Effect of Foliar Application of CaCl_2 on Lettuce Growth and Calcium Concentrations with Organic and Conventional Fertilization

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Abstract. Calcium-rich vegetables in diet could minimize calcium deficiency and maximize good health and well-being. The aim of the present study was to determine the effect of different levels of foliar application of CaCl_2 on lettuce (*Lactuca sativa* L.) growth and calcium concentrations with the application of organic and conventional fertilizers. Pot experiments were conducted with three calcium levels (60, 120, and 180 $\text{mg}\cdot\text{L}^{-1}$ of CaCl_2) of an organic fertilizer (3N–0.8P–3.5K) and commercial conventional fertilizer (15N–15P–15K). Calcium in whole oven-ashed samples of shoots was analyzed by atomic absorption spectrometry. Nine lettuce cultivars including butterhead, romaine, and loose-leaf phenotype were tested. These results revealed that the calcium concentration in lettuce significantly increased as calcium levels increased from 60 to 180 $\text{mg}\cdot\text{L}^{-1}$. Elevated calcium concentrations in organic and conventional fertilizers increased the concentration of calcium in lettuce from 1.82% at 60 $\text{mg}\cdot\text{L}^{-1}$ to a mean of 2.15% at 120 and 180 $\text{mg}\cdot\text{L}^{-1}$. The concentration of calcium in the loose-leaf phenotype was 2.17%, 2.47%, and 3.80% higher than that in the butterhead and romaine phenotypes at 60, 120, and 180 $\text{mg}\cdot\text{L}^{-1}$, respectively. Furthermore, the significant difference in calcium concentration among cultivars ranged from 1.27% to 3.05%. ‘Perilla Green’, ‘Breen lettuce’, and ‘Salinas’ had the highest calcium concentrations followed by ‘Jericho lettuce’, ‘Salad Bowl’ and ‘Crisp’, and ‘Kaiser’, whereas ‘Valmaine’ and ‘Rosa Green’ had the lowest calcium concentrations. The present study revealed that selecting fertilizers and cultivars with high calcium concentration can increase the total calcium content of lettuce.

Mineral nutrients required in the human body are mainly obtained through the consumption of vegetables. Calcium is an essential and major nutrient in vegetable. Adequate calcium intake is important for constructing and maintaining bones, the clotting of blood, and the function of hormones and enzymes (Department of Health & Human Services, 2000; Ervin et al., 2004). For most men and women, the recommended daily intake of calcium is 1000 $\text{mg}\cdot\text{d}^{-1}$ (Meacham et al., 2008). However, the required calcium intake

should total 1300–1700 $\text{mg}\cdot\text{d}^{-1}$ for the elderly (Heaney, 2001). A study suggested that it is possible to improve dietary calcium intake by increasing the consumption of calcium-rich vegetables (Bernstein et al., 2002). Calcium-rich vegetables in diet could ameliorate the potential for calcium deficiency in human nutrition. Fresh leafy vegetables such as celery, collard, chinese cabbage, and soybean sprouts contain high levels of calcium, which are major sources of minerals (Kamchan et al., 2004). Increasing the calcium content in leafy vegetables could further improve its nutritional benefits to consumers, considering that calcium is the mineral nutrient most commonly deficient in modern diets (Grusak, 2002).

Lettuce (*L. sativa* L.) is an annual plant of the family Asteraceae. It is an important leafy vegetable that has high market value, it is grown around the world and is used particularly as the base for salads. It can be easily

cultivated because it has a short vegetation period. Lettuce is a good source of vitamin A and potassium, as well as a minor source of several other vitamins and nutrients (Niari et al., 2012), and its nutritional characteristics have been studied throughout the world (Ashkar and Ries, 1970; Keat et al., 1999). Calcium has been considered an important nutrient in lettuce. The calcium content of lettuce is affected by calcium uptake. This is associated with the water uptake of its roots and in turn affects the calcium distribution in the shoots (Collier and Tibbitts, 1984; Schlagnhauer et al., 1987).

The aim of the present study was to determine the effect of the foliar application of CaCl_2 on lettuce growth and calcium concentrations with organic and conventional fertilizer applications and determine whether the calcium content could be increased through nutritional regimes.

Materials and Methods

Experimental site and plant materials. Nine lettuce cultivars, including butterhead (‘Salinas’, ‘Crisp’, and ‘Kaiser’), romaine (‘Breen lettuce’, ‘Jericho lettuce’, and ‘Valmaine’) and loose-leaf (‘Perilla Green’, ‘Salad Bowl’, and ‘Rosa Green’) phenotypes, were tested. All seeds were obtained from Hubei Eshu Agricultural Technology Co. Ltd. (Wuhan, China). Peregriated seeds were sown in seedling trays to produce uniform seedlings. Seedlings at the three leaf stage were manually transplanted on 4 Feb. 2016. These seedlings were transplanted on a hill spaced at 20 × 20 cm.

Experimental design and management. The experiments were conducted using three calcium levels (60, 120, and 180 $\text{mg}\cdot\text{L}^{-1}$ of CaCl_2) and two fertilizer forms, viz. organic fertilizer (3N–0.8P–3.5K, with micronutrients reported; Wuhan Green Chemical Co., Ltd., Wuhan, China) and commercial conventional fertilizer (15N–15P–15K, with micronutrients reported; Wuhan Green Chemical Co., Ltd.). These fertilizers were manually broadcast during basal application. These experiments were laid out in a randomized complete block design with four replications for each treatment. The foliar application of CaCl_2 fertilizers was performed in three concentrations, and plants were sprayed three times every 20 d, and watered

Table 1. The fresh and dry weights of heads of the cultivars and phenotypes of lettuce.

Cultivar	Phenotype	Head wt ^a (g/head)	
		Fresh	Dry
Perilla Green	Loose leaf	174 a	12.00 a
Breen lettuce	Romaine	171 ab	11.60 a
Salinas	Butterhead	166 ab	11.50 a
Jericho lettuce	Romaine	166 ab	11.30 ab
Crisp	Butterhead	163 abc	11.40 ab
Salad Bowl	Loose leaf	163 abc	10.80 bc
Kaiser	Butterhead	163 abc	10.20 cd
Valmaine	Romaine	150 d	9.80 de
Rosa Green	Loose leaf	125 e	9.40 de

^aMeans of the fresh or dry weights of cultivars, and the lower case letters represent the significant differences identified by Duncan’s new multiple range test ($P = 0.05$).

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Table 2. The fresh weight, dry weight, calcium concentration, and total calcium content of the three phenotypes with organic and conventional fertilizers and calcium levels.

Measurement	Phenotype			Fertilizer		Calcium level (mg·L ⁻¹)		
	Butterhead	Romaine	Loose leaf	Organic	Conventional	60	120	180
Fresh weight (g/head)	156.00 ab	158 a	154 b	171	167 ^{NS}	147 b	176 a	173 ab
Dry weight (g/head)	9.60 ab	9.80 a	9.50 b	10.90	10.90 ^{NS}	10.40 b	11.70 a	10.70 b
Proportion of calcium (dry weight)	1.74 c	2.09 b	2.17 a	1.76	2.15*	1.82 c	2.21 a	2.10 b
Total calcium content (g/head)	0.16 b	0.15 c	0.17 a	0.15	0.18*	0.17 c	0.21 a	0.19 b

^{NS}, *The difference in these measurements were not statistically significant ($P > 0.05$) or the difference was statistically significant as identified by F test ($P \leq 0.05$). For phenotypes and calcium levels: The lower case letters represent the significant differences identified by Duncan's new multiple range test ($P = 0.05$).

Table 3. The interaction of phenotypes and fertilizers in terms of the head fresh weights of lettuce.

Cultivar	Phenotype			
	Butterhead	Romaine	Loose leaf	Mean
	Fresh weight (g/head)			
Organic	134	151	150	145
Conventional	142	146	130	139 ^{NS}

The least significant difference (0.05) for the interaction between fertilizers and phenotypes was 6.

^{NS}The difference in these fertilizers was not statistically significant as identified by F test ($P > 0.05$). The means of the phenotypes are presented in Table 2.

Table 4. The interaction between fertilizers and calcium concentration in terms of fresh weight of lettuce are listed in descending order according to the mean weights of cultivars.

Cultivar	Type of fertilizer							
	Organic				Conventional			
	Calcium level				Calcium level			
	60	120	180	Mean	60	120	180	Mean
	Fresh wt (g/head)							
Perilla Green	180	202	157	180	145	157	202	168
Breen lettuce	191	197	153	180	136	153	197	162
Salinas	181	201	136	173	138	136	201	158
Jericho lettuce	180	192	149	174	132	149	192	158
Crisp	160	173	168	167	136	168	173	159
Salad Bowl	182	163	162	169	147	162	163	157
Kasier	170	165	161	165	153	161	165	160
Valmaine	176	152	133	154	152	133	152	146
Rosa Green	140	142	107	130	109	107	142	119

The least significant difference (0.05) for the interaction among fertilizers, calcium concentrations, and cultivars is 6. The lower case letters represent the significant differences identified by Duncan's new multiple range test ($P = 0.05$). These means are presented in Table 1, while the means of the calcium concentrations of fertilizers are presented in Table 2.

Table 5. Interactions between calcium concentrations and fertilizers in terms of the mean dry weights of cultivars.

Cultivar	Type of fertilizer							
	Organic				Conventional			
	Calcium level				Calcium level			
	60	120	180	Mean	60	120	180	Mean
	Dry wt (g/head)							
Perilla Green	12.50	11.60	9.80	11.30	9.70	13.30	14.90	12.60
Breen lettuce	13.00	12.70	9.30	11.70	8.70	12.80	13.20	11.60
Salinas	12.60	13.50	8.70	11.60	9.30	11.40	13.70	11.50
Jericho lettuce	12.40	13.30	8.90	11.50	8.80	11.80	12.80	11.10
Crisp	12.40	11.10	10.60	11.40	9.70	11.50	13.20	11.50
Salad Bowl	11.80	12.30	9.00	11.00	8.50	12.60	10.70	10.60
Kasier	10.50	11.20	8.30	10.00	8.30	11.50	11.60	10.50
Valmaine	9.90	9.50	9.60	9.70	10.00	9.30	10.20	9.80
Rosa Green	10.80	10.80	7.20	9.60	7.90	9.60	10.10	9.20

The least significant difference (0.05) for the interaction among fertilizers, calcium concentrations, and cultivars is 0.7. The lower case letters represent the significant differences identified by Duncan's new multiple range test ($P = 0.05$). The means are presented in Table 1, while the means of the calcium concentrations of fertilizers are presented in Table 2.

once every 3 d at 5:00 PM. Standard cultural management practices were followed to achieve maximum yield.

Samplings and measurements. After harvesting the plants, the fresh weights were

recorded. Then, the sampled plants were washed with tap water, oven-dried to a constant weight at 70 °C, and the oven dry weight was recorded. Afterward, calcium concentration and total calcium content were analyzed

by atomic absorption spectrometry (Meagy et al., 2013).

Statistical analysis. The statistical analysis included analysis of variance, and Statgraphics version 3.1 for Windows was used. The means were separated by Duncan's new multiple range test for the main effects of treatments and by least significant difference for the interactions of treatments (Steel and Torrie, 1980).

Results

Head weights. The fresh and dry weights of heads varied with cultivars, as shown in Table 1. The mean fresh weight and mean dry weight of the three largest headed cultivars was 22.80% and 33.20% larger than the three smallest headed cultivars, respectively. Furthermore, among cultivars, 'Perilla Green', 'Breen lettuce', and 'Salinas' were classified with the highest dry weights, whereas 'Kasier', 'Valmaine', and 'Rosa Green' were classified with the lowest dry weights.

There was a significant difference in fresh weight and dry weight between the romaine and loose-leaf phenotypes (Table 2). The romaine phenotype had the highest fresh weight (158 g/head), followed by butterhead (156 g) and loose leaf (154 g). The dry weights in these phenotypes significantly differed in the same order as the fresh weights. The organic and conventional regimes of fertilization had no significant effect on head weight and calcium concentration (Table 2). The fresh and dry weights were slightly higher at 120 mg Ca/L than at 60 or 180 mg Ca/L (Table 2). Calcium concentration in the loose-leaf phenotype was 2.17%, 2.470%, and 3.80% higher than that in butterhead and romaine phenotypes at 60, 120, and 180 mg Ca/L, respectively. Calcium concentration and total calcium content was the highest at 120 mg Ca/L followed by 180 mg Ca/L and 60 mg Ca/L. The elevated calcium levels in organic and commercial conventional fertilizers increased the calcium concentration in lettuce from 1.82% at 60 mg·L⁻¹ to a mean of 2.15% at 120 and 180 mg·L⁻¹.

The type of fertilizer and the interaction between fertilizer and phenotype had no effect on the head weights (Table 3). Furthermore, there was no significant difference in the interaction of phenotypes and fertilizers in terms of the head fresh weights of lettuce. However, the 60 or 120 mg Ca/L concentration in conventional fertilizer

resulted in high fresh or dry weights compared with 180 mg Ca/L (Tables 4 and 5). With the organic regime, the 180 mg-Ca/L concentration suppressed the growth relative to the low supply of calcium.

Calcium concentration. As presented in Table 6, significant differences in calcium concentration occurred among individual cultivars, which ranged from 1.22% to 2.52% with organic fertilizers and from 1.47% to 3.53% with conventional fertilizer. A significant difference was found in the interaction of fertilizers by variety, but the ranking of cultivars did not differ with regard to these fertilizers. Total calcium content varied widely among cultivars, ranging from 0.17 to 0.13 g/head with organic fertilizers and from 0.16 to 0.19 g/head with conventional fertilizers (Table 7). Furthermore, there was no significant difference in the interaction of fertilizers by variety. However, there was a significant difference in the interaction of cultivars with calcium concentration, in which cultivars exhibited different trends in total calcium content when calcium concentration was increased (Table 8).

Total calcium content. The interaction of cultivars and calcium concentration with calcium accumulation in lettuce is illustrated in Table 9. Among these cultivars, total calcium content varied from 0.13 to 0.28 g/head. Cultivars with notably high calcium content were ‘Perilla Green’, ‘Breen lettuce’, and ‘Salinas’, whereas cultivars with notably low calcium content were ‘Kasier’, ‘Valmaine’, and ‘Rosa Green’. Hence, there is a significant interaction between cultivars and calcium concentration. This shows that the response of cultivars increase according to the increase in calcium concentration. That is, total calcium content reached a peak at 120 mg Ca/L when the calcium concentration increased from 60 to 180 mg Ca/L (Table 9).

Discussion

Because of genetic improvements in plant head size, the romaine phenotype had larger fresh and dry weights than the butterhead and loose-leaf phenotypes. In the present study, results revealed that the calcium concentration did not differ in overall plant head size, although the total calcium content (g/head) varied. That is, total calcium content increased as plant dry weight increased. This conclusion is consistent with the study conducted by Meagy et al. (2013). Furthermore, there was no significant difference in plant head weight between the organic and chemical nutritional regimes. Moreover, plant head size significantly increased as calcium concentration increased from 60 to 180 mg Ca/L in the chemical regime. However, little effect was observed in the organic regime. ‘Perilla Green’ and ‘Breen’ lettuce had larger fresh weights compared with other cultivars, and may be attributed to the difference in transpiration and the resulting nutrient content (Mou, 2009). Loose-leaf lettuce cultivars contained higher calcium concentration and total calcium content compared with the butterhead

Table 6. The interaction between cultivars and fertilizers in terms of the calcium concentration of lettuce.

Cultivar	Fertilizer		Cultivar mean
	Organic	Conventional	
	Calcium concn (% dry wt)		
Perilla Green	2.52	3.53	3.03 a
Breen lettuce	2.50	3.42	2.96 a
Salinas	1.85	2.13	1.99 b
Jericho lettuce	1.75	1.96	1.86 bc
Salad Bowl	1.55	1.98	1.77 bcd
Crisp	1.63	1.69	1.66 de
Kasier	1.49	1.64	1.57 de
Valmaine	1.34	1.56	1.45 ef
Rosa Green	1.22	1.47	1.35 f

The least significant difference (0.05) for the interaction is 0.31. The lower case letters represent the significant differences identified by Duncan’s new multiple range test ($P = 0.05$). The means for these fertilizers are presented in Table 2.

Table 7. The interaction between cultivars and fertilizers in terms of total calcium content in lettuce cultivars.

Cultivar	Fertilizer		Cultivar mean
	Organic	Conventional	
	Total calcium content (g/head)		
Perilla Green	0.17	0.19	0.18 a
Salinas	0.15	0.18	0.17 b
Salad Bowl	0.15	0.19	0.17 b
Crisp	0.14	0.19	0.17 b
Rosa Green	0.15	0.18	0.17 c
Jericho lettuce	0.14	0.17	0.16 b
Kasier	0.15	0.17	0.16 bc
Breen lettuce	0.13	0.16	0.15 b
Valmaine	0.14	0.16	0.15 c

The least significant difference (0.05) for the interaction is 0.31. The lower case letters represent the significant differences identified by Duncan’s new multiple range test ($P = 0.05$). The means of these fertilizers are presented in Table 2.

or romaine lettuce cultivars. It is possible that loose-leaf phenotypes transpired more water than the romaine and butterhead phenotypes because of morphology, thereby increasing nutrient uptake transport and exhibiting higher growth.

There were significant differences in nutritional value among cultivar phenotypes. Compared with butterhead and romaine cultivars, loose-leaf cultivars had higher calcium concentration and total calcium content. According to Collier and Tibbitts (1982, 1984), this transpiration affects the delivery and distribution of calcium to lettuce leaves because young leaves with developing heads can develop tipburn. There reason for this is that older leaves transpire more than young leaves, thereby depriving young leaves of calcium. Loose-leaf cultivars are less sensitive to tipburn than romaine or butterhead cultivars, showing the different magnitudes of calcium delivery to young leaves by transpiration (Hylmö, 1953).

Significant variations in calcium content were observed among cultivars. ‘Perilla Green’, ‘Breen lettuce’, and ‘Salinas’ had the highest calcium concentration. The possible reason is that cultivars allow the transpiration of water into the plant head, contributing to the increase in calcium concentration. By contrast, among romaine cultivars, Jericho lettuce, Salad bowl lettuce, and Crisp lettuce accumulate moderate levels of calcium concentration. This may be because of the thick and semi-open leaves of

Table 8. The interaction between cultivars and calcium concentrations in terms of calcium concentrations in lettuce.

Cultivar	Calcium concn		
	60	120	180
Calcium concn (% dry wt)			
Perilla Green	2.75	3.68	2.72
Breen lettuce	2.45	3.21	3.37
Salinas	1.73	1.97	2.32
Jericho lettuce	1.58	1.92	2.53
Salad Bowl	1.57	1.98	1.69
Crisp	1.65	1.87	1.57
Kasier	1.74	1.78	1.38
Valmaine	1.45	1.79	1.69
Rosa Green	1.43	1.71	1.62

The least significant difference (0.05) for the interaction between cultivars and calcium concentrations is 0.39. The means of the main effects of cultivars and calcium concentrations are presented in Table 2.

romaine plant heads, which obstruct the transpiration of water, thereby leading to lower calcium concentration, when compared with loose-leaf and butterhead cultivars (Collier and Tibbitts, 1984). Furthermore, ‘Kasier’, ‘Valmaine’, and ‘Rosa Green’ had lower calcium concentrations compared with other cultivars. Moreover, ‘Kasier’, ‘Valmaine’, and ‘Rosa Green’ have partially closed heads, whereas others have open heads. It is possible that these closed heads obstruct the transpiration of water, resulting in lower calcium concentration in the leaves (Barta and Tibbitts, 1991).

Table 9. The interaction between cultivars and calcium concentrations in terms of total calcium content in lettuce listed in descending order.

Cultivar	Calcium level			Cultivar mean
	60	120	180	
	(g calcium/head)			
Perilla Green	0.25	0.36	0.24	0.28 a
Breen lettuce	0.20	0.24	0.25	0.23 b
Salinas	0.19	0.24	0.23	0.22 b
Jericho lettuce	0.16	0.20	0.21	0.19 c
Salad Bowl	0.18	0.18	0.17	0.18 c
Crsip	0.15	0.20	0.17	0.17 cd
Kasier	0.14	0.16	0.17	0.16 cd
Valmaine	0.13	0.16	0.12	0.14 d
Rosa Green	0.12	0.15	0.11	0.13 d

The least significant difference (0.05) for the interaction between cultivars and calcium concentrations is 0.05. The lower case letters represent the significant differences identified by Duncan's new multiple range test ($P = 0.05$). The means of the calcium concentrations are presented in Table 2.

Conclusions

Fresh and dry weight was higher in the romaine phenotype than in the butterhead and loose-leaf phenotypes. Furthermore, loose-leaf cultivars had higher calcium concentrations and total calcium content when compared with butterhead or romaine cultivars. Furthermore, total calcium content was higher when commercial conventional fertilizers than when organic fertilizers. These cultivars differed widely in total calcium content. 'Perilla Green', 'Breen lettuce', 'Salinas', and 'Perilla Green' had the highest calcium concentrations among all cultivars. A wide range of variability in calcium concentration occurred among the different cultivars

of lettuce, including the different phenotypes and introductions. Therefore, breeding and selection can potentially improve nutrient density in lettuce. Enhancing the mineral nutrition levels of lettuce can improve nutrient uptake without the need to increase produce consumption.

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