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Biochemistry and Molecular Physiology of Tomato and Pepper Fruit Ripening

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ABSTRACT

Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes in the metabolites, colour, texture, and flavour of the fruit. In the present paper, we survey recent findings in the areas of fruit chlorophyll degradation, carotenoid biosynthesis, volatiles, cell wall metabolism and central metabolism shift during tomato and pepper ripening. Moreover, the latest research on molecular aspects of the ethylene response is presented.

Keywords: carotenoids, cell walls, chlorophyll, ethylene, starch, volatiles
Abbreviations: **ACC**, 1-amino-cyclopropane-1-carboxylic acid; **ACS**, 1-amino-cyclopropane-1-carboxylate synthase; **AGPase**, ADP-glucose pyrophosphorylase; **AEDA**, aroma extract dilution analysis; **CCS**, capsanthin-capsorubin synthase; **DET-1**, De-etiolated-1; **ER**, endoplasmic reticulum; **ERF**, ethylene-response factor; **HRGCO**, high resolution gas chromatography-olfactometry; **MAPK**, mitogen activated protein kinase; **NR**, never-ripe; **PG**, polygalacturonase; **PME**, pectin methylesterase; **TCA**, tricarboxylic acid cycle

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INTRODUCTION

Ripening can be defined as the summation of changes in tissue metabolism rendering the fruit organ attractive for consumption by organisms that assist in seed release and dispersal. Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes in fruit metabolites, colour, texture, and flavour of the fruit (Seymour *et al*. 1993). Ripening is influenced by internal and external cues, including developmental gene regulation, hormones, light and temperature.

Fruits with different ripening mechanisms can be divided into two groups: climacteric and non-climacteric. In climacteric fruit, ripening is accompanied by a peak in respiration and a concomitant burst of ethylene, the levels of which decline during the subsequent course of ripening. In tomato (*Lycopersicon esculentum* Mill.), which is thought to be a climacteric fruit, the ethylene burst is required for normal fruit ripening, whereas in pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.), which are non-climacteric, it is not. In tomato, molecular analysis of fruit ripening focused on the roles of cell-wall metabolizing

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Invited Review

and structural proteins (Goff and Klee 2006), and on the genetic basis of ethylene synthesis (Cara and Giovannoni 2008). In pepper, studies have mainly centered on colour changes during fruit ripening (Barry *et al.* 2008) and carotenoid biosynthesis (Ha *et al.* 2007), and in eggplant on fruit phenolics (Whitaker and Stommel 2003).

In the present paper we survey some aspects of research on tomato and pepper biochemistry and the molecular physiology of ripening. It should be noted that whereas experimental work on tomato is abundant (Passam *et al.* 2007) that on pepper is much less extensive, while eggplant has not so far been researched at this level.

In recent years, the molecular biology of ripening has turned to genomic approaches to reveal insights into primary ripening control upstream of ethylene ripening-related signal transduction systems and downstream metabolic networks. These advances have been facilitated by increasingly efficient positional cloning in tomato, by the development of a model for ethylene signal transduction from *Arabidopsis* and by improved metabolic profiling technologies. The result has been the opening of a new frontier in ripening molecular biology that is focused on upstream transcriptional control and on the characterization of hormonal and environmental signaling mechanisms.

COLOUR CHANGE

Colour change is a dramatic event that occurs in fleshy fruits as they begin to ripen. In many fruits, including tomato and pepper, there is a sharp decrease in chlorophyll content and a concomitant increase in the synthesis of carotenoids as a result of the conversion of chloroplasts into chromoplasts (Seymour *et al.* 1993). While the degradation of chlorophyll is correlated with the reprogramming of cellular metabolism at the onset of fruit ripening, these two events are not necessarily interdependent.

Chlorophyll degradation in tomato and pepper

The chlorophyll degradation pathway follows the steps: chlorophyll *b* → chlorophyll *a* → chlorophyllide *a* → pheophorbide *a* → red chlorophyll catabolite → fluorescent chlorophyll catabolite → non-fluorescent chlorophyll catabolite (Hortensteiner 2006).

In higher plants chlorophyll *b* is initially converted to chlorophyll *a* by the action of chlorophyll *b* reductase, which in rice has been proposed to be a chloroplast short-chain dehydrogenase / reductase (Kusaba *et al.* 2007). The enzyme chlorophyllase catalyzes the conversion of chlorophyll *a* into chlorophyllide and phytol and this is thought to be the rate limiting step within the chlorophyll catabolite breakdown pathway (Jacob-Wilk *et al.* 1999; Tsuchiya *et al.* 1999; Harpaz-Saad *et al.* 2007). Pheophorbide *a* oxygenase is an Fe-dependent monooxygenase (Pruzinska *et al.* 2003) and converts pheophorbide *a* into red chlorophyll catabolite, which is in turn converted into fluorescent chlorophyll catabolite by red chlorophyll catabolite reductase (Wurthrich *et al.* 2000).

In chlorophyll retention base and general senescence phenotypes, stay green mutants are grouped into several classes (Thomas and Howarth 2000). In these mutants, chlorophyll retention and senescence phenomena are not always interconnected. In class C, for example, of stay green mutants, chlorophyll degradation is inhibited, but other aspects of senescence proceed normally. A group of class C mutants have reduced pheophorbide *a* oxygenase activity and stable pigment-protein complexes within the chloroplast (Thomas and Howarth 2000; Park *et al.* 2007; Ren *et al.* 2007; Sato *et al.* 2000). These loci encode a family of novel chloroplast proteins that may promote chlorophyll degradation via destabilization of protein-pigment complexes (Armstead *et al.* 2007; Jiang *et al.* 2007).

Fruits of the *green-flesh* mutants (Kerr 1956) of tomato on ripening display a muddy brown color due to the accumulation of lycopene coupled with a lack of chlorophyll degradation. In addition to the retention of chlorophyll, the thylakoid grana and light harvesting chlorophyll binding proteins, the Rubisco small subunit and the 33 kDa oxygen evolution protein also persist in mutant fruits (Cheung *et al.* 1993). As senescence-associated marker genes appear to display normal expression patterns in mutants (Akhtar *et al.* 1999) the above mentioned phemomena cannot be attributed to an inhibition of senescence, but rather are thought to result from the inhibition of chlorophyll degradation. Moreover, like *green-flesh* of tomato, fruit of the chlorophyll retainer mutant of pepper have ripe fruits that are brown in color due to an inhibition of chlorophyll degradation during ripening.

Carotenoid biosynthesis

Tomato

Among the most appreciated attributes of fruit are the possession of colour and flavour components, and their importance as a source of minerals, vitamins, fibres and antioxidants. For this reason a fuller comprehension of the biosynthetic pathways for the production of these components is of both applied and fundamental importance. During tomato fruit ripening, a massive accumulation of lycopene occurs as a result of the conversion of chloroplasts to chromoplasts. In addition, phytoene, ζ-carotene and phytofluene accumulate, while xanthophylls decrease (Fraser *et al.* 1994). Thus, lycopene accumulation in tomato fruits arises from an increased flux through the initial stages of the pathway and a restriction by end-products that are typically found in vegetative tissues (**Fig. 1**). In tomato, two phytoene synthase genes, Psy-1 and Psy-2, have been clarified (Giorio *et al.* 2007). Psy-1 is mainly expressed in ripening fruits. Overexpression of Psy-1 under a constitutive promoter in tomato elevated the carotenoid content, which indicates that this phytoene synthase exerts the greatest control of precursor flux into the carotenoid pathway (Fray *et al.* 1995), while cyclisation is reduced (Ronen *et al.* 2000; Fraser *et al.* 2002). The regulation of carotenoid formation in tomato fruits is thought to be controlled mainly at the transcriptional level (Fraser *et al.* 1994).

Isotope labeling and functional genomics have demonstrated that the geranylgeranyl pyrophosphate utilized in the formation of carotenoids is derived from a plastid localized desoxyxylulose 5-phosphate pathway and not from mevalonate pathways functioning in the cytoplasm (Rodriguez-Conception and Boronat 2002). The first carotene formed in this pathway is phytoene, which results from the condensation of two geranylgeranyl pyrophosphate molecules catalyzed by phytoene synthase. The six double bonds are introduced through three successive reactions resulting in prolycopene. Phytoene desaturase and ζ-carotene desaturase are involved in this procedure. The product of the desaturation reactions must be finally isomerised by carotene isomerase to all-*trans* lycopene (Isaackson *et al.* 2002). The cyclisation reactions of all-*trans* lycopene introduce b-ionone end groups. The reaction is catalysed by lycopene cyclase-b yielding β-carotene.

To address the question of the role of sugars in controlling carotenoid accumulation, tomato pericarp discs from mature green fruits were cultured *in vitro* in the presence of various sucrose concentrations (Telef *et al.* 2006). Sucrose limitation delayed and reduced lycopene and phytoene ac-

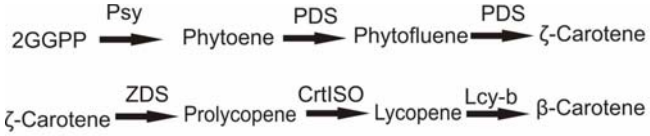

Fig. 1 A simplified scheme of β-carotene biosynthesis. GGPP, geranylgeranyl pyrophosphate; Psy, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CrtISO, carotene isomerase; Lcy-b, lycopene cyclase b.

cumulation, with no significant effect on other carotenoids. Chlorophyll degradation and starch catabolism were not affected by variations in sucrose availability. The reduction of lycopene synthesis observed under sucrose-limited conditions was mediated through metabolic changes characterised by reduced hexose accumulation levels.

Pepper

Similar to tomato, a quantitative and qualitative change in carotenoid composition arises as ripening proceeds (Camar *et al*. 1995). Capsanthin and capsorubin, two pepper carotenoids of major biological importance, are produced from antheraxanthin or violaxanthin respectively by the action of capsanthin-capsorubin synthase (CCS). The CCS gene is activated specifically during the final stages of pepper fruit ripening (Ha *et al.* 2002) and seems to produce capsaicinnoids only in the fruits (Estada *et al.* 2002).

Ripe pepper fruits can display a range of colours from white to deep red. Red peppers accumulate increasing levels of total carotenoids during ripening, whereas non-red peppers accumulate lower levels of total carotenoids of varying composition. The expression levels of the phytoene synthase, phytoene desaturase, and CCS genes are high in peppers with high levels of total carotenoids, whereas one or two of these genes are not expressed in peppers with lower levels of total carotenoids. The red colour of pepper fruit is determined by the y+ dominant allele and the yellow colour by the y recessive allele (Lefebvre *et al.* 1998). The CCS gene is present in two *Capsicum* varieties whose ripe colour is yellow, but CCS gene transcripts are absent (Ha *et al.* 2007). Sequence analysis of the CCS gene revealed two structural mutations in yellow peppers that may result in either a premature stop-codon or a frame-shift. This could suggest that nonsense-mediated transcriptional gene silencing of CCS, and not the deletion of this gene, is responsible for the yellow colour in *Capsicum*. Chromoplast proteome analysis of bell pepper fruits resulted in the identification of 150 proteins (Siddique *et al.* 2006). The majority of the identified proteins are related to plastid carbohydrate and amino acid metabolism. Among the most abundant proteins is CCS, suggesting a chromoplast-specific metabolic network.

Genetic engineering for carotenoid content and composition

An excellent review on genetic engineering for carotenoid biosynthesis has been presented by Sandmann *et al.* (2006). Much of the relevant research focused on transgenic plants. High β-carotene formation has been achieved by over-expression of an endogenous lycopene β-cyclase gene in tomato under a constitutive promoter (Rosati *et al.* 200; Dharmapuri *et al.* 2002; d'Abrosio *et al.* 2004). Phenotypes are stable over numerous generations with these non homologous genes. Moreover, the fruit specific silencing of *DET-1* (*De-etiolated-1*) gene in tomato has led to significant increases in carotenoids and other flavonoids (Davuluri *et al.* 2005). Similar findings have been reported by over-expression of the cryptochrome 2 gene product in tomato (Giliberto *et al.* 2005). Canthaxanthin and astaxanthin, are high nutritional value substances that are used as feed supplements. Gene products for astaxanthin formation have been expressed in higher plants (Mann *et al.* 2002; Stalberg *et al.* 2003; Morris *et al.* 2004; Ralley *et al.* 2004; Gerjets and Sandmann 2005).

VOLATILES

Flavour, formed in the intact fruit during ripening or upon tissue disruption, is the product of a complex mixture of sugars, acids, amino acids and volatile compounds (Baldwin *et al*. 1991).

Aroma extraction

Steam distillation is among the oldest techniques used to separate volatile from non-volatile material. Nickerson and Likens (1966) developed a versatile distillation unit for simultaneous extraction of steam distillates by solvents. Although aroma extracts can be obtained very fast and simply by this method, the elevated temperatures applied during distillation may lead to artifact formation, in particular when sugars and free amino acids are present in the food sample. In order to reduce the possibility of artifact formation, Weurman *et al*. (1970) developed a high vacuum distillation technique suitable for distilling the food its self or solvent extracts. The idea was to "transfer" the volatiles in an evacuated system to non-volatile material. Based on this high vacuum transfer technique, Schieberle and Grosch (1985) proposed a high vacuum sublimation equipment. However, the method has certain drawbacks such as partial condensation of aroma compounds with higher boiling points inside the tubing before reaching the traps, and only diethyl ether and dichloromethane extracts can be used.

Aroma extract dilution analysis (AEDA) (Ullrich and Grosch 1987) screens the odorants boiling higher than the solvent used for extraction of the food. This procedure starts with high resolution gas chromatography-olfactometry (HRGCO) of the original extract containing the volatiles. The extract is then concentrated stepwise by distilling off the solvent, and, after each step, an aliquot is analysed by HRGCO. To identify the highly volatile potent odorants, gas chromatography-olfactometry of headspace samples is also carried out (Holscher and Steinhart 1992). Guth and Grosch (1993) used AEDA analysis to identify acetic acid, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, *trans*-4,5-epoxy-(E)-2decanal, and eugenol as important fresh tomato odorants. The results of AEDA are expressed as a flavour dilution factor, which is the ratio of the concentration of the odorant in the initial extract to its concentration in the most diluted extract in which the odour can be detected by HRGCO. Consequently, the flavour dilution factor is a relative measure of the odour potency of a compound in a food extract (Grosch 1993).

A variation of this technique has been employed for the quantitative assay of major C_5-C_9 tomato volatiles using Tenax trapping and $CaCl_2$ enzyme deactivation (Buttery *et al.* 1987). A high vacuum was applied to the solvent-assisted flavour evaporation apparatus by means of a diffusion pump. From the vapour spray, which forms immediately, the volatiles and the solvent are transferred to the distillation head. The distillate enters a liquid nitrogen cooled flask. Volatiles, water and other solvents are condensed along the walls of the vessel. The identities of components are then confirmed by GC-MS methods.

Tomato

Tomato flavor has been extensively studied and more than 400 volatile compounds have been identified in tomato fruits (Buttery *et al.* 1971; Servili *et al.* 2000). Concentrations of selected odorants in three tasty (BR-139, FA-624 and FA-612) and two less tasty (R-144 and R-175) tomato cultivars are presented in **Fig. 2**. However, new constituents of sensory importance continue to be characterized (Mayer *et al.* 2008). Full favored tomatoes are characterized (Tandom *et al.* 2003) by a low level of acidity, a high content of total sugars and soluble solids, and an intermediate content of hexanal, *cis*-3-hexenal, 2- and 3-methyl-1-butanol, *trans*-2-hexenal, *cis*-3-hexenol, geranyl acetone, β-ionone, and 1-penten-3-one. The most common free volatiles (hexanal, 3-methylbutanol, *trans*-2-hexenal, 1-hexanol, *cis*-3-hexenol, quaiacol, benzyl alcohol, 2-phenylethanol, and eugenol) occur in concentrations of between 100-300 μg/l of tomato juice (Ortiz-Serrano and Gil 2007). The concentrations of volatile compounds of fruits can be increased by enzymatic hydrolysis of non-volatile precursors (Buttery *et al.* 1990, Baldwin *et al.* 2000). Most of precursor compounds in fruits

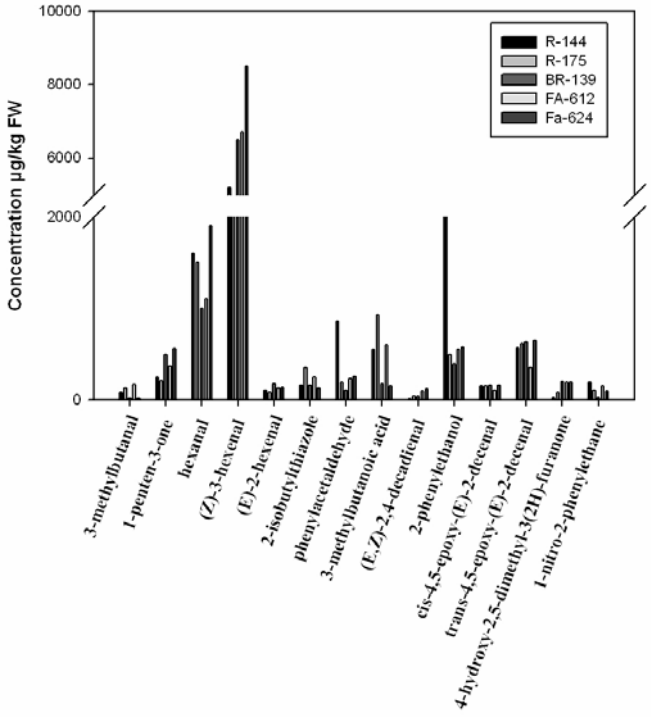

Fig. 2 Concentrations of selected odorants in three tasty (BR-139, FA-624 and FA-612) and two less tasty (R-144 and R-175) tomato cultivars. (Adapted from Mayer *et al.* 2008).

are glycosides, mainly *O*-β-D glycosides or *O*-diglycosides. The glucose moieties are attached to aglycones through a β-glycosidic linkage. Aglycones include monoterpenes, C_{11}/C_{13}-norisoprenoids, benzene derivatives and linear alcohols. In diglycosides, the glucose moiety is further substituted by various sugars such as α-L-arabinofuranose, α-L-arabinopyranose, α-L-rhamnopyranose, β-D-glucopyranose, β-D-apiofuranose, or β-D-xylopyranose (Williams 1993; Sarry and Günata 2004). The enzymatic release of volatiles from glycosides is catalyzed by β-glycosidases. Enzymatic hydrolysis of diglycosilate precursors can take place in one step by diglycosidases (Ogawa *et al.* 1997), or in two steps (Günata *et al*. 1988). However, the effect of glycosides on tomato flavor is still not completely understood (Sarry and Günata 2004).

Pepper

In samples of 13 different species of pepper and peppercorn, more than 300 volatile compounds have been characterized (Cardeal *et al.* 2006). Alpha thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, *o*-cymene, limonene, γ-terpinene, terpinen-4-ol, α-terpineol, carvone, α- and β-cubebene, α-copaene, *allo*-aromanderene and β-elemene were detected in all samples analyzed.

CELL WALL METABOLISM

Tomato

Fruit texture is among the principal quality traits determining the preferences of consumers and shelf life (Knee and Miller 2002) and is dependent on the integrity of the fruit cell walls. Fleshy fruits are predominantly composed of thin-walled parenchyma cells. The highly hydrophilic cell wall of tomato fruits is composed of pectin, cellulose, and hemicelluloses. The middle lamella is composed mainly of pectic substances cross-linked by calcium (Seymour and Gross 1996). Sugar phosphates and sugars are the precursors of pectic and hemicellulose polysaccharides (Scheible and Pauly 2004), which account for 90% of the cell wall (Redgwell and Fisher 2002). Pectins contain different structural domains that are classified as homoglacturans, type I rhamnogalacturans and type II rhamnogalacturans. Homogalacturans contain 100-200 uninterrupted galacturonates linked with 1-4 *a*-glycositic bonds (Willats *et al.* 2001a, 2001b; Bonnin *et al.* 2002) and can be methylated at position 6, acetylated at position 2 and/or 3 (Quéméner *et al.* 2003) or substituted by xylose (Le Goff *et al.* 2001), apiose or short xylose side chains on O-2 and /or O-3 (Oechslin *et al.* 2003). Type I rhamongalacturans are 1-4-*a*-linked galacturonic acid, interrupted by the insertion of 1-2 linked *a*-L-rhamnose and type II rhamongalacturans are complex structures with diverse sugars and linkages (Willats *et al.* 2001a).

During tomato fruit ripening a number of enzymes which are involved in cell wall modification are up regulated. The precise action of these enzymes, however, is not completely understood (Seymour *et al.* 2002; Brummell 2006). Pectic substances are reported to be hydrolyzed by a number of enzymes involving polygalacturanases, rhamnogalacturonases, β-galactosidases and pectin methylesterases. During tomato fruit ripening the activity of polygalacturonases, the enzymes that hydrolyze the linear polygalacturan backbones, increases dramatically (Della Penna *et al*. 1986). Among other hydrolytic enzymes that show high activity in fruits are rhamnogalacturonase and β-galactosidase (Gross *et al.* 1995). Although a number of tomato β-galactosidases are expressed during ripening (Smith and Gross 2000), the precise role of each is not known. Of the three genes (*TBG1, 3* and *4*) used in transgenic experiments in tomatoes only the repression of *TBG4* decreased fruit softening (Smith *et al.* 2002). Pectin methylesterases catalyze the de-esterification of pectins. In tomato three pectin methylesterases are expressed (Tucker and Zhang 1996). Down-regulation of a fruit specific methylesterase (PME2) resulted in an unaltered degree of fruit softening upon ripening but in reduced fruit firmness after 7 weeks at room temperature (Tieman *et al.* 1992).

Cellulases degrade carboxymethylcellulose. Their activity is generally associated with softening in tomato fruits, but the antisense suppression of a fruit-specific gene (Brummell *et al*. 1999) caused no change in the pattern of softening. Moreover, xyloglucan endotransglycosylase, which cleaves xyloglucans, is thought to be involved in ripening-related changes in cell wall of tomato fruit (Maclachlan and Brady 1994).

Expansins, a class of cell wall proteins, have been implicated in tomato fruit ripening. During fruit development an expansin is co-expressed with xyloglucan endotransglycosylase and cellulose encoding genes (Catala *et al.* 2000), while most other expansin genes are expressed during fruit development (Bertin 2005). The role of expansins in fruit ripening remains obscure.

Pepper

Depolymerization of non-xyloglucan matrix glycans is the prominent cell wall change observed during pepper ripening. Suppression of a ripening-related endo-1-4-β-glucanase in transgenic pepper fruit did not prevent depolymerization of cell wall polysaccharides during ripening (Harpster *et al.* 2002). Genetic evidence showed that polygalacturonase (PG1) is the candidate gene for the soft flesh and deciduous fruit mutation in *Capsicum*. Accumulation of PG1, mRNA and protein was detected in the fruit and it increased during ripening from the breaker to the red stage (Rao and Paran 2003). Therefore, the fruit-specific endo-polygalacturonase gene is thought to control polygalacturonase-mediated fruit softening, which is a major fruit ripening process. Recent evidence (Ogasawara *et al.* 2007) showed that during bell pepper fruit ripening, β-galastosidase activity increased markedly in comparison with other glycosidases and its pattern of activity follows the accumulation of polygalacturonase. A marked decrease in galactose content in the pectic fraction during ripening was observed, a fact that shows a major role of PG1 and β-galastosidase in fruit ripening (Ogasawara *et al.* 2007).

It is thus likely that in the coming years, our understanding both of the coordination of cell wall metabolism during fruit development and the consequences of temporal changes in wall metabolism on fruit ripening, and morphology in general, will be furthered.

CENTRAL METABOLISM SHIFT

After the start of flowering, developing fruits become important sinks. Fruit development comprises a cell-division phase, which follows pollination and usually lasts for two weeks (Bunger-Kibler and Bangerth 1983), followed by a cell-enlargement phase. Cell division and its regulation appear to be directly affected by the level of available carbohydrates and the form in which they are present (Francis and Halford 2006). During the cell enlargement phase, the fruit shows maximum growth rate and increase in size up to the mature green stage. Fruit ripening, however, is not accompanied by further growth (Gillapsy *et al.* 1993)

Sink strength of tomato fruit is principally affected (Waker and Ho 1977) by: (a) unloading of sucrose by the phloem, (b) hydrolysis and uptake of sugars, (c) biosynthesis and storage of carbohydrates (Ho *et al.* 1983). The regulation of primary carbohydrate metabolism and of the enzymes involved plays, therefore, an important role in determining the carbohydrate composition and level, and may a have large effect on the growth and the strength of sinks (Koch 2004).

Young tomato fruits undergo a transient period of starch accumulation (**Fig. 3**) (Ho and Hewitt 1986). Starch accumulation is heavy in the inner pericarp and columella tissue of the developing fruit (Wang *et al.* 1994) and may amount to *circa* 20% of dry weight in the young fruits, but is negligible in red ripe fruits. It has been proposed that transient starch functions as a carbohydrate reservoir during fruit development and contributes to soluble hexose levels in mature fruit (Dinar and Stevens 1981). The harvestable yield of tomato appears to be regulated among other factors by the rate of carbohydrate import into individual fruit and sink activity (Yelle *et al.* 1988). High accumulation of soluble solids can significantly increase the quality of the tomato, sugars being the major components and comprising approximately 65% of the soluble solids.

Sucrose, glucose and fructose are the major sugars found in tomato fruits, with high hexose accumulation being characteristic of domesticated tomato (*S. lycopesricum*) whereas some wild tomato species (*S. chmielewskii*) accumulate mostly sucrose (Yelle *et al.* 1991). Any discussion on sucrose metabolism of fruits should consider the route by which carbon enters the fruit. Tomato plants translocate sucrose (Waker and Ho 1997) which can be hydrolyzed via either invertase or sucrose synthase. Sucrose synthase is often associated with sucrose hydrolysis in starch metabolism (Quick and Schaffer 1996), and in tomato fruits its activity is correlated with transient starch accumulation (Beckles *et al.* 2001). However, sucrose synthase activity is not essential for starch synthesis, because its inhibition resulted in a reduced unloading capacity of sucrose in the initial stages of fruit development, but had only a small effect during ripening (D'Aoust *et al.* 1999). The action of sucrose synthase in the carbon metabolism of fruit during early development seems to be that of providing hexose phosphates (Roessner-Tunali *et al.* 2003). The enzyme ADP-glucose pyrophosphorylase catalyzes the synthesis of ADP-glucose in starch-synthesizing tissue. Its activity (Robinson *et al.* 1988) also follows the transient starch accumulation pattern. On the other hand, invertases hydrolyze sucrose into glucose and fructose. Three types of invertases have been purified so far in higher plants: the acid invertases which are ionically bound to the cell wall, the acid invertases localized in the vacuole (both of which show an optimal pH range of 4.5-5.0), and the cytosolic alkaline invertases, whose optimal pH range is 7.0-7.8 (Koch 2004; Roitsch and Gonzales 2004). Unlike the cytosolic isoforms, which appear to specifically hydrolyze sucrose, the vacuolar and cell wall invertases also hydrolyze other β-fructanosides, such as raffinose and stachyose. Apart from undergoing transcriptional control, the cell wall and vacuolar invertases seem to be controlled by post-translational mechanisms, such as developmentally regulated proteolytic degradation and the activity of proteinaceous inhibitors (Rausch and Greiner 2004). In tomato, a cell wall invertase (LIN5) (Fridman *et al.* 2004) is considered to be important for the establishment of sink strength and for apoplastic phloem unloading. In addition, it is thought that the invertase activity in the unloading zone leads to favorable conditions for the maintenance of mitotic activity and enhanced growth potential (Roitsch and Gonzales 2004). The expression pattern of this enzyme suggests that it is restricted to fruits and flowers (Fridman and Zamir 2003). Invertase antisense plants showed increased sucrose and decreased hexose concentrations in the fruits and 30% smaller fruits than those of the control plants (Klann *et al.* 1996). A detailed biochemical characterization of vegetative and fruit tissues of the introgression line carrying the Lin5 wild allele was reported by Baxter *et al*. (2005).

Fig. 3 Starch accumulation in tomato (blue to black color): (**A**) an immature green fruit, and (**B**) a late breaker fruit.

Starch formation and degradation

Earlier studies of the sucrose to starch transition in the tomato fruit suggested that fructokinase, sucrose synthase, and AGPase are likely to share in the control of the rate of starch accumulation (Schaffer and Petreikov 1997). Two different isoforms of fructokinase, exhibiting temporal and spatially dinstict expression patterns, have been detected (Kanayama *et al.* 1998). However, although both isoforms have been shown to play a role in floral initiation and abortion, seed number, and stem and root growth in tomato plants (Odanaka *et al.* 2002), their role in fruit metabolism has received far less attention. On the other hand, the recent application of the theory of metabolic control analysis to the same pathway in potato tubers suggested that only AGPase exhibited considerable control of starch synthesis (Davies *et al.* 2005; Geigenberge *et al.* 2005).

In spite of the fact that during ripening, massive starch hydrolysis occurs, virtually nothing is known about the enzymes involved, although work in our laboratories points to the involvement of β-amylase.

Metabolite changes

Through the analysis of over 70 primary metabolites, it was possible to distinguish three developmental stages of tomato fruits (green, orange and red) and follow the influence of hexose phosphorylation through fruit development by analyzing transgenic plants constitutively over-expressing an *Arabidopsis* hexokinase (AtHXK1) (Roessner-Tunali *et al.* 2003). Moreover, in a recent study, integrated analysis of metabolite and transcripts levels during tomato fruit development was performed (Carrari and Fernie 2006). Data from these studies show that glucose, fructose (**Fig.4**), mannose and maltose accumulate in ripe fruit, while the levels of minor sugars also displayed major shifts. Rhamnose and fucose are both rapidly and equally depleted during ripening, while galactose, xylose and arabinose display an in-

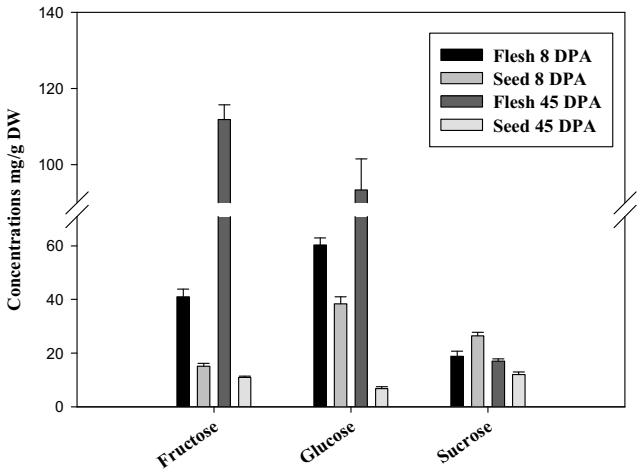

Fig. 4 Concentrations of fructose, glucose and sucrose in the flesh and seed of developing and red ripe tomato fruits. (Adapted from Mounet *et al.* 2007).

verse behaviour. Sugar alcohol levels tend to decline during development, although the levels of mannitol recover somewhat at later stages of ripening.

Levels of organic acids (Carrari and Fernie 2006) that are not associated with the TCA cycle generally display a different behaviour with respect to the developmental stage. Ascorbate, dehydroascorbate, *t*-caffeate, galacturonate, and galactonate-1-4-lactone increase either gradually or rapidly during the later stages of fruit development, whereas maleate and gulonate-1-4-lactone display variable behavior.

The levels of ascorbic acid (vitamin C) increased during ripening in all tissues, though its increase was generally largest between the green to breaker or the breaker to turning stages (**Fig. 5A**). When red fruit was compared to green, the ascorbic acid content was found to increase by nearly 10-fold in the placenta (Mounet *et al.* 2007).

Tocopherols are present at different concentrations in diverse parts of the tomato fruit (**Fig. 5B, 5C**). Vitamin E (α-tocopherol) is the most abundant tocopherol in all tissues and at all stages of fruit development, being lowest in the pericarp. Gamma-tocopherol, which is the biosynthetic precursor of α-tocopherol, was highest in the locular parenchyma and seeds of the tomato fruit (Mounet *et al.* 2007). The ratio α- to γ-tocopherol clearly differs between tissues, suggesting tissue-dependent differences in the activity of the corresponding γ-tocopherol methyltransferase. The levels of δ-tocopherol are relatively low in all tissues, while β-tocopherol is not detectable.

The total fatty acid content of tomato fruit (arachidic, behenic, linoleic, lignoceric, oleic, palrmitic and stearic acids) amounts to 0.09% flesh DW (Mounet *et al.* 2007). Whatever the tissue and the developmental stage, linoleic acid is always the major fatty acid (**Fig. 6**), followed in the flesh and seeds by palmitic and linolenic acids, which constitute the main fatty acids at 8 DPA. At 45 DPA, the major fatty acids did not change in the flesh, but in the seeds the picture is modified since palmitic and oleic acids are the most abundant after linoleic acid.

The level of amino acids (**Fig. 7**) is also highly variable during development. A gradual decline in metabolite levels was observed for GABA, β-Ala, Arg, Asn, Gln, pyroglutamate, Orn, Leu, and Val, while the levels of Ser, Ala and Pro decreased rapidly. In contrast, Trp, Cys, Glu, Asp, Lys, Met, and putrescine increased to a peak at fruit ripening. One of the most prominent changes associated with ripening tomatoes is a two-fold increase in Glu content in the tomato pericarp (Carrari and Fernie 2006). The aforementioned changes were broadly similar to those reported in earlier less extensive studies (Boggio *et al.* 2000; Chen *et al.* 2001), with major changes occurring between the green and red fruit. There was also a large increase in glucose and fructose within the cell wall components, as well as the aro-

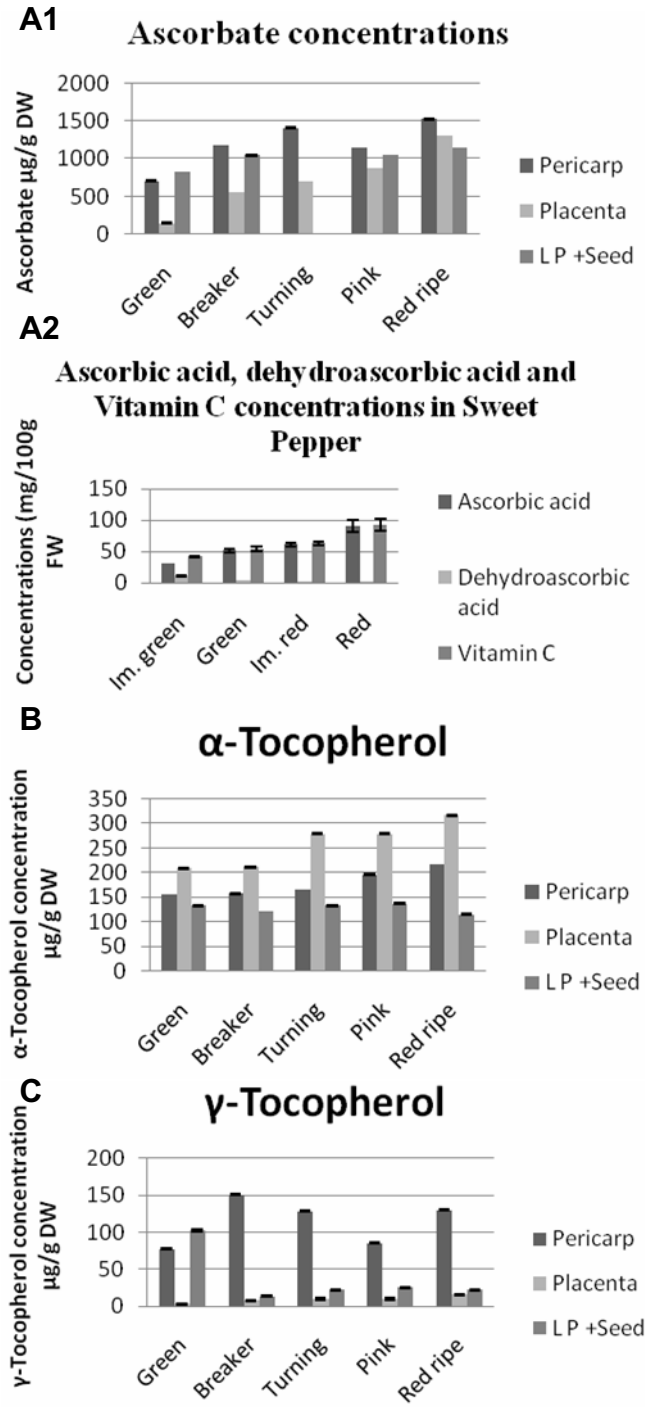

Fig. 5 Concentrations of ascorbic acid (**A1**), α-tocopherol (**B**) and γ-tocopherol (**C**) in the pericarp, placenta and locular parenchyma+seeds (LP+Seed) during tomato fruit development (Adapted from Moco *et al.* 2007). (**A2**) Concentrations of ascorbic acid, dehydroascorbic acid and vitamin C in sweet pepper during fruit development (Adapted from Marin *et al.* 2004).

matic amino acids, Asp, Lys, Met, and Cys. As might be expected there was also an increase in all pigments other than chlorophyll.

Organic acids

Although it is of central importance to the tomato fruit, relatively is currently known concerning the regulation of glycolysis and the biosynthesis of organic acids. Similarly, although organic acids are of fundamental importance both at the cellular and at the whole organism level, their study has received much less attention than that of sugars. Indeed, the TCA cycle in plants is very poorly characterized and although the structure of the cycle is well known, its regu-

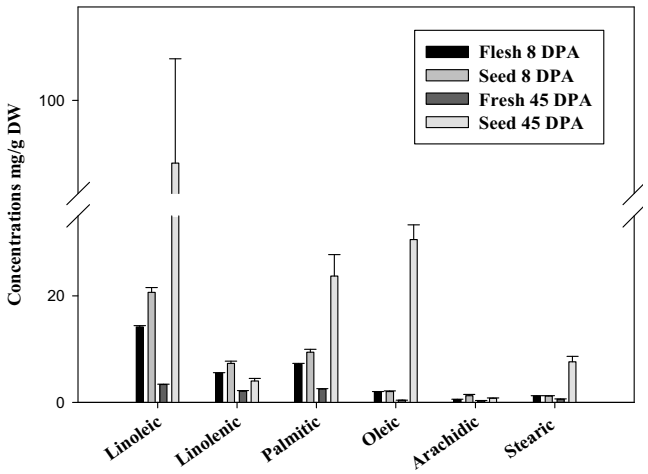

Fig. 6 Concentrations of the main fatty acids in the flesh and seed of red ripe tomato fruits. (Adapted from Mounet *et al.* 2007).

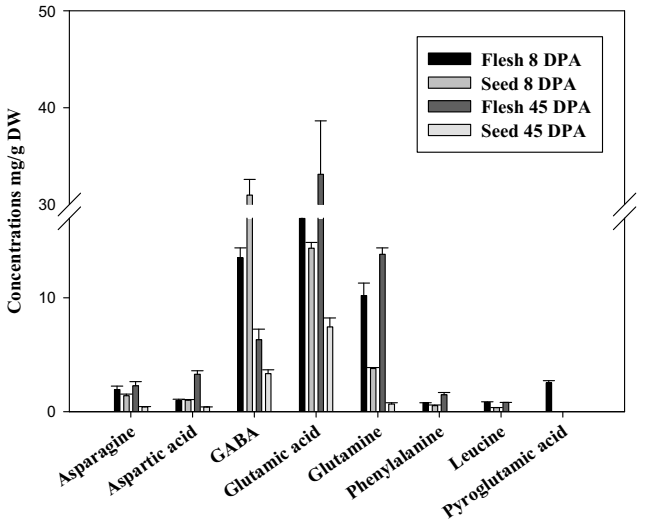

Fig. 7 Concentrations of selected amino acids in the flesh and seed of red ripe tomato fruits. (Adapted from Mounet *et al.* 2007).

lation is not (Fernie *et al*. 2004). The concentrations of organic acids of the TCA cycle (Carrari and Fernie 2006) showed a peak at around 56 days after anthesis. For the majority of organic acids of the TCA, there was a relative minor increase, but for citrate the increase was substantial. These changes could be attributed to the changes in activities of TCA cycle enzymes (Jeffrey *et al.* 1986). Although there are differences between American and European cultivars, the concentrations of citrate and isocitrate remain high until the later stages of fruit development. Because NADP-isocitrate dehydrogenase activity peaks in the ripe pericarp (Gallardo *et al.* 1995), it would be plausible to hypothesize that this enzyme supplies 2-oxoglutarate for amino acid biosynthesis and ammonia assimilation (Galvez *et al.* 1999).

MOLECULAR ASPECTS OF EHTYLENE RESPONSE IN TOMATO

Ethylene is a gaseous phytohormone that controls many processes of plant growth and development including fruit ripening, germination, organ senescence and stress responses. Among the processes controlled or influenced by ethylene, fruit ripening is one the most important to agriculture.

The role of ethylene in fruit ripening has been intensively studied in a number of plant species. However, tomato is an important model for the study of fleshy fruit development, essentially because this is the species for which well-characterized mutant stocks, efficient transient and stable transformation, extensive expressed sequence tags and microarrays resources, and high density genetic maps are available. In addition, over 30% of the tomato genome has been sequenced in an ongoing effort. Fruits with different ripening mechanisms can be divided into two groups: climacteric in which ripening is accompanied by a peak in respiration and a concomitant burst of ethylene release, and non-climacteric, in which respiration shows no dramatic change and ethylene production remains at a very low level. Tomatoes as climacteric fruits are characterized by an increase in respiration and a concomitant increase in ethylene biosynthesis just prior to the initiation of ripening.

Ethylene biosynthesis

Ethylene is formed from methionine which is converted to *S*-adenosyl-L-methionine by the enzyme 1-amino-cyclopropane-1-carboxylate synthase (ACS), then to methylthioadenosine and 1-amino-cyclopropane-1-carboxylic acid (ACC), the precursor of ethylene. ACC is oxidized to CO_2, HCN and ethylene by ACC oxidase (ACO). Tomato has at least eight ACS gene family members (*LeACSA1A*, *LeACSA1B*, *LeACSA2-7*), four of which are differentially regulated fruit ripening and wounding elicitors (Alexander and Grierson 2002). Four ACO genes have been identified in tomato so far; three of them (*LeACO1*, *LeACO3* and *LeACO4*) have been shown to be differentially expressed during fruit ripening. *Le*ACO3 transcripts are transiently accumulated at the breaker, pink, red and full-ripe stages and then disappear, whereas *LeACO1* and *LeACO4* transcripts are accumulated during the process of ripening (Nakatsuka *et al.* 1998; Cara and Giovannoni 2008).

The molecular basis of ethylene perception in tomato fruit

The skin of the tomato fruit is relatively impermeable to ethylene, so the gas builds up to high internal levels throughout the fruit. However, ethylene is readily diffusible within the confines of the fruit. It has been suggested that tomato fruits possess a capacity to measure cumulative ethylene through development and upon achievement of a certain cumulative exposure to ethylene, ripening is initiated (Klee 2004).

To achieve full ripening, climacteric fruits, such as tomato, require synthesis, perception and signal transduction of ethylene. Investigations into the ethylene response of ripening fruit have concentrated on the characterization of tomato homologues of *Arabidopsis* ethylene signal transduction genes. Ethylene is perceived by a family of membrane-localized receptors, of which at least six ethylene receptors have been identified in tomato (*LeETR1*, *2*, *4–6* and Never-ripe [NR], also called *LeCTR3*) (Klee and Tieman 2002). Based on gene and protein structures, the ethylene receptors have been divided into two subfamilies, subfamily I (*LeETR1*, *2* and *3*) contains the conserved kinase residues whereas subfamily II (*LeETR4*, *5* and *6*) lacks some conserved kinase residues. The receptors are disulfide-linked dimers, and ethylene binding is mediated by a copper co-factor (Cara and Giovannoni 2008).

The patterns of expression of the tomato ethylene receptors have been characterized. Each gene has a distinct pattern of expression in ripening fruit, and transcripts have also been found in other tissues, e.g. roots and leaves (Klee 2004). Genetic analysis in tomato and *Arabidopsis* has shown that the receptors act as negative regulators of the ethylene response pathway. In the absence of the hormone, receptors actively suppress ethylene responses. Upon ethylene binding, suppression is removed and the response occurs. In tomato, loss of a single subfamily II receptor, LeETR4, results in increased ethylene sensitivity. Antisense *LeETR4* plants show phenotypes consistent with a constitutive ethylene response, including significantly earlier fruit ripening. This mutant phenotype can be restored to wild-type by over-expression of the subfamily I receptor, *NR* (Tieman *et al.* 2000). It has been observed that in transgenic

tomato plants, where *NR* expression is reduced by antisense inhibition, expression of *LeETR4* increases proportionally. It appears, therefore, that somehow the tomato plant compensates for the loss of *NR* by increasing the expression of *LeETR4*. This phenomenon, referred to as functional compensation, has not been observed in *Arabidopsis* (Tieman *et al.* 2000; Kevany *et al.* 2007). Recent work on the tomato ethylene receptor family has demonstrated that receptor levels during fruit development determine the timing of ripening (Kevany *et al.* 2007). Protein levels are at their highest level during immature fruit development and decrease significantly at the onset of ripening, facilitating ethylene-mediated ripening processes. Ethylene treatment of immature fruit causes receptor degradation and earlier fruit ripening (Kevany *et al.* 2007). Fruit-specific suppression of the ethylene receptor *LeETR4* results in early-ripening tomato fruit (Kevany *et al.* 2008).

The molecular basis of the ethylene signaling pathway downstream to ethylene receptors in tomato fruit

In *Arabidopsis*, the ethylene signaling pathway downstream from the ethylene receptors (*CTR1*, *EIN3*, *EIL* and *ERF*) is well understood (Adams-Phillips *et al.* 2004a; Guo and Ecker 2004). Detailed knowledge of the ethylene signaling pathway defined in *Arabidopsis* enables comparative analyses to be carried out in other important crop species, such as tomato, where ethylene is critically involved in the fruit ripening process.

In tomato, ethylene signaling components have been defined, including a CTR-like gene (*LeCTR1*), through complementation of a *ctr1* mutant of *Arabidopsis* to function in ethylene signaling (Leclercq *et al.* 2002). *Arabidopsis* CTR1 has been assigned to a subclass of Raf-like mitogen-activated protein kinases (MAPK) (Cara and Giovannoni 2008). Antisense silencing of the *LeCTR1* gene resulted in plants with constitutive ethylene phenotypes, suggesting its physiological role in negatively regulating ethylene responses in tomato (Liu *et al.* 2002). Additional CTR (*LeCTR2*, *3* and *4*) genes have been identified in tomato (Adams-Phillips *et al.* 2004b). Recent studies using a yeast two-hybrid interaction assay have shown that the tomato receptors *LEETR1*, *LEETR2*, and *NR* can interact with multiple *LECTRs* (Zhong *et al.* 2008).

Homologues of *Arabidopsis EIN3*, *EIL* and *ERF* genes have also been identified and characterized in tomato. Four new members of the *ERF* (ethylene-response factor) family of plant-specific DNA-binding (GCC box) factors were isolated from tomato fruit (*LeERF1-4*). Four tomato *EIL* (ethylene insensitive) genes were identified and have been proposed to be functionally redundant positive regulators of multiple ethylene responses (Tieman *et al.* 2001; Yokotani *et al.* 2003). Recently, a novel gene (GR) was identified in tomato which is associated with the ethylene signaling pathway. Constitutive over-expression of *GR* in transgenic plants recreates the *Gr* mutant phenotype (ripening inhibition) but does not result in plants that display whole plant ethylene insensitivity (Barry and Giovannoni 2006). Tomato hosts at least two additional gene GR-family members, *GR1* and *GR2* (Cara and Giovannoni 2008).

CONCLUSIONS AND FUTURE PERSPECTIVES

Tomato ranks very high among the vegetables that are industrially produced and distributed throughout the world. They are perceived by the consumers as healthy and tasty vegetables. The properties of tomatoes beneficial to health are attributed to antioxidants, in particular lycopene, and their high content of vitamins, such as vitamin A and C. Consumer choice is driven by organoleptic quality (taste, aroma and color), origin of production, size and shape, agricultural production conditions and price.

Continuous research efforts have revealed a complex regulatory network involved in the developmental regulation of ripening in these fleshy fruits. Therefore, an increased understanding of the biochemistry and physiology of the fruit with the aid of new advents in functional genomics may contribute to the further improvement of tomato quality traits. Recently, major efforts by seed companies and researchers are being directed towards the improvement of quality traits (taste, flavor and health benefits) by conventional breeding or genetic engineering, without losing important agricultural characteristics or compromising consumer demand. Knowledge of the metabolic pathways permits the genetic construction of folate- and lycopene-fortified tomatoes. However, genetic modification approaches need to be carefully integrated with studies of the biochemistry and physiology of the fruit, as well as conventional breeding.

REFERENCES

Adams-Phillips L, Barry C, Giovannoni J (2004a) Signal transduction systems regulating fruit ripening. *Trends in Plant Science* **9**, 1360-1385

Adams-Phillips L, Barry C, Kannan P, Leclercq J, Bouzayen M, Giovannoni J (2004b) Evidence that CTR1-mediated ethylene signal transduction in tomato is encoded by a multigene family whose members display distinct regulatory features. *Plant Molecular Biology* **54**, 387-404

Akhtar M, Goldschmidt E-E, John I, Rodoni S, Matile P, Grierson D (1999) Altered patterns of senescence and ripening in *gf*, a stay-green mutant of tomato (*Lycopersicon esculentum* Mill.). *Journal of Experimental Botany* **50**, 1115-1122

Alexander VL, Grierson R (2002) Ethylene biosynthesis in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany* **53**, 2040-2055

Armstead I, Donnison I, Aubry S, Harper J, Hortensteiner S, James C, Mani J, Moffet M, Ougham H, Roberts L, Thomas A, Weeden N, Thomas H, King I (2007) Cross-species identification of Mendel's/locus. *Science* **315**, 73

Baldwin EA, Scott JW, Shewmaker CK, Schuch W (2000) Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *HortScience* **35**, 1013-1022

Baldwin EA, Nisperos-Carriedo MO, Baker R Scout JW (1991) Qualitative analysis of flavor parameters in six Florida tomato cultivars. *Journal of Agriculture and Food Chemistry* **39**, 1135-1140

Barry CS, Giovannoni JJ (2006) Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. *Proceedings of the National Academy of Sciences USA* **103**, 7923-7928

Barry CS, McQuinn RP, Chung MY, Besuden A, Giovannoni JJ (2008) Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the *green-flesh* and *chlorophyll retainer* mutations of tomato and pepper. *Plant Physiology* **147**, 179-187

Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AR, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. *Plant and Cell Physiology* **46**, 425-437

Beckles DM, Craig J, Smith AM (2001) ADP-glucose pyrophosphorylase is located in the plastid in developing tomato fruit. *Plant Physiology* **126**, 261-266

Bertin N (2005) Analysis of the tomato fruit growth response to temperature and plant fruit load in relation to cell division, cell expansion and DNA endoreduplication. *Annals of Botany* **95**, 439-447

Boggio SB, Palatnik JF, Heldt HW, Valle EM (2000) Changes in amino acid composition and nitrogen metabolizing enzymes in ripening fruits of *Lycopersicon esculentum* Mill. *Plant Science* **159**, 125-133

Bonnin E, Dolo E, Le Goff A, Thibault JF (2002) Characterisation of pectin subunits released by an optimised combinaison of enzymes. *Carbohydrate Research* **33**, 1687-1696

Brummell DA, Hall BD, Bennett AB (1999) Antisense suppression of tomato endo-1,4-beta-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Molecular Biology* **40**, 615-622

Brummell DA (2006) Cell wall disassembly in ripening fruit. *Functional Plant Biology* **33**, 103-119

Bunger-Kibler S, Bangerth F (1983) Relationship between cell number, cell size and fruit size of seeded fruits of tomato (*Lycopersicum esculentum* Mill.) and those induced parthenocarpically by the application of plant growth regulators. *Plant Growth Regulation* **1**, 143-154

Buttery RG, Seifert RM, Guadagni DG, Ling LC (1971) Characterization of additional volatile components of tomato. *Journal of Agriculture and Food Chemistry* **19**, 524-529

Buttery RG, Takeoka G, Teranishi R, Ling LC (1990) Tomato aroma components: Identification of glycoside hydrolysis volatiles. *Journal of Agriculture and Food Chemistry* **38**, 2050-2053

Buttery RG (1993) Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In Acree TE, Teranishi R (Eds) *Flavor Science: Sensible Principles and Techniques*, American Chemical Society, Washington

DC, pp 259-286
Camara B, Hugueney P, Bouvier F, Kuntz M, Moneger R (1995) Biochemistry and molecular biology of chromoplast development. *International Review of Cytology* **163**, 175-247
Cara B, Giovannoni J (2008) Molecular biology of ethylene during tomato fruit development and maturation. *Plant Science* **175**, 106-113
Cardeal ZL, Gomes da Silva MDR, Marriott PJ (2006) Comprehensive two-dimensional gas chromatography/mass spectrometric analysis of pepper volatiles. *Rapid Communications in Mass Spectroscopy* **20**, 2823-2836
Carrari F, Fernie AR (2006) Metabolic regulation underlying tomato fruit development. *Journal of Experimental Botany* **57**, 1883-1897
Catala C, **Rose JKC**, **Bennett AB** (2000) Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. *Plant Physiology* **122**, 527-534
Chen GP, **Wilson ID**, **Kim SH**, **Grierson D** (2001) Inhibiting expression of a tomato ripening-associated membrane protein increases organic acids and reduces sugar levels of fruit. *Planta* **212**, 799-807
Cheung AY, McNellis T, Piekos B (1993) Maintenance of chloroplast components during chromoplast differentiation in the tomato mutant green flesh. *Plant Physiology* **101**, 1223-1229
D'Ambrosio C, Giorio G, Marino I, Merendino A, Petrozza A, Salfi L, Stigliani AL, Cellini F (2004) Virtually complete conversion of lycopene into *β*-carotene in fruits of tomato plants transformed with the tomato lycopene *β*-cyclase (t*lcy-b*) cDNA. *Plant Science* **166**, 207-214
D'Aoust MA, Yelle S, Nguyen-Quoc B (1999) Antisense inhibition of tomato fruit sucrose synthase decreases fruit setting and the sucrose unloading capacity of young fruit. *The Plant Cell* **11**, 2407-2418
Davies HV, **Shepherd LVT**, **Burrell MM**, **Carrari F, Urbanczyk-Wochniak E, Leisse A, Hancock RD, Taylor MA, Viola R, Ross HA, McRae D, Willmitzer L, Fernie AR** (2005) Modulation of fructokinase activity of potato (*Solanum tuberosum*) results in substantial shifts in tuber metabolism. *Plant and Cell Physiology* **46**, 1103-1115
Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J, Bramley PM, Pennings HMJ, Bowler C (2005) Fruit-specifc RNAi-mediated suppression of *DET1* enhances tomato nutritional quality. *Nature Biotechnology* **7**, 825-826
Della Penna D, Alexander DC, Bennett AB (1986) Molecular-cloning of tomato fruit polygalacturonase – analysis of polygalacturonase messenger-RNA levels during ripening. *Proceedings of the National Academy of Sciences USA* **8**, 6420-6424
Dharmapuri S, Rosati C, Pallara P, Aquilani R, Bouvier F, Camara B, Giuliano G (2002) Metabolic engineering of xanthophyll content in tomato fruits. *FEBS Letters* **519**, 30-34
Dinar M, Stevens MA (1981) The relationship between starch accumulation and soluble solids content of tomato fruit. *Journal of the American Society for Horticultural Science* **106**, 415-418
Estrada B, Bernal MA, Diaz J, Pomar F, Merino F (2002) Capsaicinoids in vegetative organs of *Capsicum annuum* L. in relation to fruiting. *Journal of Agriculture and Food Chemistry* **50**, 1188-1191
Fernie AR, **Carrari F**, **Sweetlove LJ** (2004) Respiration: glycolysis, the TCA cycle and the electron transport chain. *Current Opinion in Plant Biology* **7**, 254-261
Francis D, Halford NG (2006) Nutrient sensing in plants. *Plant Molecular Biology* **60**, 981-993
Fraser PD, Truesdale RM, Bird CR, Schuch W, Bramley PM (1994) Carotenoid biosynthesis during tomato fruit development: evidence for tissue-specific gene expression. *Plant Physiology* **105**, 405-413
Fraser PD, Romer S, Shipton CA, Mills PM, Kiano JW, Misawa N, Drake RG, Schuch W, Bramley PM (2002) Biochemical evaluation of transgenic tomato plants expressing an addition phytoene synthase in a fruit-specific manner. *Proceedings of the National Academy of Sciences of the USA* **99**, 1092-1097
Fray RG, Wallace A, Fraser PD, Valero D, Hedden P, Bramley PM, Grierson D (1995) Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *The Plant Journal* **8**, 693-701
Fridman E, Zamir D (2003) Functional divergence of a synthetic invertase gene family in tomato, potato, and *Arabidopsis*. *Plant Physiology* **131**, 603-609
Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* **305**, 1786-1789
Gallardo F, Gálvez S, Gadal P, Cánovas FM (1995) Changes in $NADP^+$-linked isocitrate dehydrogenase during tomato fruit ripening. *Planta* **196**, 148-154
Gálvez S, Lancien M, Hodges M (1999) Are isocitrate dehydrogenases and 2-oxoglutarate involved in the regulation of glutamate synthesis? *Trends in Plant Science* **4**, 484-490
Geigenberger P, Regierer B, Nunes-Nesi A, Leisse A, Urbanczyk-Wochniak E, Springer F, van Dongen JT, Kossmann J, Fernie AR (2005) Inhibition of *de novo* pyrimidine synthesis in growing potato tubers leads to a compensatory stimulation of the pyrimidine salvage pathway and a subsequent increase in biosynthetic performance. *The Plant Cell* **17**, 2077-2088
Gerjets T, Sandmann G (2005) Potato tuber as a transgenic production system for keto carotenoids. *Carotenoid Science* **9**, 3139-3645
Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, Tavazza M, Giuliano G (2005) Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. *Plant Physiology* **137**, 199-208
Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. *The Plant Cell* **5**, 1439-1451
Giorio G, Stigliani AL, D'Ambrosio C (2007) Agronomic performance and transcriptional analysis of carotenoid biosynthesis in fruits of transgenic HighCaro and control tomato lines under field conditions. *Transgenic Research* **16**, 15-28
Goff SA, Klee J (2006) Plant volatile compounds: sensory cues for health and nutritional value? *Science* **311**, 815-819
Gross KC, **Starrett DA**, **Chen HL** (1995) Rhamnogalacturonase, β-galactosidase and α-galactosidase: potential role in fruit softening. *Acta Horticulturae* **398**, 121-130
Günata Z, Bitteur S, Brilloute JL, Bayonove C, Cordonnier R (1988) Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research* **184**, 139-149
Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. *Current Opinion in Plant Biology* **7**, 40-49
Guth H, Grosch W (1993) Identification of potent odorants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour and Fragrance Journal* **8**, 173-178
Ha SH, Kim, JB, Park JS, Lee SW, Cho KJ (2007) A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *Journal of Experimental Botany* **58**, 3135-3144
Hapster MH, Brummell DA, Dunsmuir P (2002) Suppression of a ripening-related endo-1,4-β-glucanase in transgenic pepper fruit does not prevent depolymerization of cell wall polysaccharides during ripening *Plant Molecular Biology* **50**, 345-355
Harpaz-Saad S, Azoulay T, Arazi T, Ben-Yaakov E, Mett A, Shiboleth YM, Hortensteiner S, Gidoni D, Gal-On A, Goldschmidt EE, Eyal Y (2007) Chlorophyllase is a rate-limiting enzyme in chlorophyll catabolism and is posttranslationally regulated. *The Plant Cell* **19**, 1007-1022
Ho LC, Hewitt JD (1986) Fruit development. In: Atherton JG, Rudich J (Eds) *The Tomato Crop, a Scientific Basis for Improvement*. Chapman and Hall, London, pp 201-240
Ho LC, Sjut V, Hoad GV (1983) The effect of assimilate supply of fruit growth and hormone levels in tomato plants. *Plant Growth Regulation* **1**, 155-171
Holscher W, Steinhart H (1992) Investigation of roasted coffee freshness with an improved headspace technique. *Zeitschrift für Lebensmitteluntersuchung und Forschung A* **195**, 33-38
Hortensteiner S (2006) Chlorophyll degradation during senescence. *Annual Review of Plant Biology* **57**, 55-77
Isaacson T, Ronen G, Zamir D, Hirschberg J (2002) Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for production of *β*-carotene and xanthophylls in plants. *The Plant Cell* **14**, 333-342
Jacob-Wilk D, Holland D, Goldschmidt EE, Riov J, Eyal Y (1999) Chlorophyll breakdown by chlorophyllase: isolation and functional expression of the Chlase1 gene from ethylene treated *Citrus* fruit and its regulation during development. *Plant Journal* **20**, 653-661
Jeffery D, Goodenough PW, Weitzman PDJ (1986) Enzyme activities in mitochondria isolated from ripening tomato fruit. *Planta* **168**, 390-394
Jiang HW, Li MR, Liang NB, Yan HB, Wei YL, Xu X, Liu JF, Xu Z, Chen F, Wu GJ (2007) Molecular cloning and function analysis of the stay green gene in rice. *Plant Journal* **52**, 197-209
Kanayama Y, **Granot D**, **Dai N**, **Petreikov M**, **Schaffer A**, **Powell A**, **Bennett AB** (1998) Tomato fructokinases exhibit differential expression and substrate regulation. *Plant Physiology* **117**, 85-90
Kenavy BM, Tieman DM, Taylor MG, Dalin V, Klee HJ (2007) Ethylene receptor degadation controls the timing of ripening in tomato fruit. *Plant Journal* **51**, 458-467
Kenavy BM, Taylor MG, Klee HJ (2008) Fruit-specific suppression of the ethylene receptor LeETR4 results in early-ripening tomato fruit. *Plant Biotechnology Journal* **6**, 295-300
Kerr EA (1956) Green flesh, gf. *Report of the Tomato Genetics Cooperative* **6,** 17
Klann EM, Hall B, Bennett AB (1996) Antisense acid invertase (*TIV1*) gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiology* **112**, 1321-1330
Klee HJ (2004) Ethylene signal transduction. Moving beyond Arabidopsis. *Plant Physiology* **135**, 660-667
Klee HJ, Tieman DV (2002) The tomato ethylene receptor gene family: form and function. *Physiologia Plantarum* **115**, 336-341
Knee M, Miller AR (2002) Mechanical injury. In: Knee M (Ed) *Fruit Quality and its Biological Basis*, Sheffield Academic Press, Sheffield, UK, pp 157-180
Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles

in sugar sensing and plant development. *Current Opinion in Plant Biology* **7**, 235-246

Kusaba M, Ito H, Morita R, Iida S, Sato Y, Fujimoto M, Kawasaki S, Tanaka R, Hirochika H, Nishimura M, Tanaka A (2007) Rice NON-YELLOW COLORING1 is involved in light harvesting complex II and grana degradation during leaf senescence. *The Plant Cell* **19**, 1362-1375

Leclercq J, Adams-Phillips LC, Zegzouti H, Jones B, Latche A, Giovannoni J, **Pech JC, Bouzayen M** (2002) LeCTR1, a tomato CTR1-like gene, demonstrates ethylene signaling ability in Arabidopsis and novel expression patterns in tomato. *Plant Physiology* **130**, 1132-1142

Lefebvre V, Kuntz M, Camara B, Palloix A (1998) Capsanthin-capsorubin synthase gene: a candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Molecular Biology* **36**, 785-789

Le Goff A, Renard CMGC, Bonnin E, Thibault JF (2001) Extraction, purification and chemical characterisation of xylogalacturonans from pea hulls. *Carbohydrate Polymers* **45**, 325-334

Liu Y, Schiff M, Dinesh-Kumar SP (2002) Virus induced gene silencing in tomato. *Plant Journal* **31**, 777-786

Maclachlan G, Brady C (1994) Endo-1,4-beta-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. *Plant Physiology* **105**, 965-974

Mann V, Harker M, Pecker I, Hirschberg J (2000) Metabolic engineering of astaxanthin production in tobacco flowers *Nature Biotechnology* **18**, 888-892

Marrin A, Ferreres F, Thomas-Barberan FA, Gil MI (2004) Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L). *Journal of Agriculture and Food Chemistry* **54**, 3861-3869

Marlatt C, Ho C, Chien MJ (1992) Studies of aroma constituents bound as glycosides in tomato. *Journal of Agriculture and Food Chemistry* **40**, 249-252

Mayer F, Takeoka GR, Buttery RG, Whitehand LC, Naim M, Rabinowitch HD (2008) Studies on the aroma of five fresh tomato cultivars and the precursors of *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenals and methional. *Journal of Agricultural and Food Chemistry* **56**, 3749-3757

Morris WL, Ducreux L, Griffiths DW, Stewart D, Davies HV, Taylor MA (2004) Carotenogenesis during tuber development and storage in potato. *Journal of Experimental Botany* **55**, 975-982

Nakatsuka A, Murachi S, Okunishi H, Shiomi S, Nakano R, Kubo Y, Inaba A (1998) Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. *Plant Physiology* **118**, 1295-1305

Nickerson GB, Likens ST (1966) Gas chromatographic evidence for the occurrence of hop oil components in beer. *Journal of Chromatography A* **21**, 1-5

Odanaka S, **Bennett AB**, **Kanayama Y** (2002) Distinct physiological roles of fructokinase isozymes revealed by gene-specific suppression of Frk1 and Frk2 expression in tomato. *Plant Physiology* **129,** 1119-1126

Ogawa K, Ijima Y, Guo W, Watanabe N, Usui T, Dong S, Tong Q, Sakata K (1997) Purification of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves (cv. Shuixian) to be processed to oolong tea. *Journal of Agricultural and Food Chemistry* **45**, 877-882

Oechslin R, Lutz MV, Amado R (2003) Pectic subtances isolated from apple celulosic residue: structural characterization of a new type of rhamnogalacturonan I. *Carbohydrate Polymers* **51**, 301-310

Ortiz-Serrano P, **Gil JV** (2007) Quantitation of free and glycosidically bound volatiles in and effect of glycosidase addition on three tomato varieties (*Solanum lycopersicum* L.) *Journal of Agricultural and Food Chemistry* **55**, 9170-9176

Ogasawara S, Abe K, Nakajima T (2007) Pepper β-Galactosidase 1 (PBG1) plays a significant role in fruit ripening in bell pepper (*Capsicum annuum*). *Bioscience, Biotechnology and Biochemistry* **71**, 309-322

Park JS, Yu JW, Park JS, Li J, Yoo SC, Lee NY, Lee SK, Jeong SW, Seo HS, Koh HJ, Jeon JS, Park YI, Paek NC (2007) The senescence-induced stay-green protein regulates chlorophyll degradation. *The Plant Cell* **19**, 1649-1664

Passam HC, Karapanos JC, Bebeli PJ, Savvas D (2007) A review of recent research on tomato nutrition, breeding and post-harvest technology with reference to fruit quality. *European Journal of Plant Science and Biotechnology* **1**, 1-21

Pruzinska A, Tanner G, Anders I, Roca M, Hortensteiner S (2003) Chlorophyll breakdown: Pheophorbide a oxygenase is a Rieske-type iron-sulfur protein, encoded by the accelerated cell death 1 gene. *Proceedings of the National Academy of Sciences of the USA* **100**, 15259-15264

Quéméner B, Cabrera Pino JC, Ralet M-C, Bonnin B, Thibault J-F (2003) Assignment of acetyl groups to O-2 and/or O-3 of pectic oligogalacturonides using negative Electrospray-Ion Trap Mass Spectrometry. *Journal of Mass Spectrometry* **38**, 641-658

Quick WP, Schaffer AA (1996) Sucrose metabolism in sources and sinks. In: Zamski E, Schaffer AA (Eds) *Photoassimilate Distribution in Plants and Crops*, Marcel Dekker, New York, USA, pp 115-156

Ralley L, Enfissi EM, Misawa N, Schuch W, Bramley PM, Fraser PD (2004) Metabolic engineering of ketocarotenoid synthesis in higher plants. *The Plant Journal* **39**, 477-486

Rao GU, Paran I (2003) Polygalacturonase: a candidate gene for the soft flesh and deciduous fruit mutation in *Capsicum*. *Plant Molecular Biology* **51**, 135-141

Rausch T, Greiner S (2004) Plant protein inhibitors of invertases. *Biochimica et Biophysica Acta* **1696**, 253-261

Redgwell RJ, Fischer M (2002) Fruit texture, cell wall metabolism and consumer perceptions. In: Knee M (Ed) *Fruit Quality and its Biological Basis*, Sheffield Academic Press, Sheffield, UK, pp 46-88

Ren GD, An K, Liao Y, Zhou X, Cao YJ, Zhao HF, Ge XC, Kuai BK (2007) Identification of a novel chloroplast protein AtNYE1 regulating chlorophyll degradation during leaf senescence in arabidopsis. *Plant Physiology* **144**, 1429-1441

Robinson NL, Hewitt JD, Bennett AB (1988) Sink metabolism in tomato fruit. I. Developmental changes in carbohydrate metabolizing enzymes. *Plant Physiology* **87**, 727-730

Rodríguez-Concepción M, **Boronat A** (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. *Plant Physiology* **130**, 1079-1089

Roessner-Tunali U, Hegemann B, Lytovchenko A, Carrari F, Bruedigam C, Granot D, Fernie AR (2003) Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. *Plant Physiology* **133**, 84-99

Roitsch T, Gonzalez MC (2004). Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science* **9**, 606-613

Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. *Proceedings of the National Academy of Sciences of the USA* **97**, 11102-11107

Rosati C, Aquilani R, Dharmapuri S, Pallara P, Marusic C, Tavazza R, Bouvier F, Camara B, Giuliano G (2000) Metabolic engineering of beta carotene and lycopene content in tomato fruit. *The Plant Journal* **24**, 413-419

Sandmann G, Römer S, Fraser PD (2006) Understanding carotenoid metabolism as a necessity for genetic engineering of crop plants. *Metabolic Engineering* **8**, 291-302

Sarry J, Günata Z (2004) Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. *Food Chemistry* **87**, 509-521

Sato Y, Morita R, Nishimura M, Yamaguchi H, Kusaba M (2007) Mendel's green cotyledon gene encodes a positive regulator of the chlorophyll-degrading pathway. *Proceedings of the National Academy of Sciences of the USA* **104**, 14169-14174

Schaffer AA, Petreikov M (1997) Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. *Plant Physiology* **113**, 739-746

Scheible WR, Pauly M (2004) Glycosyltransferases and cell wall biosynthesis: novel players and insights. *Current Opinion in Plant Biology* **7**, 285-295

Schieberle P, Grosch W (1985) Photolyse von 13(S) Hydroperoxy-S(Z), 11(E)-octadecadiensluremethylester in Gegenwart von Sauerstoff: Analyse der nidermolekularen Reaktionprodukte. *Fette Seifen Anstrichm* **87**, 76-80

Servili M, Selvaggini R, Taticchi A, Begliomini AL, Montedoro G (2000) Relationships between the volatile compounds evaluated by solid phase microextraction and the thermal treatment of tomato juice: optimisation of the blanching parameters. *Food Chemistry* **71**, 407-415

Seymour GB, Taylor JE, Tucker GA (1993) *Biochemistry of Fruit Ripening*, Chapman and Hall, London, UK, 464 pp

Seymour GB, Gross KC (1996) Cell wall disassembly and fruit softening. *Postharvest News and Information* **7**, 45-52

Seymour GB, Manning K, Erikson E, Popovich A, King G (2002) Genetic identification and genomic organization of factors affecting fruit texture. *Journal of Experimental Botany* **53**, 2065-2071

Siddique MA, Grossmann J, Gruissem W, Baginsky S (2006) Proteome analysis of bell pepper (*Capsicum annuum* L.) chromoplasts. *Plant and Cell Physiology* **47**, 1663-1673

Smith DL, Gross KC (2000) A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. *Plant Physiology* **123**, 1173-1183

Smith DL, Abbott JA, Gross KC (2002) Down-regulation of tomato beta-galactosidase 4 results in decreased fruit softening. *Plant Physiology* **129**, 1755-1762

Stalberg K, Lindgren O, Ek B, Höglund AS (2003) Synthesis of ketocarotenoids in the seed of *Arabidopsis thaliana*. *The Plant Journal* **36**, 771-779

Tandon KS, Baldwin EA, Scott JW, Shewfelt RL (2003) Linking sensory descriptors to volatile and non-volatile components of fresh tomato flavor. *Journal of Food Science* **68**, 2366-2371

Telef N, Stammitti-Bert L, Mortain-Bertrand A, Maucourt M, Carde JP, Rolin D, Gallusci P (2006) Sucrose deficiency delays lycopene accumulation in tomato fruit pericarp discs. *Plant Molecular Biology* **62**, 453-469

Thomas H, Howarth CJ (2000) Five ways to stay green. *Journal of Experimental Botany* **51**, 329-337

Tieman DV, Taylor MG, Ciardi JA, Klee HJ (2000) The tomato ethylene receptor NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. *Proceedings of the National Academy of Sciences of the USA* **100**, 352-357

Tieman DM, Ciardi JA, Taylor MG, Klee HJ (2001) Members of the tomato LeEIL (EIN3-like) gene family are functionally redundant and regulate ethy-

lene responses throughout plant development. *The Plant Journal* **26**, 47-58

Tieman DM, Harriman RW, Ramamohan G, Handa AK (1992) An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. *The Plant Cell* **4**, 667-679

Tsuchiya T, Ohta H, Okawa K, Iwamatsu A, Shimada H, Masuda T, Takamiya K (1999) Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: finding of a lipase motif and the induction by methyl jasmonate. *Proceedings of the National Academy of Sciences of the USA* **96**, 15362-15367

Tucker G, Zhang J (1996) Expression of polygalacturonase and pectinesterase in normal and transgenic tomatoes. In: Visser J, Voragen AGJ (Eds) *Progress in Biotechnology: 14. Pectins and Pectinases*, Elsevier, Amsterdam, The Netherlands, pp 347-353

Walker AJ, Ho LC (1977) Carbon translocation in the tomato: carbon import and fruit growth. *Annals of Botany* **43**, 813-823

Wang F, Smith AG, Brenner ML (1994) Temporal and spatial expression pattern of sucrose synthase during tomato fruit development. *Plant Physiology* **104**, 535-540

Whitaker BD, Stommel JR (2003) Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *Journal of Agricultural and Food Chemistry* **51**, 3448-3454

Willats WG, McCartney L, Mackie W, Knox JP (2001a) Pectin: cell biology and prospects for functional analysis. *Plant Molecular Biology* **47**, 9-27

Willats WGT, Orfila C, Limberg G, Buchholt HC, Gert-Jan W, van Alebeek M, Voragen AGJ, Susan E, Tove M, Christensen MIE, Mikkelsen JD, Murray BS, Knox JP (2001b) Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls. Implications for pectin methyl esterase action, matrix properties, and cell adhesion. *Journal of Biological Chemistry* **276**, 19404-19413

Williams PJ (1993) Hydrolytic flavor release in fruit and wines through hydrolysis of non-volatile precursors. In: Acree TE, Teranishi R (Eds) *Flavor Science: Sensible Principles and Techniques*, American Chemical Society, Washington DC, USA, pp 287-308

Wüthrich KL, Bovet L, Hunziker PE, Donnison IS, Hortensteiner S (2000) Molecular cloning, functional expression and characterisation of RCC reductase involved in chlorophyll catabolism. *The Plant Journal* **21**, 189-198

Yelle S, Hewitt JD, Robinson NL, Damon S, Bennett AB (1988) Sink metabolism in tomato fruit. III. Analysis of carbohydrate assimilation in a wild species. *Plant Physiology* **87**, 737-740

Yelle S, Chetelat RT, Dorais M, Deverna JW, Bennett AB (1991) Sink metabolism in tomato fruit. IV. Genetic and biochemical analysis of sucrose accumulation. *Plant Physiology* **95**, 1026-1035

Yokotani N, Tamura S, Nakano R, Inaba A, Kubo Y (2003) Characterization of a novel tomato EIN3-like gene (*LeEIL4*). *Journal of Experimental Botany* **54**, 2775-2776

Zhong S, Lin Z, Grierson R (2008) Tomato ethylene receptor–CTR interactions: visualization of NEVER-RIPE interactions with multiple CTRs at the endoplasmic reticulum. *Journal of Experimental Botany* **59**, 965-972