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Invited Review

Metabolomics-Inspired Insight into Developmental, Environmental and Genetic Aspects of Tomato Fruit Chemical Composition and Quality

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Tomato was one of the first plant species to be evaluated using metabolomics and remains one of the best characterized, with tomato fruit being both an important source of nutrition in the human diet and a valuable model system for the development of fleshy fruits. Additionally, given the broad habitat range of members of the tomato clade and the extensive use of exotic germplasm in tomato genetic research, it represents an excellent genetic model system for understanding both metabolism per se and the importance of various metabolites in conferring stress tolerance. This review summarizes technical approaches used to characterize the tomato metabolome to date and details insights into metabolic pathway structure and regulation that have been obtained via analysis of tissue samples taken under different developmental or environmental circumstance as well as following genetic perturbation. Particular attention is paid to compounds of importance for nutrition or the shelf-life of tomatoes. We propose furthermore how metabolomics information can be coupled to the burgeoning wealth of genome sequence data from the tomato clade to enhance further our understanding of (i) the shifts in metabolic regulation occurring during development and (ii) specialization of metabolism within the tomato clade as a consequence of either adaptive evolution or domestication.

Keywords: Fruit ripening • Metabolomics • Primary metabolite • Secondary metabolite • Tomato metabolism.

Abbreviations: FT-ICR, Fourier transform ion cyclotron resonance; GC-MS, gas chromatography–mass spectrometry; IL, introgression line; ILH, line heterozygous for the introgression; LC-MS, liquid chromatography–mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; NMR, nuclear magnetic resonance; qPCR, quantitative real-time PCR; QTL, quantitative trait locus; RNA-seq, RNA sequencing; TILLING, targeting induced local lesions in genomes; TOF-MS, time-of-flight-mass spectrometry; UPLC, ultra-performance liquid chromatography; VIGS, virus-induced gene silencing.

Introduction

Tomato, *Solanum lycopersicum*, a member of the Solanaceae family, has long been cultivated. It and its wild relatives originate from the Andean region of South America, and cherry tomato (*S. lycopersicum* var *cerasiforme*) which was probably domesticated from *S. pimpinellifolium* (Ranc et al. 2012), was the likely ancestor of modern-day cultivars. In the 16th century the conquistadors brought tomatoes to Europe, and subsequent migration and extensive selection considerably reduced the diversity of the crop (Lin et al. 2014). Today tomato is considered the leading vegetable crop, with a global yield in excess of 160 Mt in 2012 (http://faostat.fao.org/), with a net value of >US$55 billion (Vincent et al. 2013). It is also a model system for understanding fleshy fruit development (Klee and Giovannoni 2011), with massive recent progress being made towards understanding the gene regulatory circuitry (Rohrmann et al. 2011, Seymour et al. 2013, Karlova et al. 2014) and metabolic shifts (Carrari and Fernie 2006, Tohge et al. 2014) underlying this process. In addition to these important features, looking beyond the domesticated species, wild species tomato have adapted to highly diverse habitats, with different members of the clade being able to grow in arid desert-like conditions while others grow in the tropical rainforest (Peralta et al. 2008). Breeders and plant geneticists have started to tap this diversity as a means to re-introduce biotic and abiotic stress tolerance (Zamir 2001, Takeda and Matsuoka 2008, Frary et al. 2010) and fruit size and shape variation (Frary et al. 2000, Tanksley 2004). This approach has also been used, in parallel to transgenic approaches (Frary et al. 2000, Tieman et al. 2010), as a means by which to engineer metabolite content (Mutschler et al. 1996, Fridman et al. 2004, Schauer et al. 2006, Schilmiller et al. 2010, Perez-Fons et al. 2014). In this review, we will highlight how metabolomics has been brought to bear to address fundamental questions in each of these important research areas. However, before we do so, we will provide a brief technical overview of the metabolic profiling methods currently employed in research in tomato (**Fig. 1**).

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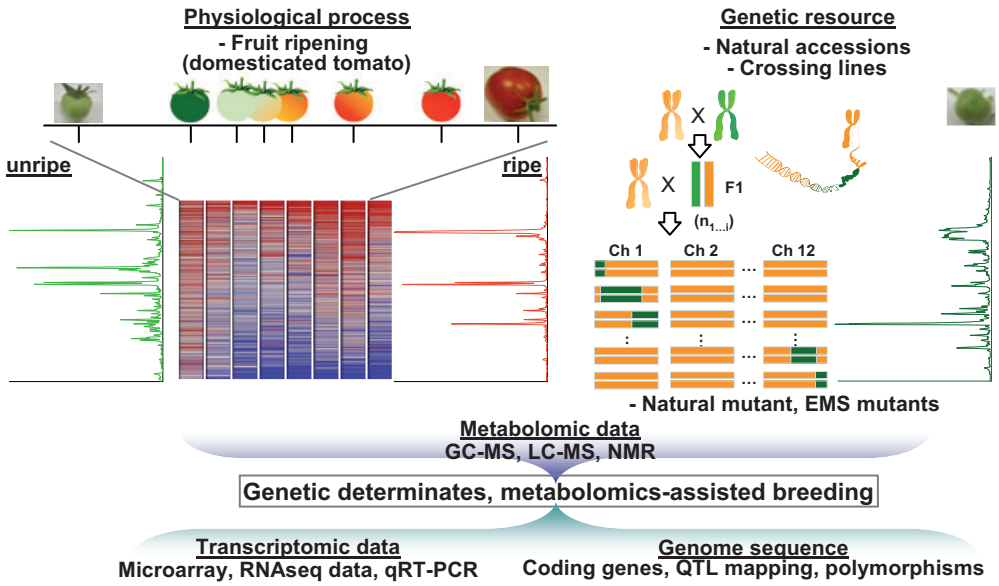

Fig. 1 Schematic overview of recent metabolomics-based approaches on tomato research.

Technical Approaches to Assess the Metabolome

While the ultimate goal of metabolomics is the quantification of the entire metabolomic complement of an organism, current approaches fall some way short of this. It has been estimated that the plant kingdom contains >200,000 metabolites (Dixon and Strack 2003, Fernie et al. 2004, Yonekura-Sakakibara and Saito 2009), ranging from primary metabolites such as sugars and amino acids which provide the monomers needed for the generation of proteins, starches and cell wall comp.onents as well as lipophilic compounds. Moreover, plants produce a wide diversity of secondary or specialized metabolites synthesized either to protect against biotic or abiotic stresses or as attractors, in order to induce pollination, and it is this class of metabolites that comprises the vast majority of the compounds estimated to be present in the plant kingdom. Three major technologies are currently being used for tomato metabolomics, namely gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR). In the following sections, we will briefly describe features of these methods and review the coverage each affords. For this purpose, **Supplementary Table S1** provides an inventory of metabolites reported in tomato using each of these techniques. The first metabolomics studies on tomato were published in 2003 when papers utilizing GC-MS, NMR and LC-MS were published (Burns et al. 2003, Le Gall et al. 2003, Roessner-Tunali et al. 2003). We will return to the biological findings reported in these papers below, but first present a brief overview of the major technologies used for metabolomics in tomato fruit as well as surveying recent developments in the field.

GC-MS is arguably the most widely used technique for plant metabolomics research to date. For this technique, polar metabolites are derivatized to render them volatile (unless they are naturally volatile) and then they are separated by GC. For detection, time-of-flight (TOF)-MS has become the method of choice because of advantages including fast scan times, which give rise to either improved deconvolution or reduced run times for complex mixtures, and relatively high mass accuracy (Obata and Fernie 2012). The crucial advantage of this technology lies in the fact that it has long been used for metabolite profiling; thus, there are stable protocols for machine set-up and maintenance as well as chromatogram evaluation and interpretation (Fernie et al. 2004, Halket et al. 2005, Lisec et al. 2006). The robustness of the protocol means that libraries of retention time and mass spectra data for standard compounds can be shared among laboratories (Schauer et al. 2005a). There are several metabolite databases available including the NIST (http://www.nist.gov/), FiehnLib (Kind et al. 2009), Golm metabolic databases, GMD (Kopka et al. 2005), KOMICs (Sakurai et al. 2014) and MoTo DB (Moco et al. 2006), which facilitate rapid peak annotation. Additionally, the short running time and relatively low running cost are strong advantages of GC-MS. However, the use of GC-MS is limited to thermally stable volatile compounds, making the analysis of high molecular weight compounds (>1 kDa) difficult. Due to the characteristics described above, GC-MS facilitates the identification and robust quantification of a couple of hundred metabolites in plant samples including sugars, sugar alcohols, amino acids, organic acids and polyamines, resulting in fairly strong coverage of the central pathways of primary metabolism. In contrast to GC-MS, general LC-based metabolomic analysis does not require prior sample treatment. On the other hand, some secondary metabolites are not stable under high temperature and light conditions; extraction step needs to be performed as rapidly as possible (Tohge et al. 2011).

LC separates the components in the liquid phase. The choice of columns, including reverse phase, ion exchange and hydrophobic interaction columns, allows the separation of various metabolites based on different chemical properties. Therefore, LC has the potential to analyze a wide variety of metabolites in

plants. The recent development of ultra-performance liquid chromatography (UPLC) renders the technique more powerful because of its sharper separation resulting in higher detection sensitivity, reproductivity and throughput than conventional HPLC (Rogachev and Aharoni 2012). Many types of MS, including quadrupole TOF, triple quadrupole, ion trap, linear trap quadrupole-Orbitrap and Fourier transform ion cyclotron resonance (FT-ICR)-MS, are used depending on the sensitivity, mass resolution and dynamic range required (for details, see Allwood and Goodacre 2010, Lei et al. 2011). The flexibility of the method thus allows us to identify highly divergent metabolite types, but also causes difficulty in establishing large mass spectral libraries for peak identification which are dependent on the instrument-type reflected retention time and mass spectra (Moco et al. 2006), and forces each research group to create its own 'in-house' LC-MS reference library. That said, there are a number of websites that aid in mass spectral analyses (reviewed in Tohge and Fernie 2009). Furthermore, isotope labeling as a means of confirming the identity of peaks has recently been proposed and has been demonstrated to allow the identification of approximately 1,000 metabolites using the FT-ICR-MS approach (Giavalisco et al. 2009). To date, LC-MS is mainly used with a reverse phase column to analyze secondary metabolites because of its ability to separate compounds with similar structure and to detect a wide range of metabolites. However, specialized protocols for determining phosphorylated intermediates, which are not readily detected by LC-MS, have also been developed (Arrivault et al. 2009, Szecowka et al. 2013), as have methods for the comprehensive analysis of phytohormones (Kanno et al. 2010). NMR spectroscopy offers an entirely different analytical approach to that afforded by MS-based techniques, being based on atomic interaction. In strong magnetic fields, atoms with non-zero magnetic moments including the biologically relevant ^{1}H, ^{13}C, ^{15}N and ^{31}P absorb and re-emit electromagnetic radiation. This emission is characterized by its frequency (chemical shift), intensity, fine structure and magnetic relaxation properties, all of which reflect the precise environment of the detected nucleus. Therefore, atoms in a molecule give a specific emission spectrum that can readily be used for identification and quantification of metabolites within a complex biological sample. The sensitivity of this method is much lower than that of MS-based techniques, and the number of compounds that can be detected in a single analysis is limited to one to several dozen (Krishnan et al. 2005, Kim et al. 2010). That said, given that it is so simple to identify and quantify peaks, it is the ideal tool for broad-range profiling of abundant metabolites, while studying changes in non-annotated profiles is highly useful for metabolite fingerprinting of extensive experiments (Lommen et al. 1998, Dixon et al. 2006, Obata and Fernie 2012). Two further techniques have recently been applied in tomato which warrant discussion, namely LC linked to multiplexed non-selective collision-induced dissociation (Wang and Jones 2014) and chemical imaging using contact printing and laser desorption/ionization MS (Li et al. 2014). The first of these represents a powerful method to perform non-biased quantitative assessment of labeling by using non-selective collision-induced dissociation (i.e. fragmentation by measuring whole specialized metabolites as well as their substructures). It can be anticipated that this approach will prove highly useful as an extension of labeling methods, in order to evaluate metabolic fluxes into specialized metabolites beyond what is already known (Giavalisco et al. 2009, Antonio et al. 2013, Nakabayashi et al. 2013). The second approach is radically different and involves contact transfer of tissue content to a pencil-lead-coated glass slide prior to matrix-assisted laser desorption ionization (MALDI)-TOF analysis (Li et al. 2014). This method improves on other metabolite imaging technologies, since it does not suffer from artifacts introduced by matrix or solvent and could readily be transferred to the crop field. This initial proof-of-concept study additionally demonstrated that it was able to provide similar results to LC-MS for a wide range of acylsugars. It thus seems likely that widespread adoption of either of these techniques will probably be highly informative aids in our understanding of the tomato metabolome.

Development of Other Post-Genomic Technologies in Tomato

Before detailing advances in tomato biology that were facilitated by the application of metabolomics, we felt it important to provide some background knowledge concerning the state-of-the art regarding various other molecular analyses in the species. Unlike Arabidopsis and rice (Alonso et al. 2003, Karlova et al. 2014), there are currently no knock-out collections for tomato; however, given that tomato has a long history of genetic research, a wide range of classical mutants exist (Klee and Giovannoni 2011). Several mutant libraries of tomato mutagenized lines have been developed for analyzing the mechanisms underlying mutant phenotypes (Menda et al. 2004, Dan et al. 2007, Saito et al. 2011). Alongside development of libraries of such mutagenized tomato lines, TILLING (targeting induced local lesions in genomes) platforms were also developed as a high-throughput reverse genetic strategy to screen for point mutations in specific regions of targeted genes (McCallum et al. 2000a, McCallum et al. 2000b, Colbert et al. 2001, Minoia et al. 2010). The first outputs of TILLING platforms, key genes for fruit pigments, fruit shelf-life, ethylene responses and phenolics accumulation in tomato fruit, are beginning to emerge (Minoia et al. 2010, Okabe et al. 2011, Di Matteo et al. 2013, Okabe et al. 2013). Tomato was additionally amongst the earliest plants subjected to transgenic approaches, and robust protocols exist for transforming both nuclear and plastid genomes of the species (Jongsma et al. 1987, Ruf et al. 2001). In addition, virus-induced gene silencing (VIGS) protocols are now well established for tomato (Orzaez et al. 2009, Quadrana et al. 2011), and novel genome editing methods such as CRISPR are beginning to be reported for the species (Brooks et al. 2014). In parallel, a broad number of genetics resources based on classical breeding have been generated, including both interspecies crosses and intraspecific crosses, wherein the cultivated tomato have been crossed with one (or more) of its wild relatives (Eshed and Zamir 1995, Lecomte et al. 2004, Prudent

et al. 2009, Alseekh et al. 2013, Pascual et al. 2015), and association mapping panels have been established for tomato (Ranc et al. 2012, Sauvage et al. 2014). Complementing these resources over the last 5 years, a massive research effort has been made in genome sequencing of tomato. The genome of the cultivated tomato and its closest relative *S. pimpinellifolium* was published in 2012 (Tomato Genome Consortium), with that of *S. pennellii* being published in 2014 (Bolger et al. 2014), together with genomic sequencing of approximately 360 accessions (Aflitos et al. 2014, Lin et al. 2014). A major facilitator of this immense data collection was the recent advances in sequencing technologies which have also massively improved transcriptomics approaches in tomato (Martin et al. 2013). Early transcriptomic studies relied on microarrays (Fouts et al. 2002, Frick and Schaller 2002, Zhang et al. 2004, Alba et al. 2005), and these provided several important observations including global profiling of gene expression response to *Pseudomonas syringae*, the fungal toxin fusicoccin, cold treatment and of ethylene control during tomato fruit development. Additionally, quantitative real-time PCR (qRT-PCR) was used to survey the changes in transcription factor expression across a ripening time course in wild-type and mutant tomato, revealing a global overview of general transcriptional–metabolic changes and light response mutant-specific biphasic changes during fruit ripening (Rohrmann et al. 2011), whereas changes in plastidial gene expression, over a similar development course, were evaluated by means of a custom microarray (Kahlau and Bock 2008). Such approaches have recently been superseded by RNA sequencing (RNA-seq; Matas et al. 2011, Chitwood et al. 2013, Koenig et al. 2013) which has been used to compare specific tissues and/or tomato wild accessions investigating differences in developmental architecture, domestication and characterization of a cuticle on the inner surface of the pericarp. Furthermore, epigenetic aspects have been comprehensively characterized in recent studies of the fruit methylome (Zhong et al. 2013), while these aspects of regulation have also received a lot of attention in more targeted approaches centered on tocopherol and lignin metabolism and tomato ripening (Manning et al. 2006, Shivaprasad et al. 2012, Quadrana et al. 2014). Finally, considerable research has been carried out utilizing proteomics notably focusing on plastid maturation and defense responses (Yeats et al. 2010, Barsan et al. 2012). In the next sections, we detail advances in our understanding of small molecule metabolism at the environmental, developmental and genetic levels on a compound class by compound class basis.

Metabolite Profiling of Primary Metabolites

Sugars, organic and amino acids

The advent of metabolomics in plants (in fact arguably in all species) was the application of GC-MS. The term metabolome was coined by Steven Oliver and colleagues in a review in 1998 (Oliver 1998), and while it was used to describe 2D chromatographic evaluation of *Escherichia coli* by Tweeddale's group (Tweeddale et al. 1998), this study presented little in the way of compound identification. While metabolite profiling was used as a technique to classify herbicide mode of action, in an unfortunately overlooked paper, in 1991 (Sauter et al. 1991), the manuscript by Fiehn et al. in 2000 wherein >300 distinct peaks were quantified (Fiehn et al. 2000) is viewed by many as the first metabolomics paper. In this study, the authors established an analytical method based on GC-MS for evaluation of biological variation of metabolic changes in Arabidopsis ecotype and mutant lines. Two years later, the same approach was taken in order to understand confounding results from strategies attempting to metabolically engineer an enhanced potato tuber biosynthesis (Roessner et al. 2001). As mentioned above, the initial reports of metabolomics approaches were carried out in 2003—two of which were focused on primary metabolites (Le Gall et al. 2003, Roessner-Tunali et al. 2003). The first of these looked for unintended effects of manipulating flavonol metabolism by genetic engineering screening sugars, amino acids and phenolic compounds, and revealing changes in the levels of 15 metabolites in addition to the target compounds (Le Gall et al. 2003). The other study also analyzed transgenic lines—in this case lines overexpressing hexokinase—and revealed that the influence of this activity on the fruit primary metabolome decreases across development (Roessner-Tunali et al. 2003). Given that these studies are both over a decade old, it is probably not surprising that considerable research has subsequently been published in both areas.

Primary metabolic shifts during development

The evaluation of metabolic shifts, reflecting changes in the metabolic flux and a general decrease in metabolic activity in primary metabolism during fruit ripening, has similarly received much attention. Particularly notable are the studies of Carrari et al. (2006) and Mounet et al. (2009) which were mainly reliant on GC-MS and NMR, and LC-MS, respectively. Interestingly, both studies carried out transcript profiling in parallel and were thus able subsequently to assemble gene–metabolite networks during the progression from early to late developmental stages of fruit ripening. In the first of these studies, GC-MS-based profiling of primary metabolites alongside GC-MS-based profiling of cell walls was carried out together with HPLC-based pigment analyses, providing data for in excess of 90 metabolites across a dense sampling kinetic. Some features of the metabolite data alone provided some interesting insight into the metabolic shifts occurring on ripening, including the ability to address the operational feasibility of the various pathways of ascorbate biosynthesis in this tissue. When correlation analyses was used to assess if there were any metabolic cues or responses to known signature genes of ripening, it was found that most metabolites exhibited very little correlation, with the exception of sugar phosphates, pigments and intermediates of the tricarboxylic acid (TCA) cycle. The causal link between TCA cycle intermediates and aspects of fruit ripening was demonstrated and later reinforced by studies which revealed that alterations in malate content resulted in reciprocal changes in the level of transitory starch, soluble sugars and post-harvest properties of tomato fruits (Centeno et al. 2011, Osorio et al. 2013b). The results of the study by Mounet et al. (2009) produced similar conclusions, identifying 37 direct gene to metabolite correlations involving regulatory genes such as bZIP and

MYB transcription factors, and it can be anticipated that work on these genes will further enhance our understanding of the role of metabolic shifts in tomato fruit development (Carrari and Fernie 2006). Interestingly, considerable further experiments have been carried out which characterize primary metabolism during fruit development and ripening (Alba et al. 2005, Enfissi et al. 2010, Karlova et al. 2011) and peach (Borsani et al. 2009, Zhang et al. 2010, Lombardo et al. 2011), as well as in non-climacteric fruits (those not characterized by an ethylene-driven respiratory burst), such as strawberry (Fait et al. 2008, Bombarely et al. 2010, Osorio et al. 2011b), pepper (Osorio et al. 2012, Liu et al. 2013) and grape (Deluc et al. 2007, Grimplet et al. 2007). Recently developed statistical methods have already been employed in a proof-of-concept study aimed at identifying conserved and non-conserved patterns of metabolite change during ripening (Klie et al. 2014). This study identified that the pattern of change in the levels of malate, serine, threonine and aspartate discriminated climacteric from non-climacteric fruits. It relied on data not only from the species mentioned above but also from the well-defined ripening mutants of tomato *non-ripening* (*nor*), *ripening-inhibitor* (*rin*) and *Never-ripe* (*Nr*) (Osorio et al. 2011a). Metabolic profiling of these mutants revealed marked shifts in the abundance of metabolites of primary metabolism which lead to decreases in metabolic activity during ripening. When combined with transcriptomic and proteomic data, several aspects of the regulation of metabolism during ripening were revealed. First, correlations between the expression levels of genes and the abundance of their corresponding proteins were infrequently observed during early ripening, suggesting that post-transcriptional regulatory mechanisms play an important role in these stages; however, this correlation was much greater in later stages. Secondly, we observed very strong correlation between ripening-associated transcripts and specific metabolite groups, such as organic acids, sugars and cell wall-related metabolites, underlining the importance of these metabolic pathways during fruit ripening. These results thus further revealed multiple ethylene-associated events during tomato ripening, providing new insights into the molecular biology of ethylene-mediated ripening regulatory networks.

The genetic determinants of primary metabolite accumulation

In the past few years, several studies have been carried out at the metabolomic level to identify the compositional quality of genetic determinants in several plant species including Arabidopsis, tomato, wheat, rice, sesame, broccoli and mustard (Schauer et al. 2006, Meyer et al. 2007, Rowe et al. 2008, Fernie and Schauer 2009, Fernie and Klee 2011, Kusano et al. 2011, Hu et al. 2014, Wen et al. 2014). These studies have led to a far richer description of the natural variation of chemical composition in these species, facilitating the identification of important sources of allelic variance for metabolic engineering (Fernie and Schauer 2009). In this vein, 10 papers concerning research in tomato are perhaps the most relevant so we will dedicate this section to describing them. In an early experiment, Schauer et al. (2005b) inventorized the primary metabolite content of fruits and leaves of cultivated tomato and five of its wild species relatives, namely *S. pimpinellifolium*, *S. neorickii*, *S. chmielewskii*, *S. habrochaites* and *S. pennellii*. This study showed that there were many differences in metabolites such as hexoses and proline which may reflect adaptation to stressful growth habits, whereas several other metabolites such as essential amino acids and vitamins are of nutritional importance and thus this information may be of importance for breeding strategies. Given this fact, studies in our laboratory have focused largely on a population of 74 *S. lycopersicum* × *S. pennellii* introgression lines (ILs). We initially used the established GC-MS method describe above over two independent harvests, being able to identify 889 quantitative trait loci (QTLs) governing the accumulation of 74 metabolites. Interestingly, although in many cases the metabolite content was increased, this was often associated with a yield penalty (Schauer et al. 2006). In order to establish whether these traits were heritable, we grew the *S. pennellii* introgressions for a third harvest, alongside lines that were heterozygous for the introgression (ILHs), enabling the evaluation of hereditability and the QTL mode of inheritance (Schauer et al. 2008). These studies revealed that the mean hereditability of the metabolite QTLs was of a range that would be regarded as intermediate. The comparative study of the tomato ILs and ILHs, however, revealed that most of the metabolic QTLs were dominantly inherited, with a considerable number displaying an additive or recessive mode of action and only a negligible amount displaying the characteristics of overdominant inheritance. Interestingly, the mode of inheritance was quantitatively different between diverse classes of compounds, with, for example, sugars and acids displaying significantly different patterns of inheritance. Moreover, several metabolite pairs belonging to the same pathway displayed a similar mode of inheritance at the same chromosomal loci, indicating that the variation in both metabolites is probably mediated by enzymes involved in their interconversion. However, the association between morphological and metabolic traits was far less prominent in the ILHs than in the ILs, which has wide implications for breeding strategies. The possibility of uncoupling enhanced metabolite content from any penalties with respect to plant performance and fecundity, and redevelopment of hybrid genetic material could prove an important advance in the use of genomics-driven breeding approaches in breeding programs; the fact that metabolite heritabilities of 25–35% are commonly estimated bodes well for the addition of this technique in future breeding strategies. The metabolomics data provided in these studies confirmed previously identified metabolic QTLs such as the Brix QTLs on chromosome 9 (Fridman et al. 2004) as well as identifying novel QTLs which have subsequently been cloned and/or tested by reverse genetic strategies, such as those involved in tocopherol (vitamin E) and branched chain amino acid content (Maloney et al. 2010, Quadrana et al. 2014). In addition, a *S. chmielewskii* IL population was profiled via the same method; however, the sink–source relationship was also altered by reduction of the fruit load (Do et al. 2010). This study, which also represents important research into the environmental influence of the metabolome, provided support for the earlier finding that harvest index had a major impact on metabolism. A further study

using intraspecific introgressions revealed that metabolite content of primary metabolites could be as strongly influenced by introducing variant alleles from within the species (Zanor et al. 2009), most probably highlighting the rigidity of primary metabolism. A final study that is important to discuss here is the first description of the use of metabolomics for genome-wide association studies in tomato (Sauvage et al. 2014). The authors used a core collection of 163 tomato accessions composed of *S. lycopersicum*, *S. lycopersicum* cv. *cerasiforme* and *S. pimpinellifolium* to map loci controlling variation in fruit metabolites, with fruits being phenotyped for a broad range of metabolites including amino acids, sugars and ascorbate. In parallel, the accessions were genotyped with almost 6,000 single nucleotide polymorphism markers spread over the genome, allowing for the identification of 44 loci that were significantly associated with a total of 19 traits including sucrose, ascorbate, malate and citrate levels, revealing this strategy also to be a powerful approach in the definition of candidate genes for crop compositional improvement.

Metabolic Profiling of Volatile Organic Compounds

Tomato volatile organic compounds

Tomato flavors are primarily defined by sugars such as glucose and fructose, by acids such as citrate, malate and glutamate, the profiling of which is described above, as well as multiple less defined volatiles (Baldwin et al. 2000, Tieman et al. 2012). Intriguingly, of the 400 volatiles detectable in tomato fruits (Tikunov et al. 2005), only 16 were predicted to contribute to flavor. Indeed the most important volatiles seem almost exclusively to derive from essential nutrients such as phenylalanine, leucine, isoleucine or linolenic acid. Many of these compounds accumulate as glycosylated precursors in a non-volatile form which are rendered volatile by the action of glycosidases. A recently identified glucosyltransferase adds a third sugar to the conjugate on the onset of ripening, resulting in a drastic reduction in the release of these volatiles (Tikunov et al. 2010, Tikunov et al. 2013), reminiscent of the behavior of other bitter metabolites such as α-tomatine (see below). It is now becoming clear that not only these 16 metabolites contribute to aroma across the entire spectrum of tomato fruits, and some metabolites, such as, for example, guaiacol, are present in considerable amounts in certain types of tomato fruits (Mageroy et al. 2012). Conversely, recent studies using prediction models have suggested that some of the 16 metabolites, such as, for example, β-damascenone, apparently have no contribution to tomato flavor (Tieman et al. 2012). The relative levels of volatiles have been demonstrated to vary greatly in different commercial hybrids (Tikunov et al. 2005), in heirloom varieties (Tieman et al. 2012) as well as in wild breeding populations (Causse et al. 2002, Mathieu et al. 2009); we will document this variation below. It is important to note that the ripening of fruit includes a dramatic change in its volatile profile (Ortiz-Serrano and Vicente Gil 2010). While, as mentioned above, some of this change is probably due to the action of glycosidases, it is anticipated that much of it is due to the transcriptional changes which occur on ripening (Rambla et al. 2014). Nevertheless, despite the presence of detailed expression maps of transcription factor abundance during tomato fruit ripening (Rohrmann et al. 2011), relatively little is known concerning the action of these, with a few exceptions such as the gene encoding SlODO1 (Orzaez et al. 2009). That said, the classical ripening transcription factor mutants *rin* and *nor* have additionally been documented to be impaired in the emission of a subset of flavor volatiles (Kovacs et al. 2009). These facts suggest that combined metabolomics and transcriptomics studies could provide further candidate genes for developmentally regulated improvement of flavor. Similarly, it has been noted that the synthesis of very many flavor volatiles increases concomitantly with ethylene production (Tieman et al. 2006a) and that their synthesis is thus blocked in the ethylene-insensitive mutant *Nr* (Kovacs et al. 2009). Moreover, epigenetic changes are also an important component of ripening, as demonstrated by the *Colorless non-ripening* (*Cnr*) mutant (Manning et al. 2006), and more recent studies have revealed the programs of DNA methylation occurring during the ripening process (Zhong et al. 2013).

The genetic determinants of volatile organic compounds

In tomato, several broad screenings of genetic populations at the level of their volatile content have been performed using the strategies above. In this section we will describe these on an approach by approach basis starting with those displaying the narrowest genetic diversity. In 2005, Tikunov et al. profiled a total of 322 compounds using solid phase methyl ester GC-MS across a set of 94 contrasting tomato genotypes covering the variation in the germplasm of commercial varieties (Tikunov et al. 2005). The study revealed that levels of volatiles of a certain chemical class behave similarly, a fact exemplified by the phenylpropanoid-derived volatiles and their derivatives. This is a highly interesting observation given that phenylpropanoid metabolism is known to contribute to plant stress responses (Dixon and Paiva 1995) while methyl salicylate has been demonstrated to be an airbourne signaling agent in pathogen resistance (Shulaev et al. 1997, Tieman et al. 2010). Evaluation of the volatile content of 19 heirloom varieties revealed massive differences in the levels of the individual volatiles, which varied between 12- and in excess of 3,000-fold (Tieman et al. 2012). This study also employed tasting panels as well as modeling studies in order to better understand the factors conferring a tomato with flavor. Arguably, the most informative strategy to date was the adoption of the QTL approach as described above for primary metabolites. A broad profiling of fruit volatiles in the *S. pennellii* ILs yielded 100 QTLs that were conserved across harvests (Tieman et al. 2006b). Metabolic and flux profiling of one of these QTLs was instrumental in defining the pathway for synthesis of important phenylalanine-derived aromatic compounds in the fruit (Tieman et al. 2006a). Some 30 additional QTLs were identified in a second population of ILs derived from a a cross between the elite cultivar with the wild species *S. habrochaites* (Mathieu et al. 2009). Whilst these approaches were reviewed recently (Fernie and Klee 2011), several studies of note have been published in the meantime, and recent

estimates state that >50 volatile QTLs have now been reported in tomato (Alseekh et al. 2013, Klee and Tieman 2013). A QTL located on chromosome 1 affects multiple volatile esters, with the *S. pennellii* introgression conferring up to 20-fold increases in these metabolites; intriguingly, these compounds correlate negatively with human taste preferences, suggesting that the esters may be linked to palatability. A retrotransposon insertion into the promoter of a carboxylesterase gene of the red-species progenitor led to a massive up-regulation of expression of this gene and consequent reductions in this suite of metabolites (Goulet et al. 2012). These results were recently complemented by a study of a ripening-related alcohol acyltransferase (AAT1) which was found to be far more efficient in *S. pennellii* than in *S. lycopersicum*, leading to the conclusion that the two species have evolved to adjust their volatile content precisely by careful modulation of the synthesis and degradation of esters (Goulet et al. 2015). In addition, the importance of a lipoxygenase and a glucosyltransferase to volatile content has also been demonstrated using transgenic approaches coupled to targeted metabolite profiling (Tikunov et al. 2013, Shen et al. 2014). In the first of these studies, the gene non-smoky glycosyltransferase, a glycosyltransferase acting on phenylpropanoids which are the precursor molecules for smoky flavor, which had previously been identified to associate with this flavor (Menendez et al. 2012), was proven to prevent the damage-induced release of the smoky aroma-associated phenylpropanoid-derived volatiles in ripening tomato fruit by means of structural modification of their glycoconjugates (Tikunov et al. 2013). The other study targeted the 13-lipoxygenase, TomloxC, revealing that this enzyme is important for the synthesis of both C5 and C6 flavor volatiles (Shen et al. 2014), thereby confirming it as an important target for improving flavor. Additionally of interest with regard to manipulating volatile content is the supply of precursors for their production. The study of Zanor et al. (2009), described above, profiled the volatile content alongside that of primary metabolism, indicating that the links between the class of metabolites were not very tight. Similarly, in tomato, the levels of phenylpropanoids (Tieman et al. 2006a, Dal Cin et al. 2011) and branched chain amino acids (Kochevenko et al. 2012) did not correlate with the levels of volatiles derived from them. As yet, association mapping studies of tomato volatiles have not been documented; however, they will probably provide an important avenue for research in the future. Whilst interrogation of published and soon to be published next-generation sequencing resources for the genomes of tomato wild species (Aflitos et al. 2014, Lin et al. 2014) and cultivars, and RNA-seq data sets (Matas et al. 2011, Chitwood et al. 2013, Koenig et al. 2013, Bolger et al. 2014) will probably also provide important clues toward flavor improvement.

Metabolic Profiling of Non-Volatile Secondary Metabolites

Phenylpropanoids, flavonoids, glycoalkaloids, pigments and acyl-sugars

Given that they have been very recently reviewed (Slimestad and Verheul 2009, Tohge et al. 2014), we will not cover studies into the metabolic regulation of secondary metabolism in any great detail here. In addition to the volatile organic compounds described above, tomato fruits are known to produce a wide variety of secondary metabolites such as polyphenols, carotenoids and alkaloids. The dynamic levels of many of these metabolites vary in a manner like that of the 400 detectable volatiles described above, in order to minimize the attractiveness of the fruit prior to maturity and maximize its attractiveness thereafter. Despite not going into detail concerning the dynamics of these compound classes, it is pertinent to describe the various technical methods utilized in their evaluation. The vast majority of these are based on LC-MS; however, before covering these approaches, it is worth mentioning a handful of studies utilizing earlier technologies. For example, NMR using purified chemicals from plant extracts has been utilized to profile positional assignments between C-H in substitution of specific acyl groups, and locations of branching (Ghosh et al. 2014). Similarly, it has been demonstrated that MALDI/TOF-MS can be utilized to acquire mass spectra of carotenoids effectively (Fraser et al. 2007). The technique has been applied in vivo to the analysis of carotenoids in isolated plant cells and in vitro, as well as in a preliminary QTL analysis described below. Finally, a recent combinatorial approach that was mainly based on direct infusion MS allowed the evaluation of some 2000 metabolic signatures (Perez-Fons et al. 2014). The vast majority of studies, however, rely on the combination of LC and MS (Moco et al. 2006, Iijima et al. 2008, Mintz-Oron et al. 2008, Rohrmann et al. 2011). A total of approximately 250 annotatable metabolites have been observed and reported in tomato fruit using such methods (**Supplementary Table S1**). It is difficult to obtain exact confirmation given the limitation of available commercial standards; however, a combination of approaches including use of literature- and web-based resources (Moco et al. 2006, Iijima et al. 2008, Mintz-Oron et al. 2008, Tohge and Fernie 2009, Sakurai et al. 2014, Schwahn et al. 2014, Tohge et al. 2014) as well as the use of biological standards, i.e. well characterized samples which can be used to aid in the identification of a novel unstudied sample (Farag et al. 2007, Suzuki et al. 2008, Tohge and Fernie 2010, Saito et al. 2013, Yang et al. 2014). The utilization of high-resolution MS can allow the determination of the exact chemical formula of an analyte (Iijima et al. 2008, Mintz-Oron et al. 2008, Weber et al. 2011, Allwood et al. 2012) which is highly useful, but, especially in the case of secondary metabolites, falls some way short of providing structural information. Large (>1,000 *m*/z) secondary N- and S-containing metabolites, such as highly modified glycoalkaloids, are difficult to use in this approach, because higher abundance of each C, N and S monoisotopic peaks results in less accuracy of mass detection. Such an approach, however, is a very powerful tool to distinguish CHO formed and other types of metabolites. A further approach that merits discussion, however, is the use of whole-plant stable isotope labeling which has been applied to both secondary metabolites and lipids (Giavalisco et al. 2009, Bromke et al. 2014), since this approach is highly effective in distinguishing biologically derived analytes from laboratory-derived artifacts. In tomato, several screens of carotenoids were carried out in the 1980s and 1990s, and metabolic

regulation of this pathway was dissected via the cloning of a range of color mutants (Bird et al. 1991, Thompson et al. 1999, Ronen et al. 2000, Isaacson et al. 2002, Galpaz et al. 2008, Kachanovsky et al. 2012), with a similar approach also allowing the identification of regulatory elements of the skin phenylpropanoid underlying the *y* (*colorless fruit epidermis*) mutant (Adato et al. 2009). The first metabolomics approach targeting fruit secondary metabolism was, however, that of Aharoni and co-workers who used direct infusion FTICR-MS to classify secondary metabolite groups which show a different expression profile during fruit development in strawberry (Aharoni et al. 2002). This study did not however return exact identifiers for the composite metabolites. As stated in **Supplementary Table S1**, 36 acyl-sugars, 122 flavonoids, 56 hydroxycinnamates and 49 glycoalkaloids have, to date, been annotated to be present in the tomato clade.

A range of polyphenols, namely phenylpropanoids and flavonoids, have been reported in tomato leaf, skin and pulp (Slimestad et al. 2008, Tohge et al. 2014). Because of the pleiotropic health-beneficial effects of dietary plant polyphenols, several polyphenols, for example chlorogenic acids (Hermann 1979, Schuster et al. 1986), sinapic acid derivatives (Wardale 1973), vanillic acid (Schmidtlein and Herrmann 1975, Fleuriet and Macheix 1976), hydroxycinnamate derivatives (El Khatib et al. 1974), naringenin and quercetin-3-*O*-rhamnoside (Wu and Burrell 1958), naringenin-glycosides (Miki and Akatsu 1972, Galensa and Herrmann 1979) and rutin (Rivas and Luh 1968), have been investigated using domesticated tomatoes in early studies. In recent studies, using MS-based metabolomics approaches (Moco et al. 2006, Iijima et al. 2008, Mintz-Oron et al. 2008, Dal Cin et al. 2011, Rohrmann et al. 2011), in total >170 polyphenols have been detected and annotated (**Supplementary Table S1**). Several studies have been carried out using the transgenic approach promoting higher production of flavonoid, by Petunia chalcone synthase (PhCHI; Muir et al. 2001), Petunia *Del* and *Ros1* (Butelli et al. 2008), maize *LC* and *C1* (Bovy et al. 2002) and an activation tagging line (ANT1; Mathews et al. 2003). In general, phenylpropanoids and flavonoids are present in all seed plants and in almost all plant organs. In addition it is known that their structural diversity and quantities vary considerably within and between plant species and accessions (Saito et al. 2013, Tohge et al. 2013). Recent studies on tomato glandular trichomes using *S. lycopersicum*, *S. pennellii*, *S. pimpinellifolium* and *S. habrochaites* elucidated several methyltransferase involved in glandular trichome-specific flavonoids (Schmidt et al. 2011, Schmidt et al. 2012, Kim et al. 2014). However, as yet metabolomic profiling of common flavonoid derivatives using a wide variety of tomato accessions has not been documented; the chemical diversity of flavonoids will be determined in future studies.

As mentioned above, there is a ripening-dependent conversion of tomatine to esculeoside glycoalkaloids via a series of hydroxylation and glycosylation reactions (Fujiwara et al. 2005, Iijima et al. 2008, Katsumata et al. 2011). Given that the former are toxic while the latter confer nutritive properties (Friedman et al. 2000), it has been proposed that they dissuade animals from eating the immature fruit. Changes in the level of α-tomatine and dehydrotomatine have been widely documented to be dependent on genotype, tissue and growth conditions (Friedman and Levin 1998, Kozukue et al. 2004, Iijima et al. 2008, Iijima et al. 2009, Itkin et al. 2011). In addition, glycoalkaloid profiling of *rin*, *nor* and *Nr* revealed that the tomatine to esculeoside conversion is regulated by ethylene-dependent fruit maturation (Itkin et al. 2009). By application of recently developed LC-MS metabolome platforms, >100 steroidal glycoalkaloids have been described in various tomato tissues (Moco et al. 2006, Iijima et al. 2008, Mintz-Oron et al. 2008, Itkin et al. 2011, Rohrmann et al. 2011). In the tomato pericarp of *S. lycopersicum*, some 12 steroidal alcohol glycoalkaloids were putatively annotated (Rohrmann et al. 2011). However, metabolic profiling of tomato outer epidermis revealed 40 (Moco et al. 2006), 13 (Mintz-Oron et al. 2008) or 93 (Iijima et al. 2008) steroidal glycoalkaloids, respectively. Additionally some 85 steroidal glycoalkaloids were identified in 21 tomato tissue types (Itkin et al. 2011), whereas 123 were found in fruit extracts of eight different accessions including wild species (Iijima et al. 2013). Moreover, a recent co-expression analysis-based study revealed the presence of steroidal glycoalkaloid gene clusters on chromosomes 7 and 12, while silencing of GLYCOALKALOID METABOLISM 4 (GAME4) resulted in a lower abundance of steroidal glycoalkaloids (Itkin et al. 2013). Within the last year, the complexity of the tomato steroidal glycoalkaloid network has been further expanded with a detailed study that identified 169 putative steroidal glycoalkaloids found in eight tomato accessions (*S. lycopersicum*, *S. pimpinellifolium*, *S. cheesmaniae*, *S. chmielewskii*, *S. neorickii*, *S. peruvianum*, *S. habrochaites* and *S. pennellii*) and four tissue types. The combined data were used for correlation analysis and were able to make a valuable contribution towards annotation and classification of steroidal glycoalkaloids as well as detecting novel putative biosynthetic branch points (Schwahn et al. 2014). This study also highlighted that understanding and construction of a scaffold of a whole biosynthetic pathway can be applied for integration analysis with other omics data such as a transcriptome data set for a further functional genomics approach. As for the primary metabolites and volatiles, wide breeding populations have begun to be utilized in the study of secondary metabolism. To date, the majority of this work has focused on either pigments or acyl-sugars (Schilmiller et al. 2012, Perez-Fons et al. 2014); however, a recent study has expanded this to include phenylpropanoids and glycoalkaloids (Alseekh et al. 2015).

The genetic determinants of secondary metabolites

While, as mentioned abov, the majority of our knowledge concerning pigmentation of fruits comes from work with spontaneous or experimentally derived mutants (Bird et al. 1991, Wilkinson et al. 1995, Thompson et al. 1999, Ronen et al. 2000, Isaacson et al. 2002, Galpaz et al. 2008, Kachanovsky et al. 2012), a process which has recently been accelerated by the development of TILLING platforms for tomato (Jones et al. 2012), ILs have also been used to study these pathways. In the proof-of-concept paper detailed above for the utilization of

MALDI-MS for plant carotenoids, QTLs were identified within a subset of the *S. pennellii* ILs for canthaxanthin and D-lycopene (Fraser et al. 2007). Similarly, analysis based on color alone revealed many QTLs within this population, but also revealed that there were factors influencing this trait beyond the structural genes of carotenoid metabolism (Liu et al. 2003). In an attempt to identify such factors, the group of Giovannoni took a network-based approach wherein they performed HPLC-based profiling of carotenoid and lycopene contents across the *S. pennellii* ILs alongside expression analysis, with guilt-by-association then being used to identify transcription factors involved in carotenoid biosynthesis (Lee et al. 2012). Using this approach, the authors identified SlERF6 as a potential regulator and confirmed this hypothesis by modifying the expression of this gene by a transgenic approach and verifying changes in the gene expression and metabolite levels involved in carotenoid biosynthesis. In an analogous approach, the tomato fruit gene regulatory network was generated using artificial neural network inference analysis and transcription factor gene expression profiles derived from fruits sampled at various points during development and ripening. One of the transcription factor gene expression profiles with a sequence related to the *Arabidopsis thaliana* ARABIDOPSIS PSEUDO RESPONSE REGULATOR2-LIKE gene (APRR2-Like) was up-regulated at the breaker stage in wild-type tomato fruits and, when over-expressed in transgenic lines, increased plastid number, area and pigment content, enhancing the levels of Chl in immature unripe fruits and carotenoids in red ripe fruits (Pan et al. 2012). Returning to the structural genes, their action was studied in a recent elegant VIGS study whereby nine genes were independently targeted and the effect of their silencing on 45 carotenoid isomers was evaluated (Fantini et al. 2013). The recent discoveries that the phytohormones strigolactone and carlolactone are derived from carotenoids (Vogel et al. 2010, Alder et al. 2012) provide a further reason for optimism that metabolomics approaches such as those described in this review retain considerable value in advancing our understanding of these metabolic pathways. Plant hormones themselves have as yet received relatively little attention at a global level in tomato (Klee and Giovannoni 2011), although the metabolomes of plants altered in their hormonal machinery have been discussed in several publications (Wilkinson et al. 1995, Tieman et al. 2000, Barry and Giovannoni 2006, Vogel et al. 2010, Osorio et al. 2013a, Kumar et al. 2014), as has the cross-talk between primary metabolism and hormone levels within the fruit (Araújo et al. 2012, Araujo et al. 2014).

Acyl-sugars are known as an interesting metabolite for the investigation of cross-talk between primary or secondary metabolism. Solanaceae acyl-sugars, also known as sugar-polyesters, consist of aliphatic acyl groups of varying chain length esterified to the hydroxyl groups of sugars. The acyl-sugars in tomato fruits generally are of low abundance in *S. lycopersicum* and *S. pennellii* (Alseekh et al. 2015), but several studies looking at elucidation of acyl-sugar metabolism have been performed using leaf glandular trichomes. The fact that tomato acyl-sugars are found in glandular trichomes of the wild tomato species, a trait often at lower abundance in domesticated tomato species, is traditionally known by their biological functions against aphids (Goffreda and Mutschler 1989, Rodriguez et al. 1993), leafminer (Hawthorne et al. 1992), whitefly (Kisha 1981, Liedl et al. 1995) and worms, including fruit worm (Williams et al. 1980, Juvik et al. 1994, Dias et al. 2013). Despite lacking high acyl-sugar production in current domesticated tomatoes, F_1 plants from a cross between *S. lycopersicum* and *S. pennellii* produce moderate amounts (Resende et al. 2002). Using a combination of metabolic QTL analysis, cross-species comparison and genome-scale gene analysis, several key genes encoding BAHD acyl-transferases (ATs; from *S. pennellii*; Schilmiller et al. 2012) and *S. habrochaites* (Kim et al. 2012), and 3-ketoacyl-acyl carrier protein synthase (KAS; from *S. pennellii*; Slocombe et al. 2008) have been found and characterized. As has been stated, acyl-sugars are derived by sugar metabolism and acyl-CoA metabolism which is synthesized from fatty acid metabolism and branched amino acid (BCAA) catabolism (Slocombe et al. 2008, Schilmiller et al. 2012); thus metabolic engineering of higher production of acyl-sugars is a complex process requiring the presence of multiple genes and is difficult due to its complexity, quantitative inheritance and a lack of metabolic cross-talk. However, natural variation of acyl-sugars has been documented in some accessions; largely different acyl-sugar profiles are found between *S. habrochaites* (Kim et al. 2012, Ghosh et al. 2014) and *S. pennellii* (Shapiro et al. 1994). In addition to being of interest with regard to understanding the framework of biosynthetic branches and key genes involved in species/accession-specific biosynthetic steps, it will provide new insights into evolution of acyl-sugar metabolism. Further investigation of key gene discovery, the cross-talk between primary metabolism and flux analysis will probably promote better understanding of acyl-sugar metabolism and provide new important insights for metabolic-assisted breeding.

A very recent study has extended the evaluation of secondary metabolism in tomato considerably (Alseekh et al. 2015). In this study, the pericarp of the same samples of *S. pennellii* ILs used for analyzing primary metabolism (Schauer et al. 2008) were profiled using Orbitrap MS and 145 metabolites including flavonols, phenylpropanoids, glycoalkaloids and acyl-sugars. In total, 679 mQTLs (metabolomic QTLs) were detected across the 76 ILs. As for the primary metabolites, comparison of the metabolite abundances between plants expressing the introgression homo- or heterozygously revealed that the mode of inheritance of the majority of the mQTLs was dominant or additive. Heritability analyses revealed that mQTLs of secondary metabolism were perhaps surprisingly less affected by the environment than mQTLs of primary metabolism. However, it is important to note that while on average there are fewer QTLs per metabolite for the secondary metabolites, the magnitude of the secondary metabolite QTLs is much greater, with certain metabolites displaying up to 10,000-fold variation across the population (Alseekh et al. 2015). The study additionally applied the recently established qRT-PCR platform of Rohrmann et al. (2011) to gain insight into putative transcriptional control mechanisms of a subset of the mQTLs including those for hydroxycinnamates, acyl-sugars, naringenin chalcone and a

range of glycoalkaloids. Finally, VIGS was used to confirm the candidate genes encoding glycosyltransferase as important for creating glycoalkaloid diversity. As stated above, interrogation of RNA-seq data alongside genome sequence data will probably greatly accelerate progress in cloning the genes underlying these QTLs. In addition, as previously stated by Zamir (2013), further meta-analyses of the massive data sets reviewed here will probably prove instrumental in identification of novel links between traits and thus provide important information for plant breeding.

Conclusions

Within the last 15 years, metabolomics-based approaches have facilitated the acquisition of huge data sets and allowed the uncovering of many previously unknown relationships. In addition, the coverage of methods currently employed sums to approximately 350 metabolites, which is far greater than that of the earliest studies and would most probably have been inconceivable some 20 years ago. While metabolite profiling is one of the tools that is of high relevance for substantial equivalence testing of novel crops as well as for quality testing of foodstuffs, it is also an immensely powerful tool for fundamental research. In tomato fruit research its use is largely focused in two areas (i) understanding of the metabolic shifts that occur during development and (ii) understanding the genetic architecture underlying accumulation of metabolites. As we have described above, tomato is one of the pre-eminent species for addressing these questions, with metabolomics playing a central role as a tool in addressing these questions. Not to downplay its importance, it is, however, important to note that integrative approaches using metabolomics alongside other post-genomic profiling methods appear to offer even greater scope with regard to the biological questions which can be tackled. Looking to the future, the grand challenge for metabolomics remains to improve its comprehensiveness (Fernie et al. 2004); in addition, methods for assessing subcellular compartmentation of metabolism are likely to be crucial (Sweetlove and Fernie 2013, Sweetlove et al. 2014), as is the further development of isotope-based methods for flux profiling (Dal Cin et al. 2011, Szecowka et al. 2013). That said, research in the last 11 or so years has provided great advances in our understanding of the structure and evolution of secondary metabolic pathways in tomato, as well as a better understanding of the influence of primary metabolism on plant growth and development.

Supplementary data

Supplementary data are available at PCP online.

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Disclosures

The authors have no conflicts of interest to declare.

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