

## Identification of *malodorous*, a Wild Species Allele Affecting Tomato Aroma That Was Selected against during Domestication

YAAKOV TADMOR,<sup>\*,†</sup> EYAL FRIDMAN,<sup>‡</sup> AMIT GUR,<sup>‡</sup> OLGA LARKOV,<sup>†</sup>  
 ELENA LASTOCHKIN,<sup>†</sup> UZI RAVID,<sup>†</sup> DANI ZAMIR,<sup>‡</sup> AND EFRAIM LEWINSOHN<sup>†</sup>

Institute of Field and Vegetable Crops, Neve Ya'ar Research Center, Agricultural Research Organization, P.O. Box 1021, Ramat Yishay, Israel, and Department of Field and Vegetable Crops, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel

Vegetable cultivation favored the inclusion of pleasant aromas in the produce, whereas unpleasant aromas were selected against. Introgression lines, generated by hybridization of a cultivated tomato (*Lycopersicon esculentum*) to its wild relative *L. pennellii*, were used to map quantitative trait loci (QTL) that influence tomato aroma. A marked undesirable flavor was detected by taste panelists in *L. pennellii* fruits and was related to an introgressed segment from the short arm of chromosome 8. Analysis of the ripe fruits' volatiles of chromosome 8 introgressed lines revealed an up to 60-fold increase in the levels of 2-phenylethanol and phenylacetaldehyde, as compared to the cultivated tomato. This effect was associated with a 10 cM segment originating from the wild species. Although 2-phenylethanol and phenylacetaldehyde have favorable contribution to tomato aroma when present at low levels, phenylacetaldehyde has a nauseating objectionable aroma when present in levels >0.005 ppm. The loss of the ability to produce high levels of phenylacetaldehyde contributed to the development of desirable aroma of the cultivated tomato. The findings provide a genetic explanation for one of the aroma changes that occurred during the domestication of the tomato.

**KEYWORDS:** Tomato; *Lycopersicon esculentum*; *L. pennellii*; mapping; flavor; aroma; 2-phenylethanol; phenylacetaldehyde

### INTRODUCTION

Tomato domestication from its wild relatives involved the exclusion of undesirable traits such as those affecting palatability of the fruits. The early steps of selection eliminated unfavorable alleles that adversely affect aroma and flavor because most of the wild progenitors are inedible and some of them are even poisonous (1, 2).

The characteristic taste of tomato and its products is mainly due to reducing sugars (fructose and glucose), free acids (mainly citric), and hundreds of volatile compounds that constitute the unique aroma of tomato (3, 4). Not only may the levels and thresholds of individual volatile compounds and their synergistic properties be important in aroma perception, but there are also many cases in which the same volatile molecule can be perceived as agreeable or disagreeable according to its relative concentration (5). More than 400 different volatile compounds have been found in tomato and its industrial products. They include hydrocarbons, alcohols, phenols, ethers, aldehydes,

ketones, carboxylic acids and esters, lactones, and sulfur, as well as nitrogen-containing compounds (3). Nevertheless, several studies have indicated that among the compounds that have a great impact in tomato aroma are the small- and medium-chain aldehydes and alcohols, such as 2-hexenal and *cis*-3-hexen-1-ol, as well as the acyclic monoterpene linalool, the norisoprenoids such as  $\beta$ -damascenone and  $\beta$ -ionone, and the phenolic derivatives 2-phenylethanol, phenylacetaldehyde, methyl salicylate, and eugenol (3, 4, 6–8).

One of the most abundant volatile compounds often present in tomato fruits is 2-phenylethanol, a compound with a pleasant flowery aroma, that can be found also in other fruits, such as apples, apricots, bananas, persimmons, grapes, guavas, and melons (9). 2-Phenylethanol is particularly abundant in cinnamon and cassia barks and in the fragrance of roses and other flowers (10). 2-Phenylethanol is also present in wines, liquors, and other alcoholic beverages such as tequila, imparting floral notes (11). Phenylacetaldehyde has a more pungent aroma as compared to 2-phenylethanol, and in high concentrations, it has a nauseating, unpleasant odor (10). Low levels of phenylacetaldehyde are normally present in tomatoes (4, 7) and in other fruits, food products, and scents of flowers (12–14). Although low concentrations of phenylacetaldehyde are associated with a typical sweet floral note or honey, at concentrations >0.005

\* Address correspondence to this author at the Department of Plant Science, Cook College, Rutgers University, 59 Dudley Rd., New Brunswick, NJ 08901-8520.

<sup>†</sup> Agricultural Research Organization.

<sup>‡</sup> The Hebrew University of Jerusalem.

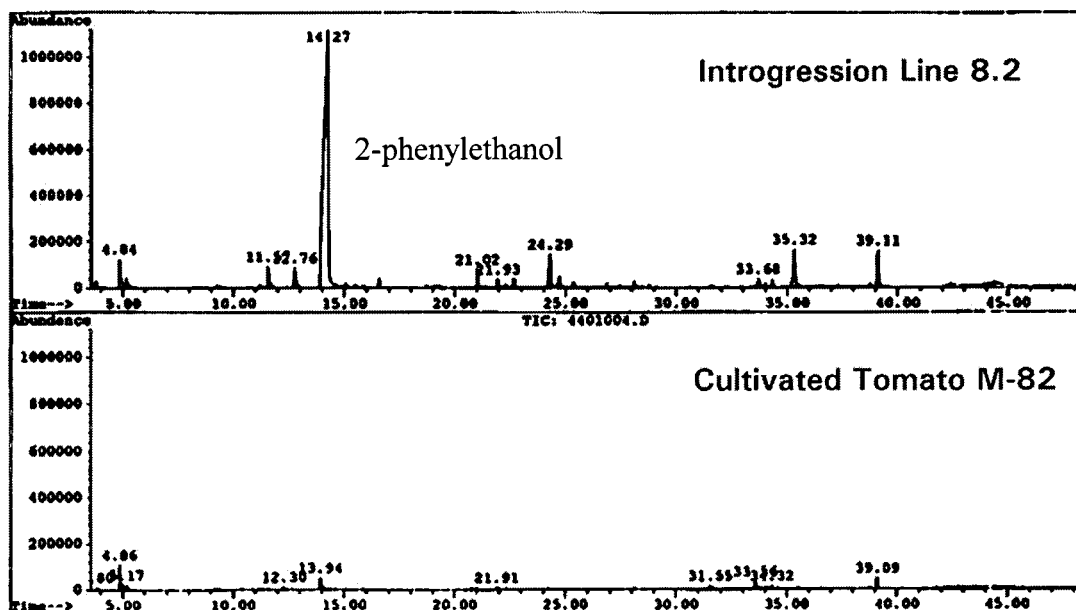


Figure 1. GC-MS analyses of the volatiles of introgression line IL8-2 (top) and parental line M-82 (bottom).

ppm, phenylacetaldehyde is considered to be detrimental to the quality of tomato juice, due to its harsh undesirable flavor (3).

A set of nearly isogenic lines (NILs) was developed from a cross between the wild green-fruited species *Lycopersicon pennellii* and the cultivated tomato (*Lycopersicon esculentum* CV-M82; 15). Each of the NILs contained a single RFLP defined *L. pennellii* chromosome segment introgression, and together the lines provide complete coverage of the genome. The nearly isogenic nature of this genetic resource enables clear association of a wild species introgression with any phenotypic effects under study by comparing the phenotype of the introgression line with the cultivated tomato M82. Using this resource, it was possible to identify QTLs that affect yield-associated traits and to study their epistatic and environmental interactions (16, 17). The utilization of introgression line analysis combined with chemical, biochemical, and molecular analyses has allowed resolution of some of the genetic factors that influence the formation of sesquiterpenes in tomatoes (18).

Some *L. pennellii*-introgressed tomato lines displayed an unpleasant aroma, reminiscent of the wild relative *L. pennellii*, notably absent from the cultivated tomato. This study describes the utilization of the *L. pennellii* ILs for the identification of a wild species chromosomal region affecting the accumulation of 2-phenylethanol and phenylacetaldehyde in ripening tomato fruits.

## MATERIALS AND METHODS

**Plant Material.** The *L. pennellii* (LA716) introgression lines IL8-1, IL8-2, and IL8-3 were previously described for their marker data (15, 19). IL8-2 was crossed to M82, and the resulting F<sub>2</sub> plants of the selfed hybrid were screened with the flanking RFLP markers for recombination events within the introgression. The identified recombinants were selfed to obtain sub-ILs of IL8-2 with shorter introgressions. One of these sub-ILs containing the *L. pennellii* introgression (TG330-CT77; 31.4 cM) was designated IL8-2-1.

**Experimental Design.** M82, IL8-1, IL8-2, IL8-2-1, and IL8-3 were transplanted in the field at Akko (northern Israel) and Rehovot (central Israel) in a completely randomized design (six replicates each). Each replicate consisted of 35 plants that were planted in a 10 m<sup>2</sup> plot. Ten fruits of mature-green, break, and red stages were collected from each plot and transferred to the Neve Ya'ar Center for biochemical analysis.

*L. pennellii* fruits were obtained from plants grown in the Faculty of Agriculture greenhouses in Rehovot.

**Metabolite Analysis.** For extraction of metabolites from tomato fruits ~200 g of fresh fruits was homogenized with a blender and filtered (Whatman No. 1). The serum was saturated with NaCl, and 50 mL of it was extracted with 4 volumes of dichloromethane. The lower organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated with a stream of N<sub>2</sub> to 0.5 mL, and analyzed by GC-MS as follows.

Samples consisting of 1.0  $\mu$ L of concentrated dichloromethane extracts were analyzed on a Hewlett-Packard EI-GC-MS (GCD system, 70 eV) equipped with an HP5 (30 m  $\times$  0.25 mm) fused-silica capillary column. Helium (1 mL/min) was used as a carrier gas. Injector temperature was 270  $^{\circ}$ C. Conditions: 70  $^{\circ}$ C for 2 min, 70–200  $^{\circ}$ C at 4  $^{\circ}$ C/min, hold for 10 min. Identification of the main components was done by co-injection of authentic standards and comparison of the MS obtained with computerized libraries (20).

Untrained taste panelists evaluated the fresh fruits' flavor for sweetness, acidity, and unique aromas.

## RESULTS AND DISCUSSION

**Flavor and Chemical Characterization of *L. pennellii* Introgression Lines.** We have shown the utility of *L. pennellii* genes introgressed into the cultivated tomato both to enhance yield and to improve other agronomical and quality characteristics of tomatoes (16). Such an approach has proven to be particularly suited for mapping and isolation of genes underlying quantitative variation such as fruit size and sugar content (17, 21). Tomato aroma is a trait that is influenced by many genes and backgrounds and therefore was considered to be a QTL in our genetic characterization.

*L. pennellii* has a very disagreeable aroma. We evaluated all introgression line populations (ILs) for flavor preferences (data not shown). Two of the ILs, IL8-1 and IL8-2, had a very strong disagreeable characteristic aroma. We further compared the components of the volatile fractions of M82, IL8-1, IL8-2, and IL8-3. GC-MS analysis of ripe mature fruits indicated the presence of up to 60-fold higher levels of 2-phenylethanol in introgression line IL8-2 as compared to the volatiles found in cultivated tomato M82 (Table 1; Figure 1). Moreover, phenylacetaldehyde, practically absent from this cultivated tomato line, was also very prominent in IL8-2. IL8-1 also displayed elevated levels of 2-phenylethanol and phenylacetaldehyde (Table 1).

**Table 1.** 2-Phenylethanol and Phenylacetaldehyde Levels in Parents and in Selected Introgression Lines of *L. esculentum* and *L. pennellii*<sup>a</sup>

line analyzed		2-phenyl-ethanol ( $\mu\text{g/g}$ of FW)	phenyl-acetaldehyde ( $\mu\text{g/g}$ of FW)
parents	<i>L. esculentum</i> M-82	0.033 $\pm$ 0.007	0.000 $\pm$ 0.000
	<i>L. pennellii</i>	0.65	1.6
introgression lines	IL8-1	0.990 $\pm$ 0.593	0.240 $\pm$ 0.095
	IL8-2	2.203 $\pm$ 1.317	0.241 $\pm$ 0.117
	IL8-3	0.055 $\pm$ 0.02	0.00 $\pm$ 0.00
	IL8-2-1	1.937 $\pm$ 0.547	0.261 $\pm$ 0.58

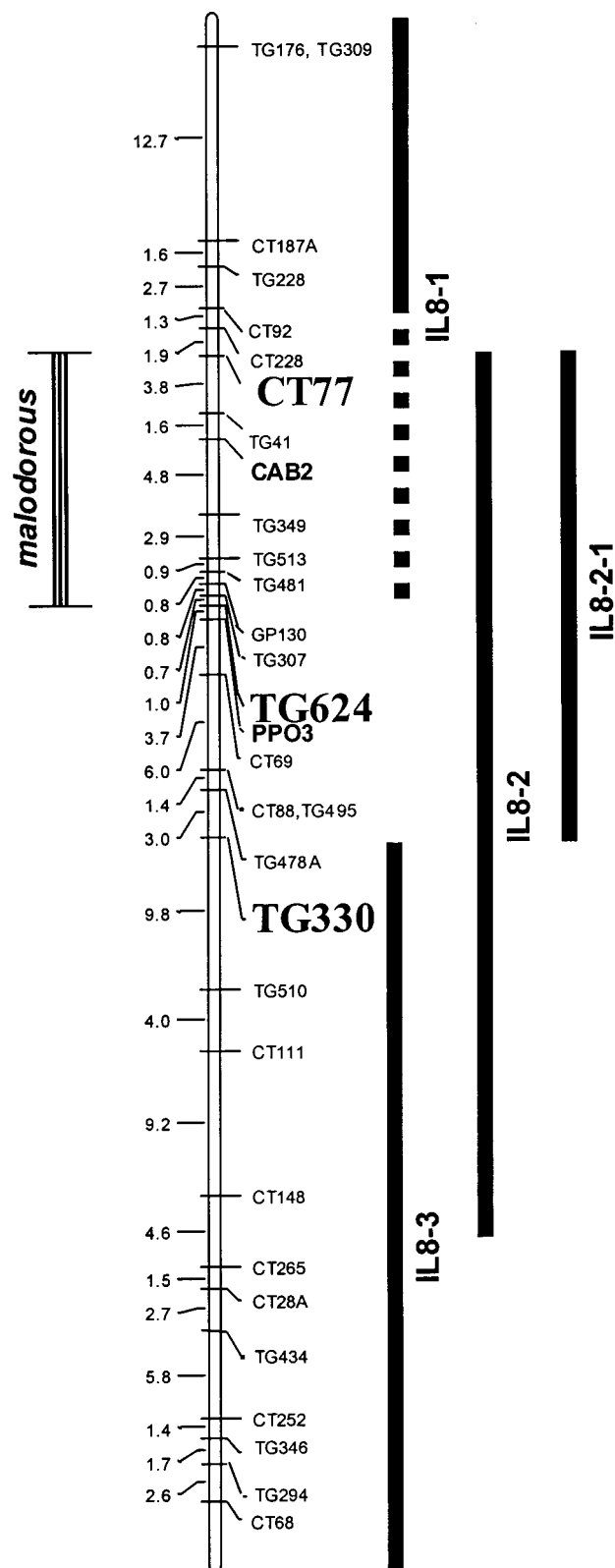
<sup>a</sup> Means and standard errors of at least three independent determinations (only one for *L. pennellii*) are shown.

On the contrary, IL8-3, which proximally overlaps IL8-2, displayed levels of 2-phenylethanol and phenylacetaldehyde similar to those of the cultivated line M82. IL8-1 is partially overlapping with IL8-2 in the distal arm (**Figure 2**). This indicated that the locus coding for elevated levels of 2-phenylethanol and phenylacetaldehyde is located in the overlapping region of both introgression lines IL8-1 and IL8-2 (**Figure 2**). We designated this locus *malodorous*.

*L. pennellii* fruits also contain elevated levels of 2-phenylethanol and phenylacetaldehyde (**Table 1**), but in contrast to what was found in the chromosome 8 ILs, the level of phenylacetaldehyde is >2-fold higher than the level of 2-phenylethanol in *L. pennellii*. The high levels of phenylacetaldehyde are probably partially responsible for the disagreeable taste of *L. pennellii* fruits. The lack of any report in the literature of unpleasant flavor characteristics of 2-phenylethanol minimizes the possibility that this compound also contributes to the undesirable flavor found in the introgression lines. The different ratio between phenylacetaldehyde and 2-phenylethanol observed in the introgression lines as compared to *L. pennellii* could be due to a different genetic background in the cultivated tomato and might be caused by different levels of phenylethanol dehydrogenase, putatively present in *L. pennellii*, as compared to the cultivated tomato. Our results suggest that the levels of this activity already present in the cultivated tomato suffice to convert phenylacetaldehyde to 2-phenylethanol.

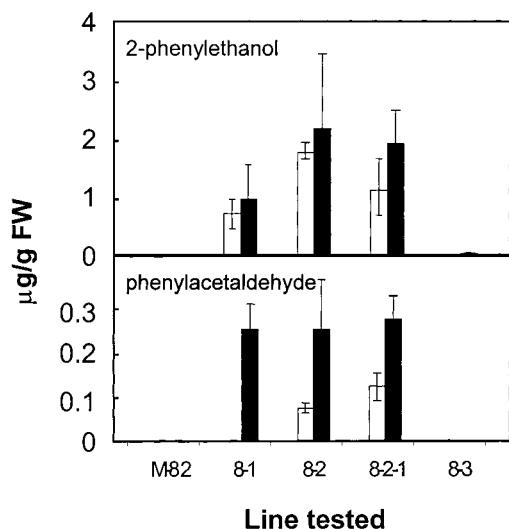
#### Fine Mapping and Mode of Inheritance of *malodorous*.

To fine map the *malodorous* locus, we utilized sub-ILs of IL8-2. An F<sub>2</sub> population, derived from selfing the hybrid IL8-2xM82, was screened for recombination between the flanking markers CT77 and CT148. One of these sub-ILs, IL8-2-1 (**Figure 2**), also had a disagreeable odor and therefore was examined for its levels of 2-phenylethanol and phenylacetaldehyde. GC-MS analysis of this line showed a volatile profile similar to that of IL8-2 (**Table 1**; **Figure 1**). These results map *malodorous* to the 15cM overlapping chromosomal region of IL8-2-1 and IL8-1, between CT77 and TG624 (**Figure 2**). When we compared the levels of phenylacetaldehyde and 2-phenylethanol in the analyzed ILs, we noticed that the levels of phenylacetaldehyde are similar in all three ILs, whereas the level of 2-phenylethanol in IL8-1 is half of the levels observed in IL8-2 and IL8-2-1 (**Table 1**). IL8-1 was reported previously as being heterozygote in the overlapping region with IL8-2, due to a gametophytic factor (19). This heterozygosity shows that the 2-phenylethanol level is inherited in a partially dominant mode, whereas the phenylacetaldehyde level is governed dominantly. However, this also might suggest that this locus is composed of two linked loci: one that resides on IL8-1 and regulates 2-phenylethanol, and another locus that determines phenylacetaldehyde content. Our data are not sufficient to



**Figure 2.** Genetic map of tomato chromosome 8. The *L. pennellii* introgressions are presented as lines on the right side of the chromosome. A solid line indicates homozygosity for the introgression, whereas a dotted line indicates heterozygosity for the introgression. The interval on which *malodorous* resides (CT77–TG624) is presented on the left side of the chromosome.

conclude whether the accumulation of these compounds is regulated by a single pleiotropic locus or by two tightly linked loci.



**Figure 3.** Accumulation of 2-phenylethanol (top) and phenylacetaldehyde (bottom) during tomato fruit maturation in tomato introgressed lines. White bars represent values of fruits at the breaker stage; black bars represent matured red fruits.

Saliba-Colombani et al. (22) have recently performed a whole genome analysis of 18 volatiles governing tomato aroma in a recombinant inbred population derived from a cross between a cherry tomato, characterized by desired aroma and flavor, and a regular tomato. A single QTL that modulates 2-phenylethanol was mapped to the same genomic region as the overlapping region of IL8-1 and IL8-2. It seems that the major locus controlling 2-phenylethanol accumulation in the tomato fruit resides in this region.

**Volatile Accumulation in Developing Tomato Fruits.** The accumulation of high levels of 2-phenylethanol and phenylacetaldehyde takes place during fruit maturation of the introgressed lines. At the green stage, the major volatiles found in M-82 and in all introgression lines analyzed are the phenolic compounds guaiacol and methyl salicylate, typical of unripe tomato fruits (not shown). No 2-phenylethanol or phenylacetaldehyde is detected at this stage (Figure 3). Interestingly, neither guaiacol nor methyl salicylate was found in green but ripe *L. pennellii*. In the breaker stage, 2-phenylethanol and phenylacetaldehyde are already very prominent in IL8-1, IL8-2, and IL8-2-1, but absent in the control line M-82 and in IL8-3. The levels continue to increase upon full maturation (Figure 3). This is in accordance with previous observations in which increased levels of volatile compounds were found during tomato fruit ripening (8). Our results indicate that the gene or genes that affect 2-phenylethanol and phenylacetaldehyde accumulation are primarily expressed late during fruit development.

**Conclusions.** The genetic potential of the wild tomato relatives in improving the cultivated tomato has been extensively studied and has afforded many agronomical advances (2). Introgression line analysis has enabled the identification of novel sesquiterpene synthase genes involved in plant defense against mites (18), also lost during the tomato domestication processes. We have described here the utilization of introgression lines to enlighten our understanding of one of the factors that contributed to the improvement of tomato aroma during domestication and also study the genetic control of 2-phenylethanol and phenylacetaldehyde formation in tomato fruits. We have shown that increased expression of 2-phenylethanol and phenylacetaldehyde was detrimental to fruit flavor and quality and provide a possible evolutionary explanation as to why these traits were left out

during tomato domestication. Similar introgressions utilizing *Lycopersicon hirsutum* resulted in comparable undesirable taste (Tadmor et al., unpublished results). Using a similar approach, we are undertaking a study to map other genes that control the formation of key aroma compounds in tomato fruits. This research highlights the power of the approach of using exotic genetic variation and specifically designed population structures to explore the genetics and biochemistry of natural plant metabolites.

#### ABBREVIATIONS USED

EI-GC-MS, electron impact gas chromatograph–mass spectrometer; IL, introgression lines; QTL, quantitative trait loci; NILs, nearly isogenic lines.

#### LITERATURE CITED

- Rick, C. M.; DeVerna, J. W.; Chetelat, R. T.; Stevens, W. A. Potential contributions of wide crosses to improvement of processing tomatoes. *Acta Hort.* **1987**, *200*, 45–55.
- Rick, C. M.; Chetelat, R. T. Utilization of related wild species for tomato improvement. *Acta Hort.* **1995**, *412*, 21–38.
- Petro-Turza, M. Flavor of tomato and tomato products. *Food Rev. Int.* **1987**, *2*, 309–351.
- Maul, F.; Sargent, S. A.; Balaban, M. O.; Baldwin, E. A.; Huber, D. J.; Sims, C. A. Aroma volatile profiles from ripe tomatoes are influenced by physiological maturity at harvest: an application for electronic nose technology. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 1094–1101.
- Ohloff, G. Recent developments in the field of naturally-occurring aroma components. *Fortsch. Chem. Org. Natur.* **1978**, *35*, 431–527.
- McGlasson, W. B.; Last, J. H.; Shaw, K. J.; Meldrum, S. K. Influence of the non-ripening mutants *rin* and *nor* on the aroma of tomato fruit. *HortScience* **1987**, *22*, 632–634.
- Krumbein, A.; Auerswald, H. Important aroma compounds in tomato determined by instrumental and sensory analysis. *Agric. Food Qual.* **1999**, *2*, 303–305.
- Baldwin, E. A.; Nisperos-Carriedo, M. O.; Moshonas, M. G. Quantitative analysis of flavor and other volatiles and for certain constituents of two tomato cultivars during ripening. *J. Am. Soc. Hortic.* **1991**, *116*, 265–269.
- Horvat, R. J.; Senter, S. D.; Chapman, Jr., G. W.; Payne, J. A. Volatile compounds from the mesocarp of melons. *J. Food Sci.* **1991**, *56*, 262–263.
- Burdock, G. A., Ed. *Fenaroli's Handbook of Flavor Ingredients*, 3rd ed.; CRC Press: Boca Raton, FL, 1995.
- Benn, S. M.; Peppard, T. L. Characterization of tequila flavor by instrumental and sensory analysis. *J. Agric. Food Chem.* **1996**, *44*, 557–566.
- Ramaswamy, R.; Ramaswamy, P.; Ravichandran, R.; Parthiban, R. The impact of processing techniques on tea volatiles. *Food Chem.* **1998**, *62* (3), 347–353.
- Wong, K. C.; Tan, C. P.; Chow, C. H.; Chee, S. G. Volatile constituents of the fruit of *Tamarindus indica* L. *J. Essent. Oil Res.* **1998**, *10* (2), 219–221.
- Brunke, E. J.; Hammerschmidt, F. J.; Schmaus, G. Headspace analysis of hyacinth flowers. *Flavour Fragrance J.* **1994**, *9* (2), 59–69.
- Eshed, Y.; Zamir, D. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* **1995**, *141*, 1147–1162.
- Eshed, Y.; Zamir, D. Less-than-additive epistatic interactions of quantitative trait loci in tomato. *Genetics* **1996**, *143*, 1807–1817.

- (17) Fridman, E.; Pleban, T.; Zamir, D. A recombination hotspot delimits a wild species QTL for tomato sugar content to 484-bp within an invertase gene. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4718–4723.
- (18) Van der Hoeven, R. S.; Monforte, A. J.; Breeden, D.; Tanksley, S. D.; Steffens, J. C. Genetic control and evolution of sesquiterpene biosynthesis in *Lycopersicon esculentum* and *L. hirsutum*. *Plant Cell* **2000**, *12*, 2283–2294.
- (19) Liu, Y. S.; Zamir, D. Second-generation *L. pennellii* introgression lines and the concept of bin mapping. *Tomato Genet. Coop.* **1999**, *49*, 26–30.
- (20) Shalit, M.; Katzir, N.; Tadmor, Y.; Larkov, O.; Burger, Y.; Shalekhet, F.; Lastochkin, E.; Ravid, U.; Amar, O.; Edelstein, M.; Karchi, Z.; Lewinsohn, E. Acetyl CoA: Alcohol acetyl transferase activity and aroma formation in ripening melon fruits. *J. Agric. Food Chem.* **2001**, *49*, 794–799.
- (21) Frary, A.; Grandillo, S.; van der Knaap, E.; Cong, B.; Liu, J.; Meller, J.; Elber, R.; Alpert, K. B.; Tanksley, S. D. A Quantitative Trait Locus Key to the Evolution of Tomato Fruit Size. *Science* **2000**, *289*, 85–88.
- (22) Saliba-Colombani, V.; Causse, M.; Langlois, D.; Philouze, J.; Buret, M. Genetic analysis of organoleptic quality in fresh market tomato: 1. Mapping QTLs for physical and chemical traits. *Theor. Appl. Genet.* **2001**, *102*, 259–272.

---

Received for review September 24, 2001. Revised manuscript received January 9, 2002. Accepted January 9, 2002. Contribution from the ARO, The Volcani Center, Bet Dagan 50250, Israel, No. 138-2001.

JF011237X