Genetic Variation in Carotenoid Concentrations among Diploid and Amphidiploid Rapid-cycling *Brassica* Species

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Abstract. Vegetable crops can be significant sources of nutritionally important dietary carotenoids, and *Brassica* are sources that also exhibit antioxidant and anticarcinogenic activity. The family Brassicaceae contains a diverse group of plant species commercially important in many parts of the world. The six economically important *Brassica* species are closely related genetically. Three diploid species (*B. nigra*, *B. rapa*, *B. oleracea*) are the natural progenitors of the amphidiploid species (*B. juncea*, *B. napus*, *B. carinata*). The objective of this study was to characterize the accumulation of important dietary carotenoid pigments among the genetically related *Brassica* species. High-performance liquid chromatographic quantification revealed significant differences in carotenoid and chlorophyll pigment concentrations among the *Brassica* species. *Brassica rapa* accumulated the highest concentrations of antheraxanthin [0.79 mg/100 g fresh weight (FW)], lutein (8.89 mg/100 g FW), and zeaxanthin (0.75 mg/100 g FW). The highest concentrations of β -carotene (4.41 mg/100 g FW) and total chlorophyll (125.9 mg/100 g FW) were found in *B. juncea*. *Brassica nigra* accumulated the highest concentrations of 5,6-epoxylutein (0.41 mg/100 g FW) and violaxanthin (2.28 mg/100 g FW), whereas *B. oleracea* accumulated the highest concentrations of neoxanthin (2.10 mg/100 g FW). For many of the pigments analyzed, the amphidiploids *B. carinata* and *B. napus* accumulated significantly less carotenoid concentrations than the diploid species and *B. juncea*. *Brassica* convey unique health attributes when consumed in the diet. Identification of genetic relationships among the *Brassica* species would be beneficial information for improvement programs designed to increase carotenoid values.

The family Brassicaceae (Cruciferae) represents a diverse group of plant species commercially important in many parts of the world. The plants produce condiment mustard; leafy, stored, processed, and picked vegetables; seed oils for margarine, salad oils, cooking oils, and industrial uses; animal feeders; and green manure crops (Williams and Hill, 1986). The six economically important *Brassica* species in world production are genetically related. Three diploid species—*B. nigra*, *B. rapa*, *B. oleracea*—are the natural progenitors of the amphidiploids species: *B. juncea*, *B. napus*, and *B. carinata*. The “cole crops,” such as broccoli, brussels

sprouts, cabbage, cauliflower, curly kale, and kohlrabi, make up *B. oleracea*. Oil seed types, as well as Chinese cabbage, turnip, and pak-choi are found within *B. rapa*. *Brassica nigra*, popularly known as black mustard, is used for its seed oil content. The cross between *B. nigra* and *B. oleracea* produces *B. carinata*. It is a tall, leafy plant mainly restricted to Ethiopia. The cross between *B. oleracea* and *B. rapa* produces *B. napus*. Rutabagas are a variety of *B. napus*, as well as the economically important oilseed rape. The cross between *B. rapa* and *B. nigra* produces *B. juncea*, the group known as mustard. The mustards are consumed in the southern United States as mustard greens, or are used as a condiment or spice (Williams and Hill, 1986).

The three common diploid species in the genus *Brassica* have haploid numbers of 8 (*B. nigra*), 9 (*B. oleracea*), and 10 (*B. rapa*) (Nwankiti, 1970). It is believed that these numbers represent a phylogenetically ascending series (Manton, 1932). The higher chromosome number species in the genus are

believed to be amphidiploids and have haploid numbers of 17 (*B. carinata*), 18 (*B. juncea*), and 19 (*B. napus*). The genetic arrangement of the diploid and amphidiploid *Brassica* species is credited to the U (1935). The diagram is referred to as the triangle of U (Nwankiti, 1970) (Fig. 1). Study of interspecific hybridization and subsequent pairing within the genus led to the designation of two distinct groups: 1) monogenomic diploids of *B. nigra* ($2n = 16$; BB), *B. oleracea* ($2n = 18$; CC), and *B. rapa* ($2n = 20$; AA); and 2) amphidiploids of *B. juncea* ($2n = 36$; AABB), *B. carinata* ($2n = 34$; BBCC), and *B. napus* ($2n = 38$; AACC) (Prakash and Hinata, 1980).

Carotenoids are C_{40} isoprenoid polyene plant secondary compounds that form lipid-soluble yellow, orange, and red pigments (Zaripheh and Erdman, Jr., 2002). The carotenoids can be divided into two groups: 1) hydrocarbon carotenes ($C_{40}H_{56}$) and 2) their oxygenated derivative, referred to as xanthophylls. Carotenoids span the thylakoid membranes of chlorophyll (Chl) complexes and function in accessory roles for light harvesting, photoprotection, and structural stabilization (Demmig-Adams et al., 1996; Tracewell et al., 2001). Carotenoid pigments protect photosynthetic structures by quenching excited triplet Chl (^3Chl) to dissipate excess energy (Frank and Cogdell, 1996) and by binding singlet oxygen ($^1\text{O}_2$) to inhibit potential oxidative damage (Demmig-Adams et al., 1996; Tracewell et al., 2001). Carotenoids are produced in the plastids and are derived from isopentenyl diphosphate (IPP). In the first step in biosynthesis, IPP is isomerized to dimethylallyl diphosphate, which becomes the substrate for the C_{20} geranylgeranyl diphosphate (GGPP) (Bramley, 2002). The first step unique to carotenoid biosynthesis is the condensation of two molecules of GGPP to form the first C_{40} carotenoid, phytoene, via phytoene synthase (Gross, 1991). The carotenoid pathway branches at the cyclization reactions of lycopene to produce carotenoids with either two β -rings (e.g., β -carotene, zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin) or carotenoids with one β -ring and one ϵ -ring (e.g., α -carotene and lutein) (Cunningham, 2002) (Fig. 2).

Fig. 1. Cytogenetic relationships between the diploid and amphidiploid *Brassica* species. Adapted from U (1935).

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Fig. 2. A simplified version of the carotenoid biosynthetic pathway in plants. DMAPP, dimethylallyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate. Ethyl beta-apo-8'-carotenoate used as internal standard.

There has been increased interest in the nutritional and medicinal importance of dietary carotenoids (Faulks and Southon, 2005; Frazer and Bramley, 2004; Yeum and Russell, 2002). Dietary intake of carotenoids has been associated with reduced risk of lung cancer and chronic eye diseases, including cataract and age-related macular degeneration (Johnson et al., 2000; Le Marchand et al., 1993). Studies have indicated that consumption of a variety of vegetables providing a mixture of carotenoids was more strongly associated with disease reduction than individual carotenoid supplements (Johnson et al., 2000; Le Marchand et al., 1993). Plants can be significant sources of carotenoids in the diet, and *Brassica* vegetables are relatively abundant sources that exhibit antioxidant and anticarcinogenic activity (Kurilich et al., 1999). Therefore, the objective of this study was to characterize the variation in

accumulation of important dietary carotenoids among the six genetically related *Brassica* species.

Materials and Methods

Seeds of rapid-cycling populations of *B. nigra*, *B. rapa*, *B. oleracea*, *B. juncea*, *B. napus*, and *B. carinata* (Crucifer Genetics Cooperative, Department of Plant Pathology, The University of Wisconsin, Madison, Wisc.) were sown into 2.5 × 2.5-cm growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and germinated in a growth chamber room (Environmental Growth Chambers, Chagrin Falls, Ohio) at 23 °C/16 °C day/night under a 16-h photoperiod on 2 Dec. 2004 and again on 11 Jan. 2005. Photosynthetically active radiation at the canopy height was 1000 μmol·m⁻²·s⁻¹ (LI-188B; LI-COR, Inc., Lincoln, Neb.). The first true leaves appeared

4 to 7 d after planting (DAP). Nutrients were applied as needed with 200 mg·L⁻¹ Peter's 20N-6.9P-16.6K water-soluble fertilizer (The Scotts Company, Marysville, Ohio). At 14 DAP, the plantlets were transferred to 11-L containers (Rubbermaid Inc., Wooster, Ohio) filled with 9 L of a nutrient solution (Hoagland and Arnon, 1950). Elemental concentrations of the nutrient solutions were N (105 mg·L⁻¹), P (15.3 mg·L⁻¹), K (117.3 mg·L⁻¹), Ca (80.2 mg·L⁻¹), Mg (24.6 mg·L⁻¹), S (32.0 mg·L⁻¹), Fe (0.5 mg·L⁻¹), B (0.25 mg·L⁻¹), Mo (0.005 mg·L⁻¹), Cu (0.01 mg·L⁻¹), Mn (0.25 mg·L⁻¹), and Zn (0.025 mg·L⁻¹). Solutions were aerated with an air blower (model 25E133W222; Spencer, Winsor, Conn.) connected to air stones. Water was added daily to maintain initial solution volumes in each container.

Each experimental replication consisted of three randomized blocks, with plots

consisting of individual containers holding six plants of one *Brassica* species, each placed into 2-cm round holds set at 10.6×9.5 -cm spacing on the lid. Plants were harvested just before anthesis 27 DAP (*B. rapa*, *B. juncea*, *B. nigra*) and 34 DAP (*B. carinata*, *B. napus*, *B. oleracea*). Leaf tissue was removed from six plants for each *Brassica* species and was immediately stored at -80°C before lyophilization. The frozen plant samples were lyophilized for a minimum of 72 h (model 6L FreeZone; LabConCo, Kansas City, Mo.).

Carotenoid/chlorophyll analysis. Plant pigments were extracted from freeze-dried tissues according to Kopsell et al. (2004) and analyzed according to Emehiser et al. (1996). A 0.10-g subsample was rehydrated with 0.8 mL ultra pure H_2O at 40°C for 20 min. After incubation, 0.8 mL of the internal standard ethyl- β -8'-apo-carotenolate (Sigma Chemical Co., St. Louis) was added to determine extraction efficiency. A total of 2.5 mL tetrahydrofuran (THF) stabilized with 25 ppm 2,6-Di-*tert*-butyl-4-methoxyphenol was also added. The sample was homogenized in a Potter-Elvehjem (Kontes, Vineland, N.J.) tissue grinding tube using ≈ 25 insertions with a pestle attached to a drill press (Sears, Roebuck and Co., Hoffman Estates, Ill.) set at 540 rpm. During homogenization, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical centrifuge for 3 min at $500 g_n$. The supernatant was removed and the sample pellet was resuspended in 2 mL THF and homogenized again with the same extraction technique. The procedure was repeated for a total of four extractions to obtain a colorless supernatant. The combined supernatants were reduced to 1.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation, Berlin, Mass.) in a water bath set at 40°C , and was brought up to a final volume of 5 mL with methanol (MeOH). A 2-mL aliquot was filtered through a $0.2\text{-}\mu\text{m}$ polytetrafluoroethylene (PTFE) filter (model Econofilter PTFE 25/20; Agilent Technologies, Wilmington, Del.) using a 5-mL syringe (Becton, Dickinson and Company, Franklin Lakes, N.J.) before analysis using high-performance liquid chromatography (HPLC).

An Agilent 1100 series HPLC unit with a photodiode array detector (Agilent Technologies, Palo Alto, Calif.) was used for pigment separation. Chromatographic separations were achieved using an analytical scale ($4.6\text{ mm i.d.} \times 250\text{ mm}$) of $5\text{ }\mu\text{m}$ and a $200\text{-}\text{\AA}$ polymeric C_{30} reverse-phase column (ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, Pa.), which allowed for effective separation of chemically similar carotenoid compounds (Fig. 3). The column was equipped with a guard cartridge ($4.0\text{ mm i.d.} \times 10\text{ mm}$) and holder (ProntoSIL), and was maintained at 30°C using a thermostat column compartment. All separations were achieved isocratically using a binary mobile phase of 11% methyl *tert*-butyl ethanol, 88.9% MeOH, and 0.1% triethylamine (v/v). The flow rate was $1.0\text{ mL}\cdot\text{min}^{-1}$, with a run time of 53 min, followed by a 10-min

Fig. 3. High-performance liquid chromatogram of *Brassica juncea* at 453 nm using a C_{30} column. Retention times for the pigments were violaxanthin, 5.520 min; neoxanthin, 5.813 min; antheraxanthin, 7.586 min; 5,6-epoxylutein, 7.894 min; chlorophyll b, 8.511 min; lutein, 9.334 min; zeaxanthin, 11.309 min; chlorophyll a, 13.900 min; ethyl- β -8'-apo-carotenolate (internal standard), 19.326 min; and β -carotene, 51.463 min. High-performance liquid chromatographic conditions are described in the text.

equilibration before the next injection. Eluted compounds from a $20\text{-}\mu\text{L}$ injection loop were detected at 453 nm (carotenoids and internal standard), 652 nm (Chl *a*), and 665 nm (Chl *b*), and data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Internal standard percent recovery ranged from 62% to 100%, with a mean for all samples at 80%. Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards (antheraxanthin, neoxanthin, 5,6-epoxylutein, lutein, violaxanthin, zeaxanthin from Carotenature, Lupsingen, Switzerland; β -carotene, Chl *a*, Chl *b* from Sigma Chemical Co.). The concentration of the external pigment standards were determined spectrophotometrically using the following $E_{1\text{cm}}^{1\%}$ values: antheraxanthin, 2349 in ethanol (ETOH), $\lambda_{\text{max}} = 446\text{ nm}$; neoxanthin, 2270 in ETOH, $\lambda_{\text{max}} = 438\text{ nm}$; 5,6-epoxylutein, 2463 in ETOH, $\lambda_{\text{max}} = 440\text{ nm}$; lutein, 2468 in ETOH, $\lambda_{\text{max}} = 440\text{ nm}$; violaxanthin, 2499 in ETOH, $\lambda_{\text{max}} = 441\text{ nm}$; zeaxanthin, 2540 in ETOH, $\lambda_{\text{max}} = 452\text{ nm}$; β -carotene, 2592 in hexane, $\lambda_{\text{max}} = 452\text{ nm}$; Chl *a*, 819 in ETOH, $\lambda_{\text{max}} = 665\text{ nm}$; and Chl *b*, 441 in ETOH, $\lambda_{\text{max}} = 649\text{ nm}$ (Davies and Köst, 1988). Spinach standard reference material (Slurried Spinach 2385, National Institute of Science and Technology, Gaithersburg, Md.) was used for method validation.

Data sets were analyzed by the GLM procedures of SAS (Cary, N.C.) with species means separated by Duncan's multiple range test ($\alpha = 0.05$). Data were recorded on both a fresh weight (FW; in milligrams per 100 g) and dry weight (DW; in milligrams per gram) basis.

Results and Discussion

Carotenoid pigment accumulation in leaf tissues differed among the *Brassica* species (Table 1). Lutein accumulation ranged from $8.89\text{ mg}/100\text{ g FW}$ (*B. rapa*) to $5.65\text{ mg}/100\text{ g FW}$ (*B. carinata*). Leaf tissue lutein averaged $8.64\text{ mg}/100\text{ g FW}$ among the diploid *Brassica* species (*B. rapa*, *B. nigra*, *B. oleracea*)

and $7.35\text{ mg}/100\text{ g FW}$ among the amphidiploid species (*B. juncea*, *B. carinata*, *B. napus*). Carotenoid 5,6-epoxides are widespread in nature. Their conversion is mediated by a mixed-function oxygenase, which requires nicotinamide adenine dinucleotide phosphate (NADPH) and O_2 as cofactors (Goodwin, 1980). The accumulation of 5,6-epoxylutein ranged from $0.41\text{ mg}/100\text{ g FW}$ (*B. nigra*) to $0.25\text{ mg}/100\text{ g FW}$ (*B. carinata*). The accumulation of 5,6-epoxylutein averaged $0.40\text{ mg}/100\text{ g FW}$ for the diploid *Brassica* species and $0.33\text{ mg}/100\text{ g FW}$ for the amphidiploid species.

The accumulation of β -carotene was similar for *B. rapa*, *B. juncea*, *B. nigra*, and *B. oleracea*, and was significantly less for *B. carinata* and *B. napus* (Table 1). The accumulation of β -carotene averaged $3.93\text{ mg}/100\text{ g FW}$ for the diploid *Brassica* species and $3.14\text{ mg}/100\text{ g FW}$ for the amphidiploid species. Insertion of the oxygen function in the carotenoid biosynthetic pathway occurs after the cyclization of lycopene (Cunningham and Gantt, 1998). Zeaxanthin forms from the hydroxylation of β -carotene. Only one *Brassica* species (*B. rapa*) accumulated significantly higher zeaxanthin concentrations than all the other species. The accumulation of zeaxanthin averaged $0.46\text{ mg}/100\text{ g FW}$ for the diploid *Brassica* species and $0.28\text{ mg}/100\text{ g FW}$ for the amphidiploid species. Epoxidation of zeaxanthin, via antheraxanthin, results in the formation of violaxanthin. The rearrangement of the epoxide group is the proposed mechanism for the conversion of violaxanthin to neoxanthin (Gross, 1991). Antheraxanthin accumulation ranged from $0.79\text{ mg}/100\text{ g FW}$ (*B. rapa*) to $0.41\text{ mg}/100\text{ g FW}$ (*B. napus*). The accumulation of antheraxanthin averaged $0.62\text{ mg}/100\text{ g FW}$ for the diploid *Brassica* species and $0.55\text{ mg}/100\text{ g FW}$ for the amphidiploid species.

The built-in ability for photoprotective energy dissipation within the photosynthetic apparatus of higher plants occurs through reversible changes in the violaxanthin content in the leaves. The xanthophyll, or violaxanthin, cycle is involved in the deepoxidation of violaxanthin in high light conditions, and

the reoxidation of zeaxanthin in dark conditions via antheraxanthin (Demmig-Adams and Adams, 1996; Gross, 1991). The accumulation of violaxanthin in *B. juncea* and *B. nigra* was significantly higher than the other *Brassica* species. The accumulation of violaxanthin averaged 1.71 mg/100 g FW for the diploid *Brassica* species and 1.47 mg/100 g FW for the amphidiploid species. The accumulation of neoxanthin also differed among the *Brassica* species. The accumulation of neoxanthin averaged 2.00 mg/100 g FW for the diploid *Brassica* species and 1.68 mg/100 g FW for the amphidiploid species.

The *Brassica* species accumulated much higher concentrations of Chl pigments. Chlorophyll pigments differed among the *Brassica* species (Table 2). Chlorophyll *a* accumulation ranged from 100.1 mg/100 g FW (*B. juncea*) to 52.4 mg/100 g FW (*B. carinata*). Leaf tissue Chl *a* averaged 93.4 mg/100 g FW among the diploid *Brassica* species and 73.6 mg/100 g FW among the amphidiploid species. Chlorophyll *b* accumulation ranged from 28.8 mg/100 g FW (*B. oleracea*) to 18.3 mg/100 g FW (*B. carinata*). The accumulation of Chl *b* averaged 26.2 mg/100 g FW for the diploid *Brassica* species and 22.5 mg/100 g FW for the amphidiploid species. Total Chl concentrations ranged from 125.9 mg/100 g FW (*B. juncea*) to 71.7 mg/100 g FW (*B. carinata*). Total leaf tissue Chl averaged 119.5 mg/100 g FW among the diploid *Brassica* species and 96.1 mg/100 g FW among the amphidiploid species.

Vegetable tissues can be dried and encapsulated for use as lutein and β -carotene dietary antioxidant supplements. Pool-Zobel et al. (1997) and Müller et al. (1999) conducted experiments to determine whether carotenoids could protect against DNA damage and oxidative stress. The researchers used dried spinach powder (obtained from Völpel GmbH, Königsmoos, Germany) to assess antioxidant potential. The lutein concentration for the dried spinach was 11.3 mg/10 g, whereas the β -carotene concentration was 3.1 mg/10 g. The dried spinach increased serum lutein and β -carotene twofold over the 2-week feeding intervention, and significantly reduced the number of DNA strand breaks in human subjects. This research suggests nutritional impacts from consuming carotenoids from dried plant materials (Müller et al., 1999). Therefore, carotenoid accumulations for the *Brassica* species in the current study were expressed on a DW basis (Table 3). Lutein accumulation ranged from 0.87 mg·g⁻¹ DW (*B. rapa*) to 0.52 mg·g⁻¹ DW (*B. carinata*). The accumulation of 5,6-epoxylutein ranged from 0.04 mg·g⁻¹ DW (*B. nigra*, *B. juncea*, *B. rapa*) to 0.02 mg·g⁻¹ DW (*B. carinata*). The accumulation of β -carotene ranged from 0.43 mg·g⁻¹ DW (*B. juncea*) to 0.22 mg·g⁻¹ DW (*B. carinata*). Zeaxanthin accumulation ranged from 0.04 mg·g⁻¹ DW (*B. rapa*) to 0.02 mg·g⁻¹ DW (*B. napus*, *B. carinata*). Antheraxanthin accumulation ranged from 0.08 mg·g⁻¹ DW (*B. rapa*) to 0.04 mg·g⁻¹ DW (*B. napus*). Violaxanthin

Table 1. Mean values for carotenoid pigments (measured in milligrams per 100 g fresh weight) for the six genetically related *Brassica* species grown in a controlled environment.^z

<i>Brassica</i> species	Lutein	5,6-epoxylutein	β -carotene	Zeaxanthin	Antheraxanthin	Violaxanthin	Neoxanthin
<i>B. rapa</i>	8.89 a	0.40 a	4.03 a	0.75 a	0.79 a	1.35 b	1.93 ab
<i>B. juncea</i>	8.79 a	0.39 a	4.41 a	0.36 b	0.67 ab	2.17 a	2.03 ab
<i>B. nigra</i>	8.55 a	0.41 a	3.93 a	0.36 b	0.56 bc	2.28 a	1.97 ab
<i>B. carinata</i>	5.65 b	0.25 b	2.45 b	0.25 b	0.57 bc	1.11 b	1.32 c
<i>B. oleracea</i>	8.48 a	0.39 a	3.85 a	0.28 b	0.51 bc	1.51 b	2.10 a
<i>B. napus</i>	7.63 a	0.36 a	2.57 b	0.22 b	0.41 c	1.12 b	1.69 b
Mean	8.00	0.37	3.54	0.37	0.59	1.59	1.84

^zComposition of leaf samples from six replications, six plants each. Mean separation within columns by Duncan's multiple range test, $\alpha = 0.05$.

Table 2. Mean values for chlorophyll pigments (measured in milligrams per 100 g fresh weight) for the six genetically related *Brassica* species grown in a controlled environment.^z

<i>Brassica</i> species	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll
<i>B. rapa</i>	99.8 a	25.3 ab	125.1 a
<i>B. juncea</i>	100.1 a	25.8 ab	125.9 a
<i>B. nigra</i>	87.1 a	24.4 b	111.5 a
<i>B. carinata</i>	52.4 c	18.3 c	70.7 c
<i>B. oleracea</i>	93.2 a	28.8 a	121.9 a
<i>B. napus</i>	68.4 b	23.3 b	91.7 b
Mean	83.5	24.3	107.8

^zComposition of leaf samples from six replications, six plants each. Mean separation within columns by Duncan's multiple range test, $\alpha = 0.05$.

Table 3. Mean values for carotenoid pigments (measured in milligrams per gram dry weight) for the six genetically related *Brassica* species grown in a controlled environment.^z

<i>Brassica</i> species	Lutein	5,6-epoxylutein	β -carotene	Zeaxanthin	Antheraxanthin	Violaxanthin	Neoxanthin
<i>B. rapa</i>	0.87 a	0.04 a	0.40 a	0.07 a	0.08 a	0.13 b	0.19 ab
<i>B. juncea</i>	0.86 a	0.04 a	0.43 a	0.04 b	0.07 ab	0.21 a	0.20 a
<i>B. nigra</i>	0.83 ab	0.04 a	0.38 a	0.04 b	0.05 c	0.22 a	0.18 ab
<i>B. carinata</i>	0.52 c	0.02 c	0.22 b	0.02 b	0.05 bc	0.10 b	0.12 c
<i>B. oleracea</i>	0.80 ab	0.04 ab	0.36 a	0.03 b	0.05 c	0.14 b	0.20 a
<i>B. napus</i>	0.71 b	0.03 ab	0.24 b	0.02 b	0.04 c	0.10 b	0.16 bc
Mean	0.77	0.03	0.34	0.04	0.06	0.12	0.18

^zComposition of leaf samples from six replications, six plants each. Mean separation within columns by Duncan's multiple range test, $\alpha = 0.05$.

accumulation ranged from 0.22 mg·g⁻¹ DW (*B. nigra*) to 0.10 mg·g⁻¹ DW (*B. napus*, *B. carinata*). Neoxanthin accumulation ranged from 0.20 mg·g⁻¹ DW (*B. juncea*, *B. oleracea*) to 0.12 mg·g⁻¹ DW (*B. carinata*). For each of the carotenoid pigments analyzed on a DW basis, the diploid *Brassica* species accumulated higher concentrations, on average, than the amphidiploid species (Table 3). Dry weight carotenoid values measured in the *Brassica* species would make them comparable sources to previously used supplements (Müller et al., 1999; Pool-Zobel et al., 1997).

Genomes are subject to change through mutation of the genetic systems that control meiosis and chromosome morphology. Interspecific hybridization between the diploid *Brassica* species is easily accomplished and can be used to make comparisons of meiotic behaviors among diploid, amphidiploid, and amphidiploid hybrids. Attia and Röbbelen (1986) studied cytogenetic relationships within cultivated species of *Brassica* and discovered a high degree of pairing and chiasmata formation for the amphihaploid *B. napus*. *Brassica carinata* had very low meiotic pairing and chiasmata compared with *B. napus*, with slightly higher pairing

observed for *B. juncea*. Morphological (Prakash and Hinata, 1980) and chromosomal (Armstrong and Keller, 1982) differentiation identified *B. napus* to be the most distantly related of the genomic species. Cunha et al. (2004) used chloroplast DNA (cpDNA) to identify polymorphisms between the six genetically distinct *Brassica* species. The study concluded that cpDNA polymorphisms could discriminate among the three diploid *Brassica* species, but were unable to identify polymorphisms among the three amphidiploid species. Research shows that *B. rapa* and *B. oleracea* are very closely related species (Harberd and McArthur, 1980). In most cases for the current study, carotenoid pigment accumulation among these two *Brassica* species did not differ (Table 1).

Earlier studies revealed structural divergence among the monogenic *Brassica* species was based upon reductions in chromosome pairing in the haploids and production of hybrids (Prakash and Chopra, 1993). Based upon a phylogenetic tree, the amphidiploid species *B. carinata* is more closely related to its maternal diploid donor *B. nigra* than to the paternal donor *B. oleracea*, *B. juncea* is more closely related

to the maternal donor *B. rapa* than to the paternal donor *B. nigra*, and *B. napus* is almost equally related to the maternal and paternal donors of *B. rapa* and *B. oleracea* respectively. The results suggest that the maternally donated cytoplasmic genomes have some effect on nuclear genome evolution in the amphidiploid species (Cunha et al., 2004; Song et al., 1996). Large genomic diversity was found among and within the three cultivated amphidiploid species of *Brassica* (Song et al., 1996).

In the current study, genetic differences were identified for carotenoid and Chl pigment concentrations within and among the diploid and amphidiploid *Brassica* species. *Brassica* species also produce glucosinolate secondary compounds that have important anticarcinogenic properties in humans. Quantitative and qualitative differences in glucosinolate production have been linked to genome differences among rapid-cycling *Brassica* species (Hill et al., 1988). For many of the pigments analyzed in the current study, the amphidiploids *B. carinata* and *B. napus* accumulated significantly less carotenoid concentrations than the diploid species and *B. juncea*. The six genetically related *Brassica* species share common evolutionary ancestry, and breeding techniques are currently used for interspecific hybridizations and species improvement. *Brassica* species convey unique health attributes when consumed in the diet, and are shown to accumulate high levels of carotenoids (USDA, 2005). Therefore, identification of the genetic relationships that exist among the *Brassica* species for carotenoid concentrations would be beneficial information for improvement programs designed to increase nutritional values.

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