Effect of nitrogen, potassium and calcium concentrations on growth, yield and nutritional quality of green oak lettuce

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Abstract

Plant production with adaptation of nutrient application rates may provide benefits to consumers, giving improved nutritional benefits and food functionality. This research studied the effect of nitrogen (N) potassium (K) and calcium (Ca) levels on yields and nutritional quality of green oak lettuce. Plants were grown in a NFT hydroponics system and supplied with nutrient solutions containing different levels of nitrogen (7.5, 15.0 and 22.5 mM L^{-1}), potassium (3.0, 6.0 and 9.0 mM L^{-1}) and calcium (2.5, 5.0 and 7.5 mM L^{-1}). At the harvest stage (four weeks after starting treatment), plant growth and yield for plant height, number of leaves $plant^{-1}$, total fresh weight, total dry weight and leaf area were recorded. The nutritional quality in terms of nutrient concentration (N, P and K), vitamin C, total carotenoids, total chlorophyll content, phenolic content, and total soluble solids were determined. The result showed that plant growth varied, due to the different concentrations of nitrogen, potassium, and calcium. For the nitrogen treatments, plants supplied with nitrogen at 15.0 mM L^{-1} had the highest number of leaves $plant^{-1}$. Plants supplied with nitrogen at 22.5 mM L^{-1} recorded the lowest of leaf area. For the potassium treatments, the total fresh weight and leaf area were highest when the plants were supplied with potassium at 6.0 mM L^{-1} compared to plants supplied with potassium at 3.0 and 9.0 mM L^{-1}. For the calcium treatments, the highest number of leaves $plant^{-1}$ and plant width was when plants were supplied with calcium at 5.0 mM L^{-1}. The results on nutritional quality i.e., vitamin C, total chlorophyll, plant mineral (NPK), total carotenoids, phenolic content and total soluble solids are discussed.

Keywords: nitrogen, potassium, calcium, nutritional quality, concentration, lettuce

INTRODUCTION

Mineral fertilization influences vegetable quality (Kaur and Kapoor, 2001). Understanding plant responses to different plant nutrients allows us to adjust fertilizer use to improve growth and their nutritional quality. Mozafar (1996) reported nitrate accumulation in spinach was reduced when vitamin C content was increased. This occurred when plants were transferred to a nitrogen-free solution for 2-3 days before harvest. In addition, Flores et al. (2004) studied the effect of different fertilization levels of Ca^{2+}, K^{+} and NO_3^{-} on the bioactive nutrient content in red pepper (*Capsicum annuum* L.) fruit. The results showed lycopene and β-carotene content in pepper increased with increasing Ca^{2+} and NO_3^{-} concentrations in the nutrient solution, while vitamin C, sugar and total phenolic acid content were not affected by Ca^{2+} or NO_3^{-} treatment.

Nitrogen (N), potassium (K) and calcium (Ca) are important nutrient elements affecting growth, yield, and quality of plants. Soundy and Cantliffe (2001) reported that shoot growth in lettuce plantlets increased as N concentration increased from 0 to 60 mg L^{-1} in a floating irrigation system. For tomato plants, it was reported that the supply of nutrient solution with high proportion of potassium increased fruit dry matter, total soluble solids and the lycopene

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content in tomato fruit, whereas a high proportion of calcium improved tomato fruit yield (Fanasca et al., 2006). Moreover, lycopene concentration in tomato supplied with 8 mM L^{-1} of potassium concentration were 40% higher than tomato provided with 0 mM L^{-1} of potassium concentration (Trudel and Ozbun, 1971). Ruamrungsri and Inkham (2017) studied the effect of the addition of calcium nitrate on growth and bulb quality of *Hippeastrum.* They reported plants supplied with calcium nitrate at 150 and 200 mg L^{-1} had higher leaf number plant^{-1} than plants supplied with calcium nitrate at 0 and 50 mg L^{-1}. In addition, Yuan et al. (2018) found increasing the total calcium content in lettuce cultivars from 60 to 180 mg L^{-1} was achievable by selecting and applying fertilizers with high calcium concentrations.

There is great interest in growing plants by adjusting nutrient concentrations to improve their growth and nutritional quality. The objective of this study is to determine the effects of nitrogen potassium and calcium concentrations on growth, yields and nutritional quality of green oak lettuce grown hydroponically.

MATERIALS AND METHODS

The experiments were conducted in a plastic greenhouse at Mae-Hia Agricultural Research, Demonstrative and Training Centre, Faculty of Agriculture, Chiang Mai University. Green oak lettuce (*Lactuca sativa* L.) seedlings were prepared by germinate seeds in sponge cubes. The seedlings were fertilized with Hoagland's standard nutrient solution for two weeks until the three true leaves stage. Seedlings were then randomly selected and transplanted into a nutrient film technique (NFT) hydroponic system. Seedlings were fertilized with different concentrations of nitrogen, potassium and calcium nutrient solutions. The experiment was divided into three sub-experiments: 1) N experiment; plants were fertilized with three levels of nitrogen (7.5, 15.0 and 22.5 mM L^{-1}) with potassium and calcium levels maintained at 6.0 and 5.0 mM L^{-1}, respectively, based on Hoagland's standard solution formula; 2) K experiment: plants were fertilized with three levels of potassium (3.0, 6.0 and 9.0 mM L^{-1}) with nitrogen and calcium levels maintained at 15.0 and 5.0 mM L^{-1}, respectively; 3) Ca experiment: plants were fertilized with three levels of calcium concentrations (2.5, 5.0 and 7.5 mM L^{-1}) with nitrogen and potassium levels maintained at 15.0 and 6.0 mM L^{-1}, respectively (Table 1). Treatments in each sub-experiment arranged in a complete randomized design (CRD) with three replicates and 30 plants per replication. The pH of all treatments was maintained at 6.0-6.5.

Table 1. Concentrations of the chemical compounds supplying the macronutrients (mM L^{-1}) and micronutrients (µM L^{-1}) in the nutrient solution of each treatments.

Nutrient sources	Treatments								
	Nitrogen (mM L^{-1})			Potassium (mM L^{-1})			Calcium (mM L^{-1})		
	7.5	15	22.5	3.0	6.0	9.0	2.5	5	7.5
Macronutrients (mM L^{-1})									
KNO_3	3.3	5.0	5.0	2.0	5.0	5.0	5.0	5.0	5.0
$Ca(NO_3)_2.4H_2O$	2.1	5.0	5.0	5.0	5.0	5.0	2.5	5.0	5.0
$MgSO_4.7H_2O$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NH_4NO_3	0.0	0.0	3.7	1.5	0.0	0.0	2.5	0.0	0.0
KCl	1.7	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0
$CaCl_2.7H_2O$	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2
$CaSO_4$	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
Micronutrients (µM L^{-1})									
H_3BO_3	46.3	46.3	46.3	46.3	46.3	46.3	46.3	46.3	46.3
$MnSO_4.4H_2O$	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
$ZnSO_4.7H_2O$	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
$CuSO_4.5H_2O$	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
FeEDTA	90.2	90.2	90.2	90.2	90.2	90.2	90.2	90.2	90.2

At harvest (four weeks after starting treatments), plant growth measurements were recorded, including plant height, number of leaves plant^{-1} and estimation of chlorophyll content (using a portable SPAD-502 Plus chlorophyll meter; Konica Minolta, Japan), total fresh weight, total dry weight, and leaf area (using LI-3100 leaf area meter; LI-COR, USA). The nutritional quality, concentration of nitrogen, potassium and phosphorus, total soluble solids, total carotenoids, total phenolic content, vitamin C and chlorophyll content determined.

Data analysis included analyses of variance (ANOVA) using generalized linear models by means of Statistic 8 analytical software package (SXW Tallahassee, FL). The significance of treatment effects presented as, not significant (ns), or significant at $p<0.05$. In the case of significant treatment effects, the comparison of means was performed by LSD at a significance level of 0.05.

RESULTS AND DISCUSSION

Growth and yields

At harvest, the nitrogen fertilizer concentration of 15 mM L^{-1} resulted in plants with significantly increased plant height, number of leaves plant^{-1}, total fresh weight and total dry weight compared to plants supplied with nitrogen concentrations of 7.5 and 22.5 mM L^{-1} (Table 2; Figure 1). This indicates a nitrogen concentration of 15 mM L^{-1} is the optimum level for growth and yield of green oak lettuces grown in hydroponic system. In addition, plants fertilized with a high concentration of nitrogen (22.5 mM L^{-1}) produced plants with the highest leaf color intensity but had the lowest growth and yield (Table 2; Figure 1).

Table 2. Plant growth and yields of Green Oak lettuce treated with different nitrogen, potassium and calcium concentrations at harvest stage (four weeks after starting treatments).

Treatments	Plant height (cm)	Plant width (cm)	No. of leaves plant^{-1}	Leaf color intensity (SPAD unit)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)
Nitrogen (mM L^{-1})							
7.5	21.1b	26.5a	27.1b	16.3b	2,134.5a	190.3b	3.4b
15.0	25.3a	27.3a	31.0a	17.0b	2,395.1a	239.4a	5.2a
22.5	18.8c	23.1b	26.0b	25.0a	1,253.6b	104.0c	3.9b
$LSD_{0.05}$	*	*	*	*	*	*	*
Potassium (mM L^{-1})							
3.0	24.2a	26.8	24.3b	18.7	1,603.7c	148.8b	3.1b
6.0	25.3a	27.3	31.0a	17.0	2,786.1a	239.4a	5.2a
9.0	20.1b	26.6	25.4b	18.3	1,893.2b	177.3b	2.6b
$LSD_{0.05}$	*	ns	*	ns	*	*	*
Calcium (mM L^{-1})							
2.5	30.7a	27.0ab	24.4b	16.0b	1,808.3c	141.1	3.1
5.0	24.5b	27.2a	26.7a	20.3a	2,395.1b	139.0	2.9
7.5	26.7b	25.5b	22.5b	14.0c	2,842.1a	135.4	2.8
$LSD_{0.05}$	*	*	*	*	*	ns	ns

*significant different between means at 0.05 level of probability, according to analysis of variance. ns = not significant.
Means within the same column followed by different letters are significantly different.

Findings from this experiment and the high leaf color intensity found in plants due to fertilizing with a high nitrogen concentration supports the role nitrogen plays in increasing chlorophyll intensity. Hokmalipour and Darbandi (2011) also found increased nitrogen, significantly increased chlorophyll content. However, this experiment found that excessively high nitrogen concentrations lowered yields, possibly due to nutrient toxicity. Sheikh and Ishak (2016) reported that high concentration of nitrogen in the tissue of plants may cause mineral toxicity and reduce physiological responses.

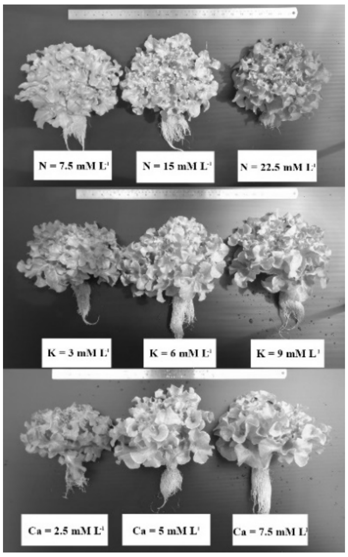

Figure 1. Growth of green oak lettuce treated with different nitrogen, potassium and calcium concentrations at harvest (four weeks after starting treatments).

Plants fertilized with potassium at 6.0 mM L^{-1} showed an increased leaf number $plant^{-1}$, leaf area and total fresh weight compared to those plants fertilized with 3.0 and 9.0 mM L^{-1} potassium concentrations (Table 2; Figure 1). These results indicate a potassium concentration at 6.0 mM L^{-1} may be an appropriate level to maximize growth and yield of green oak lettuces.

The different levels of calcium concentrations provided during this experiment had no effect on total plant fresh weight and total plant dry weight. However, this research found plants fertilized with a calcium concentration of 5 mM L^{-1} resulted in increased leaf number $plant^{-1}$, leaf color intensity and leaf area compared to plants fertilized at 2.5 mM L^{-1} (Table 2; Figure 1). Calcium is an essential element for physiological activity, particularly when cell division is occurring. Calcium is also required for cell wall structure and membrane permeability (Marschner, 1995). The supplement of calcium may have contributed to the significant increments in plant growth under high calcium concentration compared to the low concentration.

Nutritional quality

The nutritional quality of the green oak lettuces was analyzed for each treatment at harvest (four weeks after starting treatments). The results indicated the nitrogen application rates, even at the highest rate (22.5 mM L^{-1}) had no effect on phosphorus and potassium levels in green oak lettuce (Table 3). There was a decreasing trend for total soluble solids with increasing nitrogen concentrations from 7.5 to 15.0 mM L^{-1} (Table 3). Similar results were found by Xia and Cheng (2004) who reported sugar content decreased with increasing nitrogen levels. This is possibly due to the relationship between nitrogen and carbohydrate in plants. Evans (1983) described nitrogen as a direct factor which regulates carbon balance that is the basic element for sugar construction.

Plants fertilized with a high potassium concentration of (9.0 mM L^{-1}) showed the best results for potassium concentration in plant at harvest. The different application levels of potassium had no effect on nitrogen content in the plants of oak lettuce (Table 3). Plants fertilized with the highest calcium concentration (7.5 mM L^{-1}) showed significantly lower potassium content than plants fertilized with calcium concentrations of 5.0 mM L^{-1} (Table 3). These findings are possibly due to the antagonism between the nutrients, calcium, and potassium. Nguyen et al. (2017) reported that potassium, calcium, and magnesium are strongly antagonistic to each other. In case of excess concentration of one element, the uptake of the other elements is inhibited.

Table 3. Nitrogen (N), phosphorus (P), potassium (K) and total soluble solids (TSS) contents in green oak lettuces treated with different nitrogen, potassium and calcium concentrations at harvest (four weeks after starting treatments).

Treatments	N (mg gDW^{-1})	P (mg gDW^{-1})	K (mg gDW^{-1})	TSS (°Brix)
Nitrogen (mM L^{-1})				
7.5	57.31b	30.50	75.32	1.85ab
15.0	59.87b	36.10	71.08	1.50b
22.5	75.07a	36.41	49.49	2.05a
$LSD_{0.05}$	*	ns	ns	*
Potassium (mM L^{-1})				
3.0	81.76	51.37a	30.16c	1.95a
6.0	75.07	36.10b	71.08b	1.50b
9.0	80.81	46.01a	0.87a	1.95a
$LSD_{0.05}$	ns	*	*	*
Calcium (mM L^{-1})				
2.5	60.62b	49.21a	58.38ab	2.20ab
5.0	81.67a	36.10c	71.08a	1.50a
7.5	31.96c	42.19b	39.33b	1.90b
$LSD_{0.05}$	*	*	*	*

*significant different between means at 0.05 level of probability, according to analysis of variance.
ns = not significant. Means within the same column followed by different letters are significantly different.

In addition, there was less nitrogen content in the lettuce fertilized with the high calcium rate of 7.5 mM L^{-1} than the low calcium rates of 2.5 and 5.0 mM L^{-1} (Table 3). This is possibly due to secondary effects of calcium on the senescence pattern in leaves. Pal and Laloraya (1973) reported that the soluble-nitrogen content is generally less in high calcium plants.

The total carotenoid and vitamin C content in the oak lettuce did not differ with increasing nitrogen applications. However, total chlorophyll content was higher in plants fertilized with a nitrogen rate of 22.5 mM L^{-1} compared to the lower nitrogen treatments rates of 7.5, and 15.0 mM L^{-1} (Table 4). This result is understandable, as nitrogen is a structural element of chlorophyll. In the experiment, there was a dramatic decrease of total phenolics when the nitrogen concentration increased from 15.0 to 22.5 mM L^{-1} (Table 4). This is a result of excess nitrogen uptake by the plants. Li et al. (2008) reported under high nitrogen levels, the synthesis of phenols, phenylalanine is preferentially applied into chain protein synthesis rather than for phenol compounds.

Plants fertilized with potassium at 6.0 mM L^{-1} showed the highest results in total phenolic at harvest, while plants fertilized with potassium at 9.0 mM L^{-1} had the highest chlorophyll content (Table 4).

The calcium fertilizer rate of 5.0 mM L^{-1} showed the highest total phenolic content compared to the calcium rate of 2.5 mM L^{-1}. Furthermore, plants fertilized with a calcium rate of 2.5 mM L^{-1} had the highest vitamin C content compared to plants fertilized with calcium rates of 5.0 and 7.5 mM L^{-1} (Table 4). Plants fertilized with low calcium rates, showed high vitamin C levels. This is possible due to the increase in ascorbic acid, which improves tolerance to nutrient deficiency stress. As an antioxidant, ascorbic acid may affect tolerance to environmental stress (Gallie, 2013). Furthermore, increasing ascorbic acid content provides greater tolerance to other environmental stresses. Wang et al. (2010), reported *Arabidopsis* with increased ascorbic acid content and redox state, was a result of increased dehydroascorbate reductase expression, which retained more ascorbic acid and chlorophyll with lower membrane damage following exposure to high light and temperature.

Table 4. Total carotenoid, total phenolic, vitamin C and total chlorophyll contents in green oak lettuces treated with different nitrogen, potassium, and calcium concentrations at harvest (four weeks after starting treatments).

Treatments	Total carotenoid ($\mu g\ g\ FW^{-1}$)	Total phenolic ($\mu g\ g\ FW^{-1}$)	Vitamin C ($mg\ 100\ g\ FW^{-1}$)	Total chlorophyll ($mg\ 100\ g\ FW^{-1}$)
Nitrogen ($mM\ L^{-1}$)				
7.5	4.05	656.64ab	2.76	0.17b
15.0	3.23	723.61a	2.76	0.13c
22.5	4.08	556.94b	2.30	0.22a
$LSD_{0.05}$	ns	*	ns	*
Potassium ($mM\ L^{-1}$)				
3.0	4.05a	520.16b	2.76	0.13b
6.0	3.23b	730.04a	2.76	0.14b
9.0	3.88ab	446.09c	2.76	0.23a
$LSD_{0.05}$	*	*	ns	*
Calcium ($mM\ L^{-1}$)				
2.5	4.58	355.56b	3.37a	0.19b
5.0	4.38	534.57a	2.76ab	0.14c
7.5	4.53	479.01ab	2.33b	0.27a
$LSD_{0.05}$	ns	*	*	*

*significant different between means at 0.05 level of probability, according to analysis of variance. ns = not significant. Means within the same column followed by different letters are significantly different.

CONCLUSIONS

Nitrogen applied at 15 mM L^{-1} and/or potassium at 6 mM L^{-1} increased the fresh plant weight yield of green oak lettuce. The supply of different levels of nitrogen and potassium concentrations did not affect the vitamin C content. Whereas plants supplied with calcium at 2.5 mM L^{-1} had higher vitamin C levels then plants fertilized with calcium at 7.5 mM L^{-1}. This study revealed that nitrogen and/or potassium content of green oak lettuce plants increased with the application of high nitrogen and/or potassium applications.

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