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Review

The metabolic shifts underlying tomato fruit development

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Abstract Tomato (*Solanum lycopersicum*) became a model crop species to study development and maturation of fleshy fruits. The network of information and resources recently established by the International Solanaceae Genome Project (SOL www.sgn.cornell.edu) will not only provide valuable sequence information but also new tools applicable to large scale profiles at different levels of cell organization. This will surely render to the discovery of new potential targets for manipulation of fruit compositional traits. To date, a large amount of knowledge has been gathered on ethylene biosynthesis and response and cell wall metabolism during tomato fruit development and ripening. Less attention, however, has been given to the central metabolism that underpins these responses. Here we review recent reports focussed on the identification of key points on the metabolic regulation underlying tomato fruit development. Additionally, an overview of the combined application of metabolic and transcriptional profiling, aimed at identifying candidate genes for modifying metabolite contents, is discussed in the context of the usefulness for tomato breeding programs.

Key words: Fruit metabolism, *Solanum* genus.

Historical view of metabolic influence on tomato fruit ripening

Nutritional value of foodstuffs is of increasing important to consumers. This is, at least in part, due to the increasing transfer of scientific information from this research field to the popular press. Amongst the most appreciated fruit attributes are the possession of colourful and flavour components and also 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 nutrients is of applied as well as fundamental importance. Whilst plant model systems such as Arabidopsis may be a suitable starting point in the search for key regulatory mechanisms acting in fruit development and ripening (Liljegren et al. 2004), it must be borne in mind that the term fruit encompasses an enormous diversity of different kinds of organs. Thus, although fundamental development processes might be shared among different plant species, this cannot be blithely assumed. Indeed there are dramatic developmental differences across species, even those of the same family (Fernie and Willmitzer 2001). This fact is one of the main reasons of which considerable effort is being put into genomic and post-genomic study of plant species other than Arabidopsis (Mueller et al. 2005; Desbrosses et al. 2005; Carrari et al. 2004; Goff et al. 2002). One example of this is the use of tomato (*Solanum lycopersicum*), as a model system for plants bearing fleshy fruits. Several features of the tomato fruit make it a highly interesting system to study: all of them linked to the dramatic metabolic changes that occur during development. The most obvious of these changes is the transition from partially photosynthetic to fully heterotrophic metabolism that occurs coincidentally with the differentiation of chloroplasts into chromoplasts (Bartley et al. 1994), marked shifts in cell wall composition (Rose et al. 2004; Scheible and Pauly 2004), and the strict hormonal control of climacteric ripening (Barry et al. 2005; Alba et al. 2005).

Climacteric fruits such as tomato are distinguished from those that are non-climacteric by their increased respiration and ethylene biosynthesis rates during ripening. This is one of the major reasons that the majority of biochemical research has concentrated on this hormone. Initial molecular studies focussed on the isolation of ethylene-regulated genes which include those encoding the ethylene biosynthesis enzymes (S-adenosylmethionine –SAM-synthase, 1-aminocyclopropane carboxylic acid –ACC-synthase and ACC oxidase), and cell wall disassembling enzymes such as endo-polygalacturonase (PG) and pectin methylesterase (PME) (reviewed by Redgwell and

Fischer 2002). Later studies demonstrated that either lowering the amount of ethylene produced or delaying its production constituted a successful strategy to extend the shelf life of fruits (Grierson 1992) and to improve their flavour as was reported in melon fruit (Ayub et al. 1996). Biochemical evidence suggests that ethylene production may well be influenced or regulated by interactions between its biosynthesis and other metabolic pathways. One such example is provided by the fact that S-adenosylmethionine is not only substrate for the polyamine pathway but also for 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 (MET) cycle directly links ethylene biosynthesis to the central pathways of primary metabolism.

Another approach to study ethylene actions in regulating tomato ripening come from the use of mutants in terms of responsiveness to the hormone. These mutants fail to undergo an increase in ripening-related ethylene production. An example of this are *rin* (ripening inhibitor) and *nor* (non-ripening) mutants. Molecular cloning of the *rin* locus revealed that it compromise MADS box genes, carpel homeotic genes that are key determinants of carpel development, but only one of which was necessary for ripening (Vrebalov et al. 2002). *Nor* encodes a transcription factor (Giovannoni et al. 2001) but the exact molecular mechanism of its operation remains unknown. Other fruit ripening mutants identified on the basis of their insensitivity to ethylene are *Never ripe* (*Nr*) (Lanahan et al. 1994), *green ripe* (*Gr*) and *Never ripe 2* (*Nr2*) (Barry et al. 2005). Since the first demonstration that the *Nr* locus encodes an ethylene receptor (Wilkinson et al. 1995) a broad gene family of receptors has been cloned and their expression analysed in several species (for review see Adams-Phillips et al. 2004). However, the analysis of transgenic plants with reduced *Nr* levels showed that this gene is not necessary for the ripening program to proceed (Hackett et al. 2000) suggesting that the other fruit specific member of the receptor family can compensate for its deficiency (Tieman et al. 2000). The *Gr* and *Nr2* spontaneous mutants were identified in the early 80s (Jarret et al. 1984; Kerr 1981, 1982), and recently physiologically characterized (Barry et al. 2005). In addition to the reduction in the rate of ripening due to ethylene insensitivity of the fruits, this phenotype was found to be extended to other unrelated ripening processes such as floral senescence, abscission and root elongation. Following their genetic mapping these loci were found to be tightly linked on the long arm of chromosome 1. When taken together with the similar dominant phenotypes of the mutants these results suggested that they might be allelic. This speculation was recently confirmed, beyond doubt, by Barry and Giovannoni (2006). A further mutant worthy of mention is colorless non-ripening (*Cnr*), which results in mature fruits with colorless pericarp tissue showing excessive loss of cell adhesion (Thompson et al. 1999). This mutant showed an altered expression and activity of a wide range of cell wall-degrading enzymes during development and ripening. Furthermore, microarray experiments demonstrated that the *Cnr* mutation had a profound effect on many aspects of ripening-related gene expression. In some degree the program of gene expression in *Cnr* resembles to that found in dehiscence or abscission zones prompting the authors to speculate that there is a link between events controlling cell separation in tomato, a fleshy fruit, and those involved in the formation of dehiscence zones in dry fruits (Erickson et al. 2005). In a very recent study Manning and co-workers used positional cloning and virus-induced gene silencing to demonstrate that a SBP-box (SQUAMOSA promoter binding protein-like) gene resides at the *Cnr* locus and that the phenotype results from a spontaneous epigenetic change in the SBP-box promoter (Manning et al. 2006).

A model for ethylene perception and metabolism has been proposed by Klee (Klee 2002), where the receptor also acts as a negative regulator of downstream responses, in the absence of ethylene, receptors actively suppress expression of ethylene responsive genes. This model is supported by the fact that loss-of-function mutants, removes the active suppression of ethylene response. Partial loss-of-function mutants should require less ethylene than wild type to achieve an ethylene response since there is less receptor on a molar basis to inactivate. However, Tieman et al. (2000) reported that transgenic tomato with reduced LeETR4 gene expression exhibits accelerated fruit ripening. A plausible explanation given by Klee is that NR can function *in vivo* to compensate for the loss of LeETR4, despite them being less than 50% identical (Klee 2002).

Recently, several further ethylene inducible genes have been identified in tomato including mitochondrial translation elongation factors (Benichou et al. 2003) and CTR-1 (Adams-Philips et al. 2004; Leclercq et al. 2002). It seems likely, given the development of microarray resources for tomato that significant advances will be made in the understanding signal transduction following ethylene perception. Examples of this have been already embarked by using the first tomato cDNA microarray containing 12,000 unique elements encoding 8,500 genes covering a range of metabolic and developmental processes (http://bti.cornell.edu/CGEP/CGEP.html) (Fei et al. 2004; Baxter et al. 2005b). In a recent study (Carrari et al. 2006) we have carried out an integrated analysis of metabolites and transcript levels during

tomato fruit development. In this analysis, ethylene pathway associated genes clearly displayed a large degree of both positive and negative correlations with the other ripening associated genes. Interesting observations can be mentioned such as the opposite behaviour observed for the transcript corresponding to the ethylene receptor 1 respect to the rest of the ethylene receptor transcripts. Whilst this observation is currently difficult to interpret, since both ethylene receptors 1 and 2 exhibit constitutive and stable expression patterns (Alba et al. 2005; Tieman et al. 2000), it is nevertheless highly interesting and may open a new avenue for identification of components of the ethylene perception pathway.

Tomato fruit development is marked by significant changes in the cell wall components and a handful of polysaccharides degrading enzymes have received much attention over the last fifteen years. The activity of these enzymes is directly linked to the shelf-life of the fruits, one of the crucial characteristics in tomato market. Fleshy fruits as tomatoes are predominantly composed by parenchyma cells walled by an unlignified layer of cellulose microfibrils suspended in a matrix of glycoproteins, water and pectic and hemicellulose polysaccharides. The latter accounts for 90% of the cell wall (Redgwell and Fischer 2002), with cell wall polysachharides largely derived from sugars, sugar phosphates (Scheible and Pauly 2004).

Endo-polygalacturonase (PG) has been the most studied among other enzymes involved in cell wall metabolism. PG catalyses the hydrolysis of the linear α-1,4-D-galacturonan backbone of pectic polysaccharides and along side the mRNA level its activity increases dramatically during tomato ripening (Della Penna et al. 1986). Rhamnogalacturonase (RGase) together with β-galactosidase (TBG) are enzymes which depolymerise branched pectins resistant to attack by endo-PG and have been found to be highly active in tomato fruits (Gross et al. 1995). At least seven tomato TBG genes are expressed during fruit development (Smith and Gross 2000). Furthermore the functionality of three of these genes (TBG1, 3 and 4) has been assessed in tomato via transgenesis. The reduction of the expression of these genes displayed different results, whilst TBG1 did not show changes in texture or cell wall composition (Carey et al. 2001), TBG3 gene led to an increase in wall galactosyl content, an increased proportion of insoluble solids and slightly increased viscosity (de Silva and Verhoeyen 1998) and TBG4 reduction resulted in increased fruit cracking, reduced locular space, and a doubling in the thickness of the fruit cuticle (Moctezuma et al. 2003), in addition to a decreased fruit softening (Smith et al. 2002). Pectin methylesterase (PME) catalyses the de-esterification of pectin. In tomato, PME arises from the expression of three genes (Tucker and Zhang 1996). The reduced pectin depolymerization by down regulation of fruit specific PME (PME2) had no interfered with the ripening process of tomato fruit (Tieman et al. 1992). Endo-β-1,4-glucanases (or cellulase, EGase) are a class of enzymes which degrade carboxymethylcellulose. EGases are encoded by a seven-member gene family (Brummell et al. 1999) and antisense suppression of a fruit specific member caused no change in the pattern of softening, but the abscission zones of the transgenic fruit were strengthened. In addition, xyloglucan endotransglycosylase (XET) which cleaves and religates the xyloglucan molecules of the wall has been implicated in ripening-related changes to the fruit cell wall in tomato (Maclachlan and Brady 1994). A further complexity arises when non-enzymatic mechanisms of cell wall changes are considered. One such example are the expansins—small proteins that catalyse cell wall extension and for which at least 10 distinct genes have been identified in the tomato. A member of this family (*LeExp2*) is co-expressed with XET and an EGase encoding genes during fruit development (Catala et al. 2000), suggesting a cross-talk between hormone and cell wall metabolism. Surprisingly, another α-expansin gene from tomato (*LeExp1*) was found to be specifically and abundantly expressed in ripening fruit where cell expansion was supposed not to occur (Rose et al. 1997). Fruits in which EXP1 protein accumulation was suppressed to 3% that of wild-type levels were firmer than controls throughout ripening and those overexpressing high levels of this protein were much softer than controls (Brummell et al. 1999). Again, this protein has been shown to be ethylene-induced in tomato fruits and other species and differentially regulated in the *rin* (ripening inhibitor) and in the ethylene receptor *Nr* (Never ripe) mutants (Rose et al. 2000). As cell division and ripening are physiologically distinct, the role played by expansins during these processes remains obscure (Bertin 2005).

As exemplified above to date the major focus on metabolic change during fruit development were placed on hormonal regulation (Lanahan et al. 1994; Adams-Phillips et al. 2004; Barry and Giovannoni 2006), aspects of pigmentation (Fraser et al. 1994; Giuliano et al. 1993; Ronen et al. 2000) or sugar and cell wall metabolism (Yelle et al. 1991; Rose et al. 2004; Fridman et al. 2004) with only a handful of studies looking at more general aspects of metabolism. Since this fruit was initially used as dessert, selection was orientated to sweetness with sugars representing up to 60% of the total dry weight. Sucrose, glucose and fructose are the major sugars found in tomato fruits with high hexose accumulation being characteristic of domesticated tomato (*Solanum lycopersicum*) whereas some wild tomato species (i.e. *S. chmielewskii*) accumulate mostly sucrose (Yelle et al. 1991). The variance in relative levels of sucrose and hexoses is most likely due to the relative activities of the

enzymes responsible for the degradation of sucrose—invertase and sucrose synthase. The genetic basis of the sucrose-accumulation trait of wild species tomato has been highly studied by means of introgressing wild germplasms into domesticated cultivars (Yelle et al. 1991; Fridman et al. 2000; Fridman et al. 2004), and a role for an apoplastic invertase in regulating sucrose metabolism in the tomato fruits has long been postulated. Other lines of evidence also support the role of this enzyme in regulating the sugar composition in tomato fruits and suggest that changes in composition contribute to alterations in fruit size. Utilizing the reverse genetic approach, Klann et al. (1996) reported that invertase antisense plants had increased sucrose and decreased hexose sugar concentrations in the fruits and 30% smaller fruits than those from control plants.

The other enzyme with a proposed central role in developing tomato fruits is sucrose synthase (SuSy). The role of this enzyme in growing tomato fruits by means of silencing a fruit specific isoform was not essential for starch synthesis (D'Aoust et al. 1999). However, its inhibition leads to a reduced unloading capacity of sucrose in the initial stages of fruit development (7 DAA) but only a small effect from 23 DAA onwards. The influence of SuSy in the carbon metabolism runs in parallel with the highest demand for hexose phosphates (Roessner-Tunali et al. 2003), the rapid accumulation of starch and the highest levels of ADP-glucose pyrophosphorylase activity (Beckles et al. 2001). Thus, the reduced fruit set observed in SuSy antisense plants may be explained, at least in part, by the control exerted by Susy on the fruit unloading capacity. Moreover, a Susy locus has been mapped on the tomato genome and co-localise with a sugar content QTL (Causse et al. 2004). These evidences allow postulating Susy as a good candidate to explore among the natural genetic variation found in the *Solanum* species.

Together with SuSy and HXK, fructokinase (FRK) forms the pool of hexose phosphates subsequently used as substrates for respiration and starch biosynthesis. Two different FRK isoforms (FRK1 and 2) (Kanayama et al. 1997, 1998) 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 the fruit metabolism has received far less attention to date. A study of the sucrose to starch transition suggested that the activities of sucrose synthase, fructokinase and AGPase are likely to share control of the rate of starch accumulation (Schaffer and Petreikov 1997). However, 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 (Geigenberger et al. 2005; Davies et al. 2005).

Although clearly of central importance to the tomato fruit relatively little is currently known concerning the regulation of glycolysis and the conversion of hexose phosphates into organic acids. Furthermore, especially in red fruits which contain little starch, glycolysis and respiration represent the dominant carbon fluxes in the fruit (Carrari et al. 2006; Rontein et al. 2002). Interestingly, the relative fluxes through the central metabolic pathways do not alter massively through the life cycle of suspension cultured tomato cells, whilst those of anabolic pathways such as starch synthesis and the biosynthesis of amino acids and cell wall polysaccharides are low and variable (Rontein et al. 2002). Indeed the TCA cycle in plants is very poorly characterised in general and although the structure of the pathway is well known, its regulation is not (Fernie et al. 2004). As part of this ongoing project to determine the role of the mitochondrial TCA cycle in plants, we first concentrated efforts on the illuminated leaf. Biochemical analysis of the aconitase mutant (*Aco*1) revealed that it exhibited a decreased flux through the TCA cycle, decreased levels of TCA cycle intermediates and enhanced carbon assimilation (Carrari et al. 2003a), these plants were characterised by a dramatically increased fruit weight. In other study where the mitochondrial malate dehydrogenase (mMDH) was repressed via antisense and RNA interference techniques (Nunes-Nesi et al. 2005) in the elite cultivated species *S. lycopersicum* also resulted in enhanced photosynthetic activity and in an increment in fruit dry weight. Transcript and metabolite profiling of leaf material from these lines suggest that some of the increase in photosynthetic capacity is due to an elevated expression of genes associated with photosynthesis (Urbanczyk-Wochniak et al. 2006).

The nitrogen metabolism in tomato fruit is also important in ripening and is close related to the central carbon metabolism. Interesting pattern of amino acid contents in tomato fruit has been observed during several stage of ripening. At earlier stages of development GABA, glutamine, alanine, asparagine, arginine, valine and proline were found as predominant amino acid and their concentrations decrease at later stages of development. In contrast glutamate, cysteine, aspartate, tryptophan, methionine and putrescine increase at the later stage of fruit development and ripening (Carrari et al. 2006; Boggio et al. 2000). Biochemical studies on enzyme involved in amino acid biosynthesis in tomato fruit explained, at least in part the aminoacid kinetic in ripening (Boggio et al. 2000). In this work enzyme activity at three different development stages was determined and the results showed mainly two groups of pattern expression. One group is expressed mainly in green fruit, such us glutamine synthetase (GS), glutamate decarboxylase (GAD), alanine aminotransferase and NADP-malic enzyme, that

correlates with glutamine and alanine content. Another group in red fruits was represented by NADH-GDH, aspartate aminotransferase and NAD-malate dehydrogenase. The increase in the relative content of glutamate in ripe fruits related to green fruits is correlated with an increase in the levels of glutamate dehydrogenase (GDH), aspartate aminotransferase and NAD-malate dehydrogenase, and also with a decline of GS in ripe. The decline in GS and the induction of GDH in ripe fruits, suggest a reciprocal regulation of both enzymes during tomato fruit ripening. Glutamate not only is implicated in improving of tomato fruit taste but also it seems to be implicated in long shelf life attribute (SL). These evidence arise from a study where GS, GDH activities and glutamate and glutamic contents were measured in wild genotypes with a short and middle SL such us the genotype *Caimanta* and genotypes *LA1385* and *LA1673* respectively as well as in mutants with longer shelf life such us *nor* and *rin* mutants (Pratta et al. 2004). These results showed a significant negative correlation between glutamate content and SL and reciprocal induction pattern of GS and GDH, while long SL is associated with lower relative glutamate content and simultaneously with the high activity of both these enzymes in the pericarp of tomato fruits. These results suggest that there may be a way to regulate the amount of glutamate and GS and GDH during ripening in tomato fruits, which is related to the final SL of fruits. In a recently works were reported that GS is regulated at posttranslational levels by phosphorylation pathways (Lima et al. 2006) or by photooxidative strees (Palatnik et al. 1999) and also at the post-transcriptional level (Ortega et al. 2001). However the precise mechanisms that regulate glutamine/glutamate and their link to tomato ripening require further detailed investigation in order to allow full comprehension of the importance of these reactions to the ripening process.

The domestication of tomato, as that of most species, is characterized by the enhancement of a small number of characteristics at the cost of a dramatic reduction in allelic variance present in cultivated crops. The reintroduction of this allelic variance by wide crosses including wild germplasm is currently being investigated as a mechanism to reverse this trend (Maloof 2003; Koornneef et al. 2004; Rodriguez et al. 2006; Fernie et al. 2006) and will furthermore likely represent an important future route for crop improvement. An approach of exploring natural genetic variation by utilizing metabolic genomics was described by Schauer et al. (2006). Approximately 900 metabolite QTLs and more than 300 yield associated traits (YALs) were uncovered in this study. By correlation analysis of this data set a large proportion of the fruit metabolite QTLs were found to be strongly associated with whole-plant phenotypes. The harvest index (HI), a measure of the efficiency in partitioning of assimilated photosynthate to harvestable product, was identified as a main hub in the combined network of metabolic and whole-plant phenotypic traits. Experiments performed with *self pruning* (*sp*) recessive mutants permitted to rationalize that HI is a regulator of the metabolite content of the mature fruit pericarp (Figure 1). Thus important agronomic traits were identified in addition to fundamental insight into the metabolic network of tomato fruit and the interactions of this network with YALs were assessed.

Application of genomics tools to increase understanding of the developmental process

The most apparent problem of the reductionist philosophy that prevailed in biological sciences during the last decades are metabolic complexity and the fact that biological processes are rarely controlled by a single molecular entity but rather that control is generally shared. Recent technological advances allowed the emergence of high-throughput genomic tools allowing the cataloguing of changes in metabolites, transcripts and proteins in parallel with significant advances in our ability to quantify metabolic flux (Fernie et al. 2005; Ratcliffe and Shacher-Hill 2005) offer fresh hope for successful application of more holistic approaches.

In plants the development of systems biology has followed a similar trajectory to that of non-plant systems and thus is dominated by gene expression studies (Harmer et al. 2000; Espinosa-Soto et al. 2004; Thimm et al. 2004). Dynamic and coordinated changes at transcriptional levels have also been observed in tomato fruits. A detailed analysis of transcript changes focussed on the cell expansion phase in different fruit cell types showed that the expansion of locular cells is concomitant with the expression of genes controlling water flow, organic acid synthesis, sugar storage, and photosynthesis and suggest that hormones (mainly auxin and gibberellin) regulate this process (Lemaire-Chamley et al. 2005). The same observations come out from a work published by Carbone et al. (2005) suggesting that light responsiveness during fruit development may be modulated by a wide fluctuations in light signalling transcript abundance. Despite the dominance of data at the level of the transcript several interesting observations have been made on the correlative behavior of metabolites, both in isolation and in combination with information concerning other molecular entities. The measurement of many metabolites in parallel gives insights into the complex regulatory circuits that underpin metabolism. A recently paper published by Gibon et al. (2006) gives new insights on photosynthetic metabolism. Utilizing a robotized enzyme assays system

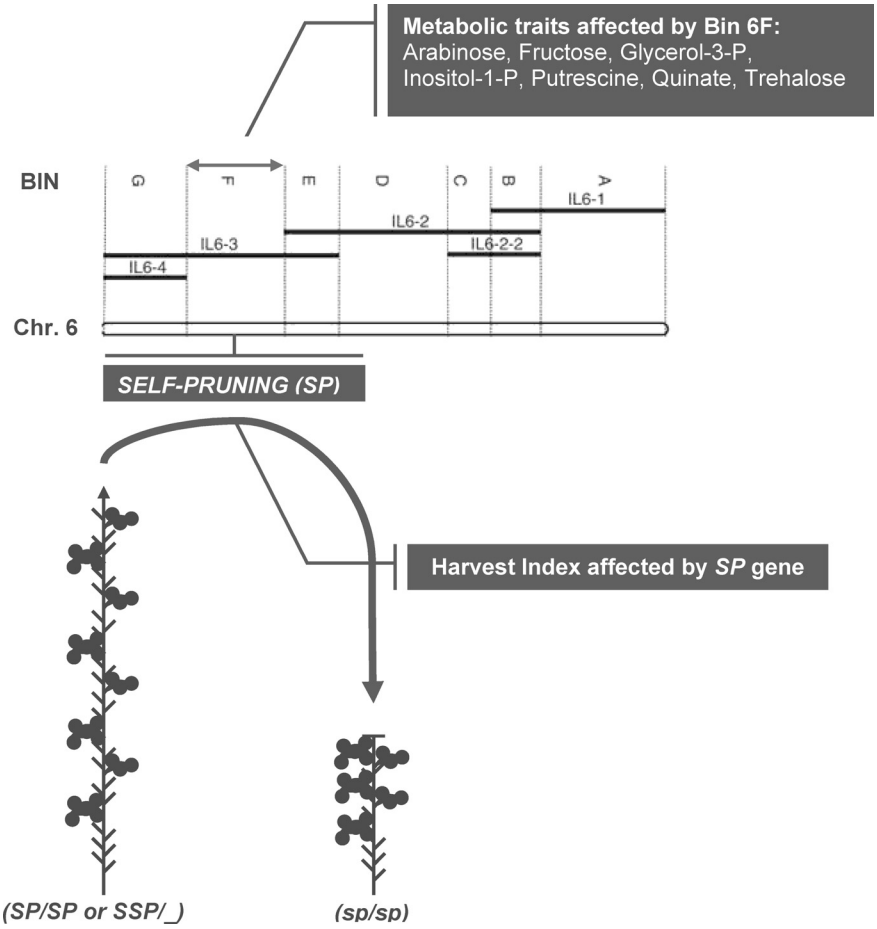

Figure 1. Harvest index (HI) as a regulator of tomato fruit metabolite content. Schematic representation of tomato chromosome 6 showing the map position of *self pruning* gene (SP) on bin 6F. Introgression line (IL) 6-3 is markedly affected by HI and seven metabolites were affected by this IL. Six of these seven metabolites were determined to be highly correlated with HI. Recessive *sp* mutation affects plant architecture and consequently HI. (Adapted from Schauer et al, 2006 and Fridman et al, 2002).

for measuring activities of key enzymes in central carbon metabolism this work adds a new dimension to a multiparallel analysis of transcripts and metabolites. An important conclusion emerged from this work; the slow response of enzyme activities in comparison with changes at transcriptional and metabolite levels might implies that correlations between transcripts and metabolites are likely to reflect a regulatory impact of metabolites on gene expression, rather than the impact of changes of gene expression on metabolism (Gibon et al. 2006). This study highlights the difficulty of interpreting datasets that are complied merely at the transcript level.

Despite the complexities inherent in analyzing datasets of this scale they do permit sophisticated network analysis. Such studies allow the actions of a small theoretical network on the strength of correlations between the metabolites that constitute these networks. A handful of examples in tomato fruits have been recently reported as first steps integrating data from gene expression and metabolite profiles. Transcriptome profiling in developing tomato pericarp in parallel with phenotypic and targeted metabolite measurements showed that transcript accumulation was extensively coordinated and often completely dependent on ethylene (Alba et al. 2005). Moreover, the comparative analysis between wild type and Never-ripe mutants (*Nr*, an ethylene receptor) illustrated about the profound alteration of gene expression exerted by the ethylene signaling pathway. About 40% of all genes expressed during a time-series including 10 stages of tomato fruit development changed their pattern as a consequence of this single gene mutation indicating that ethylene governs multiple aspects of development both prior to and during fruit ripening in tomato. In a recent study (Carrari et al. 2006) we took a similar approach, but broadened the scale of metabolites measured (to encompass primary and secondary metabolites, pigments, cell wall monosacharides) in parallel to transcript levels. A comprehensive picture of changes in gene expression was also obtained and this analysis gave important insight into metabolic and transcriptional programs underlying this process. An example was ascorbate biosynthesis that to date four putative biosynthetic pathways has been postulated in plants

(Ischikawa et al. 2006). Evidence presented in the current study suggest that the D-galacturonic acid and myo-inositol routes are unlikely to be major precursors for ascorbate biosynthesis in the tomato given their kinetic profiles relative to that of ascorbate, dehydroascorbate and threonate. The presence of homologs of all enzymes of the Smirnoff-Wheeler pathway in tomato strengthens the suggestion that the GDP-mannose pathways are the predominant route of ascorbic acid biosynthesis in the tomato (Carrari et al. 2006).

Whilst the above mentioned reports exemplified the powerful of transcript profiles as a tool for understanding one of the dimensions of the networks that governs fruit metabolism, the quantitative analysis of a large portion of a plant proteome is not yet within reach. However, efforts have begun to establish proteomics technical platforms in order to characterize the differences in wall structure and composition that occur during tomato fruit development and ripening (see Rose et al. 2004). Even when technological differences situate proteomic approaches in a narrower window of that offered by surveying mRNA expression, transcript and protein profiling are currently complementary, rather than equivalent fields and are typically used to answer different biological questions.

Cloning of genes important to quality traits encompassing fruit ripening, post-harvest physiology and agronomic yield.

Crop improvement has begun since man established and domesticated few plant species. This has had an enormous impact by one side, but also has contributed to the shrinking of the genetic bases that modern breeding sustains to today. These facts are not only important for the classical aims that breeding has focused on (i.e. improving crop yield and disease resistance) but also to the necessity in improving crop compositional quality for human health. This need is being evidenced by recent medical research that illustrates the rapidly increasing concern about food nutritional compositions (Demmig-Adams and Adams 2002; Spencer et al 2005). In this line, recent effort has shifted toward exploring natural biodiversity for crop quality improvement, particularly those traits related to chemical composition (for review see: Fernie et al. 2006). Particularly in fleshy fruits, tomato (*Solanum lycopersicum*) constitutes a model species because of the large number of biochemical studies already carried out on this crop, its modest-sized diploid genome and tolerance to inbreeding. Herein, we will highlight a handful of recent reports that account for the recompense of using wild germplasm introgressed into cultivated varieties aiming at improving crop qualities. The first QTL dissected to the level of a single gene in plants (fw2.2; Frary et al. 2002) is known for the control exerted to the fruit size. Despite the obvious economical impact this finding has on breeding programs, it have recently found evidences about the mechanism by which FW2.2 mediates cell division (Cong and Tanksley 2006). This protein physically interacts at or near the plasma membrane with the regulatory betha subunit of a CKII kinase. These kinds of kinases are well-known for their involvement on cell cycle related signaling pathway. The importance of starch accumulation as a factor determining the soluble solids content of tomato fruits (Brix: a desirable trait in tomato canned industry) (Dinar and Stevens 1981; Schaffer and Petreikov 1997) has been re-marked recently by the finding that an introgression line carrying an apoplastic invertase (Lin5) from *Solanum pennellii* (IL9-2-5) resulted in increased Brix content (Baxter et al 2005; Fridman et al. 2004). Another recent example of the potentiality of wild germplasm is given by a comparative analysis of the metabolite composition in leaves and fruits from six tomato species reported by Schauer et al. (2005). The tremendous variance observed in this inventory dataset may eventually prove useful in the selection of breeding material as an alternative to current transgenesis-based metabolic engineering strategies (Carrari et al. 2003b). More recently, the importance of the newly deciphered pathway of the volatile compounds 2-phenylacetaldehyde and 2-phenylethanol in tomato fruits has been achieved by prospecting the genetic variation observed in a single introgressed portion of chromosome 8 derived from the wild relative *Solanum pennellii* (Tieman et al. 2006; Tadmor et al. 2002). This discovery has important implications in the possibility to manipulate flavor and aroma volatiles in food products. Control of ethylene responsiveness in tomato is of commercial importance to reduce senescence, overripening, and postharvest deterioration of fruit (for review see: Giovannoni 2004). By means of a mapping population derived from a cross between *Solanum lycopersicum*×*Solanum cheesmaniae* carrying homozygous mutations on two linked loci (Green-ripe-Gr- and Never-ripe 2-Nr-2-), Barry and Giovannoni (2006) proposed the GR protein as a potential regulator of ethylene responses (Gr). Trasgenic tomato plants overexpressing GR protein proved to be useful for reducing the impact of the less desirable consequences of ethylene on tissues such as ripe fruit whilst maintaining normal plant vigour.

Future Prospects

Along with a recent published review (Carrari and Fernie 2006) we have updated here a few recent examples on the progresses that have been made in understanding the developmental control exerted on tomato fruit

metabolism. As compilation of large datasets is only just beginning alongside with new development of tools for producing them, it is expected that in the middle term rational combination of these platforms will allow a broader systems-orientated view of metabolism of the developing fruit. A collaborative project in our laboratories has recently been initiated aiming at dissecting a large set of metabolic QTLs (QML—quantitative metabolic loci). In one approach we are performing sequence analysis of introgressed genomic regions of the Zamir *Solanum pennellii* introgression lines (Eshed and Zamir 1995) aimed at finding novel genes involved in determining metabolic variations. In parallel we intend to follow a candidate gene approach to decipher the allelic variation affecting central carbon metabolism in tomato fruits. Together with the sequence data from the tomato sequencing consortium this should render valuable information with high impact in areas as diverse as functional genomic and evolutionary studies and ultimately in breeding.

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References

Adams-Phillips L, Barry C, Giovannoni J (2004) Signal transduction systems regulating fruit ripening. *Trends Plant Sci* 9: 331–338

Alba R, Payton P, Fei Z, McQuinn R, Debbie P, Martin G, Tanksley SD, Giovannoni JJ (2005) Transcriptome and selected metabolite analysis reveal multiple pointsd of ethylene regulatory control during tomato fruit development. *Plant Cell* 17: 2954–2965

Ayub R, Guis M, BenAmor M, Gillot L, Roustan JP, Latche A, Bouzayen M, Pech JC (1996) Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. *Nat Biotechnol* 14: 862–866

Barry CS, Giovannoni JJ (2006) Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signalling. *PNAS* 103: 7923–7928

Barry CS, McQuinn RP, Thompson AJ, Seymour GB, Grierson D, Giovannoni JJ (2005) Ethylene insensitivity conferred by the Green-ripe and Never-ripe 2 ripening mutants of tomato. *Plant Physiol* 138: 267–275

Bartley GE, Scolnik PA, Giuliano G (1994) Molecular Biology of carotenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol* 45: 287–301

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 Cell Physiol* 46: 425–437

Baxter CJ, Sabar M, Quick WP, Sweetlove LJ (2005) Comparison of changes in fruit gene expression in tomato introgression lines provides evidence of genome-wide transcriptional changes and reveals links to mapped QTLs and described traits. *J Exp Bot* 56: 1591–1604

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

Benichou M, Li ZG, Tournier B, Chaves A, Zegzouti H, Jauneau A, Delalande C, Latche A, Bouzayen M, Spremulli LL, Pech JC (2003) Tomato EF-Ts-mt, a functional mitochondrial translation elongation factor from higher plants. *Plant Mol Biol* 53: 411–422

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. *Ann Bot* 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 Sci* 159: 125–133

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 Mol Biol* 40: 615–622

Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, Dunsmuir P (1999) Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *Plant Cell* 11: 2203–2216

Carbone F, Pizzichini D, Giuliano G, Rosati C, Perrotta G (2005) Comparative profiling of tomato fruits and leaves evidences a complex modulation of global transcript profiles. *Plant Sci* 169: 165–175

Carey AT, Smith DL, Harrison E, Bird CR, Gross KC, Seymour GB, Tucker GA (2001) Down-regulation of a ripening-related beta-galactosidase gene (TBG1) in transgenic tomato fruits. *J Exp Bot* 52: 663–668

Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor MI, Nunes-Nesi A, Nikiforova1 V, Centero D, Ratzka A, Pauly M, Sweetlove L, Fernie AR (2006) Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. *Plant Physiol* 142(4): 1380–1396

Carrari F, Nunes-Nesi A, Gibon Y, Lytovchenko A, Loureiro ME, Fernie AR (2003a) Reduced expression of aconitase results in an enhanced rate of photosynthesis and marked shifts in carbon partitioning in illuminated leaves of wild species tomato. *Plant Physiol* 133: 1322–1335

Carrari F, Fernie AR (2006) Metabolic regulation underlying tomato fruit development. *J Exp Bot* 57: 1883–1897

Carrari F, Fernie AR, Iuesum N (2004) Heard it through the grapevine. ABA and sugar cross-talk: The ASR story. *Trends Plant Sci* 9: 57–59

Carrari F, Urbanczyk-Wochniak E, Willmitzer L, Fernie AR (2003b) Engineering central metabolism in crop species: learning the system. *Metab Eng* 5: 191–200

Catala C, Rose JKC, Bennett AB (2000) Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. *Plant Physiol* 122: 527–34

Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. *J exp Botany* 55: 1671–1685

Cong B, Tanksley SD (2006) FW2.2 and cell cycle control in developing tomato fruit: a possible example of gene co-option in the evolution of a novel organ. *Plant Mol Biol* published online Aug 29

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. *Plant Cell* 11: 2407–2418

Davies HV, Shepherd LVT, Burrell MM, Carrari F, Urbanczyk-Wochniak E, Leisse A, Hancock RD, Taylor M, Viola R, Ross H, McRae D, Willmitzer L, Fernie AR (2005) Modulation of Fructokinase activity of potato (*Solanum tuberosum*) results in substantial shifts in tuber metabolism. *Plant Cell Physiol* 46: 1103–1115

Della Penna D, Alexander DC, Bennett AB (1986) Molecular-cloning of tomato fruit polygalacturonase—analysis of polygalacturonase messenger-RNA levels during ripening. *Proc Natl Acad Sci USA* 83: 6420–6424

Demmig-Adams B, Adams WWR (2002) Antioxidants in photosynthesis and human nutrition. *Science* 298: 2149–2153

Desbrosses GG, Kopka J, Udvardi MK (2005) Lotus japonicus metabolic profiling. Development of gas chromatography-mass spectrometry resources for the study of plant-microbe interactions. *Plant Physiol* 137: 1302–1318

de Silva J, Verhoeyen ME (1998) Production and characterization of antisense-exogalactanase tomatoes. In: Kuiper HA (ed) *Report of the Demonstration Programme on Food Safety Evaluation of Genetically Modified Foods as a Basis for Market Introduction*. Ministry of Economic Affairs, The Hague, The Netherlands, pp 99–106

Dinar M, Stevens MA (1981) The relationship between starch accumulation and soluble solids content of tomato fruits. *J Am Soc Hortic Sci* 106: 415–418

Erikson EM, Bovy A, Manning K, Harrison L, Andrews J, De Silva J, Tucker GA, Seymour GB (2004) Effect of the *Colorless non-ripening mutant* on cell wall biochemistry and gene expression during tomato fruit development and ripening. *Plant Physiol* 136: 4184–4197

Eshed Y, Zamir D (1995) An Introgression Line Population of *Lycopersicon pennellii* in the Cultivated Tomato Enables the Identification and Fine Mapping of Yield-Associated QTL. *Genetics* 141: 1147–1162

Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER (2004) A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. *Plant Cell* 16: 2923–2939

Fei ZJ, Tang X, Alba RM, White JA, Ronning CM, Martin GB, Tanksley SD, Giovannoni JJ (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. *Plant J* 40: 47–59

Fernie AR, Geigenberger P, Stitt M (2005) Flux an important, but neglected, component of functional genomics. *Curr Opin Plant Biol* 8: 174–182

Fernie AR, Carrari F, Sweetlove LJ (2004) Respiration: glycolysis, the TCA cycle and the electron transport chain. *Curr Opin Plant Biol* 7: 254–261

Fernie AR, Tadmor Y, Zamir D (2006) Natural genetic variation for improving crop quality. *Curr Opin Plant Biol* 9: 196–202

Fernie AR, Willmitzer L (2001) Update on tuber formation, dormancy and sprouting. Molecular and biochemical triggers of potato tuber development. *Plant Physiol* 127: 1459–1465

Fraser P, Truesdale M, Bird C, Schuch W, Bramley P (1994) Carotenoid biosynthesis during tomato fruit development: Evidence for tissue-specific gene expression. *Plant Physiol* 105: 405–413

Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu JP, Meller J, Elber R, Alpert KB, Tanksley SD (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289: 85–88

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. *Proc Natl Acad Sci USA* 97: 4718–4723

Fridman E, Liu YS, Carmel-Goren L, Gur A, Shoresh M, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. *Mol Genet Genomics* 266: 821–826

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

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. *Plant Cell* 17: 2077–88

Gibon Y, Usadel B, Blaesing OE, Kamlage B, Hoehne M, Trethewey R, Stitt M (2006) Integration of metabolite with transcript and enzyme activity profiling during diurnal cycles in Arabidopsis rosettes. *Genome Biol* 7: R76

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. *Plant Cell* 16: S170–S180

Giovannoni J (2001) Molecular biology of fruit maturation and ripening. *Annu Rev Plant Physiol Plant Mol Biol* 52: 725–749

Giuliano G, Bartley G, Scholnik P (1993) Regulation of carotenoid biosynthesis during tomato fruit development. *Plant Cell* 5: 379–393

Goff SA, Ricke D, Lan TH, Presting G, Wang RL, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchinson D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong JP, Miguel T, Paszkowski U, Zhang SP, Colbert M, Sun WL, Chen LL, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu YS, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296: 92–100

Good X, Kellogg JA, Wagoner W, Langhoff D, Matsumura W, Bestwick RK (1994) Reduced ethylene synthesis by transgenic tomatoes expressing s-adenosylmethionine hydrolase. *Plant Mol Biol* 26: 781–790

Grierson D (1992) Control of ethylene synthesis and ripening by sense and antisense genes in transgenic plants. *P ROY SOC EDINB B* 99: 79–88

Gross KC, Starrett DA, Chen HL (1995) Rhamnogalacturonase, β-galactosidase and α-galactosidase: potential role in fruit softening. *Acta Hortic* 398: 121–130

Hackett RM, Ho CW, Lin ZF, Foote HCC, Fray RG, Grierson D (2000) Antisense inhibition of the Nr gene restores normal ripening to the tomato Never-ripe mutant, consistent with the ethylene receptor-inhibition model. *Plant Physiol* 124: 1079–1085

Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science*

290: 2110–2113
Ishikawa T, Dowdle J, Smirnoff N (2006) Progress in manipulating ascorbic acid biosynthesis and accumulation in plants. *Physiol Plantarum* 126: 343–355
Jarret RL, Tigchelaar EC, Handa AK (1984) Ripening behavior of the green ripe tomato mutant. *J Am Soc Hortic Sci* 109: 712–717
Kerr E (1981) Linkage studies of green ripe and never ripe. *TGC Reports* 31: 7
Kerr E (1982) Never ripe-2 (Nr-2) a slow ripening mutant resembling Nr an Gr. *TGC Reports* 32: 33
Klann EM, Hall B, Bennett AB (1996) Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiol* 112: 1321–1330
Klee HJ (2002) Control of ethylene-mediated processes in tomato at the level of receptors. *J Exp Bot* 53: 2057–2063
Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu Rev Plant Biol* 55: 141–172
Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1994) The never ripe mutation blocks ethylene perception in tomato. *Plant Cell* 6: 521–530
Leclercq J, Adams-Phillips LC, Zegzouti H, Jones B, Latche A, Giovannoni JJ, Pech JC, Bouzayen M (2002) LeCTR1, a tomato CTR1-like gene, demonstrates ethylene signaling ability in *Arabidopsis* and novel expression patterns in tomato. *Plant Physiol* 130: 1132–1142
Lemaire-Chamley M, Petit J, Garcia V, Just D, Baldet P, Germain V, Fagard M, Mouassite M, Cheniclet C, Rothan C (2005) Changes in Transcriptional Profiles Are Associated with Early Fruit Tissue Specialization in Tomato. *Plant Physiol* 139: 750–769
Liljegren SJ, Roeder AHK, Kempin SA, Gremski K, Ostergaard L, Guimil S, Reyes DK, Yanofsky MF (2004) Control of fruit patterning in *Arabidopsis* by INDEHISCENT. *Cell* 116: 843–853
Lima L, Seabra A, Melo P, Cullimore J Carvalho H (2006) Post-translational regulation of cytosolic glutamine synthetase of *Medicago truncatula*. *J Exp Bot* 57: 2751–2761
Maclachlan G, Brady C (1994) Endo-1,4-beta-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. *Plant Physiol* 105: 965–974
Maloof JN (2003) QTL for plant growth and morphology. *Curr Opin Plant Biol* 6: 85–90
Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, J Giovannoni J, Seymour (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat Genet* 38: 948–952
Moctezuma E, Smith DL, Gross KC (2003) Antisense suppression of a beta-galactosidase gene (TBG6) in tomato increases fruit cracking. *J Exp Bot* 54: 2025–2033
Mueller LA, Tanksley SD, Giovannoni JJ, van Eck J, Stack S, Choi D, Kim BD, Chen MS, Cheng ZK, Li CY, Ling HQ, Xue YB, Seymour G, Bishop G, Bryan G, Sharma R, Khurana J, Tyagi A, Chattopadhyay D, Singh NK, Stiekema W, Lindhout P, Jesse T, Lankhorst RK, Bouzayen M, Shibata D, Tabata S, Granell A, Botella MA, Giullano G, Frusciante L, Causse M, Zamir D (2005) The Tomato Sequencing Project, the first cornerstone of the International Solanaceae Project (SOL). *Comparative and Functional Genomics* 6: 153–158
Nunes-Nesi A, Carrari F, Lytovchenko A, Smith AMO, Loureiro ME, Ratcliffe RG, Sweetlove LJ, Fernie AR (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. *Plant Physiol* 137: 611–622
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 Physiol* 129: 1119–1126
Ortega JL, Temple SJ, Sengupta-Gopalan C (2001) Constitutive overexpression of cytosolic glutamine synthetase (gs1) gene in transgenic alfalfa demonstrates that gs1 may be regulated at the level of rna stability and protein turnover1. *Plant Physiol* 126: 109–121
Palatnik JF, Carrillo N, Valle EM (1999) The role of photosynthetic electron transport in the oxidative degradation of chloroplastic glutamine synthetase1. *Plant Physiol* 121: 471–478
Pratta G, Zorzoli R, Boggio SB, Picardi LA, Valle EM (2004) Glutamine and glutamate levels and related metabolizing enzymes in tomato fruits with different shelf-life. *Sci Hort* 100: 341–347
Ratcliffe RG, Shachar-Hill Y (2005) Revealing metabolic phenotypes in plants: inputs from NMR analysis. *Biol Rev Camb Philos Soc* 80: 27–43
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, Chp 3 pp 46–88
Rodriguez GR, Pratta GR, Zorzoli R, Piccardi LA (2006) Evaluation of plant and fruti traits in recombinant imbred lines of tomato from a cross between *Lycopersicom esculentum* and *L pimpinellifolium*. *Cie. Inv Agr* 33: 111–118
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 Physiol* 133: 84–99
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. *Proc Natl Acad Sci USA* 97: 11102–11107.
Rontein D, Dieuaide-Noubhani M, Dufourc EJ, Raymond P, Rolin D (2002) The metabolic architecture of plant cells—Stability of central metabolism and flexibility of anabolic pathways during the growth cycle of tomato cells. *J Biol Chem* 277: 43948–43960
Rose JKC, Lee HH, Bennett AB (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc Natl Acad Sci USA* 94: 5955–5960
Rose JKC, Cosgrove DJ, Albersheim P, Darvill AG, Bennett AB (2000) Detection of expansin proteins and activity during tomato fruit ontogeny. *Plant Physiol* 123: 1583–1592
Rose JK, Bashir S, Giovannoni JJ, Jahn MM, Saravanan RS (2004) Tackling the plant proteome: practical approaches, hurdles and experimental tools. *Plant J* 39: 715–733
Schaffer AA, Petreikov M (1997) Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. *Plant Physiol* 113: 739–746
Schauer N, Zamir D, Fernie AR (2005) Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. *J Exp Bot* 56: 297–307
Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J, Willmitzer L, Zamir D, Fernie AR (2006) Comprehensive metabolic profiling and

phenotyping of interspecific introgression lines for tomato improvement. *Nature Biotech* 24: 447–454

Scheible WR, Pauly M (2004) Glycosyltransferases and cell wall biosynthesis: novel players and insights. *Curr Opin Plant Biol* 7: 285–295

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

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

Spencer JPE, Kuhnle GGC, Hajirezaei M, Mock HP, Sonnewald U, Rice-Evans C (2005) The genotypic variation of the antioxidant potential of different tomato varieties. *Free Radic Res* 39: 1005–1016

Tadmor Y, Fridman E, Gur A, Larkov O, Lastochkin E, Ravid U, Zamir D, Lewinsohn E (2002) Identification of malodorous, a wild species allele affecting tomato aroma that was selected against during domestication. *J Agric Food Chem* 50: 2005–2009

Thimm O, Blässing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J* 37: 914–939

Thompson AJ, Tor M, Barry CS, Vrebalov J, Orfila C, Jarvis MC, Giovannoni JJ, Grierson D, Seymorur GB (1999) Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. *Plant Physiol* 120: 383–390

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

Tieman DV, Taylor MG, Ciardi JA, Klee HJ (2000) The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. *Proc Natl Acad Sci USA* 97: 5663–5668

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ (2006) Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci USA* 103: 8287–8292

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

Urbanczyk-Wochniak E, Usadel B, Thimm O, Nunes-Nesi A, Carrari F, Davy M, Blasing O, Kowalczyk M, Weicht D, Polinceusz A, Meyer S, Stitt M, Fernie AR (2006) Conversion of MapMan to allow the analysis of transcript data from Solanaceous species: effects of genetic and environmental alterations in energy metabolism in the leaf. *Plant Mol Biol* 60: 773–92

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (Rin) locus. *Science* 296: 343–345

Weckwerth W (2003) Metabolomics in system biology. *Ann Rev Plant Biol* 54: 669–689

Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1995) An ethylene-inducible component of signal-transduction encoded by never-ripe. *Science* 270: 1807–1809

Yelle S, Chetelat RT, Dorais M, Deverna JW, Bennett AB (1991) Sink Metabolism In Tomato Fruit. 4. Genetic and biochemical-analysis of sucrose accumulation. *Plant Physiol* 95: 1026–1035

Zamir D (2001) Improving plant breeding with exotic genetic libraries. *Nat Rev Genet* 2: 983–989