

Genetic challenges of flavor improvement in tomato

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In many instances, the intensive breeding of crops over the past half century with a focus on yield has indirectly led to reductions in flavor and nutrient content. Largely, this deterioration of quality relates directly to the genetic and biochemical complexity of such traits. Here, we describe challenges associated with quality improvement, emphasizing tomato fruit flavor. Flavor improvement is particularly problematic because of the difficulty of assessing the phenotype as well as a lack of fundamental knowledge about the chemicals driving consumer preferences, the pathways for their synthesis, and the genes regulating the output of these pathways. Recent breakthroughs from a systematic analysis of these factors and the availability of a tomato genome sequence have led to significant progress in our understanding of flavor. However, the need to deliver improved flavor in the context of high yield and long postharvest shelf life still present major challenges.

Flavor versus yield

It is generally accepted by consumers that many modern commercially produced fruits have lost their flavor. In addition, there is a growing body of evidence that suggests the nutrient content of fruits and vegetables has declined over the past half century of intensive breeding. This review addresses the evidence for these claims, the underlying causes for this deterioration, and the challenges to quality improvement. Flavor, in particular, exemplifies the complexity of understanding and manipulating quality traits. Although this review focuses on tomato flavor quality in particular, the challenges are similar for most fruit crops and, more broadly, the genetics of flavor illustrate the challenge of understanding a crop trait that is multigenic and environmentally influenced.

A survey of US Department of Agriculture (USDA) food composition data for 43 fruits and vegetables between 1950 and 1999 indicated significant reductions in minerals, protein content, and vitamins over that period [1]. Such comparisons, however, are inherently difficult. Modern analytical methods are often more sensitive and reliable, which can skew the results. Agricultural practices have also changed over the past half century. Nevertheless, there is a substantial and growing body of evidence to support the claim that nutrient content of intensively bred crops has dropped as yield has increased and time to harvest has decreased [2–4]. Modern cultivars have been

continuously selected for rapid growth and yield and, for the most part, have not been selected or even screened for nutrient content.

The tomato is no exception to this trend. According to the United Nations Food and Agriculture Organization yields of fresh market tomatoes have increased 300% since 1970 in the USA (<http://faostat3.fao.org/home/index.html>). Consumer liking for the modern commercial salad tomato is not high, and they report that fruits taste worse than many older 'heirloom' varieties [5]. Much of the consumer dissatisfaction with modern hybrids is probably associated with the harvest of immature fruits and subsequent post-harvest handling [6,7]. However, modern cultivars also have fewer of the most important contributors to flavor than varieties that pre-date the post World War II period of intensive breeding [5,8]. Because the tomato is grown as an annual, breeding has been much more intensive than for other fruits grown as perennials. In some instances, selection for certain traits has had unintended negative consequences on flavor. For example, determinate varieties have a more concentrated fruit set over a shorter period of time than indeterminate varieties. This is a desirable trait in fresh market tomatoes that are hand harvested because labor costs are less. However, determinate varieties, because of the heavier fruit load, have fewer soluble solids and are less sweet [9]. Similarly, most modern cultivars contain a mutation that inactivates the UNIFORM transcription factor [10]. Wild type fruits ripen from the blossom end toward the stem end and have what is referred to as a green shoulder; the tissue surrounding the pedicel is dark green and only turns red very slowly. Fruits from *uniform* (*u*) mutant plants ripen more uniformly, lack the pronounced green shoulder, and are deemed more attractive. However, *u* fruits have fewer chloroplasts, lower levels of carotenoids, and significantly fewer soluble solids, all of which contribute to the fruit flavor profile [10]. Thus, the widespread introduction of this mutation to make the fruits more visually appealing had the unintended consequence of making them less flavorsome.

Flavor quality improvement is an example of the real-world challenges that breeders face. For the most part, growers are paid on the basis of pounds of product in the box with no added value for taste quality. Because breeders' customers are growers rather than consumers, they emphasize yield and associated traits such as disease resistance, fruit size, and physical appearance. The challenge to flavor quality improvement is to bring the consumer back into the selection process without

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compromising the needs of the grower. We must define the genetics of flavor quality and develop a molecular toolbox to assist the breeder in maintaining and ultimately improving flavor and nutrient quality within the context of high yield.

Flavor is a somewhat subjective phenotype that integrates multiple sensory systems, ultimately manifesting itself at brain level. Although appearance and texture certainly influence perceived flavor [11], the underlying chemistry can be reduced to molecules that interact with taste and olfactory receptors [12]. The genetic challenge is to track those chemicals reliably and translate their contents into consumer preferences (i.e., phenotype). The first step is simply identifying the metabolites that are important for taste. There is a large amount of variation in metabolite composition in most crops between seasons and sites [13], and the tomato is no exception (Figure 1). This makes careful replication of every metabolome study essential [14]. Tomato flavor, in particular, is the sum of sugars, acids, and many volatile chemicals [15]. These volatiles are derived from a diverse set of precursors that include branched-chain and aromatic amino acids, fatty acids, and carotenoids. Fifty or more quantitative trait loci (QTLs) affecting volatile levels have been identified [16–18]. Because there are so many chemicals that contribute to flavor, altering one or a few of them is unlikely to have a

major effect on consumer preferences. The task can, however, be somewhat simplified by identifying important regulators of pathways that lead to multiple chemically related volatiles. For example, phenylalanine is the precursor of four important volatiles with a common, rate-limiting first step catalyzed by a family of aromatic amino acid decarboxylases [19] (Box 1). Overexpression of one of these enzymes causes tenfold or higher increases in all of the downstream volatiles [19]. However, the effects of increasing only these Phe-derived volatiles on consumer preferences have not been evaluated.

The tomato is an excellent model in which to dissect flavor at the molecular level. There is a large body of analytical work addressing the chemistry of the fruit and the functions of sugars, acids, and volatiles influencing flavor perception [20,21], providing a good catalogue of potential flavor active chemicals. This catalog is further expanded by the large set of tomato germplasm with diverse chemical content and a wide range of flavors [5]. The crop is grown worldwide and is readily transformable. Finally, the genomes of tomato and related species have recently been determined [22]. Thus, genetic and molecular manipulations make the isolation of novel genes straightforward, and their functions can easily be validated *in vivo*. Although flavor in a tomato is complex, many of the volatile chemicals contributing to its flavor also

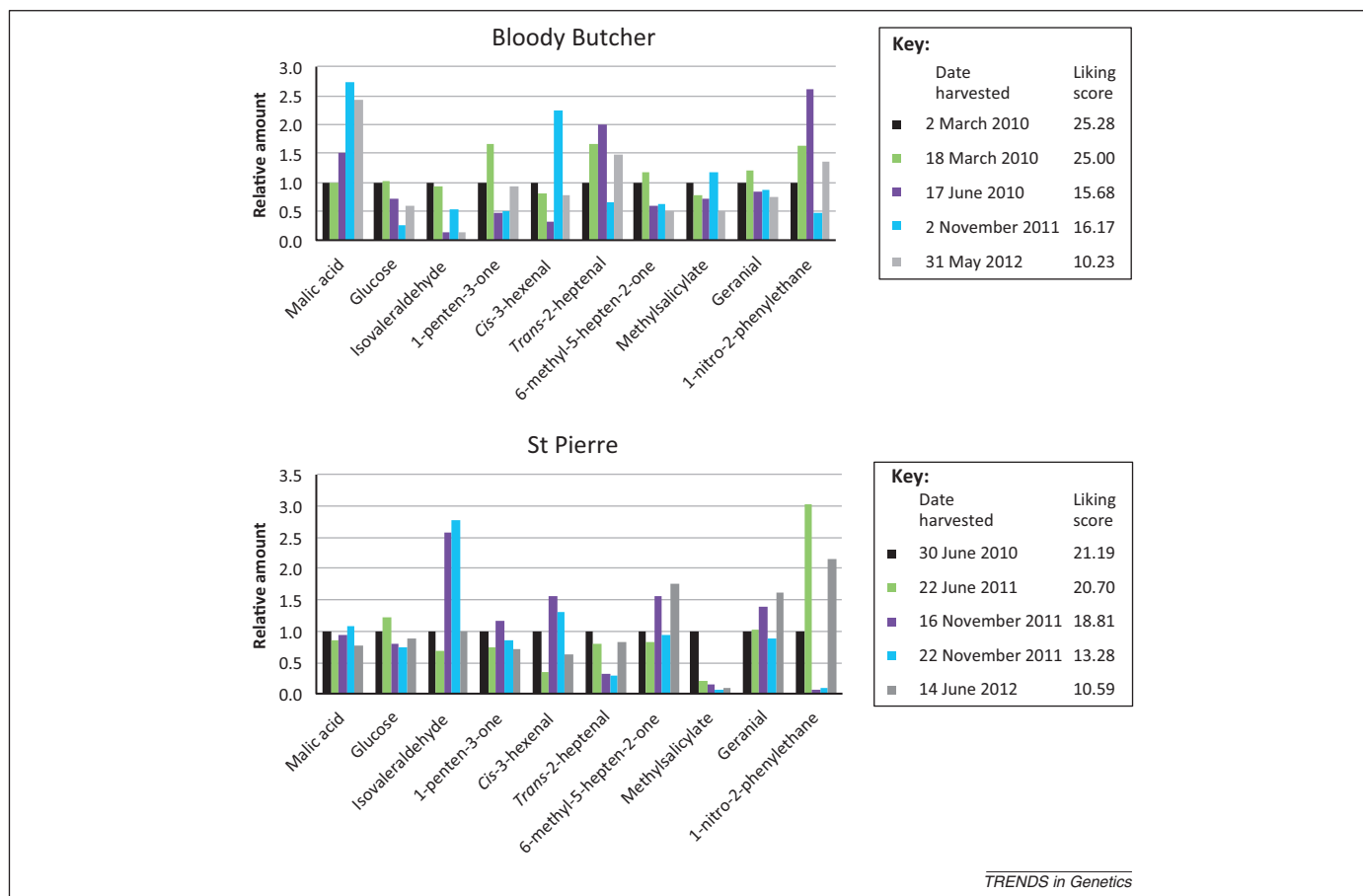


Figure 1. Seasonal variation in flavor-associated chemicals and consumer preferences. Variation in flavor-associated chemicals within the cultivars Bloody Butcher and St Pierre. Five separate consumer panels were performed between March 2010 and June 2012. The values for each chemical are set at a value of 1.0 for the first panel and expressed as a value relative to that number for each subsequent panel. Colors of bars correspond to the liking score from each panel shown on the right. Liking scores for ~100 different varieties over all seasons ranged from a high of 33 to a low of 2.

Box 1. Genes encoding tomato volatile biosynthetic enzymes

A number of genes with validated functions in tomato volatile metabolism as well as the volatiles associated with their functions have been identified (Table 1). In every case, functionality has been validated with transgenic plants. CCD1 cleaves multiple carotenoid precursors at the 5,6 and 9,10 positions to generate several flavor volatiles. CXE1 cleaves a variety of acetate esters to produce the corresponding free alcohols and acetic acid. Generally, three approaches have been used to identify genes. CG indicates a candidate gene approach where homology to genes with known function led to a tomato gene. BP indicates a screen for genes encoding enzymes that might function in a given synthetic pathway. QTL indicates a gene that was identified by linkage to a QTL affecting a target volatile pathway. Examples of the approaches are illustrated with *LoxC*, *AADC*, and *CXE1*. *LoxC* was initially identified as a cDNA with homology to known lipoxygenases that were highly expressed in ripening fruits. *AADC* was identified by a hybrid approach. Characterization of the metabolic pathway indicated that the first step in synthesis was probably an aromatic amino acid decarboxylation. A major 2-phenylethanol QTL, malodorous, was located on chromosome 8. A screen of expressed sequences identified members of a candidate gene family mapping to the vicinity of the QTL. *CXE1* was identified by chromosome walking to a QTL regulating the levels of a group of several acetate esters in fruits.

contribute to flavors of other fruits [23]. Thus, identification of the pathways and key regulatory factors controlling their output are probably translatable to future molecular improvements of other species.

Flavor improvement can be broken down into a series of specific challenges:

- What are the most important chemicals that contribute to consumer preferences (liking) either positively or negatively?
- What are the genes that control the synthesis of those chemicals?
- What are the appropriate alleles of the most important genes?

Only after these questions have been answered can one think about actually introducing a suite of genes into suitable germplasm. Modern commercial varieties must deliver high yield and postharvest shelf life year-round, properties that are essential to the grower, and it is therefore imperative that every manipulation of the genes influencing flavor should maintain these properties.

The most important flavor chemicals

The first challenge associated with flavor improvement is the assay, which requires a clear definition of flavor. With

so many genes influencing flavor chemistry, inheritance of good flavor requires the screening of large populations. Also, the chemical composition of a fruit is hugely influenced by the environment (Figure 1). Fruits from a single plant harvested on different days can have very different taste properties. Seasonal and site variations can be larger than the plant-to-plant variation observed within a single season. All of these factors make it virtually impossible for screening based on taste alone. Even objective chemical analysis of flavor chemistry is not practical, because it is expensive, technically demanding, and subject to the same environmental variation as taste.

Key to solving the flavor challenge is the need to understand the chemistry of flavor preferences. Sugars (glucose and fructose) and acids (principally citrate and malate) interact with taste receptors in the mouth, providing a foundation for good flavor. Volatiles are also essential to good flavor; removing them greatly reduces flavor intensity [15]. Volatiles can be perceived either orthonasally or retronasally. Orthonasal olfaction involves volatile uptake through the nose, whereas retronasal olfaction occurs when volatiles are released from the food in the mouth and are forced from the back of the oral cavity to the olfactory epithelium [12]. Volatiles delivered by these two pathways are not perceived by the brain in the same way. It is retronasal olfaction, and not orthonasal olfaction, that is essential to flavor. Over 400 different volatiles have been detected in tomato fruits [20,21]; however, it is likely that a much smaller subset of these most significantly contribute to flavor and consumer preferences. Historically, rank ordering of volatile contributions has been determined using odor units [21] – the ratio of the concentration of a chemical in a food to its odor threshold. The odor threshold, however, is defined as the minimum concentration at which an individual can detect the presence of a volatile and is typically determined orthonasally. If the concentration exceeds the odor threshold then that volatile must contribute to flavor and if it falls below the threshold the volatile is unimportant. Odor unit values permit rank ordering of volatiles for their contributions to flavor. Thus, the highly abundant six carbon volatile *cis*-3-hexenal with a high odor unit value is predicted to be the most important volatile contributor to tomato flavor, whereas geranial, which has a negative log odor unit value, is predicted to be insignificant in terms of flavor [21]. This approach has some merit in terms of roughly approximating the most important of the ~400 volatiles in a tomato fruit. However, there are major problems with the approach. First, it tells us nothing about consumer preferences. A given

Table 1. Genes associated with flavor-imparting volatiles in tomato

Gene	Synthesized volatile(s)	Identification method	Refs
<i>AADC</i>	Phenylacetaldehyde, 2-phenylethanol, 1-nitro-2-phenethane, 2-phenylacetoneitrile	BP/QTL	[19]
<i>PAR</i>	2-Phenylethanol	BP	[49]
<i>LoxC</i>	Z-3-Hexenal, Z-3-hexenol, hexanal, hexanol	CG	[32]
<i>SAMT</i>	Methylsalicylate	BP	[50]
<i>CTOMT</i>	2-Methoxyphenol	BP	[51]
<i>CXE1</i>	Multiple alcohols	QTL	[41]
<i>CCD1</i>	Multiple apocarotenoids	CG	[52]

Abbreviations: AADC, aromatic amino acid decarboxylase; PAR, phenylacetaldehyde reductase; LoxC, 13-lipoxygenase; SAMT, salicylic acid methyltransferase; CTOMT, catechol-O-methyltransferase; CCD1, carotenoid cleavage dioxygenase; CXE1, carboxylesterase.

volatile may be perceived as being present in the fruit but it may not impact consumer preferences at all. Further, the odor unit is at best an approximation. Odor thresholds differ widely among individuals and change with age and experience [24,25]. They are also dependent upon the matrix in which the chemical is delivered [20]. Finally, when detecting volatiles in a complex mixture of chemicals, the measured odor threshold of a pure compound can be misleading. An olfactory receptor can recognize multiple chemicals and each chemical is recognized by multiple different receptors. Combinations of different volatiles can act synergistically or antagonistically, changing the levels of detection for the individual components in the mix [26,27]. Thus, it is almost impossible to predict how a complex mixture of chemicals will be perceived by an individual or how much that individual will like the product.

One approach to understanding the flavor in a whole food is the exploitation of genetic and biochemical diversity within a species. If there is sufficient diversity in the flavor chemical composition of fruits, as there is in tomato, one can empirically establish correlations between composition and consumer preferences. Levels of primary metabolites such as glucose, fructose, citrate, and malate can vary by several-fold, whereas concentrations of flavor volatiles can vary by 1000-fold or more among heirloom varieties [5]. Even within elite commercial tomato varieties, there is substantial chemical variation [28]. In addition, seasonal and site compositional variation can be exploited to understand the underlying chemistry of consumer preferences (Figure 1). A combination of targeted metabolomics and natural variation within 66 heirloom varieties was used to create a predictive and testable model of liking [5]. The results indicate how little is known about the chemistry of human preferences. The most significant contributor to consumer liking is sugar, with a strong positive correlation between perceived sweetness and overall liking. Volatiles also make significant positive or negative contributions to liking. Surprisingly, some of the most abundant volatiles, such as *cis*-3-hexenal, do not impact consumer preferences, whereas other less abundant volatiles, including geranial, significantly impact preferences [5]. Some of the volatiles positively correlated to liking make significant contributions to the perception of sweetness [5,29]. Thus, increasing the levels of these volatiles makes a fruit appear to taste sweeter independently of sugar content. Critically, this study identified the most important target molecules that drive consumer liking. It is important to note that human preferences are influenced by genetics and cultural background, which vary person to person. A large consumer panel provides information about an average population. Nevertheless, as the tomato study shows, one cannot necessarily predict the most important contributors to flavor and consumer liking based solely on odor units. Even the average measure of consumer preferences provides insights into the flavor chemistry of a whole food product. Where sufficient chemical diversity exists in a species, for example strawberry [30] or melon [31], such screens are highly informative.

Gene identification

Armed with a ranked list of the factors that affect consumer liking, the next step is to identify the genes involved in

the synthesis and regulation of these chemicals. In many cases, these are secondary (specialized) metabolites that are valuable not only as flavorings but also as fragrances, pharmaceuticals, and industrial raw materials. Despite their importance to the human endeavor, the synthetic pathways for many of the most important chemicals have not yet been established. It is rare that the genes encoding the biosynthetic enzymes are known and even rarer that the regulation of the biosynthetic pathways is understood. Thus, manipulation of secondary metabolic pathways (i.e., synthetic biology) is an imprecise science.

There are multiple approaches for identifying the genes that regulate the synthesis of any secondary metabolite in a plant (Box 1). The fundamental goal is to define the biosynthetic pathway and the factors that regulate flux through that pathway. One can use the classical approach of defining the chain of metabolic reactions, make predictions about the responsible enzymes, and identify genes encoding those enzymes [19,32,33]. Although this approach does identify genes with products that are essential for metabolite synthesis, it may not reveal the ones that are regulatory or rate-limiting. For flavor volatiles, regulation can, in theory, take several forms. Many of the important flavor volatiles are ripening-associated [17]. Thus, the enzymes responsible for their synthesis are controlled, either directly or indirectly, by the same transcription factors that control all aspects of ripening, including RIN (ripening inhibitor) and NOR (non-ripening) [34]. Indeed, *rin* and *nor* mutant fruits [35] as well as those from heterozygous *RIN/rin* plants, which are widely used commercially to extend shelf life [20], are all impaired in the synthesis of a subset of flavor volatiles. Tomato is a climacteric fruit, meaning that ripening is ethylene-dependent. Synthesis of many flavor volatiles increases concomitantly with ethylene production [17] and that synthesis is blocked in the ethylene-insensitive *Nr* (Never ripe) mutant [35]. An additional layer of complexity is the epigenome. The first hint that epigenetic changes are an important component of tomato fruit ripening occurred with the characterization of the dominant *Cnr* (colorless non-ripening) mutation [36]. The basis of the mutation, which affects fruit color and texture, was determined to be the consequence of altered methylation within the transcriptional promoter of a SQUAMOSA binding protein. Recently, it has been shown that ripening tomato fruits undergo a massive demethylation of ripening-associated transcriptional promoters at the onset of ripening [37]. This result demonstrates that methylation state is not static within a plant and actually has an essential function to control organ maturation (i.e., ripening). These results clearly reshape the way in which we consider the events controlling ripening-associated gene expression and highlight the many-faceted nature of flavor.

At the level of metabolism, many volatiles are converted to non-volatile glycosides that do not contribute to flavor. The glycoside pool for many volatile alcohols can be significant, representing 30–40% of the compound [38,39]. There may be reversible cleavage of these glycosides as well, although little is known about glycoside metabolism. This lack of knowledge is at least partially due to the complexity of the process; there are over 100 glycosyltransferases in sequenced plant genomes, including tomato [40]. Each enzyme generally recognizes multiple substrates and multiple

enzymes can act upon a single substrate. Thus, genetically defining roles for individual glycosyltransferases can be a challenge. Finally, although highly unusual, volatiles can also be catabolized. For example, the tomato has very low concentrations of volatile esters because a ripening-specific esterase cleaves them to their corresponding free alcohols [41]. In total, the regulation of volatile content is determined by the balance of synthesis, reversible modification, and catabolism.

Although there is great value in mechanistically understanding an entire metabolic pathway, breeders need the genes that control the end product. The most direct path to those genes is empirical. Populations of plants containing either natural or mutation-induced variations in chemical composition are crucial tools needed to identify genes regulating synthesis of target chemicals. For example, introgression lines (ILs) containing fixed segments of the genomes of wild relatives of tomato [42] are a rich source of QTLs affecting primary and secondary metabolites [43]. Over 50 flavor-volatile-associated QTLs have been identified [16–18]. Alleles of some of these QTLs are likely to be important flavor-associated markers that will ultimately lead to improved flavor. Analysis of several QTLs with major effects on volatile content has led to identification of important regulators of volatile content. For example, a QTL located at the bottom of chromosome 1 affects multiple volatile esters. Green-fruited species contain much higher levels of these esters than red-fruited species and introduction of the *Solanum pennellii* (a wild green-fruited species) allele into *Solanum lycopersicum* causes up to 20-fold higher levels of these esters in ripe fruits. These esters are negatively correlated with human tomato preferences and the large reductions in ester content may be linked to palatability. A retrotransposon insertion into the transcriptional promoter of a carboxylesterase gene occurred in the ancestor of the red-fruited species [41]. The retrotransposon resulted in substantially higher expression of the carboxylesterase and consequent large reductions in volatile esters. Thus, mapping of the ester-associated QTL led to the discovery of an important enzyme regulating levels of these volatiles. It must be noted that, in this case, gene identification was greatly facilitated by a complete genome sequence and knowledge of the relevant metabolic pathway. In this manner, a QTL can direct us to a specific, ideally small, segment of a chromosome that can then be examined for candidate genes based on knowledge of the pathway as well as the pattern of gene expression or correlation with the target metabolite [44] (Box 1).

The approach of linking QTLs to the content of important flavor volatiles is not limited to tomato. In strawberry, a mapping population resulting from a cross between two varieties with distinctly different flavors led to the identification of many flavor-associated QTLs [45]. Some of these QTLs are likely to be useful as markers for improving or maintaining strawberry flavor. QTLs that associate with flavor volatiles have also been identified in apple [46] and grape [47,48]. By definition, a QTL modulates the output of the target metabolic pathway. By choosing QTLs with the largest effects on the most important flavor chemicals one can prioritize efforts to characterize the genes with the largest impact on consumer preferences.

Concluding remarks and future perspectives

Completion of the tomato genome sequence as well as those of several members of the genus [49] has greatly accelerated the rate of gene discovery. RNA-Seq methods have permitted precise mapping of introgressed segments of genomes from wild species, particularly for the extensively used *S. pennellii* population (Z. Fei and J. Giovannoni, unpublished). An available *S. lycopersicum* genome scaffold also provides an opportunity to assemble draft genome sequences of many heirloom cultivars rapidly and cheaply, in turn facilitating introgression of desirable alleles of flavor-associated genes into commercial lines.

In the current US system for large-scale tomato production, growers are paid on the basis of yield and appearance. With the exception of a few branded products or locally produced heirlooms, growers are not paid to deliver a product with improved flavor. Most commercial growers cede control long before the consumer sees the product. Improper handling can destroy the flavor of even the best tomato. In that context, commercial breeders have set a high standard with regard to yield. The only way to achieve widespread adoption of significantly improved flavor quality is to introduce flavor into something equivalent to the current commercial cultivars. However, high yield may be part of the problem, particularly with determinate varieties, and it may be impossible to achieve great taste without sacrificing yield. Still, significant improvement in flavor quality should be possible. Many of the most important flavor volatiles, such as the apocarotenoids, significantly impact consumer liking at nanomolar concentrations in the fruit. Substantial increases in synthesis of these molecules should be possible without impacting yield.

The chemistry of tomato flavor is complex; improved tomato flavor demands alterations of sugars, acids, and volatiles synthesized through multiple parallel pathways. This chemical complexity means that several genes will probably need to be introduced to achieve the proper balance of flavor. The immediate challenge is to identify the most important genes regulating flavor-associated chemical accumulation and desirable alleles of those genes. Fortunately, the remarkable chemical diversity observed within the species suggests that those desirable alleles do exist. Although we are not likely to produce a commodity tomato with heirloom flavor without sacrificing some yield, it is reasonable to assume that we can assemble a molecular toolbox for improved flavor and implement significant progress in the foreseeable future.

More promising for consumers in the long term is the prospect that breeders will incorporate flavor genetics into their programs at the earliest stages. As we identify the most important flavor chemicals and elucidate the pathways for their synthesis, molecular markers that track the most desirable alleles can be incorporated into germplasm development. The end user can and should be brought into the selection process. It is the job of the molecular biologist to provide the tools to facilitate that connection.

References

- 1 Davis, D.R. *et al.* (2004) Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *J. Am. Coll. Nutr.* 23, 669–682

- 2 Garvin, D.F. *et al.* (2006) Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *J. Sci. Food Agric.* 86, 2213–2220
- 3 Murphy, K.M. *et al.* (2008) Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. *Euphytica* 163, 381–390
- 4 White, P.J. and Broadley, M.R. (2005) Historical variation in the mineral composition of edible horticultural products. *J. Horticult. Sci. Biotechnol.* 80, 660–667
- 5 Tieman, D.M. *et al.* (2012) The chemical interactions underlying tomato flavor preferences. *Curr. Biol.* 22, 1–5
- 6 Maul, F. *et al.* (2000) Tomato flavor and aroma quality as affected by storage temperature. *J. Food Sci.* 65, 1228–1237
- 7 Baldwin, E. *et al.* (2011) Effect of 1-methylcyclopropene on tomato flavour components, shelf life and decay as influenced by harvest maturity and storage temperature. *J. Sci. Food Agric.* 91, 969–980
- 8 Goff, S.A. and Klee, H.J. (2006) Plant volatile compounds: sensory cues for health and nutritional value? *Science* 311, 815–819
- 9 Emery, G. and Munger, H. (1970) Effects of inherited differences in growth habit on fruit size and soluble solids in tomato. *J. Am. Soc. Hort. Sci.* 95, 410–412
- 10 Powell, A. *et al.* (2012) Uniform ripening encodes a Golden 2-like transcription factor regulating tomato fruit chloroplast development. *Science* 336, 1711–1715
- 11 Verhagen, J. and Engelen, L. (2006) The neurocognitive bases of human multimodal food perception: sensory integration. *Neurosci. Biobehav. Rev.* 30, 613–650
- 12 Shepherd, G. (2006) Smell images and the flavour system in the human brain. *Nature* 444, 316–321
- 13 Harrigan, G.G. *et al.* (2007) Impact of genetics and environment on nutritional and metabolite components of maize grain. *J. Agric. Food Chem.* 55, 6177–6185
- 14 Fernie, A.R. and Klee, H.J. (2011) The use of natural genetic diversity in the understanding of metabolic organization and regulation. *Front. Plant Sci.* 2, 1–10 article 59
- 15 Baldwin, E.A. *et al.* (2008) Interaction of volatiles, sugars, and acids on perception of tomato aroma and flavor descriptors. *J. Food Sci.* 73, S294–S307
- 16 Causse, M. *et al.* (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. *J. Exp. Bot.* 53, 2089–2098
- 17 Tieman, D.M. *et al.* (2006) Identification of loci affecting flavor volatile emissions in tomato fruits. *J. Exp. Bot.* 57, 887–896
- 18 Mathieu, S. *et al.* (2008) Flavor compounds in tomato fruits: identification of loci and potential pathways affecting volatile composition. *J. Exp. Bot.* 60, 325–337
- 19 Tieman, D.M. *et al.* (2006) Aromatic amino acid decarboxylases participate in the synthesis of the flavor and aroma volatiles 2-phenylethanol and 2-phenylacetaldehyde in tomato fruits. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8287–8292
- 20 Baldwin, E.A. *et al.* (2000) Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control of important aroma components. *Hortscience* 35, 1013–1022
- 21 Buttery, R.G. and Ling, L.C. (1993) Volatiles of tomato fruit and plant parts: relationship and biogenesis. In *Bioactive Volatile Compounds from Plants* (Teranishi, R. *et al.*, eds), pp. 23–34, Washington, DC, ACS Books
- 22 Sato, S. *et al.* (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* 485, 635–641
- 23 Lasekan, O. and Abbas, K. (2012) Distinctive exotic flavor and aroma compounds of some exotic tropical fruits and berries: a review. *Crit. Rev. Food Sci. Nutr.* 52, 726–735
- 24 Plotto, A. *et al.* (2006) Specific anosmia observed for β -ionone, but not for α -ionone: significance for flavor research. *J. Food Sci.* 71, S401–S406
- 25 Tempere, S. *et al.* (2011) The training level of experts influences their detection thresholds for key wine compounds. *Chemosens. Percept.* 4, 99–115
- 26 Rospars, J.P. *et al.* (2008) Competitive and noncompetitive odorant interactions in the early neural coding of odorant mixtures. *J. Neurosci.* 28, 2659–2666
- 27 Ferreira, V. (2012) Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 1: intensity and detectability. A review. *Flavour Fragr. J.* 27, 124–140
- 28 Tikunov, Y. *et al.* (2005) A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiol.* 139, 1125–1137
- 29 Vogel, J. *et al.* (2010) Carotenoid content impacts taste perception in tomato. *J. Sci. Food Agric.* 90, 2233–2240
- 30 Ulrich, D. *et al.* (2007) Diversity of aroma patterns in wild and cultivated *Fragaria* accessions. *Genet. Resour. Crop Evol.* 54, 1185–1196
- 31 Harel-Beja, R. *et al.* (2010) A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. *Theor. Appl. Genet.* 121, 511–533
- 32 Chen, G. *et al.* (2004) Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiol.* 136, 2641–2651
- 33 Raab, T. *et al.* (2006) FaQR, required for the biosynthesis of the strawberry flavor compound 4-hydroxy-2,5-dimethyl-3(2H)-furanone, encodes an enone oxidoreductase. *Plant Cell* 18, 1023–1037
- 34 Klee, H.J. and Giovannoni, J.J. (2011) Genetics and control of tomato fruit ripening and quality attributes. *Annu. Rev. Genet.* 45, 41–59
- 35 Kovács, K. *et al.* (2009) Effect of tomato pleiotropic ripening mutations on flavour volatile biosynthesis. *Phytochemistry* 70, 1003–1008
- 36 Manning, K. *et al.* (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet.* 38, 948–952
- 37 Zhong, S. *et al.* Epigenetic control of tomato fruit development. *Nat. Biotechnol.* <http://dx.doi.org/10.1038/nbt.2462>, (in press)
- 38 Tikunov, Y. *et al.* (2010) A role for differential glycoconjugation in the emission of phenylpropanoid volatiles from tomato fruit discovered using a metabolic data fusion approach. *Plant Physiol.* 152, 55–70
- 39 Ortiz-Serrano, P. and Gil, J. (2010) Quantitative comparison of free and bound volatiles of two commercial tomato cultivars (*Solanum lycopersicum* L.) during ripening. *J. Agric. Food Chem.* 58, 1106–1114
- 40 Bowles, D. *et al.* (2006) Glycosyltransferases of lipophilic small molecules. *Annu. Rev. Plant Biol.* 57, 567–597
- 41 Goulet, C. *et al.* (2012) Role of an esterase in flavor volatile variation within the tomato clade. *Proc. Natl. Acad. Sci. U. S. A.* 109, 19009–19014
- 42 Eshed, Y. and Zamir, D. (1994) A genomic library of *Lycopersicon pennellii* in *Lycopersicon esculentum* – a tool for fine mapping of genes. *Euphytica* 79, 175–179
- 43 Schauer, N. *et al.* (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nat. Biotechnol.* 24, 447–454
- 44 Lee, J.M. *et al.* (2012) Combined transcriptome, genetic diversity and metabolite profiling in tomato fruit reveals that the ethylene response factor *SIERF6* plays an important role in ripening and carotenoid accumulation. *Plant J.* 70, 191–204
- 45 Zorrilla-Fontanesi, Y. *et al.* (2012) Genetic analysis of strawberry fruit aroma and identification of *O*-methyltransferase FaOMT as the locus controlling natural variation in mesifurane content. *Plant Physiol.* 159, 851–870
- 46 Dunemann, F. *et al.* (2012) Functional allelic diversity of the apple alcohol acyl-transferase gene MdaAT1 associated with fruit ester volatile contents in apple cultivars. *Mol. Breed.* 29, 609–625
- 47 Batilana, J. *et al.* (2010) The 1-deoxy-d-xylulose 5-phosphate synthase gene co-localizes with a major QTL affecting monoterpenes content in grapevine. *Theor. Appl. Genet.* 118, 653–669
- 48 Duchene, E. *et al.* (2010) A grapevine (*Vitis vinifera* L.) deoxy-d-xylulose synthase gene colocalizes with a major quantitative trait loci for terpenol content. *Theor. Appl. Genet.* 118, 541–552
- 49 Tieman, D.M. *et al.* (2007) Tomato phenylacetaldehyde reductases catalyze the last step in the synthesis of the aroma volatile 2-phenylethanol. *Phytochemistry* 68, 2660–2669
- 50 Tieman, D.M. *et al.* (2010) Functional analysis of a tomato salicylic acid methyl transferase and its role in synthesis of the flavor volatile methyl salicylate. *Plant J.* 62, 113–123
- 51 Mageroy, M.H. *et al.* (2012) A *Solanum lycopersicum* catechol-*O*-methyltransferase involved in synthesis of the flavor molecule guaiacol. *Plant J.* 69, 1043–1051
- 52 Simkin, A.J. *et al.* (2004) The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles β -ionone, pseudoionone, and geranylacetone. *Plant J.* 40, 882–892