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Fruits: A Developmental Perspective

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ONTOGENIC RELATIONSHIPS BETWEEN FRUİT AND LEAF

- Most fruits develop from a gynoecium that contains one or more carpels.
- Fruit development would be defined as the differentiation of a preexisting organ.
- In pseudocarpic fruit, organs other than the gynoecium (e.g., receptacle bracts, the floral tube, or the enlarged axis of the infloresence) participate in the formation of the fruit

It appears that the size (i.e., the number of cells) of the internal layer (L3) in the shoot apical meristem determines the floral meristem size and carpel number in tomato (and other fruits).

Size of the floral meristem during carpel initiation and final carpel number are determined by the genotype of L3 but not L1 or L2.

The bulk of the tissue in the pericarp appears to be derived from cells in L3, whereas L1 and L2 contribute to the outer and inner epidermal cell layer and to a layer of small cells immediately adjacent to the epidermal cell layers, respectively.

The ontogenic relationship between fruits and leaves is evident from the cytological appearance of the cells in a cross-section of the carpel and fruit pericarp.

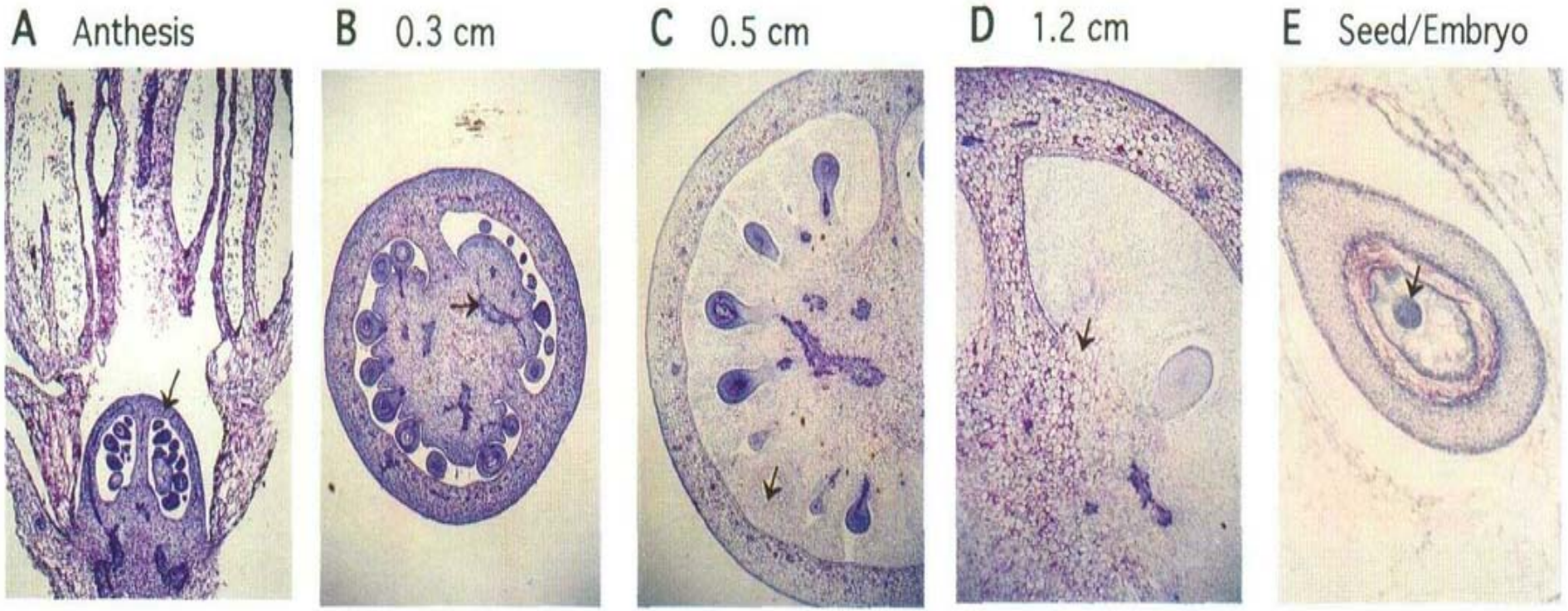

Figure 1. Tomato Fruit Development.

Tissue from VFNT cherry tomato was fixed in formalin plus acetic acid, embedded in paraffin, cut into 10-µm sections, and stained with toluidine blue. Sections were photographed with bright-field illumination at a magnification of ×6.25 **(A)** to **(D)** and ×400 **(E)**.

(A) Longitudinal section through the ovary within the flower at anthesis. Arrow indicates the pericarp.

(B) Cross-section of a fruit 0.3 cm in diameter. Arrow points to vascular tissue within the placenta.

(C) Cross-section of a fruit 0.5 cm in diameter. Arrow indicates the presence of locular tissue, which has differentiated from the placenta.

(D) Part of a cross-section through a fruit 1.2 cm in diameter. Arrow points to the gradient zone of differentiation between placenta and locular tissue.

(E) Cross-section through a developing seed from a fruit 1.2 cm in diameter. Arrow points to the developing embryo within the seed.

- The pericarp is covered on the outside by a thin cuticle that thickens as the fruit ages.
- The skin of the pericarp further consists of an epidermal layer and three to four layers of collenchymous tissue.
- The outer epidermal cells contain little to no starch and no stomata, but the inner pericarp cells contain many starch grains.
- In tomato, the cells that contribute to most of the carpel and fruit pericarp are large and vacuolated, and they are morphologically similar to leaf palisade cells. They contain most of the chloroplasts that give the developing fruit its green appearance.
- Cells in the outer and inner epidermal layer are small and have fewer chloroplasts.
- Thus, the carpel can be viewed as **a modified leaf that has folded into a tubular structure that encloses the ovules**.
- The fusion of two or more carpels in fruits such as tomato results in complex morphological structure in which it is difficult to discern the ontogenical relationships of cells in the fusion zones.

Genetic analysis of flower development has demonstrated that the antagonistic action of homeotic genes is required for normal carpel formation.

In Arabidopsis, mutant strains of *apetela-2* and *agamous* display a homeotic conversion with the reduction of carpels to leaflike structures, suggesting that homeotic genes have been recruited during evolution for the modification of leaves into the specialized carpel structure that develops into the fruit.

Cells in developing fruit often contain photosynthetically active chloroplasts and express nuclear and plastid genes for photosynthetic proteins.

This is consistent with the above discussed ontogenic relationship between cells in leaf and fruit.

However, the expression pattern of genes for photosynthetic proteins in fruit can vary from that in leaves, as has been shown for the gene family that encodes the small subunit of ribulose-1,5-bisphosphate carboxylase *(rbcS)* in tomato. Only two of the five *rbcS* genes are expressed during fruit development.

However, the interaction of DNA binding proteins with the promoter regions of all five tomato *rbcS* genes is similar in leaf and developing fruit, suggesting that the inactivation of a subgroup of *rbcS* genes in fruit must be regulated at a level other than DNA-protein interactions alone, presumably through signal transduction pathways that are specific to fruit cells.

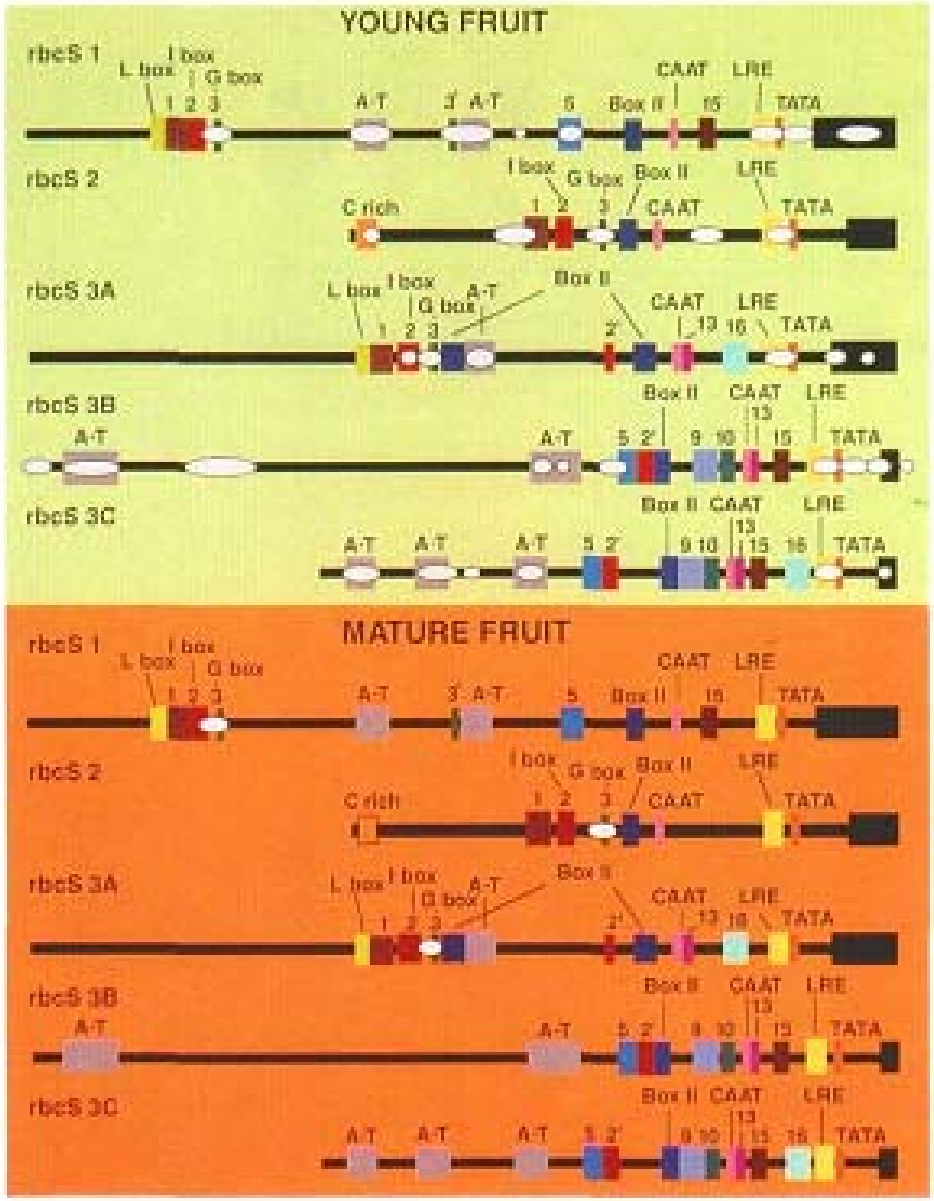

Figure 3. Developmental Changes in *rbcS* Promoter–Protein Interactions.

- ***rbcS* expression pattern in fruit is regulated by the physiological sink state of fruit cells rather than by a fruit developmental program.**

Compared to the photosynthetically active young fruit, most of the DNA-protein interactions are undetectable in ripening fruit, as shown in Figure 3, which is consistent with the transcriptionally inactive state of all *rbcS* genes during this phase of development.

The expression of genes for proteins such as TPRF-F1 and 2A11 is high in carpels and during early tomato fruit growth but is very low or undetectable in other organs.

- These examples demonstrate that, although ontogenic relationships exist between cells in leaf and fruit that can be demonstrated at the genetic level, fruit cells have also evolved a unique gene expression program that reflects their difference in function

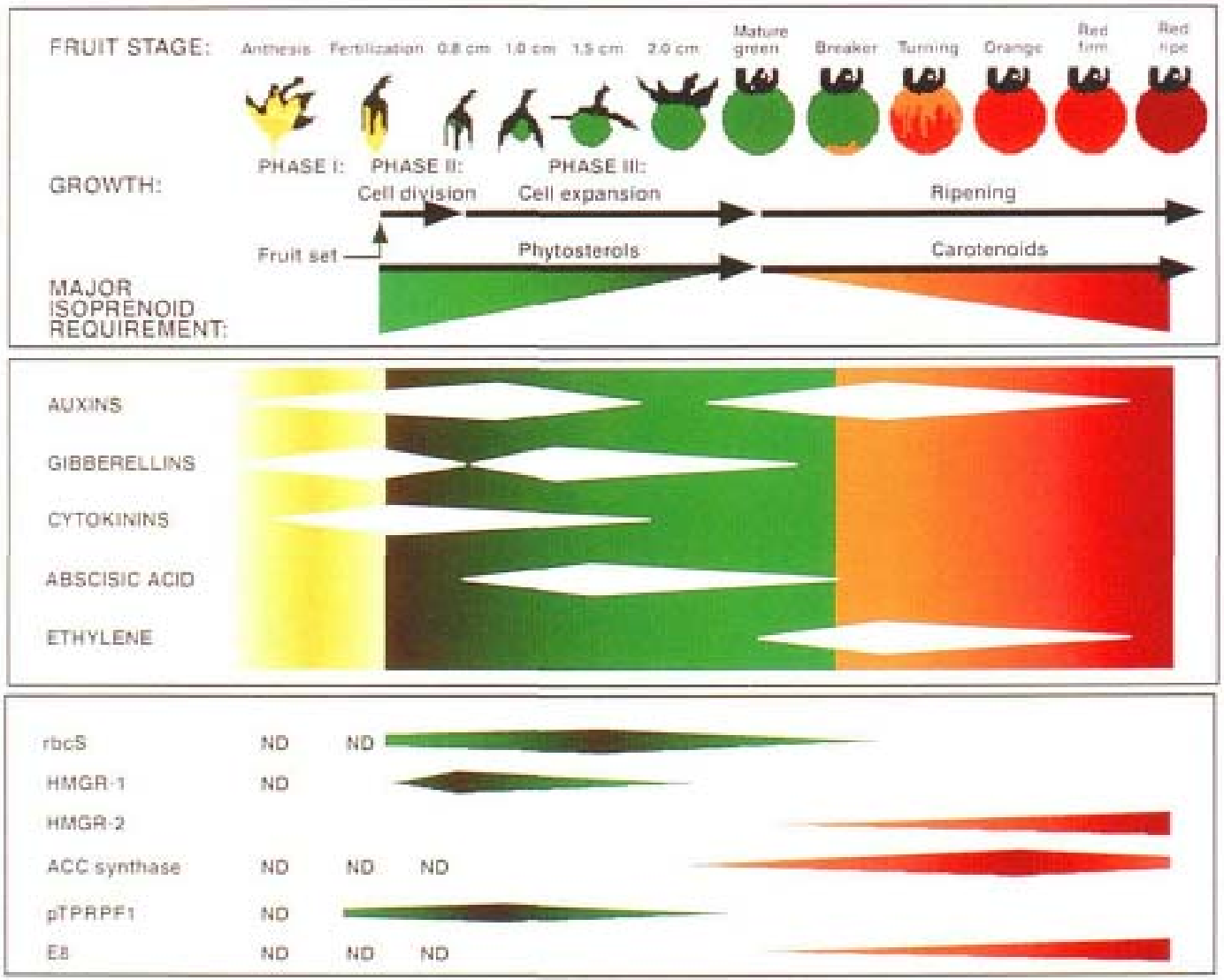

Figure 2. Hormonal Changes and Differential Gene Expression during Fruit Development.

Changes in hormone levels throughout tomato fruit development are indicated by white diamonds. Changes in steady state levels of selected mRNAs are indicated by green (phase II and III) and red (fruit ripening) diamonds.

Ripening is an aspect of development that is unique to fruit and that is initiated after seed maturation has been completed.

FRUIT DEVELOPMENT PHASES

In most plants, early fruit development can be divided into three phases.

Phase I: Ovary Development, Fertilization, and Fruit Set

- It is now well established that the precise spatial and temporal synthesis and action of auxin, cytokinin, and gibberellins are required for most or all of the normal (and visible) fruit developmental program.

- Factor(s) produced by the sporophytic tissue surrounding the developing ovary are required for triggering and maintaining cell division in the fruit primordia until the ovary has reached its mature size. At this time, cell division activity is reduced temporarily until fertilization has been completed.

Phase 2: Cell Division, Seed Formation, and Early Embryo Development

Following fertilization in tomato, cell division is activated in the ovary and continues for -7 to 10 days.

As the cell division phase ends, individual cells enlarge, as does the entire fruit, for the next 6 to 7 weeks.

Before the cell enlargement phase, dividing cells in the developing fruit are small, tightly compressed, and rich in cytoplasmic substances and have small vacuoles.

As cells enlarge, the primary cell wall and the cytoplasmic layer become relatively thinner, and vacuoles occupy a greater proportion of the cell volume.

- during phase **II,** cell division activity is highest in pericarp and placental tissues.

In the very early stages of phase II, mitotic activity is higher in the outer pericarp than in the inner pericarp.
Cell divisions in the developing seeds occur at the peripheral integument layers rather than in the embryos.
Cells within the columellar and placental tissue, which most likely represent vascular cells, also show high mitotic activity.
Four to six days after anthesis, cell division is still occurring in the fruit. At this time, mitotic activity in the pericarp is confined mostly to the outer layer, and mitotic activity within the placenta is localized to cell layers peripheral to the seeds.
Vascular tissues and developing seeds also show mitotic activity at this time. At the end of phase II and overlapping with phase III, mitotic activity is restricted to the outer pericarp cell layer and the outer placental cell layer.

Cell proliferation and differentiation in the fruit tissue are temporally coordinated with the mitotic activity in the developing seed and in the developing embryo. Research on signals that coordinate cell division and differentiation in the developing fruit with that in the seed and embryo will benefit further from the increased availability of plant genes for cell cycle regulatory proteins such as p34cdc2, cyclins, MAP kinase, and the nuclear GTP binding RAN protein

Phase 3: Cell Expansion and Embryo Maturation

After the period of cell division, fruit growth is due mostly to an increase in cell volume.

The number and timing of cell divisions can vary significantly in different fruits, and both contribute to its final size; in most plants, however, the increase in cell volume makes by far the greatest contribution to the final size of the fruit.

Cell expansion commonly increases fruit size by a factor of 100-fold or more.

In tomato fruit, the volume of cells in the placenta, locular tissue, and mesocarp tissue can increase by more than 10-fold, but cells that comprise the exo- and endocarp, which continue to divide, expand less.

- The auxin content of the tomato fruit peaks twice during development.
- During tomato fruit development, there are two peaks of gibberellin accumulation, which coincide with activation of cell division early in phase **II** and cell expansion in phase **III.**
- During tomato seed development, the endogenous ABA concentration peaks at the stage of cell enlargement and then declines at later stages of fruit development

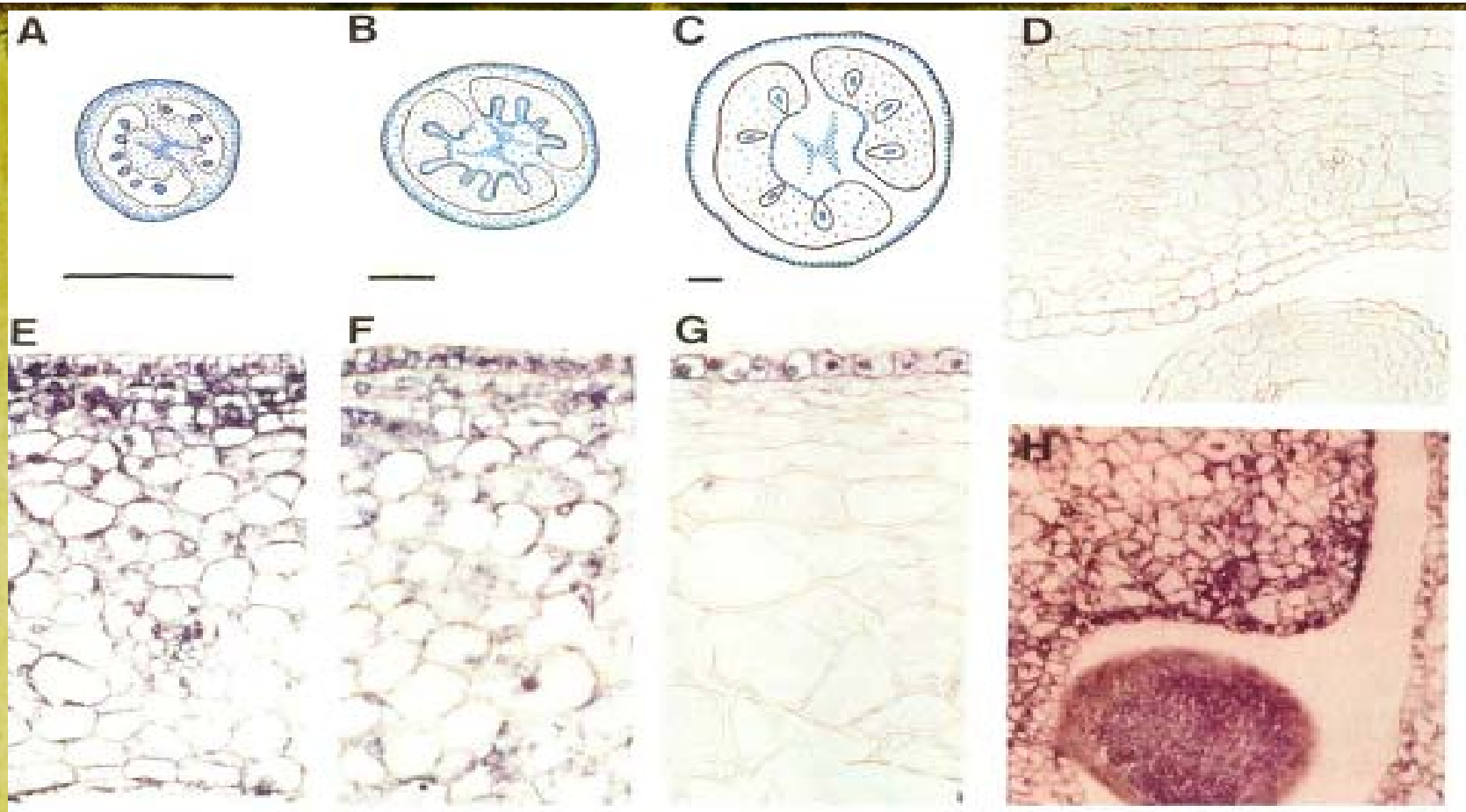

Figure 4. Mitotic Activity in Phase II and III of Tomato Fruit Development.

(A) to (C) Schematic summary of tomato fruit cell division; blue dots represent areas of mitotic activity. Bars = 0.2 cm.
(A) Cross-section of an early phase II fruit.
(B) Cross-section of a later phase II fruit.
(C) Cross-section of an early phase III fruit.
(D) to (H) Data from PCNA immunocytochemistry experiments. Tissues were prepared as described in Figure 1 and reacted with a monoclonal antibody that recognizes PCNA (Santa Cruz Biotechnology, Inc.). After washes and subsequent incubation with a secondary antibody conjugated to alkaline phosphatase (Oncogene Science), sections were reacted with substrates and washed, and coverslips were mounted with an aqueous medium (Polysciences). Sections were photographed under bright-field illumination.
(D) A cross-section from a fruit 0.3 cm in diameter reacted with secondary antibody alone (control). Under these conditions, no staining of nuclei is detectable in the pericarp, vascular tissues, or seeds. Magnification ×200.
(E) Pericarp from fruit 0.3 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×200.
(F) Pericarp from fruit 0.5 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×200.
(G) Pericarp from fruit 1.2 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×200.
(H) Placenta and seed tissue from a fruit 0.5 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×100.

Fruit Development and Seed Dispersal

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Annual Plant Reviews

Chapter 8

FACTORS INFLUENCING THE RIPENING AND QUALITY OF FLESHY FRUITS

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1 Introduction

Fruit consumption ensures that seeds are dispersed in a nutrient rich media.

In order for frugivore-assisted seed dispersal to occur, dramatic biochemical changes are initiated

Ripening-associated changes are conserved in many diverse fruit species and include the conversion of starch to sugars, alterations in texture, the accumulation of brightly coloured pigments, and the synthesis of volatile aroma compounds.

- **Plant secondary metabolites**, including aroma volatiles, appear to be key in the interaction of frugivores and ripening fruits, serving as both feeding attractants and deterrents.
- Carotenoids
- Ethanol
- Capsaicin
- Glycoalkaloids

2 Control of fruit ripening

Fleshy fruits display a broad range of phenotypic and chemical diversity. However, despite this diversity there are several common features that accompany the onset of fruit ripening.

This suite of common biochemical changes suggests that diverse fruits may share common pathways that mediate ripening.

Although the factors that control the onset of ripening are not fully understood, this represents an area of intense research activity.

Table 8.1 Classical mutants of tomato displaying altered fruit phenotypes associated with ripening and quality

Locus	Gene product/ function	Fruit phenotype	Chromosome	Reference
Apricot (*at*)	Unknown	Altered carotenoids	5	Jenkins and Mackinney, 1955
Beta (β)	Lycopene β-cyclase	Altered carotenoids	6	Ronen *et al.*, 2000
Colourless epidermis (*y*)	Unknown	Reduced flavonoids in peel	1	Rick and Butler, 1956
Colourless non-ripening (*Cnr*)	SBP-box transcription factor	Severe ripening inhibition	2	Manning *et al.*, 2006
Cuticular water permeability (*Cwp*)	Novel, unknown function	Fruit cracking and shrivelling	4	Hovav *et al.*, 2007
Delayed fruit deterioration (*dfd*)	Unknown	Altered fruit cuticle properties	Unknown	Saladie *et al.*, 2007
Delta (*Del*)	Lycopene epsilon cyclase	Altered carotenoid profile	12	Ronen *et al.*, 1999

		profile		
Dwarf (*d*)	Cytochrome P450, brassinosteroid biosynthesis	Delayed fruit ripening, pleiotropic effects	2	Lisso *et al.*, 2006
Green-flesh (*gf*)	STAY-GREEN homologue	Altered fruit pigmentation	8	Barry *et al.*, 2008
Green-stripe (*gs*)	Unknown	Striped fruit epidermis	7	Larsen and Pollack, 1951
Green-ripe (*Gr*)	Ethylene signalling	Reduced ethylene responsiveness	1	Barry and Giovannoni, 2006
High-pigment-1 (*hp-1*)	DDB1 homologue	Enhanced fruit pigmentation	2	Liu *et al.*, 2004
High-pigment-2 (*hp-2*)	DET1 homologue	Enhanced fruit pigmentation	1	Mustilli *et al.*, 1999
High-pigment-3 (*hp-3*)	Zeaxanthin epoxidase	Enhanced fruit pigmentation	2	Galpaz *et al.*, 1999
Lecer6	β-ketoacyl-CoA synthase	Shrivelling on the vine	Unknown	Vogg *et al.*, 2008
Never-ripe (*Nr*)	Ethylene receptor	Reduced ethylene responsiveness	9	Wilkinson *et al.*, 1995

Table 8.1 (*Continued*)

Locus	Gene product/ function	Fruit phenotype	Chromosome	Reference
Non-ripening (*nor*)	Transcription factor	Severe ripening inhibition	10	Giovannoni, 2001
Ripening-inhibitor (*rin*)	MADS-box transcription factor	Severe ripening inhibition	5	Vrebalov *et al.*, 2002
Tangerine (*t*)	Carotenoid isomerase	Altered carotenoid profile	10	Isaacson *et al.*, 2002
Yellow-flesh (*r*)	Phytoene synthase	Altered carotenoid profile	3	Fray and Grierson, 1993

3 Transcription factors serve as master regulators of fruit ripening

The characterization of tomato mutants with impaired fruit ripening has proven to be an effective strategy for gaining insight into the mechanisms that control ripening.

- The *ripening inhibitor* (*rin*)
- *non-ripening* (*nor*)
- *Colourless non-ripening* (*Cnr*)

display severe **inhibition of fruit ripening** manifest through **inhibited ethylene synthesis**, greatly **reduced carotenoid synthesis** and **reduced fruit softening**

The *rin* locus maps to the long arm of tomato chromosome 5 and is tightly linked to the *macrocalyx* (*mc*) locus that causes the production of large sepals.

The fruit pericarp of mature *Cnr* fruit is white and exhibits reduced cell–cell adhesion resulting in fruits with a mealy texture. Analysis of the cell wall properties of the *Cnr* mutant revealed several changes when compared to wild type including stronger pericarp cell walls in mature *Cnr* fruit, a 50% increase in intercellular spaces, reduced calcium-binding capability of homogalacturonan in the middle lamella and altered deposition of (1→5)--L-arabinan in mutant fruit cell walls.

4 Hormonal control of fruit ripening

All of the major plant hormones have been shown to influence aspects of fruit development and ripening. Hormone levels change during cell division following fertilization, cell expansion during fruit growth and at the onset of ripening, influencing the expression of a multitude of genes implicated in these processes (Srivastava and Handa, 2005). Discussion of hormonal regulation in this chapter will focus purely on the effects of hormones on ripening and fruit quality.

4.1 Ethylene

Fleshy fruits have traditionally been classified based upon their ripening behaviour. **Climacteric fruits**, including tomato, avocado, apple and banana produce a burst of respiration and display increased ethylene synthesis at the onset of ripening whereas in **non-climacteric fruits** such as grapes, strawberries and citrus these changes are not evident

- Chemicals that block either ethylene synthesis or perception can inhibit fruit ripening.

- Similarly, genetic control of ethylene synthesis through reduction of ACC levels or ACC oxidase activity also leads to inhibition of ripening.

- Mutations or transgenic approaches that disrupt the function of genes involved in the ethylene-signalling pathway also disrupt ripening.

Variation in climacteric ethylene production and ethylene responsiveness has been observed in several fleshy fruit species including apple, melon, peach, plum, pepper and, Asian pear, leading to different fruit quality and ripening characteristics.

In apple, peach and Chinese pear, the reduced ethylene biosynthesis observed in cultivars with extended shelf life has been linked to reduced expression levels of ACC synthase isoforms that are typically expressed during ripening.

In apple and Asian pear cultivars structural differences have been observed in different ACS isoforms and these correlate with ethylene production and postharvest shelf life.

Tremendous variation for fruit morphology and ripening traits also exists within the Cucurbitaceae family and in particular, melons display both climacteric and non-climacteric ripening behaviour.

- Cantaloupe melons display a climacteric ripening phenotype
- Honey dew melons produce little ethylene

non-climacteric character was controlled by two recessive loci

Fruits that have **traditionally been classified as non-climacteric** have recently been re-evaluated for the possible role of ethylene in regulating various aspects of the ripening process.

the possibility that **citrus** fruits may display climacteric-like behavior following harvest.

stimulate the accumulation of anthocyanin, and the expression of ripening-related genes in **grape**

differential expression of components of the ethylene signaling pathway have been demonstrated in several non-climacteric fruits including **strawberry**, **citrus** and **grape.**

4.2 Brassinosteroids

- Several lines of evidence point to a role for brassinosteroids (BRs) as potential promoters of fruit ripening in **grape**.

Genetic evidence of a role for BRs in mediating ripening and quality attributes in tomato has come from characterization of the *dwarf* (*dx*) mutant of tomato. The *dwarf* mutation lacks a functional cytochrome P450, required for the synthesis of castasterone and exhibits delayed fruit ripening and altered quality attributes including reduced levels of starch and sugars and elevated amino acids.

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Plant Physiology

The mechanism for brassinosteroids suppressing climacteric fruit ripening

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The effect of BR on ethylene biosynthesis and fruit ripening has been documented. For example, BR-treated jujube (*Zizyphus jujuba*) fruit, which is categorized as a climacteric fruit, shows significantly reduced ethylene production during storage (Zhu et al., 2010), while strawberry (*Fragaria ananassa*), a nonclimacteric fruit, shows delayed fruit ripening after application of epibrassinolide (EBR; Chai et al., 2012). In tomato, another climacteric fruit, treatment with brassinolide promotes the expression of *SlACS* and *SlACO* genes, as well as ethylene production (Zhu et al., 2015). More interestingly, overexpression of the BR biosynthetic gene *DWARF* in tomato results in increased level of endogenous BRs and ethylene production and earlier ripening (Li et al., 2016b), indicating endogenous BR can affect fruit ripening.

The plant hormone ethylene is important for the ripening of climacteric fruit, such as pear (*Pyrus ussuriensis*), and the brassinosteroid (BR) class of phytohormones affects ethylene biosynthesis during ripening via an unknown molecular mechanism. Here, we observed that exogenous BR treatment suppressed ethylene production and delayed fruit ripening, whereas treatment with a BR biosynthesis inhibitor promoted ethylene production and accelerated fruit ripening in pear, suggesting BR is a ripening suppressor. The expression of the transcription factor *BRASSINAZOLE-RESISTANT 1PuBZR1* was

The conserved brassinosteroid-related transcription factor BIM1a negatively regulates fruit growth in tomato

Kentaro Mori ✉, Martine Lemaire-Chamley, Joana Jorly, Fernando Carrari, Mariana Conte, Erika Asamizu, Tsuyoshi Mizoguchi, Hiroshi Ezura, Christophe Rothan

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To date, the BR-signalling network has been thoroughly characterized in the model plant Arabidopsis. BRs are perceived by the plasma membrane receptor kinase BRASSINOSTEROID INSENSITIVE 1 (BRI1). BR binding to BRI1 activates BRI1 kinase activity …

Here, we investigated the function of SlBIM1a, which is highly expressed in fruit. SlBIM1a overexpressing lines displayed severe plant and fruit dwarfism, and histological characterization of different transgenic lines revealed that SlBIM1a expression negatively correlated with fruit pericarp cell size, resulting in fruit size modifications.

Tomato SlBIM1a brassinosteroid-related transcription factor is involved in the regulation of fruit growth by negative regulation of pericarp cell expansion, possibly at the crossroad with auxin and light signalling.

4.3 Auxin

- In tomato, auxin levels increase early during fruit development during the cell division phase and subsequently decline before increasing at the onset of ripening.

Under low auxin conditions, the Aux/IAA proteins bind to the ARFs and inhibit transcription. In contrast, when auxin levels increase, binding of auxin to the receptor, an F-box protein designated SCFTIR1, targets the Aux/IAA proteins for degradation and releases their inhibitory effect on auxin-inducible gene expression.

The expressions of several Aux/IAA and ARF genes are induced at the onset of ripening in both tomato and peach and show regulation by ethylene, suggesting possible interplay between ethylene and auxin in mediating ripening-related gene expression

Transgenic manipulation of the ARF gene *DR12* in tomato causes a range of pleiotropic phenotypes that includes:

dark green immature fruits that when mature exhibit a blotchy ripening pattern.

Pericarp cells also possess an unusual pattern of cell division

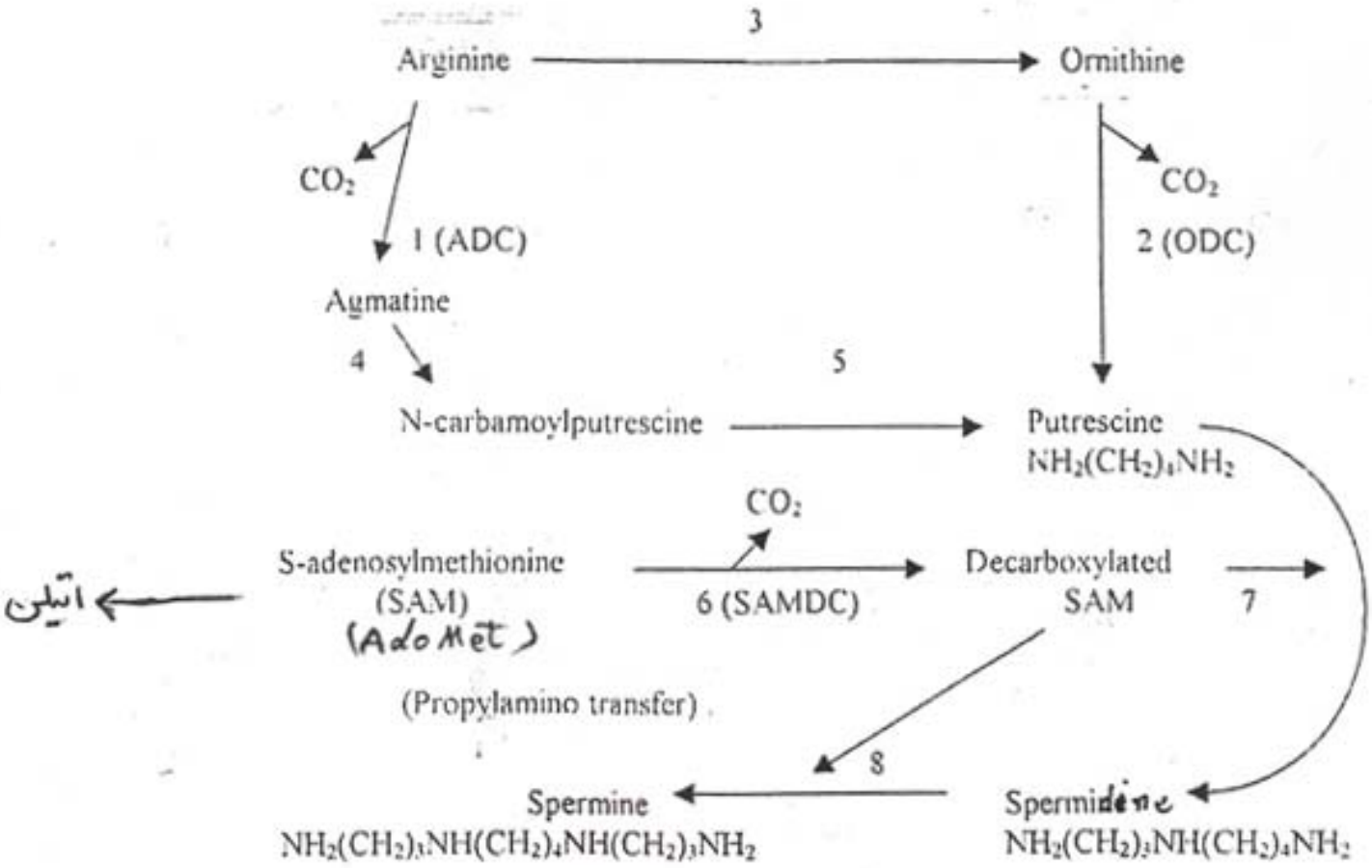
a higher number of small cells in the outer pericarp that contributes to an increased overall thickness of the pericarp.

DR12 suppressed fruit displays enhanced firmness that may result from the increased thickness of the pericarp or subtle changes that are observed in pectin composition in the transgenic lines

4.4 Polyamines

The transgenic lines with elevated PA levels exhibited a range of beneficial phenotypes including **increased vine life**, **higher carotenoid levels** and **increased juice viscosity**. The transgenic fruit also produced **elevated levels of ethylene**.

This result was unexpected given that both PAs and ethylene share SAM as a common precursor as it was anticipated that diverting SAM towards PA biosynthesis would reduce ethylene levels.

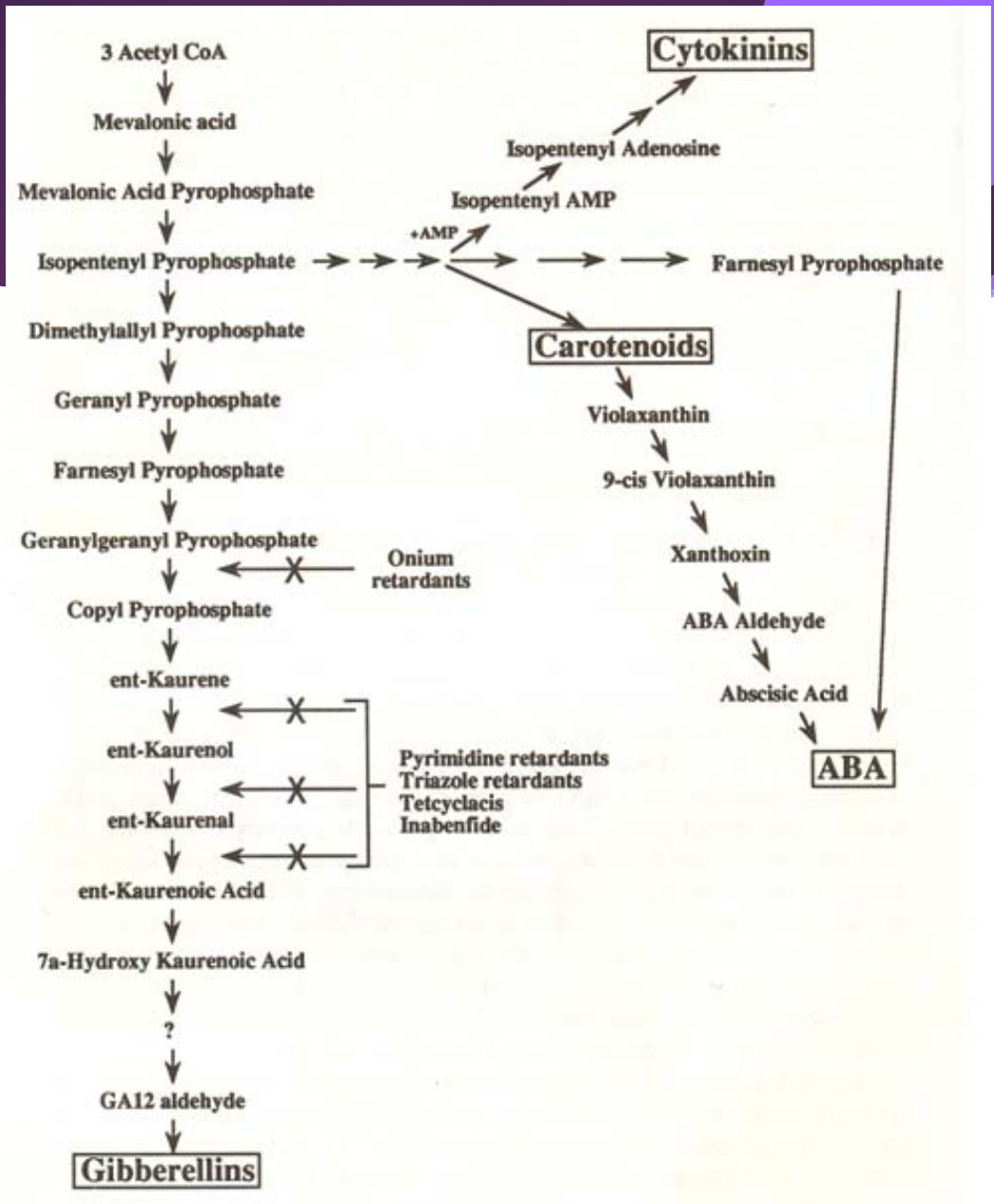

3
Arginine
Ornithine
CO_2
1 (ADC)
CO_2
2 (ODC)
Agmatine
4
5
N-carbamoylputrescine
Putrescine
$NH_2(CH_2)_4NH_2$
CO_2
S-adenosylmethionine
(SAM)
(AdoMet)
6 (SAMDC)
Decarboxylated
SAM
7
اتیلن
(Propylamino transfer)
8
Spermine
$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
Spermidine
$NH_2(CH_2)_3NH(CH_2)_4NH_2$

5 The influence of light on fruit quality

- The accumulation of brightly coloured pigments in fruits is one of the most dramatic events that accompanies ripening.

Fleshy fruits predominantly accumulate **carotenoids**, **anthocyanins** and **flavonoids** and the de novo synthesis of these compounds at the onset of ripening is preceded by, or occurs concomitantly with, the degradation of chlorophyll.

An exception to this generalization occurs in banana where the degradation of chlorophyll at the onset of ripening leads to the unmasking of the yellow-pigmented xanthophylls that are already present in immature fruit.


3 Acetyl CoA
Mevalonic acid
Mevalonic Acid Pyrophosphate
Isopentenyl Pyrophosphate
Dimethylallyl Pyrophosphate
Geranyl Pyrophosphate
Farnesyl Pyrophosphate
Geranylgeranyl Pyrophosphate
Copyl Pyrophosphate
ent-Kaurene
ent-Kaurenol
ent-Kaurenal
ent-Kaurenoic Acid
7a-Hydroxy Kaurenoic Acid
?
GA12 aldehyde
Gibberellins
Onium retardants
Pyrimidine retardants
Triazole retardants
Tetcyclacis
Inabenfide
+AMP
Isopentenyl AMP
Isopentenyl Adenosine
Cytokinins
Carotenoids
Violaxanthin
9-cis Violaxanthin
Xanthoxin
ABA Aldehyde
Abscisic Acid
Farnesyl Pyrophosphate
ABA

Ethylene plays a significant role in regulating carotenoid synthesis in tomato.

light-signalling pathways in influencing carotenoid, flavonoid and anthocyanin accumulation during ripening in several species including tomato, apple and grape.

The manipulation of downstream components of the light-signalling pathway can also elicit changes in the pigment content of tomato fruit. Silencing of *LeHY5*, a positive regulator of light responses and *LeCOP1-like*, a negative regulator of light signalling, resulted in fruits that accumulated lower and higher levels of carotenoids than non-transformed controls, respectively

manipulation of light-signalling components appears to be an effective strategy to modify the phytonutrient content of fleshy fruits.

The Molecular Biology and Biochemistry of Fruit Ripening

Edited by
GRAHAM B. SEYMOUR
MERVIN POOLE
JAMES J. GIOVANNONI
GREGORY A. TUCKER

1 Biochemistry of Fruit Ripening

Sonia Osorio and Alisdair R. Fernie

Introduction

The quality of fruit is determined by a wide range of desirable characteristics such as

- nutritional value,
- flavor,
- processing qualities,
- shelf life.

The list of fruits and vegetables traded throughout the world is both long and diverse. The FAO lists over 100 “lines” of which 60 are individual fruits or vegetables or related groups of these commodities.

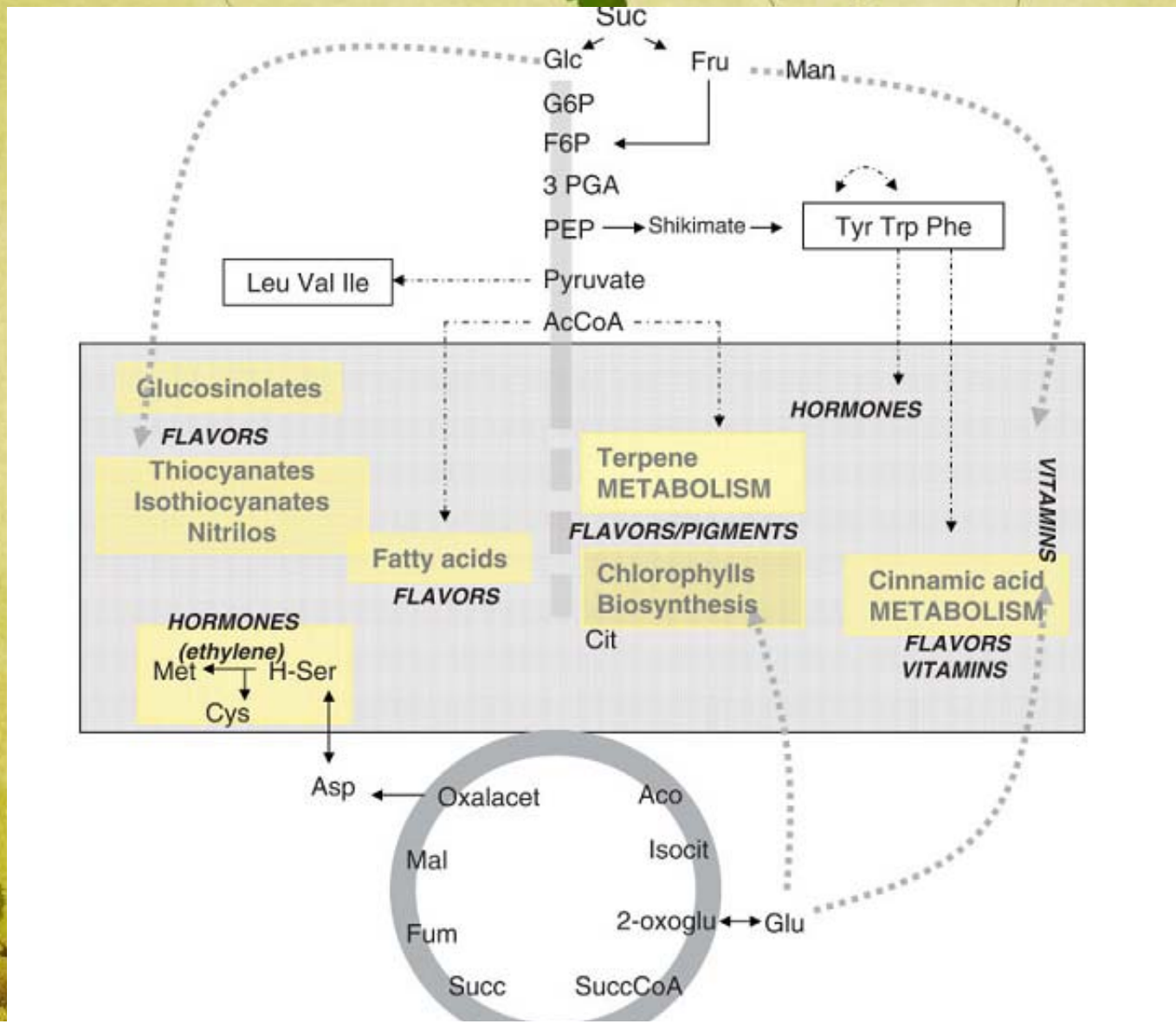
Table 1.1 Global production, consumption, and net export of the five major (million tons) fruit commodities in 2002–2004. Data from European Commission Directorate-General for Agriculture and Rural Development (2007).

Commodity	Production	Consumption	Net Export
Banana	71	58	12.9
Tomato	119	103	2.1
Apple	59	56	3
Grape	64	59	1.7
Orange	63	53	2.5

- Fruit ripening is highly coordinated, **genetically** programmed, and an irreversible **developmental process** involving specific biochemical and physiological attributes that lead to the development of a soft and edible fruit with desirable quality attributes.

The main changes associated with ripening include:

- **color** (loss of green color and increase in nonphotosynthetic pigments that vary depending on species and cultivar),
- **firmness** (softening by cell-wall-degrading activities),
- **taste** (increase in sugar and decline in organic acids),
- **odor** (production of volatile compounds providing the characteristic aroma).


Suc
Glc
Fru
Man
G6P
F6P
3 PGA
PEP
Shikimate
Tyr Trp Phe
Leu Val Ile
Pyruvate
AcCoA
Glucosinolates
FLAVORS
Thiocyanates
Isothiocyanates
Nitrilos
Fatty acids
FLAVORS
HORMONES
(ethylene)
Met
H-Ser
Cys
HORMONES
Terpene
METABOLISM
FLAVORS/PIGMENTS
Chlorophylls
Biosynthesis
Cit
Cinnamic acid
METABOLISM
FLAVORS
VITAMINS
VITAMINS
Asp
Oxalacet
Aco
Isocit
Mal
Fum
2-oxoglu
Glu
Succ
SuccCoA

Central Carbon Metabolism

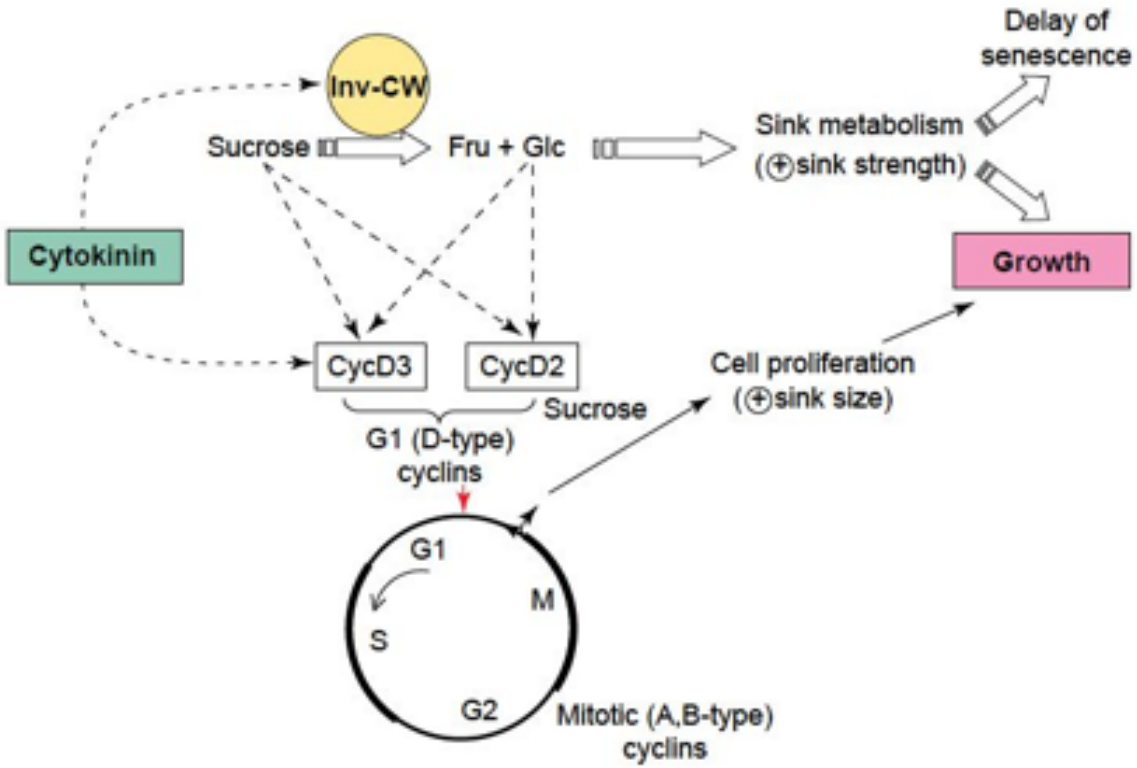
Sucrose, glucose, and fructose are the most abundant **carbohydrates** and are widely distributed food components derived from plants.

The sweetness of fruits is the central characteristic determining fruit quality and it is determined by the **total sugar content** and by **their ratios** among those sugars.

- in domesticated tomato (*Solanum lycopersicum*) only a high accumulation of the two hexoses is observed, whereas some wild tomato species (i.e., *Solanum chmielewskii*) accumulate mostly sucrose

- unload into the sink organ

The rate of photoassimilate transport in plants is mainly determined by sink strength, which consists of sink size and sink activity (Smith et al. 2018). Sink size is a physical parameter determined by the cell numbers and cell size, whereas sink activity is a physiological parameter influenced by many processes such as carbon metabolism, assimilate transport and storage by the sink cells (Morey et al. 2018). Phloem unloading constitutes a range of cell-to-cell transport steps that convey phloem-mobile components from phloem to the sink organs. Potent transport pathways are mainly determined by the occurrence of apoplasmic barriers and the whole plant sink/source ratios for photoassimilates (Milne et al. 2018). Sink/source ratio may be increased by partial defoliation or decreased by fruit thinning. Change in the sink-source dynamics is one of the criteria considered to adjust the sink activity and allow a greater supply of photoassimilates for the growth of fruits (Pereira et al. 2017). Changes in the sink/source ratio may have a great effect on various important cellular processes including carbohydrate metabolism, and the secondary metabolites synthesis and transport (Pastore et al. 2011).



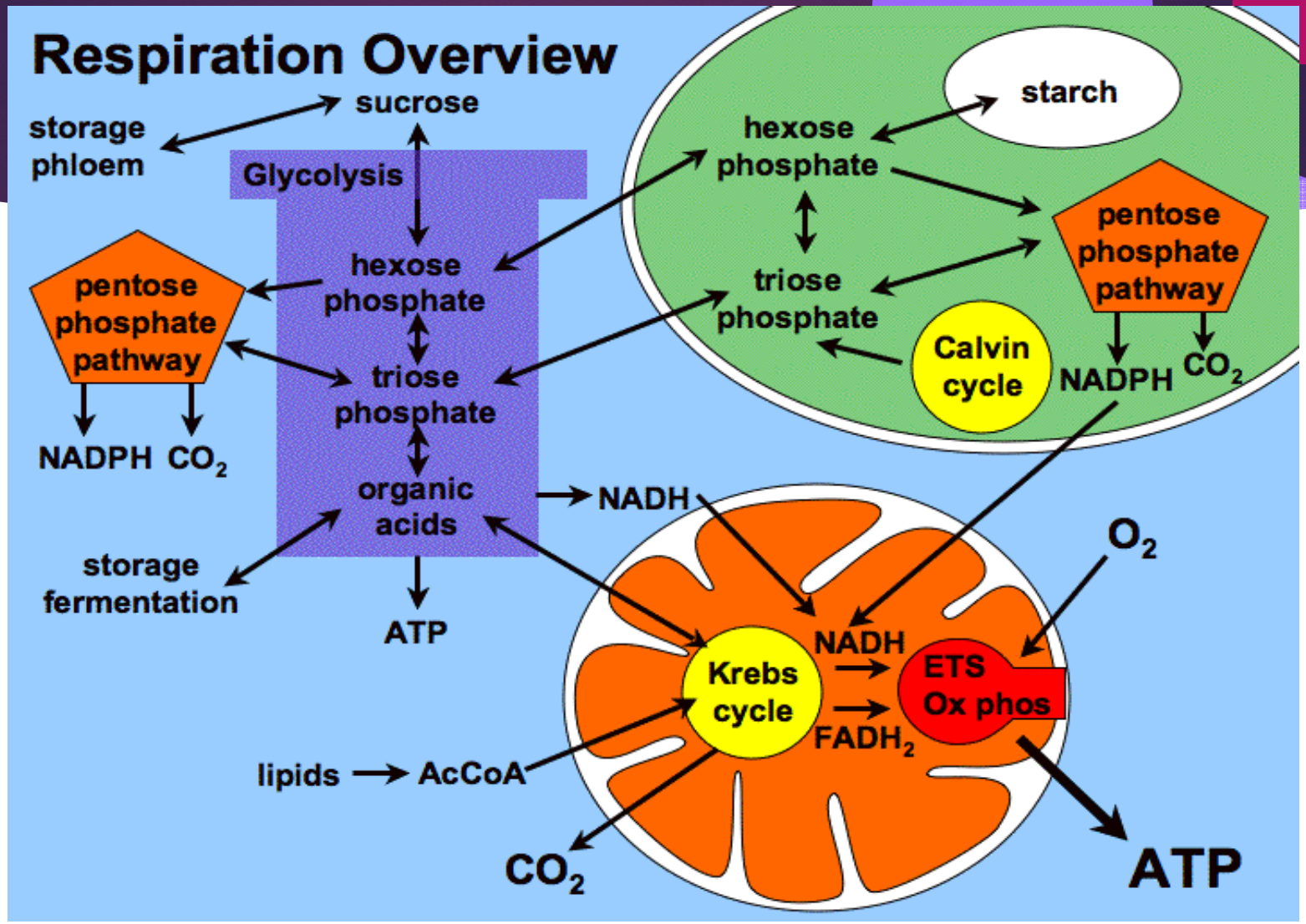
شکل ۲-۳- ایتورتاز و تنظیم چرخه سلولی: نتایج نشان داده که سایتوکینین فعال کردن یکی از سیکلین‌های نوع D (CycD3) را بر عهده دارد. این سیکلین انتقال از فاز G1 به S تقسیم میتوز سلولی را کنترل می‌کند و نقطه مرکزی پراوری سلول‌ها (پیکان قرمز) را بر عهده دارد. افزایش Inv-CW القا شده توسط سایتوکینین منجر به افزایش قدرت مخزن شده و جریان اسیلات‌ها به بافت‌های مخزن را افزایش می‌دهد. علاوه بر این، سوکروز و گلوکز با القا سیکلین‌های نوع D (CycD2 و CycD3)، باعث پراوری سلولی و افزایش اندازه مخزن می‌شوند. در نتیجه افزایش اندازه و قدرت مخزن، رشد و تمایز بافت مقصد پیشرفت می‌کند (۶).

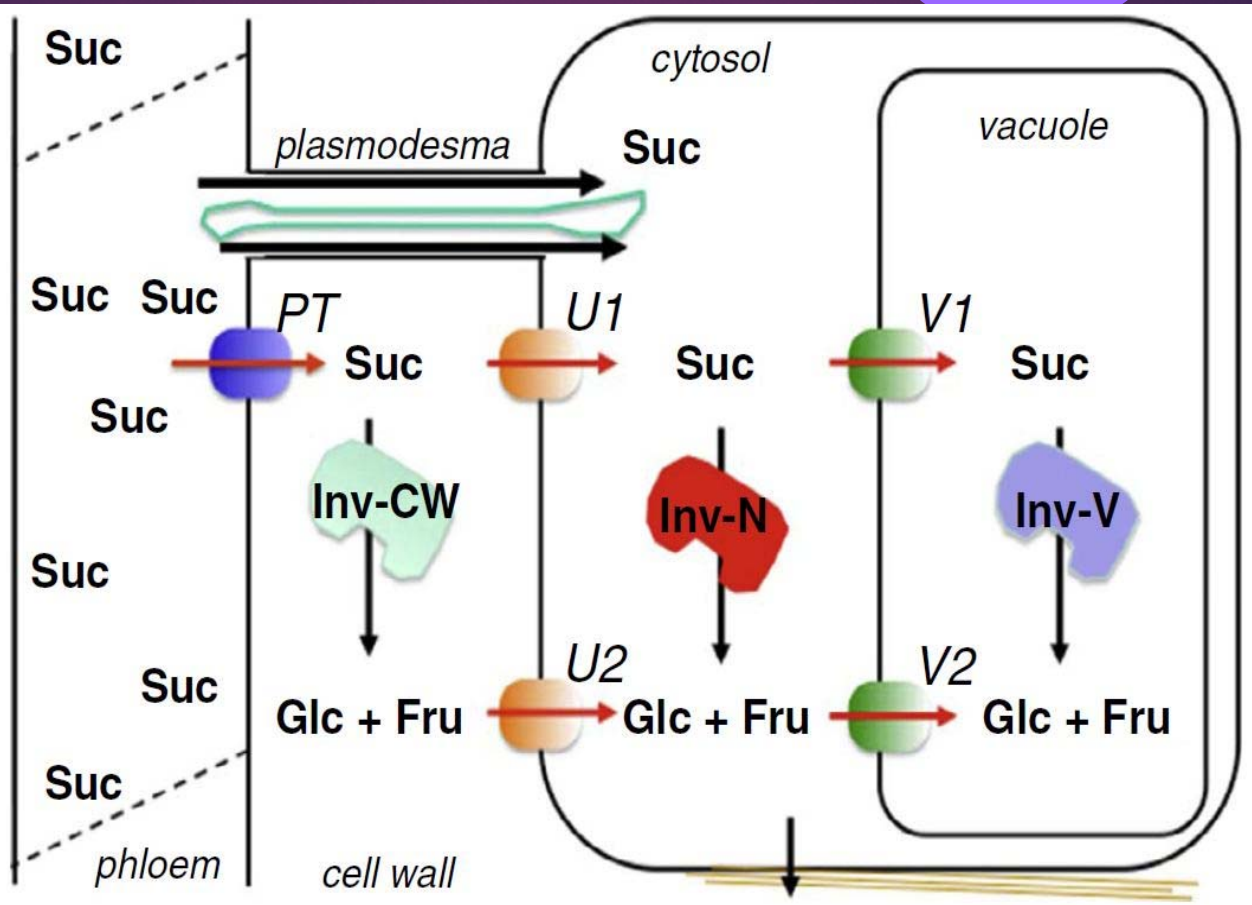
Suc is the main product of photosynthesis and the form in which most of the carbohydrate is transported between cells and throughout the plant.

It is perfectly matched to act as a signaling molecule modulating sink-source relationships and the use of resources (Zhang et al. 2018).

Generally, Suc transfer from the sieve element-companion cell complex to the surrounding cells can occur symplastically via the plasmodesmata, or through an apoplastic unloading pathway via transporters (Cheng et al. 2015).

Suc may be either directly pumped into the sink parenchyma cell by plasma-membrane localized Suc transporters (SWEET transporters) or hydrolyzed by Inv-CW into Glu and Fru and then transported into storage cells by hexose transporters (Wang et al. 2019).


Respiration Overview
sucrose
storage
phloem
Glycolysis
hexose
phosphate
pentose
phosphate
pathway
triose
phosphate
NADPH
CO2
organic
acids
storage
fermentation
ATP
NADH
starch
hexose
phosphate
triose
phosphate
pentose
phosphate
pathway
Calvin
cycle
NADPH
CO2
O2
NADH
Krebs
cycle
ETS
Ox phos
FADH2
lipids
AcCoA
CO2
ATP


Suc
cytosol
plasmodesma
Suc
vacuole
Suc Suc
PT
U1
V1
Suc
Suc
Suc
Suc
Suc
Inv-CW
Inv-N
Inv-V
Suc
U2
V2
Suc
Glc + Fru
Glc + Fru
Glc + Fru
Suc
phloem
cell wall

Enzymatic hydrolysis of Suc by Inv-CW within sink organs increases the contribution of carbohydrates to the osmotic potential, thereby contributing to the regulation of the carbohydrate import in the sink tissues (Patrick and Offler 2001).

In certain sink tissues, such as roots, phloem unloading usually involves the symplastic pathway; however, it has been shown that in some fruits which accumulate high concentrations of soluble sugars, the apoplastic unloading route is also employed over fruit development course (Cheng et al. 2015).

The uptake of hexoses from the apoplast into the sink cells is an outcome of the hydrolysis of the phloem-imported Suc; this is achieved by the HTs.

The inhibition of the Glu transport by silencing LeHT1 could result in a 55% decrease in the fruit hexose accumulation (McCurdy et al. 2010). Plant HTs are involved in energy production, pathogen defense and osmoregulation in the unloading zone, facilitating the Suc transport to the sink tissues (Slewinski 2011).

The present study showed that decreasing the sink/source ratio via fruit thinning increased the Suc and hexose contents in the tomato fruit pulps, also causing higher ST accumulation in the pericarp of the tomato fruits (Table 2). Since ST content was the highest at the end of the cell division (15 DAT), it was clear that it was degraded during the tomato fruit development. Biais et al. (2014) also reported the decreasing trends of ST until the red ripe stage of tomato fruits.

tomato introgression lines containing an exotic allele of LIN5, a cell wall invertase that is exclusively expressed in flower (mainly ovary but also petal and stamen) and in young fruit and it has been demonstrated that alterations in the efficiency of this enzyme result in significantly increased partitioning of photosynthate to the fruit and hence an enhanced agronomic yield.

LIN5 antisense plants had decreased glucose and fructose in the fruit proving *in planta* the importance of LIN5 in the control of the total soluble solids content.

Aslani et al. 2020

all three studied LeHTs displayed the highest expression levels at the first developmental stage (i.e., 15 DAT), while the lowest ones were detected at 30 DAT (Fig. 3). The higher expression levels of LeHT1, LeHT2, and LeHT3 at 15 DAT were coincident with rapid fruit growth due to the active cell division, reflecting the LeHTs pivotal role in the regulation of hexoses released to maintain the fruit cell metabolism. On the other hand, the subsequent downregulation of these genes at 30 DAT (i.e., in the second examined phase of fruit development) could be ascribed to the beginning of Suc accumulation into the vacuole of parenchyma cells, where the absence of Inv-V and Inv-N activities (Fig. 2) paved the way for its storage. In the third phase of fruit development (i.e., 45 DAT), the expression of LeHT1 and LeHT2 was increased. It has been previously observed that an increase in the expression level of LeHT2 occurs during the final stage of fruit development (Reuscher et al. 2014), but LeHT1 and LeHT3 tend to be highly expressed in the young fruits (Dibley et al., 2005). The results obtained herein and those reported by the above-mentioned studies suggest that LeHT1 and LeHT2 are the most likely candidates facilitating the transport and accumulation of sugar within the pericarp of the mature tomato fruits.

Mature tomato fruit employs apoplastic Suc unloading (via sugar transporters) in the sink cells; then, Suc is mainly converted to Glu and Fru by Inv-CW in the cell wall space, where the hexose transport into parenchyma cells is facilitated by HTs (Wang et al. 2019). The activity of the Inv-CW was highly increased from 30 to 45 DAT in the fruits of the examined tomato cultivars. This increase depicted a shift from the symplastic unloading to the apoplastic one in the maturing tomato fruits. This finding provides further evidence for apoplastic unloading at the onset of ripening, showing agreement with the above-mentioned literature. The concurrence of apoplastic unloading and the enhanced expression of LeHT1 and LeHT2 at the foregone stage of fruit growth reinforce the proposal that the activity of HTs mediates the transport of monosaccharides down a concentration gradient along the phloem path (Murcia et al. 2017). Moreover, during the final stage of fruit growth, in addition to the hexose sugars derived from unloaded Suc, more Glu or Fru would be passively leaked from cells into the extracellular space which needs to be taken up by the HTs (Wang et al. 2019). This is consistent with the higher level of LeHT1 and LeHT2 expression observed in the present study.

Suc may be directly imported into the sink cells, where it is transported from the cytosol into the vacuole. Then, it may be stored as Suc, or hydrolyzed by Inv-V to produce Glu and Fru (Koch 2004). Cytosolic Suc can also be hydrolyzed by Inv-N into Glu and Fru, whereby it may be metabolized or transported into the vacuole (Lalonde et al. 2003). The enzymes present in the vacuole and cytosol control the Suc/hexose ratio in the cell (Baxter et al. 2005); hexose accumulation is often accompanied by high Inv-V and Inv-N activities (Koch 2004). Not only does enzymatic hydrolysis of Suc by invertases within sink organs facilitate the contribution of carbohydrates to maintain the osmotic potential, but it also contributes to the regulation of carbohydrate imported into the sink tissues (Patrick et al. 2001).

Aslani et al. 2020

In general, in all treatments applied to decrease the sink/source ratio, two phases of Inv-N and Inv-V activities were distinguished. The first phase involved rather minimal changes in enzyme activities. Enzyme activities can be compensated by coordinated changes in other enzymes (Pascual et al. 2013). Sucrose synthesis is another enzyme associated with Suc utilization that showed different responses to invertase during tomato fruit development (Steinhauser et al. 2010), maybe the activity of this enzyme is the reason for some difference between hexoses content and Inv-V and Inv-N activities from 15 to 30 DAT. During this phase, the Suc is localized in the vacuole, where the absence of Inv-V and Inv-N activities permits its storage (Schaffe et al. 1999). Due to a growing need for hexoses and a progressive Suc-driven increase in the osmotic potential, which facilitated cell expansion, there was an increase in the Inv-V and Inv-N activities, as compared with the early stage of fruit growth, leading to the second phase of the activity of the latter enzymes. Unlike the first phase, induction of the Inv-N and Inv-V activity in this second phase indicated their putative role in the accumulation of Glu and Fru (Xue et al. 2018).

A role for **apoplastic invertase** in the control of sink size has been postulated previously in other species; the apoplastic invertase-deficient *miniature1* mutant of maize exhibits a dramatically decreased seed size as well as altered levels of phytohormones.

- regarding the regulation of carbon partitioning in fruits

- Starch is another carbohydrate that undergoes modifications during ripening.

The tomato introgression lines containing the exotic allele of LIN5 (IL 9-2-5) accumulated significantly more starch in both, pericarp and columella tissues

- starch accumulation plays an important role in determining the soluble solids content or Brix index of mature fruit.

- **Organic acid** manipulation is highly valuable from a metabolic engineering perspective.

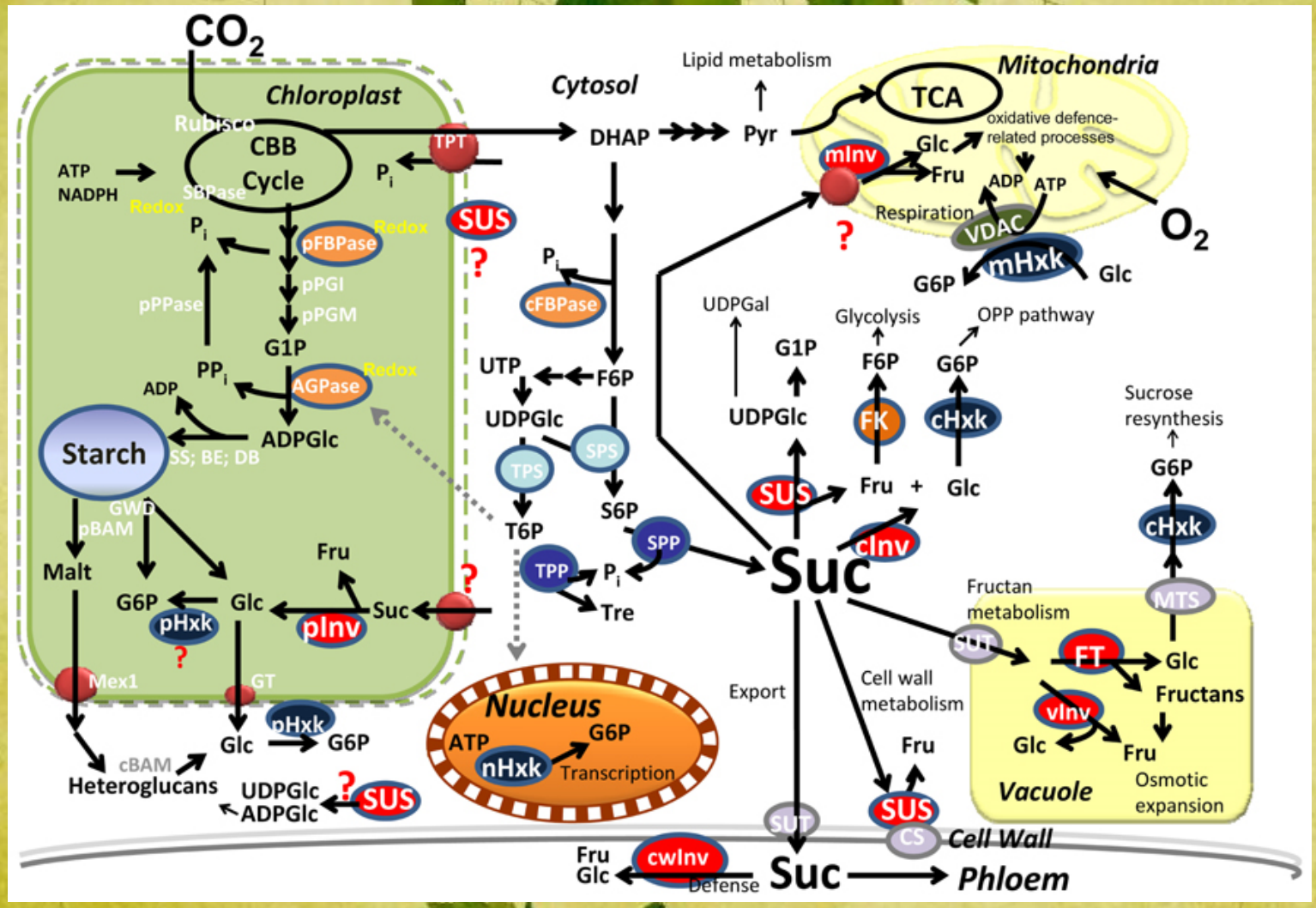
Malate is the predominant acid in many fruits, both climacteric, and nonclimacteric.

Interestingly, levels of both citrate and malate were also highly correlated to many important regulators of ripening.

Patterns of malate accumulation differ between plant species and even cultivars.

In fruits, patterns of malate accumulation and degradation cannot be explained by the classification of species as climacteric or nonclimacteric, nor can they be attributed to changes in overall respiration rates.

Some climacteric fruits such as plum and tomato appear to utilize malate during the respiratory burst, while others such as banana and mango continue to accumulate malate throughout ripening, even at the climacteric stage


CO2
Chloroplast
Rubisco
CBB Cycle
ATP
NADPH
Redox
SBPase
TPT
Pi
SUS
pFBPase
pPGI
pPGM
pPPase
G1P
PPi
AGPase
ADP
ADPGlc
Starch
SS; BE; DB
GWD
pBAM
Malt
G6P
Glc
Fru
Suc
pHxk
pInv
Mex1
GT
cBAM
Heteroglucans
UDPGlc
ADPGlc
Cytosol
DHAP
Pyr
Lipid metabolism
cFBPase
UTP
F6P
UDPGlc
TPS
SPS
T6P
S6P
TPP
SPP
Tre
Nucleus
ATP
nHxk
G6P
Transcription
Export
Suc
Mitochondria
TCA
oxidative defence-related processes
mInv
Glc
Fru
ADP
ATP
Respiration
VDAC
mHxk
O2
G6P
Glc
UDPGal
G1P
UDPGlc
Glycolysis
OPP pathway
F6P
G6P
FK
cHxk
Fru + Glc
SUS
cInv
Sucrose resynthesis
G6P
cHxk
MTS
Fructan metabolism
SST
FT
Glc
Fructans
vInv
Glc
Fru
Vacuole
Osmotic expansion
Cell wall metabolism
Fru
SUS
CS
Cell Wall
SUT
Fru
Glc
cwInv
Defense
Suc
Phloem

Biochemical analysis of the *Aco1* mutant revealed that it exhibited a decreased flux through the TCA cycle, decreased levels of TCA cycle intermediates, enhanced carbon assimilation, and dramatically increased fruit weight.

Reduced mMDH activity, showed an increment in fruit dry weight likely due to the enhanced photosynthetic activity and carbon assimilation in the leaves, which also led to increased accumulation of starch and sugars, as well as some organic acids (succinate, ascorbate, and dehydroascorbate)

Ethylene in Ripening

The role of ethylene in ripening of climacteric fruits has been known for more than 50 years.

Since then, considerable effort has been focused on the studies of:

- **ethylene biosynthesis** (S-adenosylmethionine, SAM; SAM synthetase; 1-aminocyclopropane carboxylic acid; ACC synthase; and ACC oxidase),
- **ethylene perception** (ethylene receptors, ETRs);
- **signal transduction** (ethylene response factor, ERFs);
- and **ethylene-regulated genes** such as cell-wall disassembling genes (endopolygalacturonase; pectin methyl esterase, PME; and pectate lyase).

six ethylene receptors have been isolated in tomato
(*ETHYLENE RECEPTOR1*, *LeETR1; ETHYLENE RECEPTOR2*, *LeETR2; ETHYLENERECEPTOR5*, *LeETR5; NEVER-RIPE, NR; ETHYLENE RECEPTOR4*, *LeETR4;* and *ETHYLENE RECEPTOR6*, *LeETR6*)

compared to

five members in *Arabidopsis*
(*ETHYLENE RECEPTOR1*, *ETR1; ETHYLENE RECEPTOR2*, *ETR2; ETHYLENE R*
ESPONSE SENSOR1, ERS1; ETHYLENE RESPONSE SENSOR2, ERS2; and ETHYLENE INSENSITIVE4, EIN4).

Five of the six tomato receptors have shown to bind ethylene but expression studies have been shown different profiles. Transcript levels of *LeETR1*, *LeETR2*, and *LeETR5* change little upon treatment of ethylene in fruit, where *NR*, *LeETR4*, and *LeETR6* are strongly induced during ripening

The existence of tomato-ripening mutants confirmed the genetic basis of ripening. The *Neverripe (Nr)* and *ripening inhibitor (rin)* fruit shown in Figure 3.1 were photographed 6 months after control fruit ripened and remained in good condition for a year or more, provided they were prevented from becoming dehydrated. After 1 year the *rin* seeds began to germinate but the flesh had still not changed their color, although there had been some softening.

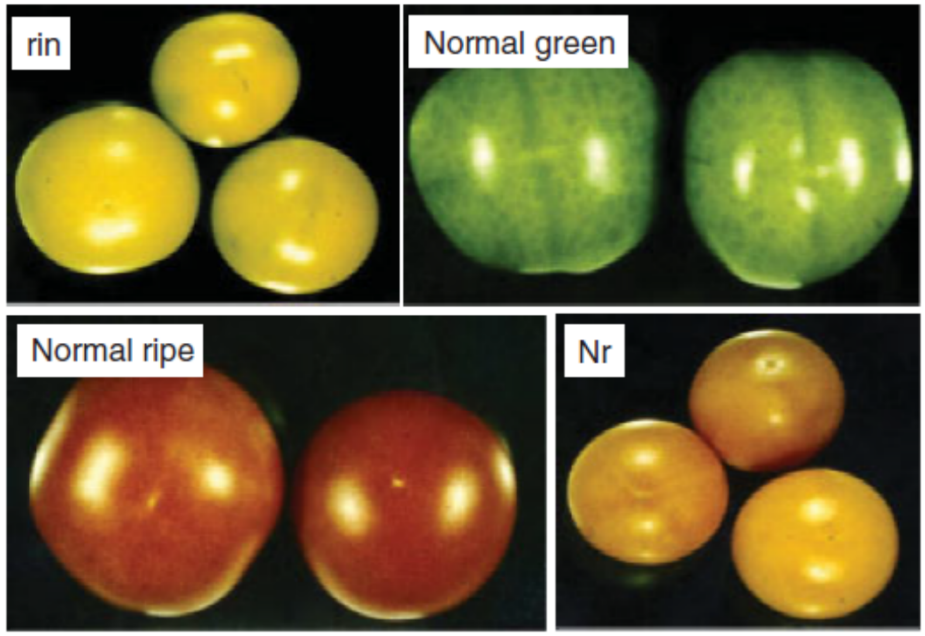


Figure 3.1 Mutants of ethylene action and ripening. Mature green and ripe Ailsa Craig tomato fruits, photographed at approximately 40 and 50 days respectively, together with fruit from near-isogenic lines of the *ripening inhibitor* (*rin*) *and Neverripe* (*Nr*) mutants, photographed at around 6 months old. As discussed in the text, the Nr mutation bred into the Pearson tomato cultivar (not shown) has a more severe phenotype and the fruits remain green.

The Nr mutation was shown to be due to a crucial amino acid change in a tomato ethylene receptor and the two other mutants had drastically reduced ethylene production, although ripening could not be restored by adding ethylene externally.

Ethylene plays an important role in ripening

This indicates that ripening and the eventual deterioration and rotting of normal fruit is a regulated process; it is not unavoidable event, but is genetically determined.

A range of fruit color mutants, in which no other aspect of ripening appeared to be affected, indicated that the ripening pathway had several independent branches (Fig. 3.2).

The production of individual enzymes involved in cell wall metabolism (e.g., polygalacturonase (PG), pectin esterase (PE), pigment synthesis (phytoene synthase (PSY)), and volatile production could each be inhibited independently by gene silencing without affecting other ripening attributes.

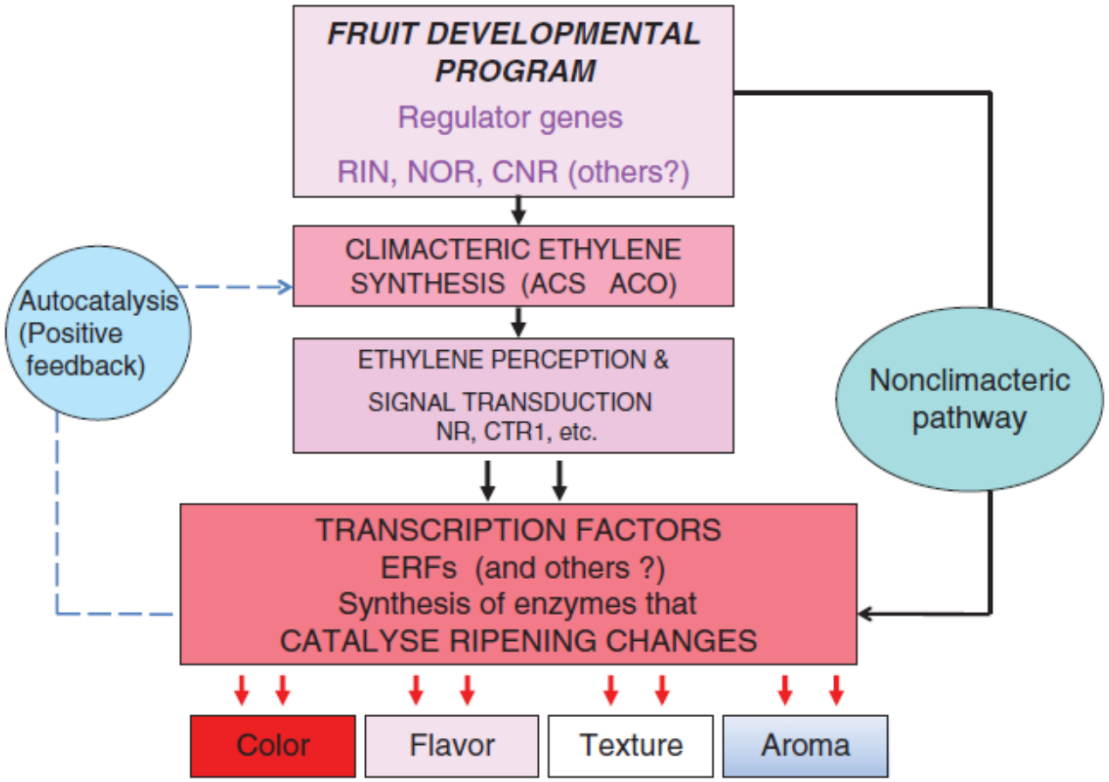


Figure 3.2 Control of ripening. The main features of the ripening process are indicated, based on studies in tomato. Note the regulator genes, the feedback loop promoting autocatalytic ethylene production, ethylene synthesis, perception and signaling mechanisms, and branches leading to separate responses controlled by transcription factors.

Thirty years before ethylene receptors were cloned and sequenced, Burg and Burg (1967) predicted the presence of a metal ion in the receptor based on the metal affinity of compounds that have ethylene-like or ethylene-antagonistic activities. For example, Ag(I), prevents ripening, by interfering with the ethylene-receptor interactions.

Figure 3.3 Ag^+ inhibits ethylene perception or action and consequently prevents ripening. Silver thiosulfate, which is a translocatable form of Ag +, was introduced to one side of the mature green fruit pedicel while it was still attached to the plant. A needle was used to insert a thread asymmetrically into the fruit stalk to act as a wick for the uptake of the solution. Ag + was only transported to half of the vascular tissue and delivered to one-half of the fruit, preventing those cells from responding to ethylene and they did not undergo ripening changes. See Davies et al. (1988), (1990).

Ethylene and Climacteric and Nonclimacteric Fruits

All plants produce some ethylene during their life cycle.

Often there is a **low** basal level, in tomato this is around 0.05 $nL.g^{-1}.h^{-1}$, which can **increase** 100-fold or more at particular stages of the life cycle, for example in response to wounding or pathogen attack, ripening, senescence, or abscission.

Increased respiration and a burst of ethylene biosynthesis are characteristics of the ripening process in many fleshy fruits, such as tomato, avocado, apple, melon, and banana, and these are called climacteric fruits

In other fruits, such as strawberry, grape, and citrus, ripening control was **originally!** thought to be independent of ethylene.

It is **becoming** clear, however, that ethylene may be involved in the development and aspects of ripening of some nonclimacteric fruits, such as citrus, white grape, and strawberry, although it seems that only low levels of ethylene are required.

There is recent evidence for small changes in ethylene biosynthesis genes, and ethylene production during ripening of strawberry (Trainotti et al., 2005).

Fei et al. (2004) compared the ripening genes of climacteric tomato fruits and nonclimacteric grapes and showed that the two species shared a subset of common ripening transcription regulators, including members of the MADS-box, zinc finger, and bZIP transcription factor families, suggesting that these genes have been conserved for ripening control during evolution, and are active in both climacteric and nonclimacteric fruits.

Thus, it has gradually become clear that the distinction between climacteric and nonclimacteric fruits, and the view that ethylene is only involved in controlling ripening of climacteric fruits, is an oversimplification. In general, fruit with the highest respiration rate tend to ripen most rapidly. Ethylene hastens the ripening of climacteric fruits and the increase in ethylene production associated with the respiratory rise is autocatalytic. There is clear evidence that, at least in some fruits, ethylene causes the climacteric rise in respiration. Climacteric and nonclimacteric types can be found in the same species, however, suggesting that small genetic differences underlie the climacteric trait, and in other cases different fruit parts, such as skin and pulp, may behave differently. It is likely, therefore, that as our molecular understanding increases, and the ripening behavior of a wider range of fruits is investigated at the genetic level, we may need to modify our classification of fruit types. The view that is emerging is of a basic underlying genetic program that controls climacteric and nonclimacteric ripening, which is modulated by a range of factors, including ethylene, particularly in the climacteric fruit types, but also involving other regulators (Fig. 3.2).

Biochemical evidence suggests that ethylene production may be influenced or regulated by interactions between its biosynthesis and other metabolic pathways. One such example is provided by the fact that SAM is the substrate for both the polyamine pathway and the nucleic acid methylation; the competition for substrate was demonstrated by the finding that the overexpression of a SAM hydrolase has been associated with inhibited ethylene production during ripening (Good et al., 1994). On the other hand, the methionine cycle directly links ethylene biosynthesis to the central pathways of primary metabolism.

Many fruits can ripen on the plant or tree, but some, such as avocado, only ripen after they are picked. A "**tree factor" that inhibits ripening** has been postulated to explain this effect.

Also, in strawberry it was recognized over 50 years ago that auxin, coming probably from the developing achenes, actually delayed the onset of ripening.

Harvesting can also hasten the ripening of some fruits and in such cases wound- or dehydration stress-ethylene from the calyx or calyx scar may also stimulate ripening.

It would be wrong to assume, however, that ethylene is the only hormone that affects ripening.

Auxin inhibits the ripening of strawberry, which is nonclimacteric.

Auxin is also involved in the regulation of some ethylene response factors (ERFs, transcription factors), which are important in climacteric fruit ripening, and there is some evidence of interactions between auxin and ethylene signaling during ripening.

Fruits need to be mature before they can effectively respond to ethylene—that is, they develop a competence to respond to ethylene and to ripen.

An intriguing observation, made many years ago is that "ethylene shortens the green life" (of banana). When bananas are picked green they will take a certain number of days to start to ripen.

If they are *transiently* exposed to ethylene after picking, the time that elapses before they eventually ripen is reduced.

Similarly, picked green tomatoes do not respond to ethylene when they are very immature, and they take a progressively shorter time to respond to external ethylene when they are picked closer and closer to the time when they would naturally ripen.

This tells us several things: firstly, ethylene synthesis is normally delayed until the fruit is ready to ripen, and secondly, either the ethylene response system is not developed, or is inhibited, until close to maturity. It is possible that other hormones play a role in these processes.

A Molecular Explanation for System-1 and System-2 Ethylene

Ethylene synthesis via the Yang cycle requires the action of two key enzymes:
ACC synthase (ACS)
and
ACC oxidase (ACO) (Fig. 3.5).

Reduction in the expression of tomato ACS2 and ACO1 by expressing antisense genes in transgenic plants inhibited or delayed ripening and spoilage in tomato and melon (Figs. 3.4, 3.5, 3.6, and 3.7).

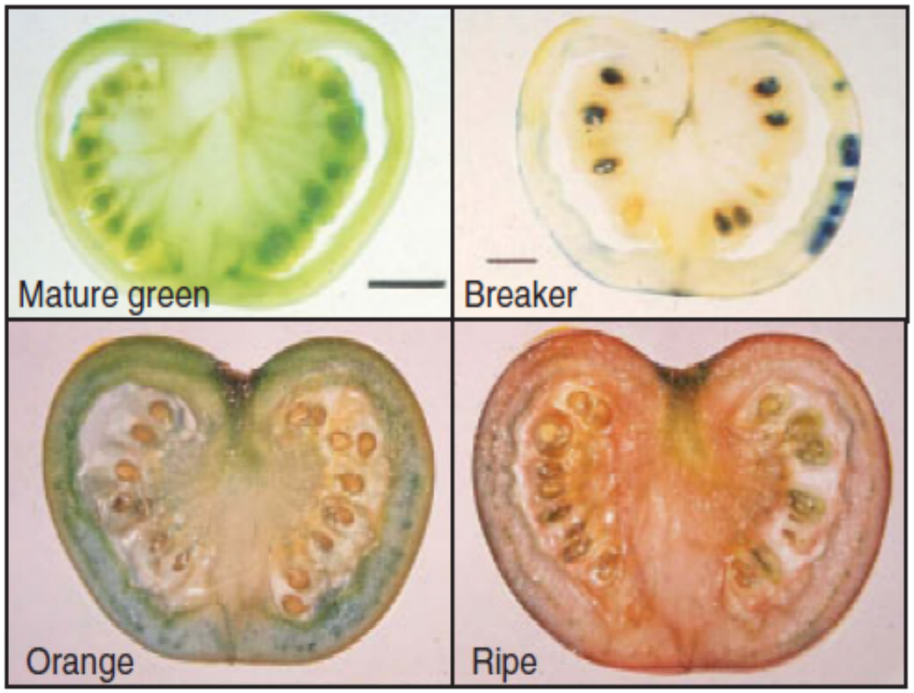


Figure 3.4 ACO1 promoter-Gus expression in transgenic tomatoes. The ACO1 gene promoter was fused to the GUS (β-glucuronidase) reporter gene, transformed into Ailsa Craig plants and tomatoes examined during fruit development and ripening. GUS activity, indicated by the blue staining, was evident around the vascular tissue at the breaker stage of ripening (first sign of color change) and was more uniformly distributed in orange fruits. Note that, although GUS staining is not really quantitative, the intensity of blue color corresponds approximately to the ethylene production curve during normal ripening (Fig. 3.5). See Blume

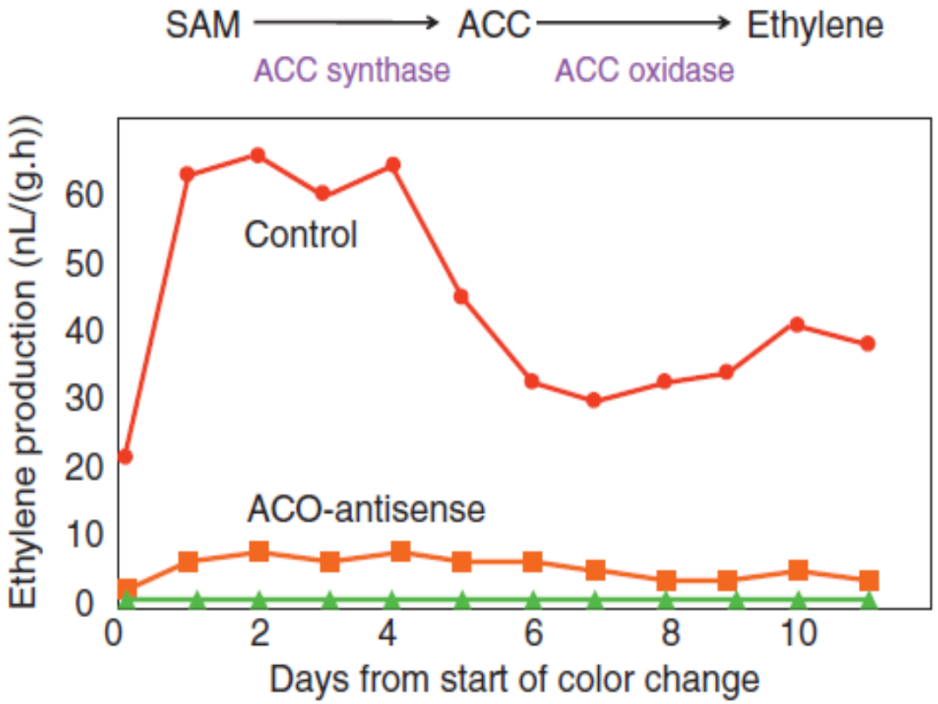


Figure 3.5 Gene silencing of ACO1 inhibits ethylene production in ripening fruit. Ethylene is synthesized from S-adenosyl methionine (SAM), to produce 1-amino-cyclopropane-1-carboxylic acid (ACC) by ACC synthase, which is then converted to ethylene by ACC oxidase. Before ACC oxidase was discovered, a possible candidate clone from tomato, TOM13, was used to construct an antisense gene under the control of the Cauliflower mosaic virus 35S promoter and transformed into tomato plants. Primary transformants were selected for those with the transgene and selfed, to produce progeny which were selected for 0, 1, or 2 transgenes and ethylene measured during fruit maturation and ripening. Control: red circles; one antisense gene: orange squares; two antisense genes: green triangles. The fruit with 2 antisense genes produced only 5% of the normal ethylene. They still ripened, but over-ripening and deterioration were strongly delayed. See Hamilton et al. (1990) and Figure 3.6. Expression of TOM13 in yeast confirmed that it encoded ACC oxidase (see Hamilton et al., 1991).

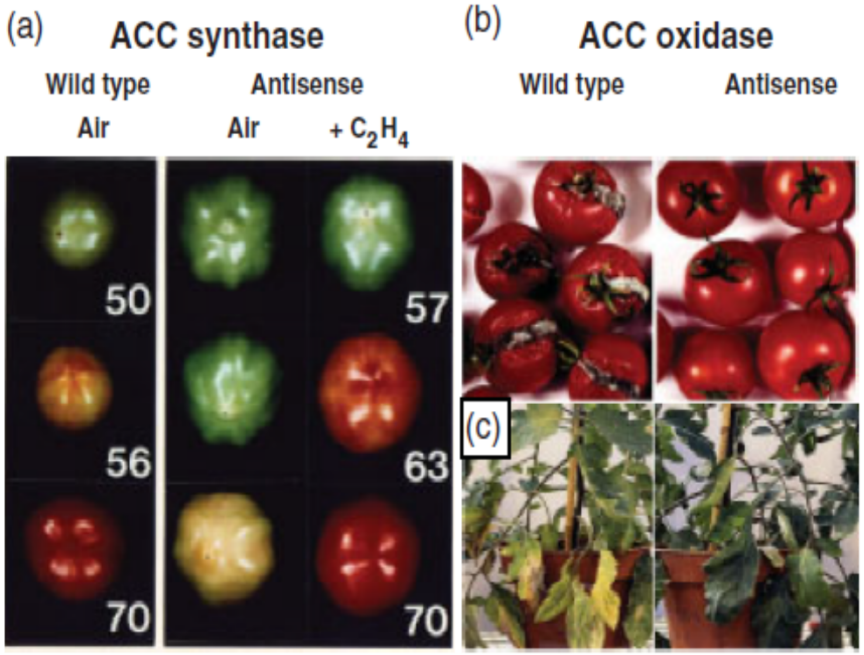


Figure 3.6 Antisense silencing of ACS (a) and ACO (b) inhibits ripening and senescence (c). Fruit expressing an ACS2 (ACC synthase 2) antisense gene, together with controls, are shown in (a). Note that in air the antisense fruit did not ripen, but ripening could be restored by adding external ethylene. The ACO1 (ACC oxidase) antisense gene only inhibited ethylene synthesis by approximately 95%: fruit ripened, but over-ripening and deterioration were greatly reduced (b). Also, leaf senescence was delayed (c). See Oeller et al. (1991); Picton et al. (1993), and Figure 3.5.

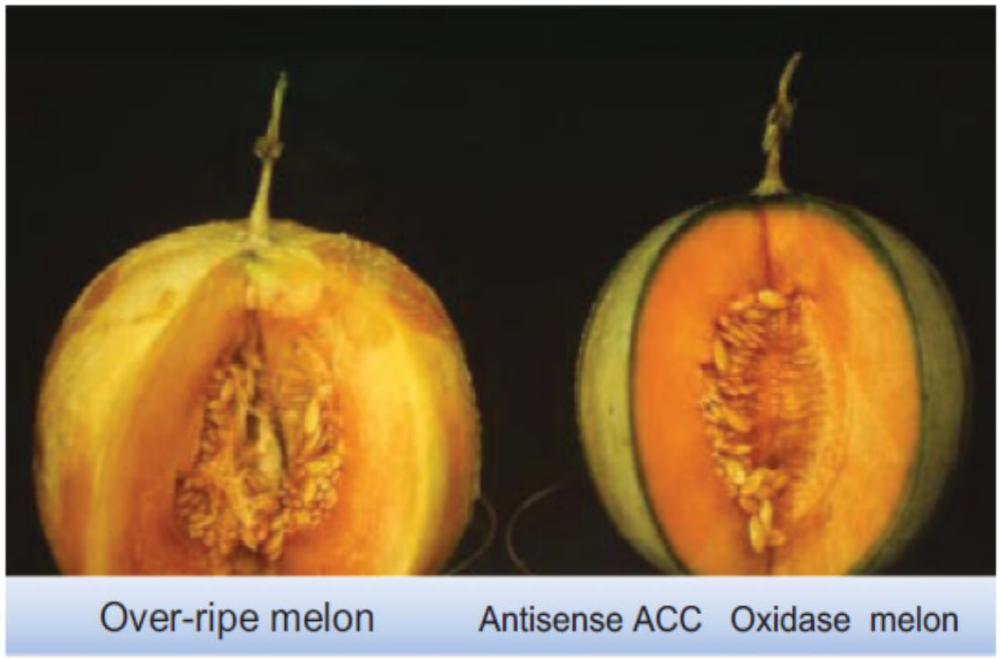


Figure 3.7 Inhibition of ripening of low ethylene melon by silencing the ACO1 gene. Transgenic melon expressing an ACO1 antisense gene produced extremely low levels of ethylene, and remained firm for much longer than the controls. In addition, the rind remained green and fruit remained attached to the plant for longer, because separation at the abscission zone was delayed, allowing fruit to accumulate more sugar. See Ayub et al. (1996).

- Low ethylene fruits have a much longer shelf life, because the expression of some ripening genes that lead to over-ripening, infection, and decay are reduced.
- Ethylene is also required for the full development of flavor and aroma, however and biotechnological control of ethylene needs to strike a balance between enhancing shelf life while preserving flavor and aroma of fruits.

- We now know that there are at least 14 ACS and ACO genes in plants such as Arabidopsis and tomato, and these are expressed at different times in the life cycle and in response to different developmental and environmental cues.

- In addition, some ethylene receptors and signaling molecules show different expression patterns during development.

A characteristic feature of ethylene synthesis in climacteric fruits is that some parts of the plant, including unripe fruits, synthesize low levels of ethylene, which is regulated by feedback inhibition but, after the onset of ripening, unrestrained or autocatalytic ethylene production begins and a major burst of ethylene evolution occurs, and this stimulates ripening. McMurchie et al. (1972) explained this autocatalytic rise in ethylene in climacteric fruits by proposing that **two systems (System-1 and System-2)** were involved in ethylene biosynthesis (reviewed by Lelievre et al., 1998). **System-1 functions** during normal vegetative growth, is autoinhibited by ethylene, and is responsible for producing the basal levels of ethylene that are synthesized by all plant tissues. Nonclimacteric fruit may be considered to be locked in the System-1 stage and only produce low levels of ethylene. **System-2** comes into play during the ripening of climacteric fruits, and during senescence, and requires the induction of new isoforms of ACS and ACO. These are regulated differently, give rise to much higher levels of ethylene, without autoinhibition (feedback inhibition) by ethylene.

In tomato, some *ACO* and *ACS* genes are expressed in unripe fruits while others show ripening-related expression, and the accumulation of their mRNAs can be either stimulated or inhibited by ethylene and developmental cues.

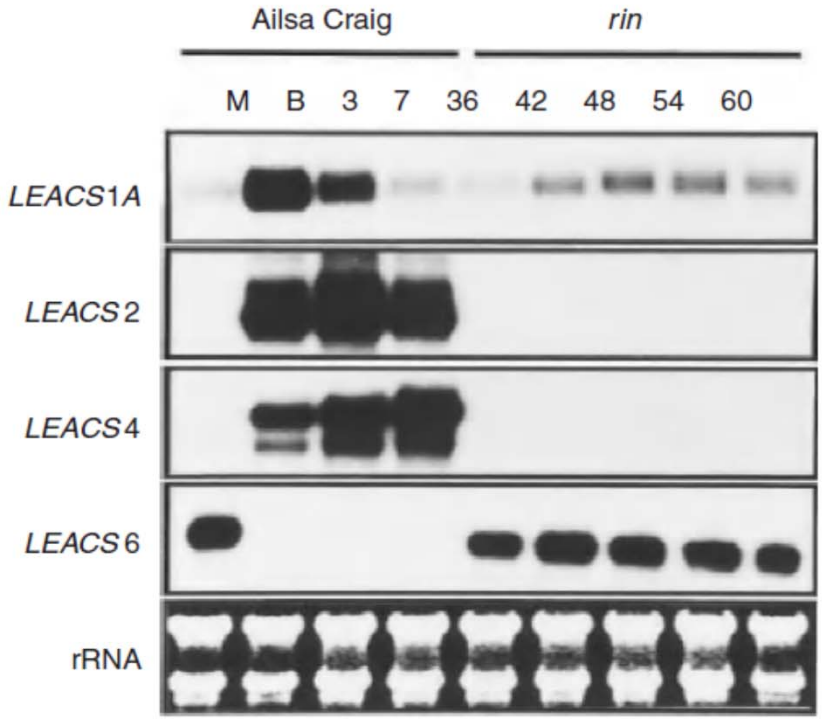


Figure 3.8 Expression of different ACS genes in developing tomato fruit. Gene-specific probes were hybridized with RNA from normal and *rin* fruit in the Pearson background at the mature green (M) and breaker (first sign of color change) stages, and at different days thereafter. In the case of *rin,* which does not ripen, fruits were studied for up to 60 days. Note that ACS2 and ACS4 are the most strongly expressed and ACS1A is transiently expressed during normal ripening, and these changes are greatly reduced or prevented in *rin* fruit. ACS6, on the other hand, is repressed during normal ripening but not in rin fruit. See Barry et al. (2000).