessed for allergenicity, but none has been found to date. There is also concern about introduced genetic material, such as the marker genes that confer resistance to antibiotics. Studies showed that the product of the marker gene used (neomycin phospho-

transferase, type II gene, NPTII) is rapidly degraded like other dietary proteins during mammalian digestion, is not toxic, and is not likely to transfer antibiotic resistance and/or reduced efficacy of antibiotics (IFT, 2000b).

This colloquium explores the use of biotechnology as it relates to harvested horticultural crops in terms of shelf life, nutrition, decay, abscission, and flavor. We have had examples like the Flavr Savr™ tomato, which was specifically engineered to reduce softening and thus extend shelf life. Although this product was not as successful as anticipated, it set an example and other genetically engineered fruits and vegetables will surely follow. New technologies are often potentially hazardous and promising at the same time. Good examples are nuclear power and agrochemicals. The benefits of these new technologies outweigh the risks, for both are used around the world. And so it is with molecular biology. For better or worse, this technology is not likely to go away, so we had best use it wisely.

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Biochemical and Genetic Determinants of Cell Wall Disassembly in Ripening Fruit: A General Model

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Fruit ripening is accompanied by textural changes that are derived, at least in part, from structural changes in the cell wall. These textural changes collectively contribute to fruit softening, to increased pathogen susceptibility, and ultimately to deterioration of fruit tissue. Blocking ethylene production or perception has been shown to maintain fruit firmness and to greatly extend the shelf life of fruit (Klee, 1993; Murray et al., 1993; Picton et al., 1993). However, ethylene control also retards ripening-associated biochemical processes that contribute to the ultimate quality of the fruit. Thus, there is a continuing need to dissect the molecular mechanisms that underlie fruit softening so that these processes can be regulated independently of other aspects of fruit ripening.

Structural models of the plant cell wall remain controversial but can be viewed in the simplest form as a core structure of cellulose microfibrils embedded in two coextensive networks of pectin and hemicellulose (Carpita and Gibeau, 1993). Recent cell wall models emphasize the degree of non-covalent associations between polymers, such as the extensive hydrogen bonding between cellulose and xyloglucan that occurs in dicot primary cell walls. This cell wall matrix provides structural support to the plant body but also must respond dynamically to developmental processes that require changes in cell shape and, in some cases, cell separation (Rose and Bennett, 1999). Thus, highly regulated disassembly of cell wall components is a critical component of plant development, and one of its most dramatic

manifestations is in ripening fruit. In most cases, cellulose microfibrils are not disassembled during normal plant developmental processes, including fruit ripening. However, there is extensive evidence that both of the major matrix polymers, pectins and hemicellulose, are extensively disassembled during fruit ripening (Crookes and Grierson, 1983; MacLachlan and Brady, 1994; McCollum et al., 1989; Sakurai and Nevins, 1993). This paper reviews recent data, which suggests a general model for ripening-associated cell wall disassembly that provides experimental guidance to regulate fruit softening as a strategy to improve fruit quality.

RESOLUTION OF TWO SEQUENTIAL STAGES OF CELL WALL DISASSEMBLY

It has been recognized that fruit softening and the underlying cell wall structural changes must be complex (Fischer and Bennett, 1991). The complex patterns of cell wall disassembly may reflect cooperative biochemical actions that collectively contribute to the overall textural changes that occur in ripening fruit. Alternatively, these complex patterns of cell wall disassembly may reflect relatively distinct events that occur sequentially with each event contributing to separate and distinct aspects of fruit softening. Experiments attempting to resolve temporal events in cell wall disassembly were carried out in ripening 'Charentais' melons to assess the possibility that cell wall disassembly could be resolved into distinct sequential events (Rose et al., 1998). These experiments took advantage of the rapid ripening of climacteric 'Charentais' melon and the ability to sample fruit at specific points in

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