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# The inheritance of volatile phenylpropenes in bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae) chemotypes and their distribution within the plant

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ABSTRACT

The volatile phenylpropenes estragole and *t*-anethole are the major constituents of the oleoresin of the aerial parts of bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae). The levels of estragole and *t*-anethole varied during plant development, being maximal in flowers and developing mericarps. Still the ratio between estragole and *t*-anethole remained constant throughout development. Estragole-rich types were hybridized with *t*-anethole rich types to examine the genetic basis of this polymorphism. A reverse correlation between estragole and *t*-anethole content was evident and the action of a biallelic gene with partial dominance for high estragole content was inferred. Understanding phenylpropene inheritance might explain chemical polymorphism in wild bitter fennel populations, sheds light on the molecular mechanisms that lead to chemotypes evolution and is crucial for breeding fennel varieties with desired chemical compositions.



## 1. Introduction

The phenylpropene derivatives estragole and *t*-anethole are the major constituents of bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae) oleoresin (Karlsen et al., 1969; Bernath et al., 1996; Barazani et al., 1999). Estragole and *t*-anethole are used as food flavorants, in alcoholic beverages and in mouth care products (Karlsen et al., 1969). The mericarps (commonly referred as seed) are the most aromatic and widely used part of the plant, but all aerial parts contain the typical oleoresin. Various studies carried out in fennel from different geographic origin have indicated that the content of estragole in mericarps varies from 2 to 86% and that of *t*-anethole from 0 to 89% (Pank et al., 2003). In Israel, indigenous populations of bitter fennel occur in diverse habitats and a previous study examining 11 indigenous populations indicated a reversed association between the content of estragole and *t*-anethole, suggesting genetic control of this trait (Barazani et al., 1999).

Associations between volatile phenylpropenoid composition and environmental parameters (e.g. annual rainfall and temperature) have been conjectured (Barazani et al., 1999). Still, irrigation, fertilization and other cultivation practices had no significant effect on the concentration of *t*-anethole in the essential oil of cultivated bitter fennel (Buntain and Chung, 1994; Barazani et al., 2002).

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Cluster analysis of the composition of phenylpropenoid derivatives among indigenous Israeli bitter fennel populations and their cultivated representatives yielded two different chemotypes as follows: a "*t*-anetholic" chemotype, represented by populations in the Negev desert and in the northern coastal plain, and an "estragolic" chemotype of a northeastern population of Mt. Dov (Barazani et al., 2002). A chemotype is defined as a "locally adapted population of a widespread species; such populations show minor changes of morphology and/or physiology (and/or phytochemistry) which are related to habitat and are genetically induced. Nevertheless, they can still reproduce with other ecotypes/chemotypes of the same species" (Barazani et al., 2002). A chemotype in this sense is a taxonomic entity similar to an ecotype (Allaby, 1998).

Our knowledge on the patterns of inheritance of the oleoresin constituents in bitter fennel is limited and information on the chemical composition of roots is scarce. Accordingly, the aim of this study was to determine the volatile phenylpropene content in different parts of the plant as well as to uncover the genetic basis that determines the phenylpropene content and the chemical variation in bitter fennel chemotypes and populations.

## 2. Materials and methods

### 2.1. Plant material

Bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae) chemotypes from Israel, Mt. Katharina in Sinai Desert (Egypt) and Mersin (Turkey) were examined (Table 1) (Barazani et al., 1999; Gross et al., 2002). Fruits collected from these populations were field grown at the Newe Ya'ar Research Center in Israel under standard agricultural practices as before (Gross et al., 2002). Preliminary tests indicated that the cultivated individuals exhibited the same chemical differences as seen in wild representatives growing in their natural habitats. Phenotypes were defined based on Muckensturm et al. (1997): individual plants displaying 90–100% estragole out of the total volatile phenylpropene fraction were defined "estragolic" (E), whereas plants with 0–10% estragole out of the total phenylpropene fraction were defined "anetholic" (A). Individuals displaying 50–80% estragole in the volatile phenylpropene fraction were defined as "estragolic/anetholic" (EA).

### 2.2. Extraction of volatiles

Samples from different tissues of the adult plants and from seedlings (offspring) as indicated were analyzed for estragole and *t*-anethole content according to Gross et al. (2002). The samples were crushed using a mortar and pestle and extracted in a rotor shaker (150 rpm) with 5 ml hexane for 2.5 h at room temperature. The extract was filtered through a layer of glass wool and dried with sodium sulfate (Gross et al., 2002).

### 2.3. Analysis of volatiles

Flower and fruit extracts were diluted five-fold with hexane whereas extracts from leaves, stalks and roots, were concentrated to 1 ml by evaporation with a gentle stream of nitrogen before analyses. One μl of such hexane extracts were injected to the GC–MS for analysis (Barazani et al., 2002). Identification of the main components was done by comparison of a mass spectra and retention time data with those of authentic samples. The quantitative analyses was performed by employing ethyl myristate (10–50 μg) as an internal standard (Gross et al., 2002).

### 2.4. Crosses

Parental plants differing in volatile phenylpropene composition were subjected to controlled hybridizations. The volatile phenylpropenes are seemingly synthesized and stored in the oil ducts derived from the maternal tissue of the mericarps (Gross et al., 2006). Thus, segregation analysis was performed on offspring plants (seedlings) and not on the mericarps themselves. Parental plants were reciprocally crossed to get $F_1$ hybrids, which were then either self pollinated or crossed to either of the parental types yielding the $F_2$ and BC segregating populations. The crossing technique included the following steps: Selected flowers in the yellow bud stage (Gross et al., 2008) from one parent were emasculated and the rest of the flowers in the umbel

**Table 1**
Estragole and *t*-anethole content (mg/g fw) and percent estragole/volatile phenylpropenes in the oleoresin of mericarps and seedlings (3-week old) were sampled of different bitter fennel populations.

| Population | Fruits (mericarps) | | | Seedlings | | | Phenotype |
|---|---|---|---|---|---|---|---|
| Locality | Estragole | *t*-Anethole | % estragole/volatile phenylpropenoids | Estragole | *t*-Anethole | % estragole/volatile phenylpropenoids | |
| Beer Hail, Israel (BH) | 0.65 ± 0.1 | 14.1 ± 1.5 | 4.4 ± 0.1 | 0.0 ± 0.0 | 0.9 ± 0.1 | 4.9 ± 0.2 | A |
| Mt. Dov, Israel (MD) | 5.14 ± 0.4 | 0.1 ± 0.1 | 97.6 ± 2.1 | 0.7 ± 0.1 | 0.0 ± 0.0 | 99.9 ± 0.0 | E |
| Mt. Katharina, Egypt (S) | 0.28 ± 0.0 | 6.6 ± 0.7 | 4.0 ± 4.6 | 0.0 ± 0.0 | 0.7 ± 0.1 | 5.1 ± 0.1 | A |
| Mersin, Turkey (T) | 5.12 ± 0.4 | 0.0 ± 0.0 | 99.7 ± 0.1 | 0.5 ± 0.0 | 0.0 ± 0.0 | 99.8 ± 0.1 | E |

Average ± SE; $n = 10–14$ each population. A = "anetholic"; E = "estragolic".