Genetic Variation of Beta-carotene and Lutein Contents in Lettuce

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ABSTRACT. There is increasing medical evidence for the health benefits derived from dietary intake of carotenoid antioxidants, such as β-carotene and lutein. Enhancing the nutritional levels of vegetables would improve the nutrient intake without requiring an increase in consumption. A breeding program to improve the nutritional quality of lettuce (Lactuca sativa L.) must start with an assessment of the existing genetic variation. To assess the genetic variability in carotenoid contents, 52 genotypes including crisphead, leaf, romaine, butterhead, primitive, Latin, and stem lettuces, and wild species were planted in the field in Salinas, Calif., in the Summer and Fall of 2003 with four replications. Duplicate samples from each plot were analyzed for chlorophyll (a and b), β-carotene, and lutein concentrations by high-performance liquid chromatography (HPLC). Wild accessions (L. serriola L., L. saligna L., L. virosa L., and primitive form) had higher β-carotene and lutein concentrations than cultivated lettuces, mainly due to the lower moisture content of wild lettuces. Among major types of cultivated lettuce, carotenoid concentration followed the order of: green leaf or romaine > red leaf > butterhead > crisphead. There was significant genetic variation in carotenoid concentration within each of these lettuce types. Crisphead lettuce accumulated more lutein than β-carotene, while other lettuce types had more β-carotene than lutein. Carotenoid concentration was higher in summer than in the fall, but was not affected by the position of the plant on the raised bed. Beta-carotene and lutein concentrations were highly correlated, suggesting that their levels could be enhanced simultaneously. Beta-carotene and lutein concentrations were both highly correlated with chlorophyll a, chlorophyll b, and total chlorophyll concentrations, suggesting that carotenoid content could be selected indirectly through chlorophyll or color measurement. These results suggest that genetic improvement of carotenoid levels in lettuce is feasible.

Carotenoids are a diverse group of lipid-soluble pigments synthesized in plants, fungi, and bacteria. Beta-carotene, a hydrocarbon carotene, and lutein, an oxygenated xanthophyll, are two nutritionally important plant-derived carotenoids. Beta-carotene is the most potent provitamin A; its deficiency can result in xerophthalmia, blindness, and premature death (Mayne, 1996). It is estimated that 124 million children worldwide are deficient in vitamin A, and improved vitamin A nutrition could prevent 1.2 million deaths annually among children aged 1–4 years (Humphrey et al., 1992). Lutein offers protection against the occurrence of age-related macular degeneration that is a leading cause of blindness and vision impairment among Americans 55 years or older (Seddon et al., 1994). Epidemiological studies suggest that the onset of chronic diseases such as coronary heart disease, certain cancers, and eye diseases including cataract can be reduced by high dietary intakes of carotenoid-rich foods (Johnson et al., 2000; Sies and Krinsky, 1995). Dietary intake of carotenoids like lutein, β-carotene, and lycopene has been associated with reduced risk of lung cancer (Le Marchand et al., 1993), prostate cancer (Giovannucci, 1999), and colon cancer (Slattery et al., 2000) due to their antioxidant activities.

In photosynthetic tissues, carotenoids, along with chlorophyll a and b, function in light harvesting and play important roles in photoprotection by quenching free radicals, singlet oxygen, and other reactive species (Siefermann-Harms, 1987). The biogenesis of carotenoids takes place in chloroplasts where the carotenoid exists in photosynthetic membranes as chlorophyll-carotenoid-protein complexes (Gross, 1991). High correlations between carotenoid and chlorophyll accumulations have been reported for kale (Brassica oleracea L. var. acephala; Kopsell et al., 2004), swiss chard (Beta vulgaris L.; Ihl et al., 1994), and other crop species (Grunwald et al., 1977; Terry and Abadia, 1986).

Vegetables play an important role in human diet and nutrition. Lettuce is the most important vegetable crop produced for fresh market in the United States in terms of acreage, production, and market value [National Agricultural Statistics Service (NASS), 2005]. In the United States, ≈75% of lettuce is produced in California (NASS, 2005). Most carotenoids produced in lettuce are β-carotene and lutein (Hart and Scott, 1995; USDA, 2004). About two-thirds of lettuce production and consumption in the United States is of the crisphead type (NASS, 2005). Compared to leaf or romaine types, crisphead lettuce is known to have much lower β-carotene and lutein contents (USDA, 2004). However, there is very limited information about varietal differences in carotenoid content, especially within the crisphead type, as available nutrient data were mostly obtained by analyzing samples from supermarkets. Izaki et al. (1986) determined β-carotene concentration of nine lettuce cultivars grown in Japan (including one crisphead cultivar) to be between 270 and 3900 μg/100 g fresh weight. Simonne et al. (2002) evaluated carotenoid content in 17 lettuce cultivars (including two crisphead cultivars) under the warm spring conditions of the southeastern United States, and found that β-carotene levels ranged from 124 to 900 μg/100 g fresh weight while lutein varied from 387 to 2709 μg/100 g.
In addition to genetic differences, carotenoid content may also be influenced by environmental factors. Light promotes the rapid synthesis of carotenoids in etiolated plants previously grown in the dark, being constituents of the photosynthetic apparatus, and generally the optimal temperature for carotenogenesis in plants is relatively low (Gross, 1991). Bureau and Bushway (1986) found that the β-carotene content of crisphead lettuce was higher in November than in July. Carotenol levels of butterhead lettuce produced by various cultivation methods ranged from 1360 to 3190 μg/100 g fresh weight in soil-grown samples and from 1810 to 2760 μg/100 g in hydroponically grown samples (Kobayashi et al., 1989). Benoit et al. (1984) found that plastic-covered and uncovered lettuce plants contained 11.0 and 16.6 μg carotene per gram fresh weight, respectively. There was a positive relation between N fertilizer doses and carotenol levels, while deficiency of P and K caused an increase in carotenol content in spinach leaves (Sengwald, 1959). Bottcher (1988) found that carotenol loss of head lettuce increased linearly with storage time, whereas the rate of loss was greater at higher storage temperatures.

Because plant-based foods contain numerous health-promoting phytochemicals that may act synergestically, dietary intake of carotenoids is generally viewed as more effective than the use of supplements. Despite the known benefits, efforts by public health organizations and the produce industry to increase the consumption of fruits and vegetables have met limited success due to dietary habits and cultural reasons. Over 70% of North Americans do not eat the recommended levels of fruits and vegetables, and it has been reported that consumption of vegetables would need to increase by over 300% in order to meet minimum recommendations (McNamara et al., 1999). Enhancing the nutritional levels of vegetables would improve the nutrient intake without requiring an increase in consumption. A breeding program to improve the nutritional value of lettuce must start with an assessment of the existing genetic variation in the germplasm including wild species. The objectives of this study were to assess the genetic variability in carotenol content in different types of lettuce and wild relatives, especially in the crisphead type, to examine the relationship among β-carotene, lutein, and chlorophyll concentrations, and to evaluate the interactions of lettuce genotypes with environmental factors such as growing seasons and plant position in the field.

Materials and Methods

Experiments were conducted at the Agricultural Research Station of the USDA, Salinas, Calif. Fifty-two genotypes from the lettuce germplasm collection maintained at the station, including crisphead, green leaf, red leaf, romaine, butterhead, stem, Latin, Batavia, and primitive forms of lettuce, a chlorophyll-deficient mutant, and wild species (L. serriola, L. saligna, L. virosa) from different geographic areas, were evaluated in Summer and Fall 2003. Seeds were planted in Sunshine Plug 5 Growing Mix (Sun Gro Horticulture, Bellevue, Wash.) in plastic transplanting trays (128 cells, 3 × 3 × 5 cm in length × width × height) in a greenhouse on 29 Apr. and 15 July for the summer and fall experiments, respectively.

Four weeks after planting, plants were transplanted in the field in a randomized complete-block design with four replications and each plot consisting of two rows of six plants each at the commercial spacing of 30 cm between plants and 35 cm between rows on a 1-m-wide double-row bed. The orientation of the rows was east-west for the summer experiment and north-south for the fall experiment. Preplant fertilizer was incorporated into the soil 1 week prior to transplanting as a combination of monoammonium phosphate, diammonium phosphate, and potassium sulfate (6N–8.8P–16.6K; Western Farm Service, Fresno, Calif.) at the rate of 336.6 kg·ha⁻¹. Nitrogen was sidedressed twice as ammonium sulphate at the rate of 67.2 kg·ha⁻¹ 2 weeks and 5 weeks after transplanting. Sprinkler irrigation was supplied twice per week to ensure adequate soil moisture for plant growth.

For each growing season, lettuce cultivars were harvested when they reached commercial size. Wild species were harvested when plants reached full size. Two plants in the middle of the rows were harvested from each plot in the morning with one from each side of the bed. Base- and wrapper-leaves were removed from crisphead and butterhead lettuce plants at harvest. The harvested plants were put on ice, transported to the lab, and weighed. A 0.3-g sample was taken from the 7th leaf from the outside of the head for each crisphead and butterhead lettuce plant, and from a leaf in an intermediate (neither inside nor outside) position on the plant for each other types of plant. The samples were taken from the edge of the leaves to avoid major ribs and were stored on ice prior to the extraction of carotenoids. The plants were then cut into four quarters and oven-dried at 70 °C for 48 h before being weighed for dry weight.

Carotenol and chlorophyll pigments were extracted and analyzed following the procedure of Norris et al. (1995) with minor modifications. The 0.3-g lettuce sample was placed in a 1.5-mL microcentrifuge tube and ground with a pellet pestle (Kontes Glass Co., Vineland, N.J.) mounted on an electric drill in 200 μL of 80% acetone. Ethyl acetate (120 μL) was added, and the mixture was vortexed. Water (140 μL) was added, and the mixture was vortexed and centrifuged at 15,700 g, for 5 min. The carotenol and chlorophyll containing upper phase was then transferred to a fresh tube. The sample was extracted two more times by adding ethyl acetate (120 μL), vortexing, centrifugation at 15,700 g, for 5 min, and removing the upper phase. The combined upper phases were then vacuum dried in a centrifugal evaporator (SpeedVac, Savant Instruments, Farmingdale, N.Y.) and stored at −80 °C under nitrogen until analysis. The dried extract was resuspended in 1.5 mL ethyl acetate, filtered through a 0.45-μm syringe-driven Nylon filter (Millex-HN; Millipore Co., Bedford, Mass.), and analyzed by reverse phase high-performance liquid chromatography (HPLC). The HPLC system (Alliance, Waters Co., Milford, Mass.) consisted of a separation unit (model 2695), a 4.6 × 10 mm-guard cartridge (S5 ODS1), a 4.6 × 250-mm, 5-μm packing C₄₅ column (S5 ODS1, Waters Spherisorb), and a photodiode array detector (model 2996). Extracts were kept in a 4 °C sample cooler before a 10-μL sample was injected into a 35-min gradient of ethyl acetate (0% to 35%) in acetonitrile-water-triethylamine [9:1:0.01 (v/v/v)], at a flow rate of 1 mL·min⁻¹. Carotenol and chlorophyll pigments were identified by comparing the retention time and absorption spectra of individual peaks with the standards. The concentration of individual pigment in lettuce samples was determined using peak areas relative to the corresponding standards at 440 nm wavelength. Beta-carotene, lutein, chlorophyll a, and chlorophyll b standards were obtained from Sigma Chemical Co. (St. Louis).

Data were analyzed by analysis of variance (ANOVA) using the general linear model procedure of JMP version 5 (SAS Institute, Cary, N.C.). Lettuce type and genotype were considered fixed effects, and replication and season were considered random effects. A crisphead cultivar, ‘Climax’, bolted before transplanting in the fall and therefore was excluded in the analyses for the fall season.
and across seasons. For comparisons between genotypes, least significant differences (LSD) were calculated with an error rate of \( P = 0.05 \). A correlation matrix for genotypes within each season was calculated for all variables using the multivariate platform of JMP. Spearman’s coefficients of rank correlation (Steel and Torrie, 1980) were calculated to test differences in rank order among the genotypes between the two growing seasons.

**Results and Discussion**

Beta-carotene and lutein concentrations differed significantly among lettuce types, genotypes, and seasons \((P < 0.01)\). This demonstrates that there is genetic variation in carotenoid accumulation among the lettuce genotypes tested, despite the influence from environments. There was also a significant genotype × season interaction \((P < 0.001)\). This suggests that the genotypes responded differently to the different environments. However, rank orders of carotenoid content did not significantly change for the genotypes from summer to fall season \([\text{Spearman’s rank correlation } (r_s) = 0.948 \text{ and } 0.930 \text{ for } \beta\text{-carotene and lutein concentrations, respectively, } P < 0.001]\). Mercadante and Rodriguez-Amaya (1991) reported seasonal variation between winter and summer production in \( \beta\text{-carotene and lutein} \) levels between two kale cultivars grown in Brazil. Kopsell et al. (2004) also found yearly variation in \( \beta\text{-carotene} \) and lutein concentrations among 23 *Brassica oleracea* cultivars, but the interaction of year and cultivar was not significant.

Carotenoid concentration in vegetables is usually reported on a fresh weight basis in literature. On fresh weight bases, wild lettuces, including *L. serriola*, *L. saligna*, *L. virosa*, and primitive forms of lettuce, generally had higher carotenoid levels than cultivated lettuces \( (\text{Table 1}) \). Among the major types of cultivated lettuce, carotenoid concentration followed the order of: green leaf or romaine > red leaf > butterhead > crisphead. The synthesis or absorption of many nutrients in plants is light dependent, and it has been demonstrated that the lower nutritional value of crisphead lettuce is largely due to the enclosure of its leaves in the head structure \((\text{Mou and Ryder, 2004})\). Romaine type had similar or higher carotenoid concentration than green leaf lettuce in summer, but green leaf had significantly higher carotenoid concentration than romaine in the fall \( (\text{Table 1}) \). Crisphead lettuce accumulated more lutein than \( \beta\text{-carotene} \), while other lettuce types had more \( \beta\text{-carotene} \) than lutein.

When expressed on a dry weight basis, romaine and green leaf lettuces had significantly higher carotenoid concentration than wild lettuces \( (\text{Table 1}) \). The major difference is that the wild lettuces had much lower moisture content \((\approx 90\%)\) than cultivated lettuces \((95\% \text{ to } 97\%)\). When expressed on fresh weight bases, therefore, the carotenoid concentration of cultivated lettuces was diluted by their high water content.

Among major cultivated lettuces, crisphead lettuce had highest head weight, followed by romaine, butterhead, green leaf, and red leaf types \( (\text{Table 2}) \). Chlorophyll \( a \), chlorophyll \( b \), and total chlorophyll concentrations generally followed the same rank order as carotenoid among different types of lettuce. There was also great variation in plant weight and chlorophyll concentration within the types and between the seasons. ‘Merlot’ and ‘Ruby’ both have intense red color but had higher chlorophyll concentration than the two cultivars with a mixture of green and red colors, ‘Lolla Rossa’ and ‘Prizehead’ \( (\text{data not shown}) \). This suggests that the green color of chlorophyll was masked by large amount of anthocyanin in ‘Merlot’ and ‘Ruby’.

Although crisphead lettuce had lower carotenoid level than other lettuce types, there were significant differences among genotypes within the crisphead type whether on a fresh weight or dry weight basis \( (\text{Table 2}) \). Three modern cultivars, ‘Legacy’, ‘Salinas 88’, and ‘Top Gun’, consistently had higher carotenoid concentrations in summer and fall \( (\text{averaging 471 and 592 } \mu\text{g/100 g of } \beta\text{-carotene and lutein on a fresh weight basis, respectively})\) than other cultivars. Three older cultivars, ‘Great Lakes’, ‘Green Lake’, and ‘Imperial 44’, had the lowest carotenoid concentrations in both seasons \( (\text{averaging 168 and 182 } \mu\text{g/100 g of } \beta\text{-carotene and lutein on a fresh weight basis, respectively})\). These results attest to the achievements of modern lettuce breeding, although nutritional improvement has not received great attention in the past \((\text{Ryder, 1986})\).

Two butterhead cultivars of ‘Bibb’ type \( (\text{‘Bibb’ and ‘Buttercush’) had significantly higher carotenoid concentration in both seasons \( (\text{averaging 2434 and 2179 } \mu\text{g/100 g of } \beta\text{-carotene and lutein on a fresh weight basis, respectively})\) than three butterhead cultivars of “Boston” type \( (\text{‘Dark Green Boston’, ‘Dynamite’, and ‘Epic’}) \); averaging 832 and 746 \( \mu\text{g/100 g of } \beta\text{-carotene and lutein on a fresh weight basis, respectively})\); Table 3). The top of the head for “Boston” type of lettuce is closed, while the top of “Bibb” type of lettuce is more open. The semi-open head of “Bibb” type allows easier penetration of sunlight into the head.

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**Table 1.** Means of moisture, \( \beta\text{-carotene} \) and lutein concentration, expressed on fresh weight and dry weight bases \((\mu\text{g per unit leaf wt})\), for different types of lettuce grown in the field in Salinas, Calif., in Summer and Fall 2003.³

| Type³ | No. of genotype | Moisture (%) | Beta-carotene | Lutein |
|-------|-----------------|--------------|---------------|--------|
|       |                 | Summer | Fall | Summer | Fall | Summer | Fall | Summer | Fall | Summer | Fall |
| Crisphead | 22           | 96.7 a | 97.0 a | 328 f | 319 h | 102 f | 108 f | 416 f | 372 i | 131 e | 131 f |
| Butterhead | 5            | 96.5 ab | 96.2 b | 1418 e | 1527 f | 410 e | 415 d | 1303 e | 1335 g | 382 d | 367 e |
| Romaine | 5             | 95.6 cd | 95.4 c | 4029 b | 3228 d | 954 a | 749 a | 3985 b | 2868 d | 946 a | 654 ab |
| Green leaf | 5           | 94.5 e | 94.7 e | 4038 b | 3860 c | 748 b | 714 a | 3338 c | 3106 c | 613 b | 574 c |
| Red leaf | 4             | 95.3 d | 95.4 cd | 2464 d | 2231 e | 545 d | 494 bc | 2138 d | 1961 f | 473 c | 430 d |
| Batavia | 1             | 96.5 ab | 94.9 de | 1364 e | 1054 g | 406 e | 204 d | 1021 e | 938 h | 346 d | 178 f |
| Latin | 1              | 96.1 bc | 96.0 b | 3121 c | 2323 e | 819 b | 568 b | 2274 d | 2178 e | 620 b | 596 bc |
| Stem | 1              | 94.2 e | 94.4 e | 4237 b | 4136 b | 715 b | 708 a | 3629 c | 4316 a | 636 b | 700 a |
| Wild | 7              | 88.9 f | 90.3 f | 6609 a | 4472 a | 607 c | 472 c | 5578 a | 3707 b | 510 c | 397 de |
| Chl deficient | 1        | 96.9 a | 96.6 ab | 249 f | 161 h | 83 f | 41 f | 305 f | 204 i | 106 e | 50 g |
| Mean |                | 95.2 | 95.4 | 2302 | 1915 | 409 | 362 | 2060 | 1682 | 380 | 329 |

²Means in the same column followed by different letters indicate significant differences at \( P < 0.05 \).

³Wild type includes *L. serriola*, *L. saligna*, *L. virosa*, and primitive forms of lettuce. Chl, chlorophyll.
Table 2. Means of plant weight, chlorophyll (Chl) \( a \) and \( b \), and total chlorophyll (Chl \( a \) + Chl \( b \)) concentration on a fresh weight basis (\( \mu \text{g per 100 g leaf} \)) for different lettuce types grown in the field in Salinas, Calif., in Summer and Fall 2003.\(^{z}\)

| Type\(^{y}\) | Summer | Fall | Summer | Fall | Summer | Fall | Summer | Fall |
|-------------|--------|------|--------|------|--------|------|--------|------|
| Plant wt (g) |        |      |        |      |        |      |        |      |
| Crisphead   | 1037 a | 829 a| 2936 g  | 4146 e| 1760 g  | 2036 ef| 4695 f  | 6182 h|
| Butterhead  | 406 c  | 312 c| 16459 f | 21410 e| 6376 e  | 6957 d | 22873 e | 28367 f|
| Romaine     | 650 b  | 508 b| 45076 cd | 54263 b| 26473 b | 19445 a| 71549 f | 73708 b|
| Green leaf  | 273 d  | 304 c| 49248 bc | 51846 b| 17002 c | 14873 b| 66249 b | 66719 c|
| Red leaf    | 291 d  | 228 d| 33691 e  | 27761 d| 9806 d  | 8025 d | 34397 d | 35786 d|
| Batavia     | 332 cd | 233 cd| 40832 de | 37728 c| 13305 cd| 11130 c| 54138 c | 48857 d|
| Latin       | 210 de | 307 cd| 52703 b  | 67655 a| 14881 c | 19779 a| 67584 b | 87435 a|
| Stem        | 94 e   | 87 e | 78414 a  | 65139 a| 30865 a | 18878 a| 109279 a| 84017 a|
| Chl deficient| 647 b  | 560 b| 2084 g   | 1513 g | 1129 fg | 720 f | 3212 f  | 2233 h|
| Mean        | 636    | 512  | 27335    | 27682 | 11114   | 8799  | 38498   | 36481 |

\(^{z}\)Means in the same column followed by different letters indicate significant differences at \( P < 0.05. \)

\(^{y}\)Wild type includes \( L. \text{serriola, L. saligna, L. virosa,} \) and primitive forms of lettuce.

Table 3. Means of moisture, \( \beta \)-carotene and lutein concentration, expressed on fresh weight and dry weight bases (\( \mu \text{g per unit leaf wt})\), for 52 lettuce genotypes grown in the field in Salinas, Calif., in Summer and Fall 2003.

| Genotype   | Type\(^{y}\) | Moisture (%) | Fresh wt (\( \mu \text{g/100 g)} \) | Dry wt (\( \mu \text{g·g}^{-1} \)) | Fresh wt (\( \mu \text{g/100 g)} \) | Dry wt (\( \mu \text{g·g}^{-1} \)) |
|------------|-------------|--------------|----------------------------------|---------------------------------|----------------------------------|---------------------------------|
| Bronco     | Crisphead   | 97.2         | 96.9                             | 340                             | 316                             | 140                             | 101                             |
| Calmar     | Crisphead   | 96.2         | 97.6                             | 318                             | 262                             | 87                              | 101                             |
| Climax     | Crisphead   | 97.5         | ----                             | 398                             | ----                             | 177                             | ----                             |
| Empire     | Crisphead   | 96.5         | 98.2                             | 260                             | 221                             | 69                              | 124                             |
| Francisco  | Crisphead   | 96.5         | 97.0                             | 222                             | 248                             | 87                              | 101                             |
| Glacier    | Crisphead   | 96.4         | 97.6                             | 538                             | 273                             | 132                             | 117                             |
| Great Lakes| Crisphead   | 97.1         | 96.5                             | 167                             | 181                             | 47                              | 50                              |
| Green Lake | Crisphead   | 97.1         | 97.3                             | 203                             | 179                             | 82                              | 72                              |
| Ice Cube   | Crisphead   | 96.2         | 96.0                             | 311                             | 347                             | 69                              | 84                              |
| Imperial 44| Crisphead   | 97.3         | 97.3                             | 105                             | 175                             | 42                              | 78                              |
| King Crown | Crisphead   | 95.8         | 97.5                             | 311                             | 301                             | 78                              | 122                             |
| Legacy     | Crisphead   | 97.5         | 96.5                             | 477                             | 408                             | 201                             | 109                             |
| Mohawk     | Crisphead   | 96.0         | 96.3                             | 236                             | 316                             | 63                              | 99                              |
| Monterey   | Crisphead   | 96.2         | 96.3                             | 340                             | 379                             | 65                              | 94                              |
| Niner      | Crisphead   | 97.0         | 98.0                             | 217                             | 245                             | 80                              | 88                              |
| Salinas 88 | Crisphead   | 96.4         | 96.9                             | 528                             | 509                             | 154                             | 161                             |
| Sniper     | Crisphead   | 96.6         | 97.2                             | 402                             | 350                             | 113                             | 135                             |
| Thompson   | Crisphead   | 97.1         | 97.1                             | 271                             | 230                             | 97                              | 81                              |
| Tiber      | Crisphead   | 97.2         | 97.3                             | 532                             | 342                             | 206                             | 127                             |
| Top Gun    | Crisphead   | 96.4         | 97.0                             | 458                             | 444                             | 102                             | 143                             |
| Vanguard 75| Crisphead   | 95.6         | 96.4                             | 227                             | 520                             | 41                              | 134                             |
| Yuma       | Crisphead   | 97.0         | 96.6                             | 355                             | 463                             | 129                             | 166                             |
| Bibb       | Butterhead  | 96.5         | 96.4                             | 2819                            | 2847                            | 751                             | 802                             |
| Buttercrunch| Butterhead  | 96.6       | 96.1                             | 2122                            | 1946                            | 687                             | 505                             |
| Dark Green Boston| Butterhead | 97.0  | 95.8                             | 731                             | 851                             | 194                             | 219                             |
| Dynamite   | Butterhead  | 96.6         | 97.2                             | 650                             | 630                             | 205                             | 217                             |
| Epic       | Butterhead  | 95.9         | 95.5                             | 768                             | 1361                            | 212                             | 362                             |
| Darkland   | Romaine     | 94.8         | 96.4                             | 5242                            | 3690                            | 1000                            | 1086                            |
| Heart’s Delight| Romaine | 95.3       | 95.3                             | 2379                            | 1427                            | 550                             | 340                             |
| Parris Island| Romaine    | 95.3       | 95.1                             | 4208                            | 3492                            | 953                             | 704                             |
| Tall Guzmaine| Romaine    | 96.5       | 95.4                             | 3573                            | 3818                            | 1022                            | 836                             |
| Valmaine   | Romaine     | 96.3         | 95.0                             | 4741                            | 3711                            | 1245                            | 778                             |
| Grand Rapids| Green leaf  | 94.3         | 95.1                             | 2927                            | 2272                            | 565                             | 436                             |
| Greengo    | Green leaf  | 94.1         | 95.3                             | 5088                            | 5184                            | 831                             | 1044                            |
| PI 206963  | Green leaf  | 92.6         | 93.7                             | 5693                            | 5508                            | 752                             | 915                             |
| Salad Bowl | Green leaf  | 95.4         | 93.8                             | 2873                            | 2907                            | 668                             | 460                             |
| Waldmann’s Green leaf | Green leaf | 96.0      | 95.3                             | 3609                            | 3426                            | 922                             | 715                             |

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which probably contributed to its higher carotenoid content.

A romaine cultivar, ‘Heart’s Delight’, had much lower carotenoid level in both seasons (averaging 1903 and 2118 μg/100 g of β-carotene and lutein on a fresh weight basis, respectively) than other four romaine cultivars (averaging 4059 and 3753 μg/100 g of β-carotene and lutein on a fresh weight basis, respectively; Table 3). ‘Heart’s Delight’ is a cultivar for romaine heart production and forms a closed head, while other romaine cultivars have open heads. The closed head obstructs the penetration of sunlight, probably leading to its lower carotenoid content.

Significant differences in carotenoid concentration were also found among genotypes within green leaf and red leaf types (Table 3). PI 206963 and ‘Greengo’ had higher carotenoid levels than other leaf lettuces in both seasons. Simonne et al. (2002) also reported that ‘Greengo’ was the highest in β-carotene among 17 lettuce cultivars tested. ‘Merlot’ had the highest carotenoid concentration among red leaf cultivars.

Along with genetic variability, there also appears to be an environmental influence on carotenoid accumulation in lettuce. Carotenoid concentration and plant weight were significantly higher in summer than in the fall (Table 4). Some wild, primitive, and romaine lettuces especially showed large reductions in carotenoid level in the fall (Table 3). The summer experiment was harvested in July when average solar radiation and air temperature were 294 W·m–2 and 16.2 °C, while the fall experiment was harvested in October when average solar radiation and air temperature were 173 W·m–2 and 15.1 °C, respectively [California Irrigation Management Information System (CIMIS), 2003]. As the synthesis of carotenoids is light dependent, the reduced solar radiation in the fall may have contributed to the lower carotenoid content in lettuce. There was also an overall reduction in chlorophyll b and total chlorophyll concentrations from summer to fall, although there was an increase in chlorophyll a and total chlorophyll levels in the fall for crisphead, butterhead, romaine, green leaf, and stem lettuces (Table 2). These results suggest that environmental manipulations during production, in conjunction with the selection of cultivars, may be necessary to optimize the carotenoid levels of lettuce crops.

It is a standard practice to grow lettuce on double-row raised beds in California. There was no difference in carotenoid concentration whether plant samples were from the north or south side of the bed in summer or from the east or west side of the

Table 4. Mean values of carotenoids (expressed on a fresh weight basis) and plant weight as affected by season and plant position on bed for lettuce genotypes grown in the field in Salinas, Calif., in Summer and Fall 2003. a

| Season | Bed position | Beta-carotene (μg/100 g) | Lutein (μg/100 g) | Plant wt (g) |
|--------|--------------|--------------------------|------------------|-------------|
| Summer | South        | 1901 a                   | 1742 a           | 689 a       |
| Summer | North        | 1907 a                   | 1729 a           | 671 a       |
| Fall   | West         | 1848 b                   | 1622 b           | 542 b       |
| Fall   | East         | 1841 b                   | 1630 b           | 495 c       |

aPI 206963, PI 251247, PI 490999, and PI 491181 were excluded from analysis as bed position data for these genotypes were not collected. Means in the same column followed by different letters indicate significant differences at P < 0.05.

bIndicating plant samples were harvested from which side of the double row beds.
have a large population of plants to screen. Indeed, this may therefore be more suitable for lettuce breeders who usually select for high green leaves (Table 3). 801277-1 is a chlorophyll deficient mutant of lettuce with a recessive gene (L. var. L. sativa) and is highly negative compared with moisture content and low pigment levels (Tables 1 and 2). Likewise, there were highly negative correlations between moisture content and pigment levels on a fresh weight basis, partly because crisphead lettuce generally had high moisture content and low pigment accumulation (Tables 1 and 2). Beta-carotene and lutein concentrations were highly correlated with chlorophyll \(a\), chlorophyll \(b\), and total chlorophyll concentrations in both seasons on a fresh weight basis (Table 5). On a dry weight basis, beta-carotene and lutein concentrations were also highly correlated with chlorophyll \(a\), chlorophyll \(b\), and total chlorophyll concentrations in both seasons, with correlation coefficients ranging from 0.93 to 0.99. This suggests that carotenoid levels in lettuce may be selected for higher levels of one carotenoid would likely lead to the increase of the other carotenoid. Plant weight was negatively correlated with beta-carotene, lutein, and chlorophyll concentrations, probably because crisphead cultivars tend to have high plant weight and low pigment levels (Tables 3 and 3). Furthermore, the higher carotenoid concentration in wild lettuces may not be transferable to cultivated lettuces that have higher water content. Coupled with the potential use of chlorophyll content or green color as selection markers, genetic improvement of carotenoid content in lettuce seems feasible.

| Traits \(a\) | Plant wt | Moisture (%) | Beta-carotene (fresh wt) | Lutein (fresh wt) | Chl \(a\) | Chl \(b\) | Total Chl | Beta-carotene (dry wt) | Lutein (dry wt) |
|----------------|--------|-------------|-------------------------|-----------------|--------|--------|----------|---------------------|----------------|
| Plant wt      | --     | 0.677      | -0.744                  | -0.705          | -0.770 | -0.587 | -0.741   | -0.622              | -0.516         |
| Moisture (%)  | 0.773  | --         | -0.826                  | -0.789          | -0.838 | -0.684 | -0.821   | -0.381              | -0.299         |
| Beta-carotene (fresh wt) | -0.746  | -0.771  | --                     | 0.992           | 0.948 | 0.943 | 0.984    | 0.800               | 0.746         |
| Lutein (fresh wt) | -0.722  | -0.735  | 0.985                   | --              | 0.937 | 0.962 | 0.982    | 0.818               | 0.788         |
| Chl \(a\)     | -0.684 | -0.725    | 0.985                   | 0.986           | --    | 0.828 | 0.983    | 0.777               | 0.717         |
| Chl \(b\)     | -0.632 | -0.677    | 0.962                   | 0.964           | 0.987 | --    | 0.917    | 0.794               | 0.783         |
| Total Chl     | -0.674 | -0.716    | 0.982                   | 0.983           | 0.999 | 0.992 | --       | 0.813               | 0.767         |
| \(\beta\)-Carotene (dry wt) | -0.582 | -0.396  | 0.863                   | 0.865           | 0.866 | 0.887 | 0.873    | --                  | 0.978         |
| Lutein (dry wt) | -0.536  | -0.339  | 0.826                   | 0.854           | 0.846 | 0.869 | 0.853    | 0.986               | --            |

\(a\) (fresh wt) and (dry wt), carotenoid concentrations expressed on a fresh weight and dry weight basis, respectively; Chl = chlorophyll concentration expressed on a fresh weight basis.

Bed in the fall (Table 4). However, plants on the west side of the bed had significantly higher weight than plants on the east side of the bed in the fall. That was probably due to the fact that it was often foggy in the morning in the fall and plants on the west side of the bed received more sunlight in the afternoon.

During each season, beta-carotene and lutein concentrations, whether on a fresh weight or dry weight basis, were highly correlated (Table 5). This is good news for lettuce breeders as selection for higher levels of one carotenoid would likely lead to the increase of the other carotenoid. Plant weight was negatively correlated with beta-carotene, lutein, and chlorophyll concentrations, probably because crisphead cultivars tend to have high plant weight and low pigment levels (Tables 1 and 2). Likewise, there were highly negative correlations between moisture content and pigment levels on a fresh weight basis, partly because crisphead lettuce generally had high moisture content and low pigment accumulation (Tables 1 and 2). Beta-carotene and lutein concentrations were highly correlated with chlorophyll \(a\), chlorophyll \(b\), and total chlorophyll concentrations in both seasons on a fresh weight basis (Table 5). On a dry weight basis, beta-carotene and lutein concentrations were also highly correlated with chlorophyll \(a\), chlorophyll \(b\), and total chlorophyll concentrations in both seasons, with correlation coefficients ranging from 0.93 to 0.99. This suggests that carotenoid levels in lettuce may be selected for higher levels of one carotenoid would likely lead to the increase of the other carotenoid. Plant weight was negatively correlated with beta-carotene, lutein, and chlorophyll concentrations, probably because crisphead cultivars tend to have high plant weight and low pigment levels (Tables 1 and 2). Likewise, there were highly negative correlations between moisture content and pigment levels on a fresh weight basis, partly because crisphead lettuce generally had high moisture content and low pigment accumulation (Tables 1 and 2). Beta-carotene and lutein concentrations were highly correlated with chlorophyll \(a\), chlorophyll \(b\), and total chlorophyll concentrations in both seasons during the storage of head lettuce (Lactuca sativa L. var. capitata L.). Part 2. Nutritional value. Nahrung 32:27–36.

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Table 5. Correlation coefficients (r) between traits calculated from the means of 52 lettuce genotypes grown in Salinas, Calif. over two seasons in 2003. The values above the diagonal (---) lines are from the summer season, and the values below the diagonal (----) lines are from the fall season. All coefficients are significant at \(P < 0.01\).
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