

ABSTRACT

PAN, ZHIWEI. Celtuce production characteristics under nutrient and water stress. (Under the direction of Dr. Christopher C. Gunter).

Celtuce (*Lactuca sativa* L. var. *augustana*), is one of the six morphologically different lettuce (*Lactuca sativa* L.) cultivar groups. It is also known as stem lettuce, asparagus lettuce, Chinese lettuce or *woh sun* in Chinese. Celtuce was first introduced to the U.S. by W. Atlee Burpee & Co. in 1941 but has raised limited interest since then. Although three lettuce cultivar groups head, leaf, and romaine counted together with the second largest vegetable production of the U.S. in 2017, there is no documented information about celtuce production from USDA National Agricultural Statistics Service. Very few studies have been conducted on celtuce. The characteristics, habits, and growing methods of celtuce remain unclear.

In a hydroponic production study, two commercial celtuce cultivars ‘Celtuce’ and ‘Summer 38’ were selected for morphological and physiological differences that could potential affect the yield and quality. The study was conducted in 2018 and 2019 in a self-constructed hydroponics system with 32 troughs and 8 independent nutrient solution supplemental systems in the Marye Anne Fox Science Teaching Laboratory Greenhouses on North Carolina State University campus, Raleigh, NC. Plants constantly received 8 different nutrient levels as the combination of 4 nitrogen levels (50, 100, 150, 200 mg·L⁻¹) and 2 boron levels (0.5, 1 mg·L⁻¹). Photosynthetic rates were measured 5 days after transplanting to hydroponics system and then measured every 10 days. When harvested, stem fresh weight, dry weight, length, diameter, hardness, soluble solids content, nitrate content and leaf fresh weight, dry weight, number, soluble solids content, and nitrate content were measured. For cultivars, cultivar ‘Celtuce’ had overall significantly ($P \leq 0.05$) better yield and quality than ‘Summer 38’ under same condition. For nutrient treatments, higher nitrogen treatments resulted in higher photosynthetic

rates during all cultivation period. 100 and 150 mg·L⁻¹ nitrogen levels significantly ($P \leq 0.05$) increased the yield and quality of celtuce by increasing the stem fresh weight, stem length and diameter, and soluble solids content. Plants under 50 mg·L⁻¹ had significantly lower yield ($P \leq 0.05$). 200 mg·L⁻¹ nitrogen treatment, however, not only didn't improve the yield and quality but also increased the nitrate content of celtuce. 1 mg·L⁻¹ boron level significantly ($P \leq 0.05$) strengthened the quality of celtuce by increasing the diameter and hardness of stem. These results indicated that hydroponics system is feasible for celtuce and appropriate nitrogen and boron levels can increase the yield and quality of celtuce.

In the deficit irrigation study, two celtuce cultivars 'Celtuce' and 'Summer 38' were applied with 4 irrigation regimes (25%, 50%, 75%, 100% of 300 ml/pot everyday) in long-term. The study was conducted in 2018 and 2019 in pots in Fox Greenhouse in NCSU, Raleigh, NC. Both cultivars suffered significant ($P \leq 0.05$) reduction in yield and quality when under 25% irrigation regimes. 'Celtuce' could maintain similar yield and quality as full irrigation when under 75% irrigation regime while 'Summer 38' could maintain even under 50% irrigation regimes. These results indicated that deficit irrigation is potentially practical for celtuce production in saving water input and having similar yield.

The two studies together demonstrated the potential of celtuce to be cultivated domestically, either with hydroponics system for faster production or conventionally with deficit irrigation for economically friendly production.

© Copyright 2019 by Zhiwei Pan

All Rights Reserved

Celtuce production characteristics under nutrient and water stress.

by
Zhiwei Pan

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Horticultural Science

Raleigh, North Carolina
2019

APPROVED BY:

Dr. Christopher C. Gunter
Chair of Advisory Committee

Dr. Helen T. Kraus

Dr. Kathryn A. Boys

DEDICATION

To my parents Rong Pan and Dongying Zheng.

BIOGRAPHY

Zhiwei Pan was born on October 5th, 1996 to Rong Pan and Dongying Zheng as the only child of his family, in Zhejiang province of southeast china. Raised up there, studied there. During his three year of middle school, he had a small field behind the dormitory where he experienced cultivation, growing, management and harvest of sorts of vegetables. The top important stuff during that period was watering his vegetables every day. That experience strongly inspired him to get systematic knowledge about vegetable and agriculture. After passing university attending exam in 2014, Zhiwei attended Zhejiang University, had a year of general studying in agriculture and decided to major in horticulture. During the year of sophomore, Zhiwei had a chance into fruit harvest lab to conduct research on ‘The effect of Methyl jasmonate (MeJA) and controlled atmosphere in prolonging storage time and increasing aroma of peach’. After three years of university experience, he participated in ‘3+2’ cooperated program between Zhejiang University and North Carolina State University and got the chance to study abroad for the first time.

Zhiwei spent his senior year in NCSU, got his bachelor’s degree in horticulture from Zhejiang University in 2018. Now he is pursuing his master’s degree in vegetable ecology about celtuce hydroponics production under the guidance of Dr. Christopher C. Gunter.

ACKNOWLEDGMENTS

First, I am deeply grateful to my parents for their financial and mentally support all over the years. There is no way I can have the chance study abroad without their support.

My sincere appreciation goes to my committee chair Dr. Christopher C. Gunter, and committee members Dr. Helen T. Kraus and Dr. Kathryn A. Boys. The study would not be conducted without their help.

My special appreciation goes to Matthew Waldschmidt for his great help and hard work!

I must thank Blake Wently and Bryan Bayers for helping me construct the complicated hydroponics system.

I would like to thank Dr. Hernandez and his group members, Christian Collado, Hans Spalholz, and Brandon Huber for their guidance and helps in measuring lighting and photosynthesis parameters.

I thank Dr. Grady Miller for his guidance in data analysis.

I also thank Xiaonan Shi, Paige Herring, Paul and Lauren Redpath for their helps and suggestions.

Also, I would like to thank my friends Mengtao Chen and Changrui Zeng for their mentally supports and encouragements in the past two years.

TABLE OF CONTENTS

LIST OF TABLES vi

LIST OF FIGURES vii

Chapter 1: Effects of Nitrogen and Boron on Celtuce (*Lactuca sativa L. var. augustana*)

in Hydroponics Production..... 1

Introduction..... 1

Methodology..... 4

Results..... 8

Discussion..... 11

Citation..... 14

Chapter 2: The Influences of Long-term Deficit Irrigation on Yield and Quality of

Celtuce (*Lactuca sativa L. var. augustana*) 28

Introduction..... 28

Methodology..... 30

Results..... 32

Discussion..... 33

Citation..... 36

LIST OF TABLES

CHAPTER 1. Effects of Nitrogen and Boron on Celtuce (*Lactuca sativa L. var.*

augustana) in Hydroponics Production

Table 1.	Concentrations of elements injected into nutrient solutions of different treatments (tmt)	18
Table 2.	Two-way analysis of variance with orthogonal contrasts of nitrogen and boron for yield and quality data for celtuce under eight different nutrient treatments grown in hydroponics system for two consecutive trials	19
Table 3.	Main effect of cultivars ‘Celtuce’ and ‘Summer 38’ on stem and leaves characteristics for two trials (mean \pm SE)	20

CHAPTER 2. The Influences of Long-term Deficit Irrigation on Yield and Quality of

Celtuce (*Lactuca sativa L. var. augustana*)

Table 1.	Two-way ANOVA for a fully factorial arrangement (2 cultivars x 4 irrigation regimes) of treatments for the combined experimental replicates of two trials	39
Table 2.	Main effect of cultivars on stem and leaves characteristics (mean \pm SE)	40

LIST OF FIGURES

CHAPTER 1. Effects of Nitrogen and Boron on Celtuce (*Lactuca sativa L. var.*

augustana) in Hydroponics Production

- Figure 1. ‘Cracked stem’ (left) and ‘hollow stem disorder’ (right) on celtuce observed in preliminary study..... 21
- Figure 2. Celtuce in hydroponics system (A), celtuce stem with skin peeled off (B), 1 cm-thick stem samples (C), leaves samples and stem samples (D) 22
- Figure 3. CO₂ exchange rate of 5 days after transplant (DAT), 15DAT, 25DAT, 35DAT, and 45DAT, \pm SE under 50 mg·L⁻¹ N, 100 mg·L⁻¹ N, 150 mg·L⁻¹ N, 200 mg·L⁻¹ N of the first trial experiment 23
- Figure 4. Stem FW (fresh weight) (A), stem length (B), stem diameter (C), S/L (stem/leaves ratio = stem fresh weight/leaves fresh weight) (D), stem SC (soluble solids content) (E), stem NC (nitrate concentration) (F), \pm SE under 50 mg·L⁻¹ N, 100 mg·L⁻¹ N, 150 mg·L⁻¹ N, 200 mg·L⁻¹ N 24
- Figure 5. Leaf FW (fresh weight) (A), leaf number (B), leaf SC (soluble solids content) (C), leaf NC (nitrate concentration) (D), \pm SE under 50 mg·L⁻¹ N, 100 mg·L⁻¹ N, 150 mg·L⁻¹ N, 200 mg·L⁻¹ N 25
- Figure 6. Stem diameter (A) and hardness (B), \pm SE under 0.5 mg·L⁻¹ boron treatment, 1 mg·L⁻¹ boron treatment 26
- Figure 7. Stem SC (A) and NC (B), \pm SE of different part of stem of two trials. 27

CHAPTER 2. The Influences of Long-term Deficit Irrigation on Yield and Quality of

Celtuce (*Lactuca sativa L. var. augustana*)

Figure 1. The morphology changes of celtuce cultivar ‘Celtuce’ from transplanting (1 week before deficit irrigation conducted) to 0 week (deficit irrigation started) and to 5 weeks after deficit irrigation (harvest) 41

Figure 2. The morphology changes of celtuce cultivar ‘Summer 38’ from transplanting (1 week before deficit irrigation conducted) to 0 week (deficit irrigation started) and to 5 weeks after deficit irrigation (harvest)..... 42

Figure 3. Stem fresh weight (A), stem diameter (B), stem sugar content (C), leaves fresh weight (D), and leaves number (E), \pm SE under 25% irrigation regime (75 ml/pot everyday), 50% (150 ml/pot everyday), 75% (225 ml/pot everyday), 100% (300 ml/pot everyday)..... 43

Figure 4. Stem fresh weight (A), stem diameter (B), stem sugar content (C), and leaves fresh weight (D), \pm SE for two cultivars ‘Celtuce’ and ‘Summer 38’ under 100% irrigation regime (300 ml/pot everyday), 75% (225 ml/pot everyday), 50% (150 ml/pot everyday), 25% (75 ml/pot everyday)..... 44

CHAPTER 1

Effects of Nitrogen and Boron on Celtuce (*Lactuca sativa* L. var. *augustana*) in Hydroponics Production

Introduction

Celtuce (*Lactuca sativa* L. var. *augustana*), is one of the six morphologically different lettuce (*Lactuca sativa* L.) cultivar groups (Nonnecke, 1989). It is also known as stem lettuce, asparagus lettuce, Chinese lettuce or *woh sun* in Chinese (Schneider, 2001). Celtuce was first introduced to the U.S. by W. Atlee Burpee & Co. in 1941 but has received limited interest since then (Anderson and Helgeson, 1941). Combined, the three lettuce cultivar groups head, leaf, and romaine account for the second largest vegetable in production of the U.S. in 2017; however, there is no documented information about celtuce production from USDA National Agricultural Statistics Service (2018). In China, the stems of celtuce are used in salads and stir fry (personal observation) where it is valued for its high vitamin C and carbohydrate (USDA FoodCentral, 2019a & 2019b). The leaves are not eaten. Celtuce is good for cultivation for nutritional and agricultural value. However, very few studies have been conducted evaluating celtuce production; thus, the characteristics, habits, and growing methods of celtuce remain unclear.

Hydroponics is a comprehensive technique of growing plants without soil. The term was first used by Dr. W.F. Gericke of the University of California in 1937 who started commercially using this production system in 1929 (Mason, 1990). Although the term hydroponics is strictly defined as growing plants in water culture (Resh, 2004), the definition has broadened to encompass all types of soilless cultivation. Compared to traditional soil culture, hydroponics production has higher yields and superior quality; less input of water and fertilizers; faster crop

rotations and year-long production potential (Resh, 2015). Due to these advantages, the area of hydroponically grown production has expanded rapidly in the past decades. In the early 1960s, only 100 hectares (247 acres) of hydroponic production were commercially used internationally (Mason, 1990) and by the 2010s, Netherlands had more than 25,000 acres of greenhouse production, Canada had around 2800 acres, the U.S. had 1500 acres and the rapidly expanding of hydroponics in China is believed to be more than 3100 acres (Resh, 2015). Much of the research on this crop group has been conducted on lettuce production with hydroponics system; no research on the production of celtuce in hydroponics could be found. The feasibility of growing celtuce with hydroponics system is still uncertain.

Nitrogen is the second largest required element by plants, incorporated in proteins, nucleic acid, chlorophyll, co-enzymes, phytohormones and secondary metabolites for around 1-5% of total plant dry matter (Hawkesford, 2012). Nitrogen fertilizers are generously used as an agricultural practice to reach maximum yield of horticultural crops (Cardoso et al., 2015). According to Food and Agriculture Organization (FAO), around 110 million tons of nitrogen fertilizers were applied globally in 2015 to increase the yield of crop (FAO, 2017). Nevertheless, the utilization rate of applied nitrogen fertilizer by crops was less than 64% and could be as low as 8% in rapeseeds (Sylvester-Bradley and Kindred, 2009). The over applied nitrogen fertilizer not only increases unnecessary agricultural input, but also results in environmental problems. Furthermore, the over applied nitrogen is accumulated in vegetables as nitrate, among which lettuce cultivars have the highest level with the concentration more than 1000 mg·kg⁻¹ (MAFF, 1999; Wolf and Wasserman, 1972). The daily acceptable intake of nitrate is set as 0-3.7 mg·kg⁻¹ body weight by the Joint Expert Committee of the Food and Agriculture (JECFA) Organization of the FAO and WHO and as no-observed-adverse-effect-level (NOAEL) of 7 mg·kg⁻¹ body

weight by the EPA (EFSA ANS Panel, 2017; Liu et al., 2014; U.S. EPA, 1991). Consumption of vegetables with high accumulated nitrate may increase the risks of nitrate-induced syndromes and other severe pathologies (Liu et al., 2014; Stagnari et al., 2015). In hydroponics, constant and high nitrogen concentrations are used to improve plant growth, which leads to excessive nitrate absorption and accumulation by plants (Sago and Shigemura, 2018). Thus, it is essential to use appropriate levels of nitrogen fertilizers to produce vegetables, especially lettuce, as well as control the concentration of nitrate in the tissue. To date, no research has been conducted with celtuce to define the suitable nitrogen level in soil or hydroponics, nor on the nitrate accumulation of celtuce.

Boron is essential for normal growth and maturation of lettuce (McHargue and Calfee, 1932). Rather than directly synthesized into cell wall, boron influences the formation of the cell wall by affecting the incorporation of proteins, pectins and/or precursors into cell wall structure. Therefore, boron deficiency has been associated with cell wall abnormalities and negatively affects the morphology and physiology of lettuce cultivars. New growth leaves are deformed, crinkled, and stunted, while older leaves are chlorotic and with tip burn (Crisp et.al, 1976; Gunter, et.al, 2009; McHargue and Calfee, 1933). The cell wall can be severely altered and resulting in cracked stems and petioles in celery (*Apium graveolens*), stem corkiness and hollow stem order in Chinese cabbage (*Brassica chinensis*) (Broadly et al. 2012; Shorrocks, 1997). In a preliminary study, the cracked stems and hollow stem disorder on celtuce were observed in an earlier study and seriously diminished the crop commercial values (Fig. 1). Further understanding of the effects of boron on celtuce quality needs to be understood.

In an effort to understand celtuce production and nutrition, this study was conducted to 1) determine the feasibility of growing celtuce with hydroponics system; 2) establish suitable levels

of nitrogen fertilizer regime to provide low nitrate concentration, high yield and quality of celtuce products; 3) certify the functions of boron on celtuce quality.

Methodology

GROWTH CONDITIONS. A study evaluating the effect of different N and B concentration on growth of two cultivars of celtuce was conducted in the Marye Anne Fox Science Teaching Laboratory (Fox) Greenhouses on North Carolina State University (NCSU) campus, Raleigh, NC (35.787 N, 78.673 W) between 28 Nov. 2018 and 5 Feb. 2019. The second trial of this study was conducted between 10 Jan. 2019 and 22 Mar. 2019. The environment within the greenhouse was automatically maintained by integrated environmental controller (Priva Maximizer; Priva inc., Camarillo, CA) with day temperatures of 23.8 ± 3 °C and night temperature of 18.3 ± 3 °C. Humidity, while not maintained, fluctuated between 50% and 70%.

A nutrient film technique hydroponics system with 32 troughs was made with commercial PVC pipe (PVC D 2729 Sewer pipe; Charlotte Pipe, Charlotte, NC) with length of 1.22 m (48 in) and diameter of 10.16 cm (4 in), each of which could support 4 plants with a 7.62 cm (3 in) diameter hole and 22.86 cm (9 in) interval between holes. Troughs were parallel to each other with 27.94 cm (11 in) between each on the greenhouse bench, which with dimensions of 9.45 m (372 in, length) x 1.22 m (48 in, width) x 0.81 m (32 in, height). A 2 cm (0.78 in) in height wooden frame was insert under the inlet end of the troughs to give a 0.94% slope from inlet to outlet.

Four, randomly assigned troughs, shared nutrient inflow and outflow plumbing and were connected to an independent nutrient tank [a 75.71-L (20 gal) resin trash can (Vented Brute[®] 20-gal gray; Rubbermaid Commercial Products[®], Atlanta, GA)]. One submersible oil-filled pump

(model 1-A; Little Giant, Oklahoma City, OK) supplied nutrient solution to the inlet end of each trough at 2.68 L·min⁻¹.

NUTRIENT SOLUTIONS. Four different nitrogen and two different boron concentrations were evaluated for this study (Table 1). Two commercially available fertilizers: Peters[®] 5-11-26 Hydroponic Special fertilizer (Everris NA Inc., Dublin, OH) and YaraLiva[®] CALCINIT[®] 15.5-0-0 (Yara North America, Inc., Tampa, FL) were used to provide different nitrogen levels and other necessary macro- and micronutrients. Boric acid (Powder/Certified ACS) (Fisher Scientific, Fair Lawn, NJ) was used to provide different boron levels. Granular 4 mesh anhydrous calcium chloride (J.T.Baker[®], Phillipsburg, NJ) was used to adjust the nutrient solutions to provide the same calcium concentrations for all groups. All chemicals were separately dissolved as condensed solutions to avoid potential precipitants, then added into corresponding nutrient tank where the water was pre-injected. After dilution, each nutrient tank had 70 L solution. Water was added every day to maintain a constant volume of nutrient solution. Nutrient solutions were renewed every 10 days.

SEEDLINGS PRODUCTION. All celtuce transplants were produced in the Fox greenhouses. Two commercially available celtuce cultivars ‘Celtuce’ and ‘Summer 38’ (*Lactuca sativa* var. *asparagina*) (Kitazawa Seed Co., Oakland, CA) were selected for this study as they had different morphology and characteristics. In the first trial, seeds were sowed into 1.5 in rockwool plugs (A-OK starter plugs; Grodan[®], Roermond, Netherlands) one seed per plug at 28 Nov. 2018. All seedlings received constant 170 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental photosynthetically active radiation besides natural light which was composed with 50% red light and 50% blue light (Pro 325 LED lighting system; LumiGrow, Emeryville, CA) from 7 am to 7 pm every day and watered with basic nutrient solution (treatment1, Table 1) every day. In the second trial, seeds were sowed on

10 Jan. 2019. Seedlings were transplanted into hydroponics system 25 days after sowing for each trial.

EXPERIMENT DESIGN. This study was arranged in a split-plot design with the nutrient treatments (main plots) arranged in a randomized complete block design (RCBD) with four blocks. Eight nutrient treatments were a combination of four nitrogen levels (50 mg·L⁻¹, 100 mg·L⁻¹, 150 mg·L⁻¹, 200 mg·L⁻¹) and two boron levels (0.5 mg·L⁻¹, 1 mg·L⁻¹) (Table 1). Two celtuce cultivars (split-plot) were randomly assigned within each whole plot. Two plants of each cultivar were transplanted into one trough. Transplanting occurred 25 days after each sowing, on 22 Dec. 2018 and 5 Feb. 2019 respectively. Azatin® O (OHP, Inc., Mainland, PA) or BotaniGard® (BioWorks, Inc., Victor, NY) was applied to plants weekly to manage pest populations.

DATA COLLECTION. Photosynthetic rates were measured by Multiphase Flash™ Fluorometer (LI-6800 Portable Photosynthesis System; LI-COR®, Inc., Lincoln, NE) at 5 d after transplanting (DAT), 15 DAT, 25 DAT, 35 DAT and 45 DAT. For each measurement, one fully expanded leaf for each celtuce cultivar within each nutrient treatment and block was measured. The parameters of the photosynthesis system were set as 400 ppm leaf chamber CO₂, 60% leaf chamber humidity, 23.8 °C leaf chamber temperature, 380 μmol·s⁻¹ leaf chamber air flow rate, 10000 rpm fan speed. The light intensity for measurement was based on the average light intensity that plants received inside the greenhouse during the whole trial and calculated with the equations:

Light intensity = Average daily light integral of the growing period / average day time of the growing period.

Inner light intensity = light intensity x greenhouse transmission rate.

Daily light integral was determined from daily light integral and high-resolution maps of the U.S. (Faust and Logan, 2018). The transmission rate of a typical glass greenhouse was 70%. The light intensity parameters of fluorometer were set as $330 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for first trial and $455 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for second trial, consists of 50% red light and 50% blue light. The photosynthetic rates were represented as CO_2 exchange rate. However, due to a fluorometer malfunction the last three measurements of the second trial. Only the data of the first trial was used and analyzed in this study.

Harvest occurred on 45 DAT for both trials. Roots were cut free of the stem and leaves at the rockwool cube. Every leaf of a plant longer than 1 cm was counted (leaf number), removed, weighed and recorded as leaf fresh weight (FW). Next, leaves were cut into cross-sectional 0.5 cm-wide slices, from which one leave slice sample (5 g) was randomly collected (Fig. 2D). Five drops of sap were pressed from each sample with a commercial garlic press to test soluble solids content (SC) with pocket refractometer (PAL-1; ATAGO, Minato, Tokyo, Japan) and nitrate concentration (NC) with nitrate meter (LAQUAtwin- NO_3 -11; HORIBA Scientific, Kyoto, Japan). Stem length, widest diameter, and weight were also measured. Then, 2 mm-thick portion of the stem was removed (Fig. 2B) and two measurements for hardness were taken using a fruit hardness tester (GY-3; Graigar Instruments, Shenzhen, Guangdong, China) with 8 mm diameter pressure head and 10 mm insertion depth. The stem was cut out into 1 cm-thick sections, from top to bottom, each of which was cut out a 1 cm-thick stem sample (Fig. 2C & D). Three samples were tested for soluble solids content (SC) and nitrate concentration (NC) separately with pocket refractometer and nitrate meter, respectively. All leaves and stem tissues, including residuals after pressing, were collected and dried out in 71°C (160°F) oven for 48 hours for dry weight (DW).

Three heads, each of two different brands of four commercially available conventionally-grown lettuce cultivars, ‘romaine’ lettuce (Roth Farms, Bella Glade, FL), ‘red leaf’ lettuce (Roth Farms, Bella Glade, FL), ‘iceberg T’ lettuce (Tanimura & Antle Fresh Foods, Inc., Salinas, CA) and ‘iceberg B’ lettuce (Bonipak Produce Co., Santa Maria, CA) were bought from local grocery store. The outermost and inner most portion of each head were measured twice for soluble solids content (SC) and nitrate concentration (NC) with the sample method mentioned above. The SC and NC content of iceberg lettuce of two brands were close enough to be merged as one as group ‘iceberg’.

DATA ANALYSIS. All data were analyzed with the GLIMMIX procedure in SAS (v 9.4; SAS Institute, Cary, NC). Two trials were analyzed independently. The model used nutrient treatment (main plot), cultivar (split-plot) and their interaction as fixed effects, with blocks as random effects. The Satterthwaite option (Schaaljie et al., 2002) was used as degree of freedom approximation. Regression analysis was conducted to analyze the effects of nitrogen (linear, quadratic) and boron (linear). Contrast statements were used to conduct means separation tests with significant level $P \leq 0.05$ by comparing the following nitrogen treatment groups: ‘50 vs 100’, ‘50 vs 150’, ‘50 vs 200’, ‘100 vs 150’, ‘100 vs 200’, ‘150 vs 200’.

Results

In both trials, no plant died and no ‘cracked stem’ or ‘hollow stem disorder’ was observed.

CULTIVAR. Significant differences ($P \leq 0.05, 0.01, 0.001$) in stem FW, stem length, stem hardness, number of leaf, leaf SC, and stem FW to leaf FW (S/L) ratio were observed between cultivar ‘Celtuce’ and ‘Summer 38’ for both trials (Table 2). Other characteristics, including

specific stem characteristics of interest (stem diameter, stem SC and stem NC), of cultivar ‘Celtuce’ and ‘Summer 38’ were either significantly different for one trial only or had no significant differences for both trials (Table 2). However, the interaction of cultivar and nutrient treatment was not significant for any characteristics of celtuce. ‘Celtuce’ had significantly higher ($P \leq 0.05$) stem FW, stem length, leaf SC and S/L ratio than ‘Summer 38’ (Table 3). While stem hardness and leaf number of ‘Celtuce’ were significantly lower ($P \leq 0.05$) (Table 3).

NUTRIENT TREATMENT (NITROGEN). The photosynthetic rates of all celtuce plants grown with all nitrogen treatments had a general trend that first increased rapidly (before 15DAT) and then increase more slowly (after 15DAT) (Fig. 3). Throughout the growing season, celtuce supplied 50 mg·L⁻¹ N had significantly lower photosynthetic rates than celtuce supplied 150 mg·L⁻¹ and 200 mg·L⁻¹ N.

Significant differences ($P \leq 0.05, 0.01, 0.001$) in stem FW, stem length, stem diameter stem hardness; stem SC and stem NC; and S/L ratio were observed among 4 nitrogen treatments (Table 2). Basically, those characteristics were linearly correlated with nitrogen treatments, but sometimes were quadratically correlated (Table 2). The stem FW and diameter of plants grown with 50 mg·L⁻¹ N were significantly lower than other treatments in both trials (Fig. 4A & B). The stem FW and diameter of plants with 200 mg·L⁻¹ N were significantly lower than plants with 100 and 150 mg·L⁻¹ N in trial 2 but not trial 1, but in trial 2, plants grown with 100, 150 mg·L⁻¹ N had significantly higher stem diameter (Fig. 4C). Only plants fertilized with 50 mg·L⁻¹ N in trial 2 had significantly lower S/L ratio (Fig. 4D). Plants under 50 mg·L⁻¹ nitrogen treatment had higher soluble solids content (SC) than those fertilized with higher N rates (Fig. 4E). The stem SC of trial 1 was lower than commercially available lettuces while the stem SC of trial 2 was higher. The stem NC of plants with 50 mg·L⁻¹ N in trial 1 and 50 and 100mg·L⁻¹ N in trial 2 were

significantly lower than other treatments in the same trial. Plants under all nitrogen treatments of both trials had higher nitrate concentration than commercially available lettuces (Fig. 4D).

The leaf FW, leaf number, NC of leaves differed ($P \leq 0.05, 0.01, 0.001$) among 4 nitrogen treatments (Table 2). Those characteristics were linearly and quadratically correlated with nitrogen treatments. The leaf FW was significantly higher when fertilized with 100 and 150 $\text{mg}\cdot\text{L}^{-1}$ N than with 50 $\text{mg}\cdot\text{L}^{-1}$ N in both trial and with 200 $\text{mg}\cdot\text{L}^{-1}$ N trial 2 (Fig. 5A). these data support the lower S/L ratio of celtuce when fertilized with 50 $\text{mg}\cdot\text{L}^{-1}$ N. Significantly fewer leaf number were observed in plants fertilized with 50 $\text{mg}\cdot\text{L}^{-1}$ N in both trials (Fig. 5B). Even though the leaf SC were not significant different between N rates, both cultivars of celtuce had consistently lower SC than any commercially available lettuce (Fig. 5C). Oppositely, leaf NC was consistently higher, and even significantly higher when fertilized with 100, 150 and 200 $\text{mg}\cdot\text{L}^{-1}$ N (Fig. 5D).

NUTRIENT TREATMENT (BORON). Boron rate significantly affected the stem diameter and stem hardness in trial 1 but not in trial 2 (Table 2). The stem diameter and hardness of plants fertilized with 0.5 $\text{mg}\cdot\text{L}^{-1}$ boron treatment were significantly lower in trial 1 and numerically lower in trial (Fig. 6A and B). In trial 2, even though the plants under 1 $\text{mg}\cdot\text{L}^{-1}$ boron treatment had no significantly higher stem diameter and hardness, the means of those plants were still higher than plants under 0.5 $\text{mg}\cdot\text{L}^{-1}$ boron treatment.

DISTRIBUTION PATTERN IN STEM. The SC and NC levels differed within the stem (Fig. 7). The SC levels increased linearly while the NC concentrations decreased from the base to the top of the stem. The bottom part of the stem had significantly lower stem soluble solids content but higher nitrate concentration in both trials. In contrast, the top part of the stem had significantly higher SC but lower NC.

Discussion

Generally, two cultivars presented with similar responses toward same treatments. But with the same cultivation time in hydroponics, ‘Celtuce’ had higher yield and quality than ‘Summer 38’, which requires longer growing season than ‘Celtuce’ (Kitazawa Seed Co., 2008a; 2008b). The stem FW, stem length and diameter are all critical characteristics that affect the commercial value of celtuce plants, which were observed with better results in ‘Celtuce’ in the study. Remarkably, the S/L ratio was also higher in ‘Celtuce’, which demonstrates the efficiency of celtuce plants to use nutrition supplies to produce, transfer and storage photosynthetic products for stem formation. Cultivar ‘Celtuce’ displayed with physiological and agricultural advantages and may be more promising for commercial production.

The influences of nitrogen treatments were overall consistent among all indexes. The nitrogen rates of 100 and 150 mg·L⁻¹ produce higher yield and better quality of celtuce (stem hardness and soluble solids content). The high biomass accumulation of celtuce plants under these treatments was due to high photosynthetic rates. Similar findings between nitrogen application and plants photosynthetic rates were reported in the earlier study that the chlorophyll concentration of lettuce () was proved as linearly increased with increasing N application (Mahlangu et al., 2014).

When compare the soluble solids content of celtuce stems and leaves with commercially available lettuce, celtuce stems generally had higher soluble solids content and nitrate concentration than conventional lettuce when under optimal condition (trial 2) while the leaves of celtuce always had lower SC than conventional lettuce. This result that stem of celtuce has

better quality may address the reason that celtuce is mainly produced for its enlarged stem (Schneider, 2001).

The observation that celtuce plants of trial 2 had lower NC than trial 1 because plants received higher light intensity and longer photoperiod from natural light during trial 2, agreed on the report that double the light intensity from $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ could significantly reduce the nitrate concentration of lettuce by 40-55% (Zhang et al., 2018). The comparisons of NC between hydroponic-cultivated celtuce and conventional lettuce reflected the potential disadvantage of hydroponics production that plants could accumulate extremely high nitrate due to the continuous supplement of nitrogen. The method conducted by Zhou et al. (2018) to control the nitrate concentration of hydroponic-cultivated lettuce without diminish the yield and quality by reducing the nitrogen supply from 8mM to 0.5 mM in the last 7 days of cultivation before harvest may could be adapted into celtuce hydroponics production.

In the study, boron strengthened the celtuce characteristics that related with cell wall formation. Higher boron treatment ($1 \text{ mg}\cdot\text{L}^{-1}$) increased the stem diameter and stem hardness, either significantly different or not. Despite that the boron effect on celtuce was not reported in earlier studies, a few experiments were conducted to demonstrate the relation between boron deficiency/toxicity and plants leaf and stem morphology. The influence of boron on lettuce cell wall was reported as indirectly affects the auxin activity first (Crisp et al., 1976). Significant correlation was observed as boron-deficiency (with $0.001 \text{ mg}\cdot\text{L}^{-1}$ borax in nutrient solution) increased auxin-1 activity, which resulted in plants possessed with soft cell wall, macroscopically represented with necrosis, tip burn and other morphological abnormality (Crisp et al., 1976). Hajiboland et al. (2012) studied the effects of boron deficiency on the morphology of turnip, red cabbage, tobacco and celery, reporting that boron deficiency significantly

decreased the shoot dry weight of all crops, and the abnormal cell wall of celery was observed to have higher collenchyma cells. The over application of high concentration of boron, however, significantly reduced the yield of celery when the concentration was higher than $9.8 \text{ mg}\cdot\text{L}^{-1}$ and the yield of lettuce when the concentration was higher than $1.3 \text{ mg}\cdot\text{L}^{-1}$ (Francois, 1988). The boron treatment set in my study was close to the threshold of boron for lettuce in Francois's report (1988). While no toxicity of boron on celtuce was observed, the further understanding of the effect of boron on celtuce may needs to be addressed.

Citation:

Anderson, J.M., and R.C. Helgeson. 1941. Celtnce-a new vegetable for '42. *Seed world* 50 (9): 22.

Broadly, M., P. Brown, I. Cakmak, Z. Rengel and F.J. Zhao. 2012. Functions of nutrients: micronutrients, p. 191-248. In: Marschner, P (eds). *Marschner's mineral nutrition of higher plants*. 3rd ed. Academic press, Waltham, MA.

Cardoso, F.L., J.L. Andriolo, M.D. Picio, M. Piccin, and J.M. Souza. 2015. Nitrogen on growth and yield of lettuce plants grown under root confinement. *Horticultura brasileira*, 33: 422-427.

Crisp, P., G.F. Collier, and T.H. Thomas. 1976. The effect of boron on tipburn and auxin activity in lettuce. *Scientia horticulturae*, 5: 215-226.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food),

Mortensen, A., F. Aguilar, R. Crebelli, A. Di Domenico, B. Dusemund, M.J. Frutos, P.

Galtier, D. Gott, U. Gundert-Remy, C. Lambre, J-C Leblanc, O. Lindtner, P. Moldeus, P.

Mosesso, A. Oskarsson, D. Parent-Massin, I. Stankovic, I. Waalkens-Berendsen, R.A.

Woutersen, M. Wright, P. van den Brandt, C. Fortes, L. Merino, F. Toldra, D. Arcella, A.

Christodoulidou, F. Barrucci, A. Garcia, F. Pizzo, D. Battacchi and M. Younes. 2017.

Scientific Opinion on the re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. *EFSA Journal* 2017;15(6):4787, 123 pp.

<<https://doi.org/10.2903/j.efsa.2017.4787>>

FAO. 2017. World fertilizer trends and outlook to 2020. <<http://www.fao.org/3/a-i6895e.pdf>>

Faust, J., and J. Logan. 2018. Daily Light Integral: A Research Review and High-resolution Maps of the United States. *Hortscience*, 53(9): 1250–1257.

- Francois, L.E. 1988. Yield and quality responses of celery and crisphead lettuce to excess boron. *Journal of the American society for horticultural science*, 113(4): 538-542.
- Gunter, C.C., B.E. Whipker, I. McCall, and J. Garzón. 2009. *Acta horticulture*, 843: 171-176.
- Hajiboland, R., F. Farhanghi, and M. Aliasgharpour. 2012. Morphological and anatomical modifications in leaf, stem and roots of four plant species under boron deficiency conditions. *Anales de boologia*, 34: 15-29.
- Hawkesford, M., W. Horst, T. Kichey, H. Lambers, J. Schjoerring, I.S. Moller, and P. White. 2012. Functions of macronutrients, p. 135-189. In: Marschner, P (eds). *Marschner's mineral nutrition of higher plants*. 3rd ed. Academic press, Waltham, MA.
- Kitazawa Seed Co. 2018a. Celtuce. < https://www.kitazawaseed.com/seed_068-163.html>
- Kitazawa Seed Co. 2018b. Summer 38. < https://www.kitazawaseed.com/seed_554-163.html>
- Liu, C.W., Y. Sung, B.C. Chen, and H.Y. Lai. 2014. Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (*Lactuca sativa* L.). *international journal of environmental research and public health*, 11: 4427-4440.
- MAFF: Ministry of Agriculture, Fisheries and Food. 1998. Nitrate in vegetables, food surveillance information sheet no. 158. HMSO publication center, London.
- Mahlangu, R.I.S., M.M. Makobo, D. Sivakumar, P. Soundy, and J. Jifon. 2016. Lettuce (*Lactuca sativa* L.) growth, yield and quality response to nitrogen fertilization in non-circulating hydroponic system. *Journal of plant nutrition*, 39(12): 1766-1775.
- Mason, J. 1990. *Commercial hydroponics*. Kangaroo Press, Kenthurst, NSW.
- McHargue, J.S., and R.K. Calfee. 1932. Effect of boron on the growth of lettuce. *Plant physiology*, 7(1): 161-164.

- McHargue, J.S., and R.K. Calfee. 1933. Further Evidence That Boron Is Essential for the Growth of Lettuce. *Plant physiology*, 8(2): 305-313.
- Nonnecke, I.L. 1989. Vegetable production. Van Nostrand Reinhold, New York, NY, p. 75-89, 450-466.
- Resh, H.M. 2004. Hydroponics food production: a definitive guidebook of soilless food-growing methods, 6th edition. CRC Press, Boca Raton, FL.
- Resh, H.M. 2015. Hydroponics for the home grower. CRC Press, Boca Raton, FL.
- Sago, Y. and A. Shigemura. 2018. Quantitative nitrate management reduces nitrate accumulation in hydroponics butterhead lettuces grown under artificial lighting. *HortScience*, 53(7): 963-967.
- Schaaljie, G.B., J.B. McBride, G.W. Fellingham. 2002. Adequacy of approximations to distributions of test statistics in complex mixed linear models. *Journal of agricultural, biological, and environmental statistics*, 7(4): 512-524.
- Schneider, E. 2001. Vegetables from amaranth to zucchini: the essential reference. William Morrow, New York, NY, p. 461-465.
- Shorrocks, V.M. 1997. The occurrence and correction of boron deficiency. *Plant and soil*, 1993: 121-148.
- Stagnari, F., A. Galieni, and M. Pisante. 2015. Shading and nitrogen management affect quality, safety and yield of greenhouse-grown leaf lettuce. *Scientia horticultrae*, 192: 70-79.
- Sylvester-Bradley, R., and D.R. Kindred. 2009. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *Journal of experimental botany*, 60(7): 1939-1951.
- USDA, FoodData Central. 2019. Celtuce, raw. <<https://fdc.nal.usda.gov/fdc-app.html#/food-details/169990/nutrients>>

- USDA, FoodData Central. 2019. Lettuce, raw. <<https://fdc.nal.usda.gov/fdc-app.html#/food-details/342618/nutrients>>
- USDA, National Agricultural Statistics Service. 2018. Vegetables 2017 Summary. ISSN: 0884-6413 < <https://downloads.usda.library.cornell.edu/usda-esmis/files/02870v86p/5425kd81z/9019s517t/VegeSumm-02-13-2018.pdf>>
- U.S. EPA, National Center Protection Agency. 1991. Nitrate; CASRN 14797-55-8. Integrated risk information system (IRIS), <https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0076_summary.pdf>
- Wolff, I.A., and Wasserman, A.E. 1972. Nitrates, nitrites and nitrosamines. *Science*, 177: 15-19.
- Zhang, X., D.X. He, G.H. Niu, Z.N. Yan, and J.X. Song. 2018. Effects of environment lighting on the growth, photosynthesis and quality of hydroponic lettuce in a plant factory. *International journal of agricultural and biological engineering*, 11(2): 33-40.
- Zhou, W.W., Y. Chen, H.P. Xu, X. Liang, Y. Hu, C.W. Jin, L.L. Lu, and X.Y. Lin. 2018. Short-term nitrate limitation prior to harvest improves phenolic compound accumulation in hydroponic-cultivated lettuce (*Lactuca sativa* L.) without reducing shoot weight. *Journal of agricultural and food chemistry*. 66(40): 10353-10361.

Table 1. Concentrations of elements injected into nutrient solutions of different treatments (tmt)

	tmt1	tmt2	tmt3	tmt4	tmt5	tmt6	tmt7	tmt8
	50N	100N	150N	200N	50N	100N	150N	200N
	0.5B	0.5B	0.5B	0.5B	1B	1B	1B	1B
Nutrients	Macronutrients (mg·L ⁻¹)							
N-NO ₃	49.74	96.19	142.64	189.12	49.74	96.19	142.64	189.12
N-NH ₄	0.26	3.81	7.36	10.88	0.26	3.81	7.36	10.88
P	44.50	44.50	44.50	44.50	44.50	44.50	44.50	44.50
K	200	200	200	200	200	200	200	200
Ca	200	200	200	200	200	200	200	200
Mg	28.74	28.74	28.74	28.74	28.74	28.74	28.74	28.74
S	37.09	37.09	37.09	37.09	37.09	37.09	37.09	37.09
	Micronutrients (mg·L ⁻¹)							
B	0.5	0.5	0.5	0.5	1	1	1	1
Fe	2.78	2.78	2.78	2.78	2.78	2.78	2.78	2.78
Zn	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Cu	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Mn	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Mo	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09

Table 2. Two-way analysis of variance with orthogonal contrasts of nitrogen and boron for yield and quality data for celtuce under eight different nutrient treatments grown in hydroponics system for two consecutive trials

Effect	T ^z	df	Stem							Leaf						
			FW	DW	Length	Diameter	Hardness	SC	NC	FW	DW	No.	SC	NC	S/L ^y	
Nutrient (N) ^x	1	7	*	NS	NS	NS	**	***	***	*	NS	NS	NS	***	NS	
	2	7	***	NS	***	***	NS	NS	***	***	NS	*	NS	*	**	
Nitrogen	Linear	1	1	*	NS	*	NS	**	***	***	*	NS	NS	NS	***	NS
		2	1	**	NS	**	*	NS	NS	***	*	NS	*	NS	**	*
	Quadratic	1	1	*	NS	NS	NS	NS	**	**	**	*	*	NS	*	NS
		2	1	***	NS	***	***	NS	NS	NS	***	NS	*	NS	NS	**
	Boron	1	1	NS	NS	NS	*	*	**	NS	*	*	NS	NS	NS	NS
		2	1	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	**	NS
Cultivar (C) ^w	1	1	***	NS	***	NS	***	NS	NS	NS	NS	***	*	NS	***	
	2	1	***	*	***	NS	***	NS	*	*	NS	***	***	NS	***	
N × C	1	7	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
	2	7	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	

^zT= trial.

^yS/L ratio = stem FW/leaf FW.

^xNutrient treatments were the combination of 4 nitrogen levels (50, 100, 150, 200 mg·L⁻¹) and 2 boron levels (0.5, 1 mg·L⁻¹).

^wCultivar treatments represents two commercially available cultivar ‘Celtuce’ and ‘Summer 38’.

NS, *, **, ***, denote non-significant at $P > 0.05$, significant at $P \leq 0.05, 0.01, 0.001$, respectively.

Table 3. Main effect of cultivars ‘Celtuce’ and ‘Summer 38’ on stem and leaves characteristics for two trials (mean \pm SE)

Trial Cultivar	Trial1		Trial2	
	Celtuce	Summer 38	Celtuce	Summer 38
Stem FW (g)	79.1 \pm 4.6a ^z	57.0 \pm 4.5b	71.1 \pm 4.0a	44.7 \pm 4.0b
Stem DW (g)	2.05 \pm 0.16a	2.09 \pm 0.15a	1.82 \pm 0.24a	1.14 \pm 0.24b
Stem length (cm)	18.0 \pm 0.6a	12.7 \pm 0.6b	13.0 \pm 0.6a	7.9 \pm 0.6b
Stem diameter (mm)	29.0 \pm 0.7a	30.3 \pm 0.7a	31.3 \pm 0.8a	31.7 \pm 0.8a
Stem hardness (kg·cm ⁻²)	4.8 \pm 0.1b	5.2 \pm 0.1a	5.7 \pm 0.1b	6.3 \pm 0.1a
Stem SC (°Bx)	2.1 \pm 0.1a	2.2 \pm 0.1a	3.2 \pm 0.1a	3.2 \pm 0.1a
Stem NC (mg·L ⁻¹)	3264.8 \pm 158.3a	3442.9 \pm 158.1a	2935.7 \pm 97.2b	3289.3 \pm 99.1a
Leaf FW (g)	189.9 \pm 8.1a	208.1 \pm 8.0a	203.7 \pm 8.5b	222.7 \pm 8.5a
Leaves DW (g)	9.66 \pm 0.36a	9.45 \pm 0.36a	11.40 \pm 0.86a	11.44 \pm 0.86a
Leaf number (no.)	21.4 \pm 0.6b	25.2 \pm 0.6a	22.0 \pm 0.8b	26.3 \pm 0.8a
Leaf SC (°Bx)	1.9 \pm 0.1a	1.8 \pm 0.1b	2.6 \pm 0.1a	2.3 \pm 0.1b
Leaf NC (mg·L ⁻¹)	3909.8 \pm 141.6a	3872.6 \pm 140.1a	3359.5 \pm 202.1a	3452.6 \pm 202.3a
S/L (%) ^y	40.7 \pm 1.4a	26.7 \pm 1.3b	33.8 \pm 1.2a	19.2 \pm 1.2b

^zMeans \pm SE followed by the same letter within each index of each trial are not significantly different ($\alpha = 0.05$) and represent the average value and standard error of one trial, eight nutrient treatments, two plants and four blocks ($n = 64$).

^yS/L ratio = stem FW/leaf FW.



Figure 1. 'Cracked stem' (left) and 'hollow stem disorder' (right) on celtuce observed in preliminary study.



Figure 2. Celtuce in hydroponics system (A), celtuce stem with skin peeled off (B), 1 cm-thick stem samples (C), leaves samples and stem samples (D).

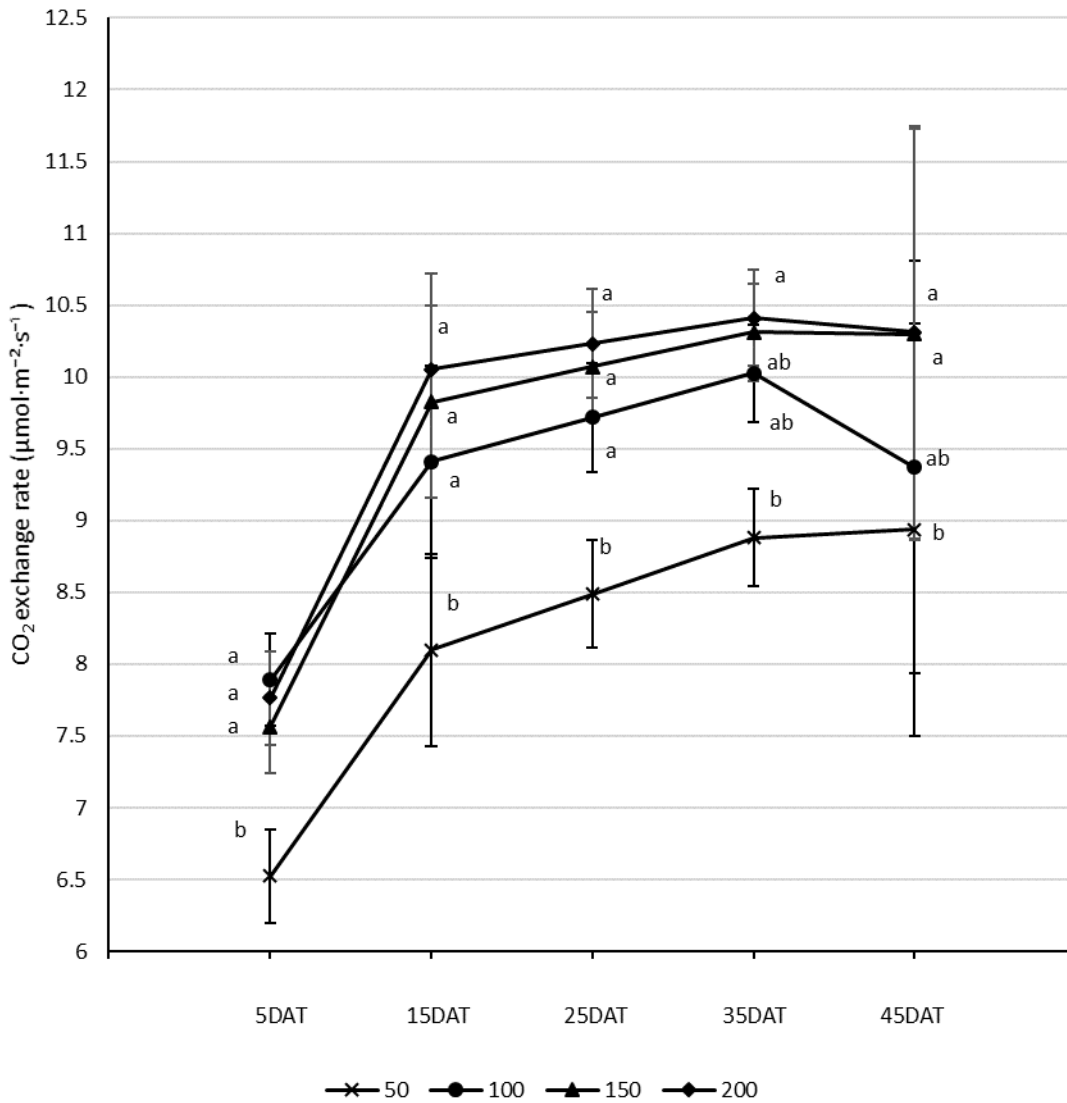


Figure 3. CO₂ exchange rate of 5 days after transplant (DAT), 15DAT, 25DAT, 35DAT, and 45DAT, \pm SE under 50 mg·L⁻¹ N, 100 mg·L⁻¹ N, 150 mg·L⁻¹ N, 200 mg·L⁻¹ N of the first trial experiment. Means with the same letter within the same measuring date are not significantly different ($\alpha = 0.05$) and represent the average value of two cultivars, two plants, and four blocks (n = 16).

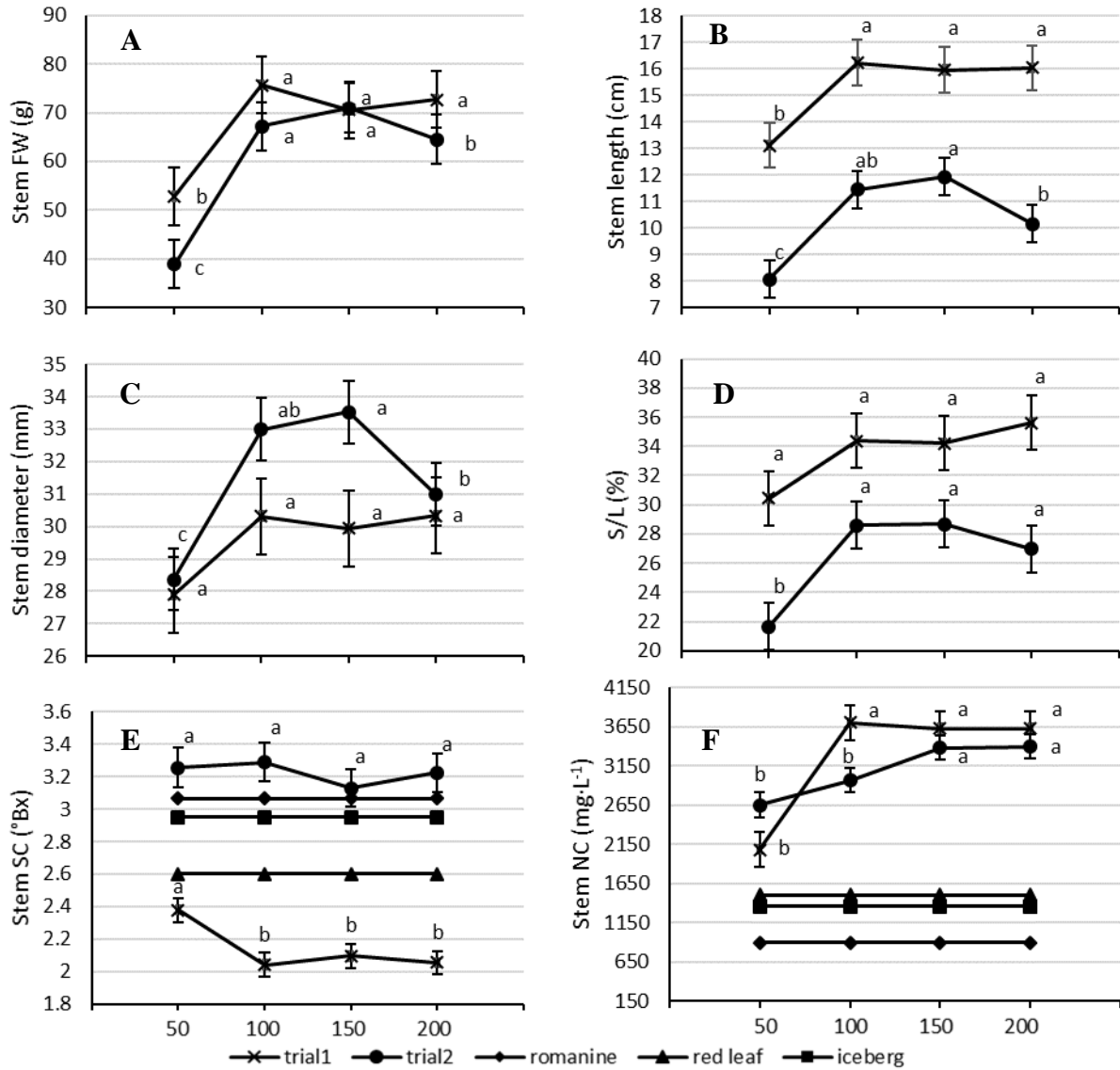


Figure 4. Stem FW (fresh weight) (A), stem length (B), stem diameter (C), S/L (stem/leaves ratio = stem fresh weight/leaves fresh weight) (D), stem SC (soluble solids content) (E), stem NC (nitrate concentration) (F), \pm SE under 50 mg·L⁻¹ N, 100 mg·L⁻¹ N, 150 mg·L⁻¹ N, 200 mg·L⁻¹ N. Means with common letters of the same trial within same index plot are not significantly different ($\alpha = 0.05$) and represents the average value of one trial, two cultivars, two boron treatments, two plants, and four blocks (n = 32). ‘Romaine’, ‘Red leaf’, and ‘Iceberg’ are commercial available products, n = 6 for ‘Romaine’ and ‘Red leaf’; n = 12 for ‘Iceberg’.

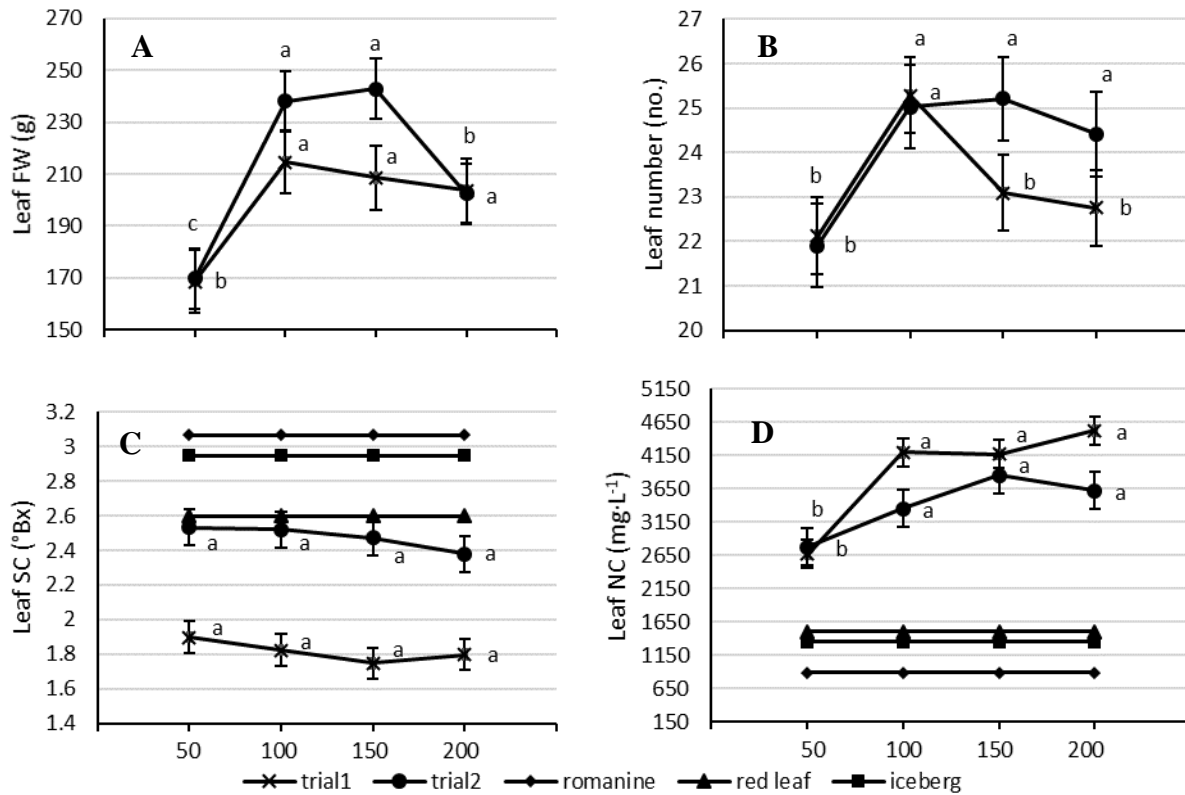


Figure 5. Leaf FW (fresh weight) (A), leaf number (B), leaf SC (soluble solids content) (C), leaf NC (nitrate concentration) (D), \pm SE under 50 mg·L⁻¹ N, 100 mg·L⁻¹ N, 150 mg·L⁻¹ N, 200 mg·L⁻¹ N. Means with common letters of the same trial within same index plot are not significantly different ($\alpha = 0.05$) and represent the average value of one trial, two cultivars, two boron treatments, two plants, and four blocks (n = 32). ‘Romaine’, ‘Red leaf’, and ‘Iceberg’ are commercial available products, n = 6 for ‘Romaine’ and ‘Red leaf’; n = 12 for ‘Iceberg’.

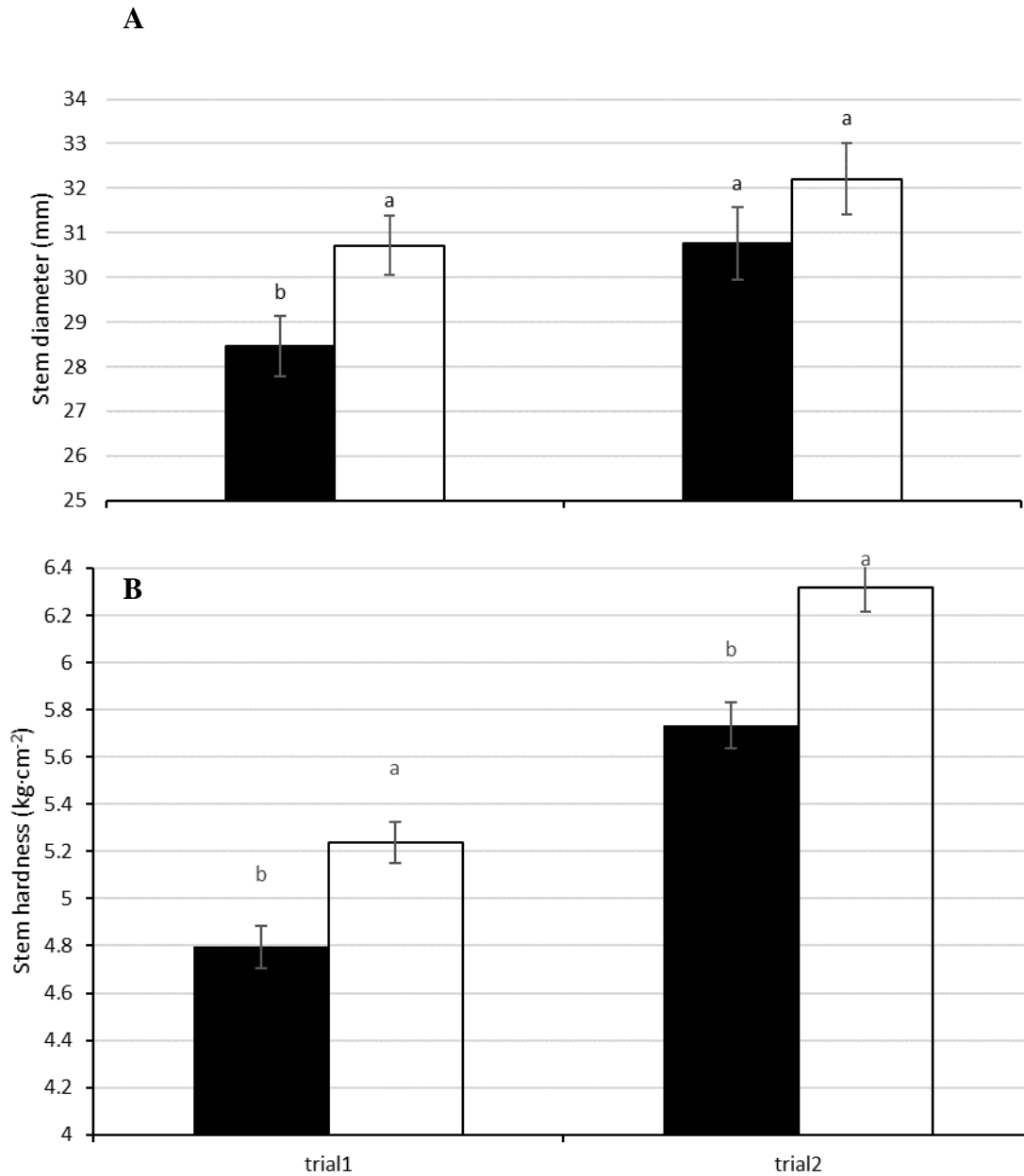


Figure 6. Stem diameter (A) and hardness (B), \pm SE under $0.5 \text{ mg}\cdot\text{L}^{-1}$ boron treatment, $1 \text{ mg}\cdot\text{L}^{-1}$ boron treatment. Means with common letters of the same trial within same index plot are not significantly different ($\alpha = 0.05$) and represents the average value of one trial, two cultivars, four nitrogen treatments, two plants, and four blocks ($n = 64$ data points for each mean).

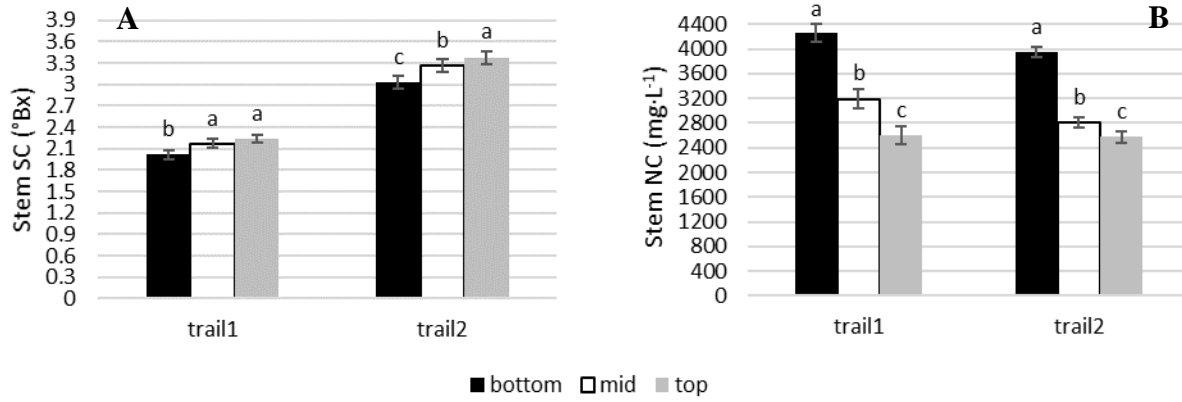


Figure 7. Stem SC (A) and NC (B), \pm SE of different part of stem of two trials. Means with common letters of the same trial are not significantly different ($\alpha = 0.05$) and represents the average value of one trial, eight nutrient treatments, two cultivars, and four blocks ($n = 128$).

CHAPTER 2

The Influences of Long-term Deficit Irrigation on Yield and Quality of Celtuce (*Lactuca sativa*

L. var. augustana)

Introduction

Celtuce (*Lactuca sativa* L. var. *augustana*), is one of the six morphologically different lettuce (*Lactuca sativa* L.) cultivar groups (Nonnecke, 1989). It is also known as stem lettuce, asparagus lettuce, Chinese lettuce or *woh sun* in Chinese (Schneider, 2001). Celtuce was first introduced to the U.S. by W. Atlee Burpee & Co. in 1941 but has raised limited interest since then (Anderson and Helgeson, 1941). Although three lettuce cultivar groups head, leaf, and romaine when counted together represent the second largest vegetable produced in the U.S. in 2017, there is no documented information about celtuce production from USDA National Agricultural Statistics Service (2018). Very few studies have been conducted on celtuce. The characteristics, habits, and growing methods of celtuce remain unclear.

Water is an essential part of any plant's life cycle as water is the major constituent of all plant tissues, maintains turgor pressure, plays key roles in photosynthesis and transpiration (Nonnecke, 1989). Lettuce cultivars including celtuce, as a major category of leafy greens, have the highest water content about 94.8% among vegetable classifications (Kramer, 1969). Water shortage at any growing stage results in loss of crop quality, this is especially critical at head developing stage (Verhallen and Roddy, 2009). Lettuce cultivars are typically shallow-rooted vegetables with roots length up to 30 cm (Verhallen and Roddy, 2009). This makes lettuce cultivars extremely sensitive to drought and easier to be affected by upper soil drought stress

(Kizil et al., 2012). Since irrigation quadratically benefits the marketable yield of lettuce (Capra et al., 2008a), adequate irrigation is applied throughout the whole rotation of lettuce production.

However, agricultural irrigation is being challenged by the shortage of water resource. Agricultural water consumption could be exhaustion to the scarce water resource, which accounts for 60 to 80% of total consumptive water use worldwide and 80% domestically in the U.S. (Huffaker and Hamilton, 2007; Suchoff et al., 2018). Because of increasing populations, irrigated area will expand over 20% by 2025 (Sojka et al., 2007) and agricultural water use will increase by 70 to 90% by 2050 (Suchoff et al., 2018). More vitally, marginal water use efficiency decreases rapidly when the amount of irrigation is close to the water requirement of crops (Capra et al., 2008a), which means over-irrigation only results the waste of water and irrigation input without any increase of yield.

Deficit irrigation (DI), known as the deliberate and designated under-irrigation regime for crops to attain the maximum yield of the crop from the specific field, was introduced in the 1970s to handle the conflict between agricultural irrigation and water resource shortage (Capra et al., 2008a). To maximize the yield of crop while reducing the amount of irrigation water, DI requires precise and systematic irrigation regime, as well as the comprehensive understanding of crop responses to water deficit (Capra et al., 2008a; 2008b).

Due to limited information regarding the ideal irrigation regime for celtuce and urgent requirement of water-saving agriculture. This study was conducted to 1) determine the influence of long-term deficit irrigation (control, deficit 25%, deficit 50%, and deficit 75%) on celtuce production, including yield and quality; and 2) Determine the optimal deficit irrigation regime to maintain similar celtuce production while reducing irrigation water.

Materials and methods

GROWTH CONDITIONS. The study was conducted in the Marye Anne Fox Science Teaching Laboratory Greenhouses on the North Carolina State University (NCSU) campus, Raleigh, NC (35.787 N, 78.673 W) between 5 Oct. 2018 and 11 Dec. 2019. The second trial of this study was conducted between 8 Dec. 2018 and 15 Feb. 2019. The growing conditions in the greenhouse were automatically maintained by integrated environmental controller (Priva Maximizer; Priva inc., Camarillo, CA) with day temperatures of 23.8 ± 3 °C and night temperature of 18.3 ± 3 °C. Humidity, while not maintained, fluctuated between 50% and 70%.

SEEDLINGS PRODUCTION. All seedlings were produced in the Fox Greenhouses on the North Carolina State University (NCSU) campus in Raleigh. Two commercially available celtuce cultivars ‘Celtuce’ and ‘Summer 38’ (*Lactuca sativa* var. *asparagina*) (Kitazawa Seed Co., Oakland, CA) were selected as they have different tolerance to temperature and water deficiency. ‘Celtuce’ has better cold tolerance while ‘Summer 38’ has strong heat tolerance as it can survive in high temperature up to 37.7 °C (100°F) for few days (Kitazawa Seed Co., 2018a; 2018b). In the first trial, seeds were sown on 5 Oct. 2018 into 48-cells black plastic plug tray (L806; Landmark Plastic, Akron, OH), each cell with dimensions of 5.97 cm (2.35 inches, length) x 3.93 cm (1.55 inches, width) x 5.92 cm (2.33 inches, height). The trays were filled with an all-purpose potting mix (Fafard® 4P Mix; Sun Gro® Horticulture, Agawam, MA). All experimental units were thoroughly watered daily, and besides natural light received constant $176 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemental photosynthetically active radiation with supplemental lighting with 50% red light and 50% blue light (Pro 325 LED lighting system; LumiGrow, Emeryville, CA) from 7 am to 7 pm every day during seedling stage. After germinating, seedlings were thinned to one plant per cell. In the second trial, seeds were sown on 8 Dec. 2018.

EXPERIMENT DESIGN. 3.79 L (1 gal) black polyethylene pots with dimensions of 19.68 cm (7¾ inches, top diameter) x 17.78 cm (7 inches, height) x 15.5 cm (6¼ inches, bottom diameter) (Blow molded – classic line C400; Nursery Supplies, Inc., Chambersburg, PA) were used as container in this experiment. Each pot was filled with 1200 g all-purpose potting mix (Fafard® 4P Mix; Sun Gro® Horticulture, Agawam, MA) and gently shaken until substrate was 16 cm height inside the pot to attain similar substrate density. In the first trial, seedlings were transplanted 0.5 cm deep into prepared pots at 30 Oct. 2018. All seedlings were randomly classified into 4 irrigation treatments, which were 100% irrigation regime (300 ml/pot everyday), 75% (225 ml/pot everyday), 50% (150 ml/pot everyday), 25% (75 ml/pot everyday) respectively. Each treatment had 5 plants as replications. In the first 7 Days After Transplantation (DAT), all treatments were irrigated with 100% irrigation regime to ensure seedlings successfully established. Differentiated irrigation were conducted 8 DAT from 6 Nov. 2018. Plants of cultivar ‘Celtuce’ were harvested at 5 Dec. 2018 and ‘Summer 38’ were harvested at 11 Dec. 2018 due to their longer growing period compared to cultivar ‘Celtuce’. In the second trial, seedlings were transplanted at 3 Jan. 2019 and treatments were conducted 8 DAT from 10 Jan. 2019.

DATA COLLECTION. Harvest occurred on 5 Dec. 2018 for first trial and 15 Feb. 2019 for second trial. At the time of each harvest, plant roots were cut off by hand. On each plant, every leaf longer than 1 cm was removed at the base, counted into total leaf number, and weight as fresh total leaf weight. Then leaves were longitudinally cut along the midrib into 0.5 cm wide slices and one leaf slices sample was pressed with a garlic press () to express 5 drops of sap to test sweetness with pocket refractometer (PAL-1; ATAGO, Minato, Tokyo, Japan). Stem length, widest diameter, and weight were measured. Then stem was peeled to a depth of 2 mm thick skin and measured hardness twice on flesh by fruit hardness tester (GY-3; Graigar Instruments,

Shenzhen, Guangdong, China) with 8 mm diameter pressure head and 10 mm insertion depth. Stem was then trisected from top to bottom based on the stem length, each of which was cut out a one-centimeter thick stem sample for sweetness measurement.

DATA ANALYSIS. Initially, the trial dates were analyzed for potential interactions, however no significant differences were found between trial dates and all data from both trials' dates were combined and analyzed with the GLIMMIX procedure in SAS (v 9.4; SAS Institute, Cary, NC). The model utilized cultivar, irrigation level, and their interaction as fixed effects, with replication nested in trial as random effects. The Satterthwaite option (Schaaljie et al., 2002) was used as degree of freedom approximation. Orthogonal contrasts were conducted to find out the relation between treatments and variables. Tukey's honest significance test (HSD) with significant level $P \leq 0.05$ was applied in all mean separation test.

Results

CULTIVAR. Significant differences ($P \leq 0.05, 0.01, 0.001$) in stem fresh weight, stem length, stem diameter, hardness and stem sugar content were observed between cultivar 'Celtuce' and 'Summer 38' (Table 1). With same irrigation treatments, 'Celtuce' had significantly higher ($P \leq 0.05$) stem fresh weight, stem length, stem diameter, and stem soluble solids content than 'Summer 38' (Table 2). While stem firmness of 'Celtuce' was significantly lower ($P \leq 0.05$) (Table 2). No significant difference ($P \leq 0.05$) in leaves fresh weight and leaves number were observed between cultivars.

IRRIGATION REGIMES. The morphological changes of the two cultivars 'Celtuce' and 'Summer 38' during the whole deficit irrigation treatments were presented as Fig.1 and Fig. 2, respectively. Significant differences ($P \leq 0.01, 0.001$) in stem weight, stem diameter, stem

soluble solids content, leaf weight and leaf number were observed among irrigation regimes (Table 1). Celtuce cultivated with 25% irrigation regime had significantly lower ($P \leq 0.05$) stem fresh weight with the mean of 13.5 g per plant and leaves fresh weight with the mean of 43 g per plant (Fig. 3A and D). Stem fresh weight and leaf fresh weight linearly increased as the irrigation increased (Table 1). Celtuce grown with the 25% irrigation regime also had significantly lower ($P \leq 0.05$) stem diameter and leaves number (Fig. 3B and E) than cultivated in other regimes. Those two characteristics quadratically increased as the irrigation increased (Table 1). A linear decrease in stem sugar content was observed as irrigation regimes increased (Fig. 3D).

INTERACTION. The cultivar \times irrigation interaction was significant ($P \leq 0.05, 0.01, 0.001$) in stem weight, stem diameter, stem soluble solids content, and leaf weight (Table 1). For cultivar ‘Celtuce’, stem fresh weight, stem diameter, and leaves fresh weight of plants under 25% irrigation regime were significantly lower ($P \leq 0.05$) than under other regimes (Fig. 4A, B, and D). Stem sugar content of plants under 25% irrigation regime was significantly higher than others (Fig. 4C). For cultivar ‘Summer 38’, stem diameter of plants under 25% irrigation regime were significantly lower ($P \leq 0.05$) while this characteristic had no differences among 50%, 75%, and 100% irrigation regime (Fig. 4B). No difference was observed in stem fresh weight, stem sugar content or leaves fresh weight among irrigation regimes of ‘Summer 38’ (Fig. 4A, C and D).

Discussion

The two celtuce cultivars ‘Celtuce’ and ‘Summer 38’ were observed with significantly different responses to deficit irrigation in various characteristics. Overall, cultivar ‘Celtuce’ responded with higher yield and quality when cultivated with deficit irrigation. This cultivar may

be more promising for commercial production for its steady growth and high-quality production characteristics. Noticeably, different than ‘Celtuce’, cultivar ‘Summer 38’ had no significant response to different deficit irrigation, indicating its insensitivity to the change of water supply from the environment. This variety has strong tolerance to heat (Kitazawa, 2018b) and may have the ability to partially maintain normal physiological metabolisms even under severe deficit-irrigated situation.

Generally, celtuce responded with lower yield and quality under less irrigation regime and higher yield and quality under larger irrigation regime. Even though no earlier study reported the influence of deficit irrigation on celtuce, but the feature is consistent with other lettuce cultivars. The marketable yield and basic quality indexes of curly lettuce (*Lactuca sativa* var. *Crispa* cv. *Bohemia*) was significantly diminished by severe deficit irrigation, as 56.8% to 71.6% loss of marketable yield compared to normal irrigation when curly lettuce was applied with long-term 20% of normal irrigation (Kuslu et al., 2008). Yazgan et al. (2008) also reported the influence of deficit irrigation on lettuce (*Lactuca sativa* var. *Olenka*) that 25% of normal irrigation not only led to 76.8% of yield loss but also caused significant reduction in plant height and head diameter with 15% and 22%, respectively. In a recent study, the effects of deficit irrigation on lettuce (*Lactuca sativa* cv. *Hazar*) was confirmed again by Kirnak et al. (2016) that 25% of normal irrigation caused 51.3% of yield loss

The responses of celtuce to different deficit irrigation levels were either linear or quadratic. When under slight deficit irrigation condition (75% irrigation regime), the reductions of all celtuce characteristics were not statistically significant. Those relations were presented in previous studies when conducted on lettuce. Strong quadratic relation between total water received and total marketable yield of lettuce (*Lactuca sativa* var. *Batavia rossa*) was exhibited

by Capra et al. (2008a) as the total marketable yield of lettuce increased gradually when the amount of received water increased before reaching the maximum yield. When treated with 75% of normal irrigation, the depletion of lettuce total marketable yield occurred but not significantly different (Capra et al., 2008a). A linear relationship was observed between seasonal evapotranspiration and marketable plant yield (Kuslu et al., 2008). Another irrigation treatment study also demonstrated that neither 50% nor 25% reduced irrigation affected the quality and shelf-life on 'Iceberg' lettuce while 'Romaine' lettuce had higher visual quality when applied with deficit irrigation (Martinez-Sanchez et al., 2012). Similarly, long-term 25% deficit irrigation regime did not affect the fresh head weight, compactness and dry matter content of 'Iceberg' lettuce (Luna et al., 2012).

Long-term deficit irrigation treatment of this study proved that slight deficit irrigation (75% of normal irrigation regime) did not cause a significant reduction in lettuce yield and quality, which may provide a brief deficit irrigation strategy for lettuce production. This strategy may be adapted into practical use once the production of lettuce established. Moreover, the long-term deficit irrigation is still a rough approach to the best deficit irrigation strategy. Understanding the influence of deficit irrigation on lettuce, the long-term treatment could be divided into phenological stages, on which the different regulated deficit irrigations are conducted in further studies.

Citation:

- Anderson, J.M., and R.C. Helgeson. 1941. Celtuce-a new vegetable for '42. Seed world 50 (9): 22.
- Capra, A., S. Consoli, A. Russo, and B. Scicolone. 2008a. Integrated Agro-Economic Approach to Deficit Irrigation on Lettuce Crops in Sicily (Italy). Journal of irrigation and drainage engineering, 134(4): 437-445.
- Capra, A., S. Consoli, and B. Scicolone. 2008b. Water management strategies under deficit irrigation. Journal of agricultural engineering, 4: 27-34.
- Huffaker, R., and J. Hamilton. 2007. Conflict, p. 3-21. In: Lascano, R.J. and R.E. Sojka. Irrigation of agricultural crops, 2nd ed., Agronomy, Monograph no 30. ASA, CSSA, and SSSA, Madison, WI.
- Kirnak H., I. Tas, Z. Gokalp and S. Karaman. 2016. Effects of different irrigation levels on yield of lettuce grown in an unheated greenhouse. Current trends in natural sciences, 5(9): 145-151.
- Kitazawa Seed Co. 2018a. Celtuce. < https://www.kitazawaseed.com/seed_068-163.html>
- Kitazawa Seed Co. 2018b. Summer 38. < https://www.kitazawaseed.com/seed_554-163.html>
- Kizil, U., L. Genc, M. Inalpulat, D. Sapolyo, and M. Mirik. 2012. Lettuce (*Lactuca sativa* L.) yield prediction under water stress using neural network (ANN) model and vegetation indices. Zemdirbyste-agriculture, 99(4): 409-418.
- Kramer, P.J. 1969. Plant & soil water relationships: a modern synthesis. McGraw-Hill, New York, NY.

- Kuslu, Y., A. Dursun, U. Sahin, F.M. Kiziloglu and M. Turan. 2008. Effect of deficit irrigation on curly lettuce grown under semiarid conditions. *Spanish journal of agricultural research*, 6(4): 714-719.
- Luna, M.C., J.A. Tudela, A. Martinez-Sanchez, A. Allende, A. Marin, and M.I. Gil. 2012. Long-term deficit and excess of irrigation influences quality and browning related enzymes and phenolic metabolism of fresh-cut iceberg lettuce (*Lactuca sativa* L.). *postharvest biology and technology*, 73: 37-45.
- Nonnecke, I.L. 1989. Vegetable production. Van Nostrand Reinhold, New York, NY, p. 75-89, 450-466.
- Schaaljie, G.B., J.B. McBride, G.W. Fellingham. 2002. Adequacy of approximations to distributions of test statistics in complex mixed linear models. *Journal of agricultural, biological, and environmental statistics*, 7(4): 512-524.
- Schneider, E. 2001. Vegetables from amaranth to zucchini: the essential reference. William Morrow, New York, NY, p. 461-465.
- Sojka, R.E., D.L. Bjornberg, and T.S. Strelkoff. 2007. Irrigation-induced erosion, p. 237-275. In: Lascano, R.J. and R.E. Sojka. *Irrigation of agricultural crops*, 2nd ed., Agronomy, Monograph no 30. ASA, CSSA, and SSSA, Madison, WI.
- Suchoff, D.H., J.R. Schultheis, M.D. Kleinhenz, F.J. Louws and C.C. Gunter. 2018. Rootstock improves high-tunnel tomato water use efficiency. *Horttechnology* 28(3): 344-353.
- USDA, National Agricultural Statistics Service. 2018. Vegetables 2017 Summary. ISSN: 0884-6413 <<https://downloads.usda.library.cornell.edu/usda-esmis/files/02870v86p/5425kd81z/9091s517t/VegeSumm-02-13-2018.pdf>>

Verhallen, A., and E. Roddy. 2009. Irrigation of vegetable crops. Ontario Ministry of Agriculture, Food and Rural Affairs. <

http://www.omafra.gov.on.ca/english/crops/facts/info_irrigation.htm#irrigation>

Yazgan, S., S. Ayas, C. Demirtas, H. Buyukcangaz and B.N. Candogan. 2008. Deficit irrigation effects on lettuce (*Lactuca sativa* var. Olenka) yield in unheated greenhouse condition. Journal of food, agriculture & environment, 6(2): 357-361.

Table 1. Two-way ANOVA for a fully factorial arrangement (2 cultivars x 4 irrigation regimes) of treatments for the combined experimental replicates of two trials

Effect	df	Stem weight	Stem length	Stem diameter	Stem hardness	Stem sugar content	Leaves weight	Leaves number
Cultivar (C) ^z	1	*	*	***	*	**	NS	NS
Irrigation (I) ^y	3	***	NS	***	NS	**	***	***
Linear	1	***	NS	***	NS	***	***	***
Quadratic	1	NS	NS	***	NS	NS	NS	**
Cubic	1	NS	NS	NS	NS	NS	NS	NS
C × I	3	***	NS	**	NS	*	*	NS

^zCultivar treatment compared two commercial cultivars ‘Celtuce’ and ‘Summer 38’.

^yIrrigation treatments were four irrigation regimes as 25% irrigation regime (75 ml/pot everyday), 50% (150 ml/pot everyday), 75% (225 ml/pot everyday), 100% (300 ml/pot everyday).

NS, *, **, *** denote non-significant at $P > 0.05$, significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 2. Main effect of cultivars on stem and leaves characteristics (mean \pm SE)

Cultivar	Celtuce	Summer 38
Stem fresh weight (g)	24.6 \pm 3.1 a ^z	20.2 \pm 3.1 b
Stem length (cm)	11.5 \pm 1.9 a	8.6 \pm 1.9 b
Stem diameter (mm)	24.0 \pm 0.5 a	20.3 \pm 0.5 b
Stem hardness (kg/cm ²)	8.0 \pm 0.2 b	8.9 \pm 0.2 a
Stem soluble solids content (°Bx)	8.6 \pm 0.5 a	6.7 \pm 0.5 b
Leaves fresh weight (g)	67.4 \pm 3.3 a	64.0 \pm 3.1 a
Leaves number (no.)	24.5 \pm 0.5 a	25.2 \pm 0.5 a

^zMeans \pm SE followed by the same letter within each index are not significantly different

(Tukey's HSD; $\alpha = 0.05$) and represents the average value and standard error of two trials, 4 irrigation treatments, and five replications ($n = 40$ data points for each means \pm SE).

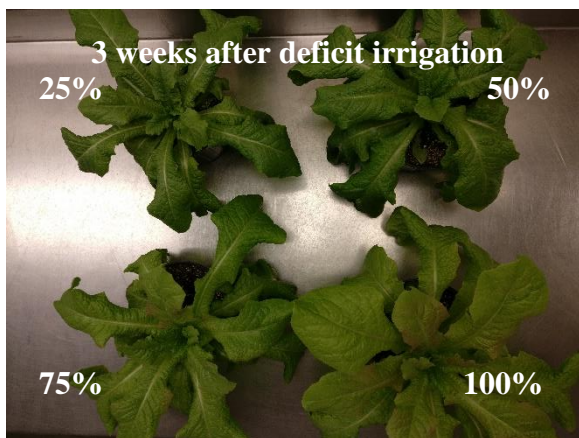
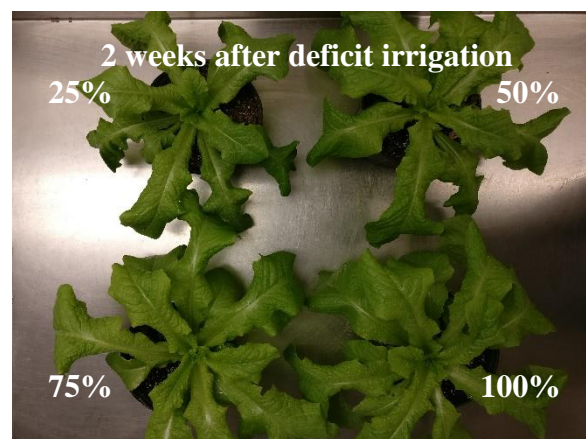
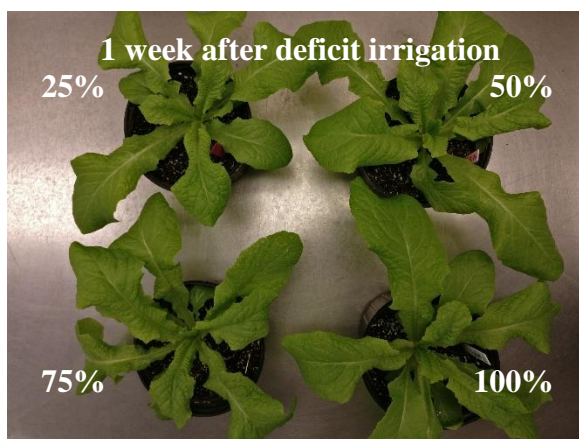
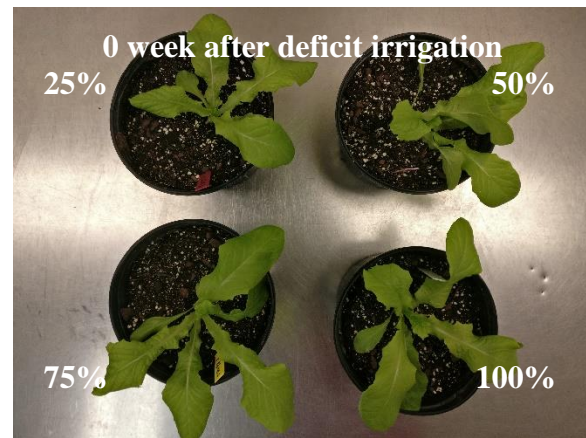


Figure. 1. The morphology changes of celuce cultivar 'Celuce' from transplanting (1 week before deficit irrigation conducted) to 0 week (deficit irrigation started) and to 5 weeks after deficit irrigation (harvest).

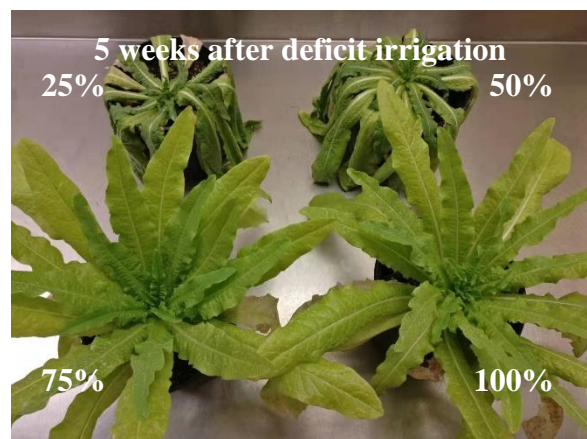
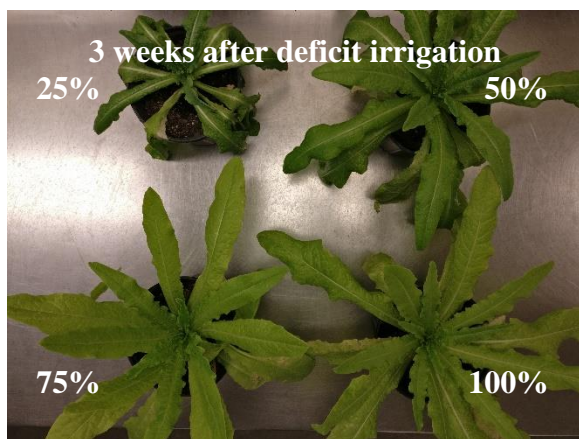
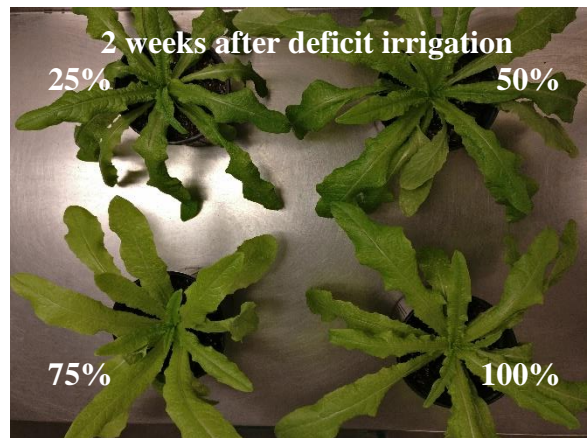
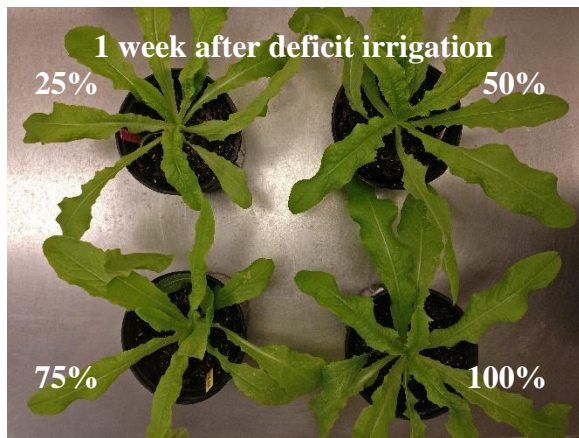
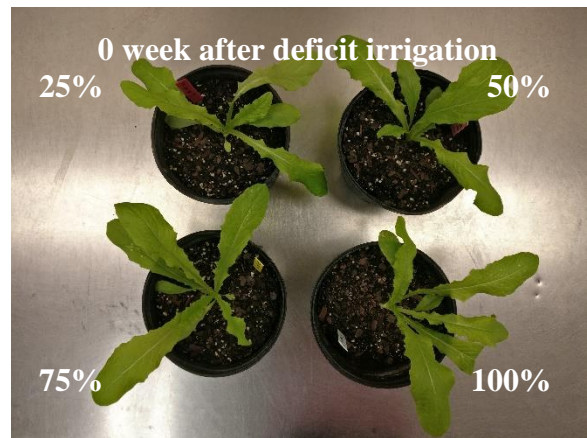
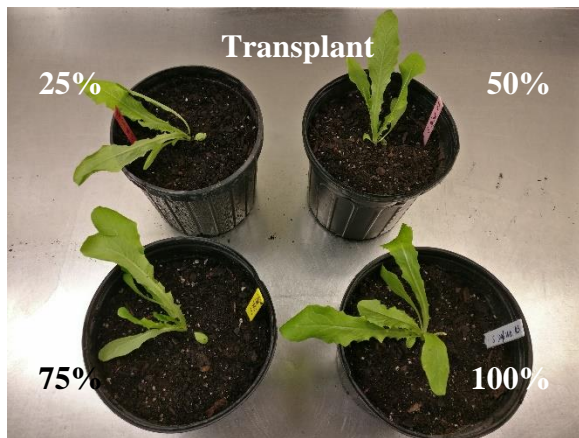


Figure. 2. The morphology changes of celuce cultivar 'Summer 38' from transplanting (1 week before deficit irrigation conducted) to 0 week (deficit irrigation started) and to 5 weeks after deficit irrigation (harvest).

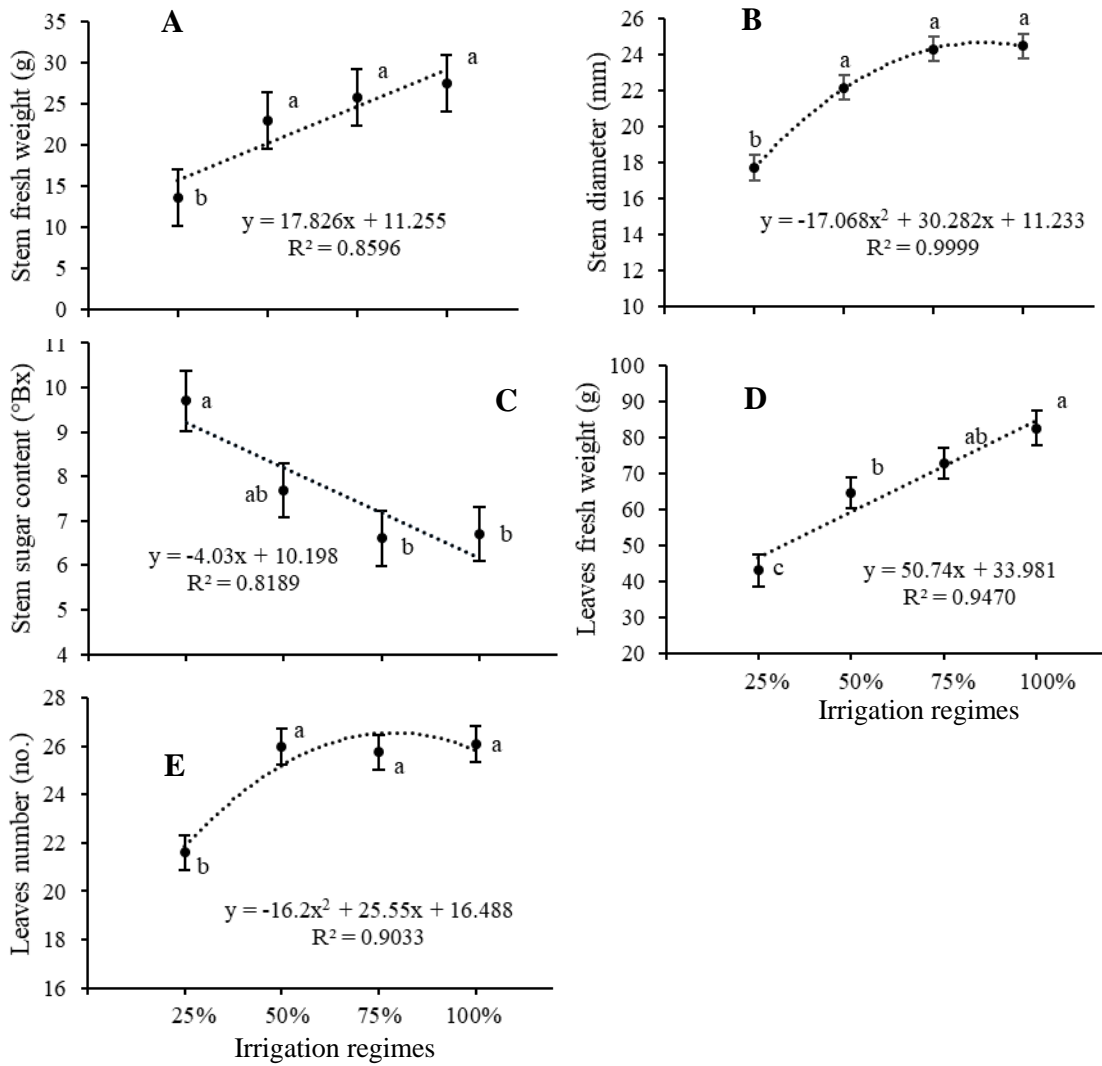


Figure. 3. Stem fresh weight (A), stem diameter (B), stem sugar content (C), leaves fresh weight (D), and leaves number (E), \pm SE under 25% irrigation regime (75 ml/pot everyday), 50% (150 ml/pot everyday), 75% (225 ml/pot everyday), 100% (300 ml/pot everyday). Means with common letters within same index plot are not significantly different (Tukey's HSD; $\alpha = 0.05$) and represents the average value of two trials, two cultivars and five replications ($n = 20$ data points for each mean). Regression lines significantly explained the relations between independent variable and corresponding dependent variable.

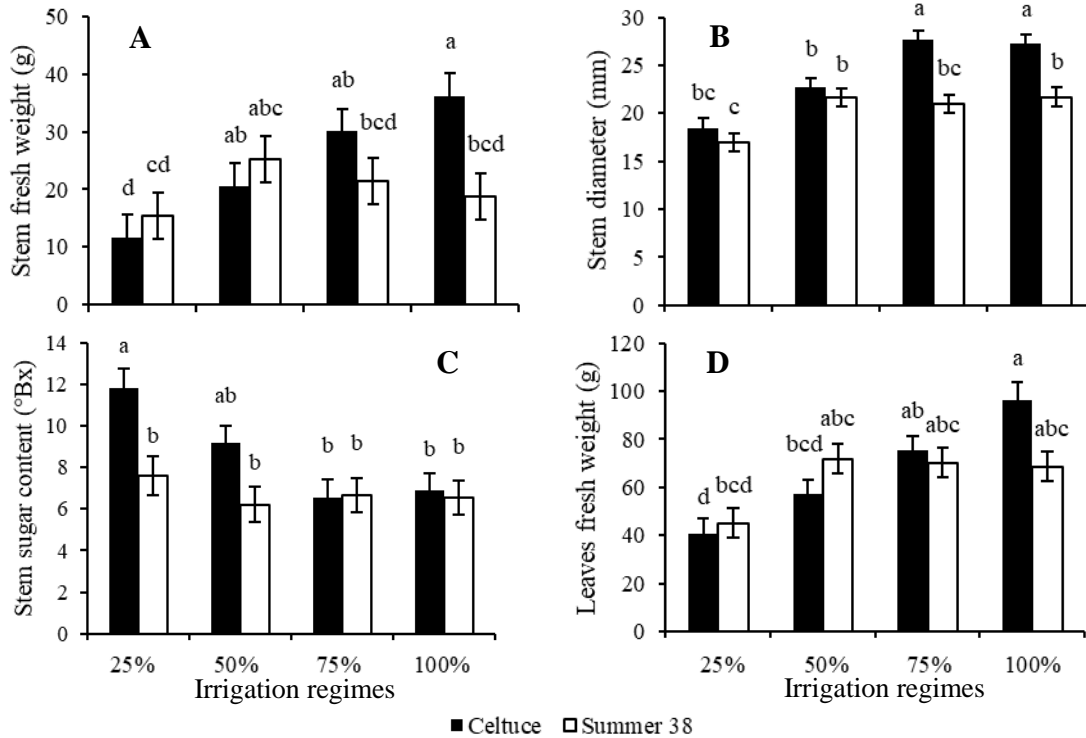


Figure. 4. Stem fresh weight (A), stem diameter (B), stem sugar content (C), and leaves fresh weight (D), \pm SE for two cultivars ‘Celtuce’ and ‘Summer 38’ under 100% irrigation regime (300 ml/pot everyday), 75% (225 ml/pot everyday), 50% (150 ml/pot everyday), 25% (75 ml/pot everyday). Means with common letters within same index plot are not significantly different (Tukey’s HSD; $\alpha = 0.05$) and represents the average value of two trials and five replications ($n = 10$ data points for each mean).