

Supplementary Methods

Vector construction and plant transformation. The *O. basilicum* *GES* coding region was cloned into the binary vector pBin19 that already contained the tomato fruit specific PG promoter (4.8 kb) and PG terminator (1.8 kb) and the kanamycin-resistance marker gene *NPTII* driven by the *CaMV 35S* promoter⁹. The binary vector was introduced into *Agrobacterium tumefaciens* strain *EHA105*, with 100 mg/l kanamycin for selection.

The first generation of kanamycin-resistant tomato plants was analyzed by PCR and Southern blot using the *GES* full-length clone as a probe. Ten transgenic lines containing one to six copies of *GES* were obtained (data not shown). Preliminary analysis of the composition of volatiles in ripe fruit showed that all transgenic lines produced geraniol. Two lines displaying the highest levels of geraniol were selfed to produce the T₂ generation. T₂ plantlets were screened by PCR for the presence of *GES*, and 10 positive progenies from each line were grown till fruit set.

Molecular analysis of transgenic plants. Genomic DNA was extracted from young leaf tissue. About 100 mg of tissue were ground in liquid N₂ with a polypropylene tip and extracted with 700 μ l of extraction buffer [0.15 M sorbitol, 0.1 M Tris-HCl pH 7.5, 2 mM EDTA, 0.83 M NaCl, 0.83 % (w/v) cetyl trimethyl ammonium bromide (CTAB), 0.83 % (w/v) N-lauroylsarcosine, 0.05 M sodium bisulfite]. Genomic DNA in transgenic plants was screened by PCR for the presence of *GES* using a set of primers specific to *GES*. The PCR was performed with a ready mix (ABgene, Surrey, UK) containing 1.5 mM MgCl₂, 100 ng of genomic DNA, and 50 μ M of the following primers: sense primer 5'-TACGCCACGCTTCTCTGCTTG-3', and antisense primer 5'-AGCCTCCGCAA ACTCCATAGCC-3' in a total volume of 25 μ l. Amplification was performed in an Eppendorf Mastercycler gradient (Eppendorf AG. 5331, Hamburg) PCR

device under the following conditions: 4 min at 94 °C and 30 cycles of: 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C, followed by 10 min at 72 °C.

Extraction of volatiles. Approximately 50 g of fresh ripe fruits (8 to 10 days after the “breaker” stage) were cut into small pieces and extracted with 100 ml of methyl *tert*-butyl ether (MTBE) by vigorous shaking on a shaker apparatus overnight¹⁴. Each sample contained 10 µg of isobutyl benzene as an internal standard. The ether phase was separated off, dried with anhydrous Na₂SO₄, and concentrated to 0.5 ml in a Turbo Vap II evaporator (Zymark Corp., Hopkinton, MA).

GC-MS analysis of volatiles. A 1-µl aliquot of the concentrated MTBE extract was injected into a GC-MSD system (Agilent, USA). The instrument was equipped with a Rtx-5 SIL column (30 m length @ 0.25 mm i.d., 0.25 µm film thickness, stationary phase 95% dimethyl- 5% diphenyl polysiloxane). Helium (0.8 ml/min) was used as the carrier gas with splitless injection. The injector temperature was 250 °C, and the detector temperature was 280 °C. The following conditions were used: initial temperature 50 °C for 1 min, followed by a ramp of 50 to 260 °C at a rate of 5 °C/min. A quadrupole mass detector with electron ionization at 70 eV was used to acquire the MS data in the range of 41 to 350 m/z. A mixture of straight-chain alkanes (C7-C23) was injected into the column under the above-mentioned conditions for determination of retention indices. The identification of the volatiles was assigned by comparison of their retention indices with those of literature and by comparison of spectral data with standards. The amount of component in each sample was calculated as: (peak area @ internal standard response factor) divided by (response factor @ internal standard peak area). Geraniol and its derivatives were identified by

comparison of the EI-MS obtained with authentic standards and complemented with computerized libraries.

Log odor unit determination. The contribution of geraniol derivatives to the aroma of transgenic tomatoes was determined according to Baldwin *et al.*². Log odor unit was calculated from the ratio of the concentration of a component to its odor threshold. Compounds with a positive log odor unit are likely to have an impact on the fruit aroma^{2,3}. Odor detection units were taken from FlavorBase 98 (FlavorBase is a trademark of Leffingwell & Associates).

Determination of carotenoids. Frozen samples of ripe fruit (Br+8 to Br+10) were homogenized with a polytron apparatus, and 0.5 g of the homogenate was extracted with hexane:acetone:ethanol (50:25:25 v/v), followed by 5 min of saponification in 8 % (w/v) KOH. The saponified material was extracted twice with 4 ml of hexane, which was then evaporated off under vacuum. The solid pellet was resuspended in 400 μ l of acetonitrile:methanol:dichloromethane (45:5:50 v/v) and passed through a 0.2- μ m nylon filter for HPLC analysis. Samples were analyzed in an Alliance photodiode array HPLC machine (Hewlett-Packard) utilizing the gradient system described previously¹⁴. The levels of lycopene, β -carotene and phytoene were quantified by comparison with calibration curves constructed from authentic standards.

Smell and taste trials. Smell trials (orthonasal route) were conducted by 34 untrained panelists differing in age and sex. Ten fresh ripe tomatoes from T₂ *GES*-transgenic and 10 control tomatoes were placed in closed storage boxes for 2 h at room temperature. Panelists were then asked to compare the smell of the two boxes on a scale of 1 to 5 and to describe

any special notes detected. Panelists were not allowed to see the tomatoes inside the boxes. The taste trials were conducted according to the requirements of the Helsinki Commission. For the taste tests, fresh ripe tomatoes from T₂ *GES*-transgenic and control plants were deseeded before serving them to the panelists. Evaluations were performed on three separate occasions and in three different locations by untrained taste panels of people varying in age and sex (total of 82 individuals). The taste trials were performed in a specialized room equipped with a dim red light. Panelists were asked to rank, using an arbitrary scale of 1 to 9, the general taste, aroma (retronasal route), sweetness and acidity attributes of three fruit samples. Each panelist evaluated two control fruit samples and one transgenic fruit sample served in random order. The differences in scores between transgenic and control fruits were determined for each panelist. To test for significance of the data, mean values were examined using Student's *t*-test. A *P*-value of $p < 0.05$ was considered to be significant.