



Article Growth, Physicochemical, Nutritional, and Postharvest Qualities of Leaf Lettuce (*Lactuca sativa* L.) as Affected by Cultivar and Amount of Applied Nutrient Solution

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Abstract: The effects of different nutrient solution quantities on growth, physicochemical, nutritional, and postharvest qualities of lettuce were investigated. Two differently pigmented Korean leaf lettuce cultivars "Geockchima" and "Cheongchima" were grown in soilless perlite culture supplied with 250, 500, 1000, and 2000 mL·d⁻¹·plant⁻¹ nutrient solutions. Several growth parameters (plant height, leaf number, fresh weight, dry matter) were evaluated. The highest lettuce growth was observed when plants were supplied with 1000 mL·d⁻¹·plant⁻¹. Cultivating lettuces in the lowest nutrient solution quantity showed higher dry matter, crude fiber, osmolality, chlorophyll, and anthocyanin contents. Upon increasing the nutrient solution, the crispiness, greenness, and levels of ascorbic acid, nitrogen, and potassium, increased, while phosphorus and magnesium were unaffected, and calcium content declined. Postharvest qualities were better maintained in lettuces irrigated with the least amount of nutrient solution, extending their shelf life. We conclude that lettuce can be grown with 1000 mL·d⁻¹·plant⁻¹ for higher yield, and short-term storage and/or transportation. However, when lettuces need to be stored for a certain period, such as long-distance shipment, they should be cultivated with a limited nutrient solution, which requires further detailed investigation. The results of this study can be applied for distributing, storing, transporting, and marketing lettuce.

Keywords: hydroponics; overall visual quality; relative fresh weight; shelf life; storage

1. Introduction

The vegetable production system has largely shifted from traditional soil culture to soilless culture in several parts of the world, especially developed countries, as it offers some clear and unique advantages, such as improved quality and safety [1–3]. The overall management and control of plant nutrition required during various growing cycle stages are also easily upheld in soilless culture. While soilless culture requires significantly higher initial capital and operational costs, it can be balanced by higher productivity and improved product quality [4]. For these reasons, greenhouse crop production is now a steadily growing agricultural sector worldwide [5]. In Korea, the total area under greenhouse cultivation has increased recently, covering about 52,444 hectares, with vegetables occupying 68.5% of the total area [6]. Lettuce production, in particular, has increased from 3387 hectares in 2016 to 3484 hectares in 2014, indicating a 4.2% increment in production areas compared to the previous year [7]. As vegetable research in Korea has traditionally prioritized the improvement of productivity and quality, commercial production of lettuce in the greenhouse system is one of the greatest challenges for maintaining those aspects, as well as the safety and postharvest behavior of the produce. Solid-medium soilless culture, one of the most



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). popular and easy methods in greenhouse cultivation, uses artificial upper soils, such as perlite and rock wool, as plant support. Since the physical properties, such as particle size, capillary space, water retention capacity, and the presence of pathogens, of these media are better than those of soil, the rhizosphere environment can be controlled, enhancing productivity [8]. Plant nutrients or specific ingredients, which cannot be easily adjusted in soil culture, can be easily controlled in soilless media. As a result, solid-medium soilless cultures are commonly used in the cultivation of vegetables, such as tomatoes, Chinese cabbage, sweet peppers, and cucumbers [9–11], and it is currently being used in Korea for the commercial cultivation of lettuce.

In horticultural crops, water supply and/or availability plays a pivotal role in processes associated with growth, productivity, and biomass, as well as having key implications on quality aspects, especially in the case of lettuce, which comprises 95% water [12]. As the edible parts of lettuce are the photosynthetic leaves, it is crucial to maintain optimal crop growth, which can be achieved through a well-scheduled irrigation program [13]. However, a previous study reported that deficiency in irrigation water could preserve the postharvest quality and shelf life of fresh-cut lettuces [14,15]. Water stress also influences the composition and concentration of nutrient-providing and biofunctional plant compounds [16]. Components, such as chlorophyll and anthocyanin, can be accumulated when plants experience different levels of water stress or drought condition [16, 17]. The application of plant nutrients, on the other hand, could play a critical role in determining the nutrient and mineral composition of the plants [18–20]. Since water is a limited natural resource, and there is an increasing pressure to optimize the water use efficiency of crop production, reduced or limited water supply to lettuce during growth could be an effective way of not only saving irrigation water, but also maintaining the postharvest qualities of the produce. One of the most significant challenges for lettuce growers, wholesalers, and retailers is preserving postharvest qualities, which depend on the type and cultivar [21,22], storage conditions [23], and postharvest treatments [24]. Therefore, improving the growth, productivity, nutritional quality, and storage potential of lettuce is a desired goal for the growers and consumers. Numerous studies have highlighted the effects of cultivation methods [2], irrigation amount and water stress condition [16,25], salinity and elevated CO_2 [26], and light quality and fertilization [27,28] on the growth and yield of lettuce; however, no study has been conducted on the effects of nutrient solution management through irrigation volume control in solid-medium soilless culture on the growth and quality aspects of lettuce at harvest, as well as their storage behavior at postharvest. Thus, the effects of various levels of applied nutrient solution on the growth, physicochemical, nutritional, and postharvest qualities of two different pigmented leaf lettuce cultivars grown in soilless perlite cultures in greenhouse conditions were investigated.

2. Materials and Methods

2.1. Plant Material, Growing Condition, and Measurement of Growth Parameters

Seeds of two Korean leaf lettuce (*Lactuca sativa* L.) cultivars, "Geockchima" (red leaf, Nongwoo Bio Co. Ltd., Gyeonggi-do, Korea) and "Cheongchima" (green leaf, Nongwoo Bio Co. Ltd., Gyeonggi-do, Korea), were sown in a plastic seedling tray containing 162 cells and filled with watered wicking material, commercially known as "*Biosangto* No. 1" (Seminis Korea, Pyeongtaek, Korea). With two months as the total duration of the trial, the seeds were sown on April 21, 2016 and on April 26. Five-day-old, seedlings were transplanted onto soilless perlite medium (particle size 5 mm, Parat No.1, Samson Co., Gyeonggi-do, Korea) in the plastic greenhouses of the National Institute of Horticultural and Herbal Science (NIHHS), Suwon-si, Republic of Korea. The average day and night air temperatures of the plastic greenhouses were maintained at 26.6 ± 2.5 °C and 17.3 ± 2.1 °C, while the relative humidity was $46.5 \pm 10.3\%$ and $74.2 \pm 9.8\%$, respectively. The beds containing perlite medium were previously prepared for growing lettuce following the Rural Development Administration guidelines [29]. The beds were covered with black polyethylene film as mulching material, with planting holes on the film ensuring plant-to-

plant distances of 20 cm. The beds contained only a single row of lettuce, and bed-to-bed distance was approximately one meter. Plants were watered, via an automated control system, with nutrient solution containing N–P–K–Ca–Mg at 14.5–3.5–9–4–2 mequiv L^{-1} , while electrical conductivity and pH were adjusted at 1.8 and 5.5–6.0 dS·m⁻¹, respectively. The nutrient solution was prepared according to NIHHS guidelines, and adapted to the local environmental conditions [29]. The nutrient solution was supplied to the base of each plant by a drip irrigation system using a pipe during the growing period, and four different irrigation volumes, 250, 500, 1000, and 2000 mL·d⁻¹·plant⁻¹, were used as the irrigation treatment. The frequency of irrigation was 10 times during the day and twice at night. The experiment was arranged in a completely randomized block design with three replications consisting of 15 plants per replicate. A preliminary experiment was conducted with limited replication and plants to observe the plant growth parameters and postharvest performances. Growth parameters, including plant height, number of leaves, and fresh weight, were measured at the time of harvest. Plant height was determined by measuring the length of the plant from the shoot tip to the surface, and leaf number was determined by counting leaves with a minimum width of 1 cm. Fresh weight was measured by weighing the living biomass of each plant that was present aboveground following the harvest.

2.2. Harvest Practice, Storage Condition, and Postharvest Quality Evaluation

Lettuce was harvested by cutting the entire plant when it reached commercial maturity, i.e., 29 days after transplanting, after which plant height, number of leaves, and shoot fresh weight were measured. With regards to conducting the storage experiment, 5–12 leaves per plant from the 3rd to 14th leaf position were randomly selected and immediately transported to the laboratory (within 10 min) using a cooled chamber and maintained at 4 °C. For the postharvest quality evaluation, a single replicate under each treatment containing about 20 leaves (approx. 150-200 g) of uniform size and color, and with no physical damage, was sampled. The leaves were packaged in transparent polypropylene bags (size 32 cm \times 22 cm, thickness 0.05 mm) with four holes (two holes each side) of 5 mm diameter and manually sealed with thread-like wire. Then, the packages were placed in plastic trays on a shelf in a 7 °C dark room, and stored for up to 12 d. Storage was terminated when samples became unusable due to visible deterioration symptoms, such as yellowing, wilting, decay, or spoiling. Fresh weight, color, and overall visual quality (OVQ) were measured at two-day intervals during storage. Three replicates were used for each evaluation day under each irrigation treatment. An OVQ analysis was performed by an eight-member panel, with ages between 28 and 52 years (five men and three women). Considering appearance, visual color, off-odor, and freshness, the OVQ was evaluated using a 9-point scale (9 = excellent, 7 = good, 5 = fair, 3 = poor, and 1 = unusable). A score of 5 was considered the marketability limit. The OVQ scoring and acceptance of marketability were adopted from Aguero et al. [30].

2.3. Color Measurement

Five leaves were randomly selected from the 3rd to 14th leaf position of one plant in each replicate immediately after harvest for color measurement using a chromameter under different irrigation treatments (Minolta CR-400, Minolta, Osaka, Japan). Similarly, five leaves were randomly selected from each pouch on each evaluation day for measuring color parameters. Three readings were taken from the left, middle, and right part of the upper region, and closer to the tip of the adaxial surface of the leaf; therefore, an average of 15 readings were taken for a single replication. Before measuring the color readings from leaf samples, the chromameter was calibrated using a standard white plate (Y 93.5, x 0.3155, y 0.3320) provided by the manufacturer. Color changes were quantified in the L^* , a^* , b^* color space. L^* refers to the lightness, and ranged from $L^* = 0$ (black) to $L^* = 100$ (white). Green and red indicate negative and positive values of a^* , respectively, while yellow and blue indicate positive and negative values of b^* , respectively. Total color difference (ΔE^*) was calculated using the formula of [21]:

$$\Delta E^* = \{ (L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2 \}^{1/2} L_0^*$$
(1)

where a_0^* and b_0^* represent the values at harvest, and L^* , a^* , and b^* indicate reading on any evaluation day.

2.4. Measurement of Dry Matter, Texture, Crude Fiber, Osmolality, and Ascorbic Acid Content

For dry matter measurement, five intact lettuce leaves were inserted into a thin paper envelope, weighed, and dried at 70 °C for 48 h to achieve a constant weight. Three envelopes were prepared from each replicate, and the average values were expressed as a percentage of dry matter. Texture analysis of the lettuce leaves was performed in terms of force required to puncture the leaves using a texture analyzer (TA Plus, Lloyd Instruments, Model LF2303, Ametek Inc., Fareham, Hants, UK). The sample was placed on the central opening of the metal holding plate, where leaf tissues (approx. 5 cm \times 5 cm) were set tightly on the stage with clips, and a 5 mm diameter flat-head stainless steel cylindrical probe was connected to the tissues for the test. The movement of the probe was adjusted to 5, 2, and 10 mm/s as the pre-test, test, and post-test speeds, respectively. The probe was run with a 20 N load cell and attached to a creep meter equipped with software (NEXYGENTMMT v 4.5, Lloyd Instruments, Ametek Inc., UK) for automatic analysis using a computer.

The crude fiber was determined following the AOAC [31] method using a crude fiber analyzer (Fibertec Systems M1020, Foss Tecator, Eden Prairie, MN, USA). The method involved the digestion of the fresh samples with boiling and via dilution in acid and alkali for the calculation for residual ash content. The osmolality of lettuce leaf sap samples was measured using a Wescor5520 vapor pressure osmometer (Wescor Inc., Logan, UT, USA) following the method of Clarkson et al. [32]. Lettuce leaves were sliced into small pieces, mixed, and 10 g was used to collect sap using a juice maker. Ascorbic acid content was determined following the method of Jagota and Dani [33]. Briefly, a 0.5 g fresh leaf sample was extracted and filtered with double-distilled water. Then, 0.2 mL homogenate was added with 0.8 mL 10% trichloroacetic acid followed by vigorous shaking, incubating in an ice bath, centrifugation, and collection of supernatant. Following this, the 2.0 mL diluted supernatant was mixed vigorously with 0.2 mL 0.2 M Folin phenol reagent. After 10 min, the absorbance of this solution was measured at 760 nm using a UV-VIS recording spectrophotometer (DU 650, Beckman Coulter™, Chaska, MN, USA). The concentration of ascorbic acid was calculated from the standard curve prepared from different concentrations of ascorbic acid following the same procedure.

2.5. Microscopic Observation of Lettuce Tissue

Samples for histological study were taken from the leaf apex at the equatorial region, at a position of 90% distance of leaf length from the base. The samples were prepared following the method of Luna et al. [34], with some modifications. Briefly, samples (about 1 mm³) were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), post-fixed with 1% osmium tetroxide, each for 2 h at 4 °C, and then held overnight in phosphate buffer. After fixation, specimens were dehydrated in a graded series of ethyl alcohol, processed through three changes of propylene oxide (for 15, 15, 30 min per change), and gradually infiltrated with embedding medium, Epon. Specimens were sectioned (1500 nm), stained with periodic acid–Schiff, and viewed under a Carl Zeiss Axioskop 2 light microscope (Carl Zeiss, Jena, Germany).

2.6. Determination of Total Chlorophyll and Anthocyanin

Total chlorophyll content was determined following the method of Lin et al. [28], with some modifications. First, 5 g fresh leaf tissues were homogenized in 20 mL of 80% acetone using an Ultra-Turrax tissue homogenizer (T 25 B, Ika Works Sdn. Bhd, Rawang, Selangor, Malaysia) at a moderate speed for about one minute. The homogenate was filtered through

four layers of cotton cloth and centrifuged at 4 °C, $15,000 \times g$ for 20 min, after which the supernatant was transferred to a volumetric flask, and the volume was determined using 80% acetone. The absorbance of the supernatant was measured at 663 and 645 nm, and total chlorophyll (4) content was calculated using the following formulae:

Chlorophyll a (chl a) =
$$12.72 \times OD663 - 2.59 \times OD645$$
 (2)

Chlorophyll b (chl b) =
$$22.88 \times OD645 - 4.67 \times OD663$$
 (3)

$$Total chlorophyll = (chl a + chl b)$$
(4)

Similarly, total anthocyanin was estimated using a spectrophotometric pH differential method following the procedure of Ferrante and Maggiore [35], with minor modifications. Five grams of fresh leaf tissue was homogenized in 20 mL methanol containing 1% HCl, incubated overnight at 4 °C in darkness, centrifuged at 4 °C 12,000× *g* for 20 min, the supernatant collected in a volumetric flask, and made up to a known volume with the same acidified methanol. An aliquot of 2 mL was diluted with 18 mL potassium chloride buffer (pH 1.0), and another 2 mL aliquot was diluted separately with 18 mL sodium acetate buffer (pH 4.5). Absorbances of these solutions were measured at 510 and 700 nm, and anthocyanin contents were calculated using the formula of [36]:

Monomeric anthocyanin pigment (mg·L⁻¹) = (A × MW × DF × 1000)/(
$$\varepsilon$$
 × 1) (5)

where A = (A510–A700) pH1.0–(A510–A700) pH4.5 with molar absorptivity (ϵ) of 26,900 (L·cm⁻¹·mol⁻¹); MW = molecular weight of cyanidin-3-glucoside (449.2); DF = dilution factor of this experiment.

Anthocyanin content (mg·L⁻¹) was then converted to mg·100 g⁻¹ fresh sample. Unless otherwise stated, all chemicals used for biochemical assay were purchased from Sigma Aldrich Co. Ltd. St. Louis, MO, USA.

2.7. Mineral Analysis

For the analysis of mineral contents and C/N ratio, the harvested leaves were dried in a circulated drying oven for 48 h at 80 °C, and subsequently ground to make a powder. The mineral contents were determined using RDA's elemental analysis methods [29]. To measure nitrogen (N) content, a finely ground leaf sample (0.5 g) was decomposed with 1 g catalyst (K_2SO_4 :CuSO₄ = 9:1) and 10 mL concentrated sulfuric acid in Kjeldahl flasks at 380 °C for 3–4 h, and then analyzed using a Kjeldahl analyzer (Kjeltec2300 analyzer unit, Foss Tecator AB, Hoganas, Sweden). For phosphorus (P) determination, a 0.5 g sample was digested in acid in a fume hood until the digested sample color became transparent. P was measured at 470 nm using a UV-visible spectrophotometer after coloring with ammonium vanadate. Potassium (K), calcium (Ca), and magnesium (Mg) were measured using atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan). Dried leaf samples (0.5 g) were decomposed with 10 mL digest solution containing HNO₃ and HClO₄ (3:1) at 180 °C for 12–16 h, filtered, and made up to a known volume. The C/N ratio was measured by the combustion method using CN corder (CNS2000, Leco, St. Joseph, MI, USA).

2.8. Statistical Analyses

Analysis of variance (ANOVA) of the data was performed by SAS software ver. 9.1 (SAS Institute, Cary, NC, USA). A combined ANOVA was performed using cultivar as a fixed variable [37]. Based on the level of significance calculated from the F-value of the ANOVA, Duncan's multiple range tests were applied at $p \le 0.05$ for mean comparisons among the various treatments.

3. Results and Discussion

3.1. Growth of Lettuce as Affected by Cultivar and Amount of Applied Nutrient Solution

Lettuce growth was significantly influenced by the applied nutrient solution amount and cultivars in perlite culture (Table 1). Regarding the effect of cultivar, Cheongchima produced more leaves ($p \le 0.05$) than Geockchima, while an opposite trend was observed for plant height. Alternatively, the amount of irrigation had a significant ($p \le 0.01$) effect on plant height, number of leaves, and fresh shoot weight. However, the irrigation volume and cultivar interaction effect on these growth parameters was not significant (Tables 1 and 2).

Table 1. Effects of the amount of applied nutrient solution on growth performance, dry matter, and crude fiber content of two leaf lettuce cultivars grown in perlite culture.

Cultivar	Amount of Applied Nutrient Solution (mL·d ⁻¹ ·plant ⁻¹)	Plant Height (cm)	No. of Leaves ^x	Fresh Weight of Shoot (g∙plant ⁻¹)	Dry Matter (%)	Crude Fiber (%)
Geockchima	250	31.50 ± 1.58 b $^{\mathrm{z}}$	$24.73\pm1.84~\mathrm{b}$	$76.75 \pm 9.50 \text{ c}$	5.60 ± 0.62 a	1.29 ± 0.06 a
	500	$32.18\pm1.87~\mathrm{b}$	$27.43\pm5.54~\mathrm{b}$	$119.35 \pm 47.90 \mathrm{b}$	$4.50\pm0.55\mathrm{b}$	1.43 ± 0.07 a
	1000	36.80 ± 1.76 a	34.33 ± 3.16 a	253.43 ± 65.51 a	$3.25 \pm 0.19 \text{ c}$	$0.91\pm0.03~{ m b}$
	2000	35.65 ± 1.91 a	33.75 ± 3.50 a	249.05 ± 53.51 a	$3.35 \pm 0.51 \text{ c}$	$0.84\pm0.01~{ m b}$
Cheongchima	250	$25.70 \pm 2.18 \text{ c}$	$31.93 \pm 1.58 \text{ c}$	$78.05 \pm 11.59 \text{ d}$	6.20 ± 0.51 a	1.31 ± 0.06 a
Ū	500	$29.93 \pm 2.57 \mathrm{b}$	$35.28 \pm 2.60 \mathrm{bc}$	$127.25 \pm 26.01 \text{ c}$	$4.75\pm0.62\mathrm{b}$	1.23 ± 0.06 a
	1000	33.65 ± 0.97 a	40.58 ± 2.68 a	284.28 ± 12.62 a	$3.72 \pm 0.36 \text{ c}$	$0.60\pm0.06~\mathrm{b}$
	2000	33.90 ± 1.50 a	$38.68 \pm 3.91 \text{ ab}$	$228.05 \pm 43.76 \mathrm{b}$	$3.65\pm0.48~{ m c}$	$0.61\pm0.03~{ m b}$
Significance	Cultivar (A)	*	*	NS	NS	*
0	Irrigation amount (B)	**	**	**	**	**
	Interaction $(A \times B)$	NS	NS	NS	NS	**

^x is the average of four plants. ^z Means with the same letter(s) within a column in each cultivar are not significantly different at $p \le 0.05$ according to Duncan's multiple range test. NS, *, ** indicate non-significant, and significant at $p \le 0.05$, 0.01, respectively.

Table 2. Photographs of the crops cultivated in the Geockchima and Cheongchima treatment zones.

	Amount of Nutrient Solution Applied (mL d^{-1} ·plant ⁻¹)							
Cultivar	250	500	1000	2000				
Geockchima	1							
Cheongchima								

The highest plant height, number of leaves, and fresh weight were obtained by irrigating lettuces with 1000 mL·d⁻¹·plant⁻¹ nutrient solution, regardless of the cultivar.

These growth performances declined slightly when plants were irrigated with 2000 mL·d⁻¹·plant⁻¹, but remained higher than for plants irrigated with 250 or 500 mL·d⁻¹·plant⁻¹. Compared with plants irrigated with the lowest amount, fresh weight increased by 55%, 230%, and 224% when irrigation amounts were 500, 1000, and 2000 mL·d⁻¹·plant⁻¹ in Geockchima, and 63%, 264%, and 192% in Cheongchima, respectively. These results corroborated those of Kirnak et al. [25], who discovered proportionally higher plant height and yield of lettuce with increased irrigation levels. Limited irrigation was associated with reduced growth and yield in lettuce [34,38], which supports the current study. Higher leaf lettuce growth was possibly achieved via increased tissue water content

coupled with cell size enlargement [39]. On comparing leaf tissue anatomy, distinct cell size differences were observed among samples grown under different irrigation regimes (Figure 1). Cellular tissues in Figure 1C,D show wide gaps owing to high fertilizer input, whereas those in Figure 1A,B are dense. Similarly, vacuoles in Figure 1C,D are larger than those in Figure 1A,B, signifying that high fertilizer input induces plant growth.



Figure 1. Leaf cross section micrographs of Geockchima (**left**) and Cheongchima (**right**) lettuce cultivars grown in the perlite culture as affected by different amount of nutrient solution. (**A**) 250 mL·d⁻¹·plant⁻¹, (**B**) 500 mL·d⁻¹·plant⁻¹, (**C**) 1000 mL·d⁻¹·plant⁻¹, and (**D**) 2000 mL·d⁻¹·plant⁻¹.

Lettuce requires sufficient water for maximum growth and yield. However, overwatering stress interrupts photosynthesis, affecting plant size and quantity [40]. In comparison, plants cultivated with the highest nutrient solution had reduced growth. Since only higher irrigation amounts did not increase growth, it is crucial to irrigate plants with the proper volume. Several studies have demonstrated how water conditions influence crop cultivation and quality [3,16,17,41,42].

3.2. Dry Matter, Crude Fiber, Texture, Osmolality, and Ascorbic Acid Content as Affected by Cultivar and Amount of Applied Nutrient Solution

The amount of irrigation significantly affected dry matter and crude fiber content in both cultivars (Table 1). With an increase in irrigation, dry matter content declined; therefore, applying 1000/2000 mL·d⁻, ·plant^{-p} nutrient solution produced lower dry matter content. Eichholz et al. [16] observed significantly higher dry matter content in lettuces grown in deficient water than those produced in well-watered or water-logged conditions, supporting the results of this study. Crude fiber content was affected by cultivar ($p \le 0.05$), irrigation amount ($p \le 0.01$), and their interaction ($p \le 0.01$). The crude fiber amount in both cultivars was inversely proportional to the applied nutrient solution amount. The highest crude fiber (1.31%) was observed when the least amount of nutrient solution was applied in Cheongchima. Fiber is involved in supporting growth, textural stability, and aerial plant structures [16].

Leaf texture and osmolality values showed no significant difference between cultivars, but were significantly reduced due to increased irrigation volumes ($p \le 0.05$) (Table 3). The

texture was measured by determining the puncture force, with its declining values (lower force) indicating higher crispiness of leaves, while stronger forces were required to puncture the flexible or leathery leaves. It is evident that the water content of leaf tissues plays a critical role in maintaining cellular turgor, which is a crucial component of the rigidity and firmness in plant tissue. When lettuce plants were irrigated with a higher amount of nutrient solution, the water content of tissues increased proportionally, decreasing dry matter and osmolality values (Tables 1–3).

Hence, the tissue structures, including cell size and intercellular space or vacuole volume, became larger (Figure 1). Luna et al. [34] also reported a similar observation in romaine lettuce. Ilker and Szczesniak [43] reported that textural characteristics exhibited by plant parenchymal tissues depend on cell shape and size, cytoplasm-to-vacuole ratio, intercellular space volume, cell wall thickness, osmotic pressure, and the solute types present in the cell. For lettuce, crispiness is a desirable characteristic associated with the consumer's perception and freshness of the leaves, which could be triggered by applying a higher amount of nutrient solution or irrigation water. However, maintaining the postharvest qualities of those samples would be a great challenge, as faster and more rapid water losses occurred in our study, along with other forms of quality deterioration (Figure 2).



Figure 2. Effect of the amount of nutrient solution on relative fresh weight of leaf lettuce cultivars Geockchima and Cheongchima during storage at 7 °C. Mean values at each evaluation day with different lowercase letters are significantly different according to Duncan's multiple range test ($p \le 0.05$), ns = non-significant.

The cell sap of the least-watered plants contained nearly 42% and 39% more solute than that of the highest-watered plants in Geockchima and Cheongchima, respectively. Similar results were reported in salt-stressed lettuce, which showed better organoleptic properties, postharvest processability, and shelf life [32].

Cultivar	Amount of Applied	Texture/Puncture Force (N⋅m ⁻²)	Osmolality (mmol·kg ⁻¹)	Ascorbic Acid	C	Color Value of Le	af	Total Chlorophyll Content	Total Anthocyanin Content (mg·100 g^{-1} FW)
	(mL·d ⁻¹ ·plant ⁻¹)			(mg·100 g ⁻¹ FW)	L^*	a*	b^*	$(mg \cdot g^{-1} FW)$	
Geokchima	250	1.8 a	492.0 a	10.28 c	39.4	-0.8 a ^z	10.5 b	1.71 a	4.07 a
	500	1.7 a	442.4 b	20.01 b	41.7	-3.6 b	11.7 b	1.58 a	3.10 b
	1000	1.2 b	415.6 c	26.03 a	42.9	−7.3 c	20.1 a	0.87 b	2.10 c
	2000	1.0 b	346.7 d	24.43 a	46.2	-10.0 d	21.2 a	0.98 b	1.49 c
Cheongchima	250	1.8 a	447.3 a	16.81 b	45.4	−12.7 a	16.4 b	2.03 a	-
0	500	1.5 ab	439.0 a	12.52 c	47.4	-13.9 ab	19.0 a	1.89 a	-
	1000	1.2 bc	372.3 b	21.14 a	46.5	$-14.8 \mathrm{b}$	20.2 a	1.32 b	-
	2000	0.9 c	321.8 c	21.52 a	47.4	-14.1 ab	19.8 a	1.42 b	-
Significance	Cultivar (A)	NS	NS	NS	NS	**	*	*	-
0	Irrigation amount (B)	**	**	**	NS	**	*	**	**
	Interaction $(A \times B)$	NS	*	*	NS	**	NS	NS	-

Table 3. Physicochemical and nutritional quality parameters of harvested leaf lettuce as influenced by cultivar and amount of applied nutrient solution in perlite culture.

^z Means with the same letter(s) within a column in each cultivar are not significantly different at $p \le 0.05$ according to Duncan's multiple range test. NS, *, ** indicate non-significant, and significant at $p \le 0.05, 0.01$, respectively.

The ascorbic acid content of lettuce ranged from 10.3 to 26.0 and 12.5 to 21.5 mg 100 g⁻¹ in Geockchima and Cheongchima, respectively (Table 3). A higher ascorbic acid content was measured during the highest growth stage of lettuce under optimum irrigation conditions. This may indicate appropriate ascorbic acid generation during normal crop growth. In Geockchima, about two times more ascorbic acid was determined when lettuces were grown with double the amount of nutrient solution than the initial amount (Table 3). This result corroborates the findings of Zeipina et al. [42], who discovered nearly half the amount of ascorbic acid in leaf lettuce grown in reduced moisture than for those cultivated in optimal moisture conditions. However, ascorbic acid content did not increase in such a trend for other irrigation amounts, especially in Cheongchima. The increase in ascorbic acid relative to the higher strength of nutrient solution was also observed in hydroponically grown leaf lettuce [44]. Additional to nutrient amount or fertilization, several factors, including plant genotype, growing season, and light intensity, can influence the ascorbic acid content in lettuce and other horticultural crops [45,46]. This could be a potential reason for the variability observed in ascorbic acid content in the cultivar Cheongchima.

3.3. Color Values, Chlorophyll, and Anthocyanin Contents as Affected by Cultivar and Amount of Applied Nutrient Solution

Color attributes (except L^* value) and total chlorophyll content of lettuce leaf were significantly affected by cultivar and irrigation amount (Table 3). When Geockchima was irrigated with the least amount, the a^* value (-0.8) was the highest value for red. However, a^* value (-14.8) of Cheongchima was the highest value for green when the irrigation amount was 1000 mL·d⁻¹·plant⁻¹. The a^* value was significantly different ($p \le 0.05$) among the treatments in Geockchima, but not in Cheongchima. However, the *b** value increased until 1000 and 500 mL·d⁻¹·plant⁻¹ applied nutrient solution in Geockchima and Cheongchima, respectively. According to the amount of irrigation, the cultivar Geockchima responded more for color development than Cheongchima. Comparing the pigment contents and color values in Geockchima, the correlation coefficient (r) between chlorophyll and a^* value was 0.91, and between anthocyanin and a^* value was 0.99 (data not shown). The changing pattern of a^* and b^* observed in Geockchima were inconsistent with a previous result for leaf lettuce grown in well-watered, moderate water deficit, and severe water deficit conditions [3]. In contrast, Fallovo et al. [19] discovered no significant differences in L^* , a^* , and b^* values when leafy lettuces were grown in a floating system with different nutrient solutions, which supports our L^* values and partially supports the color data of the cultivar Cheongchima. The variation in color parameters between the cultivars could be due to the genetic constituents of the plant, as Geockchima is red-leafed and Cheongchima is green-leafed. Consequently, a comparatively lower amount of total chlorophyll contents was discovered in the former than in the latter, possibly because of the presence of anthocyanin. Varietal differences in chlorophyll contents of lettuce leaves were observed in several previous studies [16,17,26]. The lowest amount of total chlorophyll was measured in Geockchima lettuces, which indicated the highest growth Tables 1–3. Considering the total chlorophyll contents of these lettuces (irrigated with 1000 mL·d⁻¹·plant⁻¹), about 81% and 96% higher total chlorophyll contents were measured in plants irrigated with 500 and 250 mL d⁻¹ plant⁻¹ nutrient solutions, respectively, in Geockchima, and 43% and 53%, respectively in Cheongchima. Our results are consistent with those of Eichholz et al. [16], who observed an increase in chlorophyll content in some varieties of lettuce grown in water deficit conditions compared to those grown in well-watered or water-logged conditions.

Similar to chlorophyll content, anthocyanin content declined ($p \le 0.05$) gradually, corresponding to the increased levels of applied nutrient solution (Table 3). However, anthocyanin was not detected in Cheongchima, as this cultivar is characterized by its dark green color. The highest amount of anthocyanin (4.07 mg·100 g⁻¹) was produced when lettuces were irrigated with the least amount of nutrient solution, and the declines in anthocyanin contents were about 23%, 48%, and 63%, with each respective increase in level of supplied nutrient solution. Similarly, Baslam and Goicoechea [17] observed more

anthocyanin in two lettuce cultivars when the plants were grown in water deficit or cyclic drought conditions than the control plants. The accumulated anthocyanin in water-stressed plants may prevent desiccation through osmotic effects, though the mechanism remains unclear [47]. Anthocyanins are the most important group of water-soluble pigments in plants, and may be induced by several environmental factors, including water stress [48], as a mechanism for adapting to the surrounding environment.

3.4. Mineral Contents as Affected by Cultivars and Amount of Applied Nutrient Solution

The mineral contents (except P and Mg) and C/N ratio of the lettuces were significantly affected by irrigation amount, whereas only N and Ca contents were influenced by cultivar (Table 4). The N content in leaves likely increases with irrigation amount because of the higher uptake of N from the supplied nutrient solution. Cheongchima had a comparatively higher N content than Geockchima. Consequently, the C/N ratio declined gradually in Cheongchima, but no obvious trend was found in Geockchima (Table 4).

Table 4. The mineral content of leaves of two lettuce cultivars grown in perlite culture as influenced by the amount of applied nutrient solution.

Cultivar	Amount of Applied Nutrient Solution	Mineral Content (%, DW)					
	$(\mathbf{m}\hat{\mathbf{L}}\cdot\mathbf{d}^{-1}\cdot\mathbf{p}\mathbf{lant}^{-1})$	Ν	Р	К	Ca	Mg	C/N Ratio
Geockchima	250	4.68 ab ^z	0.22	3.31 a	1.16 a	0.24	7.94 a
	500	4.40 b	0.20	3.24 a	1.04 ab	0.20	8.13 a
	1000	4.91 a	0.25	3.51 a	1.01 ab	0.22	6.93 b
	2000	4.87 a	0.19	2.60 b	0.91 b	0.14	7.22 b
Cheongchima	250	4.55 b	0.27	1.90 c	0.83 a	0.18	9.09 a
0	500	4.88 b	0.24	2.22 c	0.74 ab	0.17	8.53 a
	1000	5.40 a	0.31	3.98 b	0.62 b	0.17	6.63 b
	2000	5.48 a	0.27	5.80 a	0.61 b	0.16	6.49 b
Significance	Cultivar (A)	**	NS	NS	**	NS	NS
0	Irrigation amount (B)	**	NS	**	*	NS	**
	Interaction $(A \times B)$	**	NS	*	NS	NS	**

^z Means with the same letter(s) within a column in each cultivar are not significantly different at $p \le 0.05$ according to Duncan's multiple range test. NS, *, ** indicate non-significant, and significant at $p \le 0.05$, 0.01, respectively.

However, both cultivars showed two statistically distinct groups of C/N ratios, irrigated with a lower and higher amount of nutrient solution. These results corroborate the results of Stefanelli et al. [18], who found higher yield and N content of red oak lettuce, along with lower C:N, when nitrogen application was increased, although the trend was limited, only occurring up to certain levels of N application. Significant increases in N, P, K, and Mg contents were also reported when the concentration of nutrient solution increased in a floating culture of lettuce [20], which partly supports the current investigation. This study was based on the Carbon/Nutrient Balance (CNB) Theory's [49] N content and C:N data, and the CNB theory was validated for leaf lettuce.

P content was unaffected by all factors, while K content increased progressively as irrigation amounts increased; therefore, the highest K content (5.80%) was discovered in plants grown with the highest level of nutrient solution, and in the Cheongchima cultivar (Table 3). A similar observation was also reported in lettuce grown in floating raft culture [20]. However, the data were erratic in Geockchima, although the highest K content (3.51%) was determined in the highest growth-yielding plants, demonstrating similarity with the results of Fallovo et al. [20]. Unlike K content, the highest level of Ca was found in plants grown with the least amount of nutrient solution. Geockchima yielded more Ca ($p \le 0.01$) than Cheongchima in all treatment conditions. Mg content did not significantly differ in any measured factor, but an insignificant decline was noticed with the increased volume of nutrient solution. The decline in Ca or Mg with increasing N, Ca, and Mg application might possibly occur because of 2⁺ cation absorption–competition interactions between Ca and Mg [18].

3.5. Postharvest Qualities as Affected by Cultivars and Amount of Applied Nutrient Solution

During the storage of harvested lettuce, relative fresh weight loss declined gradually in all irrigation regimes, showing significant differences ($p \le 0.05$) among the irrigation treatments (Figure 2). The greater fresh weight losses were found in Cheongchima than in Geockchima, and samples irrigated with higher amounts than those with lower amounts. As a result, some samples, especially those irrigated with higher nutrient solution, became unusable after a few days of storage. On day 6, for instance, about 4% and 5% water losses occurred in samples grown in the least irrigation amount, while these losses were 12% and 16% in samples irrigated with the highest nutrient solution in Geockchima and Cheongchima, respectively (Figure 2). In a similar study, Lee and Chang [22] discovered greater fresh weight loss in leaf lettuce grown in higher nutrient concentrations compared to controls during storage at 5 °C. Water loss not only induces important quality, marketability, and economic losses of fresh produce, it also negatively influences the visual, compositional, and eating quality of the plant, even when weight losses are subtle [50]. The authors of the present study also conclude that the weight loss of lettuce differs between cultivars and postharvest conditions, such as temperature, humidity, gas composition, and/or type of polymeric film used for packaging the sample during storage. The total color difference (ΔE) of Cheongchima was significantly ($p \le 0.05$) influenced by irrigation amounts throughout storage, whereas minimal differences were observed in Geockchima (Figure 3). The experimental crops cultivated in the Geockchima and Cheongchima treatment zones are shown in Table 2. Relative fresh weight, total color difference, and overall visual quality score measurements were discontinued when they became worthless as products.



Figure 3. Changes in total color difference in two leaf lettuce cultivars (Geockchima and Cheongchima) grown in perlite culture with different amounts of the nutrient solution during storage at 7 °C. Mean values at each evaluation day with different lowercase letters are significantly different according to Duncan's multiple range test ($p \le 0.05$), ns = non-significant.

The variable pattern of color changes found between the two varieties could be attributed to the differences in leaf color, whereby the green-leafed Cheongchima might have faster color changes compared to the red-leafed Geockchima. It seems that the green-leafed cultivar is more vulnerable to color changes possibly because the pigment (chlorophyll) responsible for the leaf color usually declines with the progress of storage (Aguero et al., 2008). Leaf color change in Geockchima was minor, probably due to the presence of anthocyanin, which is comparatively more stable than chlorophyll during storage [35]. Leaves harvested from 1000 and 2000 mL·d⁻¹·plant⁻¹ treatment showed faster color changes compared to those of the other two treatments, especially in the cultivar Cheongchima. Overall, the effect of different nutrient solutions on color changes of Geockchima is small compared with Cheongchima. Since the color of the leaf is a crucial quality character of leafy vegetables such as lettuce at postharvest stages, the changing pattern of total color difference in accordance with irrigation treatment found in this study may suggest the necessity of further detailed studies on the color quality of lettuce at postharvest stages.

OVQ, measured by an expert panel, showed no variation among the irrigation treatments until the second day of storage, but significant differences ($p \le 0.05$) were noticed after that (Figure 4). Overall, samples grown in higher nutrient amounts showed a corresponding rapid decline in OVQ during storage, to greater degrees in Cheongchima. Consequently, samples of 2000 mL \cdot d⁻¹ \cdot plant⁻¹ treatment received lower OVQ scores, which were insufficient for maintaining marketable life after four and six days of storage, in Cheongchima and Geockchima, respectively. Our results are in accordance with the results of Hoque et al. [51], who found an increase in lettuce yield with an increased rate of nitrogen application, whereas postharvest quality declined. During the storage of fresh-cut iceberg lettuce, the highest quality was discovered in samples harvested from deficit irrigation [14]. A possible reason for the lower OVQ scores of samples irrigated with the higher nutrient solution could be the higher fresh weight loss that occurred in those samples (Figures 2 and 4). The difference in color change per the storage period of the two varieties was lower in Geockchima than in Cheongchima. In Geockchima, no difference was noted based on the amount of fertilizer input; however, a large difference was confirmed in Cheongchima. This implies that for storage-distribution in Cheongchima, fertilizer input quantity must be considered.

Nunes and Emond [50] discovered a significant correlation between weight loss and visual quality in several fresh fruits and vegetables, which supports our observation. As well as water loss, total color difference data showed that samples with higher ΔE values received lower OVQ scores, suggesting the implication of color change in OVQ scoring. Lettuce leaves harvested from 250 mL·d⁻¹·plant⁻¹ treatment, on the other hand, showed marketable life until 8 and 10 days after harvest in Geockchima and Cheongchima, respectively (Figure 4). The overall visual quality evaluation began with 9 points based on Table 2, and was performed by three researchers in the storage room. This result may not ensure the higher storage potentiality of the Cheongchima cultivar because the obtained OVQ value just reached the brink of minimum marketability value (a score of 5) on 10 days of storage, when a higher water loss was recorded compared to that of Geockchima (Figures 2 and 4). Overall, lettuce grown with lower irrigation volumes (250 and 500 mL·d⁻¹·plant⁻¹) in Cheongchima showed slightly higher OVQ scores compared to those cultivated with higher volumes. However, the differences in OVQ affected by irrigation volume were unclear in Geockchima, which might need further detailed investigation.



Figure 4. The overall visual quality scores of leaf lettuce cultivars, Geockchima and Cheongchima, grown in perlite culture with different amounts of the nutrient solution during storage at 7 °C (the dotted line corresponds to the limit of acceptability). Mean values in each evaluation day with different lowercase letters are significantly different according to Duncan's multiple range test ($p \le 0.05$), ns = non-significant.

4. Conclusions

This study clearly demonstrated the amount of applied nutrient solution that influences the growth, physicochemical, nutritional, and postharvest qualities of leaf lettuce in perlite culture. Lettuces grown in nutrient-deficient solution showed significantly lower yield and contributing characters, as well as lower ascorbic acid concentration, but a higher amount of dry matter, crude fiber, total chlorophyll, and anthocyanin contents than those grown in sufficiently or excessively supplied nutrient solution. Lettuce grown with 1000 mL·d⁻¹·plant⁻¹ nutrient solution had the highest growth and nutritional components. However, postharvest performances, such as relative fresh weight, color changes, and OVQ, were better maintained in lettuces grown with limited nutrient solutions. Based on these results, cultivation conditions advantageous for growth and yield do not always enhance storability after harvest. Therefore, the supply of plant nutrients with irrigation water during the growth of lettuce should be adjusted based on their uses, so that a higher yield could be targeted for domestic or immediate uses only, while a limited supply of nutrient solution could ensure a longer postharvest life when shipment or storage of lettuce for certain period is required. Considering these views and based on these findings, it can be concluded that leaf lettuce could be irrigated with 1000 or 250 mL·d⁻¹·plant⁻¹ nutrient solution to increase yield or maintain postharvest qualities. As a result, further studies on the proper nutrient solution control system during cultivation in relation to postharvest quality are required. Additionally, these results can be applied to lettuce distribution, storage duration requirement, transportation conditions, and can be used for lettuce consumption and marketing.

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