

# Variation in Lutein, $\beta$ -carotene, and Chlorophyll Concentrations among *Brassica oleracea* Cultigens and Seasons

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**Abstract.** Green leafy vegetables are important sources of dietary carotenoids, and members of *Brassica oleracea* L. var. *acephala* rank highest for reported levels of lutein and  $\beta$ -carotene. Twenty-three leafy *B. oleracea* cultigens were field grown under similar fertility over two separate years and evaluated for leaf lutein and  $\beta$ -carotene accumulation. Choice of *B. oleracea* cultigen and year significantly affected carotenoid levels. Lutein concentrations ranged from a high of 13.43 mg per 100 g fresh weight (FW) for *B. oleracea* var. *acephala* 'Toscano' to a low of 4.84 mg/100 g FW for *B. oleracea* var. *acephala* 343-93G1.  $\beta$ -carotene accumulations ranged from a high of 10.00 mg/100 g FW for *B. oleracea* var. *acephala* 'Toscano' to a low of 3.82 mg/100 g FW for *B. oleracea* var. *acephala* 30343-93G1. Carotenoid concentrations were significantly higher in year 2 than in year 1, but rank order among the cultigens for both lutein and  $\beta$ -carotene did not change between the years. During each year, there were high correlations between leaf carotenoid and chlorophyll pigments. Under similar growing conditions, choice of *B. oleracea* cultigen will influence carotenoid accumulation, and this may affect the health benefits of consuming these leafy green vegetable crops.

Carotenoids are secondary plant compounds that form lipid soluble yellow, orange, and red pigments. Lutein (3R,3'R,6'R  $\beta$ -carotene-3,3'-diol), a oxygenated xanthophyll, and  $\beta$ -carotene ( $\beta$ , $\beta$ -carotene), a hydrocarbon carotene, are examples of two nutritionally important plant-derived carotenoids (Zaripheh and Erdman, Jr., 2002). In green plants, carotenoids are bound to specific protein complexes of PSI and PSII, and along with chlorophyll *a* and *b*, function in light harvesting. Carotenoids also protect photosynthetic structures by quenching <sup>3</sup>Chl and <sup>1</sup>O<sub>2</sub> to inhibit oxidative damage (Tracewell et al., 2001).

Kale (*Brassica oleracea* L. var. *acephala*) ranks highest among all leafy vegetable crops for lutein and  $\beta$ -carotene content (Sommerburg et al., 1998). Lutein/zeaxanthin levels within leafy *B. oleracea* cultigens are reported to

range from 8.0 to 39.5 mg/100 g fresh tissue, while levels of  $\beta$ -carotene range from 2.8 to 14.5 mg/100 g fresh tissue (Sommerburg et al., 1998; Khachik et al., 1986). However, Kachick et al. (1986) admitted the upper end of the reported lutein and  $\beta$ -carotene ranges were obtained from one homogenous sample per cultigen, and would likely fail to be representative of material consumed nationwide in the United States.

The content and distribution of carotenoids in plants appears to be shaped by physiological, genetic, and biochemical factors (Goldman et al., 1999; Grusak et al., 1999). Mercadante and Rodriguez-Amaya (1991) observed differences in lutein and  $\beta$ -carotene content of two field-grown kale cultivars, 'Manteiga' and 'Tronchuda', in Brazil. Seasonal variation between winter and summer production in lutein and  $\beta$ -carotene levels were also observed between the two cultivars. Kurilich et al. (1999) found significant differences in  $\beta$ -carotene and  $\alpha$ -tocopherol content within and among 50 broccoli (*B. oleracea* L. var. *italica*) accessions. Klein and Perry (1982) reported significant differences in carotenoid (vitamin A) content of five different vegetables {carrot (*Daucus carota* L.), celery [*Apium graveolens* L. var. *dulce* (Mill.) Pers.], tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* L. Capitata Group), and corn (*Zea mays* L.)} purchased from six different U.S. locations, but could not conclude if differences were due to cultivar, culture, or postharvest handling procedures.

The nutritional and medicinal importance

of dietary carotenoid consumption is being established (Balentine et al., 1999). Dietary intake of lutein,  $\beta$ -carotene, and  $\alpha$ -carotene has been associated with reduced risk of lung cancer in both men and women (Le Marchand et al., 1993). Diets containing carotenoid-rich fruits and vegetables are associated with decreased risk of chronic eye diseases, including cataract and age-related macular degeneration (Johnson et al., 2000). Although direct evidence of carotenoid antioxidant and photoprotective functions in humans is lacking, dietary intake of carotenoids is still recommended (Krinsky, 2002; Olsen, 1999).

Because of the reported health benefits of consuming carotenoid-rich, green leafy vegetables, the objective of this study was to characterize the variability of lutein and  $\beta$ -carotene accumulation in 23 different *B. oleracea* cultigens suitable for production in the northeastern region of the United States. Due to previous reports of seasonal variability of carotenoid accumulation, cultigens were evaluated over two separate growing seasons.

## Materials and Methods

Twenty-three *B. oleracea* cultigens, including commercial cultivars and USDA-ARS accessions, were seeded into artificial media (Pro-mix BX; Premier Horticulture, Dorval, Quebec; Table 1) on 16 June 2001 and 18 June 2002. The medium was supplied with bottom heat (23 °C) and plants were greenhouse grown (22 °C day/14 °C night set points) for 4 weeks under natural photoperiods (lat. 43°09'N). Nutrients were applied as needed using 200 mg·L<sup>-1</sup> Peter's 20N-6.9P-16.6K water-soluble fertilizer (Grace-Sierra, Milpitas, Calif.).

Plants were transplanted into the field on or about the second week of July during both years. Fertilizer was applied 1 week prior to transplanting and cultigens were grown according to New England Cooperative Extension guidelines for minor cole crops (Howell et al., 2002). Each cultigen was planted in a plot consisting of two rows of 12 plants each at the recommended spacing of 45 cm within rows and 91 cm between rows. Plots were replicated three times in a randomized complete-block design for each year of evaluation. Irrigation was supplied during plant growth to ensure plants received a total of 2.54 cm of water per week. Nitrogen was sidedressed as NH<sub>4</sub>NO<sub>3</sub> at the rate of 67.2 kg·ha<sup>-1</sup> 3 weeks after transplanting. Plants were harvested on 25 Aug. 2001 and 27 Aug. 2002. From 15 July to 27 Aug. 2001, the average daily temperature was 19.5 °C (with 6 d reaching temperatures >32.0 °C); average daily photosynthetically active radiation (PAR) was 558  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, and total rainfall was 5.61 cm. From 15 July to 27 Aug. 2002, the average daily temperature was 22.3 °C (with 12 d reaching temperatures >32.0 °C), average daily PAR was 584  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, and total rainfall was 4.95 cm (Univ. of New Hampshire weather station, Durham). At harvest, the third most fully expanded leaf from 10 uniform plants per replicate was removed and combined for carotenoid analysis (Jones, 1972). Tissues were lyophilized for 48 h (model

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6 L FreeZone; LabConCo, Kansas City, Mo.) and stored at  $-80^{\circ}\text{C}$  prior to extraction.

Plant pigments were extracted from freeze-dried tissue according to the method of Beecher and Howard (USDA Food Composition Laboratory, Beltsville, Md.; personal communications), which is a modification of the method of Khachik et al. (1986). A 0.10-g subsample was rehydrated with 0.8 mL of  $\text{ddH}_2\text{O}$  at  $40^{\circ}\text{C}$  for 20 min. After incubation, 0.8 mL of the internal standard ethyl- $\beta$ -apo-carotenol (Sigma Chemical Co., St. Louis) and 2.5 mL of tetrahydrofuran (THF) stabilized with 25  $\text{mg}\cdot\text{L}^{-1}$  2,6-Di-*tert*-butyl-4-methoxyphenol (BHT) were added. The sample was homogenized in a Potter-Elvehjem (Kontes, Vineland, N.J.) tissue grinding tube using  $\approx 25$  insertions with a pestle attached to a drill press (model Craftsman 15 inch 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  $g$ . The supernatant was removed and the sample pellet was resuspended in 2.0 mL THF and homogenized again with the same extraction technique. The extraction procedure was repeated two more times to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL using nitrogen (model N-EVAP 111; Organomation Inc., Berlin, Mass.) at  $40^{\circ}\text{C}$  and 2.5 mL MeOH and 2.0 mL THF were added to the sample prior to HPLC analysis. Saponification was not performed on the *B. oleracea* samples because it has been found to degrade carotenoids and reduce recovery (personal observation; Kimura et al., 1990; Mercadante and Rodriguez-Amaya, 1991).

An Agilent 1100 series HPLC unit with a photo diode array detector (Agilent Technologies, Palo Alto, Calif.) was used for sample separation. All samples were analyzed using a RP C-18, 80 Å, 3.0  $\mu\text{m}$ , 300  $\times$  4.6-mm column (Adsorbosphere HS; Alltech, Deerfield, Ill.) fitted with a 7.5  $\times$  4.0-mm 5.0- $\mu\text{m}$  guard column (All Guard C-18; Alltech). The column was maintained at  $16^{\circ}\text{C}$  using a thermostatted column compartment. Eluents were: (A) 75% acetonitrile, 20% methanol, 5% hexane, 0.05% BHT, and 0.013% triethylamine (TEA) in water (v/v); and (B) 50% acetonitrile, 25% THF, 25% hexane, and 0.013% TEA in water (v/v). The flow rate was 0.7  $\text{mL}\cdot\text{min}^{-1}$  and the gradient is 100% A for 30 min, 50% A and 50% B for 2 min; 100% B for 2 min; and 50% A and 50% B for 2 min. The eluent composition was returned to 100% A and the column was equilibrated for 10 min prior to the next injection. Eluted compounds from a 20- $\mu\text{L}$  injection were detected at 452, 652, and 665 nm and data sets were collected, recorded, and integrated using 1100 HPLC ChemStation Software (Agilent Technologies). Peak assignment was performed by comparing retention times and line spectra obtained from photodiode array detection with authentic standards (lutein from Carotenature, Lupsingen, Switzerland;  $\beta$ -carotene, chlorophyll *a*, and chlorophyll *b* from Sigma Aldrich, St. Louis).

Data sets were analyzed by the GLM procedures of SAS (Cary, N.C.) with cultivar means

Table 1. List of leafy *Brassica oleracea* cultivars and sources of seed.

| Cultigen              | <i>B. oleracea</i> var. | Accession lot | Seed source/origin <sup>a</sup> | Source location |
|-----------------------|-------------------------|---------------|---------------------------------|-----------------|
| Bona                  | <i>acephala</i>         |               | Johnny's Selected Seeds         | Albion, Maine   |
| Calvolo Palmizio Nero | <i>selenesia</i>        | G 30714 92GI1 | USDA-ARS/Italy                  | Geneva, N.Y.    |
| Condor                | <i>mendolsa</i>         | G 30859 97GIU | USDA-ARS                        | Geneva, N.Y.    |
| Couve Espanhola       | <i>costata</i>          | G 29806 91GI1 | USDA-ARS                        | Geneva, N.Y.    |
| Couve Nabica          | <i>costata</i>          | G 30855 96GI1 | USDA-ARS/Portugal               | Geneva, N.Y.    |
| Couve Portuguesaa     | <i>costata</i>          | G 29805 92GI  | USDA-ARS                        | Geneva, N.Y.    |
| Crimson Garden        | <i>acephala</i>         |               | Johnny's Selected Seeds         | Albion, Maine   |
| Giant Jersey Kale     | <i>palmifolia</i>       | G 30723 92GI  | USDA-ARS/UK                     | Geneva, N.Y.    |
| NZ Thousand Head      | <i>ramosa</i>           | G 30724 96GI  | USDA-ARS                        | Geneva, N.Y.    |
| Panca de Chaves       | <i>costata</i>          | G 30858 96GI1 | USDA-ARS                        | Geneva, N.Y.    |
| Premier               | <i>selenesia</i>        | G 30729 90UO  | USDA-ARS                        | Geneva, N.Y.    |
| Redbor F1             | <i>acephala</i>         |               | Johnny's Selected Seeds         | Albion, Maine   |
| Red Russian           | <i>acephala</i>         |               | Johnny's Selected Seeds         | Albion, Maine   |
| Round Leaf Kale       | <i>alboglabra</i>       | G 30685 97GI  | USDA-ARS                        | Geneva, N.Y.    |
| S.C. Green Glaze      | <i>selenesia</i>        | G 30012 96GI2 | USDA-ARS                        | Geneva, N.Y.    |
| Shetland              | <i>acephala</i>         | G 30735 96GI1 | USDA-ARS                        | Geneva, N.Y.    |
| Siberian Improved     | <i>selenesia</i>        | G 29221 89UO  | USDA-ARS                        | Geneva, N.Y.    |
| Tall Marrowstem       | <i>medullosa</i>        | G 30717 92GI  | USDA-ARS/UK                     | Geneva, N.Y.    |
| Toscana               | <i>acephala</i>         |               | Johnny's Selected Seeds         | Albion, Maine   |
| Winterbor F1          | <i>acephala</i>         |               | Johnny's Selected Seeds         | Albion, Maine   |
| 30343-93G1            | <i>acephala</i>         | G 30343 93GI  | USDA-ARS/UK                     | Geneva, N.Y.    |
| 30661-92G1            | <i>acephala</i>         | G 30661 -92G1 | USDA-ARS/UK                     | Geneva, N.Y.    |
| 30665-96G1            | <i>acephala</i>         | G30661 96GI1  | USDA-ARS/UK                     | Geneva, N.Y.    |

<sup>a</sup>Origins not listed are either United States or unknown.

Table 2. Mean values<sup>a</sup> (mg per 100 g fresh weight) of lutein and  $\beta$ -carotene for 23 leafy *Brassica oleracea* cultivars over two growing seasons (YR1 and YR2).

| Cultigens                        | Lutein           |                  | $\beta$ -carotene |                 |
|----------------------------------|------------------|------------------|-------------------|-----------------|
|                                  | YR1              | YR2              | YR1               | YR2             |
| Bona                             | 6.96 $\pm$ 1.41  | 7.14 $\pm$ 1.29  | 4.77 $\pm$ 0.27   | 5.67 $\pm$ 0.88 |
| Calvolo Palmizio Nero            | 12.36 $\pm$ 1.03 | 13.33 $\pm$ 1.81 | 7.80 $\pm$ 0.45   | 8.07 $\pm$ 1.61 |
| Condor                           | 6.28 $\pm$ 0.65  | 6.92 $\pm$ 0.23  | 4.26 $\pm$ 0.52   | 5.97 $\pm$ 0.49 |
| Couve Espanhola                  | 5.64 $\pm$ 0.24  | 6.98 $\pm$ 0.39  | 4.39 $\pm$ 0.42   | 5.40 $\pm$ 0.28 |
| Couve Nabica                     | 7.14 $\pm$ 0.94  | 8.97 $\pm$ 0.70  | 5.13 $\pm$ 0.70   | 7.33 $\pm$ 0.82 |
| Couve Portuguesaa                | 5.31 $\pm$ 0.79  | 6.42 $\pm$ 0.53  | 4.28 $\pm$ 0.12   | 5.66 $\pm$ 0.30 |
| Crimson Garden                   | 6.66 $\pm$ 0.36  | 8.71 $\pm$ 2.93  | 4.75 $\pm$ 0.83   | 5.54 $\pm$ 0.03 |
| Giant Jersey Kale                | 5.53 $\pm$ 0.79  | 8.89 $\pm$ 0.68  | 4.99 $\pm$ 0.70   | 7.35 $\pm$ 0.46 |
| NZ Thousand Head                 | 6.41 $\pm$ 0.25  | 7.24 $\pm$ 0.40  | 4.73 $\pm$ 0.43   | 5.81 $\pm$ 0.04 |
| Panca de Chaves                  | 4.91 $\pm$ 0.25  | 6.09 $\pm$ 2.05  | 3.49 $\pm$ 0.26   | 4.32 $\pm$ 0.89 |
| Premier                          | 7.96 $\pm$ 0.64  | 9.07 $\pm$ 0.59  | 7.44 $\pm$ 1.29   | 7.67 $\pm$ 0.31 |
| Redbor F1                        | 6.52 $\pm$ 0.63  | 8.07 $\pm$ 0.28  | 5.46 $\pm$ 0.13   | 6.51 $\pm$ 0.41 |
| Red Russian                      | 7.69 $\pm$ 1.91  | 6.91 $\pm$ 1.26  | 5.82 $\pm$ 1.33   | 5.78 $\pm$ 1.13 |
| Round Leaf Kale                  | 5.98 $\pm$ 0.36  | 8.29 $\pm$ 0.89  | 4.66 $\pm$ 0.95   | 6.34 $\pm$ 0.84 |
| S.C. Green Glaze                 | 6.07 $\pm$ 0.02  | 6.27 $\pm$ 0.76  | 4.73 $\pm$ 0.70   | 4.74 $\pm$ 1.31 |
| Shetland                         | 4.94 $\pm$ 0.34  | 7.12 $\pm$ 0.39  | 3.49 $\pm$ 0.44   | 6.39 $\pm$ 0.35 |
| Siberian Improved                | 7.32 $\pm$ 0.21  | 8.88 $\pm$ 1.25  | 5.60 $\pm$ 0.42   | 7.24 $\pm$ 1.16 |
| Tall Marrowstem                  | 6.94 $\pm$ 2.02  | 8.20 $\pm$ 2.05  | 5.20 $\pm$ 0.98   | 6.60 $\pm$ 1.56 |
| Toscana                          | 11.52 $\pm$ 0.88 | 13.43 $\pm$ 0.77 | 10.00 $\pm$ 0.41  | 9.92 $\pm$ 0.57 |
| Winterbor F1                     | 7.71 $\pm$ 1.11  | 9.60 $\pm$ 0.78  | 5.33 $\pm$ 0.64   | 7.01 $\pm$ 1.13 |
| 30343-93G1                       | 4.84 $\pm$ 0.46  | 5.83 $\pm$ 0.01  | 3.82 $\pm$ 1.04   | 5.08 $\pm$ 0.23 |
| 30661-92G1                       | 5.82 $\pm$ 0.34  | 6.96 $\pm$ 1.19  | 4.59 $\pm$ 0.67   | 5.53 $\pm$ 1.07 |
| 30665-96G11                      | 6.09 $\pm$ 1.04  | 6.90 $\pm$ 1.21  | 4.71 $\pm$ 0.60   | 5.76 $\pm$ 1.35 |
| Mean                             | 6.81             | 8.10             | 5.19              | 6.33            |
| LSD <sub>0.05</sub> <sup>y</sup> | 1.47             | 1.92             | 1.63              | 1.42            |
| LSD <sub>0.05</sub> <sup>x</sup> | 1.21             |                  | 1.08              |                 |

<sup>a</sup>Composition of leaf samples from three replications, 10 plants each  $\pm$  standard error.

<sup>y</sup>LSD for differences between cultivar means within year.

<sup>x</sup>LSD for differences between cultivar means between years.

separated by least significant difference (LSD) of 0.05 within and between growing seasons. A correlation matrix for cultivars within each season was calculated for all variables tested. To test differences in rank order among the cultivars between the two growing seasons, Spearman's rank correlations were calculated (Steel and Torrie, 1980).

### Results and Discussion

Lutein accumulation differed among cultivars ( $P < 0.001$ ) and year ( $P < 0.001$ ), but

not for the interaction of cultivar and year. Total lutein concentration among the cultivars ranged from a high of 13.43 g FW ('Toscana') to a low of 4.84 mg/100 g FW (30343-93G1; Table 2). Lutein accumulation was significantly higher ( $P < 0.001$ ) during year 2. During year 1, lutein ranged from 12.36 mg/100 g FW ('Calvolo Palmizio Nero') to 4.84 mg/100 g FW (30343-93G1). During year 2, 'Toscana' displayed the highest lutein accumulation (13.43 mg/100 g FW) while the lowest was again measured in 30343-93G1 (5.83 mg/100 g FW). Previously, Mercadante and Rodriguez-

Amaya (1991) reported lutein + violaxanthin values in two field-grown kale cultivars in Brazil to range from 11.4 to 7.1 mg/100 g FW. Violaxanthin is a diepoxide xanthophyll that is converted to zeaxanthin by the enzyme violaxanthin deepoxidase under light stress (Havaux and Niyogi, 1999). It can be difficult to separate lutein, violaxanthin, and zeaxanthin analytically due to their structural similarities; therefore, many authors report them together (Khachik et al., 1991). Because violaxanthin and zeaxanthin occur in small quantities in green leafy vegetables, comparisons between studies that report these compounds combined with lutein can be approximated. Results from the current study are within reported ranges for lutein accumulation in other field grown leafy *B. oleracea* cultigens.

Rank order of lutein did not significantly change for the cultigens from year 1 to year 2 [Spearman's rank correlation ( $r_s$ ) = 0.71;  $P < 0.001$ ]. Cultigens with the highest lutein accumulation were 'Cavolo Palmizio Nero', 'Toscano', 'Winterbor F1', 'Premier', 'Siberian Improved', 'Couve Nabica', 'Crimson Garden', and 'Tall Marrowstem'. Cultigens with the lowest lutein accumulation were 30343-93G1, 'Penca de Chaves', 'Couve Portuguesa', 'Shetland', 'S.C. Green Glaze', 'Couve Espanhola', 30661-92G1, and 30665-96G11.

$\beta$ -carotene accumulation differed among cultigens ( $P < 0.001$ ) and year ( $P < 0.001$ ), but not for the interaction of cultigen and year. Total  $\beta$ -carotene accumulation among the cultigens ranged from a high of 10.00 mg/100 g FW ('Toscano') to a low of 3.82 mg/100 g FW (30343-93G1; Table 2).  $\beta$ -carotene accumulation was also significantly higher ( $P < 0.001$ ) during year 2. During year 1,  $\beta$ -carotene

ranged from 10.00 mg/100 g FW ('Toscano') to 3.82 mg/100 g FW (30343-93G1). During year 2, 'Toscano' again displayed the highest  $\beta$ -carotene accumulation, while the lowest was measured in 'Panca de Chaves'. Müller (1997) detected 7.28 mg  $\beta$ -carotene/100 g FW in field-grown kale of unknown cultigen.  $\beta$ -carotene values in our study are similar to previously reported values for other field-grown leafy *B. oleracea* cultivars (Kurilich et al., 1999; Mercadante and Rodriguez-Amaya, 1991).

Rank order of  $\beta$ -carotene also did not significantly change for the cultigens from year 1 to year 2 [Spearman's rank correlation ( $r_s$ ) = 0.74;  $P < 0.001$ ]. Cultigens with the highest  $\beta$ -carotene accumulation were 'Toscano', 'Cavolo Palmizio Nero', 'Premier', 'Siberian Improved', 'Couve Nabica', 'Giant Jersey Kale', and 'Redbor'. Cultigens with the lowest  $\beta$ -carotene accumulation were 'Penca de Chaves', 30343-93G1, 'S.C. Green Glaze', 'Couve Espanhola', 'Shetland', 'Couve Portuguesa', 30665-96G11+, and 'Condor'.

Chlorophyll *a* content differed among cultigens ( $P < 0.001$ ), year ( $P < 0.001$ ), and for the interaction of cultigen and year ( $P = 0.002$ ). Chlorophyll *b* content differed among cultigens ( $P < 0.001$ ), year ( $P < 0.001$ ), and for the interaction of cultigen and year ( $P < 0.001$ ). Chlorophyll *a* + Chl *b* also differed among the cultigens ( $P < 0.001$ ), year ( $P < 0.001$ ), and for the interaction of cultigen and year ( $P < 0.001$ ). Values for Chl *a* and Chl *b* were within previously reported ranges for kale (Table 3; Khachik et al., 1986). Rank order of Chl *a*, Chl *b*, and Chl *a* + Chl *b* did not significantly change for the cultigens from year 1 to year 2 [Spearman's rank correlation ( $r_s$ ) = 0.47,  $P = 0.01$ ; ( $r_s$ ) = 0.72,  $P < 0.001$ ;

( $r_s$ ) = 0.56,  $P = 0.003$ , respectively].

During each season, there were high correlations between carotenoid and chlorophyll accumulations (Table 4). Similarities in behavior of carotenoids and chlorophylls have been reported for other crop species (Grunwald et al., 1977; Terry and Abadía, 1986; ). Ihl et al. (1994) found chlorophylls to highly correlate with total carotenoid levels in the leaves of Swiss chard (*Beta vulgaris* L.). Our results support these correlative relationships among these *B. oleracea* cultigens (Table 4). This suggests it may be possible to use chlorophyll content, or green coloration, to estimate gross lutein and  $\beta$ -carotene concentration in leafy green vegetables.

There appears to be a strong genetic influence on carotenoid accumulation within several vegetable crop species. Kurilich et al. (1999) determined that 79% of  $\beta$ -carotene and 82% of  $\alpha$ -tocopherol variation in broccoli heads was due to genetic differences among 50 accessions. Kurilich and Juvik (1999) found significant differences for lutein, zeaxanthin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, and  $\alpha$ -tocopherol kernel content among 44 sweet and dent corn lines. Significant differences for lutein,  $\beta$ -carotene, and  $\alpha$ -tocopherol accumulation have been observed among several red pepper (*Capsicum annuum* L.) cultivars (Almela et al., 1991; Daoud et al., 1996). However, few studies to date have investigated the lutein and  $\beta$ -carotene variability among leafy *B. oleracea* cultigens, which consistently rank highest for lutein and  $\beta$ -carotene accumulation (Heinonen et al., 1989; Holden et al., 1999).

Along with genetic factors, there also appears to be an environmental influence on carotenoid accumulation. Mercadante and

Table 3. Mean values<sup>z</sup> (mg per 100 g fresh weight) of chlorophyll (Chl) *a* and *b* for 23 leafy *Brassica oleracea* cultigens over two growing seasons (YR1 and YR2).

| Cultigens                        | Chl <i>a</i>   |                | Chl <i>b</i>  |              | Chl <i>a</i> + Chl <i>b</i> |                |
|----------------------------------|----------------|----------------|---------------|--------------|-----------------------------|----------------|
|                                  | YR1            | YR2            | YR1           | YR2          | YR1                         | YR2            |
| Bona                             | 137.93 ± 11.39 | 128.47 ± 29.50 | 41.26 ± 1.10  | 30.23 ± 5.88 | 179.19 ± 11.40              | 158.70 ± 35.38 |
| Calvolo Palmizio Nero            | 278.03 ± 23.54 | 202.86 ± 21.76 | 89.52 ± 1.65  | 58.50 ± 5.60 | 367.55 ± 23.96              | 261.36 ± 26.74 |
| Condor                           | 140.92 ± 32.23 | 121.81 ± 18.00 | 39.19 ± 6.15  | 28.29 ± 0.74 | 180.11 ± 38.34              | 150.10 ± 18.72 |
| Couve Espanhola                  | 157.05 ± 4.97  | 114.81 ± 11.90 | 40.10 ± 4.72  | 28.62 ± 2.57 | 197.15 ± 9.69               | 143.43 ± 13.70 |
| Couve Nabica                     | 120.84 ± 31.06 | 131.01 ± 19.15 | 38.02 ± 4.58  | 32.82 ± 2.67 | 158.86 ± 35.43              | 163.83 ± 21.73 |
| Couve Portuguesa                 | 126.12 ± 26.58 | 114.93 ± 8.62  | 38.09 ± 5.28  | 26.42 ± 2.90 | 164.21 ± 31.61              | 141.35 ± 11.51 |
| Crimson Garden                   | 165.88 ± 20.29 | 143.61 ± 32.40 | 49.41 ± 4.56  | 39.35 ± 6.65 | 215.29 ± 24.21              | 182.96 ± 38.99 |
| Giant Jersey Kale                | 129.28 ± 32.15 | 151.92 ± 5.97  | 39.31 ± 5.64  | 35.63 ± 1.31 | 168.59 ± 37.67              | 187.55 ± 6.39  |
| NZ Thousand Head                 | 118.99 ± 8.80  | 116.84 ± 6.07  | 35.78 ± 0.96  | 27.44 ± 1.10 | 154.77 ± 9.76               | 144.28 ± 6.58  |
| Panca de Chaves                  | 123.24 ± 9.83  | 84.26 ± 18.19  | 32.13 ± 4.17  | 19.78 ± 2.72 | 155.37 ± 12.54              | 104.04 ± 20.88 |
| Premier                          | 175.77 ± 47.27 | 148.71 ± 16.82 | 50.67 ± 12.99 | 37.34 ± 3.82 | 226.44 ± 60.26              | 186.05 ± 20.21 |
| Redbor F1                        | 161.53 ± 13.70 | 133.51 ± 7.02  | 46.60 ± 3.17  | 32.30 ± 2.10 | 208.13 ± 16.70              | 165.81 ± 9.11  |
| Red Russian                      | 166.24 ± 26.83 | 113.69 ± 21.49 | 45.09 ± 13.17 | 28.25 ± 5.51 | 211.33 ± 36.80              | 141.94 ± 26.93 |
| Round Leaf Kale                  | 128.62 ± 18.98 | 135.38 ± 19.20 | 38.82 ± 0.90  | 32.48 ± 3.99 | 167.44 ± 18.84              | 167.86 ± 23.19 |
| S.C. Green Glaze                 | 146.17 ± 8.32  | 105.31 ± 16.04 | 41.04 ± 0.78  | 26.51 ± 3.53 | 187.21 ± 7.55               | 131.82 ± 19.57 |
| Shetland                         | 109.77 ± 11.42 | 132.41 ± 3.30  | 30.95 ± 1.21  | 30.56 ± 1.44 | 140.72 ± 11.63              | 162.97 ± 4.52  |
| Siberian Improved                | 143.83 ± 28.76 | 145.21 ± 6.87  | 49.90 ± 2.56  | 37.36 ± 1.76 | 193.73 ± 31.21              | 182.57 ± 7.61  |
| Tall Marrowstem                  | 170.52 ± 18.96 | 145.46 ± 32.23 | 40.27 ± 7.47  | 33.12 ± 7.60 | 210.79 ± 21.75              | 178.58 ± 39.82 |
| Toscano                          | 276.00 ± 40.84 | 207.00 ± 25.53 | 86.82 ± 5.80  | 54.92 ± 5.97 | 362.82 ± 46.32              | 261.92 ± 31.41 |
| Winterbor F1                     | 164.26 ± 28.02 | 170.66 ± 12.19 | 49.60 ± 9.39  | 39.57 ± 3.01 | 213.86 ± 37.11              | 210.23 ± 10.02 |
| 30343-93G1                       | 144.60 ± 14.46 | 106.31 ± 12.80 | 34.23 ± 3.90  | 24.11 ± 1.64 | 178.83 ± 14.36              | 130.42 ± 14.44 |
| 30661-92G1                       | 134.43 ± 14.76 | 96.48 ± 18.17  | 37.80 ± 1.11  | 26.59 ± 4.32 | 172.23 ± 15.37              | 123.07 ± 22.29 |
| 30665-96G11                      | 157.61 ± 21.07 | 116.98 ± 16.82 | 42.86 ± 7.05  | 28.36 ± 4.36 | 200.47 ± 28.11              | 145.34 ± 20.96 |
| Mean                             | 155.55         | 132.20         | 45.12         | 32.98        | 200.66                      | 165.18         |
| LSD <sub>0.05</sub> <sup>y</sup> | 40.17          | 28.72          | 9.64          | 6.44         | 46.98                       | 36.24          |
| LSD <sub>0.05</sub> <sup>x</sup> | 24.22          |                | 5.64          |              | 29.27                       |                |

<sup>z</sup>Composition of leaf samples from three replications, 10 plants each ± standard error.

<sup>y</sup>LSD for differences between cultivar means within year.

<sup>x</sup>LSD for differences between cultivar means between years.

Table 4. Correlation coefficients (*r*) between concentrations of carotenoids and chlorophylls (Chl) in leafy *Brassica oleracea* subspecies averaged across 23 cultigens over two growing seasons.

| Variable                                  | Lutein | $\beta$ -carotene | Chl <i>a</i> | Chl <i>b</i> | Chl <i>a</i> + Chl <i>b</i> |
|-------------------------------------------|--------|-------------------|--------------|--------------|-----------------------------|
| <i>YR 1: Correlation coefficients (r)</i> |        |                   |              |              |                             |
| Lutein                                    | ---    | 0.80***           | 0.59***      | 0.83***      | 0.67***                     |
| $\beta$ -carotene                         |        | ---               | 0.62***      | 0.74***      | 0.67***                     |
| Chl <i>a</i>                              |        |                   | ---          | 0.77***      | 0.98***                     |
| Chl <i>b</i>                              |        |                   |              | ---          | 0.86***                     |
| Chl <i>a</i> + Chl <i>b</i>               |        |                   |              |              | ---                         |
| <i>YR 2: Correlation coefficients (r)</i> |        |                   |              |              |                             |
| Lutein                                    | ---    | 0.86***           | 0.85***      | 0.90***      | 0.87***                     |
| $\beta$ -carotene                         |        | ---               | 0.79***      | 0.82***      | 0.80***                     |
| Chl <i>a</i>                              |        |                   | ---          | 0.93***      | 0.99***                     |
| Chl <i>b</i>                              |        |                   |              | ---          | 0.96***                     |
| Chl <i>a</i> + Chl <i>b</i>               |        |                   |              |              | ---                         |

\*\*\*Significant at  $P < 0.0001$ .

Rodriguez-Amaya (1991) reported seasonal variation between winter and summer production in lutein and  $\beta$ -carotene levels between 'Manteiga' and 'Tronchuda' kale cultivars grown in Brazil. Results from the current study indicate a year-to-year variation in carotenoid accumulation as well. Although the cultigens were grown at exactly the same time of year during season 1 and season 2, mean lutein and  $\beta$ -carotene values were higher during year 2 (Table 2). Despite these yearly variations, rank order of the cultigens for carotenoid accumulation did not change. Selecting cultivars that consistently rank high for carotenoid accumulation should allow producers to provide maximum carotenoid content for a given production year.

Assessing the genetic variability for carotenoid accumulation and identifying vegetable cultigens with maximum levels may have important health implications for consumers (Kurilich and Juvik, 1999). Recent scientific evidence describing the protective functions of lutein in the macular region of the eye has created interest in this carotenoid (Khachik et al., 1997; Olsen, 1999; Snodderly, 1995). Dietary intake of lutein and  $\beta$ -carotene is associated with decreased risks of cancer and age-related macular degeneration (Mortensen et al., 2001). Studies indicate that a high intake of a variety of vegetables, providing a mixture of carotenoids, was more strongly associated with reduced cancer and eye disease risk than intake of individual carotenoid supplements (Johnson et al., 2000; Le Marchand et al., 1993). The *B. oleracea* germplasm grown in this study exhibited a 2.4-fold difference in lutein and a 2.5-fold difference in  $\beta$ -carotene concentration. Therefore, identifying *B. oleracea* cultigens with the genetic potential for high carotenoid accumulation may hold dietary and nutritional advantages.

Significant variability existed for lutein and  $\beta$ -carotene accumulation among 23 leafy *B. oleracea* cultigens grown in the northeastern region of the United States. The carotenoid content of the *B. oleracea* cultigens demonstrated a year-to-year variation as well. However, rank order of the carotenoid content of the cultigens did not change between the two seasons of production. The *B. oleracea* cultigens that consistently ranked highest for carotenoid accumulation were 'Calvo

Palmizio Nero', 'Toscano', 'Premier', and 'Siberian Improved'. Under similar growing conditions, choice of *B. oleracea* cultigen will influence carotenoid accumulation, and this may affect the health benefits of consuming these leafy green vegetables.

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