Tomato Flavour: Lost and Found?

Tomato is the highest value fruit crop in the world (Tieman at al., 2017) and a major component of healthy diets as it provides ready sources of vitamins A, C, E and K, minerals including K and Fe and lycopene as an antioxidant. However, modern varieties are often described as having little flavour, especially in comparison to traditional or heirloom types. One of the major challenges is to breed fruit that have a long shelf life fit for the modern supply chain, but maintain excellent eating quality. The route taken by many breeders has been to introduce non-ripening mutations, such as *ripening inhibitor* (*rin*), into elite backgrounds. Hybrids containing *rin* produce firmer fruits that ripen slowly (Kitagawa et al., 2005), but they often have poor flavour, fail to develop full colour and have reduced nutritional value. Another reason for poor flavour is that the modern cultivated tomato has been selected for yield, disease resistance and size. Breeders will select for sugars and acids as these are known to be important, but there is a negative correlation between fruit weight and sugar content. Tomato flavour results from not only a complex mix of taste metabolites, but also the perception of volatile compounds (Figure 1).

FLAVOUR IS A COMPLEX MIXTURE OF SUGARS, ACIDS AND VOLATILES

Volatile compounds that influence tomato flavour have, until recently, received relatively little attention and traditional breeding has often failed to capture their value due to their high degree of complexity (Rambla et al., 2014). It has become apparent that volatiles from fruits are potential sensory cues for health and nutritional value. For instance, ripe tomato fruits produce several hundred volatile compounds and almost all the important volatiles related to flavour are derived from essential nutrients and these include essential fatty acids, essential amino acids and the coloured carotenoids found in tomato and other fruits (Goff and Klee, 2006). Concentrations of volatile compounds range from several micrograms per gram fresh weight for hexanal to nanogram per gram for β-damascenone. The perception of aroma is not due to the additive effect of the different volatile compounds, but their interaction (Goff and Klee, 2006; Rambla et al., 2014). Some of the volatiles that positively correlate to liking make significant contributions to the perception of sweetness (Klee and Tieman, 2013). There are opportunities for novel aroma profiles to improve flavour. Aroma of transgenic fruits has been enhanced with phenolic volatiles 2-phenylethanol, phenylacetaldehyde and benzaldehyde which has a preferred floral aroma, although these changes impacted the generation of other volatile components. Interestingly not all volatiles are positive and a lack of esters in cultivated lines has a positive effect on liking in tomato (Goulet et al., 2012).

Variability in tomato volatile profiles can be found in wild species and heirlooms and also in different commercial hybrids and for breeding, quantitative trait loci (QTL) for volatiles can now be successfully transferred between different genetic backgrounds. The tomato genome sequence (The Tomato Genome Consortium, 2012) has been a key tool in mapping volatile QTL and identifying the underlying genes, most of which do not fall within known pathways and are likely to encode regulatory functions. However volatiles need to be considered along with the other taste components and sometimes taste metabolites can be clustered in certain genomic regions (Liu et al., 2016). The key to solving the flavour challenge is to join all this information together to understand which compounds are the most important, which are the most preferred and where the superior alleles occur to boost the flavour potential of modern cultivars.

A NEW ROADMAP FOR IMPROVED FLAVOUR

In a landmark recent study Tieman and co-authors (Tieman et al., 2017) provide an understanding of the flavour deficiencies in modern cultivars and explain some of the steps necessary to help the commercial tomato recover its flavour.

Taking 398 modern, heirloom and wild accession of tomato the authors performed whole genome sequencing and targeted metabolome quantification of sugars, acids and volatiles that contribute to flavour. A large number of these tomato accessions were then also evaluated by a consumer panel and rated for overall liking and flavour intensity. Metabolites associated with consumer liking were then identified and these formed the basis of a genome-wide association study (GWAS) to identify the genetic basis of these attributes.

Poor flavour of the modern varieties was associated with reduced levels of sugars, organic acids and volatile compounds including β-ionone, E-2-hexenal, 6-methyl-5-hepten-2-one and phenylacetaldehyde. Analysis of the data indicated that modern varieties have lost the alleles that result in higher sugar content and flavour volatiles. In the case of sugars the study confirmed and demonstrated in detail the inverse correlation between sugars and fruit size reflecting the preferential selection of larger fruit size. Fortunately, as mentioned above, small levels of some volatile compounds improve sweetness perception without changing sugar concentration which would allow for flavour without affecting fruit size. GWAS resulted in the identification of superior alleles capable of altering some of the chemicals contributing to consumer liking including key volatile components. Interestingly the study revealed that many of the superior alleles for good volatiles were lost during modern breeding except in the case of those derived from carotenoids. These of course have been selected for as they contribute to the fruit colour and this trait was of interest for breeders; most of the volatiles are derived from non-coloured compounds. This study provides the information to lay the foundation for a step change in the potential for using molecular breeding to produce tomato fruits with better flavour.

HOW IS FLAVOUR ALTERED BY TEXTURE OR BY COLD STORAGE

The potential improvements in flavour using the new genome-enabled approaches are unlikely to be fully realised without an understanding of the interactions between flavour and texture. As mentioned earlier, long shelf life is essential for the modern supply chain. Very recently fruit softening has been uncoupled from other aspects of ripening (Uluisik et al., 2016) which should reduce or even eliminate the need to use non-ripening mutations in breeding programmes. The need to harvest fruits prior to full ripeness may also be reduced. Texture and flavour including volatile production interact as texture is related to, among other factors, the degradation of cell walls. At the simplest level bursting of cell wall will encourage contact between enzymes and substrates involved in the release of volatile compounds. Texture affects consumer preferences and its interaction with flavour perception demands further study.

Other factors compounding the challenge of maintaining good flavour include cold storage of produce including tomato fruits; a necessary procedure to maintain shelf life in many supply chains. Low flavour scores in chilled fruit are the result of reduced volatile content. The expression of 1000s of genes is reduced during low temperature storage and some of these are related to production of volatiles. Those reductions are accompanied by increases in the methylation status of the promoters of genes including those involved in volatile synthesis and ripening regulatory genes. DNA methylation is known to play an important role in tomato fruit ripening and much of the methylation induced by chilling was transient. Although the expression of some genes critical to volatile synthesis recovers on transfer back to 20^{o}C some genes do not (Zhang et al., 2016). The tomato chemical genetic road map (Tieman et al., 2017) and our understanding of the mechanistic basis of fruit ripening provide the strategy for real improvements in tomato fruit flavour and of course the approach of linking QTL to control important volatile components is not limited to tomato.

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Duoduo Wang and Graham B. Seymour[1] , University of Nottingham, School of Biosciences, Division of Plant and Crop Science, Sutton Bonington, Lough, Leics LE12 5RD, UK.

[1.] Corresponding author E-mail graham.seymour@nottingham.ac.uk, tel 44-1159-516323

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(A)

(B)

Volatile	Conc. (ppb)	Log odor units	Odor Characteristics
cis-3-Hexenal	12,000	3.7	tomato/green
ß-ionone	4	2.8	fruity/floral
hexanal	3,100	2.8	green/grassy
ß-damascenone	1	2.7	fruity
1-penten-3-one	520	2.7	fruity floral/green
2+3-methylbutanal	27	2.1	musty
trans-2-hexenal	270	1.2	green
2-isobutylthiazole	36	1.0	tomato vine
1-nitro-2-phenylethane	17	0.9	musty, earthy
trans-2-heptenal	60	0.7	green
phenylacetaldehyde	15	0.6	floral/alcohol
6-methyl-5-hepten-2-one	130	0.4	fruity, floral
cis-3-hexenol	150	0.3	green
2-phenylethanol	1,900	0.3	nutty/fruity
3-methylbutanol	380	0.2	earthy, musty
methyl salicylate	48	0.08	wintergreen

Figure 1. A list of the major tomato volatiles, their concentrations and their characteristic odors.
(A) heirloom tomato collection and (B) volatile list (from http://hos.ufl.edu/kleeweb/flavorresearch.html with permission).