Comparative Carotenoid Accumulation and Retention in Near-isogenic *rprp* and *RPRP* Inbred Carrot Lines

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ABSTRACT. The *rp* allele causes a significant reduction in total carotenoid pigmentation in carrot (*Daucus carota* L.) roots. The objective was to investigate the effect of *rp* on the composition, accumulation, and retention of carotenoids in two near-isolines of carrot, W266*RPRP* and W266*rprp*, during vegetative growth and postharvest storage. Field experiments were conducted during 1996 and 1997 in which roots were sampled weekly from 62 to 100 days after seed-sowing and biweekly during postharvest storage at 4 °C up to 386 days after sowing. Linear increases in total carotenoid concentration were observed for W266*RPRP* and W266*rprp* during vegetative growth. The average daily rate of increase in total carotenoid concentration in W266*RPRP* and in W266*rprp* was 12.7 and 1.3 mg·g⁻¹ dry weight, respectively. A linear decrease in carotenoid concentration was measured for W266*RPRP* but not for W266*rprp* during postharvest storage. At 100 days after sowing, high-performance liquid chromatography analyses showed W266*rprp* had 20-fold lower concentrations of α -carotene and 50-fold lower concentrations of β -carotene in root tissue compared to W266*RPRP*. Levels of β -carotene and lutein in the first true leaves were reduced by $\approx$ 50% in W266*rprp* compared to W266*RPRP*. Results from this investigation suggest that the *rp* allele affects the concentration of root and foliage carotenoids, as well as the rate of carotenoid accumulation and degradation in carrot roots. The impact of the *rp* allele is far greater in root tissue than in foliage, suggesting it may act as a transcription factor or structural gene affecting primarily root carotenoid biosynthesis.

Carotenoids are widely distributed in nature. They are isoprenoid polyenes found in all photosynthetic organisms and in many nonphotosynthetic organisms (Spurgeon and Porter, 1983). Carotenoids have a very important role in photosynthesis and in photoprotection as antioxidants (Gross, 1991; Van Breemen, 1996). Carotenoids also play a significant role in human nutrition because all vitamin A comes through the diet. About 50 of the 600 carotenoids identified are precursors to vitamin A (Van Breemen, 1996). Carotenoids have also been implicated by several studies as potential anticarcinogenic agents (Bendich, 1990; Krinsky, 1990) although Blumberg (1994) observed that synthetic β -carotene supplementation resulted in an increase of lung cancer in humans. No adverse effects of natural-source β -carotene, when consumed from plant tissues, has been reported.

Carrots (*Daucus carota*) are an excellent source of carotenes (Simon and Wolff, 1987). Carrot roots come in many different colors and each color is associated with the presence of certain carotenoids, xanthophylls, or anthocyanins. In orange carrots, α -carotene accounts for 15% to 40% of total carotenoid content while β -carotene accounts for 45% to 80% and ζ -carotene for 2% to 10%. The remaining 3% to 6% is distributed between lycopene and γ -carotene and β -zeacarotene (Gross, 1991).

The total amount of carotenoids present in carrot roots is affected by both genetic and environmental factors. Herbicides and other chemicals, the length of postharvest storage, plant maturity, and other environmental factors can influence the total carotenoid content present in carrot roots. Maturity and temperature influence pigmentation but variations in photoperiod, soil type, pH and fertilizer levels seem to have little effect on carotenoid content

(Laferriere and Gabelman, 1968). During growth of the carrot plant, the level of most carotenes present in the root increased up to 140 d after planting (Lee, 1986). However, in two of the three cultivars studied, maximum α - and β -carotene levels were obtained after 110 d. Pepkowitz et al. (1944) suggested carotenoid content in carrot roots reaches a maximum at $\approx$ 90 to 100 d of field growth and then decreases. The discrepancy between the studies of Lee (1986) and Pepkowitz et al. (1944) is likely due to the use of different cultivars. Both authors noted the rate of accumulation of carotenoids in carrot roots is cultivar specific, and thus may be under genetic control. Le Dily et al. (1993) showed the total carotenoid content of carrot roots stored in cold storage at 0 to 1 °C for 209 d did not change. Conversely, Phan et al. (1973) suggested the change in total carotenoid content in carrot roots is tissue specific in cold storage. These workers found a decrease in xylem carotenoid levels and little changes in phloem carotenoid levels.

A limited amount of information exists on genetic control of carotenoid biosynthesis in carrot. Twenty-one genes corresponding to defined phenotypes have been described in carrot (Peterson and Simon, 1986; Simon, 1996); of which 10 are related to the synthesis of carotenes. There are at least two genes that determine the difference between orange and red root colors (Umiel and Gabelman, 1972). The *A* gene is involved in β -carotene synthesis but does not determine the orange phenotype. The *L* gene is involved in lycopene synthesis but does not determine the red color alone. The *Y₁* and *Y₂* loci influence the amount and distribution of α - and β -carotene (Kust, 1970). The *Y* locus blocks the synthesis of α - and β -carotene and xanthophylls, whereas *Y₁* and *Y₂* only block synthesis of carotenes but not xanthophylls (Buishand and Gabelman, 1979; Simon, 1996). Each of the dominant alleles at the *Y₁*, *Y₂*, and *Y* loci prevent formation of orange color in the xylem. Thus the *y₁y₁y₂y₂* genotype results in orange-rooted carrots and *Y₂Y₂* or *Y₂y₂* genotypes result in yellow-colored carrot roots (Bradeen et al., 1997). The *Io* and *O* genes are responsible for the intensity of orange color that develops when the *yyy₁y₁y₂y₂* genotype is present. The *Io* gene and the *O* gene might be identical to the *A* gene (Umiel and Gabelman, 1972).

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Goldman and Breitbach (1996) identified a recessive allele, *rp*, that resulted from a spontaneous mutation in the inbred line W266D. The *rp* allele causes a significant reduction in total carotenoid pigmentation in carrot roots. This allele is unique with respect to carrot root color genes because all white-rooted carrots were thought to be controlled by dominant alleles before this finding (Buishand and Gabelman, 1979; Imam and Gabelman, 1968; Laferriere and Gabelman, 1968; Peters, 1981). Carrots that carry the allele in the homozygous recessive condition have white leaves during early stages of development which is caused by a reduction in chlorophyll due to a reduction in photoprotection conferred by carotenoids.

Roots of *rrpp* plants are white at the beginning of the growing season but progressively develop low levels of carotenoid pigmentation. This increase in carotenoid pigmentation is especially evident during postharvest cold storage. Due to the late onset of accumulation of carotenoid pigmentation in *rrpp* carrot root, it was hypothesized that the rate of accumulation of α - and β -carotene was the only difference between *rrpp* and *RPRP* plants because the orange color in orange-rooted carrots is mainly caused by the presence of α - and β -carotene.

Spectrophotometry has been the conventional method of identifying carotenoids by comparing ultraviolet/vis absorption spectra with reference material (Winegrath et al., 1996). High-performance liquid chromatography (HPLC) has become a very powerful tool in carotenoid research (Guillou et al., 1993). Many isocratic or gradient elution reversed-phase HPLC methods have also been used in the separation of provitamin A carotenoids in other fruits and vegetables (Khachick and Beecher, 1988; Khachick et al., 1986; Wills et al., 1988).

Both fresh market and processed carrots may be stored for long periods of time before they are purchased or processed in North America. Studies on carotenoid biosynthesis, accumulation, and retention are important in providing information on how to maximize the nutritive value of carrots as well as in elucidation of carotenoid synthesis in a major, safe dietary source of provitamin A carotenoids. Therefore, the objectives of this study were to investigate the composition, accumulation, and retention of carotenoids in roots of two near-isogenic carrot genotypes: the orange-rooted inbred line W266*RPRP* and the reduced-pigment inbred line W266*rrpp*.

Materials and Methods

GENETIC MATERIALS. The *rrpp* and *RPRP* genotypes were represented by the near-isogenic lines W266*rrpp* and W266*RPRP*, which were obtained from the carrot breeding program at the University of Wisconsin, Madison. These genotypes were described by Goldman and Breitbach (1996). W266*rrpp* and W266*RPRP* are near-isogenic lines in an elite inbred line (W266D) genetic background.

PLANT CULTURE. The experiment was conducted in a carrot production field on muck soil in Randolph, Wis. during 1996 and 1997. Standard carrot production practices for Wisconsin were followed. A randomized complete block design with four replications was used. Treatments were arranged in a split plot design with accessions assigned randomly to whole plots and harvest dates assigned randomly to subplots. Seeds were sown 28 May 1996 and 28 May 1997, at a density of 43 seeds/m. Each plot consisted of two rows 3.7 m long. Five carrot roots were harvested from each plot on six harvest dates; 62, 69, 76, 83, 93, and 100 d after planting. At each harvest date, carrots were evaluated for carotenoid content promptly following harvest. At 100 d after sowing, all remaining roots were

harvested, placed in paper bags filled with wood shavings, and stored at 4 °C. Samples were removed from cold storage for carotenoid extraction at 114, 128, 156, 183, 295, and 386 d after sowing for the 1996 field experiment only. Plant samples were also harvested from field-grown plants at 60 d after sowing, to assess carotenoid concentration in the first several leaves.

SPECTROPHOTOMETRY. Carotene extraction was carried out at 24 °C under low light conditions according to the method described by Buishand and Gabelman (1979; modified by Simon and Wolff, 1987). A 2- to 3-g sample of fresh carrot, representing a cross-section of the root, was excised, freeze-dried, and ground at 25,000 rpm in a grinder (Cyclone; Virtis, Gardiner, N.Y.) for 3 min with hexane. The extract was then filtered twice by suction filtration and passed through Na₂SO₄. The final volume was adjusted to 100 mL with hexane and stored in amber bottles at 0 °C.

The absorption spectrum of W266*rrpp* was measured, and five absorption maxima were observed: 290, 347, 368, 448, and 475 nm. Samples were measured at $\lambda = 450$ nm on a spectrophotometer (Genesys 5; Spectronic Instruments, Rochester, New York) (Simon and Wolff, 1987) and estimation of total carotenoid concentration was obtained by comparing sample absorbance to a standard curve of β -carotene (Sigma, St. Louis, MO) (Simon and Wolff, 1987; Umiei and Gabelman, 1971). From the standard curve, absorbance was converted into micrograms per gram dry weight (DW) of total carotenoids.

HPLC. Extracts prepared from roots and first true leaves of W266*rrpp* and W266*RPRP* were subjected to HPLC analyses. The primary goal of these measurements was to assess α - and β -carotene concentrations. Lutein was used as an internal standard in these measurements according to the protocols of Thayer and Bjorkman (1990). First true leaves of W266*rrpp* exhibit distinct white and green sectors, due to degradation of chlorophyll caused by the severe reduction in carotenoids (Goldman and Breitbach, 1996). Tissue extracts were prepared from green and white sectors separately for W266*rrpp*. Preparation of the carrot root sample for HPLC analysis was modified from the method of Thayer and Bjorkman (1990). All extractions were performed in the dark at 4 °C. A sample of frozen root tissue (0.3 g) was first ground in liquid nitrogen with mortar and pestle and then in ethyl acetate with an electric drill. The mixture was centrifuged at 2,000 rpm for 2 min and the supernatant was removed and reserved. Additional ethyl acetate was added to the pellet and ground and centrifuged as before. The supernatant containing the carotenoid fraction was filtered and injected promptly into a 4.6 $\times$ 250-mm nonendcapped column (Zorbax ODS; Mac-Mond Analytical, Chadds Ford, Pa.). Lutein and α - and β -carotene standards (Sigma Chem.) were dissolved in ethyl acetate and injected at known concentrations and standard curves were generated. HPLC runs were monitored on a diode detector system (model 168; Beckman Coulter, Fullerton, Calif.) with an autosampler (model 507). Solvent A was 85% acetonitrile and 15% methanol; solvent B was 68% methanol and 32% ethyl acetate. Flow rate was 1 mL·min⁻¹ at 30 °C. The absorption spectra of the carotenoids was monitored at $\lambda = 445$ nm. Elution time was 41 min and data collection stopped at 35 min.

STATISTICAL ANALYSIS. The rate of total carotenoid accumulation and retention during vegetative growth and postharvest storage was analyzed via regression analysis on plot carotenoid means. Three replicates of each tissue portion (roots and first true leaves) for each genotype were assessed via HPLC analysis. Each carotenoid was compared to a standard curve generated from known concentrations of lutein, and α - and β -carotene and presented as a mean with an accompanying SE. Rate of α - and β -carotene accumulation and

Table 1. Concentrations (mean $\pm$ SE, $n = 2$) of α -carotene, β -carotene, and lutein in W266 $rprp$ and W266RPRP carrot roots and first true leaves assessed via reverse-phase liquid chromatography.

Genotype	Tissue	Concn (pmol mg ⁻¹ FW) ^z		
		α -Carotene	β -Carotene	Lutein
W266 $rprp$	Root	0.2 $\pm$ 0.3	0.4 $\pm$ 0.7	0.8 $\pm$ 1.4
W266RPRP	Root	9.0 $\pm$ 3.9	43.0 $\pm$ 10.3	0.0 $\pm$ 0.0
W266 $rprp$	First true leaf, white sectors	6.1 $\pm$ 5.4	27.5 $\pm$ 13.2	62.5 $\pm$ 21.0
W266 $rprp$	First true leaf, green sectors	11.8 $\pm$ 5.1	34.9 $\pm$ 15.5	126.8 $\pm$ 3.8
W266RPRP	Foliage ^y	6.7 $\pm$ 3.0	52.2 $\pm$ 1.6	81.8 $\pm$ 7.1

^zCarotenoid values based on conversion factors from Thayer and Bjorkman (1990).

^yRefers to leaf tissue from the third true leaf and subsequent mature leaves.

retention was analyzed via regression based on individual root samples. Data from vegetative growth and postharvest storage were analyzed separately. Linear or quadratic models were fitted where appropriate using plot means for each year separately ($P < 0.05$). Regression models of total carotenoid concentration in W266RPRP in 1996 and 1997 and total carotenoid concentration in W266 $rprp$ in 1996 and 1997 during vegetative growth were then compared by their slopes and y-intercepts. When the slope and the y-intercept of the 2 models were not significantly different ($P < 0.05$), data were combined over the 2 years and a linear model was fitted. In instances where neither a linear nor a quadratic model could be fitted ($P < 0.05$), a paired two-sample t test was used to compare total carotenoid concentration between 62 and 100 d after sowing for vegetative growth and between 114 d after sowing and the day of termination of the postharvest storage period. The ratio of α - to β -carotene was also determined.

Results

COMPARATIVE CAROTENOID CONCENTRATION ASSESSED VIA HPLC.

Alpha and beta-carotene concentrations were estimated in roots and first true leaves of W266 $rprp$ and W266RPRP genotypes (Table 1, Fig. 1). This analysis revealed 0.2 pmol·mg⁻¹ fresh weight (FW) of α -carotene in roots of W266 $rprp$ and more than 20-fold higher concentrations (9.0 pmol·mg⁻¹ FW) of α -carotene in W266RPRP. Concentrations of β -carotene were 0.4 pmol·mg⁻¹ FW in W266 $rprp$ and more than 50-fold higher (43.0 pmol·mg⁻¹ FW) in W266RPRP. Concentrations of lutein were 0 in W266RPRP and 0.8 pmol·mg⁻¹ FW in W266 $rprp$, demonstrating a near lack of lutein in roots of these genotypes.

Concentrations of α - and β -carotene were similar in roots and foliage of W266RPRP, but drastically different in roots and foliage of W266 $rprp$. Concentrations of α -carotene were similar in first true leaves of both genotypes. Although higher concentrations of β -carotene were measured in green sectors of leaves from W266 $rprp$, large SE values suggest that β -carotene concentrations did not vary greatly between both green and white sectors from first true leaves of W266 $rprp$, and first true leaves of W266RPRP. Whereas carotenoid levels were extremely low in roots of W266 $rprp$, similar reductions were not noted in foliage. White sectors from the first true leaf of W266 $rprp$ exhibited 27.5 pmol·mg⁻¹ FW β -carotene and 62.5 pmol·mg⁻¹ FW lutein, while green sectors exhibited 34.9 and 126.8 pmol·mg⁻¹ FW, respectively, for the same compounds. These measures were closer to the range of values observed in foliage of W266RPRP, although reduced by about one-half for β -carotene and lutein in white sectors of W266 $rprp$. The ratio between α - and β -carotene varied from 1:5 to 1:6 throughout vegetative growth in W266RPRP (data not presented).

CAROTENOID ACCUMULATION DURING VEGETATIVE GROWTH. Total carotenoid concentration increased in a linear fashion in roots of both W266 $rprp$ and in the orange-rooted carrot, W266RPRP, during vegetative growth (Table 2, Fig. 2). The rate of increase and total carotenoid concentration were different between the two near-isolines. The total carotenoid concentration in roots of W266RPRP was 10 times greater than W266 $rprp$ throughout the 100 d growing season. The average daily rate of increase in total carotenoid concentration for roots of W266RPRP was 12.6 mg·g⁻¹ DW and for W266 $rprp$ was 1.3 mg·g⁻¹ DW.

CAROTENOID RETENTION DURING POSTHARVEST STORAGE. Total carotenoid concentration decreased in a linear fashion in roots of W266RPRP, but not for roots of W266 $rprp$ (Fig. 3). Despite this significant linear trend for W266RPRP, only small changes in carotenoid concentration were detected for W266RPRP between 114 and 295 d after sowing. The greatest amount of change was observed between 295 and 386 d after sowing, when carotenoid concentration decreased 60% compared to what was present at the beginning of storage (Table 3, Fig. 3). Taking into account the entire postharvest period, the average daily rate of decrease in total carotenoid concentration in W266RPRP was 2.68 mg·g⁻¹ DW.

At 386 d after sowing, average total carotenoid concentration in roots of W266RPRP was eight times greater than in W266 $rprp$. Between 114 and 386 d after sowing, the average total carotenoid concentration of roots in W266 $rprp$ dropped from 95.5 to 60.9

Fig. 1. HPLC chromatograms of α - and β -carotene peaks from root-tissue extracts of W266RPRP (top) and W266 $rprp$ (bottom) carrot at 100 d after planting. HPLC conditions described in materials and methods. Elution time in minutes. Arrows indicate elution of a peak containing both α - and β -carotene.

Table 2. Mean squares and F values from regression of total carotenoid concentration for a) W266RPRP during vegetative growth in 1996 and 1997, b) W266rprp during vegetative growth in 1996 and 1997, c) W266RPRP during postharvest storage in 1996, and d) W266rprp during postharvest storage in 1996

Source of variation	df	MS	F	P
a) Regression ²	1	1,311,041	26.8	0.0001
b) Regression	1	13,690	16.0	0.0001
c) Regression	1	894,520	17.5	0.0001
d) Regression	1	193	0.3	0.58

²Error degrees of freedom for a and b are 47. Error degrees of freedom for c and d are 21 and 17, respectively.

mg·g⁻¹ DW but this decrease in total carotenoid concentration was not significant at $P < 0.05$ according to the paired two sample *t* test.

Discussion

The primary focus of this study was to investigate the effect of the reduced pigment (*rp*) allele on accumulation and retention of carotenoids in the two near-isolines, W266RPRP and W266rprp. According to Gross (1991), in orange carrots, α - and β -carotene account for 15% to 40% and 45% to 80% of total carotenoid content, respectively. Because carotenoids other than α - and β -carotene are present in carrot roots, spectrophotometric methods may underestimate total carotenoid concentration. It should be noted that the spectrophotometric and HPLC measurements of total carotenoid concentration were only an approximation because as Craft et al. (1992) had found, the solubility of β -carotene in hexane and in acetonitrile is very low. These solvents are, however, the typical solvents used by most researchers for carotenoid extraction. Dihydroxy and more polar carotenoids also have poor solubility in hexane, thus the spectrophotometric measurements of total carotenoid concentration could have been an underestimation. β -Carotene is much more soluble in other solvents such as tetrahydrofuran (THF) and chloroform but such solvents are not used due to their volatility.

CAROTENOID IDENTIFICATION AND ACCUMULATION DURING VEGETATIVE GROWTH. Simon and Wolff (1987) showed that there is a tendency to underestimate total carotenoid content from spectrophotometry as compared to summing individual carotenoids separated with HPLC. Because total carotenoid estimation was based upon a standard curve of pure β -carotene at $\lambda = 450$ nm, they suggested that the underestimation of total carotenoid content from spectrophotometry may be due to the higher absorbance of β -carotene than all other carotenoids in carrot at $\lambda = 450$ nm; hence, all other carotenes were less efficiently estimated. It was expected that measurements of total carotenoid concentration from HPLC analyses will be consistently higher than that obtained from spectrophotometric analyses.

Spectrophotometric and HPLC analyses provided different information on the *rp* allele and its impact on carotenoid biosynthesis in carrot. Spectrophotometric analyses quantified the extent to which the *rp* allele reduces net carotenoid biosynthesis in W266rprp. A basis for this was already shown by Goldman and Breitbach (1996), however their measurement was only at 120 d after planting. W266RPRP contained about ten times more total carotenoids than W266rprp at any given point in time during vegetative growth and postharvest storage. Consistent with Pepkowitz et al. (1944), where carotenoid content in carrot roots reached a maximum at ≈ 90 to 100 d of field growth, total carotenoid concentration in both W266RPRP and W266rprp increased up to 100 d after planting (Fig. 2). Total

carotenoid concentration in W266RPRP and in W266rprp increased at a daily rate of 12.7 and 1.3 mg·g⁻¹ DW, respectively. Total carotenoid accumulation during vegetative growth between the mutant and the wild type follow the same trend but at a reduced rate in W266rprp. This suggests enzyme activity and/or substrate level is lower in W266rprp than in W266RPRP. One explanation for the difference in rate of carotenoid accumulation may be an altered binding site of a key enzyme that causes a reduction in efficiency. For example, in maize (*Zea mays* L.), a mutation at the *shrunk 2* (*SH2*) allele causes a reduction of starch levels in the endosperm due to an altered subunit of the starch synthetic enzyme ADP-glucose pyrophosphorylase (Bhave et al., 1990).

A test of the two slopes indicated the rate of increase in total carotenoid concentration in both W266rprp and W266RPRP during vegetative growth was not significantly different between 1996 and 1997. This suggests accumulation of total carotenoid in these genotypes was not subject to significant genotype $\times$ year interactions in this investigation. However, these 2 years did not represent disparate environments for Wisconsin carrot production at the locations tested, as evidenced by standard check cultivars in an adjacent hybrid trial in both years. If total carotenoid accumulation in carrot roots of inbred lines are generally not subject to significant genotype $\times$ environment interaction, and if this carries over to hybrid cultivars, then farmers can predict the nutritional value of the crop with some reliability. Based on spectrophotometric methods, total carotenoid concentration in both W266rprp and W266RPRP increased linearly during vegetative growth.

Fig. 2. Plots of total carotenoid concentration during vegetative growth in roots of (A) W266RPRP and (B) W266rprp carrot during vegetative growth in 1996 and 1997.

Table 3. Comparison of total carotenoid concentration (mean $\pm$ SE, $n = 8$) from spectrophotometric analyses during vegetative growth and postharvest storage of W266RPRP and W266rprp carrot in 1996.

Days after sowing	Total carotenoid concn ² (mg·g ⁻¹ FW)	
	W266RPRP	W266rprp
62	44.4 $\pm$ 12.5	3.1 $\pm$ 0.6
69	82.6 $\pm$ 12.5	9.5 $\pm$ 2.2
76	84.5 $\pm$ 24.7	9.4 $\pm$ 3.2
83	86.5 $\pm$ 22.0	6.6 $\pm$ 2.5
93	134.0 $\pm$ 34.3	15.5 $\pm$ 2.9
100	96.6 $\pm$ 72.1	8.6 $\pm$ 8.3
128	136.0 $\pm$ 27.7	11.5 $\pm$ 5.1
156	150.0 $\pm$ 27.3	8.1 $\pm$ 1.5
183	141.2 $\pm$ 20.1	7.9 $\pm$ 1.8

²Includes all carotenoids extracted by hexane, as described in Materials and Methods.

CAROTENOID RETENTION DURING POSTHARVEST STORAGE. Although accumulation of total carotenoids between W266rprp and W266RPRP followed the same trend, the two near-isolines behaved somewhat differently in the retention of total carotenoids during postharvest storage. Total carotenoid concentration in both isolines did not decrease appreciably during the period from 114 to 295 d after sowing (Fig. 3). Between 295 and 396 d after sowing only, a significant decrease in carotenoid concentration was noted for W266RPRP only. In general, our results agree with those of Le Dily et al. (1993), who found total carotenoid content in carrot roots stored in cold storage for 209 d did not change. Meanwhile, Lee (1986) suggested that both α - and β -carotene levels increased up to 100 to 125 d in storage at 2 °C and then decreased. Other carotenoids, such as β -zeacarotene and γ -carotene, also increased and reached a maximum level at 125 d and 50 to 70 d, respectively, in storage (Lee, 1986). Brown (1949) also suggested an increase in carotenoid content in carrot roots during storage for the first 20 weeks of storage, then remained constant until 30 weeks of storage when the study was terminated. Kopas-Lane and Warthesen (1995), on the other hand, found that α -carotene content in carrot roots stored in either lighted or dark cold storage did not change whereas 9-cis β -carotene level decreased significantly in lighted cold storage.

The discrepancy between different studies may be explained by the different storage conditions, such as temperature, humidity, and light, and of different cultivars by different authors. It should be noted that W266RPRP and its near-isoline counterpart W266rprp are inbred lines, whereas other authors used 'Nantes', 'Chantenay', and 'Tendersweet', which are open-pollinated cultivars. An inbred line is usually derived from sib-mating several plants from an open-pollinated population for several generations. Due to inbreeding depression, carrot inbreds are more uniform but less productive than their hybrid or open-pollinated counterparts. As suggested by Lee (1986), Pepkowitz et al. (1944), and Le Dily et al. (1993), accumulation and retention of carotenoids is cultivar specific and thus may be under genetic control. These contradictory results also suggest that carotenoid biosynthesis, accumulation, and retention in carrot roots is very complex and probably controlled by a number of genes.

An explanation for the decrease in total carotenoid concentration in W266RPRP is that carrot roots in postharvest storage are still respiring and carotenoids might be broken down or oxidized during this process. Oxygenases are responsible for cleavage of carotenoids in plants (Enzell, 1985). Characteristic cleavage products of carotenoids have been detected in roses (*Rosa* L. sp.) and in saffron

(*Crocus sativus* L.) (Lutz and Winterhalter, 1992). However, the degradation of carotenoids in carrot is not well understood.

CAROTENOID PROFILES OF W266rprp AND W266RPRP. Concentrations of α -carotene were more than 20-fold greater in roots of W266RPRP than in W266rprp. Concentrations of β -carotene were more than 50-fold greater in roots of W266RPRP than in W266rprp. Previous estimates by Goldman and Breitbach (1996) based on spectrophotometric data demonstrated an approximate 12-fold reduction in total carotenoids in W266rprp compared to W266RPRP. However, this measurement was based on roots sampled at 100 d after sowing. As suggested from spectrophotometric analyses in this study, carotenoid concentrations can increase appreciably throughout the growing season. Samples assessed by HPLC (Table 1) in this investigation were from plant tissue harvested at $\approx$ 60 d after sowing. Data in Table 2 indicate nearly 4-fold increases in carotenoid levels from 60 to 100 d after sowing, and this difference may be responsible for the discrepancy noted between HPLC and spectrophotometric analyses.

The primary difference between carotenoid profiles in W266RPRP and W266rprp was differential concentrations in roots and foliage for W266rprp. Carotenoid levels were more reduced in roots of W266rprp than foliage, suggesting that the *rp* allele acts primarily on root tissue. Concentrations of β -carotene and lutein in white sectors of the first true leaves of W266rprp were $\approx$ 50% as high as those from W266RPRP, which differs greatly from the 50-fold reduction in β -carotene measured for roots of W266rprp. Therefore, the *rp* allele primarily reduces the amount of carotenoids in roots, and secondarily affects concentrations of

Fig. 3. Plots of total carotenoid concentration during postharvest storage in roots of (A) W266RPRP and (B) W266rprp carrot in 1996.

carotenoids in foliage, but not in older leaves. This may be similar to the action of the *Y* allele, which can eliminate production of carotenoids in roots but not in foliage. Foliage levels of carotenoids in plants carrying various alleles at the *Y* locus have not been evaluated. The *rp* allele may be a lesion in a gene of the carotenoid biosynthetic pathway that results in a significant reduction in carotenoid levels. If carotenoid levels in foliage of plants carrying *YY* are not reduced compared to *yy* plants, it is likely that the *Y* gene is a regulatory gene controlling carotenoid biosynthesis in the roots. Given that scenario, it is possible that the *rp* allele could be a transcription factor affecting *Y*. Further investigation of carotenoid concentrations in various *RP* and *Y* genotypes is necessary to determine if this hypothesis has merit.

An intriguing possibility for the function of the *rp* allele is that carotenoid cleavage or degradation is enhanced in *rprp* genotypes compared to those carrying *RP*, and that both *rprp* and *RP* genotypes manufacture similar amounts of carotenoids. In this scenario, *rp* would not represent a lesion in carotenoid biosynthesis, but instead would constitute a mechanism for reducing carotenoids once they have been manufactured. Future experiments designed to genetically dissect the effect of *rp* on the carotenoid biosynthetic pathway may yield answers to the question of whether *rp* prevents or degrades root carotenoids.

The decrease in total carotenoid concentration in carrot roots during postharvest storage might be of importance to consumers and to processors. In North America, roots are stored for long periods of time before they are purchased or processed and as the storage period increases, the nutritive value of the carrot roots decreases. At 386 d after sowing, total carotenoid concentration in W266RPRP decreased to about the same level as 62 d after sowing. Thus, optimal carotenoid content in this inbred line was observed from 100 d to 114 d after sowing. The increase of total carotenoid concentration in W266RPRP and W266rprp during vegetative growth and maximization around 100 d suggests that, to maximize the nutritive value of carrot roots for consumers, later harvest should be favored.

Results from this investigation demonstrate the *rp* allele affects the concentration of carotenoids in the first leaves, as well as the rate of net carotenoid accumulation in carrot roots. The impact of the *rp* allele is far greater in root tissue than in foliage, suggesting it may act as a transcription factor or structural gene affecting primarily root carotenoid biosynthesis.

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