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Effect of Nutrient Solution Cooling in Summer and Heating in Winter on the Performance of Baby Leafy Vegetables in Deep-Water Hydroponic Systems

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Abstract: Hydroponics has become a popular production technology for leafy greens in greenhouses. However, year-round production of cool-season leafy greens remains challenging due to costly heating and cooling during winter and summer seasons, depending on location. Therefore, the objective of this study is to investigate the effect of nutrient solution cooling and heating in deep-water hydroponic systems on the performance of several leafy green vegetables. Two experiments of nutrient solution cooling during the summer season and another two experiments of nutrient solution heating during the winter season were conducted in Texas, USA in 2020–2021. Lettuce (*Lactuca sativa* L.) 'Bergams Green' and 'Red Mist', Pak Choi (*Brassica rapa* subsp. *chinensis*) 'Purple Magic' and 'White Stem', and spinach (*Spinacia oleracea* L.) 'Mandolin' and 'Seaside' were grown in the summer experiments, and only the two lettuce cultivars were grown for the winter experiments. For both cooling and heating studies, six deep-water culture systems were used with two treatments: cooling (23 °C) vs. no cooling, and heating (22 °C) vs. no heating, with three replications in each experiment. In the nutrient solution cooling study, spinach was the most heat-sensitive species, and 'Mandolin' was more heat-tolerant than 'Seaside,' as evidenced by its lower mortality rate in both experiments. Lettuce and pak choi grew well and solution cooling increased shoot fresh weight in both lettuce cultivars and in 'White Stem' pak choi but not in 'Purple Magic' pak choi. Conversely, during the winter season, solution heating increased shoot fresh weight of both lettuce cultivars; however, 'Red Mist' was more responsive than 'Bergams Green' lettuce. These results indicate the potential to increase crop yield by controlling nutrient solution temperature throughout the year, depending on the season. Also, there were genotypic differences in both cooling and heating experiments, indicating that more research is needed to determine the species-dependent and even cultivar-dependent nutrient solution temperature control strategies to achieve optimum year-round production.

Keywords: deep-water culture; lettuce; pak choi; spinach; root zone temperature control

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1. Introduction

The use of hydroponic systems in greenhouses to produce leafy green vegetables is increasing in popularity throughout the world and is becoming an important component of the world's fresh vegetable production system [1]. Hydroponic cultivation is very efficient due to its soilless nature and ability to recirculate water and nutrients. For example, hydroponic cultivation of lettuce (*Lactuca sativa* L.) can produce 10-fold the yield of conventional field agriculture while using 90% less water [2]. However, maintaining an optimal air temperature of a greenhouse can be challenging during summer, especially in southern regions of the United States, due to intense solar heat and high outside air temperature and humidity. For the winter season, air temperatures are typically sub-optimal for crop production, or even below freezing. This is especially true in northern regions of the U.S.

where the average outside temperature is close to freezing; thus, heating is essential for effective plant growth, but it increases production costs. Although much research has been directed towards improving the efficiency of supplemental lighting for greenhouse crop production [3,4], less attention has been paid towards alternative methods of cooling or heating that can lower the energy costs of greenhouse hydroponic production during the summer and winter for year-round production.

Baby leafy greens are young, tender, freshly harvested salad vegetables that have been increasingly favored by consumers due to their high nutritional values for human health [5]. Lettuce and spinach (*Spinacia oleracea* L.) are two of the most widely consumed baby leafy greens [6]. Pak choi (*Brassica rapa* subsp. *chinensis*), with many cultivars and different leaf colors and shapes, is a popular Asian leafy vegetable suitable for hydroponic production [7,8]. These baby leafy greens are often produced hydroponically in controlled environments, such as greenhouses, to sell at local markets [9]. However, greenhouse hydroponic production can be energy-intensive due to the inherent cost of winter heating and summer cooling [2]. It is necessary to explore an effective way to reduce the energy cost for greenhouse production during adverse seasons. One potential approach is to cool or heat the nutrient solution instead of the entire greenhouse space to reduce energy cost. For hydroponic systems, it is easy to control root-zone temperature by heating or cooling the nutrient solution in the reservoirs [10]. For example, during the winter season, baby leafy greens can be produced with an optimal nutrient solution temperature at sub-optimal greenhouse air temperature to reduce heating costs [10].

The optimal air temperature for pak choi is 18–20 °C and 17–24 °C for lettuce [3]. These cooler and somewhat narrow ranges can be limiting, particularly during the summer where high air temperature can cause plant stress in these cool-season leafy vegetables. Plant growth of spinach plants can be inhibited at air temperatures above 23 °C, while air temperatures above 35 °C can severely reduce the metabolism efficiency in spinach plants, resulting in bolting and decreased yields [11–13]. During summer greenhouse production, cooling the solution in a hydroponic system has been shown to increase dissolved oxygen levels and reduce root disease occurrence, but different plant growth responses have been reported [10,14,15]. For example, root-zone cooling at 20 °C increased biomass of aeroponically grown 'Wintergreen' lettuce at an air temperature of 24–38 °C in a tropical greenhouse [14]. In contrast, low nutrient solution temperature of 10 °C triggered stress responses in the whole plant, resulting in reduced leaf and root growth in 'Red Wave' lettuce compared to solution temperatures of 20 °C and 30 °C [10]. In red perilla (*Perilla frutescens*), plant growth was not affected by low nutrient solution temperature (10 °C) compared to the control (20 °C) under greenhouse air temperature of 25/20 °C (light/dark period) [15]. Apparently, the response to nutrient solution cooling varied with plant genotype and air temperature. However, the effectiveness of cooling nutrient solution on the performance of lettuce, spinach, and pak choi in warm climates like in Texas, USA remains unknown.

Conversely, low air temperature during the winter production season can inhibit plant growth of cool-season crops. To maintain normal yield and quality of lettuce, greenhouse air temperature needs to be heated to 24–25 °C [16]. Increasing root-zone temperature increased plant growth by promoting water and nutrient uptake [17–19]. Using a nutrient film technology (NFT) hydroponic system, shoot fresh weight of 'Marbello' and 'Bastion' lettuce increased in a heated nutrient solution (20 °C) with an air temperature of 9–17 °C, compared to the control without root-zone heating [18]. In a deep-water culture system, 'Ostinata' lettuce grown in nutrient solution of 24 °C and 31 °C produced more dry mass than at 17 °C [17]. The early yield of melon (*Cucumis melo* 'Arava') increased when grown in nutrient solution that was heated compared to a non-heated control [19]. However, the above beneficial effects of solution heating treatment were observed in mature vegetable crops, which have a longer production cycle than baby leafy greens. Also, for the same plant species, seedlings of red- and green-leaf cultivars have shown great differences in growth response to environment change [20].

Considering the above, the objectives of this study were to investigate effects of cooling the nutrient solution during the summer on the performance of lettuce, pak choi, and spinach plants, and the effects of heating the nutrient solution during the winter on the performance of red and green lettuce plants. We hypothesized that cooling the solution during hot summer and heating the solution during winter improve crop performance compared to the control. The goal was to demonstrate the potential benefits of controlling the nutrient solution temperature of hydroponic production systems instead of the whole greenhouse air space to reduce energy cost and to control the greenhouse environmental for optimum year-round production of leafy green vegetables.

2. Materials and Methods

2.1. Nutrient Solution Cooling during Summer Season

2.1.1. Plant Materials and Culture

Two experiments were conducted in a glass greenhouse at the Texas A&M AgriLife Research Center in Dallas, TX (32°59′13.2″ N 96°45′59.8″ W; elevation 131 m) from July to September of 2020 (Table 1). Leafy greens used in the study included lettuce 'Bergam's Green' and 'Red Mist', pak choi 'Purple Magic' and 'White Stem', and spinach 'Mandolin' and 'Seaside'. Seeds were sown in rockwool cubes (2.5 cm) in standard nursery trays (25 cm width × 51 cm length × 6.4 cm depth) and covered with a dome to maintain high moisture during germination. The rockwool was pre-rinsed with tap water and pre-soaked with a half strength of the nutrient solution with electrical conductivity (EC) of 1.0 mS cm^{-1} and pH adjusted to 6.0 prior to sowing. A custom nutrient solution was prepared using tap water and fertilizer salts at 10.7 (mM) N, 1.13 P, 5.38 K, 3.25 Ca, 1.44 Mg, 1.44 S, and microelements at 53.7 (mM) Fe, 27.8 B, 6.0 Mn, 1.83 Zn, 1.10 Cu, and 0.63 Mo. One pelleted seed was sown per cube, and trays were placed on an indoor propagation rack equipped with air circulation fans and automatic sub-irrigation using the same nutrient solution. After germination in darkness, the dome was removed and three white Arize LED light bars (GE Current, Boston, MA) with a photosynthetic photon flux density (PPFD) of 132 μmol m^{-2} s^{-1} were turned on for 12 hours each day. Daily average air temperature on the propagation rack was maintained between 20–23 °C. Seedlings were moved to a greenhouse 2–3 days before transplanting for hardening and were transplanted to the hydroponic system 10 days after sowing. Based on our pilot study, germination time varied among species. To synchronize the transplanting time, seeding time was different: spinach cultivars were sowed first, followed by lettuce, then pak choi.

Table 1. Greenhouse environmental conditions and solution characteristics during two experiments for both cooling and heating studies conducted in 2020 and 2021.

Expt.	DLI * (mol m^{-2} d^{-1})	Air Temp. (°C)	Solution Temp. (°C)		Solution pH		Solution EC (mS cm^{-1})		Solution DO (mg L^{-1})	
			Control	Treat.	Control	Treat.	Control	Treat.	Control	Treat.
Nutrient solution cooling										
Expt. 1	14.1	27.7	30.2	23.3	6.7	6.7	1.7	1.7	6.7	7.5
Expt. 2	12.2	26.8	29.1	22.9	6.3	6.3	1.6	1.6	6.8	7.7
Nutrient solution heating										
Expt. 1	12.7	14.3	15.7	22.0	6.2	6.3	2.0	2.1	7.9	7.0
Expt. 2	19.9	18.1	19.7	22.4	6.7	6.8	2.0	2.1	8.4	7.6

* DLI (daily light integral) is estimated by multiplying the daily average PPFD (photosynthetic photon flux density) by 0.0864. Temp. = temperature; EC = electrical conductivity; DO = dissolved oxygen.

Seedlings with two true leaves (10–14 days after sowing) were transplanted into a deep-water culture (DWC) hydroponic system consisting of a pond measuring 1.2 m × 1.2 m × 0.3 m, filled with 330 L of the nutrient solution, mentioned above, with an EC of 1.6 mS cm^{-1} and a pH of 6.0. Two floating rafts, each with 28 planting holes and a dimension of 0.6 m × 1.2 m (Beaver Plastics, Acheson, AB, Canada), were used in each system. A submersible water pump equipped with a venturi was set up inside the pond to aerate the nutrient solution. A total of six DWC systems were used for each experiment. To control algae, biofilm, and Pythium in the nutrient solution, a bactericide/fungicide (ZeroTol 2.0, BioSafe Systems, East Hartford, CT, USA) was applied weekly at the recommended rate of 0.2 mL L^{-1}.

The air temperature in the experimental greenhouse was controlled by a wet-pad-fan evaporative cooling system and a shade cloth on top of the roof. The shade cloth was removed during winter growing season; thus, the daily light integral (DLI) was the highest in the second experiment of solution heating in March of 2021 (Table 1). Throughout each experiment, greenhouse air temperature and reservoir solution temperature were measured using thermocouples, and photosynthetic active flux density (PPFD) using a quantum sensor. All sensors were connected to a data logger (Campbell Scientific Inc., Logan, UT, USA) and hourly averages were recorded. The average air and solution temperatures and average DLI during each experiment are presented in Table 1.

2.1.2. Experimental Design and Treatments

For both experiments, there were two treatments, namely control (or no cooling) and cooling treatment. A total of six DWC systems were used, three for control and three for the cooling treatment. An Active AQUA AACH25HP hydroponic water chiller (AACH25HP, ¼ HP, Hydrobuilder, Chico, CA, USA) was used to cool the solution. The chiller was connected to a water pump in each of the three DWC systems to maintain the nutrient solution temperature at a constant set point of 24 °C day and night. The nutrient solution temperature was established 24 h prior to transplanting seedlings into the systems. In each experiment, the two-factor (temperature × genotype) experiment was arranged with solution temperature as the main plot and genotype as the subplot and was replicated three times (blocks). The placement of leafy greens (lettuce, pak choi, and spinach), each with two cultivars, were randomized in each DWC system. There were 10 plants per cultivar for lettuce and pak choi and 8 plants per cultivar for spinach.

2.1.3. Data Collection

For both experiments, the treatment solutions in the reservoirs were monitored daily for EC and pH, and weekly for dissolved oxygen (DO) and solution volume. The EC and pH were measured using a combo meter (Bluelab, Tauranga, New Zealand). The DO was measured using a portable HI 98193 DO meter (Hanna Instruments, Woonsocket, RI, USA).

Plants were harvested after 21 days when they were still considered baby leafy greens instead of mature size. The following data were collected: shoot fresh weight (FW) and dry weight (DW), total leaf area (TLA), specific leaf area (SLA), relative chlorophyll content (SPAD), leaf chlorophyll fluorescence (F_v/F_m, maximal quantum yield), and performance index (PI), which quantifies the overall functionality of the electron flow through PSII [21]. Plants were cut at the substrate level and weighed immediately for shoot FW before being placed in paper bags and dried in a drying oven (Thermo Fisher Scientific, Waltham, MA) at 70 °C to measure shoot DW. Leaves were separated from the basal stem and TLA was measured using an LI-3100C Leaf Area Meter (LI-COR, Lincoln, NE). The SLA was calculated as the total leaf area divided by leaf DW. The SPAD was measured using a handheld SPAD-502Plus meter (Konica Minolta, Osaka, Japan) and the average value was recorded from readings of three mature leaves per plant. F_v/F_m and PI were measured using a PocketPea fluorimeter (Hansatech Instruments, King's Lynn, UK) on one representative mature leaf per plant following 15 min dark adaptation. Since some spinach plants were dead in both experiments, plant death percentage was recorded. Also, surviving spinach plants were small in size, so their root dry biomass was not measured.

2.2. Nutrient Solution Heating during Winter

2.2.1. Plant Materials and Culture

Two experiments were conducted in the same greenhouse from January to March of 2021 but only using the same lettuce cultivars (‘Red Mist’ and ‘Bergams Green’). The same propagation protocol was followed except for the light intensity, which had increased to a PPFD of 230 µmol m^{-2} s^{-1}. Uniform seedlings were selected and transplanted to the same six DWC hydroponic systems to start root-zone heating treatment 18 days after sowing. The floating rafts (Beaver Plastics, Acheson, AB, Canada) with the same dimension but different number of planting holes were used. Each DWC system consisted of 36 plants in this study, while the cooling study had 56 plants. The 36 plants per DWC corresponded to a planting density of 25 plants m^{-2}.

2.2.2. Experimental Design and Treatments

The same experimental design as the cooling study was followed for both Expt. 1 and Expt. 2 with two treatments: control (no heating) and solution heating. A single, submersible 500-W electric heater (HG-902, Shenzhen Mago Co., Ltd., Shenzhen, China) was placed in each heated DWC system to achieve a temperature set point of 22 °C. The unheated DWC system served as the control. The nutrient solution temperature was established 24 h prior to transplanting seedlings.

The greenhouse temperature was controlled by two heaters as well as a wet-pad-fan evaporating cooling system (cooling system acted during the second experiment on warm days). Cooling and heating set points were 22 °C and 13 °C, respectively. The cooling set point was standard for greenhouse production of lettuce, but the heating set point was intentionally set low to provide cooler-than-normal ambient air temperature, particularly at night, to test the effectiveness of the heated solution treatment.

2.2.3. Data Collection

Same plant performance except GI and environmental data were collected in both experiments in the heating study. In Expt. 1, a winter storm caused a power outage and the greenhouse air temperature dropped to below zero for several hours in the morning of Feb. 15. The freeze damage of lettuce plants was assessed several days later and calculated as percent of damaged leaves (partial or edge of the leaf) to the total leaf count per plant. However, plants grew out quickly and the freeze damage on some leaves had minimal effect on the study.

2.3. Statistical Analysis

All data were processed with analysis of variance (ANOVA) using JMP 14 (SAS, Cary, NC, USA) to determine the effect of cooling or heating varying with plant genotype. All results of Expt. 1 and Expt. 2 in both solution cooling and heating studies are presented separately due to significant differences between the two experiments. Means of different treatments were separated using Tukey’s HSD test at $\alpha = 0.05$. For the plant genotype with an observable increase in shoot FW due to solution cooling or heating, correlation analysis was performed between shoot FW and the other plant traits.

3. Results

3.1. Effect of Nutrient Solution Cooling

3.1.1. Plant Growth

Among the three species, all lettuce and pak choi plants were healthy and marketable. Spinach plants in the no solution cooling treatment started to die shortly after transplanting. In the cooling treatment some spinach plants started to die by the end of the second week, and by the end of the third week (experiment termination), 60% of ‘Mandolin’ and 80% of ‘Seaside’ plant were dead in the first experiment (Figure 1A). Cooling the nutrient solution reduced the percent of dead plants, with no observed differences between the two cultivars in Expt. 1. However, in Expt. 2, all ‘Seaside’ plants were dead in both cooling and control treatments by the end of the 3-week growth; however, most 'Mandolin' plants still survived but with low quality (not marketable) due to stunted growth (Figure 1B). 'Seaside' spinach plants died gradually in the cooling treatment, while most were dead by the end of the first week in the control.

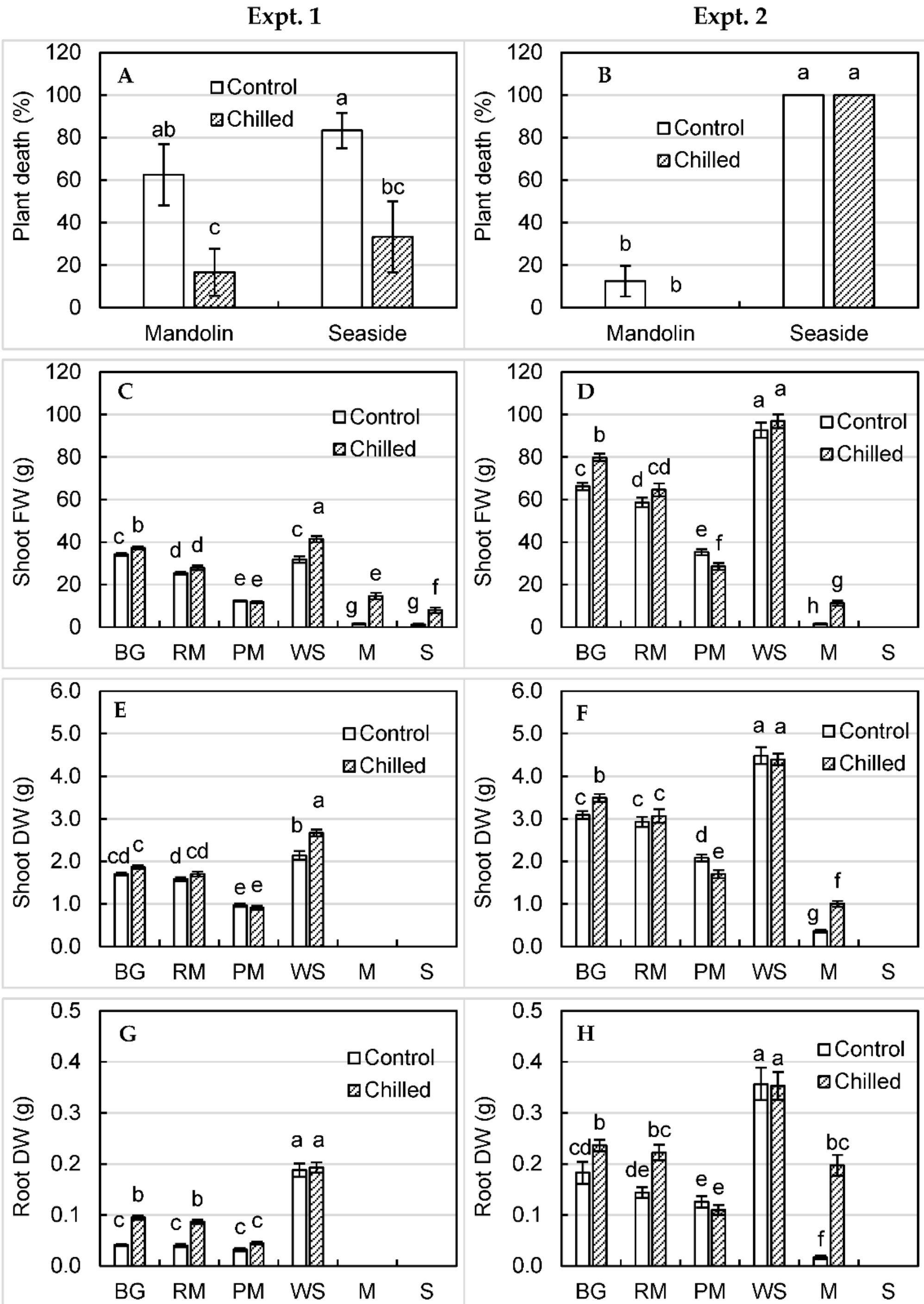

Figure 1. Plant death (mortality %) (**A**,**B**), shoot fresh weight (FW) (**C**,**D**), shoot and root dry weight (DW) (**E**–**H**) of the lettuce varieties 'Bergam's Green'(BG) and 'Red Mist' (RM), Pak Choi varieties 'Purple Magic' (PM) and 'White Stem' (WS), and spinach (*Spinacia oleracea* L.) 'Mandolin'(M) and 'Seaside' (S) grown in a deep-water culture hydroponic system with nutrient cooling or no cooling (Control). All growth data units from (**C**–**H**) are g/plant. Different letters represent significant differences between treatments according to Tukey's HSD test ($p < 0.05$).

Shoot FW was influenced by nutrient solution cooling, but response varied with genotype and experiment (Figure 1C,D) with spinach and pak choi having the greatest differences. Shoot FW in the cooling treatment increased by 9% and 21% in 'Bergam's Green' lettuce in Expt. 1 and Expt. 2, respectively, and 30% in 'White Stem' pak choi in Expt. 1 but decreased by 19% in 'Purple Magic' pak choi in Expt. 2.

Similarly, shoot DW was affected by nutrient solution cooling and varied with genotype and experiment (Figure 1E,F). In Expt. 1, nutrient solution cooling increased shoot DW by 25% in 'White Stem' pak choi, and in Expt. 2, an increase of 13% in 'Bergam's Green' lettuce and 177% in 'Mandolin' spinach was observed; however, a reduction in shoot DW of 18% was observed in 'Purple Magic' pak choi. Root DW was also affected by the nutrient solution cooling treatment compared with the control (Figure 1G,H). In lettuce, root DW increased by 131% and 30% in 'Bergam's Green', and 117% and 24% in 'Red Mist' in Expt. 1 and 2, respectively.

Nutrient solution cooling increased total leaf area (TLA) by 9% and 19% in 'Bergam's Green' lettuce in Expt. 1 and Expt. 2, respectively, and by 23% in 'White Stem' pak choi in Expt. 1 and 557% in 'Mandolin' spinach in Expt. 2 (Figure 2A,B). Specific leaf area (SLA) and leaf relative chlorophyll content index (SPAD) were not affected by the treatment in all genotypes in both experiments, except for an increase in SLA by 110% in 'Mandolin' spinach plant in Expt. 2 (Figure 2C–F).

3.1.2. Stress Evaluation

Chlorophyll fluorescence parameters F_v/F_m and PI were measured to evaluate the stress status of plants. In Expt. 1, the maximal quantum yield (F_v/F_m) decreased by 3% in the solution cooling treatment in 'Bergam's Green' and 4% in 'Red Mist' lettuce but increased by 5% in 'Mandolin' and 11% in 'Seaside' spinach (Figure 3A,B). During Expt. 2, solution cooling did not affect F_v/F_m except for a 1% decrease in 'White Stem' pak choi.

The performance Index (PI) showed a similar pattern as F_v/F_m in response to the solution cooling treatment, except for pak choi (Figure 3C,D). In Expt. 1, solution cooling decreased PI by 35% in 'Bergam's Green' and 37% in 'Red Mist' lettuce, 21% in 'Purple Magic' and 18% in 'White Stem' pak choi but increased PI by 205% in 'Mandolin' and 120% in 'Seaside' spinach. In Expt. 2, PI was not affected by the treatment except for a 17% decrease in 'White Stem' pak choi.

3.2. Effect of Nutrient Solution Heating

3.2.1. Plant Biomass

In Expt. 1, shoot FW of 'Bergam's Green' and 'Red Mist' increased by 57% and 126%, respectively, in the heated treatment (Figure 4A). In Expt. 2, shoot FW increased by 18% for 'Red Mist' in the heated treatment but was not different for 'Bergam's Green' (Figure 4B). The effect of treatment on shoot DW followed the same pattern as shoot FW (Figure 4C,D). In Expt. 1, shoot DW of 'Bergam's Green' and 'Red Mist' increased by 25% and 59%, respectively, in the heated treatment. In Expt. 2, only 'Red Mist' had a 13% increase in shoot DW. Root DW was increased by the heated treatment in Expt. 1 but not in Expt. 2 (Figure 4E,F). In Expt. 1, root DW of 'Bergam's Green' and 'Red Mist' increased by 27% and 15% in the heated treatment, respectively.

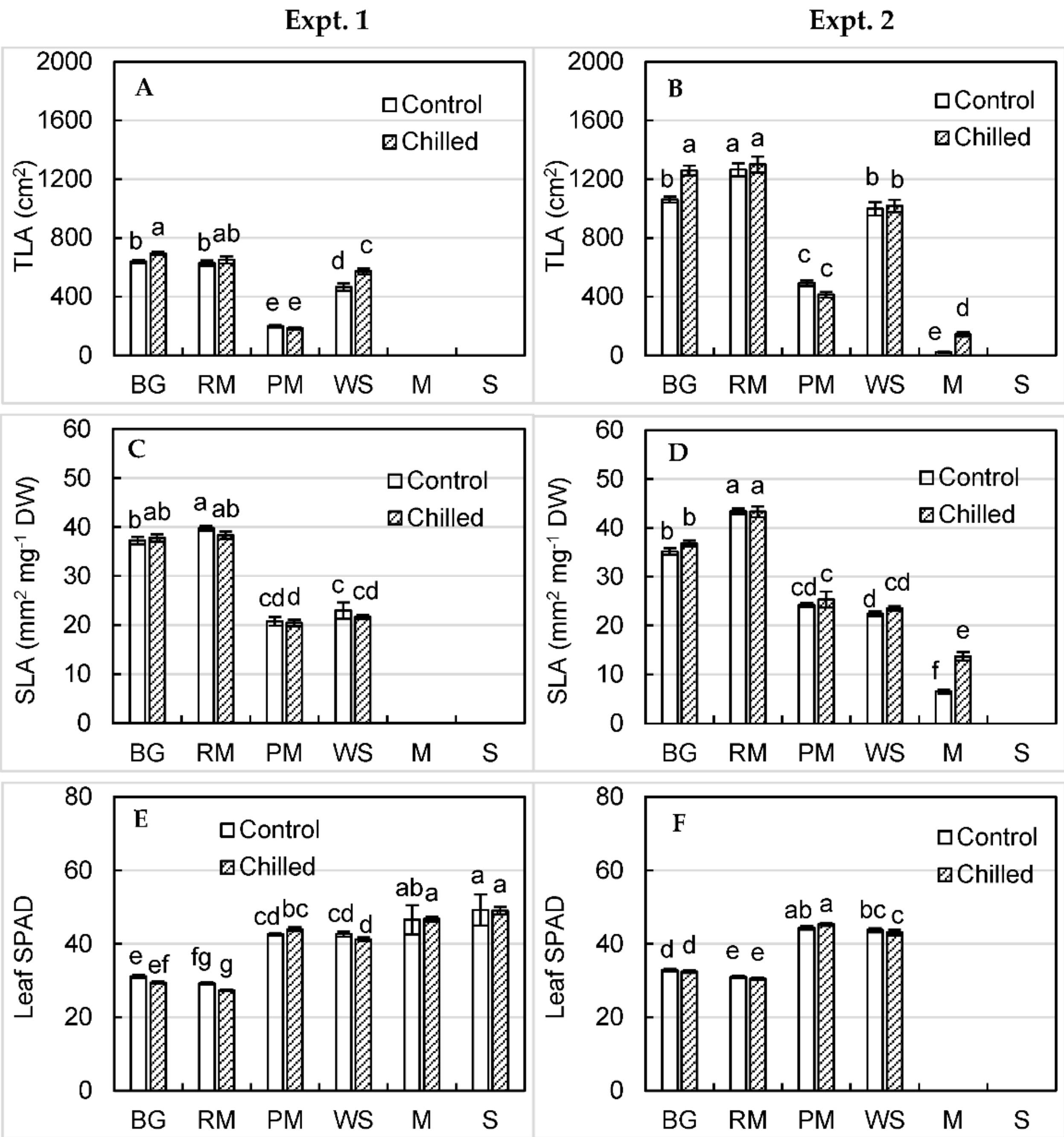

Figure 2. Total leaf area (TLA) (**A**,**B**), specific leaf area (SLA) (**C**,**D**), and leaf relative chlorophyll content (SPAD) (**E**,**F**) of the lettuce varieties 'Bergam's Green'(BG) and 'Red Mist' (RM), Pak Choi varieties 'Purple Magic' (PM) and 'White Stem' (WS), and spinach (*Spinacia oleracea* L.) 'Mandolin'(M) and 'Seaside' (S) grown in a deep-water culture hydroponic system with nutrient cooling or no cooling (Control). Different letters represent significant differences between treatments according to Tukey's HSD test ($p < 0.05$).

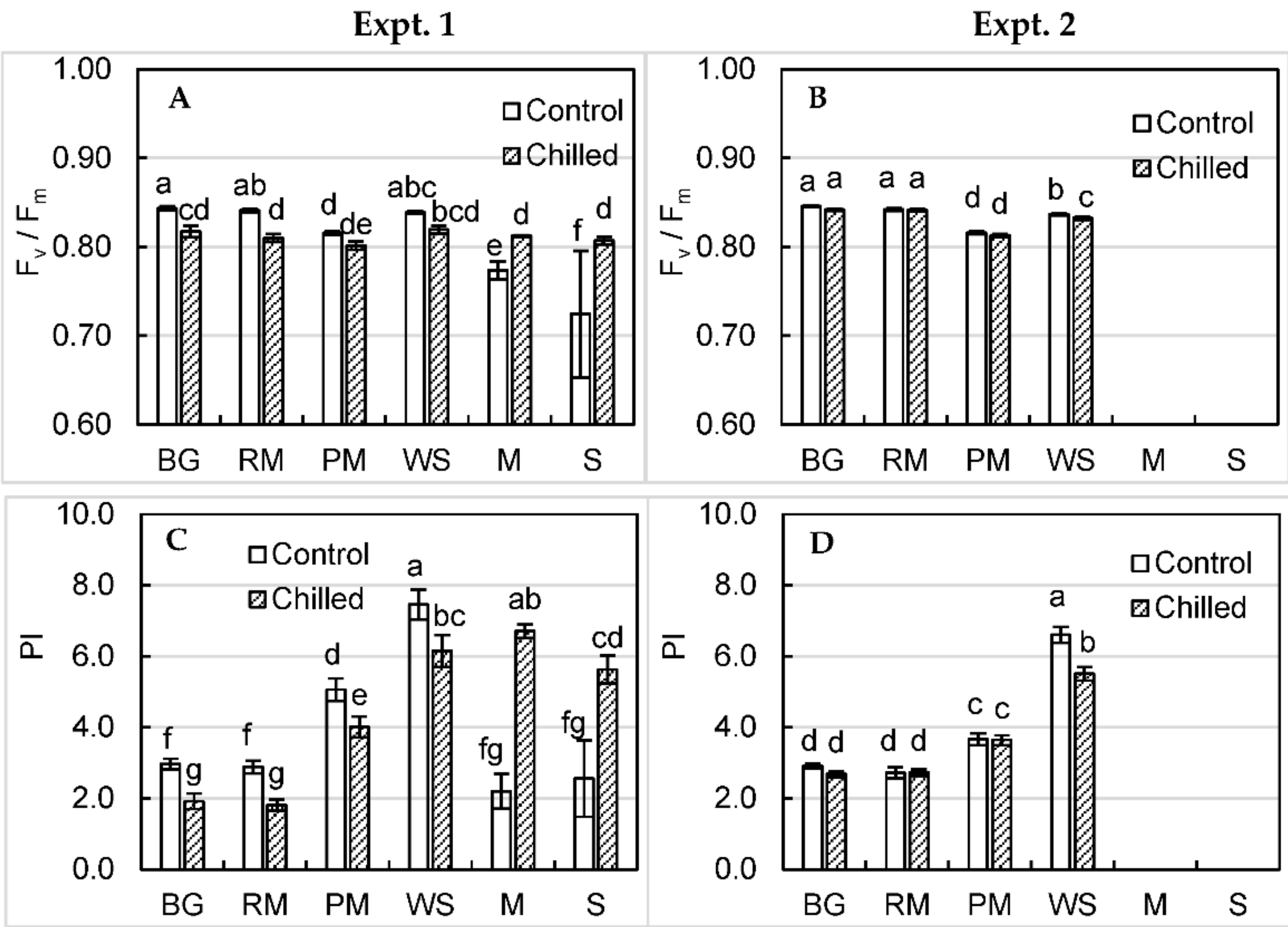

Figure 3. Chlorophyll fluorescence (F_v/F_m) (**A**,**B**) and performance index (PI) (**C**,**D**) of the lettuce varieties 'Bergam's Green'(BG) and 'Red Mist' (RM), Pak Choi varieties 'Purple Magic' (PM) and 'White Stem' (WS), and spinach (*Spinacia oleracea* L.) 'Mandolin'(M) and 'Seaside' (S) plants grown in a deep-water culture hydroponic system with nutrient cooling or no cooling (Control). Different letters represent significant differences between treatments according to Tukey's HSD test ($p < 0.05$).

3.2.2. Leaf Traits

The heating treatment increased TLA of both cultivars but the difference between treatments was larger in Expt. 1 than in Expt. 2 (Figure 5A,B). The TLA of 'Bergam's Green' and 'Red Mist' increased by 92% and 130%, respectively, in the heated treatment compared to the control in Expt. 1, while only 'Red Mist' increased by 18% in Expt. 2. The heated treatment increased SLA for both species in both experiments (Figure 5C,D). SLA increased by 56% and 44% in Expt. 1, and 10% and 5% in Expt. 2, for 'Bergam's Green' and 'Red Mist', respectively.

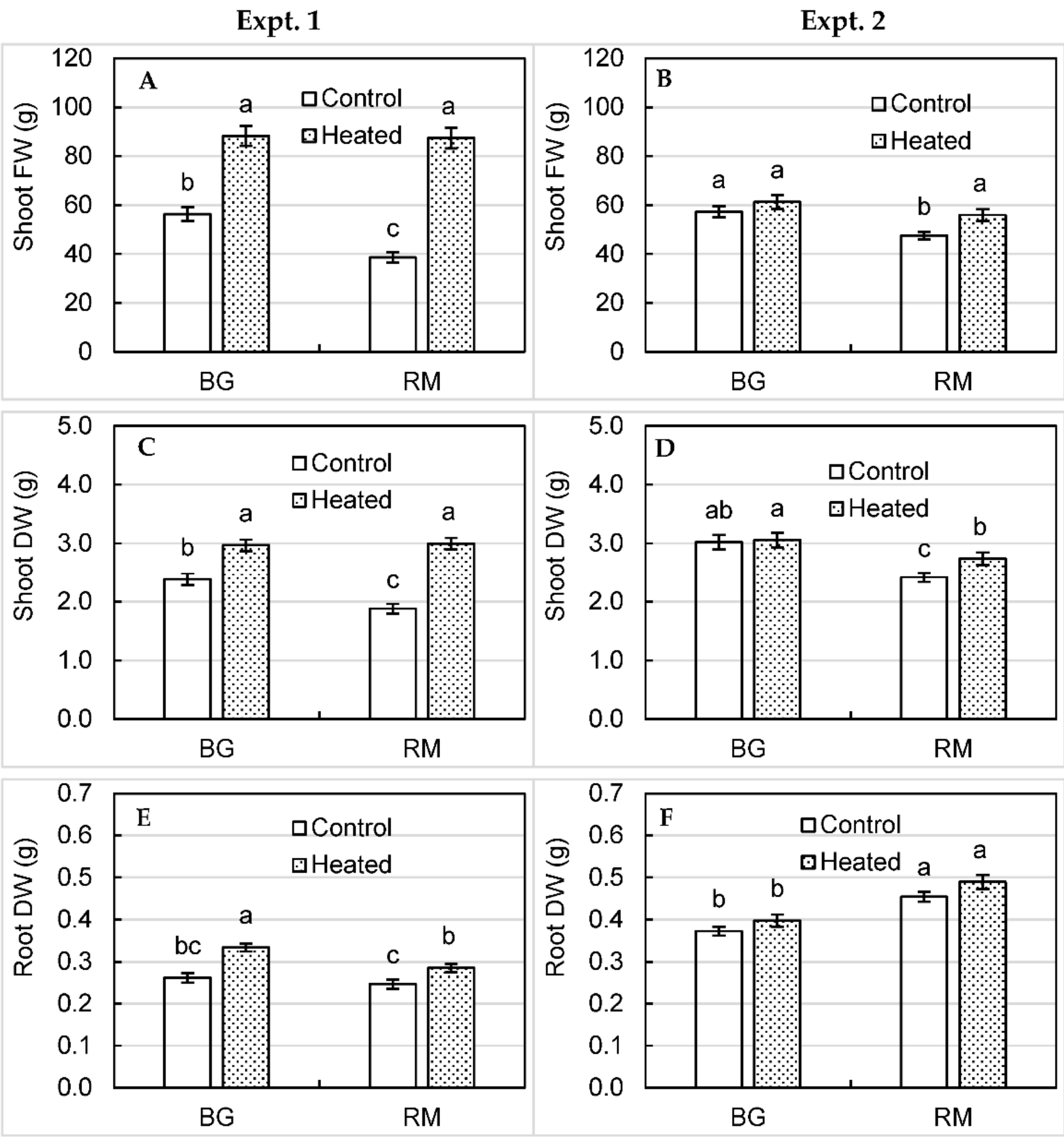

Figure 4. Shoot fresh weight (FW) (**A**,**B**), dry weight (DW) (**C**,**D**), and Root dry weight (DW) (**E**,**F**) of the lettuce varieties 'Bergam's Green' (BG) and 'Red Mist' (RM) grown in a deep-water culture hydroponic system with nutrient solution heating or no heating (control). All growth data units are g/plant. Different letters represent significant differences between treatments according to Tukey's HSD test ($p < 0.05$).

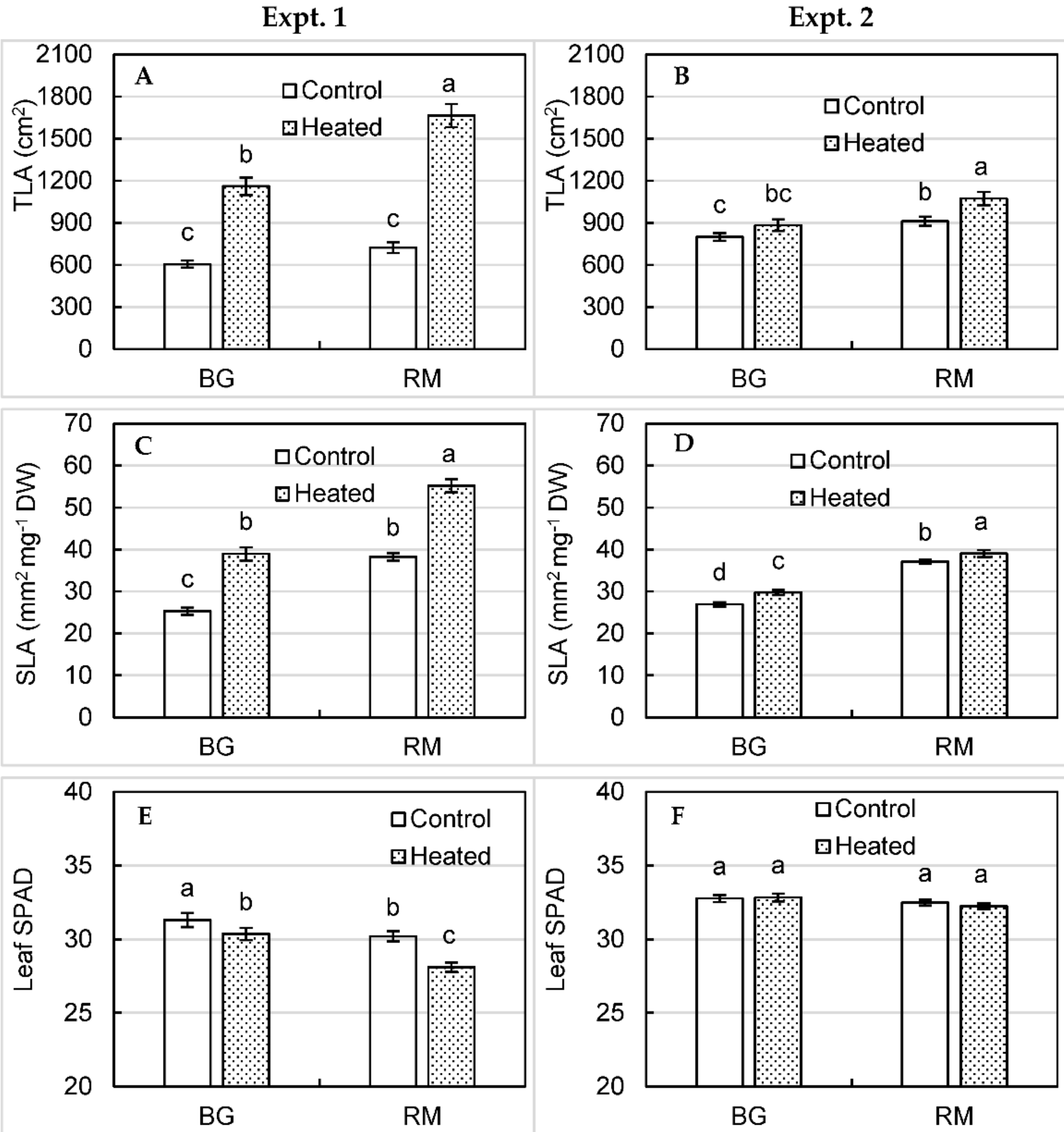

Figure 5. Total leaf area (TLA) (**A**,**B**), specific leaf area (SLA) (**C**,**D**), and leaf relative chlorophyll content (SPAD) (**E**,**F**) of the lettuce varieties 'Bergam's Green'(BG) and 'Red Mist' (RM) grown in a deep-water culture hydroponic system with nutrient solution heating or no heating (control). Different letters represent significant differences between treatments according to Tukey's HSD test ($p < 0.05$).

Leaf SPAD was affected by the heated treatment in Expt. 1, but not Expt. 2 (Figure 5E,F). In Expt. 1, the heated treatment relative to control reduced leaf SPAD by 3% and 7% in 'Bergam's Green' and 'Red Mist', respectively.

3.2.3. Stress Evaluation

The F_v/F_m and PI were generally not affected by the heated treatment except for PI in 'Red Mist' in Expt. 1 (Figure 6A–D). The PI decreased by 29% for 'Red Mist' lettuce plants grown in the heated solution compared to the control in Expt. 1.

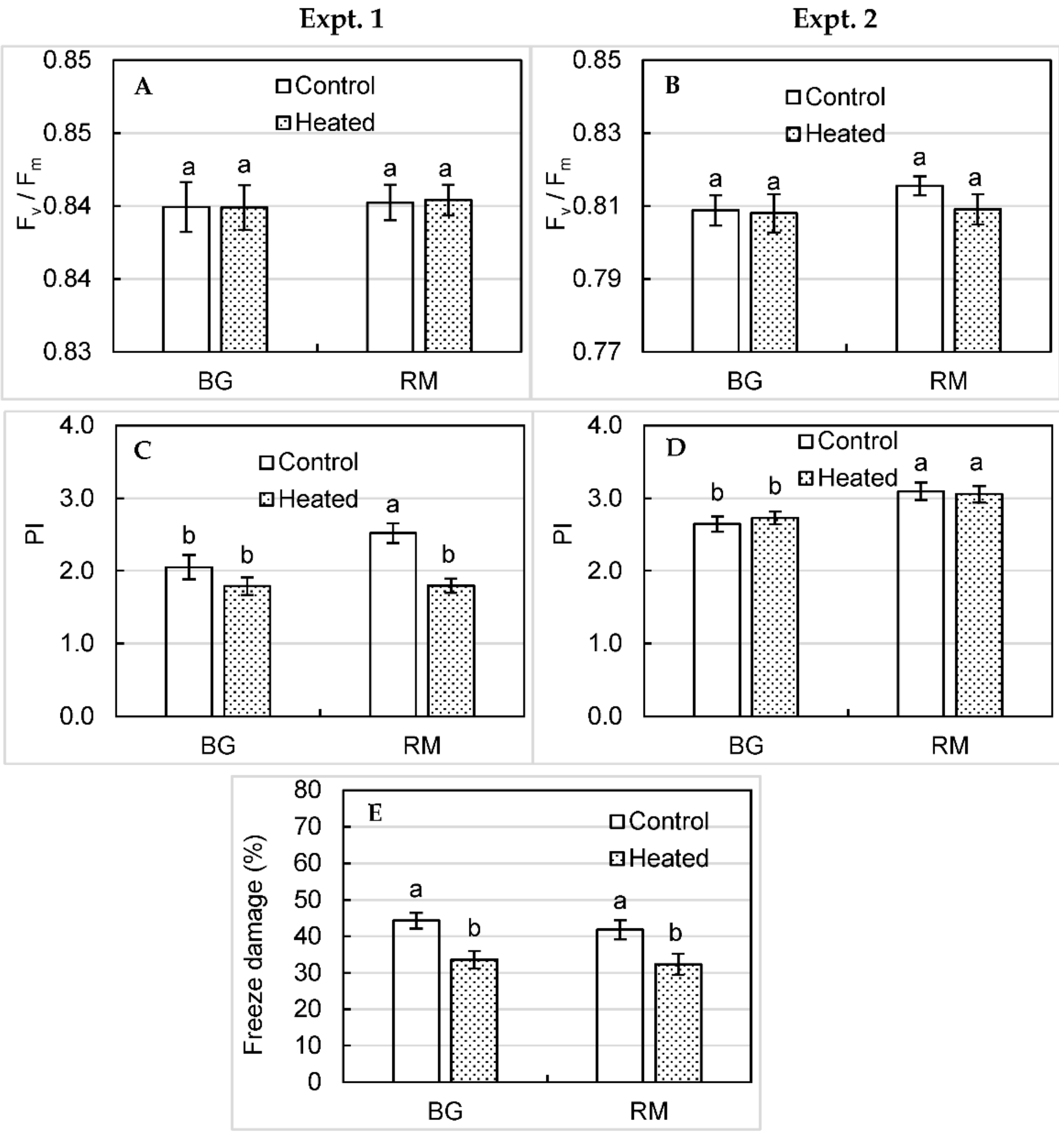

Figure 6. Chlorophyll fluorescence (F_v/F_m) (**A**,**B**), and performance index (PI) (**C**,**D**) and plant freeze damages (**E**) of the lettuce varieties 'Bergam's Green' (BG) and 'Red Mist' (RM) grown in a deep-water culture hydroponic system with nutrient solution heating or no heating (control). Different letters represent significant differences between treatments according to Tukey's HSD test ($p < 0.05$). The plant damage was caused by freezing air temperatures that occurred for several hours at night due to a power outage during the winter storm on Feb. 16, 2021 (DAT 18). Plants were visually assessed for freeze damage on the leaves a few days after the freeze event.

The short term (a few hours) below-freezing temperature due to the winter storm caused some damage on lower and older leaves. However, there was less leaf freeze damage in plants grown in the heated treatment compared to the control for both lettuce cultivars (Figure 6E). The freeze damage was observed in lower and older leaves only, while new and young leaves were not affected; thus, the damage did not stop the plants' continued growth.

4. Discussion

4.1. Root-Zone Temperature Control Has the Potential to Increase Crop Yield

Optimization of root-zone temperature through solution heating or cooling in a hydroponic system would be a more efficient way for winter or summer greenhouse production of cool-season baby leafy greens, compared with controlling the air temperature of the whole greenhouse [22]. Shoot FW in 'Bergam's Green' lettuce and 'Mandolin' spinach was increased by solution cooling in both experiments. Similarly, heating the solution increased shoot FW in 'Red Mist' lettuce in both experiments. These results indicate the potential of increasing yield through root-zone temperature adjustment in these leafy greens in winter or summer greenhouse production.

Due to the nature of glazing materials of greenhouses, the air temperature is easily affected by outside environmental conditions. Consequently, maintaining an optimal greenhouse air temperature by heating or cooling the whole greenhouse leads to a high energy cost and achieving an optimal air temperature may not be possible in hot summers [2]. On the contrary, heating or cooling the nutrient solutions may be readily achieved with a much lower energy input. By enhancing the thermal insulation of the culture bed and placing the nutrient reservoirs underground, energy costs can be further reduced.

The increased plant fresh biomass by optimization of root-zone temperature was at least partly attributed to the improved photo-assimilate accumulation through a greater leaf area (e.g., leaf expansion) to increase light interception. This is because shoot FW in heated solution was closely related to greater TLA (correlation coefficient, r of 0.988) and shoot DW (r of 0.832), and the increased shoot FW by cooling the solution was closely related to greater TLA (r of 0.934), shoot DW (r of 0.897) and root DW (r of 0.809). Apparently, for plants grown in the solution cooling treatment, in addition to increased biomass accumulation, root growth also contributed to shoot FW, since more developed root systems contributed to increased water and nutrient uptake in plants and thus improved crop yield [23]. In the solution cooling treatment, the higher dissolved oxygen, which was 7.5 mg L^{-1} vs. 6.7 mg L^{-1} in the control, might also partially contribute to the increased root growth.

4.2. Responses to Root-Zone Temperature Control Vary with Plant Genotype

Plant growth and development is dependent on the temperature surrounding the plant and each species has a specific temperature range represented by a minimum, optimum, and maximum [24]. Between minimum and optimum, plant growth rate is linearly correlated with (air) temperature. It is understandable that growth rate is also affected by nutrient solution temperature, and there might be also minimum, optimum, and maximum temperatures for nutrient solution. However, little quantitative relationship between plant growth and nutrient solution temperature is available. In this study, different plant species responded differently to root-zone cooling. For example, spinach was the most heat-sensitive species among the three species and lowering nutrient solution temperature to 24 °C was not sufficient and the air temperature during the day at (25–32 °C) was well above the optimum temperature (15–20 °C) [25]. The good news is that there was a cultivar difference: 'Mandolin' was more heat-tolerant, as evidenced by low mortality than that of 'Seaside', although plants did not grow out. This indicates that more research is needed to identify heat-tolerant spinach cultivars and the suitable range of nutrient solution temperatures to determine if it is feasible to grow spinach in a warm climate through nutrient solution cooling. For other species, differences were small and based on biomass and leaf trait parameters in response to the nutrient solution cooling. With a nutrient solution temperature of 24 °C, lettuce and pak choi can be grown in a hot summer; thus, year-round hydroponic production is possible. In the nutrient solution heating study, the two lettuce cultivars also responded differently. Similar plant response variation between different cultivars under nutrient solution heating was observed in a recent study on hydroponic lettuce [3]. These results confirmed that optimal nutrient solution temperature varies with species and even cultivar within a species.

For chlorophyll fluorescence parameters, solution cooling significantly improved F_v/F_m and PI in spinach. However, differences in these parameters were not observed in the other two species. On the contrary, there were slight decreases in the two parameters in lettuce and pak choi in the solution cooling treatment. It is worth noting that the values of F_v/F_m in lettuce and pak choi plants were still in the non-stress range of 0.79 to 0.84 [26], indicating no stress. However, spinach plants without nutrient solution cooling were stressed based on the F_v/F_m values. Compared to F_v/F_m, there were relatively bigger differences in PI between treatments and genotypes, indicating that PI was more sensitive than F_v/F_m to detect stresses.

4.3. Effect of Air Temperature

During summer greenhouse production, when outside temperature was higher, the beneficial effects of solution cooling were greater for the same plant genotype. For example, in 'White Stem' pak choi under chilling treatment relative to control, growth index, shoot FW, shoot DW, and TLA increased only in Expt. 1, but not Expt. 2. Inside the experimental greenhouse, the daily average air temperature was 27.7 °C and 26.8 °C, and control solution temperatures were 30.2 °C and 29.1 °C in Expt. 1 and 2, respectively. It appears that, at least for 'White Stem' pak choi, plants benefited more from solution cooling at higher vs. lower greenhouse air temperature. Possibly, higher greenhouse air temperature during summer increased solution temperature and resulted in worse root-zone environment such as lower DO level (6.7 mg L^{-1} without cooling vs. 7.7 mg L^{-1} with cooling) due to an inverse relationship between water temperature and DO concentration [27]. Solution cooling can increase DO level in the solutions and improve root-zone environment [22], which was confirmed in this study.

During winter greenhouse production, when outside temperature was lower, the beneficial effects of solution heating were greater for the same plant genotype. For example, in 'Bergam's Green' lettuce under heating treatment relative to control, shoot FW, shoot DW, root DW, and TLA increased only in Expt. 1, but not in Expt. 2. Inside the experimental greenhouse, the daily average air temperature was 14 °C and 18 °C, and control solution temperature was 16 °C and 20 °C in Expt. 1 and 2, respectively. It appears that, at least for 'Bergam's Green' lettuce, plants benefited more from solution heating at lower vs. higher greenhouse air temperature.

5. Conclusions

During summer or winter, greenhouse production of cool-season crops such as lettuce and pak choi using deep-water culture systems, nutrient solution cooling or heating to a target temperature of 24 °C increased shoot biomass and total leaf area compared with control. Spinach was the most heat-sensitive species and solution cooling to 24 °C was not sufficient for marketable growth. However, both lettuce and pak choi can effectively be grown during the summer with nutrient solution cooling. Likewise, nutrient solution heating during the winter season increased lettuce plant growth with a relatively low air temperature setting in the greenhouse to reduce heating cost. Overall, more research is needed for optimization of nutrient solution temperature range for both cooling and heating of various high-value baby leafy greens.

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