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Nutrient Solution Temperature Affects Growth and °Brix Parameters of Seventeen Lettuce Cultivars Grown in an NFT Hydroponic System

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Abstract: Nutrient solution temperature in a hydroponic system affects °Brix and yield of lettuce; thus, it is important to maintain the water temperature within an appropriate range. Nutrient-film technique (NFT) trials were conducted to investigate the effects of different water temperatures (18.3 °C, 21.1 °C, and ambient) on growth and °Brix of 17 cultivars from five different types (Loose leaf, Romaine, Butterhead, Salanova, and Batavian) of lettuce. The average daily water temperature for ambient treatment was recorded to be 20 to 26.5 °C. The study was conducted in a split-plot design with three replications over time. Results indicated that water temperature affected root and shoot fresh and dry weight, plant width, and °Brix for lettuce. Lettuce grown at 21.1 °C were 15% greater for shoot fresh weight than plants grown at ambient conditions. All the growth and quality parameters of lettuce were found to be affected by cultivars, with “Coastal Star” showing the best results in both growth and °Brix parameters. All the cultivars of the Romaine type showed greater growth and °Brix, while Salanova lettuce did not perform well in all treatments compared to other lettuce types. For CO₂ assimilation, the interaction between water temperature and cultivars was significant, with “Parris Island” having the greatest rate at ambient water temperature. These results suggested that maintaining water temperature at 21.1 °C produced lettuce with greater growth and biomass but had 26% lower °Brix than lettuce grown at 18.3 °C.

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1. Introduction

Hydroponics is a technique of growing plants by placing the roots in a nutrient solution with or without mechanical root support [1]. It is also referred to as “controlled environment agriculture”, or CEA, since raising plants hydroponically requires control of various environmental factors such as water temperature, light intensity and duration, humidity, and pH of the solution and mineral nutrients [2]. Hydroponics offers advantages compared to soil-based systems due to the more efficient use of fertilizers and water, greater control of the growing climate, and pest control [3]. An important characteristic of hydroponic cultivation is the need and ability to control the temperature of the nutrient solution or of the root system using heaters or a cooling spiral. It has been reported that relatively small changes in root-zone temperature can have a significant impact on root development, depending on the phenological stage of crops and duration of temperature [4].

Water temperature can affect many physiological processes during plant growth and development. Studies have shown that when the temperature is above or below an optimum level, it may influence plant metabolic activities, including the accumulation of different metabolites such as phenolic compounds, nutrient uptake, chlorophyll pigment formation, photosynthesis, and finally, the growth and development of the plant [5]. In most

plant species, nutrient uptake by roots is decreased at low temperatures [6]. In addition, the temperature of the nutrient solution also affects the amount of oxygen dissolved in the solution. Oxygen solubility has been found to be reduced at high solution temperatures [7]. At prolonged high temperatures, oxygen deficiency in the root zone can lead to poor root and plant performance and an increase in the incidence of diseases and pests [8].

Lettuce (*Lactuca sativa* L.) is a herbaceous annual belonging to the family Compositae. It is an important cool season leaf/salad vegetable having a high content of vitamin A and C and is consumed widely [9]. Lettuce often suffers from growth and physiological disorders when the nutrient solution temperature is above or below optimum [10]. Maintaining optimum root-zone temperature is crucial as it is an important factor affecting plant growth and uptake of water and nutrient [11]. The effect of cooling of the nutrient solution was studied on butterhead lettuce, and significant increases in canopy diameter, leaf number, and yield with root-zone cooling at 19 °C were reported when compared to the non-chilled condition [12]. On the other hand, heating the nutrient solution to 15 °C and 20 °C increased shoot growth for “Marbello” and “Bastion” lettuces, respectively [13]. In another experiment, heating the soil to 18 °C, thereby heating the root zone, decreased the length of the production cycle of butterhead lettuce by 14 to 17 days in the field [14]. However, the response in lettuce growth to temperature is cultivar-dependent [15].

The importance of optimal air temperature has been widely recognized, and it is equally important to optimize the water or root temperature in hydroponic crops as it is an important parameter to maximize crop growth and minimize possible damages [16]. While the effects of the root zone have not been widely studied in hydroponic lettuce, there is evidence that by optimizing water temperature, market quality lettuce can be grown all year round, even in warmer climates. It is hypothesized that lower temperatures will improve lettuce growth and °Brix. The objectives of our research were to identify the optimum water temperature needed for the greatest °Brix and yield of five different types of lettuce grown in NFT hydroponic systems.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Seeds of seventeen cultivars of lettuce, including five different types, were obtained from Johnny’s Selected Seeds (Winslow, ME, USA) on 1 March 2020. Cultivars included Black Seeded Simpson, Waldman’s Dark Green, and Panisse (Loose-leaf type), Parris Island, Jericho, and Coastal Star (Romaine type), Nancy, Optima, and Buttercrunch (Butterhead type), Butter, Sweet Crisp Red, Sweet Crisp Green, Oakleaf Red, and Oakleaf Green (Salanova type), Nevada, Cherokee, and Sierra (Batavian type). Seeds were sown in oasis cubes (Harris Seeds, Rochester, NY) of size 1.5 cm³ on 6 March 2020 and kept under the mist bench at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. Nutrients were applied once when the seedlings were 2 weeks old. The nutrient solution was prepared by mixing 4.9 g Jack’s 5-12-26 and 3.2 g calcium nitrate diluted in 10 L of water and applied over the seedlings with a watering can once 2 weeks after sowing.

Seedlings were transplanted from the mist bench to the Hydrocycle Pro NFT table (Growers Supply, Dyersville, IA, USA) on 29 March 2020. Each NFT table had 10 channels measuring 10 cm wide × 5 cm deep × 900 cm long, and each channel lid had 18 of the 2.5 cm site holes, spaced 20 cm on center. One lettuce plant per slot was planted, and 10 plants per cultivar per table were transplanted in a randomized arrangement. The tables had a slope of 2.8% approximately between the irrigation and drainage end, and the water flowing along the slope was collected in the tank and recirculated by the pump to the irrigation pipe. Nutrient solution was prepared using Jack’s 5-12-26 (J.R. Peters, Allentown, PA, USA) and calcium nitrate (American Plant Products, Oklahoma City, OK, USA) with 150 mg/L⁻¹ nitrogen, 48 mg/L⁻¹ phosphorus, 216 mg/L⁻¹ potassium, 139 mg/L⁻¹ calcium, 31 mg/L⁻¹ magnesium, 3 mg/L⁻¹ iron, 0.05 mg/L⁻¹ manganese, 0.15 mg/L⁻¹ zinc, 0.50 mg/L⁻¹ boron, 0.15 mg/L⁻¹ copper, and 0.10 mg/L⁻¹ molybdenum. Tanks

were filled to 40-gallon capacity, and 147.41 g of Jack's along with 97.52 g of calcium nitrate was added initially according to the recommended rates. The pH of the nutrient solution was maintained at 5.5–6.5, and the EC level was maintained between 1.5 and 2.5 mS/cm^{−1} throughout the growing period. pH down solution (General Hydroponics, Santa Rosa, CA, USA) was used to lower the pH because alkaline tap water and nutrient solution were added whenever the EC was low. No supplemental lighting was used in the greenhouse, and DLI levels ranged from 10 to 15 mol/m^{−2}/d^{−1}. The greenhouse temperature was set at 21/18 °C (day/night) regime, but daytime temperatures were often greater in the latter two runs. Relative humidity averaged 44%.

2.2. Water Temperature

Water temperature in the NFT nutrient tanks was set at 18.3 and 21.1 °C, and a control at ambient temperature. Water chillers (Active Aqua, Hydrofarm, Petaluma, CA, USA) were used to control water temperature. Daily water temperature in the ambient treatment ranged from 18.8 to 25.5, 23.3 to 30.4, and 16.7 to 24.7 °C during the first, second, and third replications, respectively. The average temperature for the ambient treatment was 23.6, 26.5, and 20 °C during the first, second, and third replications, respectively. Daily water temperature in the 18.3 °C chilled treatment ranged from 17.9 to 18.8, 17.7 to 19.0, and 17.6 to 18.5 °C during the first, second, and third replications, respectively. The average temperature for the 18.3 °C chilled treatment was 18.3, 18.4, and 18.3 °C during the first, second, and third replications, respectively. Daily water temperature in the 21.1 °C chilled treatment ranged from 20.5 to 21.3, 20.5 to 21.3, and 20.5 to 22 °C during the first, second, and third replications, respectively. The average temperature for the 21.1 °C chilled treatment was 21.1, 21.1, and 20.9 °C during the first, second, and third replications, respectively.

2.3. Data Collection

Data on photosynthesis rate and °Brix value were collected after 4 weeks of plants being on the NFT tables. For the °Brix value, a mature leaf from the middle of a stem was selected from each plant. The juice from the leaf was squeezed using a sap squeezer and measured by a refractometer (Cole-Parmer, Vernon Hills, IL, USA) in sunlight. One °Brix is equivalent to 1 g sugar in 100 mL of water. Photosynthesis rate was measured using a Li-cor 6400 (LI-COR, NE). The Li-cor with 6400-02B LED light source chamber was used by keeping the reference CO₂ at 400 ppm. The block temperature was set at 28 °C, and the light level was set at 1000 µmol/m^{−2}/s^{−1}. One of the top young leaves was selected per plant and was used as a non-destructive sample for the photosynthesis rate. Data were collected between 10 a.m. and 3 p.m.

SPAD, height (cm), and width (cm) of each plant were measured at the time of harvesting, which was 35 d after transplanting. Each plant was scanned using a chlorophyll meter (SPAD-502, Konica Minolta, Japan) for the measurement of leaf chlorophyll concentration. From each plant, three mature leaves at the top, middle, and bottom were selected in a non-destructive manner. Plants were harvested after 35 d of being on the table, and data were collected on the shoot and root fresh weight, then oven-dried for 2 d at 53.9 °C for dry root and shoot weight. A data logger (HOBO, Onset, Bourne, MA) was used to record the water temperature at 5 min intervals to calculate the average daily water temperature of the solution in all three tanks throughout the growing period.

2.4. Experimental Design and Statistical Analysis

The study was conducted in a split-plot design with three replications. Factors were water temperature (three levels) and cultivars of lettuce (seventeen levels). The experiment was replicated by starting the second set of seeds on 7 June 2020 and the third on 17 September 2020, and similar growth conditions as described above were followed. Statistical analysis was performed using SAS/STAT software (Version 9.4; SAS Institute, Cary, NC, USA). Tests of significance were reported at the 0.05, 0.001, and 0.0001 levels.

The data were analyzed using generalized linear mixed models methods. Tukey multiple comparison methods were used to separate the means.

3. Results and Discussion

3.1. Temperature and Cultivar Effect on Plant Growth Parameters

There was a significant cultivar $\times$ temperature interaction for carbon dioxide assimilation (Table 1). The greatest net CO₂ assimilation was recorded in Romaine type “Parris Island” within the ambient water temperature (Table 2). Temperature is said to be one of the major environmental factors affecting the physiological processes in plants, especially photosynthesis and plant growth [17]. Similar to our findings, root zone cooling has been found to affect the net photosynthetic rate (PN) and CO₂ assimilation of hydroponic lettuce and several other species [18–20]. Increased PN, stomatal conductance (GS), intercellular CO₂ concentration (Ci), and transpiration rate (Tr) in “Romaine” lettuce by root zone cooling at 25 °C were reported compared to a 30 °C control treatment, although the chlorophyll content in lettuce leaves was not affected by root zone cooling, suggesting the increase in photosynthesis was caused by improved stomatal conductance, which enabled sufficient CO₂ for photosynthesis [20]. Another study suggested that changes in photosynthesis induced by root temperatures were mainly attributed to corresponding changes of Gs since there was a similarity in the trends for PN and Gs; however, the responsiveness to root-zone temperature depends on the plant species and cultivars [21]. It was possible to prevent photoinhibitory damage in temperate lettuce plants grown in the tropics under hot ambient temperature by exposing the roots to a lower temperature of 20 °C [22]. It was concluded that the reduction in photosynthesis in lettuce plants with their roots exposed to hot ambient temperature was mainly due to the reduction in water uptake.

Table 1. Tests of effects for water temperature (18.3 °C, 21.1 °C, and ambient) and seventeen lettuce cultivars grown in NFT hydroponics systems at OSU research greenhouses in Stillwater, OK, USA.

	Cultivar	Temperature	Cultivar $\times$ Temperature
CO ₂ assimilation	*** Z	NS	*
SPAD	***	NS	NS
Height	***	NS	NS
Width	***	**	NS
°Brix	*	***	NS
Root fresh wt.	***	***	NS
Shoot fresh wt.	***	**	NS
Root dry wt.	***	***	NS
Shoot dry wt.	***	**	NS

^Z Indicates significant at or non-significant (NS) at * $p \leq 0.05$, ** $p \leq 0.001$, or *** $p \leq 0.0001$.

Table 2. Least square means for lettuce cultivar and water temperature (18.3 °C, 21.1 °C, and ambient) for net CO₂ assimilation in NFT hydroponic systems at OSU research greenhouses in Stillwater, OK, USA.

Types	Cultivars	Water Temperature (°C)	CO ₂ Assimilation ($\mu\text{mol}/\text{m}^{-2}/\text{s}^{-1}$)
Loose leaf	B.S. Simpson	18.3	18.9 abcdefgh ^z
		21.1	19.8 abcdefg
		Ambient ^y	19.7 abcdefgh
	Waldman’s Dark Green	18.3	18.7 abcdefgh
		21.1	18.8 abcdefgh
		Ambient	18.1 bcdefgh
	Pannise	18.3	16.5 gh
		21.1	16.2 h
		Ambient	15.3 h

Table 2. Cont.

Types	Cultivars	Water Temperature (°C)	CO ₂ Assimilation (μmol/m ⁻² /s ⁻¹)
Butterhead	Buttercrunch	18.3	20.3 abcde
		21.1	19.0 abcdefgh
		Ambient	18.1 abcdefgh
	Optima	18.3	18.5 abcdefgh
		21.1	18.8 abcdefgh
		Ambient	20.6 abc
	Nancy	18.3	18.8 abcdefgh
		21.1	19.3 abcdefgh
		Ambient	18.9 abcdefgh
Romaine	Jericho	18.3	18.2 abcdefgh
		21.1	20.6 abc
		Ambient	19.8 abcdefgh
	Coastal Star	18.3	19.3 abcdefgh
		21.1	19.1 abcdefgh
		Ambient	19.3 abcdefgh
	Parris Island	18.3	20.2 abcdef
		21.1	18.3 abcdefgh
		Ambient	21.5 a
Batavian	Cherokee	18.3	19.7 abcdefgh
		21.1	21.3 ab
		Ambient	19.8 abcdefgh
	Sierra	18.3	19.4 abcdefgh
		21.1	20.4 abcde
		Ambient	20.5 abcd
	Nevada	18.3	19.8 abcdefgh
		21.1	19.7 abcdefgh
		Ambient	18.9 abcdefgh
Salanova	Oakleaf Red	18.3	17.6 bcdefgh
		21.1	17.1 efgh
		Ambient	16.8 fgh
	Oakleaf Green	18.3	16.5 gh
		21.1	17.7 bcdefgh
		Ambient	16.2 h
	Sweet Crisp Green	18.3	19.4 abcdefgh
		21.1	18.5 abcdefgh
		Ambient	17.1 defgh
	Sweet Crisp Red	18.3	18.2 abcdefgh
		21.1	17.7 bcdefgh
		Ambient	17.6 bcdefgh
	Butter	18.3	17.4 cdefgh
		21.1	18.9 abcdefgh
		Ambient	17.2 cdefgh

^z Means (n = 30) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the mixed model ($p \leq 0.05$). ^y Ambient treatment indicates control water temperature, which ranged from 20 to 26.5 °C.

Plant width showed significant main effects for both cultivar and temperature (Table 1). There was no difference in plant width of lettuce grown at water temperatures of 18.3 °C and 21.1 °C, but both were greater than the ambient temperature treatment (Table 3). “Waldman’s Dark Green” had the greatest width, but was not different than “Nancy”, “Optima”, “Jericho”, “Parris Island”, “Coastal Star”, and “Sweet Crisp Green” (Table 4). Within plant types for plant width, “Waldman’s Dark Green” of Loose-Leaf type was greater than “B.S. Simpson” and “Panisse” and Salanova type “Sweet Crisp Green” was greater than “Butter” (Table 4). For root and shoot fresh and dry weight, there were significant cultivar and water temperature main effects (Table 1). The greatest root and shoot fresh and dry weights were observed when the water was at 21.1 °C (Figure 1). “Jericho” had the

greatest root weight but was not different from all other cultivars except “Oakleaf Green”, “Oakleaf Red”, and “Butter” (Table 4). “Optima” had the greatest root dry weight and was similar “Oakleaf Green”, “Oakleaf Red”, “Sweet Crisp Green”, and “Butter” (Table 4). For shoot fresh weight, “Coastal Star” and “Sweet Crisp Green” were greatest but not different than all other cultivars except “Panisse”, “Oakleaf Green”, “Oakleaf Red”, and “Butter”. Within Loose-leaf type, “Black Seeded Simpson” and “Waldman’s Dark Green” had greater fresh and dry shoot weight compared to “Panisse”.

Table 3. Least square means for water temperature on growth and °Brix of five types of lettuce grown in NFT hydroponic systems at the OSU research greenhouses, Stillwater, OK, USA.

Temperature (°C)	°Brix	Width (cm)	Shoot Fresh wt. (g)	Root Fresh wt. (g)	Shoot Dry wt. (g)	Root Dry wt. (g)
18.3	5.7 a ^z	22.5 a	86.8 ab	22.7 b	5.2 b	0.8 b
21.1	4.5 b	23.3 a	96.8 a	26.8 a	5.9 a	1.1 a
Ambient	4.3 c	20.7 b	84.1 b	22.5 b	5.0 b	0.9 b

^z Means (n = 30) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the mixed model ($p \leq 0.05$).

Table 4. Least square means for five types and seventeen cultivars on growth and quality of lettuce grown in NFT hydroponic systems at OSU research greenhouses in Stillwater, OK.

Types	Cultivars	°Brix	SPAD	Height (cm)	Width (cm)	Shoot Fresh wt. (g)	Root Fresh wt. (g)	Shoot Dry wt. (g)	Root Dry wt. (g)
Loose leaf	B.S. Simpson	4.5 b ^z	17.5 gh	25.2 a	20.5 bcd	109.3 a	27.1 a	6.7 abc	1.0 abc
	Waldman’s Dark Green	4.8 ab	22.3 gf	21.9 ab	27.5 a	118.3 a	27.3 a	7.5 ab	1.1 ab
	Panisse	4.9 ab	15.5 h	13.2 def	21.9 bcd	65.1 bcd	21.8 abcd	4.7 cdefg	0.9 abcd
Butter head	Buttercrunch	4.9 ab	30.5 bc	12.7 def	22.1 bcd	82.8 abc	21.9 abcd	4.9 cdefg	0.9 abcd
	Nancy	5.1 ab	22.8 efg	14.7 cdef	25.6 ab	104.8 a	24.8 ab	5.9 abcde	1.0 abcd
	Optima	5.1 ab	28.9 bcd	15.2 bcde	24.4 ab	92.2 abc	26.6 ab	6.2 abcd	1.2 a
Romaine	Jericho	4.5 b	24.3 def	20.4 abc	25.2 ab	122.9 a	28.9 a	7.5 abc	1.1 abc
	Parris Island	5.4 a	31.4 abc	17.3 bcd	22.7 abcd	91.2 abc	24.7 ab	5.6 abcdef	1.1 abc
	Coastal Star	4.7 ab	31.7 abc	21.7 ab	25.4 ab	123.0 a	27.9 a	7.4 abc	1.1 ab
Batavian	Cherokee	5.0 ab	25.7 def	14.7 cdef	21.7 bcd	97.6 ab	26.8 ab	5.0 cdef	1.0 abcd
	Nevada	4.6 ab	23.1 defg	14.9 bcdef	18.9 bcd	80.8 abcd	21.5 abcd	4.5 cdefg	0.9 abcd
	Sierra	5.3 a	24.5 def	14.5 cdef	21.5 bcd	85.2 abc	24.5 abc	4.4 defg	1.0 abcd
Salanova	Oakleaf Green	4.9 ab	27.2 cde	7.2 f	18.7 cd	51.3 d	18.9 d	3.5 g	0.8 d
	Oakleaf Red	4.7 ab	32.6 ab	9.2 ef	19.8 bcd	63.7 cd	20.9 bcd	4.0 fg	0.8 d
	Sweet Crisp Green	4.7 ab	33.5 ab	16.3 bcd	23.4 abc	123.0 a	22.9 abcd	7.7 a	0.9 bcd
	Sweet Crisp Red	4.9 ab	31.9 ab	13.5 cdef	20.6 bcd	89.7 abc	22.5 abcd	5.4 bcdef	0.9 abcd
	Butter	5.0 ab	35.2 a	9.1 ef	17.9 d	61.8 cd	19.1 cd	4.2 efg	0.8 cd

^z Means (n = 30) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the mixed model ($p \leq 0.05$).

Figure 1. “Parris Island” grown at 18.3 °C (left), 21.1 °C (middle), and ambient (right).

Water temperature is an important growth factor that may influence plant development in hydroponic systems. At optimum temperatures, water can nourish growth, while at lower or high levels, plant growth can be negatively affected [23]. Similar to our results, root-zone cooling at 20 °C increased the biomass of aeroponically grown lettuce from that in plants with ambient conditions (24 °C to 38 °C) in a tropical greenhouse [22]. Since the roots of plants are more sensitive to heat stress than the above-ground parts, the high root-zone temperature can easily damage the root, which can restrict the length and diameter of the stem [24]. High root-zone temperature (27.5 °C) resulted in water and nutrient loss in tomatoes (*Solanum lycopersicum* L.), which ultimately led to a reduction in plant growth [25]. In another study, high temperature in the root zone resulted in reduced leaf, stem, and fresh and dry weights of coriander (*Coriandrum sativum* L.), as a result of water deficits in the plants [26]. The temperature of the nutrient solution has a direct relation to the amount of oxygen consumed by plants and an inverse relation to the oxygen dissolved [27]. Nutrient solution cooling to 22 and 25 °C provided positive effects on the availability of dissolved oxygen levels in the nutrient solutions as well as on all growth parameters (plant height, leaf number, chlorophyll content, leaf area) and production parameters (number of fruits and yield) in cucumber (*Cucumis sativus* L.).

When the water temperature in the hydroponic system is too low, it can affect nutrient uptakes of plants. A study on red leaf lettuce showed that the exposure of lettuce roots to low temperature (10 °C) significantly reduced leaf area, stem diameter, and fresh weight of tops and roots compared to 20 °C [28]. A research study was conducted with the objective of evaluating the effect of cooling of nutrient solution on growth and development of lettuce “Vitória de Santo Antão” in hydroponics and greater fresh weight of leaves and stem, greater volume of roots, dry weight of leaves, and roots, and greater percentage of water in the plants with cooling of the nutrient solution (maximum 26 °C) were reported compared to the fluctuating ambient water temperature, which ranged from 24 to 29.9 °C [29]. Further, controlling the temperature to a maximum of 26 °C resulted in a greater amount of oxygen (9.3 mg·L⁻¹) in the solution compared to the ambient temperature (6.2 mg·L⁻¹). In another study, using 24 °C root zone temperature, lettuce crop growth was maximized, and damages minimized at various air temperatures ranging from 17 to 31 °C. Head size, leaf color, leaf thickness, and root structure were found to be best in 24 °C water compared to 17 and 31 °C regardless of the air temperature. A 31 °C water temperature resulted in the presence of root diseases in lettuce that ultimately reduced the head size, whereas poorly formed roots in 17 °C water also contributed to the reduced head size of lettuce [16]. Response of Korean mint plants (*Agastache rugose* L.) to various root zone temperatures was studied and increase in nine plant growth parameters, namely, shoot and root fresh weights, stem and root lengths, leaf length and leaf width, leaf area, and shoot and root dry weights were reported at 28 °C compared to 10 and 36 °C [24]. They concluded that too high as well as too low temperature led to water stress, ion imbalance, and growth inhibitors, and promoter imbalance in the plant. These results support our findings in

which increased root fresh weight, dry weight, and shoot dry weight at 21.1 °C compared to lower (18.3 °C) or higher ambient temperature was found (Table 3).

In the present study, plant height was found to be affected by cultivars (Table 1). “Black Seeded Simpson” had the greatest plant height and was found to be significantly similar to “Waldman’s Dark Green”, “Jericho”, and “Coastal Star” (Table 4). Within types for plant height, “B.S. Simpson” and “Waldman’s Dark Green” were greater than “Panisse” while Salanova type “Sweet Crisp Green” was greater than “Oakleaf Green”, “Oakleaf Red”, and “Butter” (Table 4). Lettuce growth response is known to be cultivar-dependent [15]. An experiment was conducted to evaluate the effects of four types (Romaine, Butterhead, Bibb, Summer Crisp) and 18 lettuce cultivars on growth and bolting, some of which were common in our experiment. Similar to our findings, large differences in growth characteristics, including the size index and head fresh weight between the cultivars of various types as well as within types was reported, and these differences were attributed to genetic diversity [30]. Another study was conducted to study the growth characteristics of nine commonly grown lettuce cultivars of four different types (Leaf, Butterhead, Romaine, and Crisphead subtype Batavia), in which Romaine and leaf lettuce showed greater plant growth rates during the initiation period of plant growth than “Butterhead” and “Batavia” lettuce [31]. They reported that the interaction between genotype and environment affects lettuce plant development and plays a critical role in determining the marketable yields of lettuce.

3.2. Temperature and Cultivar Effect on SPAD Readings and °Brix

There was a significant main effect of cultivar on SPAD readings (Table 1). The greatest mean for SPAD was observed in “Butter”, which was not different from “Sweet Crisp Green”, “Sweet Crisp Red”, “Oakleaf Red”, “Parris Island”, and “Coastal Star” (Table 4). SPAD readings and total chlorophyll content of leaves have been found to be significantly correlated [32]. Leaves with high chlorophyll concentration not only show increasing greenness visually but also indicate the sound growth of plants physiologically [33]. In a study conducted by the authors of [34], differences in chlorophyll concentration among three different cultivars of lettuce “Impuls”, “Valeria”, and “Lotto” were found and were attributed to differences in gene numbers. Irrespective of the water temperature, there was a difference in chlorophyll concentration, which supports the fact that genetic factors affecting the plant growth also influence the chlorophyll concentration in various horticultural crops [34–36].

Significant main effects of cultivar and temperature occurred for °Brix content (Table 1). For water temperature, °Brix was greatest in water temperature of 18.3 °C (Table 3). Among cultivars for °Brix, “Sierra” had the greatest value but was not different from all other cultivars except “Jericho” and “Black Seeded Simpson” (Table 4). Foods with high °Brix readings are higher in sucrose and in mineral nutrients thus serve as a general indicator of the nutritional quality of foods [37–39]. Root-zone temperature can affect the production of various plant metabolites in many plants, including leafy vegetables [40]. A study was conducted on red leaf lettuce “Red Wave”, and it was found that there was a significant increase in production of anthocyanin and total phenol content in the leaves of red lettuce when grown in 10 °C water than the ones that were grown in 20 and 30 °C [28]. There was also a significant difference in the total soluble solids among various temperatures, with the greatest °Brix found in lettuce grown at 10 °C. Similarly, low-temperature treatment of roots was found to enhance °Brix in spinach (*Spinacia oleracea* L. cv. Orai). It is suggested that low-temperature treatment induces water stress, and the plants exposed to osmotic stress such as salt or water stress induce osmoregulation by the accumulation of osmolites such as sugar, minerals, and amino acids in order to maintain the turgor pressure in leaves [40,41]. Similarly, a rapid increase in sugar contents of all plant parts, including leaves, epicotyls, hypocotyls, and roots of young cotton plants (*Gossypium hirsutum* L.), was observed at a lower root temperature of 10 °C. Not only temperature but lettuce cultivars also affect °Brix [42]. According to the sensory panel and analytical evaluations of five different

lettuce cultivars, it was demonstrated that genetic constitution had a greater impact on sensory qualities and total phenolic content. Flavor, total phenolic content, and degree of bitterness varied greatly among lettuce cultivars of different types and pigmentation but, with few exceptions, did not vary within cultivars across the growing season [43].

4. Conclusions

In the present experiment, lettuce grown at 21.1 °C chilled water were greater in terms of shoot fresh and dry weight as well as root fresh and dry weight compared to 18.3 °C and ambient water treatments, whereas greater °Brix was recorded at 18.3 °C water temperature. This shows that °Brix of lettuce increases with decreasing water temperature. All the cultivars in the Romaine type showed greater growth and °Brix, while Salanova lettuce did not perform well in all treatments compared to other lettuce types. For CO₂ assimilation, the interaction between water temperature and cultivars was found to be significant, with “Parris Island” having the greatest rate at ambient water temperature. While this study shows some possible advantages of chilling the nutrient solution in an NFT hydroponic system, further research could be performed to investigate full effects on nutritional quality, including how °Brix relates to overall quality in terms of sugars and starches. Future research should investigate other cultivars suitable for hydroponics using these water temperatures or should evaluate other temperatures for these lettuce types and cultivars.

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