Nutrient Density in Lettuce Cultivars Grown with Organic or Conventional Fertilization with Elevated Calcium Concentrations

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Additional index words. calcium accumulation, cultivar selection, fertilizer regimes, *Lactuca sativa*, lettuce genetics, lettuce phenotypes, macronutrients

Abstract. Calcium-rich vegetables in the diet could ameliorate the potential for calcium (Ca) deficiency in human nutrition. This study investigated the prospect of increasing Ca density of lettuce (*Lactuca sativa* L.) through cultivar selection and nutrient management in a greenhouse. Eighteen lettuce cultivars including butterhead, romaine, and loose-leaf phenotypes of heritage and modern genetics were tested. Organic fertilizer (3N–0.7P–3.3K) and commercial conventional fertilizer (20N–4.4P–16.6K) factored with three Ca levels (50, 100, 200 mg·L⁻¹ as CaCl₂) were the fertilizer regimes. Calcium in whole shoots was analyzed by atomic absorption spectrometry of oven-ashed samples. Heritage cultivars had a significantly higher Ca concentration (1.93% dry weight) than modern cultivars (1.54%). Loose-leaf phenotypes had the highest Ca concentration (2.06%) followed by butterhead (1.66%) and romaine (1.49%). Accumulation of Ca was higher with the conventional fertilizer (1.90%) than with the organic fertilizer (1.58%). Elevated Ca level in the fertility regimes raised the Ca concentration in lettuce from 1.56% at 50 mg·L⁻¹ to a mean of 1.82% at 100 mg·L⁻¹ and 200 mg·L⁻¹. Large differences in Ca concentration occurred among individual cultivars with ranges from 1.27% to 3.05%. ‘Salad Bowl’, ‘Red Deer Tongue’, ‘Buttercrunch’, and ‘Bronze Mignonette’ were the top in cultivar ranking with mean Ca concentration of 2.50%, whereas ‘Adriana’, ‘Austrole’, ‘Coastal Star’, and ‘Forellenschluss’ were low accumulators with a mean of 1.33%. Head size of cultivars had no correlation with Ca concentration. This experiment indicates that selection of nutrient regimes and cultivars can be used to increase Ca accumulation in lettuce.

Critical functions in the human body require mineral nutrients that are obtained through consumption of food. Calcium is required for constructing and maintaining bones, clotting of blood, and for function of hormones and enzymes (Department of Health & Human Services, 2000; Ervin et al., 2004; Karll, 2000; Krause and Mahan, 1984; National Research Council, 1989). Diets rich in Ca minimize Ca deficiency and maximize good health and well-being (Greenwald et al., 2001). The recommended daily intake of Ca for most men and women is 1000 mg·d⁻¹ (Meacham et al., 2008); however, for elderly individuals, the required Ca intake should total 1300 to 1700 mg·d⁻¹ (Heaney, 2001). A study suggested that it is possible to improve dietary Ca

intake by increasing consumption of fruits, vegetables, and Ca-rich foods (Bernstein et al., 2002). However, fresh fruits are high in vitamins but are often low in minerals (Ashmead, 1982); on the other hand, fresh leafy vegetables are major sources of minerals (Kamchan et al., 2004). Vegetables such as kale, celery, collard, Chinese cabbage, and soybean sprouts contain high levels of Ca (Kamchan et al., 2004).

Lettuce (*Lactuca sativa* L.) is an important vegetable crop with high market value, and its nutritional characteristics have been studied throughout the world (Ashkar and Ries, 1970; Keat et al., 1999). The Ca content of lettuce is affected by relative humidity, which affects Ca distribution in the shoots, thereby resulting in the deficiency disorder of tipburn (Collier and Tibbitts, 1984; Schlagnhauser et al., 1987).

Degree Brix, a measure of sucrose, is used commonly for expressing the quality of juices, fruits, and vegetables (Hale et al., 2005; Ikeda et al., 2013; Widodo et al., 1996). This use has been expanded to include the concept that foods with high Brix readings also are high in mineral nutrients and that Brix readings are a general indicator of nutritional qualities of foods (Anderson, 2009; Frank, 2013; Sullivan, 2012).

This study was conducted to determine whether the nutrient concentration of lettuce varied among cultivars and if nutritional regimes could be managed to increase the Ca contents in the produce. Brix was measured in this investigation to assess whether a correlation occurred between this reading and Ca concentration in lettuce.

Materials and Methods

Plant materials. Eighteen lettuce cultivars of heritage and modern genetics with butterhead, romaine, and loose-leaf phenotypes were studied. Seeds were obtained from Seeds of Change (Rancho Dominguez, CA) and Johnny’s Seeds (Winslow, ME). All seeds were planted in peatmoss-based medium (Fafard Growing Mix 1-PV; Conrad Fafard Inc., Agawam, MA). Seedlings at the three-leaf stage were transplanted to 15-cm round standard pots filled with the same peatmoss medium. Temperatures generally ranged from 23 to 30 °C during day and 18 to 24 °C at night. Light conditions in the greenhouse, at the University of Massachusetts, Amherst (lat. 42.37° N, long. 72.53° W), were from sunlight during the season of production in June and July 2010 with ≈13 h of daylight and 11 h of night.

Treatments. Two fertilizers factored with three elevated Ca concentrations were fertility regimes for this study. One regime was conventional, peatlite professional fertilizer (20N–4.4P–16.6K with micronutrients reported; Peters Fertilizer Products, J.R. Peters, Inc., Allentown, PA). The conventional fertilizer solution was prepared at 1 g·L⁻¹ and 4 mL 0.5 M MgSO₄ was added per liter. The second fertilizer regime was a commercial organic fertilizer (3N–0.7P–3.3K with micronutrients not reported; Pure Blend Pro Grow; Pure Blend Pro Fertilizer Products, Chandler, AZ). The organic fertilizer solution was prepared at 6.7 mL·L⁻¹ to approximate the supply from the conventional fertilizer. The factorial design consisted of three concentrations of Ca (50, 100, 200 mg·L⁻¹ as CaCl₂) with three replicates in randomized complete blocks. The two fertilizers solutions were applied at 100 mL/plant for the first week and then 200 mL/plant until harvest 3 weeks later. The nutrient solution drained through the medium during application to avoid salt accumulation in the medium and to ensure a constant supply of nutrients.

Measurements. At harvest, fresh weights of plants were recorded, and the samples were washed once in tap water and twice in deionized water and oven-dried to a constant weight at 70 °C with dry weights being recorded. For tissue analysis of Ca, 0.5 g of ground samples was ashed at 500 °C for 8 h in a furnace, and the ash was dissolved in 10% (v/v) HCl prepared with distilled H₂O and concentrated HCl. The solutions were analyzed for Ca by atomic absorption spectrometry (Kalra, 1998).

Brix. Two leaves were selected from each head, one leaf from the center of the head and one from the outside. Juice was squeezed by hand into a plastic cup, mixed, and measured

Received for publication 2 Aug. 2013. Accepted for publication 10 Oct. 2013.

This material is based on work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, the Massachusetts Agricultural Experiment Station, and the Stockbridge School of Agriculture under Projects number MAS00963 and MAS00981. Paper number 3497 in the Experiment Station journal series.

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by a refractometer in sunlight (Pocket Refractometer PAL-1; ATAGO, Tokyo, Japan). One degree Brix is equivalent to 1 g of sucrose in 100 mL water. No correlation of Brix with Ca or other nutrient contents in plants is available.

Tipburn. Tipburn was assessed just before harvest by examination of each head of lettuce and ranking the severity of tipburn with an index of 0, no tipburn; 1, slight tipburn; 2, moderate tipburn; and 3, severe tipburn based on judgment of the investigators.

Statistical analysis. The statistical analyses of data were performed by analysis of variance or regression analysis (Steel and Torrie, 1980) with data processing by SAS software (SAS Version 9.2; Cary, NC). Means were separated by Duncan's new multiple

range test for main effects of treatments and by least significant difference for interactions of treatments (Steel and Torrie, 1980).

Results

Head weights. Fresh and dry weights of heads varied with cultivars (Table 1). The five largest headed cultivars had a mean fresh weight that was 40% larger and a mean dry weight that was 60% larger than the five smallest headed cultivars. 'Tropicana', 'Cosmo-Savoy', 'Buttercrunch', 'Coastal Star', 'Adriana', and 'Two Star' were grouped with high dry weights, and 'Tom Thumb', 'Winter Density', 'Black-Seeded Simpson', 'Bronze Mignonette', and 'Focca' had the lowest dry weights among the cultivars.

Heads of heritage (143 g/head) and modern (158 g) cultivars differed in fresh weights, and modern cultivars (10.2 g/head) also had higher dry weights than heritage cultivars (8.4 g) (Table 2). Loose-leaf phenotype had the highest fresh weight (154 g/head) followed by romaine (153 g) and butterhead (145 g) (Table 2). The dry weights of the phenotypic groups differed significantly in the same order as fresh weights. The organic and conventional regimes of fertilization had no significant effect on head weights (Table 2). Fresh and dry weights were slightly higher at 100 mg Ca/L than at 50 or 200 mg Ca/L (Table 2); however, regression analysis showed no significant trend between head weights and Ca in the medium.

A significant interaction of genetic group and phenotype occurred for fresh weights and dry weights with modern cultivars of butterhead and loose-leaf phenotypes being larger than heritage cultivars, whereas romaine phenotypes of heritage cultivars were larger than modern cultivars (Table 3). The type of fertilizer and the interaction of fertilizer × phenotype had no effect on head weights (Table 3). However, the levels of 100 or 200 mg Ca/L in the chemical fertilizer resulted in higher fresh or dry weights than the 50-mg treatment (Tables 4 and 5). With the organic regime, 200 mg Ca/L suppressed growth relative to the lower supplies of Ca. The interaction of Ca level with phenotype or with genetic grouping (heritage or modern) was nonsignificant and is not reported.

Calcium concentration. Calcium concentrations varied widely among the cultivars ranging from 1.20% to 2.54% with organic fertilization and from 1.35% to 3.56% with conventional fertilization (Table 6). The interaction of fertilizer by variety was significant, although the ranking of cultivars did not differ within the fertilizers (Table 6). The Ca

Table 1. Head weight of cultivars in descending order by fresh weight and listing of genetics and phenotypes of lettuce.

Cultivar	Genetics	Phenotype	Head wt ² (g/head)	
			Fresh	Dry
Tropicana	Modern	Loose leaf	177 a	11.6 ab
Cosmo-Savoy	Heritage	Romaine	175 ab	10.8 bc
Buttercrunch	Heritage	Butterhead	171 ab	11.5 ab
Coastal Star	Modern	Romaine	169 abc	12.0 a
Adriana	Modern	Butterhead	166 abc	11.4 ab
Two Star	Modern	Loose leaf	165 abcd	11.3 ab
Forellenschluss	Heritage	Romaine	162 abcde	9.6 de
Claremont	Modern	Romaine	158 abcde	9.0 ef
Simpson Elite	Modern	Loose leaf	156 bcde	9.1 ef
Red Deer Tongue	Heritage	Loose leaf	150 cde	7.1 gh
Salad Bowl	Heritage	Loose leaf	146 def	9.4 de
Australe	Modern	Butterhead	145 ef	9.1 ef
Red Rosie	Modern	Romaine	145 ef	10.1 cd
Focca	Modern	Butterhead	145 ef	8.5 f
Bronze Mignonette	Heritage	Butterhead	143 ef	8.5 f
Black-Seeded Simpson	Heritage	Loose leaf	129 f	7.3 g
Winter Density	Heritage	Romaine	112 g	6.3 h
Tom Thumb	Heritage	Butterhead	97 g	4.9 i

²Means of cultivar fresh or dry weights with different letters in columns are significantly different by Duncan new multiple range test, $P = 0.05$.

Table 2. Fresh weight, dry weights, calcium (Ca) concentration, total Ca, and tipburn of lettuce heads as a function of modern or heritage genetics, butterhead, romaine, or loose-leaf phenotype, organic or conventional fertilization, and Ca supply of lettuce.

Measurement	Genetics		Phenotype			Fertilizer		Calcium level (mg·L ⁻¹)		
	Heritage	Modern	Butterhead	Romaine	Loose Leaf	Organic	Conventional	50	100	200
Fresh wt (g/head)	143	158*	145 b	153 a	154 a	153	149 NS	143 b	156 a	151 ab
Dry wt (g/head)	8.4	10.2*	8.9 b	9.6 a	9.3 a	9.2	9.4 NS	9.0 b	9.9 a	9.0 b
Percent Ca (dry wt)	1.93	1.54*	1.66 b	1.49 c	2.06 a	1.58	1.90*	1.56 b	1.86 a	1.79 a
Total Ca (g/head)	0.16	0.16 ^{NS}	0.15 b	0.14 b	0.18 a	0.14	0.18*	0.14 c	0.18 a	0.16 b
Brix (°)	3.68	4.01*	3.43 c	3.86 b	4.23 a	3.71	3.97*	4.02 a	4.10 a	3.40 b
Tipburn ²	1.68	1.69 NS	2.06 a	2.06 a	0.93 b	1.68	1.69 NS	1.78 a	1.67 a	1.60 a

²Visual index scale of 0, no tipburn; 1, slight tipburn; 2, moderate tipburn; and 3, severe tipburn.

For genetics and fertilizers, NS, *Means of measurements are not significantly different, $P > 0.05$, or are significantly different by F-test, $P \leq 0.05$, respectively. For phenotypes and calcium levels, means followed by different letters are significantly different by Duncan's new multiple range test, $P = 0.05$.

Table 3. Interaction of genetics and phenotypes and fertilizers on fresh weight of heads of lettuce.

Cultivar	Genetics ²							
	Heritage				Modern			
	Phenotype			Mean	Phenotype			Mean
	Butterhead	Romaine	Loose-leaf		Butterhead	Romaine	Loose-leaf	
Organic	134	152	152	146	156	154	166	159
Conventional	140	147	131	139 NS	148	160	165	158 NS

²Least significant difference (0.05) for interaction of fertilizers × genetics × phenotypes = 12.

NS Means of fertilizers are not significantly different by F-test, $P > 0.05$.

Means of phenotypes are reported in Table 2.

Table 4. Interaction of type of fertilizer and calcium (Ca) level on fresh weight of lettuce listed in descending order of mean weights of cultivars.

Cultivar	Type of fertilizer ^z								Cultivar mean ^y
	Organic				Conventional				
	Ca level			Mean	Ca level			Mean	
	50	100	200		50	100	200		
	Fresh wt (g/head)								
Tropicana	192	198	154	182	138	174	201	171	177 a
Cosmo-Savoy	181	200	159	180	147	190	170	169	175 ab
Buttercrunch	183	200	139	174	139	169	199	169	171 ab
Coastal Star	162	172	166	167	135	179	199	171	169 abc
Adriana	181	195	147	174	128	151	195	158	166 abc
Two Star	179	160	159	166	146	155	191	164	165 abcd
Forellenschluss	172	167	158	166	151	148	175	158	162 abcde
Claremont	175	153	136	154	151	170	163	161	158 abcde
Simpson Elite	162	163	130	152	126	160	195	160	156 bcde
Red Deer Tongue	162	162	146	157	146	132	149	142	150 cde
Salad Bowl	168	188	123	160	123	121	154	133	146 def
Australe	151	172	129	150	131	149	141	140	145 ef
Red Rosie	144	158	121	141	123	158	166	149	145 ef
Foceia	160	138	133	144	127	145	165	146	145 ef
Bronze Mignonette	121	166	116	135	132	153	171	152	143 ef
Black-Seeded Simpson	134	135	151	140	131	130	96	119	129 f
Winter Density	127	110	97	111	92	117	128	112	112 g
Tom Thumb	79	92	114	95	77	108	111	99	97 g

^zLeast significant difference (0.05) for interaction of fertilizers × Ca level × cultivars = 12.

^yCultivar means followed with different letters are significantly different by Duncan's new multiple range test, $P = 0.05$. These means are presented also in Table 1. Means of fertilizers calcium levels are reported in Table 2.

Table 5. Fertilizers and calcium (Ca) level interaction with cultivars listed in descending order of mean dry weights of cultivars.

Cultivar	Type of fertilizer ^z								Cultivar mean ^y
	Organic				Conventional				
	Ca level			Mean	Ca level			Mean	
	50	100	200		50	100	200		
	Dry wt (g/head)								
Coastal Star	12.6	11.8	9.9	11.4	9.8	13.1	14.8	12.6	12.0 a
Tropicana	13.1	13.1	9.1	11.8	8.6	12.7	13.1	11.5	11.6 a
Buttercrunch	12.8	13.7	8.4	11.6	9.2	11.3	13.6	11.4	11.5 a
Adriana	12.5	13.4	8.8	11.5	8.7	11.9	13.0	11.2	11.4 ab
Two Star	12.3	10.9	10.4	11.2	9.8	11.6	13.1	11.5	11.3 ab
Cosmo-Savoy	11.7	12.2	8.9	11.0	8.4	12.5	10.9	10.6	10.8 bc
Red Rosie	10.4	11.1	8.0	9.8	8.2	11.6	11.5	10.4	10.1 cd
Forellenschluss	9.8	9.4	9.4	9.5	10.1	9.1	10.1	9.7	9.6 de
Salad Bowl	10.9	10.7	7.1	9.6	7.8	9.5	10.3	9.2	9.4 de
Australe	9.7	10.9	7.8	9.5	7.6	9.8	8.9	8.7	9.1 ef
Simpson Elite	9.3	9.3	7.0	8.5	8.0	10.3	10.7	9.7	9.1 ef
Claremont	10.4	9.1	7.2	9.9	8.3	9.7	9.1	9.0	9.0 ef
Bronze Mignonette	7.1	9.3	6.3	7.5	8.1	9.9	10.6	9.5	8.5 f
Foceia	9.4	8.2	7.5	8.4	7.5	9.1	9.4	8.7	8.5 f
Black-Seeded Simpson	7.3	7.6	8.1	7.6	7.8	7.4	5.4	6.9	7.3 g
Red Deer Tongue	7.4	7.2	7.0	7.2	7.1	6.6	6.8	6.8	7.0 gh
Winter Density	7.4	6.6	5.2	6.4	5.0	7.1	6.8	6.3	6.3 h
Tom Thumb	4.5	4.7	3.9	4.4	4.1	5.6	6.4	5.4	4.9 i

^zLeast significant difference (0.05) for interaction of fertilizers × Ca level × cultivar = 0.7.

^yCultivar means followed with different letters are significantly different by Duncan's new multiple range test, $P = 0.05$. These data are presented also in Table 1. Means for fertilizers and calcium levels are reported Tables 2.

concentration was always higher with conventional fertilization but the elevation with conventional over organic fertilization ranged from 4% to 49% among cultivars. Mean Ca concentration was ≈20% higher with conventional fertilization (1.90%) than with organic fertilization (1.58%) (Tables 2 and 6). Heritage cultivars had ≈25% higher Ca concentration (1.93%) than modern cultivars (1.54%) (Table 2). Loose-leaf phenotypes had the highest Ca concentrations (2.06%) followed by butterhead (1.66%) and romaine (1.49%)

phenotypes (Table 2). The Ca concentrations with 100 or 200 mg Ca/L (mean 1.82%) were higher than that occurring at 50 mg Ca/L (Table 2). The interaction of genetics with Ca level was significant (Table 7). Heritage cultivars were always higher in Ca than modern cultivars, but the difference ranged from 14% to 32%. The interaction of cultivar with Ca level was significant with cultivars showing different trends in accumulation of Ca as Ca level increased (Table 8). Interactions other than fertilizer × cultivar, genetics × Ca level,

and cultivar × Ca level had no significant effect on Ca concentration and are not reported.

Total calcium accumulation. Total Ca accumulation among the cultivars varied from 0.07 to 0.28 g/head (Table 9). Cultivars with notably high accumulation were 'Salad Bowl', 'Buttercrunch', and 'Red Deer Tongue'. Cultivars with notably low accumulation were 'Tom Thumb', 'Winter Density', and 'Black-Seeded Simpson'. Total Ca did not differ among heritage and modern cultivars (Table 2), but loose-leaf (0.18 g/head) had higher accumulation than butterhead or romaine (mean 0.14 g/head) phenotype (Table 2). Total Ca was higher with conventional fertilization (0.18 g/head) than with organic fertilization (0.14 g/head) (Table 2). Total Ca was highest at 100 mg Ca/L (0.18 g/head) followed by the 200- (0.16 g) and 50-mg Ca/L treatments (0.14 g) (Table 2) in a quadratic trend. An interaction of genetics × Ca level was significant with heritage cultivars showing an increase in total Ca with each increase in Ca level and modern varieties showing highest Ca at 100 mg·L⁻¹ (Table 8). An interaction of cultivar × Ca level was significant showing that cultivars ranged in responses to increasing Ca supply with an increase, no increase, or a peak in accumulation at 100 mg Ca/L as Ca supply increased from 50 to 200 mg·L⁻¹ (Table 9).

Calcium accumulation and head size. Overall, Ca concentration did not differ with head dry weights of cultivars, averaging 1.74% (Fig. 1), but total Ca in the cultivars widely and significantly differed with increasing total dry weight of the cultivars and ranged from 0.07 to 0.28 g/head (Fig. 1).

Brix. Refractometer (°Brix) readings differed with cultivars (Table 10). 'Forellenschluss', 'Two Star', 'Salad Bowl', 'Adriana', and 'Costal Star' had °Brix of 4.5 or higher, whereas 'Winter Density', 'Buttercrunch', and 'Tom Thumb' had °Brix of ≈3.0 or below. Heritage (3.84) and modern (4.01) cultivars differed in °Brix (Table 2), and differences also occurred among phenotypes with loose-leaf (4.23) having the highest °Brix followed by romaine (3.85) and butterhead (3.43) cultivars. The two fertilizer groups differed significantly with the conventional (3.97) fertilizer giving higher °Brix than organic fertilizer (3.71) (Table 2). The levels of 50 or 100 mg Ca/L (4.02 and 4.10, respectively) resulted in higher °Brix than 200 mg Ca/L (3.40). Interactions were nonsignificant or presented no responses to suggest that any of these interactions were important factors in affecting °Brix in lettuce. Polynomial regression analysis showed no significant relationship of percent Ca and °Brix.

Tipburn. Cultivars differed with expression of tipburn ranging from no tipburn to severe tipburn (Table 11). Heritage and modern cultivars did not differ in expression of tipburn (Table 2), but loose-leaf cultivars (rating 0.93) had a lower ranking of tipburn than butterhead or romaine phenotypes (each rating 2.06) (Table 2). Tipburn did not differ with treatments of conventional or organic fertilization (Table 2). No interactive effects

Table 6. Interaction of cultivar and fertilizer on the calcium concentration in lettuce arranged in descending order of mean calcium concentration in the cultivars.

Cultivar	Fertilizer ^z		Cultivar mean ^y
	Organic	Conventional	
	----- Calcium concn (% dry wt) -----		
Salad Bowl	2.54	3.56	3.05 a
Red Deer Tongue	2.53	3.51	3.02 a
Buttercrunch	1.83	2.17	2.00 b
Bronze Mignonette	1.95	2.03	1.99 b
Focsa	1.71	1.79	1.75 c
Simpson Elite	1.52	1.97	1.73 cd
Two Star	1.39	1.84	1.61 cde
Winter Density	1.44	1.76	1.60 cde
Red Rosie	1.42	1.77	1.59 cdef
Claremont	1.28	1.91	1.59 cdef
Cosmo-Savoy	1.37	1.46	1.58 cdef
Tropicana	1.38	1.66	1.52 def
Tom Thumb	1.38	1.54	1.46 efg
Black-Seeded Simpson	1.40	1.49	1.44 efg
Adriana	1.22	1.54	1.38 fg
Australe	1.31	1.45	1.38 fg
Coastal Star	1.21	1.54	1.28 g
Forellenschluss	1.20	1.35	1.27 g

^zLeast significant difference (0.05) for interaction is 0.31.

^yCultivar means followed by different letters are significantly different by Duncan's new multiple range test, $P = 0.05$. Means of fertilizers are reported in Table 2.

Table 7. Interaction of genetics with calcium (Ca) levels on calcium concentration and total Ca accumulation in lettuce.

Ca level (mg·L ⁻¹)	Genetics ^z	
	Heritage	Modern
--- Ca concn (% dry wt)---		
50	1.70	1.43
100	2.05	1.66
200	2.06	1.52
----- Total Ca (g/head)-----		
50	0.14	0.14
100	0.18	0.18
200	0.17	0.15

^zLeast significant difference for Ca level × genetics interaction on percent Ca = 0.13 and on total Ca = 0.01. Means for main effects of Ca levels and modern and heritage cultivars are reported in Table 2.

Table 8. Interaction of cultivar and calcium level on calcium (Ca) concentration of lettuce.

Cultivar	Ca level ^z		
	50	100	200
-- Ca concn (% dry wt)--			
Salad Bowl	2.76	3.71	2.69
Red Deer Tongue	2.47	3.22	3.36
Buttercrunch	1.71	1.99	2.30
Bronze Mignonette	1.57	1.90	2.50
Focsa	1.58	2.00	1.68
Simpson Elite	1.69	1.91	1.58
Two Star	1.76	1.74	1.35
Winter Density	1.40	1.77	1.64
Red Rosie	1.47	1.73	1.60
Claremont	1.39	1.87	1.52
Cosmo-Savoy	1.53	1.66	1.56
Tropicana	1.38	1.48	1.71
Tom Thumb	1.37	1.46	1.56
Black-Seeded Simpson	1.32	1.48	1.53
Adriana	1.24	1.64	1.27
Australe	1.24	1.38	1.52
Coastal Star	1.14	1.24	1.47
Forellenschluss	1.19	1.25	1.39

^zLeast significant difference (0.05) for interaction of cultivars × Ca level = 0.39. Means of main effects of cultivars and levels of calcium are reported in Table 2.

Table 9. Interaction of cultivar and calcium (Ca) level on total Ca accumulation by lettuce listed in order of descending accumulation.

Cultivar	Ca level			Cultivar mean
	50	100	200	
	----- (g Ca/head) -----			
Salad Bowl	0.24	0.37	0.23	0.28 a
Buttercrunch	0.19	0.25	0.26	0.23 b
Red Deer Tongue	0.18	0.22	0.23	0.21 b
Two Star	0.19	0.19	0.16	0.18 c
Tropicana	0.15	0.19	0.20	0.18 c
Cosmo-Savoy	0.16	0.21	0.16	0.17 cd
Bronze Mignonette	0.12	0.19	0.21	0.17 cd
Red Rosie	0.13	0.20	0.16	0.16 cd
Simpson Elite	0.15	0.19	0.14	0.16 cd
Adriana	0.13	0.21	0.14	0.16 cd
Coastal Star	0.13	0.15	0.18	0.15 cd
Focsa	0.14	0.18	0.14	0.15 de
Claremont	0.14	0.18	0.13	0.15 def
Australe	0.11	0.15	0.13	0.13 efg
Forellenschluss	0.12	0.12	0.14	0.12 fg
Black-Seeded Simpson	0.10	0.11	0.10	0.10 g
Winter Density	0.09	0.13	0.10	0.10 g
Tom Thumb	0.06	0.08	0.09	0.07 h

^zLeast significant difference (0.05) for interaction of cultivar × Ca level = 0.05.

^yMeans followed by different letters for cultivars in column and fertilizers in row are significantly different by Duncan's new multiple range test, $P = 0.05$. Means for Ca level are reported in Table 2.

were significant. The relationship between percent Ca in heads and expression of tipburn was nonsignificant by regression analysis.

Discussion

Modern cultivars had higher fresh weights than heritage cultivars because of genetic improvement in head sizes. Because of their smaller head sizes, heritage cultivars often are perceived to have higher mineral nutrient contents than modern cultivars (Mou, 2005, 2009). However, results of this investigation showed that Ca concentration did not differ

overall with head size although total accumulation (g/head) varied, increasing as dry weight increased. Loose-leaf and romaine phenotypes had greater fresh weights than butterhead. Head weights did not differ in response to organic or chemical nutritional regimes. An increase in Ca nutrition from 50 to 200 mg·L⁻¹ increased head size in the chemical regime but had little effect in the organic regime. Loose-leaf cultivars Tropicana, Cosmo Savoy, Buttercrunch, Coastal Star, and Two Star had greater fresh weights than other cultivars. This difference in growth has been attributed to differences in transpiration and the resulting nutrient accumulation (Mou, 2009). Perhaps, loose-leaf and romaine phenotypes because of their morphology transpired more water than butterhead and therefore had increased nutrient uptake transport and higher growth.

The nutritional value of the lettuce cultivars differed with phenotypes. Loose-leaf lettuce cultivars contained higher Ca concentration and total Ca than butterhead or romaine types. Transpiration affects the delivery and distribution of Ca to lettuce leaves because young leaves of the developing heads can develop tipburn because the outer leaves are transpiring more than the young leaves, thereby depriving the young leaves of Ca (Collier and Tibbitts, 1982, 1984). Loose-leaf cultivars had less tipburn than romaine or butterhead phenotypes, again suggesting differences in delivery of Ca to the young leaves by transpiration (Hylmö, 1953).

A great variation occurred for Ca accumulation among cultivars. Loose leaf cultivars Salad Bowl and Red Deer Tongue had the highest Ca concentrations. Butterhead cultivars Buttercrunch and Bronze Mignonette had high Ca concentrations. The semiopen head of 'Buttercrunch' and 'Bronze Mignonette' perhaps allows transpiration of water into the head and contributed to their higher Ca concentrations. In contrast, 'Winter Density', 'Red Rosie', and 'Cosmo-Savoy' accumulated moderate levels of Ca concentration within romaine types. The thick and semiopen leaves of romaine heads possibly obstruct the transpiration of water, thereby leading to lower Ca concentration than with loose-leaf and butterhead cultivars (Collier and Tibbitts, 1984). Butterhead cultivars Adriana and Australe and romaine cultivars Coastal Star and Forellenschluss had much lower Ca concentrations than other romaine and butterhead cultivars. 'Adriana', 'Australe', 'Coastal Star', and 'Forellenschluss' have partially closed heads, whereas others in romaine and butterhead phenotypes have open heads. The closed head perhaps obstructs the transpiration of water, resulting in lower Ca concentration in the leaves (Barta and Tibbitts, 1991).

Tipburn varied among the phenotypes and cultivars. Loose-leaf phenotypes expressed a lesser index of tipburn than romaine or butterhead structures. 'Forellenschluss', a heritage, romaine phenotype, expressed the highest tipburn index, whereas 'Salad Bowl' and 'Tropicana', heritage and modern loose leaf cultivars, respectively, had the lowest symptoms.

Fig. 1. Calcium concentration (top) and accumulation total calcium (Ca) (bottom) as a function of head dry weights of lettuce. Equations are linear regression models. Calcium concentration did not vary with dry weights, whereas total accumulation of Ca increased as dry weight increased.

Table 10. Refractometer readings among cultivars with ranking from highest to lowest °Brix.

Refractometer reading ^z (°Brix)					
Forellenschluss	4.76 a	Tropicana	4.16 bcd	Cosmo-Savoy	3.58 f
Two Star	4.68 a	Simpson Elite	4.03 cde	Australe	3.57 f
Salad Bowl	4.51 ab	Black-Seeded Simpson	3.77 def	Focca	3.34 fg
Adriana	4.50 ab	Bronze Mignonette	3.67 ef	Winter Density	3.03 g
Coastal Star	4.50 ab	Red Rosie	3.65 ef	Buttercrunch	2.98 g
Red Deer Tongue	4.23 bc	Claremont	3.64 ef	Tom Thumb	2.54 h

^z°Brix followed by different letters are significantly different by Duncan's new multiple range test ($P = 0.05$).

Table 11. Expression of tipburn among cultivars with ranking from highest to lowest symptoms.

Cultivar and tipburn rating, 0 to 3 scale ^z					
Forellenschluss	3.00 a ^y	Adriana	2.17 bcd	Simpson Elite	1.25 fg
Red Deer Tongue	2.75 a	Claremont	2.17 bcd	Buttercrunch	1.08 g
Focca	2.67 ab	Red Rosie	1.92 cde	Coastal Star	0.83 g
Winter Density	2.58 ab	Cosmo-Savoy	1.83 cdef	Two Star	0.17 h
Tom Thumb	2.42 abc	Bronze Mignonette	1.75 def	Salad Bowl	0.08 h
Australe	2.25 bcd	Black-Seeded Simpson	1.33 efg	Tropicana	0.00 h

^zVisual index of 0, no tipburn; 1, slight tipburn; 2, moderate tipburn; 3, severe tipburn.

^yTipburn ratings followed by different letters are significantly different by Duncan's new multiple range test ($P = 0.05$).

Calcium concentration in the whole heads was not related to severity of tipburn. This lack of relationship is because total Ca in the entire head was determined rather than Ca being determined in the affected leaves only.

Concentration of Ca did not vary with head weight, but total Ca accumulation increased with head weight. These results are important because they demonstrate that a dilution of Ca does not occur with an increase in dry mass and that consumers will receive the same amount of Ca from servings of lettuce regardless of head weight.

Conclusions

Heritage cultivars accumulated higher Ca concentration but were not superior in accumulation of total Ca from modern cultivars resulting from modern cultivars having larger head weights. Loose-leaf cultivars accumulated higher Ca than romaine or butterhead phenotypes. Loose-leaf cultivars also had larger head fresh weights. Accumulation of Ca concentration or total Ca was higher in the chemical regime than in the organic regime. Cultivars differed widely in Ca accumulation with 'Salad Bowl', 'Red

Deer Tongue', 'Buttercrunch', and 'Bronze Mignonette' ranking in the top among all cultivars in Ca concentration. A wide range of variability in Ca concentration occurred among different cultivars of lettuce including differing phenotypes and introductions. Therefore, improvement of nutrient density with lettuce through breeding and selection is a potentiality. Enhancing the mineral nutrition levels of lettuce would improve the nutrient uptake without requiring an increase in produce consumption.

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