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Relationship of Carotenoids and Tocopherols in a Sample of Carrot Root-Color Accessions and Carrot Germplasm Carrying *Rp* and *rp* Alleles

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Carotenoids and tocopherols are powerful antioxidants synthesized in plants from a common precursor. They may offer significant health benefits to humans. Seed oils have been shown to possess high levels of tocopherols, but little is known about their levels in the edible portions of most vegetable crops. A two-year field experiment was conducted at two locations to assess levels of major carotenoids and tocopherols in carrot (*Daucus carota*) root and leaf tissue. Levels of compounds in root tissue reported on a dry weight basis were as follows: α-tocopherol, 0.04−0.18 ppm; lycopene, 0.00−52.94 ppm; α-carotene, 10.63−1504.76 ppm; and β-carotene, 26.69−1673.76 ppm. Higher levels of all carotenoids were measured in phloem tissue than in xylem. Leaf tissue levels of tocopherols measured on a dry weight basis ranged from 0.02 to 0.85 ppm, whereas levels of carotenoids ranged from 12.81 to 411.66 ppm. In xylem tissue, α-tocopherol was significantly ($P \leq 0.001$) positively correlated with α-carotene ($r = 0.65$) and with β-carotene ($r = 0.52$). This positive correlation indicates it may be possible to select for both increased α-tocopherol and carotenoids in carrot. The reduced pigment (*rp*) mutation of carrot exhibited a 96% reduction in levels of α- and β-carotene and a 25−43% reduction in α-tocopherol when compared to a near-isogenic line. In plants homozygous for *rp*, a substantial increase was observed in phytoene, a precursor to carotenoids, suggesting the location of the *rp* lesion in the carotenoid synthesis pathway.

KEYWORDS: Carrot; *Daucus carota*; HPLC; vitamin A; vitamin E; lycopene; α-carotene; β-carotene; α-tocopherol

INTRODUCTION

The roles of carotenoids and tocopherols are similar within the human diet and in plants due to their antioxidative nature. Both are related phytochemicals derived from a common precursor, geranylgeranyl-pyrophosphate (**Figure 1**). They are of great interest in the human health functionality of vegetable crops (*1*). Chemically, carotenoids are composed of long isoprenoid chains. Certain carotenoids serve as a major source of provitamin A in the human diet. Tocopherols possess a chromanol ring that helps control reactive oxygen species, with four isomers (α, β, γ, δ) being identified (*2*). In the human diet, carotenoids have recently been shown to help prevent atherosclerosis (*3*), macular degeneration (*4*), and cancer (*5*). Tocopherols have been shown to be beneficial in preventing degenerative effects as well, both cosmetically (*6*) and oncologically (*7*). α-Tocopherol is the isomer exhibiting the highest amount of antioxidant activity in humans (*8*). Carotenoids are integral to all photosynthetic organisms, preventing the formation of reactive oxygen radicals produced through photosynthesis and aiding in harvesting light (*9*). Tocopherols are important in the protection of plant membranes from oxidative stress (*2*) and in cell membrane integrity in animal systems (*10*). Both carotenoids and tocopherols are most accurately measured using liquid chromatography techniques.

Carrot root color is a result of various pigments that serve as intermediate products in the carotenoid pathway. The orange color of most modern cultivated carrots is due to α- and β-carotene (*11*), whereas the red color is due mostly to an accumulation of lycopene (*12*). Purple coloration of carrot roots, ranging in intensity from red to black, is primarily a result of anthocyanins (*13*). The yellow color in carrot roots is due to xanthophylls, oxygenated carotenoids downstream of β-carotene (*11*). White-colored roots are low in total carotenoids (*14*). Combinations of these various pigments create a plant that is not only visually appealing but also high in human nutritive value. Tocopherols do not contribute to the colors of plants but are associated with membrane integrity of plant cells (*15*).

Mutations within the carotenoid synthesis pathway can help reveal the roles of different genes and their products. A recessive allele for reduced pigment, *rp*, has been shown to decrease the carotenoid concentration in the carrot root by as much as 92%, yielding a white phloem and pale yellow xylem (*16*). This was the first description of a white or nonpigmented carrot root resulting from a recessive gene. The first several leaves of *rprp*

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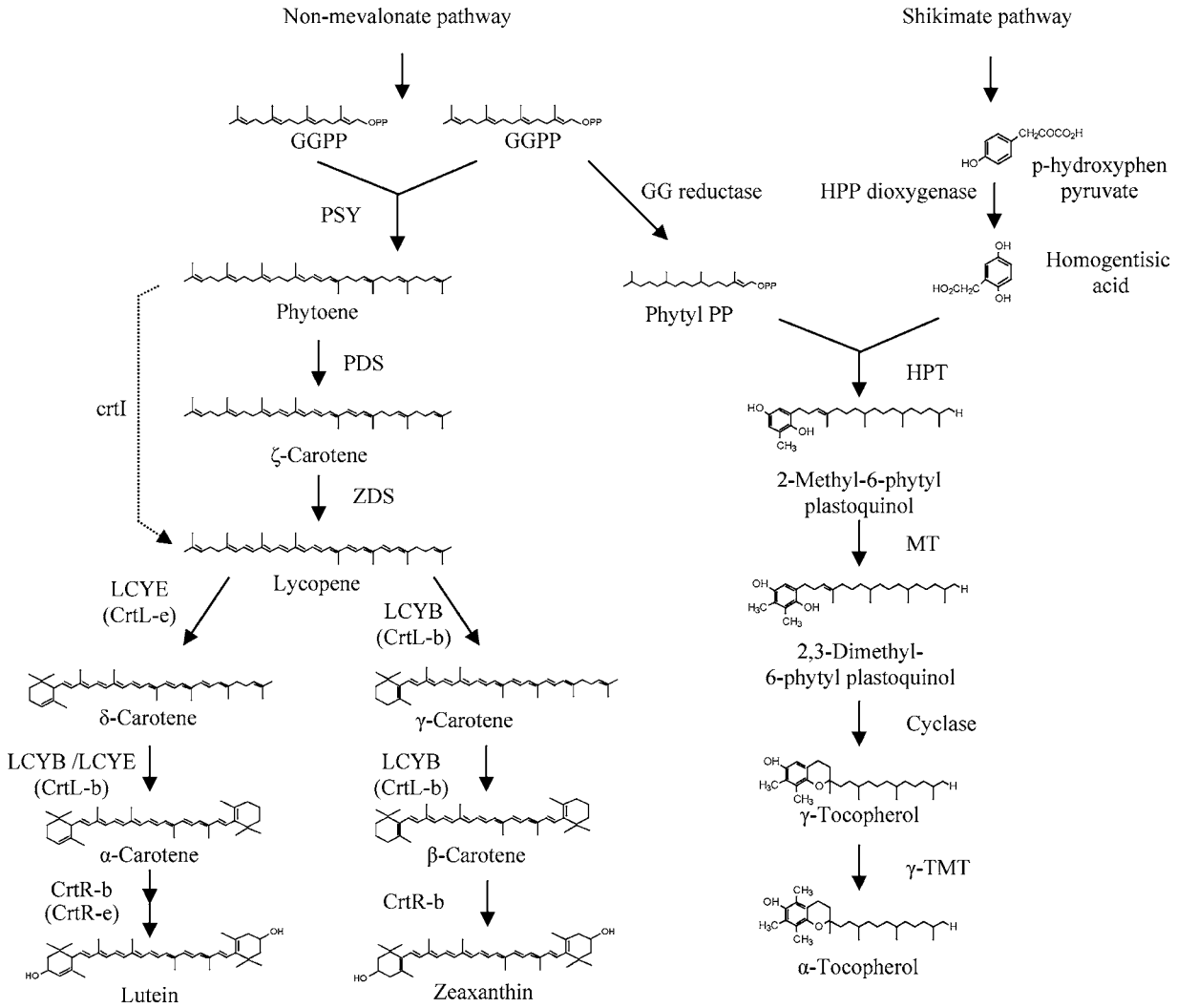

Figure 1. Pathway of carotenoid and tocopherol biosynthesis, originating from common precursor geranylgeranyl-pyrophosphate. Abbreviations: GGPP, geranylgeranyl pyrophosphate; PSY, phytoene synthase; PDS, phytoene desaturase; crtI, bacterial phytoene desaturase; ZDS, ζ-carotene desaturase; LCYE (CrtL-e), lycopene ϵ-cyclase; LCYB (CrtL-b), lycopene β-cyclase; HPP dioxygenase, *p*-hydroxyphen pyruvate dioxygenase; Phytyl PP, phytyl pyrophosphate; HPT, homogentisate phytyltransferase; MT, 2-methyl-6-phytylhydroquinone methyltransferase; γ-TMT, γ-tocopherol methyltransferase (*8*, *27*, *32*).

plants are white and speckled, indicating an effect of the *rp* allele on the chlorophyll of the plant via carotenoid reduction. Leaves possess wild-type appearance by the sixth leaf, suggesting developmental effects of the *rp* allele. The levels of various carotenoids in the roots of *rprp* plants have not been previously determined.

The purpose of this study was to measure the accumulation of different compounds along the carotenoid and tocopherol biosynthesis pathway in order to provide an explanation for the differences in color of various carrot accessions. To this end phytoene, lycopene, α-carotene, β-carotene, γ-tocopherol, and α-tocopherol levels of a sample of carrot accessions in different carrot tissue were measured. We also wished to determine the relationship of these compounds to the presence of *Rp* and *rp* alleles. These compounds all arise from a common precursor, geranylgeranyl-pyrophosphate (GGPP) (**Figure 1**). Accessions included orange-rooted accessions with a range of carotenoid levels, a red-rooted accession, and a yellow-rooted accession. Genotype × environment interactions of carotenoid and tocopherol levels were examined. In addition, an accession carrying a recessive mutation (*rp*) for reduced pigment was used to determine the effect of this mutation on carotenoid and tocopherol levels.

MATERIALS AND METHODS

Germplasm. Eight accessions were chosen to represent variation in carotenoid content and color. W266D*rprp* is a reduced carotenoid pigment carrot inbred line identical in size and shape to its near-isogenic counterpart, W266D*RpRp* (*16*). W266D*RpRp* is an orange inbred line, which served as a parent of Lucky B. W267B is an orange processing inbred line released in 1987 by the carrot breeding program of the University of Wisconsin. Danvers is a standard orange processing carrot cultivar from a population having a relatively short, conical shape (*17*). The high carotene mass population (HCM) is an orange-rooted population developed by the USDA Vegetable Crops Research Unit (*18*). Beta III is a dark orange-rooted open-pollinated cultivar released to improve carotenoid levels of home-grown carrots (*19*). Okuzawa is a red-rooted open-pollinated population obtained from the Shippo Seed Co. (Hokkaido, Japan). The yellow-rooted accession was selected from a cross of PI 379328, an accession from Yugoslavia with yellow xylem and yellow phloem, and PI 211024, an accession from Afghanistan with white xylem and yellow phloem, both of which were obtained from the North Central Regional Plant Introduction Station (Ames, IA). For the remainder of this paper this yellow-rooted accession will be referred to as "yellow type".

Field Methods. Accessions were planted in a randomized complete block design consisting of four replications on muck soil (>20% organic matter) at two locations, Randolph and Markesan, WI, during the 2001

and 2002 growing seasons. Phloem tissue is easily distinguishable from xylem tissue in carrot roots because the cambial layer is large and lacks pigment. Xylem tissue is composed by the inner core, whereas phloem tissue can be found from the cambial layer to the epidermal layer.

Samples of xylem and phloem tissue from the upper third of the carrot root and leaf tissue were collected 102 days after planting, with approximately equal amounts bulked from each of 10 random roots within each replication of each accession. Leaf tissue samples were similarly bulked. Samples were chilled overnight at 4 °C overnight before storage at −80 °C until further analysis.

Laboratory Methods. Samples were lyophilized and ground using a prechilled mortar and pestle under low light conditions. Samples were stored at −20 °C in 15 mL screw-top polypropylene centrifuge tubes until extraction. An aliquot of 200 mg was removed from each sample and extracted in 2.0 mL of heptane containing 0.05% butylated hydroxytoluene (BHT). Samples were vortexed for 2 min and centrifuged at 3000*g* at 0 °C for 10 min. A 1.5 mL extract of the supernatant was removed. Two milliliters of heptane containing 0.05% BHT was added to the carrot tissue for a second extraction according to the above method. A 1.5 mL extract was removed and combined with the first extract by vortexing for 15 s. An aliquot of 0.5 mL was filtered through a 0.2 μm nylon filter (Fisher Scientific, Pittsburgh, PA) and stored at −20 °C until high-performance liquid chromatography (HPLC) analysis. Analysis with β-apo-8′-carotenal (Fluka, St. Louis, MO) and δ-tocopherol (Matreya, Inc., State College, PA) internal standard showed nearly 100% extraction efficiency, although it is possible that some carotenoids were not extractable due to crystallization.

Samples were analyzed by using a modified HPLC method based upon that of Grela et al. (*20*). A 50 μL sample was injected a Supelguard Discovery C18 (2 cm × 4.0 mm, 5 μm) guard column connected to a Discovery C18 (25 cm × 4.6 mm, 5 μm) column (Supelco, Bellefonte, PA). The HPLC system consisted of a quaternary pump, a vacuum degasser, and a multiple-wavelength detector (Agilent 1100 series). The system was controlled by HP ChemStation software (Hewlett-Packard Co., Palo Alto, CA). The isocratic mobile phase consisted of methanol at a flow rate of 2 mL min^{-1}. Lycopene and α- and β-carotenes were detected at 454 nm, whereas α-tocopherol was detected at 296 nm (**Figure 2**). Carotenoid standards were obtained from Sigma. Tocopherol standard was obtained from Matreya, Inc. This method allowed for the simultaneous analysis of lycopene, α-carotene, β-carotene, and α-tocopherol (*21*). Elution times of compounds measured were (**Figure 2**) as follows: lycopene, 22 min (454 nm); α-carotene, 23 min (454 nm); β-carotene, 24 min (454 nm); γ-tocopherol, 3.5 min (296 nm); α-tocopherol, 5 min (296 nm); and phytoene, 23 min (296 nm). Carotenoid values and elution times were consistent with previously published data (*22*). Tocopherol elution times were consistent with previous data (*23*). Phytoene was identified through comparison of experimental data with published results (*24*). Concentrations of carotenoids and tocopherols were calculated on a dry weight basis.

Statistical Analysis. All data were analyzed using the mixed models procedure of SAS (*25*). Data were ranked prior to analysis to account for skewed, nonparametric values (*26*). Statistical analysis for differences and correlations proceeded on ranked data. Actual numeric values are presented in tables. Carrot accession, location, and year were analyzed as fixed effects, and all replicate interactions were analyzed as random effects. Pearson phenotypic correlation coefficients were calculated among all compounds using measured compound levels.

RESULTS AND DISCUSSION

Analysis of Fixed Effects. The analysis of fixed effects (**Table 1**) created with the mixed models procedure of SAS revealed highly significant interactions for accession, year, and location in all tissues. However, the accession effect was much more pronounced and highly significant ($P < 0.001$) as a result of a broad range of accessions being studied. These significant effects accounted for the wide range of compound levels detected in this study. Location effects accounted for a change in magnitude for some compounds, due perhaps to differences in moisture levels between the two sites (irrigated vs nonirrigated). Year effects also accounted for some variation in compound levels across accessions. However, for both location and year effects observed in the data the variation resulted mostly from a change in magnitude of the data and rarely a change in accession ranks. Despite these differences, accession means were pooled across year and across location for further analysis because rank changes in accession means were rarely observed between years and locations.

Carotenoid Concentration. In both root xylem and phloem, levels of α-carotene varied significantly among accessions (**Table 2**). HCM and Beta III exhibited the highest levels of α-carotene among accessions for both tissue types and were significantly different from all other accessions ($P < 0.01$). Both of these populations have been selected for elevated levels of carotenoids (*18*, *19*). HCM showed the highest levels in both xylem and phloem, at 997 and 1674 ppm, respectively. The next grouping W266D*RPRP* and W276B also showed elevated carotenoid levels over the processing standard Danvers. In both tissue types, all orange accessions showed individual accession means that were greater than those of non-orange accessions. When averaged over accessions, a difference in magnitude between tissue types was found with xylem on average containing only 69% of the α-carotene of the phloem. A much smaller range of differences between accessions was seen in the leaf tissue than in either root tissue type. HCM and Beta III again had the highest levels, but W266D*rprp*, the white-rooted accession, was not significantly different from the remaining orange carrot accessions. Leaf tissue concentrations of α-carotene were 49% lower than root phloem tissue concentrations.

Similar results were measured for β-carotene. In both root xylem and phloem, HCM showed the highest levels at 1078 and 1674 ppm, respectively. W266D*RPRP*, W276B, and Beta III exhibited elevated levels of β-carotene for both tissue types. Similar to α-carotene, all orange-rooted accessions showed individual accession means that were greater than those of non-orange accessions in both tissue types. Differences in magnitude were measured between xylem and phloem, with xylem on average containing only 67% of the β-carotene found in phloem. No clear trend existed in the leaf levels of β-carotene, although levels were only 33% that of the phloem. Okuzawa, the red-rooted accession, showed the highest levels, whereas HCM showed the lowest.

The carotenoid levels of this study agreed with results of Simon and Wolff when corrected for moisture content (*22*). Both carotenoids are responsible for the orange color in carrot roots (*11*). Selection for the orange carrot root was achieved through selection of those compounds that condition orange color, resulting in levels of carotenoids in root tissue that far exceed those in leaf tissue. Carrot accessions in this study with darker orange color contained higher levels of α- and β-carotenes. A ratio of 1:1.5 α:β-carotene was measured in the root tissue. Differences across accessions in leaf tissue were not observed to the same extent as in root tissue, perhaps due to the role of these compounds in the prevention of photo-oxidative damage in leaf tissue during photosynthesis (*27*) or the lack of artificial selection in leaf tissue for increased carotenoid levels. Plants are subject to natural selection for adequate levels of carotenoids in leaves, whereas crops such as carrot have been artificially selected for differing levels within roots for human consumption.

Phytoene and Lycopene. In an effort to explain why the root tissue of non-orange carrots showed reduced levels of α- and β-carotenes in comparison to the root tissue of orange carrots, levels of precursors to these compounds were examined (**Table 1**). Phytoene, the first committed step in the carotenoid pathway, was detected at significant levels in all non-orange

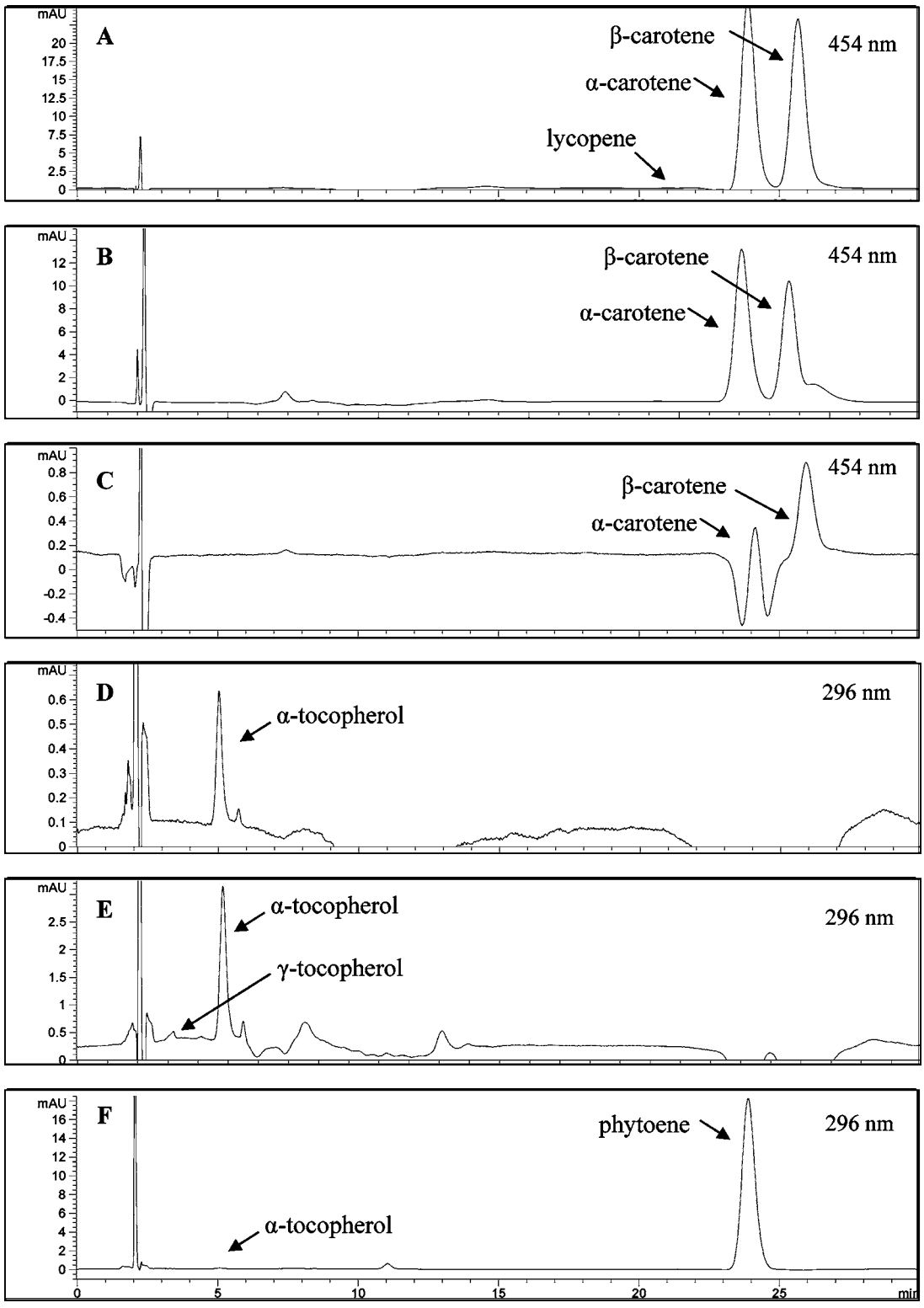

Figure 2. Typical reversed-phase HPLC chromatography profiles measured at 454 nm for major carotenoids of (**A**) xylem and phloem of orange-rooted carrot accessions, (**B**) mature leaf tissue, and (**C**) xylem and phloem of *rprp* mutant carrot root and at 296 for major tocopherols and phytoene of (**D**) xylem and phloem of orange-rooted carrot accessions, (**E**) mature leaf tissue, and (**F**) xylem and phloem of *rprp* mutant carrot root. Measured at 496 nm were lycopene, α-carotene, and β-carotene. Measured at 296 nm were γ-tocopherol, α-tocopherol, and phytoene.

carrot lines but not in any orange carrot lines. Extreme levels of this compound, not previously measured in carrot tissue, were seen in both the xylem and phloem of W266D*rprp* (476.36 and 501.11 mAu, respectively). Okuzawa and the yellow accession both exhibited detectable levels as well. Okuzawa also showed a significant level of lycopene, a precursor to α- and β-carotene downstream of phytoene, detected at levels of 53 and 54 ppm in the xylem and phloem, respectively. Minimal amounts of lycopene were observed in all other accessions. No phytoene or lycopene was detected in the leaves, however.

Tissues that accumulate α- or β-carotenes may naturally show a decreased level of the precursors to these compounds. The non-orange roots showed increased levels of these two compounds, suggesting a reduction in the production or efficiency

Table 1. Analysis of Fixed Effects of Carotenoid and Tocopherol Concentration for Xylem, Phloem, and Leaf Tissue of Carrots Using the Mixed Procedure of SAS[a]

		phytoene		lycopene		α-carotene		β-carotene		γ-tocopherol		α-tocopherol	
fixed effect	Num DF	Den DF	*F*	Den DF	*F*	Den DF	*F*	Den DF	*F*	Den DF	*F*	Den DF	*F*
						Xylem							
location (L)	1	45	0.82	6	1.03	6	1.22	6	7.71*	–[b]	–	66	62.22***
year (Y)	1	45	0.44	3	25.33*	66	51.9′**	45	115.5′**	–	–	3	32.22*
L × Y	1	45	1.29	84	0.63	66	1.11	45	2.59	–	–	66	22.05***
accession (A)	7	45	90.43***	84	80.46***	21	157.31***	42	171.69***	–	–	21	80.04***
A × L	7	45	0.73	84	1.01	66	1.93	42	3.28**	–	–	66	1.55
A × Y	7	45	0.90	84	6.15***	66	4.89***	45	5.83***	–	–	66	1.89
A × L × Y	7	45	1.25	84	1.37	66	2.54*	45	1.54	–	–	66	1.51
						Phloem							
location (L)	1	6	0.23	6	0.14	45	1.25	6	16.57**	–	–	68	39.96***
year (Y)	1	6	1.22	3	0.86	3	5.57	6	25.97**	–	–	68	4.27*
L × Y	1	6	1.03	6	1.57	41	2.72	6	6.53*	–	–	68	23.98***
accession (A)	7	45	116.07***	21	66.36***	45	217.56***	80	169.10***	–	–	21	50.48***
A × L	7	45	0.18	59	0.25	45	0.99	80	2.60*	–	–	68	2.09
A × Y	7	38	1.76	59	0.93	41	5.80***	80	3.52**	–	–	68	0.90
A × L × y	7	38	0.40	59	1.75	41	7.42***	80	2.87**	–	–	68	2.89*
						Leaf							
location (L)	1	9	4.68	–[†]	–	8	5.65*	12	6.02*	70	102.28***	5	2.43
year (Y)	I	9	6.09*	–	–	5	10.33*	12	10.36**	70	0.21	5	31.87**
L × Y	1	9	0.40	–	–	5	0.31	12	0.03	69	14.41***	41	10.21**
accession (A)	7	82	6.24***	–	–	41	61.48***	81	9.48***	23	9.00***	38	4.97***
A × L	7	82	7.38***	–	–	41	0.58	81	0.27	69	2.26*	38	0.93
A × Y	7	82	1.17	–	–	39	4.40***	81	1.16	69	1.76	41	1.46
A × L × Y	7	82	1.32	–	–	39	1.77	81	0.44	69	1.57	41	0.83

[a] Asterisks (*, **, ***) indicate significance at $P = 0.05$, 0.01, and 0.001, respectively. [b] –, γ-tocopherol in xylem, γ-tocopherol in phloem, and lycopene in leaf were not detected at measurable levels.

Table 2. Accession Means for Carotenoid Concentration of Xylem, Phloem, and Leaf Tissue of Carrots on a Dry Weight Basis

		xylem				phloem				leaf			
accession	exterior color	phytoene (mAu)[a]	lycopene (ppm)	α-carotene (ppm)	β-carotene (ppm)	phytoene (mAu)	lycopene (ppm)	α-carotene (ppm)	β-carotene (ppm)	phytoene (mAu)	lycopene (ppm)	α-carotene (ppm)	β-carotene (ppm)
W266D$_{rprp}$	white	476.36	0.03	17.00	26.69	501.11	nd[b]	33.99	60.50	0.88	nd	116.72	231.73
W266D$_{RpRp}$	orange	nd	0.64	503.48	818.86	nd	0.73	765.63	1266.25	nd	nd	99.84	253.75
W276B	orange	0.75	0.54	569.58	998.23	nd	1.08	804.85	1443.13	nd	nd	183.15	249.28
Danvers	orange	1.28	0.08	237.44	435.56	nd	0.31	517.30	969.95	nd	nd	96.63	352.14
HCM[c]	orange	nd	2.35	996.94	1077.81	nd	3.48	1504.76	1673.76	6.40	nd	308.88	205.95
Beta III	orange	nd	1.36	846.15	979.81	nd	1.71	962.32	1124.56	nd	nd	294.57	233.98
Okuzawa	red	69.45	52.94	22.36	283.07	89.23	53.95	32.07	335.68	2.22	0.01	12.81	411.66
yellow type[d]	yellow	39.28	0.03	10.63	47.26	54.78	nd	17.73	55.61	0.52	nd	45.80	343.68
mean		73.39	7.25	400.45	583.41	80.64	7.66	579.83	866.18	1.25	0.00	144.80	285.27
LSD (0.05)		12.67	1.13	52.45	79.27	19.13	1.60	54.68	85.81	4.73	0.00	36.59	56.74
LSD (0.01)		16.96	1.50	71.39	106.06	25.59	2.18	74.42	113.95	6.28	0.00	48.96	75.34
CV (%)		12.89	–[e]	22.78	22.28	17.73	–	16.95	19.25	287.56	–	28.05	30.44

[a] Milliabsorbance units under HPLC curve. [b] Not detected at measurable levels. [c] High carotene mass population. [d] Selected from a cross of P1379328 × P1211024. [e] CV for lycopene not determined due to one accession (Okuzawa) with extreme levels of lycopene compared with other accessions.

of the enzyme resulting in conversions to create carotenoids. Leaf tissue, which showed ample amounts of both α- and β-carotenes in this study, showed none of these precursors detected.

Effect of the Reduced Pigment (*rp*) Mutation. W266D*rprp* showed a dramatic decrease in both α- and β-carotene levels (96−97%) from its near-isogenic counterpart, W266D*RpRp*, in root xylem and phloem. This reduction was accompanied by an accumulation of phytoene, which was not detectable in W266D*RpRp* root tissue. No dramatic differences were noticed in mature leaf tissue for any compounds studied. Young leaf tissue of W266D*rprp* did show bleaching effects consistent with those reported by Goldman and Breitbach (*16*), but later mature leaves were identical in size, shape, and color to W266D*RpRp*. Thus, it is likely that mature leaves in W266D*rprp* plants are not significantly different in carotenoid and tocopherol profiles. Although phytoene and carotenoid levels in the young leaves were not measured, it is possible that these young leaves did have a reduction in carotenoids, resulting in the photo-oxidative bleaching damage that was observed. The near-isogenic lines W266D*rprp* and W266D*RpRp* differ only at the *RP* locus, a recessive mutation causing approximately a 93% loss of pigmentation in carrot roots (*16*). The simultaneous decrease in levels of α- and β-carotenes with an increase in phytoene suggests this allele acts to block the carotenoid pathway at the step immediately following phytoene, a reaction catalyzed by the enzyme phytoene desaturase (PDS) (*27*). Further analysis of this enzyme in *rprp* plants may support or refute this hypothesis.

Reductions in tocopherol levels in *rprp* plants compared to *RPRP* plants may indicate that mechanisms other than reduction in the efficiency of phytoene desaturase may be operative in *rprp* plants.

Table 3. Accession Means for Tocopherol Concentration of Xylem, Phloem, and Leaf Tissue of Carrots on a Dry Weight Basis

		xylem		phloem		leaf	
accession	exterior color	γ-tocopherol (ppm)	α-tocopherol (ppm)	γ-tocopherol (ppm)	α-tocopherol (ppm)	γ-tocopherol (ppm)	α-tocopherol (ppm)
W266D*rprp*	white	nd[a]	0.04	nd	0.06	0.04	0.60
W266D$_{RpRp}$	orange	nd	0.07	nd	0.08	0.04	0.63
W276B	orange	nd	0.06	nd	0.06	0.03	0.85
Danvers	orange	nd	0.10	nd	0.09	0.04	0.56
HCM[b]	orange	nd	0.18	nd	0.13	0.07	0.44
Beta III	orange	nd	0.15	nd	0.10	0.08	0.52
Okuzawa	red	nd	0.05	nd	0.04	0.06	0.52
yellow type[c]	yellow	nd	0.10	nd	0.15	0.02	0.76
grand mean		nd	0.09	nd	0.09	0.04	0.61
LSD (0.05)		nd	0.01	nd	0.01	0.02	0.20
LSD (0.01)		nd	0.01	nd	0.02	0.03	0.26
CV (%)		nd	18.43	nd	22.73	64.53	36.77

[a] Not detected at measurable levels. [b] High carotene mass population. [c] Selected from a cross of PI379328 × PI211024.

The results of this study follow the trend of recent reports of similar carotenoid-reducing mutations. The immutans (*im*) mutation of *Arabidopsis* results in a variegated leaf phenotype visually similar to that of early leaves of *rprp* in carrot. The white regions of im leaves have been shown to accumulate phytoene (*28*) in much the same way as the roots of *rprp*, suggesting both mutations involve impaired function of phytoene desaturase (PDS). In a similar way, the tangerine mutation of tomato results in a decrease in levels of lycopene in ripe fruits. Isaacson et al. (*29*) demonstrated this mutation results in a decrease in expression of CRTISO, an enzyme responsible for the conversion of phytoene to lycopene. The *rp* mutation of carrots is believed to behave like the immutans and tangerine mutations and result in decreased levels of a key enzyme in the carotenoid synthesis pathway.

Tocopherol Concentration. Significant differences in α-tocopherol concentration were measured among accessions (**Table 3**). In root xylem HCM and Beta III exhibited the highest levels of α-tocopherol among accessions, at 0.177 and 0.148 ppm, respectively. The lowest level among the accessions tested was W266D*rprp* (0.038 ppm). Similar results were measured for phloem tissue. The yellow type and HCM exhibited the highest levels, at 0.147 and 0.129 ppm, respectively. Okuzawa showed the lowest phloem level (0.036 ppm). Xylem and phloem tissues showed fairly similar levels of α-tocopherol. When both tissue types were combined, HCM, the yellow type, and Beta III exhibited the highest average levels.

Results were slightly different in the leaf tissue, with W267B and the yellow type exhibiting the highest levels at 0.853 and 0.755 ppm, respectively. HCM leaf α-tocopherol concentrations were unexpectedly the lowest among accessions (0.441 ppm). Levels of α-tocopherol in leaf tissue were >6 times that of the root tissue. γ-Tocopherol was detectable only in leaf tissues at levels only 7% that of α-tocopherol in the same tissue. Beta III exhibited the highest levels at 0.083 ppm, and Okuzawa exhibited the lowest at barely detectable levels. No patterns between orange and non-orange carrot accessions were apparent for tocopherol content. The increased levels of tocopherols in leaf over root tissue may be due to the antioxidant properties of tocopherol, perhaps aiding in photosynthesis (*1*). Tocopherols have not yet been the subjects of selection schemes in carrot. The potential for their increase in root tissue may exist, leading to increased human health functionality of the carrot root.

Correlations among Carotenoids and Tocopherols. In both root xylem and phloem tissues, α- and β-carotenes were both highly correlated with each other at $r = 0.92$ ($P < 0.001$) (**Table 4**). This may allow for simultaneous selection of both compounds to allow for a larger response to selection for nutritional value of the carrot root. Both compounds were negatively correlated with both phytoene and lycopene at various levels of significance. These negative correlations are possibly a result of both phytoene and lycopene serving as precursors to both α- and β-carotenes (**Figure 1**). These negative correlations, however, may be skewed as only a single accession contained substantial levels of phytoene and a different single accession contained substantial levels of lycopene. To draw more complete conclusions on the correlations between α- and β-carotenes and precursors, more non-orange carrot accessions would need to be tested. These tests are currently in development in breeding programs.

Table 4. Phenotypic Correlations of Accession Means for Major Carotenoid and Tocopherol Values for Xylem, Phloem, and Leaf Tissue of Carrots

	lycopene	α-carotene	β-carotene	γ-tocopherol	α-tocopherol
			Xylem		
phytoene	0.00	–0.44***	–0.50***	nd	–0.41**
lycopene		–0.29***	–0.17	nd	–0.21*
α-carotene			0.92***	nd	0.65***
β-carotene				nd	0.52***
γ-tocopherol					nd
			Phloem		
phytoene	0.05	–0.50***	–0.56***	nd	–0.20*
lycopene		–0.29**	–0.21*	nd	–0.27**
α-carotene			0.92***	nd	0.28**
β-carotene				nd	0.15
γ-tocopherol					nd
			Leaf		
phytoene	0.04	0.05	–0.05	–0.10	0.01
lycopene		–0.10	0.03	–0.08	–0.01
α-carotene			–0.04	0.55***	0.28**
β-carotene				–0.07	0.65***
γ-tocopherol					0.00

[a] Asterisks (*, **, ***) indicate significance at $P < 0.05$, 0.01, and 0.001, respectively.

In xylem tissue α-tocopherol was highly positively correlated ($P < 0.001$) with α- and β-carotenes at $r = 0.65$ and $r = 0.52$, whereas in the phloem only α-carotene showed a highly significant ($P < 0.01$) correlation ($r = 0.28$) with α-tocopherol. The significant correlation of these compounds would allow for selection of increased α-tocopherol with both α- and β-carotenes in the xylem tissue, suggesting the possibility of selecting high levels of all three compounds or using carotenoid levels as an indicator of α-tocopherol levels. In both root tissue types

α-tocopherol showed significant negative correlations with both phytoene and lycopene. In leaf tissue, α-tocopherol was highly significantly correlated ($P < 0.01$) with α-carotene ($r = 0.28$) and very highly significantly ($P < 0.001$) correlated with β-carotene ($r = 0.65$). γ-Tocopherol was found only in leaf tissue, where it was very highly significantly ($P < 0.001$) correlated ($r = 0.55$) with α-carotene. The significant correlations of tocopherols with carotenoids in leaf tissue were possibly due to a common origin of their biosynthetic pathways (**Figure 1**) or parallel response to selection pressures due to the similar antioxidant roles both play in the photosynthetic process (*1*). Correlations followed similar patterns among all tissue types analyzed.

In the present study root xylem, root phloem, and leaf tissue from a range of carrot accessions were analyzed through a single-pass extraction and detection method for the quantification of both carotenoids and tocopherols (*21*). The positive correlations observed between α-tocopherol and α- and β-carotenes suggest that it may be possible to select for higher levels of α-tocopherol without losing any of the nutritive value of increased carotenoids levels, leading to a carrot high in both vitamin E (α-tocopherol) and provitamin A (α- and β-carotenes). Selecting for carrots high in both carotenoids and tocopherols could improve the value of this crop as a food source in the diet.

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