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# GENETIC VARIATION FOR VOLATILE TERPENOIDS IN ROOTS OF CARROT, *DAUCUS CAROTA*, INBREDS AND $F_1$ HYBRIDS

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**Key Word Index**—*Daucus*; Umbelliferae; genetics; biosynthesis; terpenoids; volatile oils.

**Abstract**—Volatile terpenoid levels were determined in eight carrot inbreds and ten $F_1$ hybrids. Genetic variation between inbreds spanned a 5–10-fold range for both individual terpenes and total volatile terpenoids. Terpinolene, caryophyllene and ($E$)-$\gamma$-bisabolene were the most plentiful terpenes. Dominance for low terpinolene, caryophyllene, and total volatile terpenoids was observed in $F_1$ hybrids. The ($E$)-$\gamma$-bisabolene quantities varied independently of total volatile terpenoid levels. Genetic trends were clearer for individual compounds when terpene levels were expressed in absolute quantity per unit of root tissue rather than in percentage of total volatile terpenoids.

## INTRODUCTION

Buttery *et al.* initially investigated the terpenoid composition of carrot (*Daucus carota* var. Imperator) roots[1]. Subsequent reports have recorded volatile terpenoid levels in carrot varieties grown in several environments[2, 3]. Because mono- and sesquiterpenoids have been implicated as factors in flavor[3], herbivore palatability[4], pest resistance[5–7], and insect attraction[7–9], a better understanding of the genetic control of terpenoid formation in carrots is of interest. Such information can also aid in the elucidation of terpenoid biosynthetic pathways in this widely adapted species.

The genetics of terpene biosynthesis has been extensively considered for turpentine constituents in the Coniferae, especially *Pinus* spp., where simple genetic models can account for variation in $\alpha$-pinene, $\beta$-pinene, 3-carene, $\alpha$-phellandrene, myrcene, limonene, and camphene[10–17]. The genetics of monoterpenoid synthesis has also been investigated for interspecific hybrids of the Labiatae. In *Mentha* spp. hybrids, nine genes controlling terpenoid synthesis have been identified[18]. *Hedeoma* interspecific hybrids display simple genetic control of monoterpene synthesis for 12 compounds [19] and eugenol synthesis is dominant to thymol in *Ocimum* interspecific hybrids[20]. In addition to these two groups of plants, single genes control oct-1-en-ol and linalol levels in beans [21] and 2-isobutylthiazole, methyl salicylate, and eugenol levels in tomatoes[22].

The enzymes for the isomerization of geraniol to nerol[23] and geranyl phosphate to neryl phosphate, and for the cyclization of neryl pyrophosphate to $\alpha$-terpineol[24] have been demonstrated in carrots. To gain a better understanding of the genetics and biosynthesis of carrot volatile terpenoids, this report examines terpenoid accumulation in several diverse, unrelated inbred carrot lines and their $F_1$ hybrids.

## RESULTS AND DISCUSSION

The total volatile terpenoids in the carrot lines analyzed spanned a five-fold range, and most individual compounds demonstrated an even greater range over genotypes and environments (Table 1). The relative amounts of individual terpenoids, expressed as a percentage of the total volatile terpenoids, also varied widely. Terpinolene was usually the most abundant volatile terpene, as reported[1–3], but caryophyllene and/or ($E$)-$\gamma$-bisabolene were sometimes more plentiful. Some influence of growing location was noted, as demonstrated by B3615 × B6274 grown in Florida, Texas and California, but genetic effects were much larger than location effects. Comparing the two inbreds, B3615 × B6274, listed in Table 1, terpenoid quantities varied greatly, whereas differences in terpenoid percentages were generally much smaller. The hybrid tended to have terpenoid quantities more similar to those of the low-terpenoid parent (B6274) than the high-terpenoid parent (B3615). To draw conclusions from a larger data base, volatile terpenoid levels were determined for three carrot inbreds with high total volatile terpenoid quantities (B493, B3615, and B4367; 1400–2800 units), two with medium quantities (B6439 and F524; 1000–1100 units), three with low quantities (B6274, B9304, and B10138; 600–800 units) and 10 $F_1$ hybrids (six high × low or reciprocal, two low × medium, one high × medium or reciprocal, one high × high) grown in Florida. Comparisons of total volatile terpenoids (Table 2) and percentages (Table 3) of terpinolene, caryophyllene, and ($E$)-$\gamma$-bisabolene are presented.

Inbred carrot lines with more total volatile terpenoids generally had more terpinolene and caryophyllene (Table 2). The ($E$)-$\gamma$-bisabolene quantities were not correlated to total terpenoid quantities, with small amounts in both high (B493) and low (B10138) terpenoid inbreds. Reciprocals of the $F_1$ hybrids

Table 1. Volatile terpenoid levels in raw carrot roots

| | Inbreds | | Hybrids | | | |
|---|---|---|---|---|---|---|
| | B3615 | B6274 | B3615 × B6274 | B3615 × B6274 | B3615 × B6274 | |
| Compounds Location: | Florida | Florida | Florida | Texas | California | Range† |
| α-Pinene | 0(0)* | 0(0) | 0(0) | 0(0) | 0(0) | 0–5(0–0.5) |
| β-Pinene/sabinene | 5(0.2) | 11(1.3) | 2(0.1) | 4(0.2) | 1(0.1) | 0–10(0–1.0) |
| Myrcene | 32(1.1) | 25(3.2) | 27(2.3) | 60(3.4) | 60(4.6) | 2–83(0.1–6.9) |
| α-Phellandrene | 6(0.2) | 2(0.2) | 3(0.2) | 6(0.3) | 2(0.2) | 2–33(0.2–3.8) |
| α-Terpinene | 0(0) | 0(0) | 0(0) | 4(0.2) | 0(0) | 0–46(0–5.4) |
| Limonene | 51(1.8) | 19(2.4) | 21(1.8) | 22(1.2) | 16(1.2) | 3–77(0.2–5.8) |
| γ-Terpinene | 76(2.7) | 16(2.0) | 52(4.4) | 38(2.1) | 23(1.8) | 12–237(0.7–15.0) |
| Terpinolene | 1914(68.2) | 343(43.7) | 700(59.4) | 1034(57.9) | 712(55.0) | 135–1714(19.9–72.0) |
| Terpinene-4-ol | 5(0.2) | 0(0) | 0(0) | 0(0) | 0(0) | 0–27(0–2.5) |
| Bornyl acetate | 36(1.3) | 3(0.3) | 6(0.5) | 11(0.6) | 2(0.2) | 0–72(0–10.6) |
| Caryophyllene | 468(16.7) | 125(15.9) | 125(10.6) | 361(20.1) | 170(13.1) | 66–666(8.0–34.9) |
| (Z)-γ-Bisabolene | 31(1.1) | 29(3.7) | 18(1.5) | 22(1.2) | 23(1.8) | 0–48(0–2.4) |
| (E)-γ-Bisabolene | 183(6.5) | 277(35.3) | 225(19.1) | 232(12.9) | 289(22.3) | 14–652(0.7–36.2) |
| Total | 2807(100) | 850(100) | 1179(100) | 1794(100) | 1298(100) | 497–2824(—) |

*Reported as 1000 × ppm (% total volatiles measured).
†Range represents results from 17 inbreds or hybrids grown in 1–3 locations.

B3615 × B10138, B3615 × B9304, B4367 × B6274 and B6439 × B6274 were analysed and found to be equivalent for all terpenoids (data not presented). Therefore the direction of the hybridization is not specified in Table 2. The lack of reciprocal cross difference indicates that the genetic control of volatile terpenoid biosynthesis is nuclear rather than cytoplasmic.

Terpinolene, caryophyllene, and total terpenoid in the $F_1$ hybrids were usually equivalent to the low total volatile parent (Table 2). This biogenetic dominance toward the lower quantity parent was also characteristic of α-phellandrene, α-terpinene, limonene, γ-terpinene, terpinene-4-ol, bornyl acetate, and (Z)-γ-bisabolene (data not presented). Even when terpinolene or total terpenoid quantites in the $F_1$'s were statistically intermediate to both parents, they tended to be closer to the low parent (see quantities for B3615 × B6274 and B4367 × B6274, Table 2).

The trend or biogenetic dominance toward the parent with low quantities of terpenes observed in carrots is similar to observations in *Rubus* interspecific hybrids [25] for total volatiles, terpenes, linalol, α- and β-ionone, and in tomato hybrids [22] for methyl salicylate and eugenol. Intermediate amounts of 2-isobutylthiazole in tomatoes [22] and linalol in snap beans [21] were measured in $F_1$ hybrids of these species. Dominance for high levels of α-terpineol and geraniol was noted in *Rubus* hybrids [27], oct-1-en-3-ol in snap bean hybrids [21], and total oils in *Ocimum* interspecific hybrids [20]. Conifer terpenes are generally reported as a percentage of total terpenes rather than per unit plant weight, but genetic analysis of *Pinus monticola* indicated a dominance for high total terpene levels per unit oleoresin [11].

This is true genetic dominance for low volatile terpenoid levels in carrots and not an anomaly of hybrid vigor since the high × high hybrid (B493 × B3615) had quantities comparable to, and not less than, its parents. The conservative gene action of dominance for low mono- and sesquiterpenoid quantities is typical of other biochemical genetic systems including dominance for inhibition of carotene synthesis in carrots [26]. No biosynthetic precursors are known to over-accumulate in carrots having low amounts of volatile terpenoids.

In considering terpenoid percentages rather than quantities in carrot $F_1$ hybrids, no biogenetic dominance toward the low parent is noted for percent terpinolene and caryophyllene (Table 3). The $F_1$ hybrid had percentages intermediate to both parents (e.g. terpinolene in B3615 × B6274), below the lower parent (e.g. caryophyllene in B4367 × B6274), or above the higher parent (e.g. caryophyllene in B6439 × B6274). This was also the case for most of the other volatile terpenoids (data not presented). Dominance for intermediate to high (E)-γ-bisabolene percentage was noted in hybrids of B493, B3615, F524, and B9304 (Table 3). Dominance for high percentage pinenes and myrcene has been observed in *Pinus* [11, 13, 15, 16].

Variation for volatile terpenoid levels is large in carrot roots. This investigation of $F_1$ hybrids demonstrated a need to consider the quantities of some compounds, e.g. terpinolene and caryophyllene, and the relative percentages of others, e.g. (E)-γ-bisabolene. Dominance for low quantities of most carrot volatile terpenoids was evident in the $F_1$ generation. To determine biosynthetic relationships between volatile terpenoids, genetically segregating generations should be considered.

Table 2. Quantities of major terpenes in the roots of carrot inbreds and their $F_1$ hybrids grown in Florida

| Inbred | Quantity in inbred | Quantity in inbred × 3615 or reciprocal | Quantity in inbred × B6274 or reciprocal |
|---|---|---|---|
| Terpinolene, $LSD_{0.05} = 118$ | | | |
| B493 | 1108*e† | 1203 e | 802 d |
| B3615 | 1914 g | — | 700 cd |
| B4367 | 1426 f | — | 618 c |
| F524 | 710 cd | — | 325 b |
| B6274 | 343 b | 700 cd | — |
| B6439 | 636 c | 712 cd | 316 b |
| B9304 | 169 a | 182 a | — |
| B10138 | 350 b | 768 d | (316 b)‡ |
| Caryophyllene, $LSD_{0.05} = 109$ | | | |
| B493 | 655 e | 590 e | 427 d |
| B3615 | 468 d | — | 125 ab |
| B4367 | 300 c | — | 83 a |
| F524 | 80 a | — | 88 a |
| B6274 | 125 ab | 125 ab | — |
| B6439 | 150 ab | 220 bc | 180 ab |
| B9304 | 170 ab | 143 ab | — |
| B10138 | 177 ab | 181 ab | (234 bc)‡ |
| (*E*)-γ-Bisabolene, $LSD_{0.05} = 71$ | | | |
| B493 | 14 a | 170 de | 137 cd |
| B3615 | 183 de | — | 225 ef |
| B4367 | 255 f | — | 148 cd |
| F524 | 131 cd | — | 136 cd |
| B6274 | 222 ef | 225 ef | — |
| B6439 | 177 de | 289 f | 168 de |
| B9304 | 256 f | 320 f | — |
| B10138 | 80 abc | 100bc | (59 ab)‡ |
| Total volatile terpenoids§, $LSD_{0.05} = 191$ | | | |
| B493 | 1979 e | 2162 e | 1563 d |
| B3615 | 2807 f | — | 1179 bc |
| B4367 | 2105 e | — | 989 b |
| F524 | 1002 b | — | 653 a |
| B6274 | 785 a | 1179 bc | — |
| B6439 | 1020 b | 1294 c | 735 a |
| B9304 | 707 a | 783 a | — |
| B10138 | 686 a | 1117 bc | (598 a)‡ |

*Reported as 1000 × ppm.

†Mean values followed by the same latter are similar by Duncan's multiple range test, 5% level, for each terpene.

‡Quantities for (B10138 × B493) not (B10138 × B6274).

§Sum of all terpenoids listed in Table 1.

Table 3. Percentages of major terpenes in the roots of carrot inbreds and their $F_1$ hybrids grown in Florida*

| Inbred | % In inbred | % In (inbred × B3615) or reciprocal | % In (inbred × B6274) or reciprocal |
|---|---|---|---|
| Terpinolene, $LSD_{0.05} = 9.7$ | | | |
| B493 | 56.0 cd† | 55.6 cd | 54.5 cd |
| B3615 | 67.8 ef | — | 59.4 cde |
| B4367M | 67.7 ef | — | 62.5 def |
| F524 | 71.5 f | — | 49.8 bc |
| B6274 | 43.7 b | 59.4 cde | — |
| B6439 | 62.3 def | 55.0 cd | 49.7 bc |
| B9304 | 23.9 a | 23.2 a | — |
| B10138 | 51.0 bc | 68.8 ef | (52.8 cd)‡ |
| Caryophyllene, $LSD_{0.05} = 7.1$ | | | |
| B493 | 33.1 gh | 27.3 fg | 27.3 fg |
| B3615 | 17.2 bcde | — | 10.6 ab |
| B4367 | 14.3 abc | — | 8.4 a |
| F524 | 8.0 a | — | 13.5 abc |
| B6274 | 15.9 bcd | 10.6 ab | — |
| B6439 | 14.7 abc | 17.0 bcd | 22.8 def |
| B9304 | 24.3 ef | 18.3 cde | — |
| B10138 | 25.5 f | 16.2 bcd | (39.1 h)‡ |
| (*E*)-γ-Bisabolene, $LSD_{0.05} = 7.0$ | | | |
| B493 | 0.7 a | 7.9 bc | 8.8 bc |
| B3615 | 6.5 ab | — | 18.9 de |
| B4367 | 12.1 bcd | — | 15.0 cde |
| F524 | 12.8 bcd | — | 20.8 e |
| B6274 | 28.2 f | 18.9 de | — |
| B6439 | 17.4 de | 22.0 ef | 18.6 de |
| B9304 | 36.2 g | 40.9 g | — |
| B10138 | 11.9 bcd | 9.0 bc | (9.9 bc)‡ |

*Percentage of the summed volatile terpenoids listed in Table 1.

†Mean values followed by the same letter are similar by Duncan's multiple range test, 5% level, for each terpene.

‡Percentage for (B10138 × B493) not (B10138 × B6274).

## EXPERIMENTAL

Eight inbred carrot (*Daucus carota* L.) lines from the USDA carrot breeding program (B493, B3615, B4367, B6439, F524, B6274, B9304, and B10138) and 10 $F_1$ hybrids from these inbreds were grown in commercial carrot fields in Florida, Texas, and California during 1978–1980. These inbred lines have been self-pollinated for 4–8 generations and they represent a broad range of processing and fresh market carrot types. Roots were washed and stored at 5°C until analysis. Collection of carrot root volatiles was made by entraining raw, macerated carrot headspace volatiles over porous polymer (Tenax GC) traps and results obtained by this method were quantitatively precise and were highly correlated with those obtained by distillation [27]. Quantities are reported as 1000 × ppm which is comparable to 30 × ppm by distillation [3, 27]. Analyses were performed on a Varian Model 1840-4 GLC with dual FID and 3 m × 2.4 mm stainless steel columns packed with 5% SF-96 and 0.25% Igepal CO-880, He and $H_2$ flow at 25 ml/min, and injector temp. 190°, $O_2$ at 250 ml/min, detector temp. 230°, temp. programmed 60–200° at 3.8°/min and held at 200° for 12 min. All compounds reported were adequately separated with this GC system except β-pinene and sabinene which contributed less than 1% of the total volatiles. Compound identification was determined by cochromatography with pure compounds on SF-96 and Carbowax 20M columns as described [27].

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