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GENETIC VARIATION FOR VOLATILE TERPENOIDS IN ROOTS OF CARROT, *DAUCUS CAROTA*, BACKCROSSES AND F_2 GENERATIONS

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Abstract—Volatile terpenoid levels were measured in segregating carrot populations. Genetic analysis suggested dominance and multigenic control for low total volatile terpenoids, and low terpinolene quantities. The inheritance of caryophyllene quantities differed in different populations. Percent (*E*)-γ-bisabolene exhibited simple genetic control in several carrot populations with dominance for high levels. Compensatory shifts between the major volatile terpenes indicates an inter-relationship between the mono- and sesquiterpenoid biosynthetic pools.

INTRODUCTION

Carrots (*Daucus carota* L.) display genetic variation for volatile terpenoid quantities [1–3]. Dominance for low total volatile terpenoid quantity was found in F_1 hybrids and genetic control of individual terpene levels has been suggested [3]. This report considers genetic variation for carrot root volatile terpenoids in segregating backcross and F_2 generations.

RESULTS AND DISCUSSION

Dominance was observed for low total volatile terpenoid quantity in backcross and F_2 carrot populations (Table 1) as was noted in F_1 hybrids. A single backcross (BC_1) of (B3615 × B10138) or (B10138 × B493) to B10138 yielded total terpenoid levels indistinguishable from the B10138 parent. Backcrosses to the high parent resulted in levels approximately midway between the F_1 and the high parent. The wide range and high coefficient of variability (CV) of BC_1 and F_2 generations suggest a multigenic system for total volatile terpenoid amount. The hybrid and BC data favor dominance for low levels. All F_2 populations had individual roots with less total terpenoids than the low inbred but no roots with more than the high inbred. The difference between the high and low selection F_2s of B3615 × B10138 grown in California suggests that the carrot inbreds used were not completely homozygous for genes conditioning volatile terpenoid accumulation. This is not surprising, since no selection pressure had been put upon this trait during inbred development. In general the F_2 with more total volatile terpenoids had a smaller range and CV. This supports the idea that recessive alleles condition greater terpenoid accumulation [3] since they must be homozygous to be expressed. High terpenoid plants would be more homozygous and thereby less variable.

Trends for genetic control of individual terpenoid synthesis were usually clearer in the F_1 hybrids when expressed in absolute amount than in percentage of total volatiles [3]. This was also true in segregating generations (Tables 2–4). Dominance for low terpinolene quantity was observed for all segregating populations examined and exemplified by the California grown B3615 × B10138 families (Table 2). Multigenic control is suggested by the broad distribution of backcrosses and F_2s. Percent terpinolene of total volatiles, unlike absolute quantity, presented no clear pattern of inheritance in segregating carrot populations (data not presented).

Dominant multigenic control was observed for low caryophyllene quantity in the B3615 × B10138 (Table 3), B9304 × B3615, and B10138 × B493 populations. Unlike the terpinolene distributions, some F_2 and backcross individuals had caryophyllene quantities greater than the high parent (B3615). A weak trend for bimodality in the frequency distribution of B9304 × B3615 F_2s and backcrosses was noted. This may suggest relatively few genes controlling caryophyllene quantity. The B493 × B6274 family was unlike the others since it exhibited dominance with multigenic control for high caryophyllene quantity (Table 4). Since the B10138 × B493 populations did not indicate this trend, but B493 × B6274 did, B6274 must contribute to this reversed situation. As for terpinolene, no trends were noted for the distribution of caryophyllene percentages in segregating populations. (*E*)-γ-Bisabolene distributions were of two types. In B3615 × B10138 and B9304 × B3615 families, there was dominance for low (*E*)-γ-bisabolene quantity with large variation but some bimodality in F_2 populations (Table 5). However, there was dominance for high (*E*)-γ-bisabolene percentage in these families (Table 6). The second type of (*E*)-γ-bisabolene distribution was found in B493 × B6274 and B10138 × B6274 families where intermediate to high amounts and percentages (Table 7) were dominant. Bimodality was evident in backcross and F_2 populations and there was good indication that one dominant gene (from B6274) could condition high percent (*E*)-γ-

Table 1. Total volatile terpenoid levels in carrot inbreds, hybrids, and segregating generations

Line	Generation	Location	Mean	Range	CV
B10138	Inbred	California	685*	509–819	5†
B10138 × (B3615 × B10138)	Backcross		664	551–891	25
B3615 × B10138	F_1		773	651–905	13
B3615 × (B3615 × B10138)	Backcross		1237	1023–1441	18
B3615	Inbred		1681	1450–1880	10
B3615 × B10138	F_2‡		856	503–1368	29
B3615 × B10138	F_2§		681	273–1670	51
B10138	Inbred	Wisconsin	574	484–617	8
B3615 × B10138	F_1		928	843–996	9
B3615	Inbred		1852	1526–1970	11
B3615 × B10138	F_2§		610	342–1178	40
B9304	Inbred	Wisconsin	707	596–750	11
B9304 × B3615	F_1		783	508–825	14
B3615	Inbred		1852	1526–1970	11
B3615 × B9304	F_2		684	515–1014	26
B10138	Inbred	Wisconsin	574	484–617	8
B10138 × (B10138 × B493)	Backcross		607	501–743	15
B10138 × B493	F_1		699	575–784	13
B493 × (B10138 × B493)	Backcross		973	827–1566	30
B493	Inbred		1190	1018–1453	18
B6274	Inbred	Wisconsin	678	606–721	5
B493 × B6274	F_1		942	713–1072	12
B493 × (B493 × B6274)	Backcross		1112	936–1196	11
B493	Inbred		1190	1018–1453	18
B493 × B6274	F_2		726	522–957	29

*Reported as 1000 × ppm.

†Coefficient of variability = 100 $s/\bar{x}$.

‡F_2 from self-pollination of F_1 with terpenoid level in upper 20% of population.

§F_2 from self-pollination of F_1 with terpenoid level in lower 50% of population.

bisabolene. There was a 1:1 distribution in the backcross to B493 (5 high roots:5 low) and close to a 3:1 distribution in the F_2 (19 high roots:8 low; $\chi^2 = 0.31$, $P = 0.6$).

Besides terpinolene, caryophyllene, and (*E*)-γ-bisabolene, most volatile terpenoids in the carrot populations studied were in low quantity. Exceptions included roots high in myrcene in B3615 × (B3615 × B10138) (one root, myrcene accounted for 12% total terpenoids) and the (B493 × B6274) F_2 (six roots, myrcene accounted for 10–19% total terpenoids), high in γ-terpinene in the (B3615 × B10138) F_2s (eight roots, γ-terpinene accounted for 5–10% total terpenoids), and high in an unidentified compound with a retention time between caryophyllene and (*Z*)-γ-bisabolene in the (B3615 × B10138) F_2s (two roots, this compound accounted for 3–13% total terpenoids).

Reduction in the percentage of one monoterpene can be compensated with the increase of another in Coniferae [4]. Trends for such substitutional co-occurrence were considered in these segregating carrot populations. Clear compensatory changes were noted in approximately half of the roots with unusually large or small terpinolene, caryophyllene, (*E*)-γ-bisabolene and myrcene percentages. Nearly all of the compensatory changes were between terpinolene and either caryophyllene, (*E*)-γ-bisabolene, or both of these sesquiterpenes (data not presented). Thus roots with low percentage terpinolene usually had high percentage caryophyllene and/or (*E*)-γ-bisabolene, and vice versa. Only infrequently (5% of the roots) was substitutional co-occurrence between the two most plentiful sesquiterpenes with no involvement of terpinolene noted. Similarly, the six roots with high percentage myrcene had high caryophyllene and low terpinolene percentages. Compensation involving only monoterpenes or only sesquiterpenes would be expected if these two groups of compounds were in separate biosynthetic pools as was indicated for *Mentha piperita* and *Poncirus trifoliata* [5–7]. Since this was not observed, mono- and sesquiterpenoid synthesis may not be anatomically distinct in carrot roots [8], as has also been suggested in *Pogostemon cablin* [9].

EXPERIMENTAL

Segregating carrot populations included five backcrosses [B10138 × (B3615 × B10138), B3615 × (B3615 × B10138), B10138 × (B493 × B10138), B493 × (B493 × B10138) and

Table 2. Frequency distribution of terpinolene quantities in B3615 × B10138 carrot populations

	Terpinolene quantity*													
	0	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300
B10138	—	—	—	5†	6	—	—	—	—	—	—	—	—	—
B3615	—	—	—	—	—	—	—	—	—	—	5	7	2	—
B3615 × B10138	—	—	—	1	5	3	2	—	—	—	—	—	—	—
B10138 × (B3615 × B10138)	—	—	2	4	6	3	—	—	—	—	—	—	—	—
B3615 × (B3615 × B10138)	—	—	—	—	—	—	3	3	7	1	2	—	—	—
(B3615 × B10138) F_2‡	—	—	—	5	4	3	6	8	0	2	1	—	—	—
(B3615 × B10138) F_2§	—	4	3	12	9	4	2	0	1	—	—	—	—	—

*Reported as 1000 × ppm.
†Number of roots.
‡F_2 from self-pollination of F_1 with terpenoid level in upper 20% of population.
§F_2 from self-pollination of F_1 with terpenoid level in lower 50% of population.

Table 3. *Frequency-distribution of caryophyllene quantities in B3615 × B10138 carrot populations*

	Caryophyllene quantity*												
	0	25	50	75	100	125	150	175	200	225	250	275	300
B10138	—	—	—	1†	6	4	—	—	—	—	—	—	—
B3615	—	—	—	—	—	—	—	—	8	5	1	—	—
B3615 × B10138	—	—	—	—	—	3	5	2	1	—	—	—	—
B10138 × (B3615 × B10138)	—	—	—	4	9	2	—	—	—	—	—	—	—
B3615 × (B3615 × B10138)	—	—	—	—	2	3	5	3	2	0	0	1	—
(B3615 × B10138) F_2‡	—	1	2	2	6	1	8	1	4	2	0	2	—
(B3615 × B10138) F_2§	—	1	2	4	8	9	3	3	3	1	0	1	—

*Reported as 1000 × ppm.
†Number of roots.
‡F_2 from self-pollination of F_1 with terpenoid level in upper 20% of population.
§F_2 from self-pollination of F_1 with terpenoid level in lower 50% of population.

Table 4. Frequency distribution of caryophyllene quantities in B493 × B6274 carrot populations

	Caryophyllene quantity*											
	0	50	100	150	200	250	300	350	400	450	500	550
B6274	—	4†	5	—	—	—	—	—	—	—	—	—
B493	—	—	—	—	—	—	—	—	3	6	—	—
B493 × B6274	—	—	—	—	—	—	—	—	4	3	1	—
B493 × (B493 × B6274)	—	—	—	—	—	—	—	2	1	5	2	—
(B493 × B6274) F_2	—	—	—	—	1	5	6	7	1	5	1	1

*Reported as 1000 × ppm.
†Number of roots.

Table 5. Frequency distribution of (*E*)-γ-bisabolene quantities in B3615 × B10138 carrot populations

	(*E*)-γ-Bisabolene quantity*														
	0	25	50	75	100	125	150	175	200	225	250	275	300	325	350
B10138	—	—	5†	6	—	—	—	—	—	—	—	—	—	—	—
B3615	—	—	—	—	—	4	6	4	—	—	—	—	—	—	—
B3615 × B10138	—	1	4	2	4	—	—	—	—	—	—	—	—	—	—
B10138 × (B3615 × B10138)	—	3	6	4	2	—	—	—	—	—	—	—	—	—	—
B3615 × (B3615 × B10138)	—	2	1	3	2	2	5	1	—	—	—	—	—	—	—
(B3615 × B10138) F_2‡	—	1	9	2	4	4	5	2	2	—	—	—	—	—	—
(B3615 × B10138) F_2§	—	2	1	3	6	2	4	7	4	4	2	—	—	—	—

*Reported as 1000 × ppm.
†Number of roots.
‡F_2 from self-pollination of F_1 with terpenoid level in upper 20% of population.
§F_2 from self-pollination of F_1 with terpenoid level in lower 50% of population.

Table 6. Frequency distribution of (*E*)-γ-bisabolene percentages in B3615 × B10138 carrot populations

	% (*E*)-γ-Bisabolene										
	0	5	10	15	20	25	30	35	40	45	50
B10138	—	—	8*	3	—	—	—	—	—	—	—
B3615	—	8	6	—	—	—	—	—	—	—	—
B3615 × B10138	—	3	4	4	—	—	—	—	—	—	—
B10138 × (B3615 × B10138)	—	7	5	1	2	—	—	—	—	—	—
B3615 × (B3615 × B10138)	1	4	7	3	1	—	—	—	—	—	—
(B3615 × B10138) F_2†	1	8	7	5	8	—	—	—	—	—	—
(B3615 × B10138) F_2‡	—	2	6	6	7	3	4	7	—	—	—

*Number of roots.
†F_2 from self-pollination of F_1 with terpenoid level in upper 20% of population.
‡F_2 from self-pollination of F_1 with terpenoid level in lower 50% of population.

Table 7. Frequency distribution of (*E*)-γ-bisabolene percentages in B493 × B6274 carrot populations

	% (*E*)-γ-Bisabolene							
	0	5	10	15	20	25	30	35
B6274	—	—	—	—	9*	—	—	—
B493	2	7	—	—	—	—	—	—
B493 × B6274	—	—	4	4	—	—	—	—
B493 × (B493 × B6274)	—	1	4	0	3	2	—	—
(B493 × B6274) F_2	4	4	0	7	9	3	—	—

*Number of roots.

B493 × (B493 × B6274)] and four F_2 generations [(B9034 × B3615F_2), (B493 × B6274F_2), and two F_2s from (B3615 × B10138)]. These populations and their respective parents and F_1 hybrids were grown in commercial carrot fields in California and Wisconsin during 1979–1980. Roots were washed and stored at 5° until analysis.

Collection of raw carrot root volatiles was made by entrainment of headspace onto porous polymer (Tenax GC) traps and analyses were performed on a Varian Model 1840-4 GC 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 [3, 8].

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