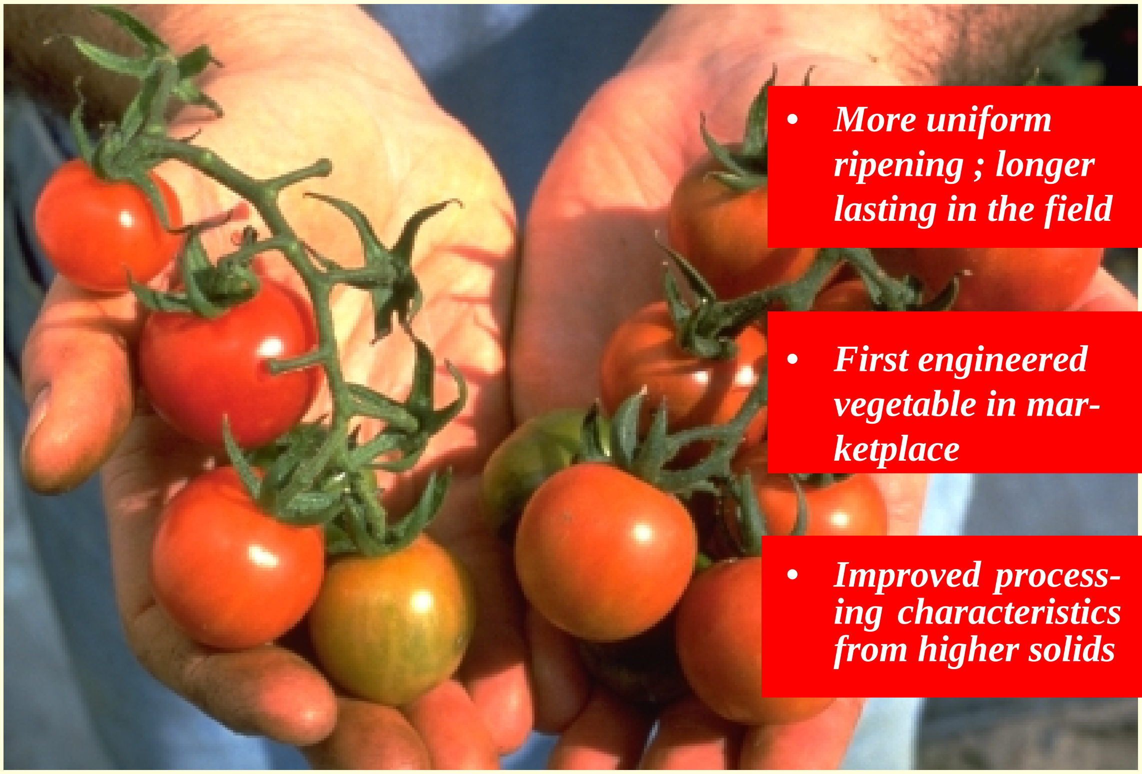
**A·B·C**

Information Series
August 1996

# AGRICULTURAL BIOTECHNOLOGY IN CALIFORNIA

# TOMATO

Tomatoes are economically important in California with over 8 million tons of processed tomatoes and 500,000 tons of fresh tomatoes being produced in 1993. Research into the development and ripening of tomato fruits has been carried out for many decades in order to learn what makes them sweet, how they ripen and what gives rise to the solid part of the tomato. These basic studies are now the foundation on which we can manipulate these traits through genetic engineering. The first genetically engineered fruits to achieve commercialization were marketed as improved-taste fresh tomatoes; this was accomplished by stopping or slowing the ripening process, allowing fruits to be picked later when they are more flavorful **(see Tomato Biotechnology)**. More recent entries in the marketplace are higher solids tomatoes, leading to better processing characteristics, and slower and more uniform ripening cherry tomatoes. These varieties and others in the research pipeline will provide farmers and processors with new ways to combat old problems.




## California's #1 in Processed Tomatoes

**MAJOR BIOTECHNOLOGY ACHIEVEMENTS**
- **Improved processing**
- **Enhanced flavor**
- **Controlled ripening**
- **Virus resistance**

**UC RESEARCH HIGHLIGHTS**
- **Sugar metabolism**
- **Pigment biosynthesis**
- **Resistance genes isolated**

**ABC's OF BIOTECHNOLOGY**
- **Make sense of antisense!**
- **What is a breaker fruit?**

# Tomato Biotechnology

What is the ideal tomato? The consumer of fresh tomatoes might say uniformly red, juicy, sweet, aromatic, slightly tart and acidic and stays at the peak of ripeness for weeks without spoiling. The processor might tell you it processes easily, lasts long periods without spoiling, is transportable over long distances without loss, and produces maximal yields of the desired product. Farmers want tomatoes that resist disease and insects, germinate uniformly, grow fast, give maximal yields under optimal and adverse conditions and is easily harvested. The truly ideal tomato, then, is all of the above and probably more.

Classical breeders have been working for decades to introduce desirable characteristics into commercial varieties. Understanding the genetics of the commercial tomato varieties and being able to interbreed cultivated varieties and outcross with wild relatives, which often have improved or novel characteristics, has led to substantial gains in growth, fruit quality, yields and disease resistance. Classical approaches, however, have the disadvantages of sometimes not being precise and often requiring many years to remove undesirable characteristics while maintaining the desirable ones from both varieties. Much research has been done on the basic processes of fruit development and ripening. Genetic engineering strategies can be used to augment classical approaches. Coupling information on the "molecular players", the genes and processes, that control fruit ripening and mediate changes in color, flavor and softening with the ability to manipulate this information by genetic engineering will allow the creation of superior varieties for both the fresh and processed tomato market.

## FRUIT SOFTENING AND FLAVOR

Polygalacturonase or PG is an enzyme that degrades pectin in fruit cell walls and, together with other enzymes, causes the softening of fruits during ripening. Fruit with decreased levels of PG activity: 1) do not get overly soft when ripe, 2) show less damage due to fungal infection and 3) have elevated levels of soluble solids. The Calgene (Davis, CA) FlavrSavr™ tomato was commercialized in the U.S. market in 1995, marketed as a more flavorful fresh tomato. This tomato was modified by turning off PG synthesis using antisense technology **(see ABC's of Biotechnology)** so that the tomatoes could be picked later, leading to better flavor and longer survival. Zeneca (U.K.), in collaboration with Peto Seeds (Woodland, CA) and Hunt Wesson (Fullerton, CA), created a similar variety, which is being marketed in England (see photo, this page) in processed tomato paste.

## FRUIT RIPENING AND FLAVOR

Growers of tomatoes are concerned with fruit ripening since this affects harvesting and ultimately determines profits. A fundamental breakthrough in understanding ripening occurred in 1989 when a UCB/USDA scientist **(see UC Research Highlights)** isolated a gene involved in the synthesis of ethylene, a natural, volatile compound synthesized at a certain point in fruit development, which leads to final ripening **(see ABC's of Biotechnology)**. Ethylene is used to treat fresh market tomatoes to give them their red color. In the genetically engineered varieties, ethylene production is blocked, so tomatoes can be picked later, at a more flavorful stage, and do not over-ripen and rot. Once picked, fruits can be ripened when commercially appropriate by simple exposure to external ethylene. One version of this strategy was used by DNA Plant Technology (Oakland CA) to produce its Endless Summer variety tomato; the strategy is also being used to control ripening in melon.

Fruit development and ripening is not a synchronous process and leads to difficulties in harvesting. To improve the uniformity of ripening both within a plant and across a field, a strategy to slow down ripening was developed in the cherry tomato. This strategy also involves manipulating ethylene but, instead of stopping it completely as described above, in this strategy production is slowed down. Using this strategy, Agritope (Beaverton, OR) has engineered and field-tested more uniformly ripening cherry tomatoes which can be picked less frequently and have more uniform color (see photo, front cover). This technology can also be used to bring more uniform ripening properties to processing tomatoes.

## VIRAL RESISTANCE

There are a number of significant viral diseases of California's commercial tomato varieties. One causing significant yield losses is cucumber mosaic virus (CMV). Several genetic engineering strategies are available to protect crops from viruses; however, the one most widely used is the coat protein, or CP, strategy. In CP-mediated resistance, the gene responsible for the "outer protective wrapper" of the virus is introduced into plant cells. The presence in plant cells of this viral gene

prevents the virus from efficiently making copies of itself thus reducing damage and yield loss. This approach has been used to create Asgrow's commercial viral-resistant yellow crookneck squash variety. Tomatoes also have been engineered with the coat protein gene from CMV (see photo, this page) and are currently being field-tested. One difficulty with the CP approach is that it is often not broad-spectrum protection and is specific for a particular strain of virus. Other strategies to protect plants against viruses are being examined that may lead to broader spectrum protection. These approaches include ones based on natural resistance genes that may protect not only against viral diseases but possibly against other pests and diseases also **(see Disease Resistance Genes: How Plants Fight Back)**.


# Disease Resistance Genes: How Plants Fight Back

**Concepts and Definitions:** Disease resistance genes form the basis of a natural genetic defense system in which a specific plant gene interacts with a specific gene from the disease-causing organism in what is called a "gene-for-gene recognition system". It is somewhat analogous to a lock and key mechanism in which the one component must fit precisely with the other component if it is to work completely and effectively. ***Disease resistance genes*** are called plant **R** or resistance **genes** which can recognize a complementary or "matching" gene in the invading pathogen. ***Avirulence genes*** are the genes in the disease-causing organism (fungal, viral, bacterial, nematode) with which the disease resistance genes "recognize" and with which they interact. When a plant with a disease resistance gene is invaded by a pathogen with an appropriate avirulence gene, then the plant undergoes what is termed a "hypersensitive response". This ***hypersensitive response*** or ***HR*** is the mechanism by which the plant defends itself from the invading plant pathogen and includes a type of "suicide strategy" whereby the invaded plant cell dies before the invading pathogen can reproduce and spread to other parts of the plant. This suicidal strategy results in small areas of cell death on the plant but an overall benefit to the plant in limiting the infection. A resistant plant reaction, a so-called ***incompatible*** plant-pathogen interaction occurs when a pathogen is recognized and the HR is engaged. In contrast, a ***compatible*** or susceptible plant-pathogen interaction is one where no recognition of the pathogen by the host occurs, the HR does not occur and the pathogen spreads throughout the plant causing damage.

**Summary:** Breeders have introgressed natural disease resistance genes into crop plants from wild relatives in order to protect them against specific pathogens. Now UC scientists (see **UC Research Highlights**) have isolated R genes for viral, bacterial and aphid resistance and are characterizing the genes to understand their precise function. Coupling an understanding of the genes with the ability to reintroduce them via genetic engineering will open the door to creating new strategies for pest resistance in plants.

## University of California Research Highlights

**Barbara Baker of UCB & USDA-ARS Plant Gene Expression Center** isolated a natural viral resistance gene from tobacco which in tomato protects it from viral attack.

**Alan Bennett of UCD** studies postharvest characteristics of tomato including the mechanisms that affect fruit softening and sugar metabolism.

**Wilhelm Gruissem of UCB** is examining the growth and regulation of lipid and lycopene (fruit pigment) synthesis in tomato fruits.

**Charles Rick of UCD** is a world expert in tomato breeding; his repository of tomato germplasm will be important for future engineering work.

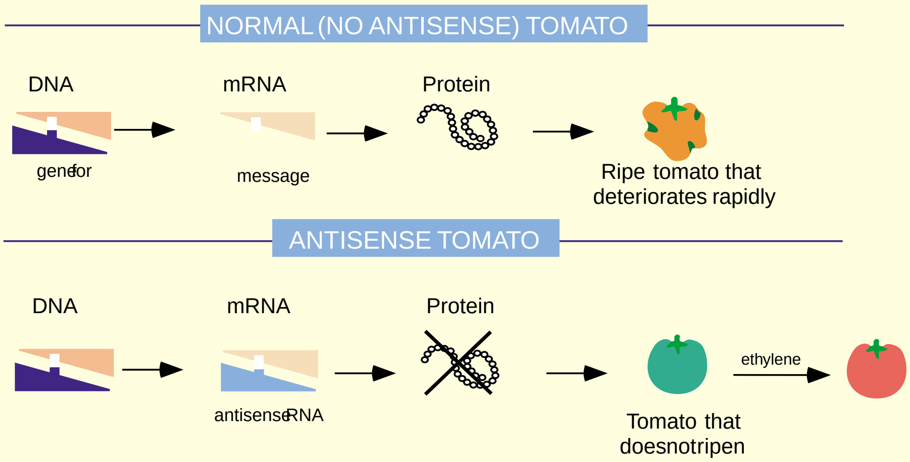
**Athanasios Theologis of UCB & USDA-ARS Plant Gene Expresssion Center** studies tomato ripening; his work led to the development of the *Endless Summer* fresh market variety with enhanced flavor characteristics.

**Valerie Williamson of UCD** wants to understand how natural nematode-resistance genes help protect tomato from attack; she has already isolated a resistance gene for aphids.

# *ABC's* OF BIOTECHNOLOGY

## Q: How Does Antisense Work?

**A:**

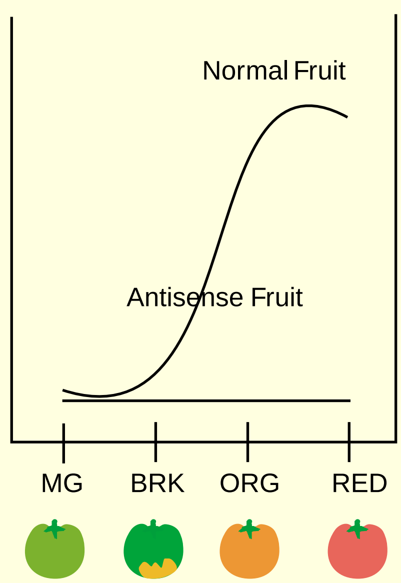



Genes exist as double-stranded complementary DNA molecules, one strand of which is used to produce the messenger RNA (mRNA) that yields the gene product (for example, for ethylene production). In the anti-sense approach, a gene is inserted so that the precise complement of the coding RNA is made inside the same plant cell as the coding strand. When this occurs, the two stands join to form a double-stranded structure that prevents the synthesis of the coding mRNA and ethylene production cannot occur. Other RNAs that are not precisely complements do not block the ethylene production.

## Q: What is a Breaker Fruit?

**A:**

A **breaker tomato fruit** is one in which ethylene biosynthesis has begun and the ripening process has initiated. Ethylene production initiates a cascade of processes in the tomato fruit including pigments and flavor characteristics.




*Selected Information on Critical Issues: A Starting Point*

**Antisense Technology In The Field-**
Vanderpan, Steve (1994) A Look Inside a New Tomato. Science of Food, Agriculture, and Environment, pp. 2-4.

**Virus Resistance-**
Proceedings of the Transgenic Virus-Resistant Plants and New Plant Viruses, National Biological Impact Assessement Program; available on-line at http://www.aibs.org

**Biotechnology Issues Specific to California-**
Genetically Engineered Fruits and Vegetables, Iowa State University Public Education Program in Biotechnology; available on-line at http://biotech.zool.iastate.edu/Biotech_Public_Ed.html


***Acknowledgements***

Trevor Suslow, Ron Voss, Kent Bradford, Ann Lorraine for critical discussions. Cover Photo courtesy of Agritope Corp., Beaverton, OR; Virus Resistant Tomato photo courtesy of Monsanto Corp., St. Louis, MO; Tomato Paste Can photo courtesy of Zeneca.
Agricultural Biotechnology in California Created by Gary Peter, PhD., Barbara Alonso, Peggy G. Lemaux, PhD.



*For further information contact:* Peggy G. Lemaux Associate Cooperative Extension Specialist Department of Plant Biology, 111 Koshland Hall, University of California, Berkeley CA 94720, Fax: (510) 642-7356
Email:lemauxpg@nature.berkeley.edu