Effects of air and root-zone temperatures on growth and quality of indoor hydroponic lettuce

by

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List of Abbreviations

°C	Degree Celsius
HFW	Head Fresh Weight
SI	Size Index
RDW	Root Dry Weight
RL	Root Length
RZT	Root Zone Temperature
RZT_T	Target Root Zone Temperature
RZT_A	Actual Root Zone Temperature
T_A	Air Temperature
g	gram
h	hours
L	Liters
ppm	parts per million

Chapter I

Introduction and Literature Review

Global Population and Agriculture

The current world population is estimated at 7.7 billion and is growing by approximately 200,000 persons per day (World Population Clock, 2019). A predicted 9.5 billion people will populate the earth by 2050, putting enormous pressure on the planet's natural resources including minerals and water. According to the United States Geological Survey, 97.4% of Earth's water is undrinkable saltwater, and the freshwater for humans to use is less than 2.6%, considering great proportion is locked in glaciers and ice caps (USGS). United Nation reported that 30% of the world's population will face water shortage problem by 2025 (UN, 2014). Although freshwater is under a shortage situation, still 70 percent of freshwater is used for agriculture globally (FAO, 2017). Currently, agriculture accounts for approximately 80% of all freshwater usage in the US (USDA-ERS, 2019). Production of nutritious food must increase with special care given to water and other resource conservation.

A water- and mineral-conservative method of agricultural food production is termed controlled environment agriculture (CEA) and includes modified growing structures such as greenhouses, high tunnels, shade houses, and indoor farms, and resource conservative technologies such as drip irrigation, nutrient recirculation, and precise environmental controls. Adoption of CEA technologies are increasing globally and in the United States. Greenhouse production is the most common form of CEA and currently, 130 countries in the world are producing greenhouse vegetables commercially. The major greenhouse crops include tomato, cucumber, lettuce, sweet pepper (capsicum), culinary herbs, eggplant (aubergine), and strawberry. The total estimated area of world greenhouse vegetable production is 496,800 ha, of which 95,000ha are soilless/hydroponic culture systems (Hickman, 2016). As a basic source of essential nutrients, vegetables can provide humans plentiful vitamins, beneficial active ingredients, cellulose, amino acids and mineral elements (Qiu et al, 2014), but vegetable cultivation is often resource intensive. In order to conserve natural resources and provide vegetables for a growing population, controlled environment agriculture (CEA) may be necessary.

Crop Information

One of the most popular vegetables grown in CEA systems is lettuce (*Lactuca sativa* L.). Lettuce is a kind of leafy vegetable belonging to Compositae family (Asteraceae). It originated from Mediterranean area and was cultivated in Egypt. The components contained in lettuce such as phenolics, ascorbic acid, carotenoids, tocopherols and glucosinolates, are beneficial to human health and can help prevent human disease from cancer, cerebrovascular and cardiovascular issues (Chutichudet et al., 2011). Lettuce is a cool-season vegetable, and they can grow vigorously at temperature from 7 to 24 °C (Sublett et al., 2018). According to Cornell hydroponic lettuce handbook, the suggested pH range is from 5.6-6.0 for CEA lettuce production and 5.8 is recommended as the optimal pH level for lettuce growth (Brechner et al., 2013), and 1.5 mS/cm is the ideal EC for hydroponic lettuce production (Domingues et al., 2012). Comestible lettuce can be categorized into six types which are crisphead, romaine, leaf, butterhead, Latin and stem (Ryder, 1999). Each type can be produced in greenhouse, and the most widely produced type of lettuce in southeastern United States is butterhead (Holmes, 2017).

Americans consume approximately 6.0 kg of lettuce per person annually, making lettuce the leading vegetable crop grown in the US in terms of value (USDA-ERS, 2018). According to the USDA National Agricultural Statistic Service (NASS, 2012), domestic lettuce was field-produced on 67,554 ha (166,800 ac) in 2015, mainly in two states: Arizona and California. Total lettuce production in the U.S. in 2015 was 3.7 billion kg (8.1 billion lbs.), which was worth nearly $1.9 billion. The vast majority of lettuce production is for domestic consumption while only 5.7% and 10.9% of domestically-produced head and leaf/romaine lettuces are exported, respectively (AgMRC, 2018).

Advantages and Disadvantages of Hydroponics

According to growing demand, more economical and efficient methods to produce vegetables such as lettuce are being developed. Hydroponics is a planting system in which plants are grown using a nutrient solution without mineral soil. Hydroponic systems are not as subject to geographic, climate, and time constraints as field-based systems. Better control and standardization can improve efficiency of energy and labor. Furthermore, in protected culture, integrated pest management (IPM) can be more easily integrated allowing for minimal use of pesticides (Ibrahim and Zuki, 2013), which is both economically and environmentally beneficial. Protected culture and hydroponics can also produce high quality products. Ibrahim and Zuki (2013) compared yield and quality of lettuce plants grown in three different production systems including a hydroponic system, aquaponics system, which combines aquaculture with hydroponics, and a field-based system. They reported that hydroponic lettuce yielded significantly higher biomass than both soil and aquaponics planting methods. Because of enough nutrients and minerals, lettuce in the hydroponic system had faster growth ratio and higher quality. Hydroponic lettuce had significantly better shape and color, flavor and texture based on tasters' sensory feedback. The authors concluded that the postharvest quality of lettuce from hydroponic planting method is higher and better than both from soil and aquaponics planting method (Ibrahim and Zuki, 2013).

Controlled environment agriculture in general, and hydroponics specifically, afford many benefits, but cost significantly more than conventional field production. Barbosa and others (2015) reported that in the arid southwestern US, hydroponic systems produced more than 11x the lettuce yield per area compared to field production but consumed more than 80x the amount of energy per kg produced. Increased yields per area were primarily achieved through benefits afforded by environmental controls which also led to the much greater energy use per kg of lettuce. The vast majority of the energy required (82% of total) in hydroponic systems was for heating and cooling loads. As expected, the highest energy consumption occurred in the coldest and hottest months of the year with December, January, July, and August accounting for 13%, 12%, 14%, and 14% of total annual heating and cooling energy usage, respectively. While indoor farms might result in a more even energy usage distribution throughout the year, for they apply precise light supplements, it is expected that heating and cooling would still account for the highest percentage of energy usage.

Required infrastructure is another added cost of CEA. Estimates range from $175,000 per ha for a single-layer polyethylene high tunnel structure to $830,000 for a glass roof greenhouse (Hickman, 2016). Infrastructure for indoor farms costs more. A staggering infrastructure cost was reported by German Aerospace Center (DLR). A single level for tomato cultivation of the vertical indoor farm jointly designed by DLR and Association for Vertical Farming (AVF) could cost $432.3 million per ha, and a single level for lettuce cultivation even cost $611.8 million per ha (Zeidler et al., 2017).

In view of such a great amount of energy and infrastructure cost, increasing indoor lettuce production profitability through improving energy efficiency, space use efficiency and crop quality is necessary. A potential way to achieve these goals is through localized temperature modification which would afford growers the benefits of controlling the plant's environment but could possibly reduce overall production costs.

Effects of Air Temperature on Plant Growth

The importance of temperature for proper plant development and optimal yield has been well-established in the literature. Sub- or supra-optimal air temperature often leads to sub-cellular level disruption, and eventually physiological disorders, on a variety of plant species. Al-Khatib and Paulsen (1999) reported that high air temperatures can lead to deleterious effects on plant photosynthetic and thylakoid membranes. Temperature also affects rubisco and other carbon metabolism enzymes which influence plant growth directly (Berry and Raison, 1981). In addition to structural and enzymatic damage, the physiological response of lettuce plants to supra-optimal ambient temperatures has been well-documented. Madariaga and Knott (1951) reported that when the ambient temperature exceeded 21.1 °C, head lettuce initiated bolting, or a rapid internode elongation followed by flower initiation. Whitaker and others (1974) reported that lettuce grown with ambient temperature higher than 21 °C promoted stalk elongation, puffy heads, and bitterness. Gent (2016) grew hydroponic lettuce under natural sunlight with two different air temperature treatments in a greenhouse (10°C/20 °C). Both relative growth rate and fresh weight under warm temperature treatment was higher than the cool treatment, but under cool temperature, dry matter content was higher. Sugar, malic acid, and potassium were significantly higher under the cool temperature than under the warm temperature. However, the concentration of nitrate was 40% higher under the warm treatment compared to the cool temperature. These results indicated that temperature changes the composition and nutrient uptake of lettuces.

To address the question of air temperature, Marsh and Albright (1991) designed a computer program, SEARCH, analyzing growth model to find the optimization of daytime air temperature. They used a computer model to analyze growth parameters, the value of crop produced, and costs of environmental modification to produce. They reported 25 °C produces the largest crop. Their model is helpful to our understanding about lettuce temperature and requirements but is now outdated in terms of production cost estimates. In addition, their model did not consider root zone temperature (RZT) which has been shown to greatly affect plant growth.

Effects of Root-zone Temperature on Plant Growth

Several researchers have reported on the effects of RZT on growth and yield of a variety of plant species. Malcolm and others (2008) analyzed the physiological responses of rootstocks of different Prunus species which were planted at and grown at RZTs of 5 °C, 12 °C and 19 °C for 6 weeks. With RZT increasing, plant leaves developed faster and more leaves of larger size were produced at higher RZT. Maaswinkel and Welles (1987) reported that in a glasshouse trial, by keeping the RZT at 14 °C throughout the iceberg lettuce growing period, fewer open heads were formed. Jensen (1985) reported that some cultivars of butterhead lettuce which were grown in hydroponic system, bolting or seed stalk formation was reduced when RZT was below 20 °C which indicated that by cooling the root-zone to optimal levels, cool-season lettuce can be grown in the tropics. In a similar experiment, Lee and Cheong (1996) tested the response of iceberg lettuce to RZT of 15 °C and 20 °C under warm tropical condition in an aeroponic system in a rooftop greenhouse. The head only formed at 15 °C RZT and low RZT correlated with shorter shoot axis, fewer leaf numbers and higher root biomass proportion. Moreover, the root system structured differently according to RZT modification. The plants grown at 15 °C formed a 'bunchy top' root system with a lot of lateral root and uniformly distributed dense root hairs. By contrast, the plants grown at 20 °C formed a 'cylindrical' root system with thin nonuniform root hairs.

As plant morphology development diversified with RZT, plant photosynthesis can be impacted by RZT as well. It is reported that low RZT decreased tomato photosynthetic capacity (Gosselin and Trudel, 1983, He et al., 2014) and accompanied by lower stomatal conductance, reduced intercellular CO_2 concentration and even growth inhibition (Ryyppö et al. 1998). High RZT stress can also impair photosynthesis by leading to stomatal closure (He et al., 2001).

In addition to affecting plant photosynthesis process, RZT has a significant effect on water uptake. Challa (1995) reported that root zone heating has a positive effect on reducing root resistance to water flow to keep crop water balance. Berry and Raison (1981) reported low temperature is correlated with water uptake inhibition which lead to immediate leaf growth inhibition. The effects of RZT on water uptake likely also influence transpiration rates and leaf temperature. Root zone temperature has also been reported to impact leaf temperature on maize and tomato (Salah and Tardieu, 1996; Ali et al., 1996). Malcolm and others (2008) reported that leaf temperature was negative correlated with RZT for Prunus 'Golden Queen'', and with RZT increasing, leaf temperature varied from warmer than environment temperature to cooler than environment. The differences in leaf temperature were due to transpiration, suggesting that transpiration decreased with reducing RZT.

The plant water uptake influenced by RZT can induce different mineral nutrient uptake response. For strawberry, fluctuation of RZT negatively affected nutrient uptake of P, K and Mg (Gonzalez-Fuentes at al., 2016). For lettuce, mineral nutrient uptake had a positive relation to RZT but higher than optimal temperature can inactivate the activity of enzymes which regulate mineral nutrient uptake. As a result, nutrient uptake rate decreased. In addition, diverse plant species response to RZT on dry matter production in different manners. Tomato leaf synthesized more nitrogen at a low RZT (Gosselin and Trudel, 1983), and produced less shoot dry mass (He et al., 2014). However, in one study pepper was reported to assimilate less nitrogen at a low RZT (Gosselin and Trudel, 1986). Similar to pepper, Prunus synthesized less nitrogen with RZT increased (Malcolm et al., 2008).

Although root-zone and air temperatures have been studied extensively on multiple species, the only experiment to date that has been conducted on lettuce to determine the combined effects of air and RZT on lettuce was conducted by Thompson and colleagues (1998). The authors used cool-white fluorescent lamps as a sole-source light resource and planted lettuce in a floating hydroponic system. The maximum dry mass was produced at 24/24 °C(air/pond) treatment, and head size and root structure were in best condition at 24 °C RZT.

Total phenolic content of hydroponic lettuce

Phenolic compounds are characterized as antioxidants (Sytar et al., 2018), which are beneficial in preventing chronic diseases in human related to oxidative stress, such as cancer (Chu et al., 2002). Lettuce contains relatively high phenolic components, and its total phenolic content can be influenced by cultivars and environmental conditions. It is reported that cultivar type has a stronger effect on the content of phenolic compared to the environment (Sytar et al., 2018). Red-leaf cultivars contain significantly higher total phenolic content than green-leaf lettuce (Sytar et al., 2018; Perez-Lopez et al., 2018). Temperature is one of the abiotic stimulations for phenolic compounds accumulating and can stimulate oxidative stress. To defend against oxidative damage, phenolic compounds are synthesized by plants (Ortega-Garcia et al., 2009). Several studies have reported temperature influence on lettuce phenolic compounds. Zhang and others (1997) reported that fruits and lettuce grown under low temperature accumulated greater amounts of phenolic compounds. Jeong and others (2015) reported that lettuce under higher night-time temperature treatment produced higher phenolic compounds. Liu and Hawrylak-Nowak reported that lettuce produced higher phenolic content under heat stress temperature conditions (Hawrylak-Nowak et al., 2018; Liu et al., 2007). Many types of research had reported about air temperature influence on lettuce phenolic content; however, the influence of root zone temperature on lettuce polyphenolic profile has not been characterized yet.

Research Objectives

Controlled environment agriculture system is energy consuming, especially on temperature control. All lettuce experiments reviewed on previous experiments were conducted in greenhouse in which the temperature and humidity were not precisely controlled. Producers need more information on environment condition for lettuce productions to produce lettuce more profitably. Objective 1 of this research is to determine which air and root-zone temperature condition combination is the most optimal for hydroponic lettuce (*Lactuca sativa* L. ‘Rex’) growth under full-spectrum LED light. Objective 2 is to determine the effects of root zone temperature on total phenolic content of hydroponic lettuce grown under full-spectrum LED at 24 °C air temperature.

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Chapter II

Effects of air and root zone temperature on growth of hydroponic lettuce (*Lactuca sativa* L. 'Rex') grown under full spectrum LED.

Abstract

An experiment was conducted in an environmental control chamber to determine the effects of air and root-zone temperature on growth of hydroponic lettuce (*Lactuca sativa* L. 'Rex'). For each replication, lettuce seeds (*Lactuca sativa* L. 'Rex') were sown in OASIS® horticubes and grew in the greenhouse for two weeks. The seedlings were transferred to environmental control chamber. There are six replications of lettuce cultivation. Three replications are under the condition set to 20°C air temperature and 65% relative humidity, and another three replications were under 24 °C air temperature and 70% relative humidity. Each replication had five nutrient solution temperature treatment (20, 22, 24, 26, and 28 °C) and a control treatment which the plant root-zone was not artificially manipulated. Full spectrum LED lights were used to supply 15 mol m^{-2} d^{-1} of photosynthetically active radiation with a photoperiod of 18 hr d^{-1}. During each replication, lettuces were harvested after 30 DAT (days after transplant), and head fresh weight (HFW), size index (SI), root dry weight (RDW) and root length were measured. Air temperature did not influence lettuce HFW, height, or root length. Root zone temperature (RZT) influenced lettuce HFW, which reached a maximum of 167 g at Actual root zone temperature (RZT_A) 25.6 °C. Lettuce plants grown at RZT_T of 22 °C, 24 °C, and 26 °C had 11%, 15%, and 11% higher HFW, respectively, than those grown under the control treatment. Lettuce SI was influenced by both T_A and RZT. Plants grown at 24 °C T_A

having a 7% greater SI compared to those grown at 20 °C T_A and SI displayed a quadratic relationship with RZT_T. Lettuce head shape would change with different RZTs, and lettuce grow at RZT_T 28 °C produced flatter head. The root system changed with increasing root zone temperature, from long, cylindrical shape to short, clustered shape. Since 25.6 °C produced the greatest HFW and relatively large-size head and 24 °C T_A produced larger lettuce head, a 24°/25°C air/nutrient solution condition was recommended for butterhead lettuce production.

Introduction

Global population is growing rapidly (World Population Clock, 2019), particularly in urban and urbanizing areas (United Nations, 2019), and is expected to reach a peak of over 9 billion persons by 2050. Food production must continue to increase to meet this rapidly rising demand without undue exploitation and inefficient use of natural resources. Currently, agriculture accounts for approximately 80% of all fresh water usage in the United States (USDA-ERS, 2019), so emerging food production technologies should seek to conserve water and other natural resources.

A water- and mineral-conservative food production technology is controlled environment agriculture (CEA) which includes modified growing structures such as greenhouses and vertical, indoor farms (vertical farms) and resource-conservative technologies including drip irrigation, nutrient and water recirculation, and precise environmental controls (Pack, 2019). Adoption of CEA is increasing globally with 130 countries reporting greenhouse vegetable cultivation (Hickman, 2016). Vertical farming is a rapidly developing CEA technology which has great potential to address food production issues in urban and urbanizing areas (Al-Kodmany, 2018). Vertical farming utilizes completely enclosed environments and allows for much higher food yields per unit area and much lower insect and disease pressures compared to traditional agriculture or even other forms of CEA (Al-Kodmany, 2018). Precisely controlled temperature, humidity, and light allow for high-quality produce and predictable yields. Because vertical farms are completely enclosed from the surrounding environment, high construction costs, expensive environmental controls, and reliance on sole-source artificial lighting lead to high fixed and variable costs which currently limit vertical farm adoption. However, vertical farms can be located virtually anywhere which may offer unique solutions to food production challenges in the future. High-quality, predictable yields are essential for continued improvement and adoption of vertical farms. Since the growing environment can be precisely controlled in such operations, prescribing optimal production factors is of utmost importance.

The effects of temperature on plant growth and development have been widely reported in the literature for a number of species including lettuce. When grown in sub-optimal RZT, tomato leaf growth and expansion was inhibited, and photosynthetic capacity decreased (Gosselin and Trudel, 1983). The tomato plants produced much less shoot dry mass at low RZT (He and Yang, 2014). According to the study of Calderon et al. (2014), with ambient temperature increasing, lettuce physiological processes get higher, such as transpiration and water absorption increasing. Besides, Marsh and others (1987) reported that young lettuce plants prefer warm temperatures which aid in leaf expansion, but effects of warm air temperatures become adverse when the plant canopy is well-established due to higher respiration rates and lower net CO_2 assimilation. Supra-optimal temperatures are often cited as primary or secondary causes of physiological disorders such as tipburn (Tibbits and Rao, 1969) and bolting (Wittwer and Honma, 1979; He and Lee, 1998).

More recent studies have focused on specific physiological responses induced in plants, including lettuce, by supra-optimal temperatures. In general, heat stress leads to oxidative stress which can negatively impact cell membrane permeability (Wahid et al., 2007), and decrease photosynthetic capacity by damaging chloroplast and thylakoid membranes, PSII, and photosynthetic pigments (Ilik et al., 2000; Wahid et al., 2007). In respiratory chain reactions, oxidative stress can excite electrons which then combine with oxygen to produce reactive oxygen species (ROS), such as singlet oxygen (${}_1O_2$), hydrogen peroxide (H_2O_2), superoxide (O_{-2}) and hydroxyl radical ($OH_.$). These free radicals are toxic to the plant, damaging proteins, DNA, lipids, and membranes (Ortega-Garcia and Peragon, 2009). Plants are either directly damaged by these free radicals and/or their growth and yield are reduced because they utilize metabolic resources to combat them. As an example, H_2O_2 is specially known to cause cell damage in plants (Dresler and Maksymiec, 2013) possibly through oxidization of -SH groups of enzymatic proteins leading to enzyme inactivity (Gill and Tuteja, 2010). However, H_2O_2 can be converted to H_2O by ascorbate peroxidase or be scavenged by enzymatic antioxidants through the ascorbate-glutathione cycle. Ascorbate peroxidase is part of the ascorbate-glutathione cycle, and ascorbate acid (AsA) is the substrate of the ascorbate-glutathione cycle. The result from study of Hawrylak-Nowak and others (2018) showed that AsA content decreased in lamb’s lettuce under heat stress. This result is consistent with the increase of H_2O_2 in plants under abiotic stress.

Manipulating root-zone temperature (RZT) has been reported in the literature often as a means to improve plant growth and yields by overcoming problematic air temperatures. For example, Lee and Cheong (1996) reported that lettuce bolting was reduced when RZT was maintained below 25 °C even though air temperatures were supra-optimal. Similarly, He and Lee (1998) reported that photoinhibitory response was prevented in lettuce grown in the hot tropics if RZT was lowered to 20 °C. Similar to root-zone chilling, root-zone heating has been used to improve growth rate and/or yields when air temperatures were sub-optimal. In an early experiment on root-zone heating, increasing soil temperature reduced field lettuce production time by up to 17 days (Boxall, 1971). Economakis and Said (2002) reported increases in dry biomass of more than 30% and, a more economically important increase in fresh biomass of 60% in an NFT system, when RZT was increased to 20 °C compared to an unheated control.

To date, most vertical farm temperature recommendations are only focused on air temperature. We designed an experiment to study the effects of air and root-zone temperatures on growth and yield of butterhead lettuce (*Lactuca sativa* L. 'Rex') with special attention paid to isolating each temperature zone.

Materials and Methods

An experiment was conducted to determine the effects of air and root-zone temperatures, at a near-constant vapor pressure deficit (VPD), on lettuce growth in an indoor, hydroponic, deep water culture (DWC) system. Various root-zone temperatures (RZTs) were achieved using either thermostat-controlled aquarium heaters (Hailea® Aquarium Heater 200W, Guangdong, China) or self-made Peltier water chillers. Air temperature and relative humidity were each held constant in an environmental control chamber (Harris Environmental System, Inc. Andover, MA) which was equipped with an HVAC system and dehumidifier (Munters HC-300 desiccant dehumidifier, Munters Corporation, Amesbury, MA) for temperature and humidity control. LED light ballasts (KIND LED, K5 Series XL750) was used as the only light supplement. The KIND LED panel is divided by three spectra channels which are red, blue and white, and each channel has an intensity percentage scale from 1-100. In the experiment the light intensity was set at 8/16/16 red/ blue/ white to supply fifteen mol m$_{-2}$ d$_{-1}$ photosynthetically active radiation (PAR) on each plant during a daily 18-hr photoperiod (Morgan, 2013). Approximately 30 L min$_{-1}$ air was supplied to plant root-zones using ceramic air stones connected to an air outlet in the environmental chamber.

A split plot experimental design was used in which air temperature (T_A) was the main plot and target root-zone temperature (RZT_T) was the subplot. Two levels of T_A were 20 °C and 24° C and five levels of RZT_T were 20 °C, 22 °C, 24 °C, 26 °C, and 28 °C. A control treatment was included in which the plant root-zone was not artificially manipulated. Six 30-d experimental replications were conducted. Three replications were under the combination of 20 °C T_A and 65% RH, and three replications were under the combination of 24 °C T_A and 70% RH resulting in VPDs of 0.82 kPa and 0.89 kPa, respectively. The VPD value was calculated by VPD Calculator (University of Arizona)

Fourteen days prior to each experimental replication, lettuce seeds (*Lactuca sativa* L. 'Rex') (Johnny's Selected Seeds, Winslow, ME) were sown in OASIS® Horticubes (2.54 cm x 3.18 cm x 3.81 cm) and grown on a greenhouse bench in Auburn, AL. Seeds were irrigated with municipal water until germination, then irrigated with a complete nutrient solution (0.6 g·L$_{-1}$ 8N-15P-36K; 0.45 g·L$_{-1}$ calcium nitrate; 0.3g·L$_{-1}$ magnesium sulfate) until transplant. Fourteen days after seeding, the third true leaf of the seedlings were developed, and plants were transferred to the previously-described environmental control chamber located in the Biosystems Engineering Research Laboratory, Auburn University, Auburn, AL.

Six 42.5-L, rectangular plastic containers (58 cm x 38 cm x 20 cm; AKRO-MILS® Multi-load Tote-42.5L capacity, Akron, Ohio) were placed on aluminum shelves in the environmental control chamber. Each container was placed directly underneath one of six LED ballasts (KIND LED, K5 Series XL750). The ballasts were suspended above the containers using adjustable chains and clips attached to the aluminum shelving, 30 cm above lettuce heads. Each container was pre-insulated with white foam boards (INSULFOAM, 3-cm thickness) which were cut to fit all five sides, including the bottom of each container, and joined together at corners with aluminum flashing tape. Prior to transplanting, each container was filled with a complete hydroponic nutrient solution containing 150, 80, 200, 150, and 35 mg·L^{-1} N, P, K, Ca, and Mg, respectively from water soluble 8N-6.5P-30K (Gramp's original hydroponic lettuce fertilizer, Ballinger, TX), calcium nitrate (15.5N-0P-K, Sylvite Company) and magnesium sulfate (10% Mg). One RZT_T treatment was randomly assigned to each container and nutrient solutions were heated or chilled to the appropriate RZT_T prior to experiment initiation.

Six rectangular rafts, which were used to provide both a stable platform for lettuce plants and insulation from temperature differences between the air and nutrient solution during experimental replications, had been previously constructed using the following methodology. First, the six rafts were cut from 2.3-cm thick polystyrene sheets (GreenGuard® XPS Extruded Polystyrene Insulation Board) to fit snugly into the interior dimensions of the top lip of the plastic containers. A hole saw bit (7.6-cm) was then used to cut six circular holes (two rows of three) which were each approximately 8 cm in diameter, due to the blade width, into the polystyrene rafts. The holes were spaced 20-cm center-to-center. Excess polystyrene was then used to create 36 piece of 8-cm diameter removeable inserts using a larger hole saw bit (8.2-cm diameter). For each removeable inserts, 2.5-cm diameter circular hole was drilled by a hole saw bit (2.5-cm diameter) in its center in order to affix one lettuce plant. Each polystyrene raft was fit snugly into the top lip of each container and was resting on top of nutrient solution inside the container so that when plant-containing inserts were placed into rafts lettuce roots were suspended in nutrient solution.

At initiation of each replication, 36 lettuce seedlings were transplanted into one of 36 polystyrene inserts. Each OASIS Horticube rootball was snugly fitted into the 2.5-cm holes in each insert and then each 8-cm diameter insert with one rootball was snugly fitted into the 8-cm holes in the polystyrene rafts. A single container was considered an experimental unit and data were collected from five plants (sub-samples) per raft. The sixth plant was located at the corner of the raft adjacent to where experimental apparatus including aquarium heaters, air tubing, and/or water chiller tubing was placed into the containers and as such was not used for data collection. The other five plants were rotated to the adjacent location in the raft every sixth day after transplant (DAT) to ensure uniformity of growing conditions throughout the experiment.

During each experimental replication air and nutrient solution temperatures were recorded every hour using temperature dataloggers (UA-001-08 HOBO 8K pendant Temperature/Alarm Data Logger Onset Computer Corporation). Air temperatures were recorded at plant level for each experimental unit. Leaf temperature was measured daily using an infrared traceable thermometer gun (Model: 12777-846 Traceable® Products). Five locations on each lettuce head were randomly selected and temperature was measured while placing the infrared thermometer on the leaf surface. Solution electrical conductivity (EC) and pH were measured for each experimental unit every other day using a handheld pH/EC meter (Hl9813-6 HANNA® instruments Inc.). Solution pH had a tendency to decrease throughout the experimental replications and was therefore maintained near 5.5 by adjusting as needed with dilute potassium bicarbonate solution (3.1g/100ml). Solution EC was maintained in the range of 1.7-2.0 mS cm^{-1} by adding either municipal water or nutrient solution when EC was above 2.0 mS cm^{-1} or below 1.7 mS cm^{-1}, respectively. Each experimental replication was terminated 30 DAT.

At termination, head fresh weight (HFW), size index (SI) ([height+width1+width2]/3), and root length were measured and recorded for each subsample (individual plant). Root dry weight (RDW) was recorded after dehydrating in forced-air dryer (The Grieve Corporation, Round Lake, Illinois USA Model SC=350) for three days at 77 °C. In addition, the fresh lettuce head was frozen by liquid nitrogen and reserved in -80 °C refrigerator for later research.

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was completely randomized, and the experimental replications were treated as a random variable. The treatment design was a 2-way factorial of air temperature and water temperature. Where plots of studentized residuals and a significant covariance test indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct treatment heterogeneity. Linear and quadratic trends over water temperatures were examined using orthogonal polynomials. The control was compared to other water temperature treatments using Dunnett's method. Differences between the two air temperatures were determined using F-tests. All significances were at $\alpha=0.05$.

Results

Root-zone temperatures fluctuated and deviated slightly from targets during the experimental periods. Table 2.1 shows average actual RZT (RZT_A) for each target RZT (RZT_T) at each air temperature, along with standard deviations.

Air temperature did not influence lettuce HFW, height, or root length (Table 2.2). Head fresh weight displayed a quadratic relationship to $\mathrm{RZT_T}$, increasing from 154 g at 20 °C to a maximum of 167 g at $\mathrm{RZT_T}$ 24 °C and decreasing as temperature increased above $\mathrm{RZT_T}$ 24 °C. Lettuce plants grown at 11%, 15%, and 11% higher HFW, respectively, than those grown under the control treatment.

Lettuce SI was influenced by both $\mathrm{T_A}$ and $\mathrm{RZT_T}$. The plants grown at 24 °C $\mathrm{T_A}$ having a 7% greater SI compared to those produced at 20 °C $\mathrm{T_A}$ (Table 2.3), and SI displayed a quadratic relationship to $\mathrm{RZT_T}$, of which developed at $\mathrm{RZT_T}$ of 22 °C, 24 °C, and 26 °C had 7%, 9%, and 6% greater SI, respectively, than those grown under the control treatment (Table 2.2). There was an interaction between $\mathrm{T_A}$ and $\mathrm{RZT_T}$ on root dry weight (RDW) in which there was a quadratic relationship between $\mathrm{RZT_T}$ and RDW at 20 °C $\mathrm{T_A}$, but no significant effect of $\mathrm{RZT_T}$ on RDW at 24 °C $\mathrm{T_A}$ (Table 2.4). At both $\mathrm{T_A}$ values, RDW was higher than the control at 28 °C $\mathrm{RZT_T}$.

Discussion

Root-zone temperature influenced lettuce HFW which reached a maximum of 167 g at $\mathrm{RZT_A}$ 25.6 °C. The quadratic trend between HFW and $\mathrm{RZT_T}$ closely matches the optimum temperature ranges cited by other authors (Thompson et al., 1998). Thompson and others conducted a research on shoot and root temperature effects on ‘Ostinata’ butterhead lettuce in glass greenhouse and reported that among RZT 17, 24, 31 °C, lettuce grown at 24 °C RZT produced greatest dry mass regardless of air temperature. Moreover, both $\mathrm{T_A}$ and RZT have a significant influence on lettuce SI. The average SI of lettuce grown at 24 °C $\mathrm{T_A}$ was 24.8, significantly greater than the SI of lettuce grown at 20 °C $\mathrm{T_A}$ which was 22.2. Berry and Raison (1981) reported that low temperature inhibits plant water uptake, leading to inhibition of leaf growth. This may lead to interpret the result that lower T_A produced lettuce with lower SI. The influence of RZT on lettuce SI was quadratic, lettuce SI up to the highest 24.5 at 27.5 °C RZT_A, and closely followed by 24 at 25.6 °C RZT_A. The SI of lettuce grown at 24, 26, 28 °C are significantly greater than control treatment (Figure 2.1, 2.2). The result of HFW and SI indicated that both sub and supra-optimal RZTs are able to impact the growth of lettuce. In addition, warmer T_A could increase leaf growth, forming larger lettuce heads, and during the experiment, we can observe the lettuce grown under 20 °C T_A had thicker leaves with darker color comparing to the lettuce grown under 24 °C T_A (data not shown); this phenomenon may also indicate the lettuce growth rate is influenced by air condition.

RZT appeared to have an effect on lettuce shoot axis growth. In Table 2.2, head height of lettuce grown at RZT_T 22, 24, 26 °C were significantly greater than control. Low RZT_T 20 °C and high RZT_T 28 °C correlates with flatter lettuce head, especially for lettuce grown at RZT_T of 28 °C (Figure 2.2), indicating that lettuce head shape would change with different RZTs. Lee and Cheong (1996). examined iceberg lettuce and found that cooler RZT of 15 °C induced short shoot axis than comparing to the plants grown at the RZT of 20 °C.

For the result of lettuce RDW, both T_A and RZT have significant influence, and there was an interaction influence on RDW. For the lettuce grown at 20 °C T_A, RDW had a significantly quadratic trend with RZT_T, and the greatest RDW was at 28 °C RZT_T. For the lettuce grown at 24 °C T_A, there was no significant trend with RZT_T. However, the RDW of lettuce grown at 28 °C RZT_T was significantly greater than that at other RZTs. The influence of RZT on root dry weight also was reported by Wright et al. (2007), their results indicated that the influence of RZT depend on plant species.

Root length was only influenced by RZT. With RZT increasing, lettuce root length showed a quadratic trend and the root length of lettuce grown at RZT_T of 24, 26, 28 °C was significantly shorter than control. The data result was consistent with the phenomenon we observed (Figure 2.3, 2.4). When the RZT was relatively lower, root-zone system formed a more lateral root and had a cylindrical shape. With the RZT increasing, root length turned to be shorter. When it came to the RZT_T at 28 °C, root system had fewer lateral roots and formed a short-clustered shape, which could interpret the data result that the lettuce with the greatest RDW had the shortest root length. The phenomenon that RZT influences root system formation was also reported by Lee and Cheong (1996), where iceberg lettuce grown under 15 °C RZT formed bunchy root cluster and lettuce grown under 20 °C RZT formed cylindrical-shaped root system. Wright et al. (2007) reported that with the RZT increasing, shorter root systems were produced by some woody ornamental plants. The shorter root systems were correlated with root elongation periods. High RZTs lead to higher cell elongation rates with shorter elongation periods. As a result, plants grown at high RZT with short root elongation period produced shorter roots (Wright et al., 2007). In addition, the root system under high RZT_T 28 °C not only formed a short bunchy shape without lateral roots but also had a brown color. This phenomenon is consistent with the result reported by He and Lee (1998), and the brown color maybe due to the death of root tissues.

In addition, we observed that under 24 °C T_A, the leaves of lettuce were thinner and normal green. However, more tipburn was noticed, but the tipburn problem was not severe. This may because promoted environmental condition induces higher lettuce growth rate (Wissemeier and Zuhlke, 2002). The tipburn was more noticeable under relatively low and high RZTs, which means adequate RZT and air temperature combination can balance the growth rate and tipburn.

Overall, with root-zone temperature increasing, root system changes from long, cylindrical shape to short, bunchy shape. And 25.6 °C produced the greatest HFW and relatively large-size head. Besides, 24 °C T_A produced larger lettuce head. In conclusion, a 24°/25°C air/nutrient solution condition was recommended for butterhead lettuce production.

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Table2.1 Actual Root-zone Temperature for Each Air Temperature Treatment

Target Temperature [z] (°C)	Air Temperature (°C)		
	20	24	Combination of 20 and 24
Control	19.4 ±0.4 [y]	21.4±0.7	20.4±1.2
20	21.2±1.1	20.3±0.7	20.8±1.1
22	25.1±0.6	21.8±0.6	23.4±1.8
24	26.6±0.5	24.6±1.1	25.6±1.3
26	27.8±0.3	27.2±1.1	27.5±0.9
28	30.4±0.8	29.4±1.3	29.9±1.2

[z] Nutrient solution was heated continuously to target temperature using an aquarium heater (Hailea® Aquarium Heater 200W, Guangdong, China).

[y] Actual temperature was measured hourly using a HOBO logger (Onset Computer Corporation)

Table 2.2 Effects of nutrient solution temperature on head fresh weight, size index, height, root length of *Latuca sativa* L 'Rex' grown in Deep Water Culture for a 30-day Period in the environmental control chamber set at both 20 and 24-degree air temperatures

Target Temperature [z]	Actual Temperature [y]	Head Fresh Weight (g)	Size Index	Height (cm)	Root Length (cm)
control	20.4	141.50	22.5	13.9	56.87
20	20.8	153.70	22.8	14.4	55.07
22	23.4	159.3*[x]	23.3	15.8***	58.70
24	25.6	167.13***	24.0***	15.3*	50.03*
26	27.5	159.03*	24.5***	16.3***	46.8***
28	29.9	142.77	23.9**	14.9	35.93***
Sign.[w]		Q***	Q***	Q*	Q***

[z] Nutrient solution was heated continuously to target temperature using an aquarium heater (Hailea® Aquarium Heater 200W, Guangdong, China).

[y] Actual temperature was measured hourly using a HOBO logger (Onset Computer Corporation)

[x] Least squares means comparisons of the control to water temperature treatments using Dunnett's method at $P < 0.05$ (*) or 0.001 (***).

[w] Significant (Sign.) quadratic (Q) trend excluding the control using using orthogonal contrasts at $P < 0.001$ (***).

Table 2.3 Effects of air temperature on size index of *Latuca sativa* L ‘Rex’ grown in Deep Water Culture for a 30-day Period in the environmental control chamber

Air Temperature (°C)	Size Index
20	22.2b[z]
24	24.8a

[z] Least squares means comparisons between air temperatures using a F-test P < 0.05.

Table 2.4 Effects of nutrient solution temperature on root dry weight of *Latuca sativa* L ‘Rex’ grown in Deep Water Culture for a 30-day Period in the environmental control chamber set at both 20 and 24-degree air temperatures

Target Temperature[z]	Actual Temperature[y]	RDW/g (Air 20)	RDW/g (Air 24)
control	20.4	0.2433	0.1880
20	20.8	0.2247	0.2007
22	23.4	0.2427	0.1880
24	25.6	0.2620a[x]	0.1707b
26	27.5	0.2847a	0.1867b
28	29.9	0.3453a***[w]	0.2441b*
Sign.[v]		Q***	NS

[z] Nutrient solution was heated continuously to target temperature using an aquarium heater (Hailea® Aquarium Heater 200W, Guangdong, China).

[y] Actual temperature was measured hourly using a HOBO logger (Onset Computer Corporation)

[x] Least squares means comparisons air temperatures for each treatment using F-tests at P < 0.05.

[w] Least squares means comparisons of the control to water temperature treatments for each air temperature using Dunnett's method at P < 0.05 (*) or 0.001 (***).

[v] Not significant (NS) or significant (Sign.) quadratic (Q) trend excluding the control using using orthogonal contrasts at P < 0.001 (***).

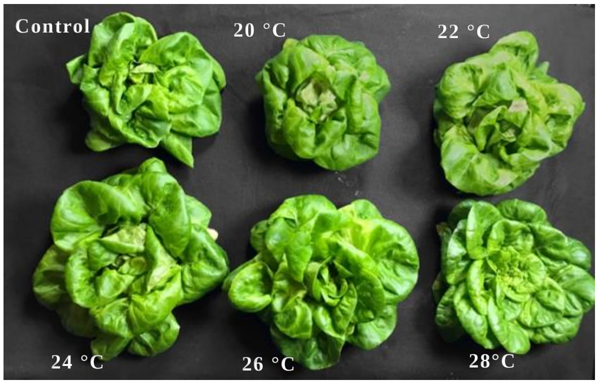

Figure 2.1. Lettuce heads grown at 20 °C air temperature. ‘Rex’ butterhead lettuce grown at root zone temperature of 24, 26, 28 °C produced larger head compared to control treatment.

Figure 2.2. Lettuce heads grown at 24°C air temperature. ‘Rex’ butterhead lettuce grown at root zone temperature of 24, 26, 28 °C produced larger head compared to control treatment.

Figure 2.3. Root system of ‘Rex’ butterhead lettuce grown at 20 °C air temperature with five levels of root zone treatment and control treatment.

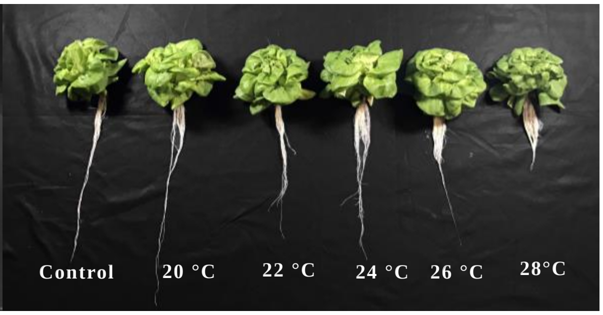

Figure 2.4. Root system of ‘Rex’ butterhead lettuce grown at 24 °C air temperature with five levels of root zone treatment and control treatment.

Chapter III

Effects of root zone temperature on total phenolic content of hydroponic lettuce (*Lactuca sativa* L. ‘Rex’) grown under full spectrum LED

Abstract

Fruits and vegetables, including lettuce, represent a source of antioxidant phenolic compounds for humans which neutralize reactive oxygen species, protecting cells against damage. Generally, accumulation of phenolic compounds in plants can be stimulated by biotic and abiotic stress, such as high light intensity, sub-optimal or fluctuating temperature condition. We conducted an experiment to determine effects of root-zone temperature on total phenolic content of hydroponic lettuce (*Lactuca sativa* L. ‘Rex’) grown under full spectrum LED in a controlled-environment chamber. Air temperature and relative humidity were maintained at 24 °C and 70%, respectively. Full spectrum LED lights were used to supply 15 mol m_{-2} d_{-1} of photosynthetically active radiation with a photoperiod of 18 hr d_{-1}. Root-zone temperature target treatments were 20, 22, 24, 26, and 28 °C. A control treatment was also used which allowed water temperature and air temperature to equilibrate. Lettuce plants were grown for 30 d in deep water culture and harvested. Total phenolic content was then assayed according to Folin-Ciocalteu method. Total phenolic content of ‘Rex’ lettuce was not affected by root-zone temperature.

Introduction

Nowadays people are much more careful about diet health and getting benefit from phytochemical contained in vegetables and fruits. Phenolic compounds can reveal plant quality and potential effects on human health (Sytar et al., 2018), and they are often considered as secondary metabolites in plants. The definition of secondary metabolites is ‘small organic molecules produced by an organism that is not essential for their growth, development, and reproduction’ (Monfil and Casas-Flores, 2014).

Phenolic compounds contribute to plant color, flavor and astringency, distributing in plant tissues (Swanson, 2003) and they are a group of small molecules which structures containing hydroxylated aromatic rings, and it is suggested that most phenolic compounds are byproducts of aromatic amino acid phenylalanine metabolism process (Swanson, 2003). Based on their chemical constitution, different subgroups can be categorized, such as phenolic acids, flavonoids, anthocyanins, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids (Gan et al., 2019; Swanson, 2003). Phenolic acids and flavonols are the main classes of phenolic compounds found in different varieties of lettuce, flavones and anthocyanins are the secondary main classes of phenolic compounds, and anthocyanins only exist in red varieties (Alarcon et al., 2016).

Phenolic compounds are characterized as antioxidants (Sytar et al., 2018). It is reported that lettuce contains relatively high phenolic components and antioxidant activity, benefiting to prevent chronic diseases related to oxidative stress, such as cancer (Chu et al., 2002). Comparing with the influence of the environment, cultivars have a stronger effect on the content of phenolic compounds in lettuce (Sytar et al., 2018). It is reported that green-leaf lettuce cultivars contain significantly lower phenolic compounds concentration compared to red-leaf cultivars (Sytar et al., 2018; Perez-Lopez et al., 2018). However, adjusting the growing condition is still a process to influence total phenolic content. Generally, accumulation of phenolic compounds can be stimulated by biotic and abiotic stress, such as high light intensity, sub-optimal or fluctuating temperature conditions (Sytar et al., 2018; Perez-Lopez et al., 2018).

For lettuce under temperature stress, oxidative stress would be stimulated, and oxidative stress excite electrons in plant respiratory chain reaction. The excited electrons combine with oxygen and produce reactive oxygen species such as singlet oxygen, hydrogen peroxide, superoxide and hydroxyl radical. These reactive oxygen species have adverse effects on plant body including damaging proteins, DNA, lipids, and membranes. To defend the oxidative damage, plant activates phenylalanine ammonia-lyase to catalyze phenylpropanoid pathway and biosynthesis of phenolic compounds such as flavonoids and phenylpropanoids started (Ortega-Garcia et al., 2009). Besides, phenolics can be degraded by polyphenol oxidase, and polyphenol oxidase can be degraded by peroxidase. Activities of both polyphenol oxidase and peroxidase can be increased under biotic and abiotic stresses (Boo et al., 2011).

Some research studies showed their result that phenolic compounds influenced by temperature. Zhang reported that fruits and lettuce grown under low temperature accumulate more anthocyanins, which are major phenolic compounds for plants (Zhang et al., 1997). Jeong et al. designed a lettuce experiment with 22 °C day-time temperature and 4, 12, 20 °C night-time temperature respectively and found lettuce treated with higher night-time temperature produced higher phenolic compounds (Jeong et al., 2015). Liu et al. (2007) also reported that higher phenolics content was produced under higher temperature conditions. Hawrylak-Nowak et al. (2018) reported that the content of phenolic compounds in lettuce leaves was higher under heat stress air condition compared with lettuce grown under normal temperature conditions.

Butterhead lettuce is one of the most commonly consumed lettuce types worldwide. Until now, the influence of air and root zone temperature on the butterhead lettuce polyphenolic profile has not been characterized yet to authors’ knowledge.

Materials and methods

Samples used in this experiment were picked from lettuce head reserved in -80 °C refrigerator (Chapter II). Three replications of lettuce grown under 24 °C air temperature and 70% relative humidity condition were measured. Each replication has 20, 22, 24, 26, 28 °C root zone temperature treatment and control samples and each treatment had five subsamples. Leaves from lettuce head were freeze-dried (HARVESTRIGHT Medium Scientific Freeze Dryer) from E. W. Shell Fisheries Center.

The total phenolic content extraction assay was followed by Li and others (2010). Freeze-dried lettuce samples had the ribs removed and were weighed for 0.25 gram into 30ml Falcon tube. 20ml of methanol, water, acetic acid mix with proportion 85:15:0.5, v/v was added into falcon tube as extraction solvent. The extraction tubes were vortexed for 30 s and then sonicated for 5 min. Tubes were incubated at room temperature for 20 min and vortexed again for another 30 s. The tubes were centrifuged at 3000 rpm for 10 min and the supernatants were transferred into 2ml centrifuge tubes with pipette and stored at -80 °C.

The total phenolic assay was based on the Folin-Ciocalteu method (Singleton and Rossi, 1965). Gallic standard stock solution was prepared by dissolving 50mg gallic acid (SIGMA-ALDRICH Company) with 50 ml HPLC grade Milli-Q water. The stock solution represented 1000mg/L gallic acid. 7.5% (w/v) sodium bicarbonate solution and 1:10 solution of Folin-Ciocalteu's Reagent (SIGMA-ALDRICH Company) was prepared. Gallic acid working solution was made (1ml Gallic acid stocking solution+9 ml HPLC water) for standard curve. One blank and 6 standards prepared as outlined in Table 3.1 were each added 40 ul into 2ml centrifuge tubes. Extracted sample solution each added 40ul into 2ml centrifuge tubes. All items for the standard curve and samples had 4 replicates. 200ul 1:10 diluted FCR and 1.5 ml Milli-Q water were added into each of the centrifuge tubes with standards or sample extraction. All the tubes were vortexed and incubated in a 40 °C water bath for 2 minutes and then vortexed again and left for 8 minutes at room temperature. 160ul of 7.5% sodium bicarbonate solution was added into each of the tubes. The tubes with mixture were vortexed again and incubated at 40 °C water bath for 30 minutes. The mixture was shaken at 25 °C and the absorbance of the sample was measured at 765nm using spectrophotometer.

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was completely randomized, and the experimental runs were treated as a random variable. The treatment design was a 2-way factorial of air temperature and water temperature. Where plots of studentized residuals and a significant covariance test indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct treatment heterogeneity. Linear and quadratic trends over water temperatures were examined using orthogonal polynomials. The control was compared to other water temperature treatments using Dunnett’s method. Differences between the two air temperatures were determined using F-tests. All significances were at $\alpha=0.05$.

Result

Table 3.2 shows the result of the total phenolic content of lettuce grown under 24 °C T_A. When the T_A was set to 24 °C, total phenolic content of lettuce grown at 22 and 24 °C RZT_T was significantly lower than control. Among all the RZT treatment, there was no significant trend between temperature and total phenolic content.

Discussion

The total phenolic content of the lettuce grown at $\mathrm{RZT_A}$ 21.4 and 24.6 °C was significantly lower compared to the control, which is consistent with the report from Zhang and others (1997). They reported that lettuce grown under low temperature accumulates more phenolic compounds. However, combining with the result that no significant phenolic content trend related to $\mathrm{RZT_T}$ indicated that RZT doesn’t have a significant influence on butterhead lettuce phenolic content biologically. According to several studies, temperature conditions affect plant total phenolic content, such as heat stress (Hawrylak-Nowak et al., 2018). It is reported that total phenolic content increased with temperature increasing (Jeong et al., 2015; Liu et al., 2007). However, green-leaf lettuce contains lower phenolic content when compared to red-leaf lettuce (Sytar et al., 2018). Lettuce cultivar ‘Rex’ chosen for the current study contain lower total phenolic content when compared to red-leaf lettuce. Visually, ‘Rex’ butterhead lettuce appeared light green and may contain lower anthocyanin concentration when compared to other green-leaf cultivars, this may explain the results of RZT which apparently did not alter total phenolic content of the current study.

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Table 3.1 Gallic Acid Standard Curve

Concentration (mg/L) GA	Working Stock (100mg/L) (ul)	Milli-Q water (ul)
0	0	200
20	40	160
40	80	120
60	120	80
80	160	40
100	200	0

Table 3.2 Effects of nutrient solution temperature on total phenolics content of *Latuca sativa* L 'Rex' grown in Deep Water Culture for a 30-day Period in the environmental control chamber set at 24-degree air temperature

Target Temperature[z] (°C)	Actual Root-Zone Temperature [y] (°C)	Total Phenolics Content (mg/100g dry weight)
Control	20.3	573.8
20	21.4	531.9*[x]
22	21.8	665.8
24	24.6	496.8**
26	27.2	561.9
28	29.4	660.9
Sign.[w]		NS

[z] Nutrient solution was heated continuously to target temperature using an aquarium heater (Hailea® Aquarium Heater 200W, Guangdong, China).

[y] Actual temperature was measured hourly using a HOBO logger (Onset Computer Corporation)

[x] Least squares means comparison of control to temperatures using the simulated method at $P < 0.05$.

[w] Non-significant (NS) trend using orthogonal contrasts at $P < 0.05$.

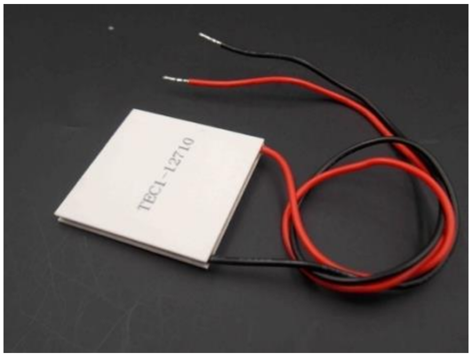
Appendix

Self-Made Peltier Cooler

The experiment running under 24 °C air temperature had five levels of root zone temperature treatment (20, 22, 24, 26, 28 °C) and a control which the root zone temperature was not manipulated artificially. The root zone temperature treatment of 20 and 22 °C required to be cooled down to target root zone temperature. We made two Peltier coolers for root zone temperature of 20 °C and 22 °C, respectively, hanging on the side of each water container, to provide lettuce a root zone condition cooler than ambient air.

Material

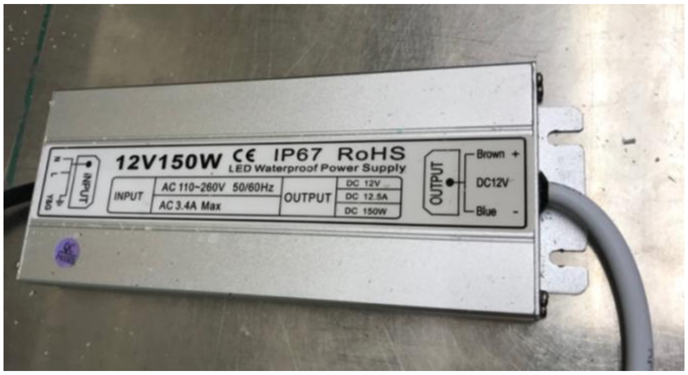
Thermoelectric cooler Peltier chip (TEC1-12710 12v/10a)



CPU Cooler (ARCTIC Alpine 11 Plus CPU Cooler)

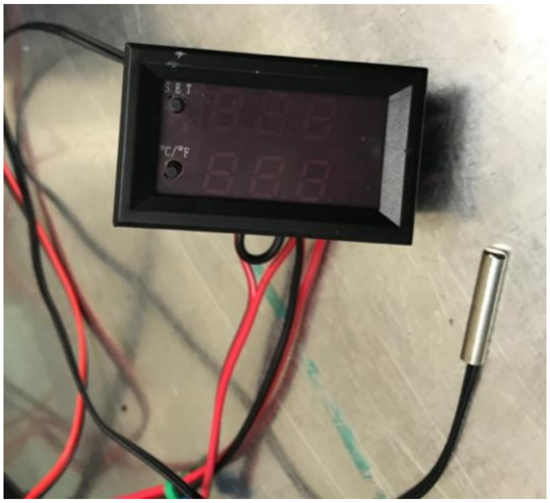
Water pump (Decdeal Submersible Water Pump DC 12V 5W Model: QR50E)

Power supply (LED Driver Waterproof IP67 Power Supply150W 12V DC)



Thermal paste (Arctic MX-4 Thermal Compound Paste)

Thermostat

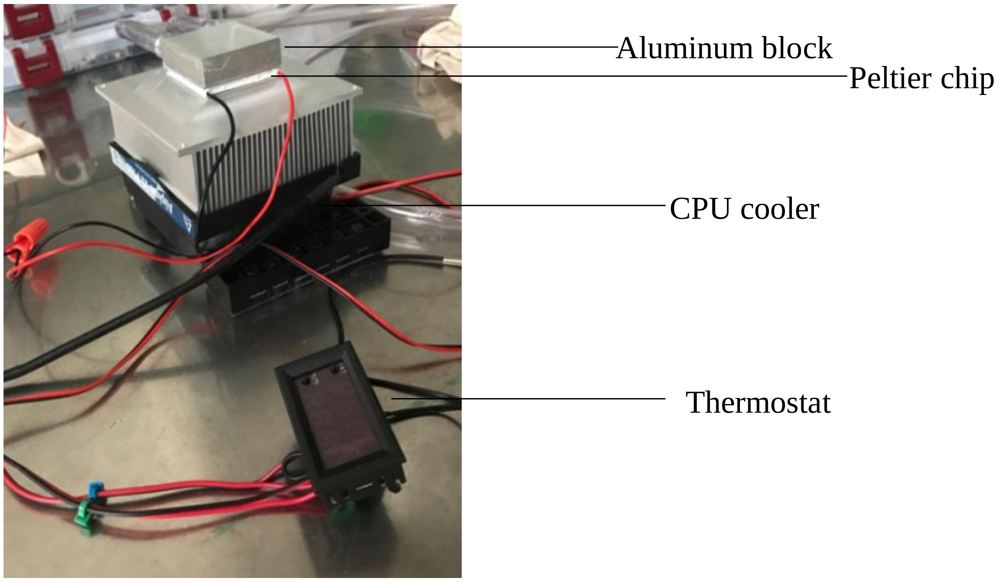


Plastic clear tube

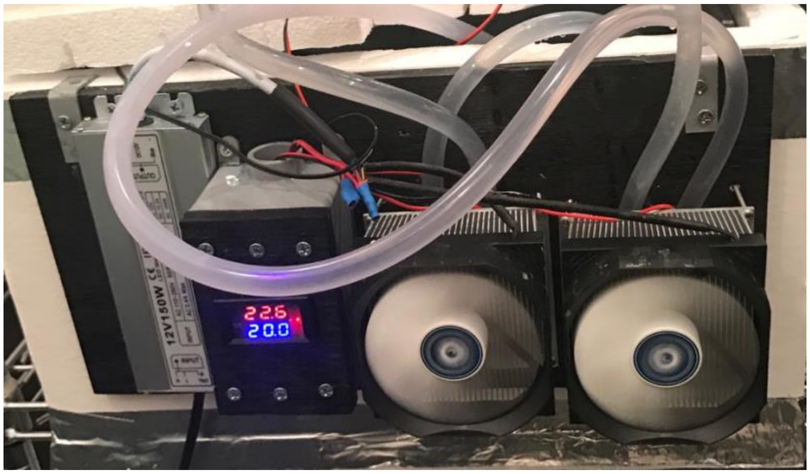
Aluminum block

Method

1. Joint the hot side of the Peltier chip with CPU cooler using thermal paste.
2. Joint the cold side of the Peltier chip with the aluminum block using thermal paste.
3. Link thermostat, pump, and power supply tandemly.
4. Link CPU cooler with power tandemly (The thermostat and pump parallelly connected with power supply)
5. Connect clear plastic tube to the pump and water-cooling aluminum block.
6. Fix the CPU cooler, thermostat, and power supply on a wooden board and hang the board on the side of the water container.
7. Fix water pump at the inside bottom of the water container corner and place the thermostat sensor close to the water pump.
8. Place another plastic clear tube end without linking to the water pump to another container side.



Self-made Peltier cooler



Self-made Peltier cooler under LED light (The number of CPU cooler depends on temperature difference between on ambient temperature and target water temperature)