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## Estimation of heritabilities and gene number in the production of provitamin A in carrots.

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### ABSTRACT

Broad sense heritabilities and estimates of the number of factors or genes involved in the production of phytoene,  $\zeta$ -carotene,  $\beta$ -carotene,  $\alpha$ -carotene, lycopene and total carotenoids were estimated in two different crosses of carrot (*Daucus carota* L.) with different backgrounds. F<sub>2</sub> plants segregating for absence of  $\alpha$ -carotene were identified in the cross B493 x QAL. HPLC chromatograms revealed that most of the increase of total carotenoids in high intensity mass selection (HCM), cycle 13, was due to increase of  $\alpha$ -carotene content than  $\beta$ -carotene. Broad sense heritabilities values were around or greater than 50% for  $\beta$ -carotene,  $\zeta$ -carotene, lycopene, phytoene and total carotenoid and around 35% for  $\alpha$ -carotene in the cross Brasilia x HCM. In the cross orange x white the heritabilities were around or greater than 90%. The estimated number of factors was 4 for  $\alpha$ -carotene, 3 for  $\beta$ -carotene and total carotenes and one for  $\zeta$ -carotene, lycopene and phytoene in the orange x dark orange cross, and 4 for  $\alpha$ -carotene, 1-2 for lycopene and total carotenes and 1 for the other carotenes in the orange x white cross.

**Keywords:** *Daucus carota*, HPLC, carotenes.

### RESUMO

#### Estimativas de herdabilidades e do número de genes envolvidos na produção de pró-vitamina A em cenoura.

Herdabilidades e estimativas do número mínimo de fatores ou genes envolvidos na produção de fitoeno,  $\zeta$ -caroteno,  $\beta$ -caroteno,  $\alpha$ -caroteno, licopeno e carotenóides totais foram estimados em dois cruzamentos de cenoura (*Daucus carota* L.). Plantas F<sub>2</sub> segregando para ausência de  $\alpha$ -caroteno foram identificadas no cruzamento B493 (cenoura laranja) x QAL (cenoura branca). Cromatogramas de HPLC revelaram que muito do aumento de carotenóides totais na população HCM (alto teor de carotenóides), 13<sup>o</sup> ciclo de seleção, foi devido ao aumento de  $\alpha$ -caroteno do que  $\beta$ -caroteno. Valores de herdabilidades no

sentido amplo foram em torno ou maior do que 50% para  $\beta$ -caroteno,  $\zeta$ -caroteno, licopeno, phitoeno e carotenóides totais e em torno de 35% para  $\alpha$ -caroteno no cruzamento Brasília (cenoura laranja) x HCM (cenoura laranja escura). No cruzamento cenoura laranja x cenoura branca os valores de herdabilidades foram em torno de 90%. O número mínimo de fatores ou genes estimados foi de quatro para  $\alpha$ -caroteno, três para  $\beta$ -caroteno e carotenóides totais e um para  $\zeta$ -caroteno, licopeno e phitoeno no cruzamento cenoura laranja x cenoura laranja escura, e de quatro para for  $\alpha$ -caroteno, um a dois para licopeno e carotenóides totais e um para os outros carotenos no cruzamento cenoura laranja x cenoura branca.

**Palavras chaves:** *Daucus carota*, HPLC, carotenos.

Vitamin A deficiency is widespread in developing countries, but also is found in poor urban populations of developed countries, among the elderly, heavy drinkers or smokers (Giuliano et al. 2000). Consumption of horticultural crops provide more than 70% of vitamin A for the World, with carrots accounting for 30% of the total vitamin A precursor in countries like the United States (Simon, 1992). Heritabilities and estimates of the number of genes involved in the production of phytoene, lycopene,  $\zeta$ -carotene,  $\beta$ -carotene,  $\alpha$ -carotene and total carotenoids are presented in two different crosses of carrot, with very distinct backgrounds. They are important estimations to relate with previous inheritance studies, to understand the evolution of this pathway and to compare with estimations from quantitative trait loci studies.

## **MATERIAL AND METHODS**

The experimental populations were developed from two different crosses between B493 x Queen Anne's Lace (QAL) and Brasilia x High intensity Mass Carotene (HCM) that have different color and carotenoid background.

Quantification of total carotene was performed as described by Simon and Wolff (1987). An estimate of "total carotenoid" concentration was obtained by comparing mean sample absorbance at 450 nm of each individual sample to a standard curve of  $\beta$ -carotene. Quantification of major carotenoids by high-performance liquid chromatography (HPLC) was performed with a using a Waters 6000A pump, a Waters WISP 710B autosampler, and a Waters 996 Photodiode Array Detector (Waters Corporation, 1994). Separations were performed using the system described by Khachik et al. (1994). Synthetic  $\beta$ -carotene (Sigma C-9750) was used as a reference standard in separate sampling.

Means, variances, standard error of means and minimum and maximum values observed within parental lines,  $F_1$  and  $F_2$  populations for all carotenoids products were estimated using the procedure Univariate (SAS, 1989). Estimates of the effective or minimum number of genes influencing all carotenoids products were determined by the methods of first

moments or also called Sewall Wright's estimator by applying the generalization presented by Lande (1981). Two estimates were obtained from the available data:  $\sigma_S^2 = \sigma_{F_2}^2 - \sigma_{F_1}^2$  (estimate 1) and  $\sigma_S^2 = \sigma_{F_2}^2 - [\frac{1}{2}\sigma_{F_1}^2 + \frac{1}{4}\sigma_{P_1}^2 + \frac{1}{4}\sigma_{P_2}^2]$  (estimate 2). An estimate of heritability for all carotenoids was calculated with  $h^2 = (\sigma_{F_2}^2 - \sigma_{F_1}^2) / \sigma_{F_2}^2$  (Burton, 1951).

## RESULTS AND DISCUSSION

The retention time in the chromatograms associated with specific carotenes in this analysis is consistent with published results by Simon and Wolff (1987), Khachik et al. (1994) and Ye et al. (2000).

F<sub>2</sub> plants segregating for absence of  $\alpha$ -carotene peak were observed in the cross B493 x QAL F<sub>2</sub>. Development of lines free of  $\alpha$ -carotene will be useful not only for genetics studies, but also to improve the provitamin A content, since, according Sharman (1983) and van der Berg et al. (2000),  $\alpha$ -carotene has lesser provitamin A activity than  $\beta$ -carotene.

Heritability values were around 50% for  $\zeta$ -carotene,  $\beta$ -carotene and total carotenoids, moderate values for lycopene, high values for phytoene and around 40% for  $\alpha$ -carotene in the cross Brasilia x HCM. The heritability values were around or greater than 90% for all characters in the cross B493 x QAL (Table 1). The estimation of the number of loci showed that characters  $\alpha$ -carotene,  $\beta$ -carotene and total carotenoid presented more genes than the other characters, in the Brasilia x HCM cross. In the B493 x QAL cross, except for  $\alpha$ -carotene and total carotenoid, all other characters showed a minimum number of genes around one (Table 1). All standard errors of minimum number of factors (Table 1) did not exceed the estimate of minimum of factors, indicating that inferences and conclusions are possible. There are evidences for two modes of inheritance of carotenoids products in carrots: discrete in a cross of orange x white and continuous in a cross of orange x dark orange.

Table 1 Estimation of broad sense heritability and minimum number of genes or factors, with standard errors of estimates, controlling the inheritance of  $\zeta$ -carotene,  $\alpha$ -carotene,  $\beta$ -carotene, phytoene, lycopene and total carotenoids (total caro) in two F<sub>2</sub> populations of carrot

Character	Brasília x HCM				B493 x QAL			
	Heritability		Number of Loci and standard error		Heritability		Number of Loci and standard error	
	1	2	1	2	1	2	1	2
$\zeta$ -carotene	0.46	0.48	0.17 ± 0.1	0.18 ± 0.1	0.92	0.98	1.13 ± 0.2	1.05 ± 0.2
$\alpha$ -carotene	0.32	0.36	4.00 ± 3.2	3.50 ± 3.0	0.83	0.95	3.93 ± 0.8	3.47 ± 0.7
$\beta$ -carotene	0.28	0.42	1.67 ± 1.3	2.52 ± 2.3	0.97	0.99	1.12 ± 0.2	1.10 ± 0.2
Phytoene	0.53	0.89	0.22 ± 0.2	0.60 ± 0.4	0.97	0.99	1.12 ± 0.2	1.10 ± 0.2
Lycopene	0.44	0.66	0.36 ± 0.2	0.53 ± 0.4	0.51	0.88	1.83 ± 0.4	1.27 ± 0.3
Total caro	0.38	0.45	2.92 ± 2.0	3.45 ± 2.4	0.98	0.89	1.68 ± 0.3	0.90 ± 0.2

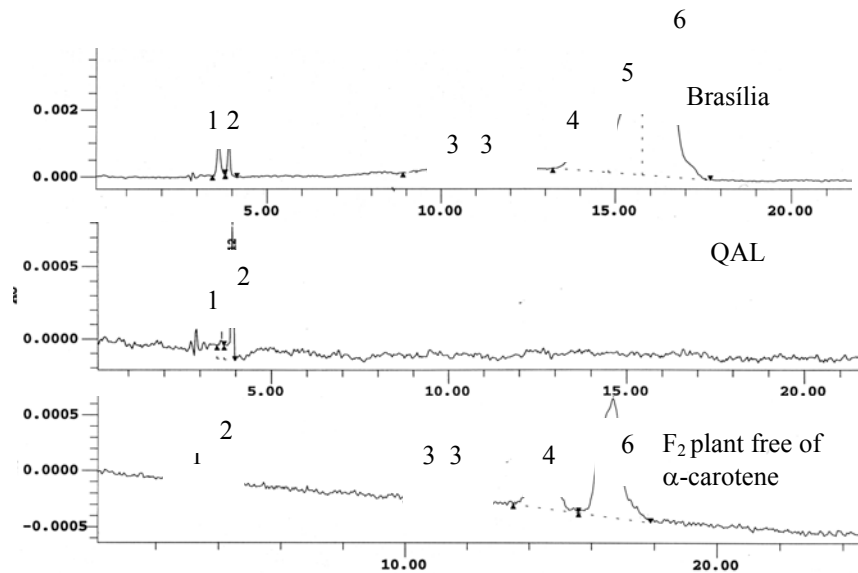


Figure 1. Representative HPLC chromatograms from roots of a typical orange (Brasília) and white (QAL) carrot at 404 nm absorbance. Peaks correspond to (1) lutein or zeaxanthin, (2) hexane noise, (3), lycopene, (4)  $\zeta$ -carotene, (5)  $\alpha$ -carotene, (6)  $\beta$ -carotene and (7) phytoene. Bottom panel is a F<sub>2</sub> plant segregating for absence of  $\alpha$ -carotene in the cross B493 x QAL.

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