replications required for min cost decreases. For instance, if a C.V. of approx 11% is desired in measuring wt of shelled fresh peas per plant, then 11 plants are required from each of 5 replicates. The same precision could be obtained by sampling 17 plants from each of only 4 replicates.

Table 4 shows the sample size and number of replications required to obtain C.V.'s of approx 13, 10 and 8% for wt of shelled fresh peas, with a cost ratio of 18:1. The C.V.'s obtained for the other characters with these replication and sample size combinations are also shown.

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Inheritance of Root Color and Carotenoid Synthesis in Carrot, *Daucus carota*, L.: Orange vs. Red[1]

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Abstract. The genetic control of root color and carotenoid synthesis in carrot, *Daucus carota* L., was studied using 3 carrot cultivars, Kintoki Heian Nagabuto, KHN: Kintoki Osaka, KOS: Kintoki Davis, KDA, and 1 inbred line (W93). Genetic models describing the inheritance of red roots in the F_2 were tested in backcross and F_3 progenies. In Kintoki cultivars the major pigment is lycopene; beta-carotene is present in smaller amounts; zeta-carotene, gamma-carotene and phytofluene were also detected. In W93 the main pigments are beta-carotene and alpha-carotene; zeta-carotene, gamma-carotene and phytofluene also are detected. The pigments were separated into carotene and carotenol fractions by partition column chromatography. The pigments in the carotene fraction were studied qualitatively and quantitatively by thin layer chromatography. Orange (W93) was dominant to red (KHN, KOS, KDA) in the F_1 progeny. The F_2 segregation indicated that at least 2 genes are responsible for the differences between orange and red. The segregation of F_3, backcrosses, and other progenies revealed the existence of dominant red as well as dominant orange, supporting the digenic composition of F_2 populations and indicating the locus with the dominant orange allele to be epistatic to the locus with the dominant red allele. The homozygous recessive would be orange also. The analysis of progenies from the crosses W93 x 'Kintoki' suggested a dominant gene for accumulation of alpha-carotene in W93 and a dominant gene for the accumulation of lycopene in 'Kintoki'.

The wide diversity of root color in carrot was reviewed and discussed previously (1, 4, 6, 15).[1,3,4] Most of the colors (whites, yellows, oranges, and reds) are a result of carotenoid pigments present in the root. In some yellow and purple cultivars the root color is due to anthochlor and anthocyanin (1). The work reported herewith is limited to carotenoid pigments only.

The carotenoids (C_{40}) can be divided into hydrocarbon pigments or carotenes, and carotenols (xanthophylls) which also contain oxygen in the molecule. In commercial carrot cultivars (orange root) about 95% of the total carotenoids are carotenes, mainly beta-carotene and alpha-carotene. Other reported carotenes include phytoene (4), phytofluene (4, 13), zeta-carotene (3, 12), lycopene (3, 4, 12), gamma-carotene (3, 4, 9, 11, 12), and delta-carotene (11). The red rooted carrot 'Kintoki', contains 150-270 μg/g total carotenoids of which about 3% are carotenols. The major pigment in this cultivar is lycopene; beta-carotene is present in smaller amounts; zeta-carotene, gamma-carotene, phytofluene and alpha-carotene also are detected (5, 12, 15).

A biosynthesis pathway for carotenoids was proposed by Porter and Lincoln (8) in 1950, based on genetic studies in tomato and molecular structure of the pigments. Since 1950 changes in Porter and Lincoln's scheme were found necessary (7, 13, 14). The linear part of the pathway from phytoene to lycopene, involving acyclic carotenes is generally agreed upon. Each step represents a stepwise dehydrogenation with removal of 2 hydrogen atoms and formation of a double bond. An acceptable pathway by which cyclic carotenoids are formed is less agreed upon (7, 8, 13, 14). In carrot the conversion of lycopene to beta-carotene by leaf chloroplasts has been demonstrated (2). Williams et al. (14) have ruled out the possible conversion of beta-carotene into alpha-carotene in orange roots.

Early attempts to study the inheritance of root color in carrot did not go beyond establishing color dominancy in the F_1 generation (for review see LaFerriere and Gabelman, ref. 6). They (6) reported the first extensive studies on the inheritance of root color in carrot, and demonstrated the complexity of the problem. Their crosses of 'White-Belgian' x 'Yellow-Belgian' suggested monogenic differences with white dominant to yellow. In progenies of the crosses between white and orange, and also between yellow and orange they noted new root

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[2]Department of Horticulture.

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[5]Takii, J. 1969. Private communication. Present address: Takii & Co., Ltd. Seed & Bulb Growers, Kyoto, Japan.

Table 1. Root colors detected in various progenies from an original cross between orange and red carrots, W 93 x 'KHN'.

Generation	No. of progenies	Colors of parents	Color classes		Chi square test			
			Orange	Red	1:1	3:1	13:3	15:1
F_1	5	Or. x Red	417	0				
F_2A	11[z]	Or.	1331	271		55.83**[x]	3.45ns	311.88**
F_2B	1[y]	Or.	136	82		18.50**	50.50**	365.00**
F_1 x KHN	3	Or. x Red	270	364	13.94**			
F_1 x (KDA x KHN)	5	Or. x Red	276	339	6.45*			
F_1 x W93	7	Or. x Or.	364	0				
F_3 (F_2A ⊗)	5	Or.	215	0				
F_3 (F_2A ⊗)	7	Or.	207	95				
F_3 (F_2A ⊗)	6	Red	0	80				
F_3 (F_2A ⊗)	5	Red	92	84				
F_2A x KHN	3	Red x Red	0	308				
F_2A x KHN	1	Or. x Red	17	33				
F_2A x KHN	1	Or. x Red	0	20				
(W93 x F_1) x KHN	8	Or. x Red	186	137				
(W93 x F_1) x KHN	2	Or. x Red	119	0				
[(F_1 x KHN) x KHN] ⊗	1	Or.	14	7				
[(F_1 x KHN) x KHN] ⊗	1	Red	15	46				
[(F_1 x KHN) x KHN] ⊗	1	Red	0	21				

[z]Homogeneity x^2 17.19ns.

[y]No F_3 or back-cross progenies were obtained from this line.

[x]Chi square significant at 5%; **, at 1%.

colors: orange tinge, intermediate-orange, and light-orange. White was dominant to yellow, orange-tinge, intermediate-orange, and orange; and at least 3 major genes determined the differences between white and orange. Evidence was found for both dominance of yellow and no dominance, at least 2 major genes between yellow and orange in 1 case, and at least 4 genes in another. They found evidence for the independent inheritance of phloem and xylem color (6).

Working with less heterozygous lines, Imam and Gabelman (4) revealed a 1 gene difference between lemon and light-orange, with lemon dominant. In other crosses, a 1 gene difference was found between light-orange and orange, with light-orange dominant.

Recently, Kust[3] looked more closely at the inheritance of root color in crosses of white and yellow carrots with the inbred orange line W93. Roots were classified visually into 24 distinct color groups on the basis of both phloem and xylem colors. Seven of the color groups were "non-tinge" (uniformly colored) and 17 color groups were "tinge;" i.e., the xylem has a different color than the phloem. Based on this classification, they were able to overcome earlier difficulties (6), and postulated a 5 gene system that accounts for the segregation of roots in progenies resulting from hybrids of white (or yellow) rooted carrots on the orange rooted carrot W93. Each of the dominant alleles of 3 genes (Y, Y_1, Y_2) prevented the formation of orange color in the xylem, while only the dominant allele Y prevented the formation of orange color in the phloem. The orange genes *(IO, O)* were responsible for the intensity of orange color that develops in the absence of the dominant alleles of Y, Y_1, and Y_2.

The red carrot 'Kintoki', was reported to be recessive when crossed to orange cultivars (5)[5].

Our objectives were to study the inheritance of color and carotenoid pigments unique to the Japanese red carrot cv. Kintoki in crosses with an inbred orange carrot W93.

Material and Methods

Plant material:

The present study employed 3 red rooted commercial carrot cultivars; Kintoki Osaka (KOS), Kintoki Heian Nagabuto (KHN), and an unnamed Kintoki cultivar (KDA). The inbred carrot line W93 was used as the orange parent. This inbred may be designated either as W93A, which is the cytoplasmic male sterile, or W93B which is the male fertile maintainer for W93A.

Roots from 'KDA' were red. 'KDA' was used in some crosses but the parent line was lost later due to failure to produce seed

Table 2. Root colors detected in various progenies from an original cross between orange and red carrots, W93 x 'KOS'.

Generation	No. of progenies	Colors of parents	Color classes		Chi square test			
			Orange	Red	1:1	3:1	13:3	15:1
F_1	7	Or. x Red	1160	0				
F_2A	5[z]	Or.	665	206		0.88ns[v]	13.96**	456.13**
F_2B	2[y]	Or.	180	26		16.84**	5.34*	13.88**
F_2C	3[x]	Or.	542	48		89.49**	44.04**	3.49ns
F_1 x KOS	1[w]	Or. x Red	95	118	2.48ns			
F_1 x (KDA x KOS)	3[w]	Or. x Red	85	109	2.97ns			
F_3	22	Or.	785	0				
F_3 (F_2A ⊗)	9	Or.	291	106				
F_3	6	Red	0	221				
F_3	1	Red	41	0				
(F_1 x KOS) ⊗	2	Red	8	56				
(F_1 x KOS) ⊗	2	Red	0	7				
F_2A x KOS	2	Or. x Red	0	64				
F_2A x KOS	2	Red x Red	0	8				
F_2A x W93	1	Or. x Or.	115	0				

[z]Homogeneity x^2 1.66ns.

[y]Homogeneity x^2 0.18ns.

[x]Homogeneity x^2 4.86ns.

[w]The same F_1 progeny was used to produce F_2B

[v]*, significant at 5%; **, significant at 1%; ns, nonsignificant

Table 3. Mean values and standard deviations for carotenoid analysis of carrot roots of the parents and profenies from the cross W93 x 'KHN'. Carotenoid concn in the root is expressed as μg per g fresh wt (ppm).

Line	% dry wt	Total carotenoids	Fraction no. 1	Fraction no. 2	Fraction no. 3	No. of plants analyzed
W93A3	10.7± 0.2	110.2± 9.1	98.5± 10.1	3.16± 0.22	0.386±0.053	5
W93-4-2M-CM-2	9.9± 0.2	89.8±11.3	79.8± 10.1	2.70± 0.30	0.349±0.129	4
KHN	9.9± 0.7	68.8±21.9	60.1± 22.1	4.90± 1.25	0.251±0.036	3
KHN-1	13.8± 1.2	91.7±33.5	79.7± 31.8	6.81± 2.57	0.918±0.536	4
W93A2 x KHN	12.4± 0.3	110.1± 8.9	97.2± 6.0	8;38± 0.71	0.401±0.093	5
W93 x KHN	11.9± 0.3	55.4± 3.9	49;2± 4.0	6.12± 0.59	0.155±0.016	5
(W93A2 x KHN) - 1	11.5± 0.2	84.7± 6.6	68.9± 5.4	6.44± 0.66	0.157±0.031	26
(W93A2 x KHN) - 1	12.5± 0.3	66.9± 6.2	56.0± 5.6	5.35± 0.80	0.139±0.019	17
(W93B x KHN) - 1	11.0± 0.4	62.8± 8.1	54.8± 7.8	5.69± 0.82	0.112±0.015	13
(W93A2 x KHN) x KHN - 1	11.1± 0.1	43.7± 2.8	38.2± 2.5	3.40± 0.25	0.307±0.028	19
W93A3 x (W93B x KHN)	10.6± 0.2	65.4± 4.8	57.8± 4.9	3.86± 0.16	0.203±0.029	18

on selfing. The roots of 'KOS' and 'KHN' were red and produced only red progenies upon self-pollination. However, differences in color intensity were noted in these progenies, with more variability in 'KOS'. Roots from the orange inbred line (W93A, W93B) had a relatively uniform orange color and produced orange progeny when sib-mated or self-pollinated.

Carrot plants were grown on a peat soil at Montello, Wisconsin from seed sown in early June. Roots were harvested in early September and stored at 40°F until classification for color. Plants to be grown for seed production were classified for root color a day or 2 before planting, usually during November and early December and a portion of the root retained for pigment analysis. The technique for pollination followed Laferriere and Gabelman (6). Most crosses were made utilizing male sterility in the seed parent; however, in some cases flowers were emasculated when both parents were male fertile.

Classification for root color:

Each root was washed, dried, bisected transversely, and the sections were rated visually for color under fluorescent light in the laboratory. Parental lines of different root colors were used as standards for comparison.

Roots of light-orange, orange, dark-orange, red-orange, and red were observed in segregating progenies. In most cases, visual classification of roots was relatively easy, but in some lines variations were continuous from light-orange to red and were difficult to separate precisely. Red roots with a small zone of orange tissue around the cambium were classified as red. Roots with red phloem, or with zones of red tissue in the phloem but with orange xylem, and roots with orange phloem and red xylem, were all classified as red-orange. Some differences in color between phloem and xylem were noted in other color groups. Some difficulties were encountered in distinguishing between dark-orange and red-orange.

Analytical methods:

The analytical procedures were described recently (12). Parental lines and progenies from the crosses W93 x KHN and

Table 4. Mean values and standard deviations for pigments present in fraction 1 of carrot roots of the parents and progenies from the cross W93 x 'KHN'. Units of measure are the number of spots detected visually for each pigment on TLC plate.

Line	Lycopene	γ carotene	ζ carotene	β carotene	α carotene	Pyto-fluence	No. of roots analyzed
W93A	2.2±0.2	1.8±0.2	3.0±0.3	6.0±0.0	4.6±0.2	4.2±0.6	5
W93-4-2M-CM-2	2.5±0.3	2.7±0.2	3.0±0.0	6.0±0.0	4.7±0.2	4.7±0.2	4
KHN	9.0±0.0	2.6±0.4	1.6±0.4	5.3±0.4	0.0±0.0	3.6±0.4	3
KHN-1	9.0±0.0	2.0±0.6	2.0±0.0	5.0±0.4	0.0±0.0	4.5±0.5	4
W93A2 x KHN	5.6±0.2	2.8±0.2	2.0±0.0	6.0±0.0	4.2±0.2	4.0±0.0	5
W93B x KHN	6.6±0.2	3.0±0.0	2.8±0.2	6.0±0.0	3.6±0.2	4.6±0.2	5
(W93A2 x KHN) - 1	5.5±0.3	3.0±0.1	3.3±0.1	6.1±0.0	3.1±0.2	4.8±0.1	26
(W93A2 x KHN) - 1	6.7±0.4	2.5±0.1	3.2±0.2	6.1±0.1	2.1±0.3	4.8±0.2	17
(W93B x KHN) - 1	6.0±0.5	2.6±0.2	3.6±0.1	6.0±0.0	2.1±0.4	4.8±0.3	13
(W93A2 x KHN) x KHN - 1	7.5±0.2	2.8±0.0	1.8±0.1	5.7±0.1	0.2±0.1	4.3±0.1	19
W93A3 x (W93B x KHN)	3.3±0.2	2.3±0.1	3.1±0.2	6.2±0.1	3.0±0.4	4.3±0.1	18

Table 5. Mean values and standard deviations for carotenoid analysis of carrot roots of the parents and progenies from the cross W93 x 'KOS'. Carotenoids concn in the root is expressed as mg. per g fresh wt (ppm).

Line	% Dry wt	Total carotenoids	Fraction no. 1	Fraction no. 2	Fraction no. 3	No. of plants analyzed
W93A3	10.7± 0.2	110.2± 9.1	98.5±10.1	3.16± 0.22	0.386±0.053	5
W93-4-2M-CM-2	9.9± 0.2	89.8±11.3	79.8±10.1	2.70± 0.30	0.349±0.129	4
KOS-1	12.8± 0.7	67.4± 9.3	59.4± 8.4	3.56± 0.79	0.184±0.023	5
W93A2 x KOS	12.2± 0.5	—	53.9± 1.3	5.55± 0.60	0.083±0.023	5
(W93A2 x KOS) - 1	13.3± 0.3	130.5±14.8	109.7±12.8	9.61± 0.87	0.175±0.023	15
(W93A2 x KOS) - 1	11.2± 0.3	57.9± 4.8	48.5± 4.2	5.10± 0.68	0.098±0.015	18
(W93A2 x KOS) - 1	—	70.3± 3.4	59.4± 3.2	5.48± 0.28	0.214±0.028	88
W93A3 x (W93A2 x KOS)	10.6± 0.5	65.4±10.3	61.0±10.4	3.69± 0.45	0.137±0.018	9
W93A3 x (W93A2 x KOS)	11.7± 0.2	106.7±20.5	94.7±20.8	9.18± 0.81	0.314±0.055	10
(W93A2 x KOS x KOS - 1	13.3± 0.6	38.9± 2.0	31.7± 1.6	4.29± 0.41	0.167±0.018	10

Table 6. Mean values and standard deviations for pigments present in fraction 1 of carrot roots the parents and progenies from the cross W93 x 'KOS'. Units of measure are the number of spots detected visually for each pigment on TLC plate.

Line	Lycopene	γ carotene	ζ carotene	β carotene	α carotene	Phyto-fluence	No. of plants analyzed
W93A3	2.2±0.2	1.8±0.2	3.0±0.3	6.0±0.0	4.6±0.2	4.2±0.6	5
W93-4-2M-CM-2	2.5±0.3	2.7±0.2	3.0±0.0	6.0±0.0	4.7±0.2	4.7±0.2	4
KOS-1	9.0±0.0	2.6±0.2	1.8±0.2	5.2±0.4	0.0±0.0	4.6±0.2	5
W93A2 x KOS	6.2±0.2	3.6±0.2	3.4±0.2	6.6±0.2	3.8±0.2	5.2±0.2	5
(W93A2 x KOS) - 1	6.0±0.3	2.7±0.2	3.0±0.1	6.2±0.1	3.4±0.3	4.3±0.3	15
(W93A2 x KOS) - 1	5.6±0.4	3.0±0.1	3.2±0.2	6.3±0.1	2.6±0.3	4.4±0.2	18
(W93A2 x KOS) - 1	5.6±0.1	2.1±0.0	2.6±0.0	6.1±0.0	1.7±0.1	2.4±0.1	88
W93A3 x (W93A2 x KOS)	3.3±0.1	2.4±0.1	1.7±0.2	6.1±0.1	4.6±0.1	2.8±0.2	9
W93A3 x (W93A2 x KOS)	4.4±0.4	2.8±0.1	3.6±0.2	6.1±0.1	4.4±0.3	5.0±0.2	10
(W93A2 x KOS) x KOS	7.2±0.4	2.7±0.2	2.2±0.3	5.9±0.1	3.0±0.4	4.5±0.2	10

W93 x KOS were analyzed for 12 variables as follows:

1. Percent dry wt.
2. Total carotenoids (measured at 450 mμ on a Bausch and Lomb spectronic 20 spectrophotometer, using a standard curve prepared with pure beta-carotene).
3. Fraction 1 (Fr-1); containing the hydrocarbon carotenoids.
4. Fraction 2 (Fr-2); containing the monohydroxy carotenoids.
5. Fraction 3 (Fr-3); carotenoid pigments that have more than one oxygen per molecule.
6. Percent recovery from column chromatography; ([Fr-1 + Fr-2 + Fr-3]/total carotenoids)
7. Lycopene.
8. Gamma-carotene.
9. Zeta-carotene.
10. Beta-carotene.
11. Alpha-carotene.
12. Phytofluene.

The last 6 variables were measured as number of visual spots seen on a thin-layer chromatography (TLC) plate using the serial dilution technique described earlier (12). Based on spectrophotometric measurements (450 mμ), Fr-1 of each sample (carotenes) was either diluted or concentrated to give a level of 100 μg/ml. Ten dilutions were prepared from the 100 μg/ml solution, with a dilution factor of $(1/2)^n$, (100, 50, 25,...,μg/ml). Using micropipettes (10 microliter) the 10 dilutions were applied on a TLC plate sequentially from left to right. A "standard solution" containing all 6 carotenes was also spotted on each plate for qualitative reference. The data were collected by determining the last dilution at which each pigment could be seen visually.

Table 7. Distribution of plants for lycopene spots on TLC Plate of the cross W93 x 'KHN' and its progenies

Gen.	Pedigree	Mean	S.D.	Spots 0	1	2	3	4	5	6	7	8	9	10	Total plants
P_1^a	W93A3	2.2	0.2			4	1								5
P_1^b	W93B-1	2.5	0.3			2	2								4
P_2^a	KHN	9.0	0.0										3		3
P_2^b	KHN-1	9.0	0.0										4		4
F_1^a	W93A2 x KHN	5.6	0.2						2	3					5
F_1^b	W93B x KHN	6.6	0.2							2	3				5
F_2	$F_1^{a\oplus}$	5.5	0.3				5	5	2	6	3	3	2		26
F_2	$F_1^{a\oplus}$	6.7	0.4				2		2	1	3	9			17
F_2	$F_1^{b\oplus}$	6.0	0.5				1	1	4	2	2	2	1		13
BCP_2	$F_1^a \times P_2^b$	7.5	0.2						1	3	5	4	6		19
BCP_1	$F_1^b \times P_1^a$	3.3	0.2			2	9	5	2						18

Results

Visual root color:

The color of F_1 roots in progeny between the 3 red rooted cultivars and the orange rooted line W93 was orange and

Table 8. Distribution of plants for alpha carotene spots of the cross W93 x 'KHN' and its progenies.

Gen.	Pedigree	Mean	S.D.	Spots 0	1	2	3	4	5	6	7	8	9	10	Total plants
P_1^a	W93A3	4.6	0.2					2	3						5
P_1^b	W93B-1	4.7	0.2					1	3						4
P_2^a	KHN	0.0	0.0	3											3
P_2^b	KHN-1	0.0	0.0	4											4
F_1^a	W93A2 x KHN	4.2	0.2					4	1						5
F_1^b	W93B x KHN	3.6	0.2				2	3							5
F_2	$F_1^{a\oplus}$	3.1	0.2	1	2	3	8	9	3						26
F_2	$F_1^{a\oplus}$	2.1	0.3	4	1	3	6	3							17
F_2	$F_1^{b\oplus}$	2.1	0.4	3	1	4	2	2	1						13
BCP_2	$F_1^a \times P_2^b$	0.2	0.1	17		2									19
BCP_1	$F_1^b \times P_1^a$	3.0	0.4	4	1	1	3	2	7						18

Table 9. Distribution of plants for lycopene spots of the cross W93 x 'KOS' and its progenies.

Gen.	Pedigree	Mean	S.D.	Spots 0	1	2	3	4	5	6	7	8	9	10	Total plants
P_1a	W93A3	2.2	0.2			4	1								5
P_1b	W93B-1	2.5	0.3			2	2								4
P_2	KOS-1	9.0	0.0										5		5
F_1	W93A2 x KOS	6.2	0.2							4	1				5
F_2	$F_1\oplus$	6.0	0.3					2	5	1	4	3			15
F_2	$F_1\oplus$	5.6	0.4				1	5	3	4	2	2	1		18
F_2	$F_1\oplus$	5.6	0.1				6	16	18	17	20	7	1		85
BCP_1	F_1 x P_1a	3.3	0.1				6	3							9
BCP_1	F_1 x P_1a	4.4	0.4			1	1	4	2	1	1				10
BCP_2	F_1 x P_2	7.2	0.4							4	2	2	2		10

uniform within the line. However, some F_1 lines were darker and some lighter in color than the orange parent. In F_2, F_3, and backcrossed progenies the roots segregated into 5 color groups; light-orange, orange, dark-orange, red-orange, and red. The data collected on the segregating progenies failed to show any consistent segregation pattern into the 5 color groups. We grouped the F_2 data in all possible combinations and the only consistent segregation pattern was obtained when light-orange, orange, and dark-orange were grouped as orange, while red-orange and red were combined as red.

Backcrossing the F_1 (which had segregated 13:3 in F_2) to the orange parent produced light-orange and orange offspring in ratios approximating 1:1. The backcross progeny of the same F_1 to the red parent segregated in ratios of 7:9, orange:red, and did not support the two gene model. The one F_2 progeny that did not fit a 13:3 ratio did fit a 9:7, orange:red. Therefore the 9:7 F_3 ratios may be in agreement with the F_2 progenies, even though the association is not evaluated critically in this paper.

In the cross W93 x 'KOS' (Table 2) the F_1 roots were orange. Three types of segregating F_2 progenies were found:

Table 10. Distribution of plants for alpha carotene spots of the cross W93 x 'KOS' and its progenies.

Gen.	Pedigree	Mean	S.D.	Spots 0	1	2	3	4	5	6	7	8	9	10	Total plants
P_1a	W93A3	4.6	0.2					2	3						5
P_1b	W93B-1	4.7	0.2					1	3						4
P_2	KOS-1	0.0	0.0	5											5
F_1	W93A2 x KOS	3.8	0.2				1	4							5
F_2	$F_1\oplus$	3.4	0.3		1	3	3	4	4						15
F_2	$F_1\oplus$	2.6	0.3	2	2	2	8	3	1						18
F_2	$F_1\oplus$	1.7	0.1	23	12	28	13	6	3						85
BCP_1	F_1 x P_1a	4.6	0.1					3	6						9
BCP_1	F_1 x P_1a	4.4	0.3			1	1	2	5	1					10
BCP_2	F_1 x P_2	3.0	0.4	1		2	2	5							10

In the F_1 of W93 x 'KHN' (Table 1) orange was dominant to red. F_3 tests of all but one of the F_2 progenies gave evidence for dominant orange, dominant red, homozygous orange, and homozygous red, all of which is consistent with digenic F_2 ratios. In the segregating F_3 progenies from orange F_2 plants, 3 segregated in ratios of 3:1, orange:red (supports digenic F_2) while 4 segregated in ratios of about 9:7, orange:red (a digenic ratio which did not support the apparent digenic F_2). 3:1, 13:3, and 15:1, orange:red, suggesting the involvement of at least 2 genes. The F_3 and other progenies gave evidence for dominant orange, dominant red, homozygous orange and homozygous red. In the segregating F_3 progenies from orange F_2 plants 3 segregated 3:1, orange:red, 2 segregated 15:1, orange:red, and 4 segregated in ratios of about 9:7, orange to red. The backcross of the same F_1 plants (which had given rise to the F_2 progenies segregating 13:3) to the orange parent gave

Table 11. Segregation for lycopene and alpha-carotene content of parents and progenies from the cross W93 x 'KHN'.

Generation	Pedigree	No. of lines	Pigments content[z] +A +L	+A -L	-A +l	-A -L	Expected ratios	X^2
P_1a	W93A3	1		5				
P_1b	W93B	1		4				
P_2a	KHN	1			3			
P_2b	KHN-1	1			4			
F_1a	W93A2 x KHN	1	5					
F_1b	W93B x KHN	1	5					
F_2	$F_1a\otimes$	1	16	9		1	9:3:3:1	8.72*
F_2	$F_1a\otimes$	1	11	2	4		9:3:3:1	1.92ns
F_2	$F_1b\otimes$	1	8	2	3		9:3:3:1	1.08ns
Sum of F_2	Sum of 3 $F_1\otimes$	3	35	13	7	1	9:3:3:1	3.94ns
BCP_1	F_1b x W93A3	1	1	13	1	3	1:1:0:0	**
BCP_2	F_1a x KHN-1	1	2		17		1:0:1:0	11.84**

[z]-A = Alpha-carotene cannot be seen on the TLC plate. -L = Not more than 4 spots of lycopene can be seen on the TLC plate.

Table 12. Segregation for lycopene and alpha-carotene content of parents and progenies from the cross W93 x KOS.

Generation	Pedigree	No. of lines	Pigments content[z] ±A ±L	±A -L	-A ±L	-A -L	Expected ratios	X^2
P_1a	W93A3	1	5	5	5			
P_1b	W93B	1		4				
P_2	KOS-1	1			5			
F_1	W93A2 x KOS	1	5					
F_2	F_1⊕	1	13	2			9:3:3:1	6.45ns
F_2	F_1⊕	1	12	4		2	9:3:3:1	4.53ns
F_2	F_1⊕	1	44	18	19	4	9:3:3:1	1.48ns
Sum of F_2	Sum of 3 F_1⊕	3	69	24	19	6	9:3:3:1	0.96ns
BCP_1	F_1 x W93A3	1		9			1:1:0:0	9.00**
BCP_1	F_1 x W93A3	1	4	6			1:1:0:0	0.40ns
BCP_2	F_1 x KOS-1	1	9		1		1:0:1:0	6.40*

[z]-A = Alpha-carotene cannot be seen on the TLC plate. -L = Not more than 4 spots of lycopene can be seen on the TLC plate.

all "orange" class, but segregated approximately 1:1, light orange:orange. The backcross of the same F_1 plants to the red parent segregated 1:1, orange:red. One F_3 progeny from a red F_2 parent gave only orange roots, which may be a case of misclassification of the F_2 root (as indicated earlier in the material and methods section).

In the cross W93 x 'KDA' the F_1 progeny were orange and the F_2 segregated 13:3, orange:red. No further information was obtained for this cross because 'KDA' was lost as a genetic line.

We also crossed the red parent 'KDA' separately with 'KHN' and 'KOS'. The F_1 and F_2 roots from these crosses were red.

Carotenoid analysis:

The average value and standard deviation for each of the 12 variables analyzed are listed in Tables 3, 4, 5, and 6. The main differences between W93 and 'Kintoki' (KHN, KOS) are in lycopene and alpha-carotene content. W93 contains only traces of lycopene, 2-3 spots on TLC plates in the present study, and sometimes 4 spots were found in earlier work (12), but its alpha-carotene content is relatively large. In 'Kintoki' (KHN, KOS), the major pigment is lycopene, and alpha-carotene was not detected (12). The distribution of progenies with respect to lycopene and alpha-carotene is given in Tables 7, 8, 9, and 10 For genetic segregation 2 phenotypes were considered with regard to each pigment. Alpha-carotene was either not detected [-A], or was present [+A]. The low lycopene phenotype [-L] included plants with 4 or fewer spots of lycopene, and high lycopene [+L] included plants with more than 4 spots.

The F_1 progeny of W93 x 'KHN' and W93 x 'KOS' contained both lycopene and alpha-carotene at an intermediate level compared to the parents. The mean values of backcross progenies shifted toward the mean of the respective backcrossed parent (Tables 4 and 6). Most of the F_2 progenies tested segregated in ratios of 9:3:3:1 (Tables 11 and 12), suggesting 2 genes controlling the differences in lycopene and alpha-carotene. However, backcross progenies did not support fully the digenic interpretation. Individual red segregants in F_2

Table 13. Distribution of total carotenoids (μ/g fresh wt) among plants of the cross W93 x 'KHN' and its progenies.

Gen.	Pedigree	Mean	S.D.	Carotenoids μg/g fresh wt 0-20	20-40	40-60	60-80	80-100	100-120	120-140	140-160	160-180	180-200	>200	Total plants
P_1a	W93A3	115.8	4.8						3	2					5
P_1b	W93B-1	89.8	11.3				2	1	1						4
P_2a	KHN	68.8	21.9			2			1						3
P_2b	KHN-1	91.7	33.5			2	1					1			4
F_1a	W93A2 x KHN	110.1	8.9					2	1	2					5
P_1b	W93B x KHN	55.4	3.9			3	2								5
F_2	F_1a⊕	84.7	6.6		3	4	4	5	7	2	1				26
F_2	F_1a⊕	66.9	6.2		1	6	5	4		1					17
F_2	F_1b⊕	62.8	8.1		2	5	4	1			1				13
BCP_2	F_1a x P_2b	43.7	2.8		7	10	2								19
BCP_1	F_1b x P_1a	65.4	4.8			10	3	5							18

Table 14. Distribution of plants for total carotenoids (μg/g fresh wt) of the cross W93 x 'KOS' and its progenies.

Gen.	Pedigree	Mean	S.D.	Carotenoids μg/g fresh wt 0-20	20-40	40-60	60-80	80-100	100-120	120-140	140-160	160-180	180-200	>200	Total plants
P_1a	W93A3	115.8	4.8						3	2					5
P_1b	W93B-1	89.8	11.3				2	1	1						4
P_2	KOS-1	67.4	9.3			2	2	1							5
F_1	W93A2 x KOS	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2	F_1⊕	130.5	14.8				3	2	5		1		2	2	15
F_2	F_1⊕	57.9	4.8		5	3	7	3							18
F_2	F_1⊕	70.3	3.4		4	30	29	12	4	3	1		1	1	88
BCP_1	F_1 x P_1a	65.4	10.3		2	2	3	1	1						9
BCP_1	F_1 x P_1a	106.7	20.5			4	1		1	1		1	2		10
BCP_2	F_1x P_2	38.9	2.0		6	4									10

Table 15. Distribution of plants for carotenols in fraction number 2 (μg/g fresh wt) of the cross W93 x 'KHN' and its progenies.

Gen.	Pedigree	Mean	S.D.	Carotenoids μg/g fresh wt 0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	>20	Total plants
P_1a	W93A3	3.16	0.22		5										5
P_1b	W93B-1	2.70	0.30		4										4
P_2a	KHN	4.90	1.25		2		1								3
P_2b	KHN-1	6.81	2.57		2		1			1					4
F_1a	W93A2 x KHN	8.38	0.71				1	4							5
P_1b	W93B x KHN	6.12	0.59			3	2								5
F_2	$F_1a^{\oplus}$	6.44	0.66		7	7	5	1	4	2					26
F_2	$F_1a^{\oplus}$	5.36	0.80	1	4	6	3	1	1	1					17
F_2	$F_1b^{\oplus}$	5.69	0.82	1	3	4	3	1	1						10
BCP_2	$F_1a \times P_2b$	3.40	0.25	2	11	6									19
BCP_1	$F_1b \times P_1a$	3.86	0.16		12	6									18

progeny which contain as much lycopene as the red parent and as much alpha-carotene as in the F_1 progeny tend to rule out the possibility that lycopene accumulation results from blocking alpha-carotene synthesis. The detection in F_2 offspring with only traces of lycopene and no alpha-carotene [-A-L], also provides evidence against this hypothesis.

The data for other pigments were arranged in distribution tables similar to the lycopene data in Tables 7 and 9. The data indicated heritable differences between W93 and Kintoki in zeta-carotene and phytofluene; however, the number and nature of genes involved was not clear. These distributions are not presented here.

Evidence for heritability of total carotenoid synthesis (Tables 13 and 14) and carotenol synthesis (Fr-2) of the roots was found (Tables 15 and 16). The wide differences found within the red cultivars may indicate heterozygosity of genetic factors controlling total carotenoids and carotenols (Fr-2), but again the nature and number of genes responsible are unknown. The skewed distribution of total carotenoids in F_2 progenies of W93 x 'KOS' suggests dominance for low levels of total carotenoids. Transgressive segregation for total carotenoids and carotenols was found in these F_2 progenies (Tables 14 and 16). Dominant genes for low total carotenoid synthesis and transgressive segregation for total carotenoids were reported previously (4, 6)[3].

One F_2 plant had a dark-orange root and 241 μg/g total carotenoids, with lycopene and alpha-carotene content at the W93 level. This points out a possible way of deriving carrot lines with higher total carotenoid levels and deeper visual root color without increasing the relative lycopene content. Another F_2 orange root had 81 μg/g total carotenoids, with only traces of lycopene and no alpha-carotene [-A-L]. Although 81 μg/g is not unusually high, it suggests the possibility of developing carrot lines reasonably high in total carotenoids, and with beta-carotene as the primary pigment.

All possible correlations between the 12 variables measured were calculated for each of the parent and progeny lines of W93 x KHN and W93 x KOS. The correlation coefficients were pooled as suggested by Steel and Torrie (10). Only pooled values which were equal to or exceeded the 1% level (0.22), and were based on a homogeneous group of correlation coefficients (10), are listed below:

Fr-2 (carotenols) and lycopene	0.22
Fr-2 (carotenols) and zeta-carotene	0.30
Fr-2 (carotenols) and phytofluene	0.30
Phytofluene and zeta-carotene	0.39
Gamma-carotene and alpha-carotene	0.30
Beta-carotene and lycopene	-0.30
Alpha-carotene and lycopene	-0.26

Discussion

At least 2 genes determined the differences in the orange and red root colors of the parents studied. The deviation from expected segregation ratios could have been a result of errors in visual classification, or the effect of additional gene(s). Support for additional gene(s) comes from the variation within the color groups as well as from differences in color between phloem and xylem (6)[3,4]. Additional support for more than 2 genes is suggested by the differences in segregation of F_2 progenies, and of F_3 progenies from orange F_2 plants. We do not exclude the possibility that differential survival in the field (poor germination, mortality, and premature bolting), or preferential selection in the greenhouse (mortality, partial male fertility, and zygote mortality) may have contributed to these deviations.

We suggest the genotype AA11 for W93, and aaLL for 'KHN' and 'KOS', as the genes responsible for the lycopene and alpha-carotene differences in these parental lines. The gene "A" for alpha-carotene synthesis does not determine the orange color phenotype, because we found some red roots that contain as much alpha-carotene as the orange F_1 roots (A-L-). Similarly,

Table 16. Distribution of plants for carotenols in fraction number 2(μg/g fresh wt) of the cross W93 x KOS and its progenies.

Gen.	Pedigree	Mean	S.D.	Carotenoids μg/g fresh wt 0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	>20	Total plants
P_1a	W93A3	3.16	0.22		5										5
P_1b	W93B-1	2.70	0.30		4										4
P_2	KOS-1	3.56	0.79		4		1								5
F_1	W93A2 x KOS	5.55	0.60			4	1								5
F_2	$F_1^{\oplus}$	9.61	0.87		2	3	4	2	3	1					15
F_2	$F_1^{\oplus}$	5.10	0.68	2	5	5	2	2	1						17
F_2	$F_1^{\oplus}$	5.48	0.28	3	20	38	11	1	2	1					88
BCP_1	$F_1x P_1a$	3.69	0.45		6	2	1								9
BCP_1	$F_1 \times P_1a$	9.18	0.81				4	2	3	1					10
BCP_2	$F_1 \times P_2$	4.29	0.41		4	5	1								10

the gene "L" does not determine the red color alone because we found some F_2 orange roots that did not have any alpha-carotene, but had 8 spots of lycopene, which is close to the red parent level. In these last cases the total carotenoid levels were low, 40-60 μg/g. This may suggest that genes for high total carotenoid synthesis may interact with "L" to produce red phenotype.

A positive correlation was found between: Fr-2 and lycopene, 0.22; Fr-2 and zeta-carotene, 0.30; Fr-2 and phytofluene, 0.30. These correlations may be interpreted as an indication of biosynthetic relationship between the 3 pigments and carotenols. The carotenols in Fr-3 represent only a minor portion of the total carotenoids and did not covariate persistently with any of the other variables. Imam and Gabelman (4) had suggested earlier that xanthophylls are probably synthesized in a different pathway than that of colorless polyenes in carrot with lemon color roots.

Some proposed biosynthetic pathways for carotenoids (7, 8, 13, 14) suggest that zeta-carotene is synthesized from phytofluene. The positive correlation found between these pigments, 0.39 and evidences for genetic factors controlling their level in the root, support either this conversion or possibly a pleiotropic effect.

The genetic complexity of root color was demonstrated repeatedly (4, 6)[3,4]. It is apparent that several factors contribute to the production of a specific root color, which is under genetic control. The visual root color of carrot is determined by the level of total carotenoids, the accumulation of specific pigments, and the distribution of pigments between phloem and xylem.

Genes affecting the level of total carotenoids in the roots had been reported (4, 6)[4]. Such genes may have little, if any, effect on the ratios between specific pigments. Earlier (12), we reported on the similarity in the pigment patterns of the orange lines W93 and WC501 which differ widely in total carotenoids, and similarly for the red lines 'KHN' and 'KOS'. These differences may have resulted from the genes controlling the level of total carotenoid synthesis. The site of action for such genes is probably in the early stages of carotenoid biosynthesis before the formation of phytoene. The 2 genes for lycopene and alpha-carotene differences are the first reported genes controlling qualitative differences of specific pigments in carrot roots.

Some genes are responsible for color differences between the phloem and the xylem (6)[4]. Kust[3] confirmed the existence of such genes, and also found evidence suggesting that some gene(s) affect simultaneously the formation of visual root color in more than one way. Their reported genes Y_1 and Y_2 affect the carotenoids in the xylem only while the gene Y affects the carotenoids in both xylem and phloem, but with stronger effect in the xylem.

Finally, we would like to note and extend the observed similarities in pigment pattern between red carrot ('KHN', 'KOS') and red tomato reported earlier (12). The carotenols of W93, WC501, 'KHN', 'KOS', and 4 tomato cultivars (12) were separated qualitatively by thin layer chromatography. 'KHN' and 'KOS' were similar to the tomato cultivars and have at least 2 additional carotenols to the 3 found in W93 and WC501. No attempt was made to identify these carotenols. These similarities justify further the use of Porter and Anderson's (7) pathway as a model for understanding carotenoid biosynthesis in carrot.

Note added in revision:

Recently, Sugano, Miya, and Nishi (Plant and Cell Physiology 12:525-531, 1971) reported their work with cultured carrot cell lines, originally derived from a "Kintoki" root. They isolated 2 interesting cell lines; line GD-2 produced lycopene as the major carotene, and line GD-1 synthesized predominantly beta-carotene. Both lines had carotenol levels close to the original cultivar. Unfortunately, they do not present any data on other carotene pigments (e.g. alpha-carotene and zeta-carotene). Their work, and utilizing adventive embryogenesis, opens a new potential approach to the genetic study of carotenoid synthesis and root color in carrot.

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