TOMATO
BIOTECHNOLOGY
Tomato Biotechnology…..

Why tomato was targeted as one of the first crops to be altered by Biotechnology?

How fruit ripening Tomato is regulated?

Approaches to altering fruit ripening by modifying ethylene biosynthesis
Tomato Biotechnology…

Tomato was an early target for genetic modification and “improvement” for a number of reasons

• Tomato is a member of Solanaceae family
  – same family as tobacco
  – many members of this family, including tomato, are easy to transform

• high value crop
Tomato Biotechnology…

More reasons for tomato biotechnology

• Several characteristics that could be improved

• fresh tomatoes for market
  – quality, taste, shelf life, seasonal availability

• Tomato processing

• product yield, reduced processing costs, improved quality
Improvement of Tomato

Tomato has been a model system for many studies in plant genetics
Lot of interesting mutants
Among these are mutations that affect ripening of fruit

rin - ripening inhibited
Nr - never ripen
nor - non-ripening
Modification of fruit ripening

Fruit ripening has been one of the primary targets for modification because

- Ripening impacts handling, shelf life, quality of fresh tomatoes
- Ripening affects processing properties
- Tomato fruit ripening is a useful model for ripening of some other fruits
- Methods developed to alter tomato ripening may be applied to other fruits
Ripening and fruit development

The penultimate stage in fruit development

Flowering

Fertilization

Fruit development and growth

Fruit ripening

Senescence
Fruit development

- Fertilization is followed by cell division and cell expansion
- It takes 40 to 50 days for the fruit to reach its maximum mature green size
- It switches from growth to ripening
- Several dramatic changes occur that are collectively referred to as ripening
Tomato fruit ripening

The following occurs:

- Gaseous hormone ethylene is produced
- Increased respiration
- Synthesis of red pigments (lycopene)
- Softening of the fruit
- Conversion of starches to sugars
- Development of flavors
Fruit ripening

• All of these processes are highly regulated; ripening is not just a random deterioration of the fruit
• Ethylene plays a central role in ripening of tomato fruits
  – and in ripening of many other fruit, e.g. banana, peach, melon
  – and in other developmental processes, e.g. flower senescence
Ethylene

Synthesis of ethylene starts with the amino acid methionine. Methionine is converted to S-adenosyl methionine (SAM). SAM leads to the formation of aminocyclopropane-1-carboxylic acid (ACC). Finally, ACC is converted to ethylene.
Ethylene and Fruit ripening

SAM → ACC synthase → ACC → ACC oxidase → Ethylene

The expressed proteins cause fruit to ripen

“ripening” genes encode proteins associated with lycopene production, softening of fruit, conversion of starch to sugar

Ethylene acts as hormone

Induces the expression of many “ripening” genes
Strategies to modify ripening

How can the over-worked and under-appreciated biotechnologist use this information to change fruit ripening?

Two approaches

Reducing the synthesis of ethylene in tomato fruits

Reducing or altering the effects of ethylene
Reducing ethylene synthesis....

SAM $\xrightarrow{\text{ACC synthase}}$ ACC $\xrightarrow{\text{ACC oxidase}}$ Ethylene

Three methods
Metabolizing SAM to something other than ACC
Metabolism of ACC so it cannot be converted to ethylene
Inactivating the genes for ethylene biosynthesis
Reducing ethylene’s effects….

Ethylene \[\rightarrow\] Fruit ripening

Three methods

1. Identifying mutants with reduced responses to ethylene (conventional)
2. Inactivating genes that cause specific ripening processes (e.g. softening) to occur
3. Making plants or specific tissues (e.g. fruits) unresponsive to ethylene
Reducing ethylene synthesis….  

SAM decarboxylase metabolizes SAM and prevents the formation of ACC  
Without ACC, no ethylene will be produced, ripening will be altered
Reducing ethylene synthesis….

Gene for SAM decarboxylase cloned from a virus that infects bacteria, T3 bacteriophage

Open reading frame of this gene was used to produce a chimeric gene

What sort of promoter should be used to drive the expression of this gene?

should be expressed in fruit when they are just starting to ripen (late mature green stage)
or a promoter from a gene that is activated in response to ethylene
Reducing ethylene synthesis

Chimeric SAM decarboxylase gene transferred into tomato by *Agrobacterium*-mediated transformation

Reduced or eliminated expression of ethylene in tomato fruits

Developed by Agritope (now part of Exelixis, a CA biotech company)

At one time, they were also applying this technique to raspberry and melon
Reducing ethylene synthesis....

Approach used by Monsanto

ACC deaminase gene identified in a bacterium, *Pseudomonas chlororaphis*

Chimeric gene transferred into plants

Reduced ethylene synthesis and delayed fruit ripening
Reducing ethylene synthesis by metabolic interference

Both methods prevent ethylene from being synthesized by interfering with the metabolic pathway

Diverting substrates away from ethylene