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The Effect of Phosphorus and Root Zone Temperature on Anthocyanin of Red Romaine Lettuce

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Abstract: In this study, phosphorus (P) strength [(full strength: $1\times$ -P, half strength: $0.5\times$ -P and zero strength: $0\times$ -P)] and root zone temperature (10 and 15 °C RZT) were investigated in red romaine lettuce to determine their effects on anthocyanin content. This experiment was conducted using a deep-flow technique (DFT) hydroponic system in a greenhouse during the winter in Gangwon province in the Korea Republic. The treatments were $1\times$ -P (15 and 10 °C RZT), $0.5\times$ -P (15 and 10 °C RZT) and $0\times$ -P (15 and 10 °C RZT). The results showed that the P (phosphorus), K (potassium) and Ca (calcium) accumulation contents were lower in the $0\times$ -P -treated red romaine lettuce leaves than in the other treatments. The maximum red romaine lettuce yields per plant, leaf number and leaf length were exhibited during the $1\times$ -P at 15 °C RZT treatment. In $0\times$ -P at 10 °C, RZT treatment showed lower leaf color values of L^* , b^* , chrome, °h and higher leaf color a^* values. Moreover, in the $0\times$ -P treatment at 10 °C RZT, the leaf chlorophyll content (SPAD), chlorophyll a, chlorophyll b and chlorophyll total were the lowest. Anthocyanin content was the highest in the $0\times$ -P treatment, followed by $0.5\times$ -P and $1\times$ -P treatments at both 15 and 10 °C RZT. Regardless of the P intensity treatment, anthocyanin content was higher at 10 °C RZT than at 15 °C RZT. In summary, the $1\times$ -P at 15 °C RZT treatment is the best for red romaine lettuce leaf yield, and the $0\times$ -P at 10 °C RZT treatment is the best for lettuce leaf anthocyanin content.

Keywords: chlorophyll; color value; deep-flow; *Lactuca sativa*; mineral

1. Introduction

Lettuce (*Lactuca sativa* L.) is a widely cultivated leafy vegetable. According to AFRA [1], the lettuce harvest area is 3387 hectares, and the production is 86128 tonnes in Korea. Lettuce cultivation in a controlled environment in winter is increasing daily due to greenhouses. However, improper treatment may lead to production costs and decreased anthocyanin content, which is beneficial to human health. Anthocyanin is associated with antioxidant, anticarcinogenic and anti-inflammatory properties that protect from the risk of cancer, heart disease, diabetes and cognitive function disorders [2].

Nutrient solution concentration is a vital factor of soil-less culture used to grow lettuce [3]. High nutrient concentrations promote ion toxicity, nutrient imbalance and osmotic stress, and low nutrient concentrations lead to nutrient deficiencies [4]. Lowering the root zone temperature in lettuce reduces the water and nutrient solution absorption [5]. P treatment may control flower stalk anthocyanin synthesis by regulating the epidermal pH value and enzymatic activity [6]. A nutrient solution with

a low ion concentration showed higher anthocyanin production in the Lollo Rosso genotype, and a medium ion concentration showed higher anthocyanin production in the Red Oak Leaf genotype of lettuce [7].

Red romaine lettuce contains anthocyanin, which is a pigment that has antioxidant activity. Cell vacuoles accumulate anthocyanin mainly in the leaves, flowers, stems, seeds and other plant tissues of fruits and vegetables [8]. Increased anthocyanin content is good for consumers due to its beneficial health compounds.

The red color of leafy vegetables is attractive to consumers who look for a functional agricultural product. Lower growing temperatures and shorter periods are important to maximize economic returns from lettuce, which is a notable source of minerals, such as calcium (Ca), iron (Fe) and magnesium (Mg) [9]. The plant growth parameters (plant yield, leaf dry matter, fresh root weight, root length, root dry matter, leaf number, leaf length, leaf width), pigments (carotenoid, chlorophyll and anthocyanin) are important factors for cost-benefit analysis. Increased yield and anthocyanin content are expected, which can fulfill the consumers' as well as the growers' demands. Anthocyanin is a pigment that may increase in leaf lettuce during production in a controlled environment. This study was conducted to determine the effect of P strength [(full strength: $1 \times -P$, half strength: $0.5 \times -P$ and zero strength: $0 \times -P$)] and root zone temperature (15 and 10 °C RZT) on anthocyanin in red romaine lettuce.

2. Materials and Methods

2.1. Lettuce Growing Environment

Lettuce (*Lactuca sativa* L. cv. romaine) was grown with a deep-flow technique (DFT) hydroponic system in a greenhouse during the winter in Gangwon province in Korea Republic. The average growing atmosphere temperature (day/night), relative humidity (day/night) and carbon dioxide concentration (day/night) were maintained at 19.29/11.41 °C (Figure 1), 28.68/34.08%, and 448.30/461.43 ppm, respectively, in the greenhouse during the treatment.

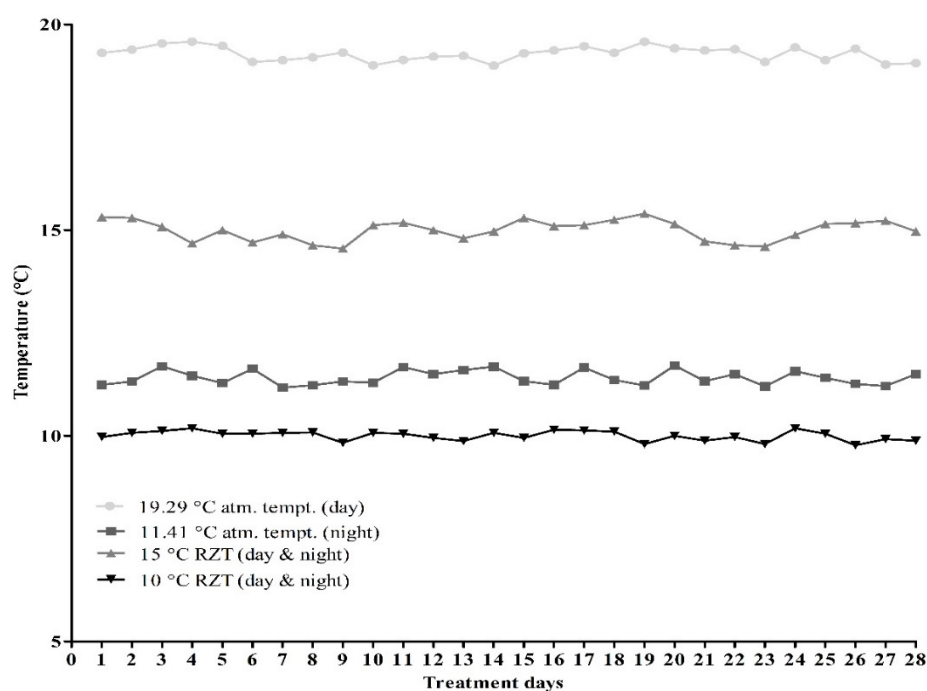


Figure 1. Lettuce growing atmosphere temperature (19.29/11.41, day/night) and treated root zone temperature (RZT 15/10, day and night) for 28 days in a deep flow technique (DFT) system during growth and development. Note: 18th January to 14th February 2018.

2.2. Lettuce and Treatments

After 30 days of plug tray sowing, the seedlings (16 seedlings in each treatment and total seedlings were 96 for 6 treatments) were transplanted to the DFT system. Three phosphate strengths [(full strength: $1\times\text{-P}$, half strength: $0.5\times\text{-P}$ and zero strength: $0\times\text{-P}$)] and two root zone temperatures (15 and 10°C RZT) were treated to confirm the proper phosphate strength and root zone temperature for enhancing the anthocyanin content of red romaine lettuce grown in hydroponics.

The nutrient solution contained $\text{NO}_3\text{-N}$ (nitrate-nitrogen) 13, $\text{NH}_4\text{-N}$ (ammonium-nitrogen) 1.17, $\text{PO}_4\text{-P}$ (phosphate-phosphorus) 3.5, K (potassium) 9, Ca (calcium) 4, Mg (magnesium) 2, $\text{SO}_4\text{-S}$ (sulfate-sulphur) 2, Fe (iron) 0.07, B (boron) 0.14, Mn (manganese) 0.018, Zn (zinc) 0.002, Cu (copper) 0.0006, and Mo (molybdenum) 0.0013 (me L^{-1}) (Table 1). In the reducing P treatment, the other nutrient contents were constant. In the $0.5\times\text{-P}$ strength, $\text{NH}_4\text{-N}$ 0.60 [NH_4Cl (ammonium chloride)] and $\text{PO}_4\text{-P}$ 1.75 [$\text{NH}_4\text{H}_2\text{PO}_4$ (ammonium dihydrogen phosphate)] (me L^{-1}) were used in the nutrient solution. Moreover, in the $0\times\text{-P}$ strength, $\text{NH}_4\text{-N}$ 0.60 [NH_4Cl] and $\text{NH}_4\text{-N}$ 0.60 [$(\text{NH}_4)_2\text{SO}_4$ (ammonium sulphate)] were used in the nutrient solution.

Table 1. Nutrient solution characteristics of low strength ion concentration ($0.4\times$) in the seedling stage in the plug tray and the hardening stage in the deep flow technique (DFT), and full ($1\times\text{-P}$), medium or half ($0.5\times\text{-P}$) and no ($0\times\text{-P}$) P ion concentrations at the growth and development stage in the DFT system of hydroponic red romaine lettuce.

Stage	Ion Concentration					
	Germination stage in plug tray	Seedling stage in plug tray	Hardening stage in DFT	Growth and development stage in DFT system		
Strength	-	$0.4\times$ (low)	$0.4\times$ (low)	$1\times\text{-P}$ (full)	$0.5\times\text{-P}$ (half)	$0\times\text{-P}$ (none)
Treatment days	Days 0–15 (15 days)	Days 16–30 (15 days)	Days 31–45 (15 days)	Days 46–73 (28 days)	Days 46–73 days (28 days)	Days 46–73 (28 days)
pH	-	6.0–6.5	6.0–6.5	6.0–6.5	6.0–6.5	6.0–6.5
EC (dS m^{-1})	-	0.75	0.75	1.80	1.80	1.80
$\text{NO}_3\text{-N}$	-	5.20	5.20	13	13	13
$\text{NH}_4\text{-N}$	-	0.46	0.46	1.17	0.60 [NH_4Cl]	0.60 [NH_4Cl] and 0.60 [$(\text{NH}_4)_2\text{SO}_4$]
$\text{PO}_4\text{-P}$	-	1.40	1.40	3.5 [$\text{NH}_4\text{H}_2\text{PO}_4$]	1.75 [$\text{NH}_4\text{H}_2\text{PO}_4$]	0
K	-	3.60	3.60	9	9	9
Ca	-	1.60	1.60	4	4	4
Mg	-	0.80	0.80	2	2	2
$\text{SO}_4\text{-S}$	-	0.80	0.80	2	2	2
Fe	-	0.02	0.02	0.07	0.07	0.07
B	-	0.05	0.05	0.14	0.14	0.14
Mn	-	0.0072	0.0072	0.018	0.018	0.018
Zn	-	0.0008	0.0008	0.002	0.002	0.002
Cu	-	0.00024	0.00024	0.0006	0.0006	0.0006
Mo	-	0.00052	0.00052	0.0013	0.0013	0.0013

Note: Values are expressed in me L^{-1} . EC—Electrical conductivity.

From germination to the seedling stage (days 0–15, 15 days), there was no additional nutrient solution added to the plug tray; only mixed peat moss and perlite (1:1, v/v) were added to the plug tray for seed germination. The $0.4\times$ nutrient solution was supplied to the seedling stage (days 16–30, 15 days) with low electrical conductivity (EC) (0.75 dS m^{-1} EC and 6.0–6.5 pH). In the hardening stage in the DFT system, $0.4\times$ nutrient solution (days 31–45, 15 days) with low EC strength (0.75 dS m^{-1}

EC and 6.0–6.5 pH) was applied. Afterwards, it was adjusted to 1.80 dS m^{−1} EC and 6.0–6.5 pH. Every week, the nutrient solution was renewed and EC and pH (1.80 dS m^{−1} EC and 6.0–6.5 pH) were maintained, supplied by the DFT system (Table 2). The treatments were 1×-P (15 and 10 °C RZT), 0.5×-P (15 and 10 °C RZT) and 0×-P (15 and 10 °C RZT).

Table 2. The pH values of the nutrient solution at the adding time and before the adding time.

P Strength	Temp.	pH Value	
		Nutrient Solution Adding Time	Nutrient Solution before Adding Time
1×-P (Full)	15 °C	6.45a ^z	5.75a
	10 °C	6.40ab	5.68a
0.5×-P (Half)	15 °C	6.33ab	5.73a
	10 °C	6.35ab	5.65a
0×-P (None)	15 °C	6.38ab	5.50a
	10 °C	6.25b	5.05b
<i>p</i> -value of St. & Temp.		*	***
<i>p</i> -value at 15 °C		NS	*
<i>p</i> -value at 10 °C		NS	***

^z Mean separation within columns by Duncan's multiple range tests (DMRT) (*n* = 4). NS, *, ***; not significant, significant at *p* ≤ 0.05 and 0.001 respectively. Same letters are significantly different to other treatment at different probability level (*p* ≤ 0.05 and 0.001). Note: St.—strength and Temp.—temperature.

The nutrients were supplied to the DFT (deep flow technique) system which included PVC (polyvinyl chloride) trays that were 4 m long, 0.15 m wide and 0.20 m deep and were regulated by an electric pump set at a rate of 60 L/min [10]. Lettuce treatment started after the 15th day of transplanting to the DFT system. This treatment continued for 28 days, and during the treatment, we analyzed the leaf number, leaf length, leaf width, chlorophyll, and color value (L*, a*, b*, chrome and °h). The lettuce experiments were conducted on days 0–30 (30 days) in plug trays, days 31–45 (15 days) in the DFT system for hardening and days 46–73 (28 days) in the DFT system during different treatments. The 73-day-old lettuce was harvested to measure the harvest time (20 °C) quality parameters.

2.3. Mineral Contents of Red Romaine Lettuce

Minerals were analyzed according to the Islam et al. [11] methods. Briefly, 0.50 ± 0.005 g of dried lettuce (80 °C) powder ashes with 10 mL of 69% nitric acid (HNO₃) and 2 mL of 30% hydrogen peroxide (H₂O₂) were dissolved in a CEM microwave (MARS 5, Microwave Reaction System, CEM Corporation, and England) for 1 h at 175 °C. Inductively coupled plasma-atomic emission spectroscopy (Integra XL Dual, GBC, and Melbourne, Victoria, Australia) was used to measure mineral content.

2.4. Growth Parameters

Plant yield, leaf dry matter, fresh root weight, root length and root dry matter were measured. Leaf and root dry matter were measured by drying the sample at 80 °C. Leaf number, leaf length, leaf width and chlorophyll were recorded. A SPAD-502 Plus chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) was used to measure the chlorophyll value. A Chroma Meter CR 400 Model (Konica Minolta Sensing, Inc., Osaka, Japan) was used to measure the lettuce leaf color value (L*, a*, b*, chrome and °h) [12].

2.5. Chlorophyll (chl *a*, chl *b* and chl Total) and Anthocyanin Contents

The chlorophyll of fresh leaf red romaine lettuce (0.10 g) was extracted with 5 mL N,N-dimethylformamide overnight. The supernatant was measured by a UV-1601 spectrophotometer

(Shimadzu, Kyoto, Japan) at 647 nm and 664 nm. Chl a and chl b values were determined according to the equations of Moran [13].

The total anthocyanin content was measured with a UV spectrophotometer (Shimadzu Corporation, Tokyo, Japan) at a 530 nm absorbance wavelength. Each 1 g leaf was extracted for 24 hours at 4 °C in dark conditions into 5 mL of 95% ethanol/1.5 N HCl (85:15, v:v). The anthocyanin was calculated according to the following formula: anthocyanin (mg/100 g fresh wt.) = (absorbance at 530 nm × volume of extraction solution × 100)/(wt. of sample of gm × 98.2). (Note, here, 98.2 is the factor of molar absorption value for the acid-ethanol solvent).

2.6. Statistical Analyses

Statistical Package for the Social Sciences (SPSS) V. 16 (SPSS Inc., Chicago, USA) was used to perform the statistical analysis of data. Differences between conditions were analyzed by a DMRT following a one-way ANOVA. In addition, Pearson correlation was analyzed between two parameters by SPSS.

3. Results

3.1. Mineral Contents of Red Romaine Lettuce

Regarding the N (nitrogen) absorption rates, the 0×-P at 15 °C RZT treatment appeared to be the highest (Table 3). Maximum N absorption occurred due to balancing the anion and cation concentrations of the root zone area. At 10 °C, the RZT (1×-P and 0.5×-P) treatments displayed low phosphorus (P) content due to the low temperature; as a result, the lettuce leaf color changed from green to reddish. High RZT showed high nutrient contents in red romaine leaf lettuce. Among the treatments, the 0×-P at 10 °C RZT treatment had the highest Fe (iron) content, which may have occurred due to the acidic condition of the nutrient solution (≤5.5 pH) during absorption. The Cl (chlorine) content was higher in the 0.5×-P and 0×-P treatments than in the 1×-P treatment because we added Cl with NH₄Cl (ammonium chloride) to adjust the NH₄ content. The PO₄-P (phosphate-phosphorus) uptake depends on temperature because 15 °C RZT is more suitable for the uptake of PO₄-P than 10 °C RZT.

Table 3. Mineral content of red romaine lettuce leaf.

P Strength	Temp.	% DW					µg/gm DW		
		N-NO ₃	N-NH ₄	P-PO ₄	K ₂ O	CaO	MgO	Fe	Cl
1×-P (Full)	15 °C	0.92d ^z	0.28cd	0.63a	0.63a	0.09a	0.013ab	6.09bc	6.72ab
	10 °C	0.72d	0.22d	0.41b	0.57ab	0.08ab	0.010b	5.23b	6.40b
0.5×-P (Half)	15 °C	1.19cd	0.43bc	0.37bc	0.54abc	0.07abc	0.017abc	8.77abc	7.66ab
	10 °C	1.59bc	0.32cd	0.24cd	0.40bcd	0.06bcd	0.020abc	7.12abc	7.15ab
0×-P (None)	15 °C	2.52a	0.67a	0.13d	0.38cd	0.05cd	0.023bc	10.31a	9.15a
	10 °C	1.95ab	0.55ab	0.10d	0.29d	0.04d	0.027c	9.49ab	8.81ab
<i>p</i> -value of St. & Temp.		***	***	***	***	***	**	***	***
<i>p</i> -value at 15 °C		***	***	***	***	***	*	**	**
<i>p</i> -value at 10 °C		***	***	***	***	***	**	***	*

^z Mean separation of columns by Duncan's multiple range tests (DMRT) (*n* = 3). *, **, ***; significant at *p* ≤ 0.05, 0.01 and 0.001, respectively. Same letters are significantly different to other treatment at different probability level (*p* ≤ 0.05, 0.01 and 0.001). Note: St.—strength, Temp.—temperature and DW—dry weight.

3.2. Plant Yield, Leaf Dry Matter, Fresh Root Weight, Root Length and Root Dry Matter

The yield of red romaine lettuce was directly proportional to the phosphorus (P) content of the nutrient solution and the root zone temperature (RZT) (Table 4). At 10 °C RZT, regardless of whether the P strength was 1×-P or 0.5×-P, yields of red romaine lettuce plants were lower than other treatments due to the effects of a low RZT. At 15 °C RZT, greater yields were displayed than at

10 °C RZT. The lower temperature and P deficiency prohibited the growth and development of roots. The 0×-P (10 °C and 15 °C RZT) treatments displayed longer root lengths than the 0.5×-P treatments (10 °C and 15 °C RZT) or even the 1×-P (10 °C and 15 °C RZT) treatments. At 15 °C, RZT (1×-P, 0.5×-P and 0×-P) treatments, root length was significantly longer than with 10 °C RZT (1×-P, 0.5×-P and 0×-P) treatments, which may have occurred due to the increased temperature. Leaf and root dry matter exhibited higher values in the 0×-P (15 °C or 10 °C RZT) treatments compared with the other treatments because low RZT probably suppressed water absorption. Plant yield and leaf dry matter showed a negatively significant ($p \leq 0.01$) correlation that may have occurred due to more nutrient content solution being absorbed by the 1×-P treatment at 15 °C RZT.

Table 4. Plant yield, leaf dry matter, root fresh weight, root length and root dry matter of red romaine lettuce at harvest day (28 days after treatment).

P Strength	Temp.	Yield/Plant (g)	Leaf Dry Matter (g)	Root Fresh Weight (g)	Root Length (cm)	Root Dry Matter (g)
1×-P (Full)	15 °C	47.68a ^z	5.80d	4.76ab	35.06b	7.81c
	10 °C	26.25b	8.03c	2.89c	23.38d	10.19b
0.5×-P (Half)	15 °C	45.40a	5.94d	4.75ab	36.06ab	7.80c
	10 °C	27.22b	9.00c	3.10bc	25.75cd	11.25ab
0×-P (None)	15 °C	13.94c	11.29b	4.85a	40.94a	7.91c
	10 °C	11.23c	13.25a	3.29abc	30.75bc	12.25a
<i>p</i> -value of St. & Temp.		***	***	***	***	***
<i>p</i> -value at 15 °C		***	***	***	***	*
<i>p</i> -value at 10 °C		***	***	***	***	***

^z Mean separation within columns by Duncan's multiple range tests (DMRT) ($n = 10$). *, ***, significant at $p \leq 0.05$ and 0.001, respectively. Same letters are significantly different to other treatment at different probability level ($p \leq 0.05$ and 0.001). Note: St.—strength and Temp.—temperature.

3.3. Leaf Number, Leaf Length, Leaf Width and Leaf Chlorophyll Content (SPAD)

In this experiment, the 15 °C RZT treatment resulted in a higher leaf number than the 10 °C RZT treatment (Table 5). In the 0×-P at 10 °C RZT treatment, the leaf length and the leaf width were the lowest because low temperature and 0×-P treatment suppresses the growth of red romaine lettuce leaf plants. There was a positively significant ($p \leq 0.01$) correlation found between leaf length and leaf width. A higher root zone temperature (15 °C RZT) resulted in a higher leaf chlorophyll content (SPAD) than a lower root zone temperature (10 °C RZT), regardless of the P strength.

Table 5. Leaf number, leaf length, leaf width and leaf chlorophyll content (SPAD) of red romaine lettuce at harvest day (28 days after treatment).

P Strength	Temp.	Leaf Number	Leaf Length (cm)	Leaf Width (cm)	Leaf Chlorophyll Content (SPAD)
1×-P (Full)	15 °C	8.70a ^z	19.30a	12.20a	27.21a
	10 °C	8.30ab	16.60ab	11.25ab	26.55ab
0.5×-P (Half)	15 °C	8.60a	18.50a	13.70a	27.03ab
	10 °C	8.40ab	17.15a	11.50ab	25.59abc
0×-P (None)	15 °C	7.80b	14.00b	9.30ab	24.35ab
	10 °C	7.70b	13.80b	8.25b	23.39c
<i>p</i> -value of St. & Temp.		*	***	***	***
<i>p</i> -value at 15 °C		*	***	**	**
<i>p</i> -value at 10 °C		*	***	**	***

^z Mean separation within columns by Duncan's multiple range tests (DMRT) ($n = 10$). *, **, ***; significant at $p \leq 0.05$, 0.01 and 0.001 respectively. Same letters are significantly different to other treatment at different probability level ($p \leq 0.05$, 0.01 and 0.001). Note: St.—strength and Temp.—temperature.

3.4. Leaf Color Value (L^* , a^* , b^* , Chrome and °h)

In the 0×-P treatment at the 10 °C root zone temperature (RZT), red romaine lettuce leaf exhibited the maximum color value of a^* and the minimum color values of L^* , b^* , chrome and °h (Table 6). The color values of b^* , chrome and °h showed significant differences in all treatments. Significant ($p \leq 0.01$) negative Pearson correlations were found between the color values of a^* and b^* which indicates that if the color value of a^* increases then the color value of b^* decreases.

Table 6. Leaf color value (L^* , a^* , b^* , chrome and °h) of red romaine lettuce at harvest day (28 days after treatment).

P Strength	Temp.	Color (L^*)	Color (a^*)	Color (b^*)	Color (chrome)	Color (°h)
1×-P (Full)	15 °C	38.90a ^z	−2.52b	5.44a	6.86a	111.87a
	10 °C	37.42ab	−1.21ab	3.29bc	3.55ab	106.80ab
0.5×-P (Half)	15 °C	38.37ab	−1.40ab	4.23ab	4.38ab	109.34ab
	10 °C	37.34ab	−1.06ab	2.70bc	2.76b	104.83ab
0×-P (None)	15 °C	36.88ab	−0.88ab	2.15c	2.23b	102.07b
	10 °C	36.12b	−0.43a	1.81c	2.04b	100.77b
<i>p</i> -value of St. & Temp.		*	**	***	***	***
<i>p</i> -value at 15 °C		NS	NS	***	**	**
<i>p</i> -value at 10 °C		NS	*	***	**	*

^z Mean separation within columns by Duncan's multiple range tests (DMRT) ($n = 10$). NS, *, **, ***; not significant, significant at $p \leq 0.05$, 0.01 and 0.001, respectively. Same letters are significantly different to other treatment at different probability level ($p \leq 0.05$, 0.01 and 0.001). Note: L^* = lightness or brightness, +a = redness, −a = greenness, b^* = yellowness, St.—strength and Temp.—temperature.

3.5. Chlorophyll (chl a, chl b and chl Total) and Anthocyanin Content

The chlorophyll (chl a, chl b and chl total) contents were lower in the 0×-P with the 10 °C RZT treatment when compared to the other treatments. Chlorophyll a, chlorophyll b and chlorophyll total had significant differences among the treatments (Table 7).

Table 7. Chlorophyll (chl a, chl b and chl total) and anthocyanin content after harvest (28 days after treatment at 20 °C) of red romaine lettuce.

P Strength	Temp.	Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)	Chlorophyll Total (µg/mL)	Anthocyanin (mg/100 g FW)
1×-P (Full)	15 °C	9.99a ^z	13.62a	23.61a	0.64d
	10 °C	8.04ab	11.77ab	19.80abc	1.44cd
0.5×-P (Half)	15 °C	8.93ab	12.77ab	21.70ab	0.77cd
	10 °C	7.87ab	11.30ab	19.17abc	2.15c
0×-P (None)	15 °C	7.78ab	10.54ab	18.32bc	4.80b
	10 °C	7.59b	9.31b	16.90c	6.65a
<i>p</i> -value of St. & Temp.		*	***	***	***
<i>p</i> -value at 15 °C		NS	*	***	***
<i>p</i> -value at 10 °C		NS	*	NS	***

^z Mean separation within columns by Duncan's multiple range tests (DMRT) ($n = 10$). NS, *, ***; not significant, significant at $p \leq 0.05$ and 0.001, respectively. Same letters are significantly different to other treatment at different probability level ($p \leq 0.05$ and 0.001). Note: St.—strength and Temp.—temperature.

Anthocyanin content was maximum in the 0×-P treatment at 10 °C RZT, followed by the treatments with 0×-P at 15 °C RZT, 0.5×-P at 10 °C RZT, 1×-P at 10 °C RZT, 0.5×-P at 15 °C RZT and 1×-P at

15 °C RZT. Moreover, the 10 °C RZT treatment showed a significantly higher anthocyanin content than the 15 °C RZT treatment. Total chlorophyll and anthocyanin contents showed a significantly negative correlation ($p \leq 0.01$).

4. Discussion

4.1. Mineral Contents of Red Romaine Lettuce

Phosphorus uptake was inhibited by the 10 °C root zone temperature (RZT) ($1\times\text{-P}$ and $0.5\times\text{-P}$). Mineral uptake requires energy for active transport by root respiration, but a low temperature suppresses root respiration [14]. Moreover, Lee et al. [15] reported that lettuce reduced evapotranspiration, and mineral uptake ($\text{P (phosphorus)} > \text{K (potassium)} > \text{NO}_3^-$) and increased electrolyte leakage at low temperatures. However, an elevated root zone temperature increased the uptake of P in melons and cucumbers [16,17]. In lettuce, an increased root zone temperature increased the mineral uptake rate [18].

Nutrient absorption is higher at high temperatures than at low temperatures in cucumbers and leafy vegetables, because low temperatures suppress water absorption [17,19]. Absorption of N, P, K, Ca, Mg, Fe and Cl is influenced by P strength and the RZT of the nutrient solution. The $\text{NO}_3\text{-N}$ (nitrate–nitrogen) absorption trends resulted from the $\text{PO}_4\text{-P}$ (phosphate–phosphorus) and the $\text{PO}_4\text{-P}$ absorption resulting from $\text{NH}_4\text{-N}$ (ammonium–nitrogen). The absorption of $\text{NH}_4\text{-N}$ increased, probably due to the increased pH (≥ 6). When $\text{NH}_4\text{-N}$ is taken up by plant roots, plant roots leak hydrogen ions and, as a result, the pH is lowered (acidity increases); when plants prefer to uptake $\text{NO}_3\text{-N}$, then hydroxyl ions are leaked by plant roots, thus the pH increases (acidity decreases) [19]. Park et al. [19] also mentioned that low temperatures promote the absorption of $\text{NH}_4\text{-N}$ and high temperatures lead to absorbed $\text{NO}_3\text{-N}$ in roots because the former lowers the pH by leaking hydrogen ions and the latter increases the pH by leaking hydroxyl ions in roots. If the temperature increases from 15 °C to 25 °C, the NO_3 content increases in lettuce plants due to nitrate reductase and glutamine synthase, which are involved in nitrogen metabolism in lettuce plants [20].

The $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, K and $\text{PO}_4\text{-P}$ uptake and assimilation are influenced by enzymes, substrate concentration, internal concentration, species and temperature [17,21–23]. Possibly, the reduction of PO_4^- in the nutrient solution will increase the uptake of other anions (mostly NO_3^- in the nutrient solution) by the plant. The reduction in the uptake rate of mineral nutrients at a low temperature was followed by reductions in potassium, nitrate and nitrogen [24]. Low P reduced leaf 3-phosphoglycerate (PGA) and ribulose-1,5-bis-phosphate, and, as a result, photosynthetic CO_2 -fixation was limited, Calvin cycle enzyme activity reduced and adenosine triphosphate (ATP)/adenosine diphosphate (ADP), nicotinamide adenine dinucleotide phosphate (NADPH)/nicotinamide adenine dinucleotide phosphate (NADP⁺) and triose-phosphate (TP)/PGA ratios were increased in plants [25]. Therefore, the optimum RZT and optimum P content can influence the mineral content uptake of red romaine lettuce plants.

4.2. Plant Yield, Leaf Dry Matter, Fresh Root Weight, Root Length and Root Dry Matter

P deficiency reduced *Brassica* cultivars and kale growth and development [6,26]. The yield of red romaine lettuce plants showed a significant difference between the P strengths and RZT treatments. High temperatures in the root zone showed high plant growth and yield compared with low temperatures [19,27]. Similarly, Boo et al. [28] reported that temperatures of 20/13 °C (day/night) are reasonable to grow lettuce. In this experiment, the 15 °C root zone temperature (RZT) (day/night) gave the maximum yield of red romaine lettuce.

The $0\times\text{-P}$ treatment had the lowest fresh root weight at 10 °C RZT, because a lower temperature promotes water stress that hampers leafy vegetable root growth [27], and a small head is produced. Moreover, P deficiency reduced the kale fresh root weight [6].

The low RZT suppressed the growth of root hair. Clarkson et al. [22] agreed that high temperature increases root length when compared to a low temperature because a low temperature inhibits the transport process of nutrients. In this experiment, low temperature treatments ($1\times-P$, $0.5\times-P$ and $0\times-P$) suppressed root growth. In cucumber, at low temperatures, the root growth is limited [17], and nutrient uptake is hampered due to decreased hydraulic conductivity and root pressure [29].

The treatment at 10 °C RZT produced more leaf dry matter than at 15 °C, regardless of the P strength. Becker et al. [30] reported that dry matter content in lettuce was higher at temperatures of 12/7 °C (day/night) when compared to 20/15 °C (day/night). In addition, Luna et al. also found that dry matter was more abundant in winter growing lettuce than in summer growing lettuce [7]. Dry matter of red romaine lettuce results from a dilution effect, [19] and this effect mainly occurs in those plant parts that receive more water. In addition, increased dry matter content probably results from the trade-off in resource allocation between the vegetative, root and fruit growth parts of the plant [31]. Therefore, $0\times-P$ and low temperature increase the leaf and root dry matter in red romaine lettuce.

4.3. Leaf Number, Leaf Length, Leaf Width and Leaf Chlorophyll Content (SPAD)

At 20/15 °C (day/night), the temperature appeared to promote a higher lettuce leaf number than at 12/7 °C (day/night), because this temperature influenced the metabolic process and quickened the growth of leaf lettuce [30]. Moreover, Costa et al. [32] reported that a 20 °C temperature promoted the maximum number of leaves in corn salad baby leaf plants compared to 15 °C or even 25 °C.

The leaf length and the leaf width were lower in the $0\times-P$ at 10 °C RZT treatment compared with other treatments. This is related to Hoque et al.'s [33] finding that suboptimal application of P causes slower leaf growth due to less cell expansion and suppression of photosynthesis. Moreover, lower leaf number and leaf lengths were observed in kale in a P-deficit treatment [6].

The nutrient solution at 20 °C exhibited a higher leaf chlorophyll content (SPAD) than the 15 °C nutrient solution, but the 25 °C nutrient solution showed the lowest leaf chlorophyll content (SPAD) among those temperatures in corn salad baby leaf [32]. Moreover, the $0\times-P$ and 10 °C RZT treatment manifested the lowest leaf chlorophyll content (SPAD) among the treatments. The energy process is difficult without P nutrients [34], and this limits photosynthesis [35]. Therefore, an elevated RZT (15 °C) increases the leaf number, leaf length, leaf width, and leaf chlorophyll content of red romaine lettuce compared with a lower RZT (10 °C).

4.4. Leaf Color Value (L^* , a^* , b^* , Chrome and °h)

The red lettuce displayed lower color values of L^* and chrome, which is in agreement with Mampholo et al. [36]. In the $0\times-P$ treatment at 10 °C RZT, the hue angle (°h) decreased in lettuce leaves. This is related to Gazula et al.'s [37] finding that if anthocyanin increases, then the hue angle (°h) decreases in lettuce leaves.

4.5. Chlorophyll (chl a, chl b and chl Total) and Anthocyanin Content

The $1\times-P$ treatment at 15 °C RZT showed elevated chlorophyll a, chlorophyll b and chlorophyll total. In kale, low and deficit P treatments exhibited lower chlorophyll contents than the normal treatment [6]. In addition, the photoreceptor (photochromic) is anthocyanin (red pigment), which prevents photoinhibition and photodamage by the absorption of solar radiation and perhaps could be absorbed by chloroplast pigments [38]. The $0\times-P$ treatment of 10 °C RZT decreased the chlorophyll (chl a, chl b and chl total) content and increased the anthocyanin content of red romaine lettuce leaf. The treatments with higher anthocyanin contents accumulated a lower P content. Temperatures of 13/10 °C (day/night) displayed higher anthocyanin contents than temperatures of 30/25 °C (day/night) when growing lettuce [28,30]. Gazula et al. [39] found that the accumulation of anthocyanin content with temperatures of 20/20 °C (day/night) was higher than with temperatures of 30/30 °C (day/night) when growing lettuce. A comparatively lower temperature most likely

leads to the accumulation of a greater anthocyanin content due to the lower rate of photosynthesis (phytochrome) and the lower accumulation of P by lettuce. *Arabidopsis thaliana* and kale exhibited increased anthocyanin content when there was a P deficit [6,40]. Anthocyanin is also influenced by chlorophyll [41]. For instance, if anthocyanin increases, then the chlorophyll content decreases. Becker et al. [30] reported that low temperatures slow the enzymatic activity of photosynthesis, as light intercepts and photosynthetic electron transport in chloroplast thylakoid membranes is interrupted. Therefore, low temperature (10 °C RZT) and 0×-P growing red romaine lettuce plants increase the accumulation and synthesis of anthocyanin.

5. Conclusions

In this experiment, the effects of P (1×-P, 0.5×-P and 0×-P) and RZT treatments on anthocyanin in red romaine lettuce were investigated. The 1×-P treatment showed elevated yields per plant, leaf number and leaf length in red romaine lettuce at 15 °C RZT. Moreover, the 0×-P at 10 °C RZT treatment displayed lower leaf color values of L*, b*, chrome and °h and higher leaf color values of a* than in other treatments. Furthermore, the 0×-P treatment at 10 °C RZT exhibited the lowest leaf chlorophyll contents (SPAD) for chlorophyll a, chlorophyll b and chlorophyll total, and the highest anthocyanin content among the treatments. In conclusion, treatments with 1×-P at 15 °C RZT and 0×-P at 10 °C RZT could be useful for the yield and anthocyanin content, respectively. These research methods and findings will help to produce enhanced red romaine lettuce leaf anthocyanin content in DFT hydroponic systems in greenhouses during the winter to fulfill consumer demands.

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