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Effects of Nutrient Composition and Lettuce Cultivar on Crop Production in Hydroponic Culture

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Abstract: A greenhouse research study was conducted at Eastern New Mexico University, Portales, NM, USA, in 2019 to examine the effects of different nutrient solutions on the growth and weight of two lettuce cultivars grown in a floating hydroponic system. Two lettuce cultivars, Buttercrunch and Black Seeded Simpson, were subjected to one of four different nutrient concentrations of N, K and Ca at 150, 100, and 150 ppm (N1), 210, 235, and 200 ppm (N2), 250, 300 and 250 ppm (N3), and 300, 350, and 350 ppm (N4), respectively. The Buttercrunch cultivar and N3 treatment proved significantly more productive than the other cultivar/treatment combinations. The greatest fresh weights were recorded in the N3 nutrient solution, 115.33 and 93.17 g/plant for Buttercrunch and Black Seeded Simpson, respectively. Buttercrunch had the greatest fresh weight, leaf number, and leaf and root length in all nutrient solutions. The nitrogen content of the solutions showed a significant positive relationship with chlorophyll content for both cultivars. It is expected that the development of a cheap and easy-to-use hydroponic system will help growers produce high-quality organic vegetables including lettuce.

Keywords: lettuce; hydroponics; Buttercrunch; Black Seeded Simpson; nutrients

1. Introduction

Lettuce (*Lactuca sativa* L.) is a green leafy vegetable belonging to the Asteraceae family. It is a cool-season vegetable which thrives in temperatures ranging from 7 to 24 °C and is commonly consumed in salad mixes [1]. Among different vegetables grown in the United States, lettuce surpasses all others except potato in terms of land devoted to production and crop value [2]. Lettuce is very nutritious and a rich source of vitamin C, minerals and fiber [3]. Lettuce has been used as a medicine for different ailments including stomach problems, inflammation, pain and urinary tract infections from ancient times due to the presence of secondary metabolites such as terpenoids, flavonoids and phenols [4]. Nowadays, consumption of organic vegetables, including lettuce, is surging because of the fast-growing human population, rapid urbanization and increased health concerns. However, one significant factor that limits vegetable cultivation is inadequate land space.

Hydroponic culture is a cheap and easy option for organic vegetable production. It is a technique that involves growing plants in water using mineral nutrients without soil [5,6]. Easy control of nutrient composition, lack of soil contamination, faster plant growth, shorter crop cycles, high product quality, and good consumer acceptance have made hydroponics an important alternative plant production technique [7–10]. In tropical climates, a lettuce crop cycle is around 70 days in soil cultivation, whereas the duration is reduced to 30 days in hydroponic culture [11]. Plants grown in hydroponics contain more minerals and chlorophyll compared to those produced in a conventional soil-based system [12,13]. The macro- and micronutrient composition of a hydroponic solution determine plant growth, leaf number, leaf area, marketable yield, and crop quality including mineral and chlorophyll content [14,15]. Furthermore, Both et al. [16] and Kang et al. [17] reported that a light intensity of 200 to 300 μmol·m^{-2} s^{-1} and a CO_2 level of 400 to 600 μmol·mol^{-1} are suitable for the hydroponic culture of lettuce.

Even though hydroponic culture can produce optimal plant growth (better yield and quality), its efficiency depends on many factors such as nutrient availability, crop genotype, growing method, and pest management [6]. Although studies have been conducted on the hydroponic culture of lettuce, the results have been inconclusive about the influence of cultivar and nutrient solution composition on lettuce performance. For example, the fresh weight reported by Li et al. [18] in a nutrient solution of 210 mg N/L was 88.8 g and 96.1 g for two lettuce cultivars. However, the fresh weight reported by Kowalczyk et al. [19] in a nutrient solution of N, P, K of 140, 50, and 300 mg/L, respectively, was 245 g and 175 g for two other lettuce cultivars. Furthermore, the increasing N concentration on a hydroponic culture of lettuce from 0 to 60 mg/L increased shoot weight from 68. 7 to 129.7 g [20]. Thus, the goal of this study was to examine the effects of different nutrient solutions on the growth and weight of two lettuce cultivars grown in a floating hydroponic system.

2. Materials and Methods

2.1. Study Site

This research was conducted in a greenhouse at Eastern New Mexico University, Portales, NM, USA (latitude: 34°10′38″ N longitude: 103°20′51″ W; altitude: 1221 m) from 1 March to 1 April 2019. The temperature, relative humidity, light intensity and ambient CO_2 level during the study were 25–30 °C, 40%–50%, 150–300 μmol m^{-2} s^{-1}, and 400–500 μmol mol^{-1}, respectively.

2.2. Lettuce Cultivars

Two lettuce cultivars, Buttercrunch and Black Seeded Simpson, were selected because of their high market demand and suitability for hydroponic culture. Seeds were sown in disposable plastic cups filled with potting soil in the greenhouse on 14 February 2019. Emerged seedlings were watered four times a week for three weeks.

2.3. Hydroponic Components

A floating hydroponic system was constructed from plastic containers (length 73 cm, width 40 cm, and depth 31 cm), Styrofoam plates (thickness 2.5 cm) and plastic cups (height 11 cm and width 8 cm). The plastic containers were filled with 20 L of water and mineral fertilizers were added. The fertilizer sources $Ca(NO_3)_2$, KNO_3, KH_2PO_4, H_3BO_3, $MgSO_4$ and $ZnSO_4$ were used to supply nutrients. Three-week-old seedlings (three to four true leaves) were transferred to the hydroponic system. The seedlings were washed to completely remove soil before placing in the hydroponic system. Twelve seedlings were placed in each container. The bare roots of the plants were continuously bathed in a stagnant nutrient solution. Plant-to-plant and row-to-row distances were maintained at 8 and 10 cm, respectively. A porous air stone was submerged in a hydroponic system to oxygenate the solution. The pH of the nutrient solution was maintained between 5.5 and 6.5 by adding NaOH or HCl as per need. The pH of the solution was checked three times a week. Furthermore, 2 L of water and nutrient solutions were added to replenish each hydroponic solution 15 days after treatment initiation.

2.4. Experimental Design

The nutrient solutions were prepared based on the full strength modified Hoagland solution. The contents of major nutrients such as nitrogen (N), potassium (K), and calcium (Ca) were varied, but other nutrients such as phosphorous (P), boron (B), zinc (Zn) and magnesium (Mg) were the same for all treatments (Table 1). The four concentrations of N, K and Ca used were 150, 100, and 150 ppm (N1), 210, 235, and 200 ppm (N2), 250, 300 and 250 ppm (N3), and 300, 350, and 350 ppm (N4), respectively. Nutrient solution N2 was similar to Hoagland solution in terms of N, Ca and K concentrations, but S, Na, Cl, Cu, and Mo were excluded, and N2 was considered the control treatment for both cultivars. The experiment was arranged in a randomized complete block design with eight treatments. Each treatment consisted of six seedlings of each lettuce cultivar and was replicated three times. There were 144 plants in total (72 for each cultivar).

Table 1. Nutrient concentrations for lettuce hydroponic culture.

Nutrients	Concentration (ppm)			
	N1	N2 (Control)	N3	N4
N	150	210	250	300
K	100	235	300	350
Ca	150	200	250	350
P	56	56	56	56
B	0.4	0.4	0.4	0.4
Zn	0.35	0.35	0.35	0.35
Mg	45	45	45	45

2.5. Data Collection

Lettuce plants were harvested 30 days after treatment initiation. The response variables measured were fresh weight, number of leaves, length of the longest root and leaf, and leaf chlorophyll content. Four leaf samples were randomly collected from each plant at harvest and mixed separately to determine chlorophyll content.

The chlorophyll content was determined by the Wellburn technique [21]. Leaf samples were extracted using 0.2 g of fresh leaves and 7 mL of 80% acetone to break down leaf cells. The optical density of the solution was measured at 645 nm (OD663) and 663 nm (OD645). The following equations were used to calculate the chlorophyll content of leaves:

$$\text{chlorophyll a (mg/g)} = (12.70 \times A663 - 2.690 \times A\ 645) \times V/(1000 \times W)$$

$$\text{chlorophyll b (mg/g)} = (22.90 \times A645 - 4.680 \times A\ 663) \times V/(1000 \times W)$$

$$\text{total chlorophyll content} = \text{chlorophyll a} + \text{chlorophyll b},$$

where V: Total volume of acetone used (L), W: Weight of fresh leaf (g), A 645: Absorbance at the wavelength of 645 nm, A 663: Absorbance at the wavelength of 663 nm.

2.6. Statistical Analysis

The data were processed in Microsoft Excel and analyzed using R studio software. Analysis of variance was used to determine differences for the main effects of cultivar and nutrient solution and for their interaction. Mean separation was performed using the least significance difference (LSD) test at $p < 5\%$.

3. Results and Discussion

3.1. Fresh Weight

Buttercrunch lettuce had the greatest fresh weight in the N3 solution followed by N2 and N4 solutions (Table 2). Black Seeded Simpson lettuce also had the highest weight in the N3 and N2 solutions. There was a statistically significant difference between lettuce cultivars for fresh weight. Interestingly, nutrient solutions N2 and N3 resulted in full-grown plants of marketable size for both cultivars. The N1 solution produced the lowest fresh weight when compared to the other treatments for both cultivars (Table 2). Thus, weight variation in lettuce could be attributed to cultivar characteristics and nutrient concentration, especially N, Ca and K in the hydroponic solutions. A previous study by Sublett et al. [1] suggested that nutrients are the primary factors that influence plant growth and biomass production in hydroponic culture. Other studies also demonstrated the lettuce response to the nutrient composition. For example, Petropelous et al. [10] reported that increasing N rate from 100 to 200 mg/L increased fresh lettuce plant weight by 23.5%–113% (from 12 to 41.9 kg/m^{-2}) in hydroponic culture. Similarly, Soundy et al. [20] reported that a P concentration range of 35 to 50 mg/L was essential for producing high-quality lettuce seedlings in hydroponic culture. Despite the highest nutrient concentrations, the N4 nutrient solution produced lettuce with a comparatively lower weight. This inferred that an excessive level of macronutrients such as N, K and Ca could have negative effects on plant growth. These findings were consistent with previous studies. Borowski and Michalek [22] reported that a high concentration of N (NH_4^+, NO_3^-) was responsible for a significant fresh weight reduction in lettuce. Similarly, Nurzyńsk [23] mentioned that nutrients including N determine the yield of leafy vegetables but an excessive nutrient concentration including nitrogen may have adverse effects on yield and leaf nitrate content of lettuce in a deep-water culture production system. Possibly, excess N in a hydroponic solution may result in high osmotic pressure around the root, thereby reducing growth. In addition, excess N may bind to the root reducing growth [24].

Table 2. Effects of nutrient solution on growth parameters of different lettuce cultivars.

Treatments			**Growth Parameter**	
Nutrient Solutions	**Fresh Weight (g)**	**Number of Leaves**	**Leaf Length (cm)**	**Root Length (cm)**
		Lactuca sativa 'Buttercrunch'		
N1	57.11 c^z	10 c	13.24 c	12.17 c
N2 (Control)	91.11 b	15 b	21.14 ab	21.94 b
N3	115.33 a	20 a	25.06 a	27.61 a
N4	82.56 b	14 b	18.94 b	19.34 b
		Lactuca sativa 'Black Seeded Simpson'		
N1	41.94 c	11 c	9.75 c	11.69 b
N2 (Control)	81.67 ab	17 ab	15.06 b	22.94 a
N3	93.17 a	21 a	19.39 a	24.67 a
N4	66.06 b	15 bc	12.22 bc	15 b
		Significance		
Nutrients (N)	** ^{y}	**	**	**
Cultivars (C)	*	NS	*	NS
N × C	*	NS	NS	NS

^{z} Means with a different letter within each cultivar are significantly different by the least significant difference (LSD) test at $p \leq 0.05$. ^{y} ** = $p < 0.001$, * = $p < 0.05$; NS, not significant at $p \geq 0.05$.

3.2. Number of Leaves

Lettuce cultivars grown in the N3 nutrient solution generally had a significantly greater number of leaves compared to the other solutions (Table 2). Differences in the number of leaves per plant could be associated with either solution composition and/or cultivar characteristics. These findings were slightly different from the findings of Petropelous et al. [10] who reported that increasing N from 150 to 200 mg/L for greenhouse lettuce production could increase the mean number of leaves from 19.1 to 22.5, respectively, after 31 days. However, Buttercrunch showed no statistically significant difference in leaf number between the N2 and N4 nutrient solutions (Table 2).

3.3. Leaf and Root Length

There were significant differences in leaf and root length between cultivars grown in the different nutrient solutions. The N3 solution generally produced the longest leaves and roots, whereas those grown in N1 had the shortest leaves and roots (Table 2). The reason for longer leaves and roots could be associated with better nutrient combinations. The leaf length in N1 for Buttercrunch and Black Seeded Simpson was 13.24 cm and 9.75 cm whereas the root length produced was 12.17 cm and 11.69 cm, respectively, likely due to lower mineral availability in the solution. Furthermore, the influence of nutrients on leaf size corresponds to several previous findings. For example, Fraile et al. [25] evaluated the growth and quality of lettuce in a hydroponic system and concluded that leaf size depended on nutrient composition, water availability and temperature. Despite the highest concentrations, the N4 solution produced relatively shorter leaves and roots inferring that an overabundance of N, K and Ca was not suitable for lettuce hydroponic culture. Previous studies supported these findings. Kay et al. [26] mentioned that high ammonium nitrogen depressed root metabolism leading to shorter roots.

Overall, the results indicated that N1 and N4 were the poorer nutrient solutions for both cultivars. The N1 and N4 content of macronutrients (N, K and Ca) resulted in lower fresh weight, fewer leaves as well as shorter leaf and root length. For both cultivars, fresh weight, number of leaves, as well as leaf and root length, were highest in the N3 nutrient solution compared to all other solutions (Table 2). Additionally, the reverse relationship was found between Buttercrunch and Black Seeded Simpson cultivars with respect to the number of leaves and leaf length. The growth of lettuce cultivars in different nutrient solutions is shown in Figure 1.

Figure 1. (**I**) Black Seeded Simpson, (**II**) Buttercrunch; (**A**–**D**) refers to nutrient compositions N1, N2, N3 and N4 respectively.

3.4. Chlorophyll Content

The contents of chlorophyll a, b and total chlorophyll were highest in N4 followed by N3 for both cultivars (Figure 2). The total chlorophyll concentration for Buttercrunch and Black Seeded Simpson in N4 were 0.55 and 0.43 mg/g fresh weight, respectively. A similar result was reported by Asimovic et al. [27] who found that chlorophyll content of lettuce was 0.583 mg/g fresh weight. However, Bohn et al. [28] reported slightly different results with a chlorophyll content of lettuce of 0.180 mg/g fresh weight. The range of lettuce chlorophyll reported by Fallovo et al. [15] was between 0.482 to 0.821 mg/g. There may be a linear relationship between N in the solution and total chlorophyll. Fraile et al. [25], Lin et al. [29] and Rambo et al. [30] also reported that N is vital to chlorophyll formation and that decreasing N content in a nutrient solution reduces chlorophyll hampering photosynthesis and crop yield. This was further supported by Fallovo et al. [15] who found that total chlorophyll content in lettuce leaves was significantly affected by nutrient solution composition. Furthermore, Mahlangu et al. [31] reported a positive relationship between N content and chlorophyll concentration in lettuce. For example, application of 30 mg N/L produced 22.8 mg chlorophyll while application of 180 mg N/L produced 27.2 mg chlorophyll. Chlorophyll is not the only factor which determines crop growth. Plant growth and yield are complex traits that depend on several factors, including cultivar, light, CO_2, plant enzymatic activity, other nutrients, and water. In light of this, we suggest that, despite higher chlorophyll concentrations, plants may have lower production. In our study, in some treatments, chlorophyll b was higher than chlorophyll a (Figure 2). The resulting low value of the chlorophyll between cultivars could have a genetic basis. Cassetari et al. [32] supported current findings stating that genetic variability affects chlorophyll in lettuce. Caldwell and Britz [33] also reported cultivar specific differences in chlorophyll content in greenhouse-grown green lettuce.

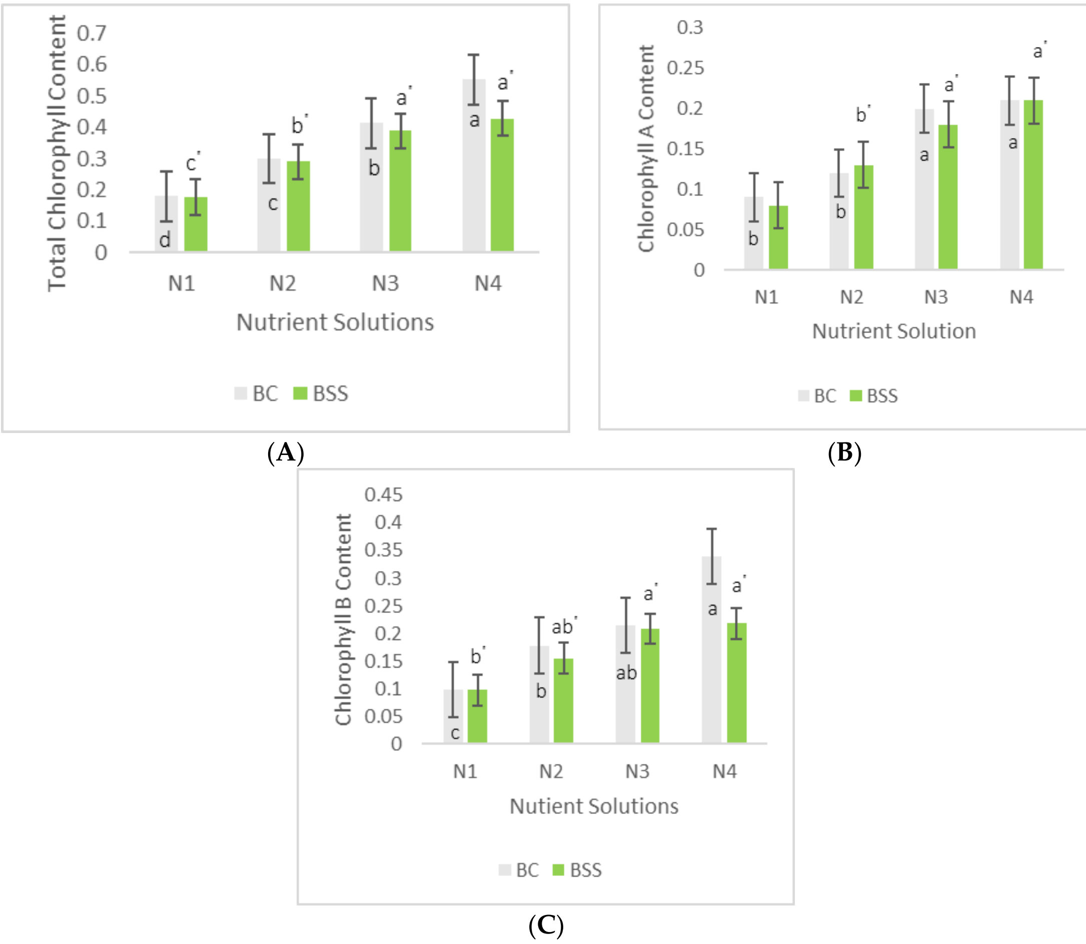

Figure 2. Chlorophyll content of two lettuce cultivars grown under different nutrients composition, (**A**) total chlorophyll, (**B**) chlorophyll a, (**C**) chlorophyll b. Means with a different letter within each cultivar are significantly different by the LSD test at $p \leq 0.05$. BC: Buttercrunch; BSS: Black Seeded Simpson cultivars.

4. Conclusions

In summary, the lettuce cultivar Buttercrunch and the N3 nutrient composition (N = 250 ppm, K = 300 ppm, and Ca = 250 ppm) should be used for the hydroponic culture of lettuce to maximize lettuce growth and weight in similar environments. It is important to know the nutrient response of each cultivar grown in hydroponics in order to optimize production. Further studies are needed to evaluate the effect of cultivar and nutrient composition on the nutritional quality of vegetables produced in hydroponic culture.

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