Metabolic engineering for flavour enhancement in tomato – Path setting for opportunities and strategies

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The recent report on altering the flavour of tomato by attempting metabolic engineering of the flavour aspects is a much needed breakthrough in the engineering of aroma constituents in fruits and vegetables[1]. Successful metabolic engineering attempts are few in the literature and specifically the study exhibits the intricacies of the networking between several pathways in plants, competing for the substrates. The metabolic engineering attempt for tomato flavour enhancement by diverting the plastidal precursors resulted in several fold increase in the pathway leading to novel aroma constituents in tomatoes.

The inter-institutional group of plant scientists in Israel and at Rutgers and the University of Michigan, USA, reported their success in transferring a gene from the basil plant into tomato plants. This particular gene geraniol synthase (*GES*) from *Ocimum basilicum*, under the control of the tomato ripening-specific polygalacturonase promoter, when expressed diverts molecules in the pathway toward becoming the red pigment lycopene, and channels instead onto the pathway that generates aroma molecules. The engineered tomato plants produced fruits enriched in several acyclic monterpenoid flavour components and also modified the appearance of the fruits that were paler than usual. Significantly, the fruit possessed a stronger aroma and smelt distinctly of perfume resembling that of rose, geranium and lemongrass, which possess geraniol, citral *cis* and *trans*, citronellol, linalool, etc., and their acetates[1], also reported as major monoterpenoids present in lemongrass, rose and geranium essential oils[2–5].

Davidovich-Rikanati *et al.*[1] report that more than half of a panel of taste-testers preferred the engineered tomato to its unengineered parent. This experiment may be a harbinger of things to come, a new era of plant modification in which flavour combinations once created by chefs, will be custom imagined for its creation by scientists in the plants themselves. Researchers from Israel's Newe Ya'ar Research Centre had systematically planned to genetically modify the flavour and aroma of tomatoes by expressing the *O. basilicum* L. *GES* gene expecting enhancement of flavour constituents, as the step would drive precursors towards acyclic flavour synthesis via geraniol synthesis[6]. The targetted *GES* gene in the tomato would divert biosynthesis of geraniol, the first C_{10} monoterpenol of the pathway and its related chemical entities would also be biosynthesized. The relevant key step of geraniol metabolism, including catalytic transformation by subsequent dehydrogenation, reduction and acylation–deacylation have been studied in some detail and reported for selected medicinal and aromatic grass plants[2–5,7].

The expression of *Ocimum GES* gene yielded[1] the fruits that smell a little like roses and lemons and was preferred by most of the untrained taste panelists to ordinary tomatoes. The finding could result in the development of new tomato-based flavours, and could also have implications for shelf-life. However, further studies are required to ascertain this aspect. The transgenic tomatoes were found to have higher levels of volatile terpenoids that display antimicrobial and antifungal qualities and it was also hypothesized that such tomatoes might possess better defence when grown under field conditions.

The team led by Efraim Lewinsohn, Israel attempted to divert the metabolic flow from the biosynthesis of the red pigment lycopene to aroma compounds. The results obtained in favour of flow of metabolites towards flavour synthesis suggest the possibility of diverting the flux in carotenoid-containing fruits and vegetables that display a similar effect if the foreign gene was expressed in them.

One drawback of the variety, however, is that the transgenic fruit also contained less lycopene, the red pigment, and antioxidant carotenoid of which tomatoes are the main natural source. There is increasing awareness about the health benefits of lycopene amongst consumers, regarding its potential to reduce risk of certain cancers and heart disease. It is also used as a natural food colouring the world over. Though commercially this study may not appear a profitable venture at a large scale owing to decrease in lycopene contents and its constituents, the hypothesis and the experimental proof that such controls are possible in the laboratory for creating designer plants with respect to flavours are exciting. It might sound easy, but till date this is the only available example where metabolic engineering attempts yielded targetted and envisaged results, though, as an additional step lycopene regulation could not be profitably controlled. Such attempts in crops like mango and banana might even yield flavoured and tastier fruits at the expense of carotene content present in the peels, but not in the edible portions.

Aroma and flavour components of tomatoes are essential parameters in defining fruit quality. Flavour volatiles are formed during fruit ripening as well as when the tissue disrupts mechanically or physiologically. It is interesting to find that in tomatoes, the volatiles have a varied route of formation such as lipid, carotenoid, amino acid, terpenoids, and lignin-related. Among these, the terpenoids pathway (especially monoterpenoids) and the oxylipin pathway have been studied in greater detail compared to others[8]. More biochemical, genetic, chemical and sensory studies are needed to improve the quality of one of the most important fresh-food products for the benefit of the health and quality conscious modern consumer. Keeping the expression of the gene under the control of wound-inducible promoter could be an attractive alternative for the induced release of the required aroma without any spin-off of lycopene in transgenic lines. Expectedly, the option would work well in salads and raw preparations, where mild traces of fine aroma emitted after cutting of the fruit is possible.

Lycopersicon species accumulate and biosynthesize secondary metabolites and terpenoids not only in fruits but in glandular trichomes present on the leaf surface, where they supposedly form the first line of defence and play a inter-

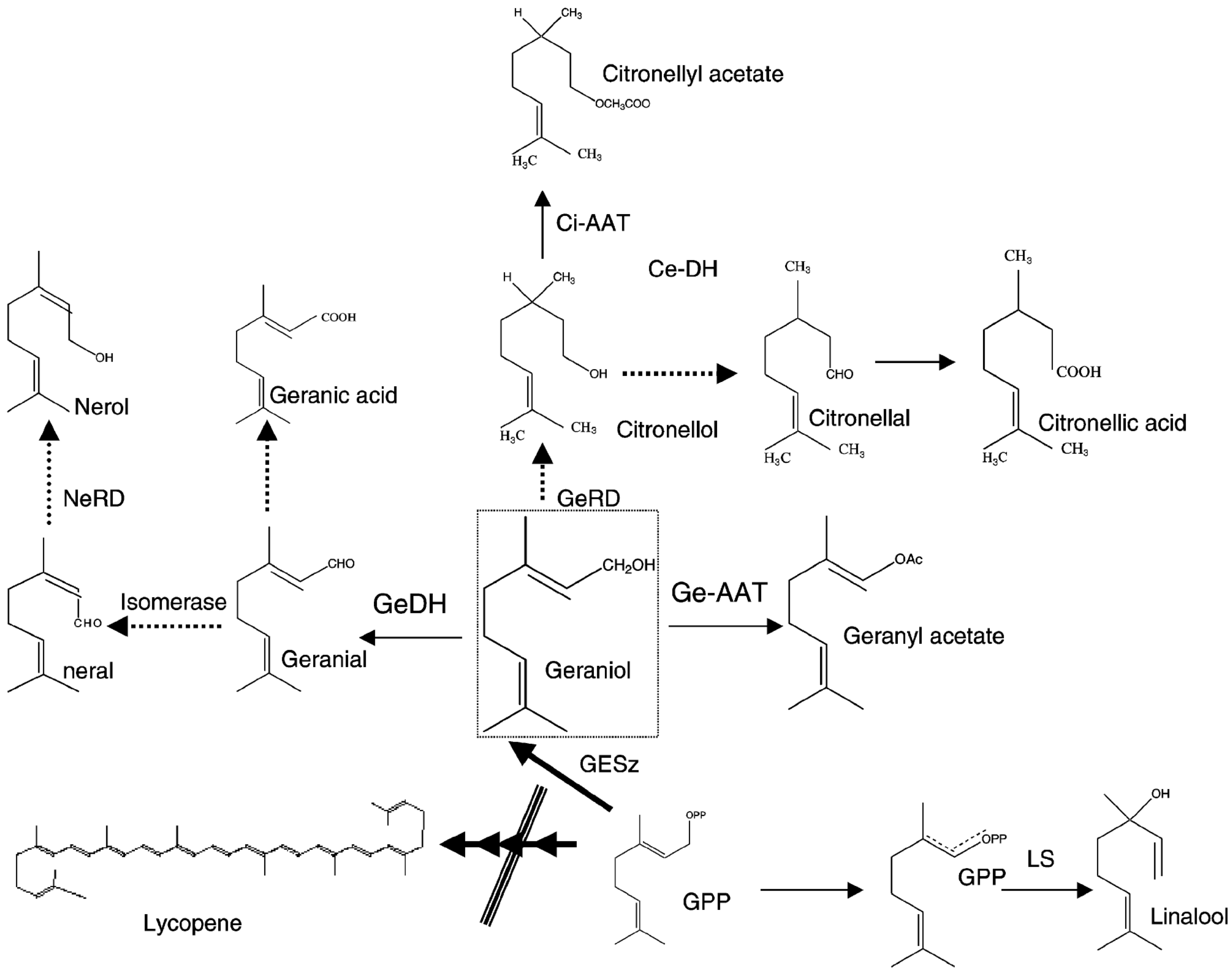

Figure 1. Pathway showing geraniol metabolism in tomato (and other acyclic monoterpenoid-bearing aromatic plants) and diversion of precursors from lycopene synthesis to monoterpenoid flavours. Geraniol molecule, after being synthesized, is terminally transformed into geanial (by GeDH; geraniol dehydrogenase), geranyl acetate (by Ge-AAT; geraniol-alcohol acetyl CoA transferase); citronellol (by putative geraniol reductase), and citronellyl acetate (by citronellyl-alcohol acetyl transferase).

active role in ecological adaptation and in defence[8].

Tomatoes have two major characters that impact the consumers: (i) lycopene, and (ii) flavours, the fine organoleptically active secondary metabolites of terpenoids origin. The Lewinsohn team attempted to improve the over-expression of the terpenoidal components by putting additional heterologous gene *GES* from *O. basilicum*. The hypothesis made was that the additional gene would provide enhanced pool of geraniol from GDP in the pathway (Figure 1). Interestingly, the GDP pool got enhanced in the *GES*-transgenic lines resulting into up-accumulation of several of the acyclic monoterpenoids in the transgenic lines such as geraniol, its isomer nerol, citronellol, geranial and neral. Several fold increase in neric acid, geranic acid and citronellic acid was recorded. Rose-oxide also appeared in transgenic lines, albeit at lower concentrations. Other monoterpene hydrocarbons myrecene, limonene and isomers of ocimene were also present in more than 100-fold concentration. Thus, the tomato transgenic fruits possessed flavours resembling that of rose and lemongrass leaves[2,3,6] and rose with respect to smell. Through the downstream geraniol synthase the synthesis of the monoterpenoids was up-regulated, the individual pattern of preferential synthesis of individual monoterpenoids appeared to the genotype preference of the pathway. As a consequence, the lycopene content in transgenic tomatoes was close to half that found in the control (48 μg/g fw compared to 97 μg/g fw for control). It is further interesting to observe the not so drastic type changes in norisoprenes and other phenolic derivatives. These remained almost unaltered, thereby indicating that the appearance of GDP and downstream geraniol type terpenoids has a co-relationship with further GGPP synthase-based lycopene synthesis, though not exactly in quantitative terms, as the terpenoids appeared in 100–1000 fold compared to the control, whereas lycopene and phytoene resulted in a decrease of about 50% and ten-fold respectively. β-Carotene remained almost unchanged.

Interestingly, the results indicate the up-accumulation of geranyl and citronellyl acetates, the sweet-smelling esters of monoterpenols contributing sweet aroma to several aromatic plants as well[6]. The results indicate clearly that several terminal monoterpenoidal enzymes of the pathway with functions and their genes might have been triggered. Enzymes such as geraniol dehydrogenase, geraniol-AAT, citronellol reductase, etc. carrying out terminal transformation for quality have been studied in our laboratory in the case of medicinal and aromatic plants, which have in fact provided one of the few reports on geraniol metabolism in the literature[2–4].

The metabolic engineering attempts previously reported in *Artemisia annua*[9] along with this attempt in tomato[1], indicate that the success in achieving metabolic engineering is high if the metabolic pathways, possible routes, networking with other end-products, physico-kinetics of the catalytic step(s) and their comparative operations are well considered before making the choice of experimentation. Such improvements, as the investigators have envisaged, are not only interesting from an academic angle, but are likely to have eco-physiological implications. For consumers, of course, it would be a sweet-flavoured treat to savour.

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