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 carotenoid 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.

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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. Carotene 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 carotene levels, while deficiency of P and K caused an increase in carotene content in spinach leaves (Sengewald, 1959). Bottcher (1988) found that carotene 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 synergistically, 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 carotenoid 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 $kg\cdot ha^{-1}$. Nitrogen was sidedressed twice as ammonium sulphate at the rate of 67.2 $kg\cdot ha^{-1}$ 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.

Carotenoid 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_n for 5 min. The carotenoid 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_n 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 C_{18} 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)], at a flow rate of 1 $mL\cdot min^{-1}$. Carotenoid 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 [Spearman's rank correlation (r_s) = 0.948 and 0.930 for β-carotene and lutein concentrations, respectively. $P < 0.001$]. Mercadante and Rodriguez-Amaya (1991) reported seasonal variation between winter and summer production in β-carotene and lutein levels between two kale cultivars grown in Brazil. Kopsell et al. (2004) also found yearly variation in β-carotene and lutein concentrations among 23 *Brassica oleracea* cultigens, but the interaction of year and cultigen 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 (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 (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 (Table 1). Crisphead lettuce accumulated more lutein than β-carotene, while other lettuce types had more β-carotene than lutein.

When expressed on a dry weight basis, romaine and green leaf lettuces had significantly higher carotenoid concentration than wild lettuces (Table 1). The major difference is that the wild lettuces had much lower moisture content (≈90%) than cultivated lettuces (95% 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 (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' (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 (Table 3). Three modern cultivars, 'Legacy', 'Salinas 88', and 'Top Gun', consistently had higher carotenoid concentrations in summer and fall (averaging 471 and 592 μg/100 g of β-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 (averaging 168 and 182 μg/100 g of β-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 (Ryder, 1986).

Two butterhead cultivars of "Bibb" type ('Bibb' and 'Buttercrunch') had significantly higher carotenoid concentration in both seasons (averaging 2434 and 2179 μg/100 g of β-carotene and lutein on a fresh weight basis, respectively) than three butterhead cultivars of "Boston" type ('Dark Green Boston', 'Dynamite', and 'Epic'; averaging 832 and 746 μg/100 g of β-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,

Table 1. Means of moisture, β-carotene and lutein concentration, expressed on fresh weight and dry weight bases (μg per unit leaf wt), for different types of lettuce grown in the field in Salinas, Calif., in Summer and Fall 2003.[z]

				Beta-carotene				Lutein			
	No. of	Moisture (%)		Fresh wt (μg/100 g)		Dry wt ($\mu g \cdot g^{-1}$)		Fresh wt (μg/100 g)		Dry wt ($\mu g \cdot g^{-1}$)	
Type[y]	genotype	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 e	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

[z]Means in the same column followed by different letters indicate significant differences at $P < 0.05$.

[y]Wild 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 (μg per 100 g leaf) for different lettuce types grown in the field in Salinas, Calif., in Summer and Fall 2003.[z]

	Plant wt (g)		Chl *a* (μg/100 g)		Chl *b* (μg/100 g)		Total Chl (μg/100 g)	
Type[y]	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall
Crisphead	1037 a	829 a	2936 g	4146 g	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 b	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	43497 d	35786 e
Batavia	630 b	472 b	17414 f	13203 f	5373 ef	3727 e	22787 e	16931 g
Latin	332 cd	233 cd	40832 de	37728 c	13305 cd	11130 c	54138 c	48857 d
Stem	210 de	307 cd	52703 b	67655 a	14881 c	19779 a	67584 b	87435 a
Wild	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. serriola, L. saligna*, *L. virosa*, and primitive forms of lettuce.

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

				Beta-carotene				Lutein			
		Moisture (%)		Fresh wt (μg/100 g)		Dry wt (μg·g^{-1})		Fresh wt (μg/100 g)		Dry wt (μg·g^{-1})	
Genotype	Type[z]	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall
Bronco	Crisphead	97.2	96.9	340	316	140	101	485	393	175	128
Calmar	Crisphead	96.2	97.6	318	262	87	101	346	299	89	136
Climax	Crisphead	97.5	----	398	----	177	----	478	----	212	----
Empire	Crisphead	96.5	98.2	260	221	69	124	347	227	99	123
Francisco	Crisphead	96.5	97.0	222	248	74	85	280	330	99	112
Glacier	Crisphead	96.4	97.6	538	273	132	117	498	338	117	159
Great Lakes	Crisphead	97.1	96.5	167	181	47	50	159	176	45	46
Green Lake	Crisphead	97.1	97.3	203	179	82	72	226	208	81	83
Ice Cube	Crisphead	96.2	96.0	311	347	69	84	548	467	132	117
Imperial 44	Crisphead	97.3	97.3	105	175	42	78	129	192	45	95
King Crown	Crisphead	95.8	97.5	311	301	78	122	306	350	76	147
Legacy	Crisphead	97.5	96.5	477	408	201	109	644	449	297	118
Mohawk	Crisphead	96.0	96.3	236	316	63	99	295	339	82	110
Monterey	Crisphead	96.2	96.3	340	379	65	94	304	375	69	97
Niner	Crisphead	97.0	98.0	217	245	80	88	294	290	109	145
Salinas 88	Crisphead	96.4	96.9	528	509	154	161	662	526	185	161
Sniper	Crisphead	96.6	97.2	402	350	113	135	563	407	159	151
Thompson	Crisphead	97.1	97.1	271	230	97	81	416	291	132	102
Tiber	Crisphead	97.2	97.3	532	342	206	127	710	398	279	159
Top Gun	Crisphead	96.4	97.0	458	444	102	143	688	582	166	196
Vanguard 75	Crisphead	95.6	96.4	227	520	41	134	302	639	58	187
Yuma	Crisphead	97.0	96.6	355	463	129	166	466	543	178	185
Bibb	Butterhead	96.5	96.4	2819	2847	751	802	2544	2394	735	682
Buttercrunch	Butterhead	96.6	96.1	2122	1946	687	505	2016	1762	641	448
Dark Green Boston	Butterhead	97.0	95.8	731	851	194	191	681	724	166	176
Dynamite	Butterhead	96.6	97.2	650	630	205	217	538	572	154	222
Epic	Butterhead	95.9	95.5	768	1361	212	362	737	1226	217	308
Darkland	Romaine	94.8	96.4	5242	3690	1000	1086	5426	3304	1111	967
Heart's Delight	Romaine	95.3	95.3	2379	1427	550	340	2627	1608	622	337
Parris Island	Romaine	95.3	95.1	4208	3492	953	704	4095	2780	876	571
Tall Guzmaine	Romaine	96.5	95.4	3573	3818	1022	836	3142	3329	900	722
Valmaine	Romaine	96.3	95.0	4741	3711	1245	778	4633	3317	1220	674
Grand Rapids	Green leaf	94.3	95.1	2927	2272	565	436	2200	1821	405	348
Greengo	Green leaf	94.1	95.3	5088	5184	831	1044	4413	4366	746	877
PI 206963	Green leaf	92.6	93.7	5693	5508	752	915	4598	4458	615	718
Salad Bowl	Green leaf	95.4	93.8	2873	2907	668	460	2195	2007	470	329
Waldmann's Green	Green leaf	96.0	95.3	3609	3426	922	715	3286	2877	829	598

continued next page

Table 3. Continued.

Genotype	Type[z]	Moisture (%) Summer	Moisture (%) Fall	Beta-carotene Fresh wt (μg/100 g) Summer	Beta-carotene Fresh wt (μg/100 g) Fall	Beta-carotene Dry wt ($\mu g \cdot g^{-1}$) Summer	Beta-carotene Dry wt ($\mu g \cdot g^{-1}$) Fall	Lutein Fresh wt (μg/100 g) Summer	Lutein Fresh wt (μg/100 g) Fall	Lutein Dry wt ($\mu g \cdot g^{-1}$) Summer	Lutein Dry wt ($\mu g \cdot g^{-1}$) Fall
Lolla Rossa	Red leaf	95.1	95.7	2010	1200	421	305	1489	1144	325	289
Merlot	Red leaf	94.7	94.8	3337	2961	646	563	3000	2456	592	456
Prizehead	Red leaf	96.2	96.1	2365	2090	608	572	2239	1977	587	523
Ruby	Red leaf	95.2	95.0	2141	2673	504	535	1823	2268	389	453
Iceberg	Batavia	96.5	94.9	1364	1054	406	204	1021	938	346	178
Little Gem	Latin	96.1	96.0	3121	2323	819	568	2274	2178	620	596
Da Ye Wo Sun	Stem	94.2	94.4	4237	4136	715	708	3629	4316	636	700
PI 251247	Primitive	90.0	90.8	9090	3437	885	397	7586	2536	737	301
PI 490999	*L. saligna*	89.8	91.2	5383	5770	533	671	4502	5763	437	683
PI 509525	*L. saligna*	88.7	90.0	5787	3049	506	308	5072	2255	420	232
PI 491181	*L. serriola*	89.8	88.8	8264	4981	798	422	7486	3638	778	317
PI 491239	*L. serriola*	89.9	91.5	5293	5295	538	626	4724	3739	454	433
PI 273597	*L. virosa*	87.5	89.9	5415	4213	441	429	4391	4035	338	401
PI 274375	*L. virosa*	86.9	89.9	7030	4561	550	450	5287	3980	408	411
801277-1	Chl deficient	96.9	96.6	249	161	83	41	305	204	106	50
Mean		95.2	95.4	2302	1915	409	362	2060	1682	380	329
$LSD_{0.05}$[y]		0.6	0.6	323	223	99	93	318	269	82	96

[z] Chl, chlorophyll.

[y] Least significant differences at $P < 0.05$.

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.[z]

Season	Bed position[y]	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

[z]PI 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 $P < 0.05$.

[y]Indicating plant samples were harvested from which side of the double row beds.

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 $W \cdot m^{-2}$ and 16.2 °C, while the fall experiment was harvested in October when average solar radiation and air temperature were 173 $W \cdot m^{-2}$ 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 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 $P < 0.01$.

Traits[z]	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
β-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	----

[z](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, β-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 β-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, β-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 indirectly by selecting for chlorophyll concentration or green color, which is much easier to do than carotenoid analysis and may therefore be more suitable for lettuce breeders who usually have a large population of plants to screen. Indeed, 'Waldmann's Green' is a green leaf lettuce selected from 'Grand Rapids' and had higher carotenoid level than 'Grand Rapids' that has yellow green leaves (Table 3). 801277-1 is a chlorophyll deficient mutant of lettuce with a recessive gene (*cd-3*; Ryder, 1996) and yellow leaves, and was low in both chlorophylls and carotenoids in both seasons (Tables 2 and 3). Similar relationships between carotenoids and chlorophylls have been reported for other leafy vegetables like kale (Kopsell et al., 2004) and swiss chard (Ihl et al., 1994).

The major product from agricultural production is food that is fundamental to human life and health. Consumers have become more demanding for safe and nutritious foods that improve physical performance, reduce risks of diseases, and increase the life span. Because of the reported health benefits of carotenoids in fruits and vegetables, there is great interest in increasing the carotenoid levels in lettuce that is the most consumed fresh vegetable in the United States. In this study, we found a wide range of genetic variability in carotenoid concentration in different types of lettuce and wild species, including the crisphead type. It may therefore be possible to increase the nutritional value of different types of cultivated lettuce through crosses with same or different types of lettuce with high levels of carotenoid and subsequent selection. Attention should be paid to moisture percentage of the plant, as 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.

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