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CROP PRODUCTION

Effects of Root-Zone Temperature on Growth, Chlorophyll Fluorescence Characteristics and Chlorophyll Content of Greenhouse Pepper Plants Grown under Cold Stress in Southern China[1]

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Abstract—In Southern China, plants are usually exposed to cold stress during winter in an unheated greenhouse, but due to the high energy consumption and costs, most of the greenhouses remain unheated. In an attempt to find a simple and affordable solution to this problem, this study was undertaken. In this research, *Capsicum frutescens* L. plants were studied to investigate the effect of different root zone temperatures on its growth and chlorophyll fluorescence characteristics under cold stress. The plants were cultivated under cold stress conditions in a root zone temperature (RZT) control system where the roots were subjected to four different root-zone temperature treatments of 20°C-T20, 25°C-T25, 45°C-T45 and a control CK group. Growth characteristics studied included plant height, stem diameter, plant width, root length, biomass accumulation. Whilst fluorescence characteristics investigated were chlorophyll fluorescence ratio F_v/F_m, photochemical quenching (qL), efficiency of Photosystem II (Y[II]) and electron transport rate (ETR). Chlorophyll content in the leaves of the plants was also investigated. The findings demonstrated that plants in the CK group suffered a detrimental effect on the growth characteristics registering the lowest values in the measured variables. Conversely, the highest values were observed in T25 RZT treatment. In fluorescence characteristics, values of F_v/F_m were maintained at between 0.8 and 0.83 but also suffered a photo-inhibitory depression in CK and T45 RZT treatments to F_v/F_m values of <0.79. This depicted that root zone heating protected the PS II of these plants from photoinactivation induced by cold stress. Similar trends were seen in the qL, Y[II], ETR values with the T20 and T25 treatments registering the highest values. Chlorophyll content was significantly higher in the leaves of the plants in the T20 and T25 group. The lowest chlorophyll content was recorded in the CK group. Plants in all the treatments accumulated more biomass in the shoot than in the roots as depicted by a significantly lower shoot to root ratio values with the exception of those in the CK group. The findings of this study suggest that pepper plants can successfully be grown in an unheated greenhouse in the Yangtze River Delta area of Southern China during winter by heating the root zone of the plants to a RZT value of 25°C, thereby providing a simple, affordable and cost-effective technique.

Keywords: *Capsicum frutescens* L., cold stress, root zone temperature, root zone heating, growth characteristics, chlorophyll fluorescence, Yangtze River Delta area of Southern China

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INTRODUCTION

Cold stress brought about by low ambient air temperatures experienced during winter in the Yangtze River Delta area of Southern China has a profound impact on plant photosynthesis, nitrogen metabolism, plant enzyme activity and plant dry matter accumulation [1]. The exposure of plants to cold stresses results in stunted growth which may severely impact yield and fruit quality [2]. In addition, cold stress conditions resulted in reduced root permeability to water thus limiting the water absorption capacity which translated to a reduction in plant water status [3]. Despite these detrimental effects of cold stress to greenhouse plants, most of the greenhouse in the Yangtze River Delta area of Southern China remain unheated during winter due to the high energy consumption and costs [4]. In a bid to find a simple and affordable solution to growing crops under cold stress in an unheated greenhouse in Yangtze River Delta area of Southern China, this study was undertaken.

[1] The article is published in the original.

Abbreviations: CF—chlorophyll fluorescence; ETR—electron transfer rate; NPQ—non-photochemical fluorescence quenching PSII—photosystem II; qL—coefficients of photochemical fluorescence quenching; RZT—root zone temperature; Y[II]—effective photochemical quantum yield of PS II.

Previous studies have shown that by simply heating the root-zone of plants, the general plant health, growth and development can be improved even though the above-soil portions are exposed to environmental stresses [5]. The productivity of numerous plant species is influenced by RZT and its effect on plant growth was possibly more than that of ambient air temperature [6]. The roots are more sensitive to temperature than the above-soil portions of the plant as roots have a small adaptability range in temperature [7]. Further, the root-zone having a higher specific heat capacity in comparison to the above-soil portion and offers the possibility of direct, precise, easy manipulation and regulation of the temperature of the root-zone layer.

In this research, pepper (*Capsicum frutescens* L.) was studied to investigate the impact of different root zone temperatures on its growth and chlorophyll fluorescence characteristics under cold stress. The plants were cultivated in a RZT control system under cold stress conditions where the roots were subjected to four different root-zone temperature treatment of 20°C-T20, 25°C-T25, 45°C-T45 and a control CK group.

In recent years, studies have been conducted to understand how to grow greenhouse plants during winter in the Yangtze River Delta area of Southern China in an unheated greenhouse. Some of the studies undertaken evaluated LED light supplementation [8] but few studies focused on root zone temperature heating. Therefore, this study aimed to find a solution that involved heating the root zone of the greenhouse plants and studying the effect of different RZTs on growth and chlorophyll fluorescence characteristics. The results could provide an insight into a simple, affordable and cost-effective technique of successfully growing greenhouse crops during winter in the Yangtze River Delta area of Southern China, offering the possibility of low energy consumption with high crop productivity.

MATERIALS AND METHODS

Plant Material

Pepper (*Capsicum frutescens* L.) is one of the principal staple vegetable crops in China and is one of the most greenhouse cultivated vegetable crop in Southern China [9] and as such was selected as the plant research material for this study.

Experimental Setup

The experiment was conducted between 27th December 2016 and 19th June 2017 in an unheated greenhouse located at the College of Engineering, Nanjing Agricultural University, Nanjing, Jiangsu Province, China (118°46′ N, 32°03′ E). Seeds of pepper (*Capsicum frutescens* L.) Sujiao no. 5 were germinated on moist cotton towels in a culture chamber at a constant temperature of 28°C and a constant humidity of 98%. After four days, the seedlings were transplanted into the respective plots on 27th of December, 2017. After the fourth true leaf had fully expanded, healthy and homogeneous seedlings were selected, and the rest were thinned out. A nutrient solution was further used to supply the dietary minerals. The experimental set-up consisted of four treatments; Control group, T20, T25, and T45. Each treatment group was consisting of nine plants.

Root Temperature Control and Measurement

To achieve different RZTs for the treatments, heat to the soil in the plots was applied by three separate carbon fiber heating boards (Hua Yu, China). The RZTs for each of the treatments were automatically controlled by an Arduino Microcontroller (Arduino, China) through four Sensirion SHT10 soil temperature sensors (Sensirion, Switzerland). Root zone temperature distribution was monitored by PT100 Soil Temperature Sensors (Teng-Hui, China) evenly positioned on the plot and set at about 10 cm in depth of medium. In total 16 PT100 sensors were used in the experiment with four sensors for each of the treatments. The THM320K; a 32-channel Temperature Recording Instrument (Teng-Hui, China) was used to log the temperature values from the sensors simultaneously.

Measurement of Abiotic Variables within the Greenhouse

The Eco-Watch ecological environment monitoring system (Dynamax, USA) was used in the monitoring and collection of microenvironmental variables within the greenhouse. The environmental variables were collected and logged at a 15-minute interval. The variables collected and logged were ambient air temperature, relative humidity, and solar irradiation.

Chlorophyll Fluorescence Analysis

All CF measurements were made with MiniPam II Chlorophyll Fluorimeter (Walz, Germany) on the attached leaves. A personal computer equipped with the WinControl-3 software was used for data acquisition. After 12 weeks, pepper plants with healthy and homogeneous canopies were selected for CF measurement. Three leaves among the most recent fully-expanded leaves were tested for each plant and an average value calculated. CF parameters; maximum photochemical quantum yield of PSII (F_v/F_m) chlorophyll fluorescence photochemical quenching (qL), non-photochemical quenching (NPQ), actual photosynthetic quantum yield (YII) and electron transport rate ETR were determined after 30 minutes of dark-acclimation of the leaves. The CF parameters were measured once every seven days as from 19:00 to

23:00. During the CF measurements, measuring light was a blue LED (470 nm), the measuring light PAR at standard settings was 0.05 μmol m^{-2} s^{-1}. The actinic light had a maximum continuous PAR of 3000 μmol m^{-2} s^{-1}, maximum PAR of saturation pulses was 6000 μmol m^{-2} s.

Growth Characteristics

Plant height, stem diameter, and plant width were measured every seven days in the different RZT treatment groups. At the end of the experiment, pepper fruits were harvested, plants were uprooted, the shoots separated from the root and the leaves were plucked from the petioles. An electronic weighing balance was used to measure the fresh weights of the roots, stems, leaves, and fruits of the respective experimental groups. Subsequently, roots, stems, leaves, and fruits from similar experimental groups were initially washed clean then put in the drying oven for 30 minutes at a temperature of 105°C. After that, a constant temperature of 80°C was applied until a constant weight of the plant/fruit was attained. The dry weights of the respective groups were also determined.

Chlorophyll Content

Chlorophyll Content measuring device, SPAD-502Plus (Konica Minolta, Japan) was applied to determine the relative chlorophyll content. The meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf. The measurements were repeated three times on four different leaves on each plant, and the values averaged to represent one observation. The leaves selected for SPAD measurements were well-exposed leaves in the upper canopy of the plants.

Statistical Analysis

Matlab was used for processing bulk data of ambient air temperature and root-zone temperature values logged by the Eco-Watch ecological environment monitoring system and THM320K respectively. It was also applied in the generation of their respective graphs. Growth and physiological characteristics data were checked for normality and analyzed by one-way ANOVA using Proc GLM procedure of the SAS statistical package version 9.2 (SAS Institute Inc., Cary, NC). Significant means at F-test were separated using Turkey's HSD (p = 0.05). JMP version 13.1.0 (SAS Institute Inc., Cary, NC) was used to model the relationship between time, measured variables, and RZT treatments. MS Excel 2016 was used in the generation of the respective graphs. The graphics programs AutoCAD 2017 was used to create artwork.

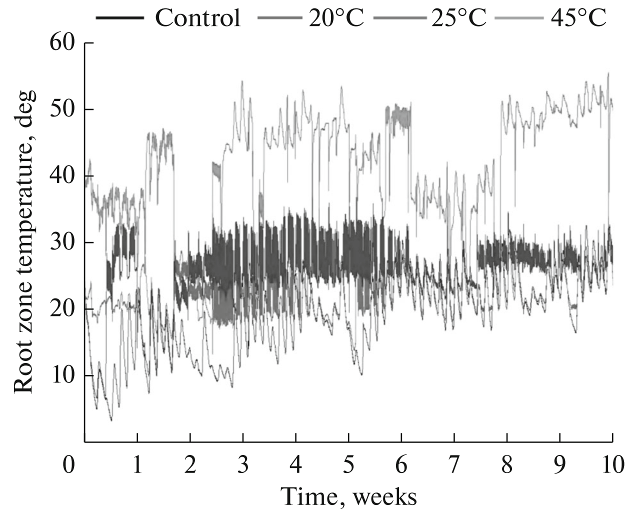

Fig. 1. Actual root zone temperatures of the different RZT treatment groups over 11 weeks of the experimental period.

RESULTS

Abiotic Variables within the Greenhouse

The ambient nighttime air temperatures frequently dropped below zero as opposed to the ambient daytime air temperatures. Ambient air temperature ranged from –2.66 to 43.01°C and the average ambient air temperature was 16.96°C. Solar irradiation ranged from 0.05 to 601.29 W/m^2, and the average solar irradiation was 270.89 W/m^2. RZT trajectories traced the desired patterns as controlled by the Arduino with the RZTs in the T20, T25 and T45 treatments varying around the respective set-point temperatures. The root temperatures of the different root zone treatments during the experimental period are as shown in Fig. 1.

Effect of Different RZTs on the Growth Characteristics of Pepper Plants

The different RZTs had both a positive and adverse impact on the growth and development of pepper plants.

Root Length Assessments

The results showed that T25 treatment had the highest root growth that was 49.02% higher than the value of the CK group, representing a significant difference ($p < 0.05$). There was no significant difference between T20 and T45 treatment (Fig. 2).

Plant Height, Stem Diameter, and Plant Width

Plants grown under T25 treatment had the highest plant height, stem diameter and plant width values with the lowest values seen in the CK group. Plant

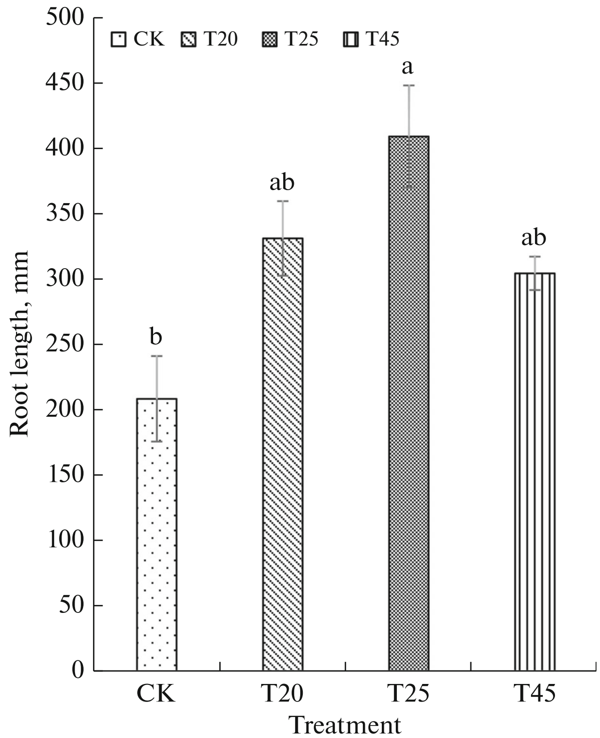

Fig. 2. Root length of pepper plants under different RZT treatments. Data is Means ± SE. Treatment means within the same column followed by different letters are statistically different based on Tukey's test at $p < 0.05$

height measurements found that plants under the T25 treatment were 53.59% higher than those in the CK group representing a significant difference at $p < 0.05$. Further, plants in the T45 treatment were 13.13% lower than those in the T25 treatment, also representing a significant difference at $p < 0.05$. There was no significant difference in plant height under the T25 and T20 treatments at $p < 0.05$ (Table 1).

Plant width assessments revealed that plants in the T25 group had the widest plant width with respect to the other treatments. The plants in the T25 group recorded values that were 54.57% higher than those of the CK group, 30.70% higher than those of the T45 treatment and 16.76% higher than those in the T20 treatment representing a significant difference at $p < 0.05$ (Table 1).

Stem diameter evaluations found that plants in the CK group recorded the lowest stem growth among the treatments and those stem growth values were 40.07% lower than those in the T25 treatment and 36.69% lower than values in the T20 treatments. This represented a significant difference at $p < 0.05$. The stem diameter values in the T45 treatment were also 14.90% lower than the values in T25 treatment, also depicting a significant difference at $p < 0.05$ (Table 1).

Biomass Accumulation

Biomass of shoot and root at T25 were significantly higher in comparison to that of CK at $p < 0.05$. In general, plants in all the treatments with the exception of plants in the control group accumulated more biomass in the shoot than in the roots as depicted by a significantly lower shoot to root ratio (Fig. 3). The dry weight of root and shoot to root ratio showed no significant difference between the treatments at $p < 0.05$ with the exception of the CK group (Figs. 4a, 4c). The shoot dry mass of T25 treatment was increased by 91.0% in comparison to those of the CK group and by 58.8% with respect to the T45 treatment representing a significant difference at $p < 0.05$. There was no significant difference in the shoot dry mass distribution values between the T25 and T20 treatments (Fig. 4b).

Chlorophyll Fluorescence Characteristics

CF variables were evaluated to determine the impact of RZT on the photosynthesis machinery. The plants in the control group had the lowest values for the maximum photochemical yield of PS II, F_v/F_m ratio while the highest values were observed in the T25 treatment (Table 2). The trend was similar for qL, Y[II] and ETR variables across all the treatments with values of these variables in the CK group significantly lower in comparison to the other treatments at $p < 0.05$.

Chlorophyll Content

Chlorophyll content estimations depicted a significant difference between T25 and both CK and T45. T25 showed the highest SPAD values which were 38.16% higher than those of the CK group and 13.96% higher than those of the T45 treatment at $p < 0.05$. There

Table 1. Plant Height, Plant Canopy Width, Stem Diameter (mm) of pepper plants under different RZT treatments

Treatment	Plant Height, mm	Plant Width, mm	Stem Diameter, mm
CK	112.05 ± 4.680	123.05 ± 2.595	3.749 ± 0.135
T20	226.3 ± 14.682	225.45 ± 13.259	5.922 ± 0.310
T25	241.45 ± 14.640	270.85 ± 10.173	6.256 ± 0.280
T45	209.75 ± 10.837	187.7 ± 8.451	5.325 ± 0.206

Data are Means ± SE. Means with different letters above the bars are statistically different at $p < 0.05$ as determined by Tukey's test.

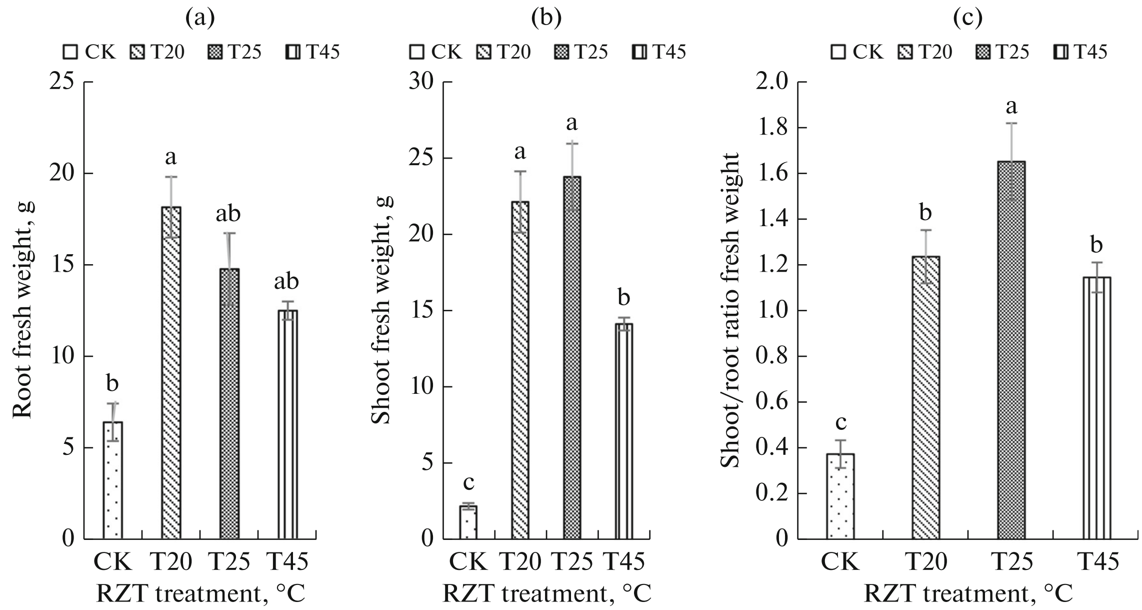

Fig. 3. Fresh weight of root, shoot and shoot to root ratio of pepper plants under different RZT treatments. Data is Means ± SE. Treatment means within the same column followed by different letters are statistically different based on Tukey's test at $p < 0.05$.

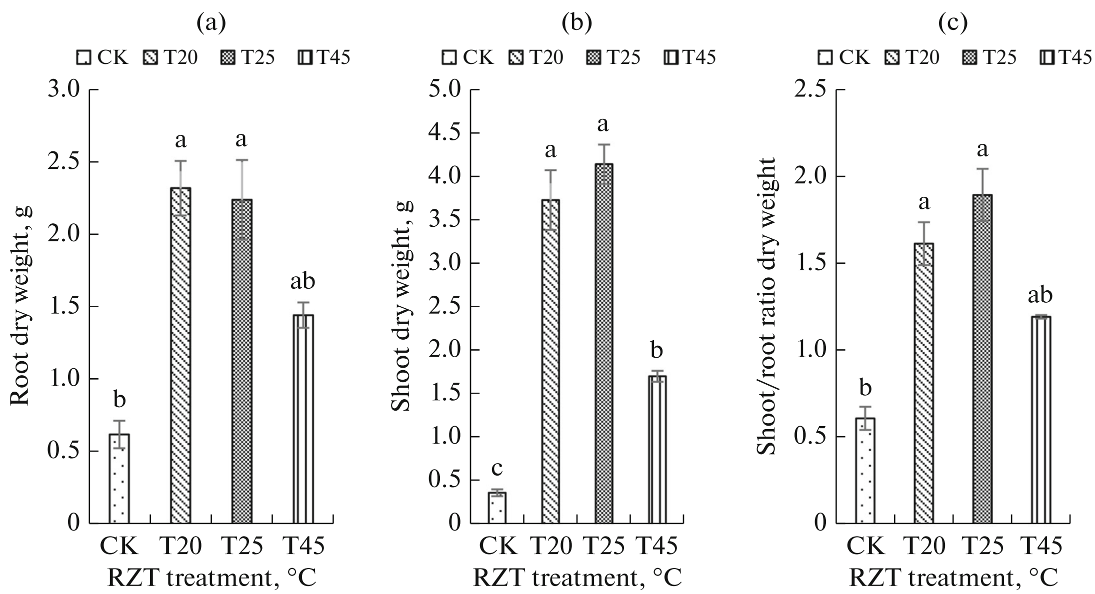

Fig. 4. Dry mass distribution of root, shoot and shoot/root ratio of pepper plants under different RZT treatments. Data is Means ± SE. Treatment means within the same column followed by different letters are statistically different based on Tukey's test at $p < 0.05$.

was no significant SPAD values difference between the T20 and T25 treatments at $p < 0.05$ (Table 3).

DISCUSSION

In this study, plant height, stem diameter and plant width values were significantly lower in plants in the CK group as compared to those in the other treatments (Table 1). This implied that cold stress had a profound effect on the growth characteristics of pepper plants in agreement with findings reported for cucumber [10] and wheat [11]. However, plant height, stem diameter and plant width values were significantly higher in the T20 and T25 groups as compared to the CK group (Table 1). From this findings, it was clear that heating of the root zone could alleviate the adverse effects of cold stress in an unheated greenhouse consistent with reports in strawberries [12],

Table 2. CF Characteristics of pepper plants under different RZT treatments

Treatment	Fv/Fm	qL	NPQ	Y(II)	ETR
CK	0.7680 ± 0.0142^{c}	0.6256 ± 0.0238^{c}	0.3303 ± 0.0350^{c}	0.5348 ± 0.0234^{c}	28.2544 ± 1.2270^{c}
T20	$0.8094 \pm 0.0071^{a, b}$	0.6648 ± 0.0268^{a}	0.4580 ± 0.0319^{b}	0.5821 ± 0.0090^{a}	31.8194 ± 0.5768^{a}
T25	0.8206 ± 0.0061^{a}	0.6643 ± 0.0096^{a}	0.4405 ± 0.0223^{b}	0.5992 ± 0.0093^{a}	31.9635 ± 0.4788^{a}
T45	0.7896 ± 0.0089^{bc}	0.6498 ± 0.0168^{b}	0.5144 ± 0.0265^{a}	0.5614 ± 0.0168^{b}	30.7322 ± 0.8771^{b}

Data is Means ± SE. Treatment means within the same column followed by different letters are statistically different based on Tukey's test at $p < 0.05$.

E. sativa [5] and lettuce [13]. It was also noted that in the T45 group, there was a significant decrease in the plant height, stem diameter and plant width in comparison to T20 and T25 treatments (Table 1). As roots are more heat sensitive than the above-ground portions of the plants, the high RZT may have resulted in injury and damage to the roots, and this could have resulted to a limitation in plant height and stem diameter as demonstrated by [14].

Root length is one of the most sensitive indicators of the effects of RZT on the root system [15].

The study revealed that plants in the CK group and the T45 group had the shortest root lengths (Fig. 2). This implied that in the CK group, cold stress had a detrimental effect on the root growth, consistent with observations by [16]. In contrast, plants in the T25 treatment had the longest root lengths (Fig. 2) depicting that root zone heating provided a favorable condition for root growth, consistent with the findings by [17].

The highest biomass accumulation in the shoot and root were recorded in the T25 treatment with the lowest shoot, root and shoot to root ratio fresh weight values seen in the CK group plants (Fig. 3). This implied that cold stress possibly impacted photoassimilate partitioning between root and shoot, with more photoassimilates partitioned to the root as opposed to the shoot. Similar conclusions were drawn by [18]. Dry mass distribution followed a similar pattern to fresh weight distribution with the highest shoot, root and shoot to root ratio dry weight values observed in the T25 treatment and the lowest values seen in the CK group (Fig. 4).

Table 3. SPAD values of pepper plants under different RZT treatments

Treatment	SPAD Value (Relative Units)
CK	31.100 ± 2.019^{c}
T20	47.455 ± 1.448^{a}
T25	50.295 ± 1.186^{a}
T45	43.275 ± 0.737^{b}

Data are Means ± SE. Means with different letters above the bars are statistically different at $p < 0.05$ as determined by Tukey's test.

The F_v/F_m ratio for healthy plants under non-stress conditions is generally maintained between 0.8 and 0.85 with optimal values of approximately 0.83, which is an estimation for majority of plant species [19]. The F_v/F_m of plants in the T20 and T25 treatment groups were maintained between 0.8 and 0.83 showing that root zone heating protected the PS II of these plants from photoinactivation that could have been induced by cold stress (Table 2). Plants in the CK group and T45 treatment showed F_m/F_v values that were <0.79 showing that the photosynthetic capacity of the plants was greatly diminished (Table 2). These observations were similar to those made by [5]. Cold stress resulted in the lowest values of qL, Y[II] and ETR as seen in plants grown in the CK group with the highest values of these variables observed for the plants in the T20 and T25 treatment (Table 2). This showed that root zone heating provided a suitable environment in the PSII reaction centers for primed photochemical reactions and electron transfer capacity which translated to enhancement of the photosynthetic performance [20]. The significantly higher values of NPQ observed in the T45 treatment were a response to heat stress as an increase in the value of NPQ reflects a photoprotective strategy of the photosynthetic machinery in response to potential damage to PS II by environmental stresses [21] (Table 2).

SPAD value estimations showed that cold stress had an adverse effect on chlorophyll content with plants in the CK group recording the lowest SPAD values. In contrast, root-zone heating had a positive effect on relative chlorophyll content with plants in the T20 and T25 treatments showing the highest SPAD values (Table 3). These observations were consistent with findings by [22, 23] that found that cold stress resulted in a decline in chlorophyll content but the degree of decline was species-dependent. Root-zone heating positively influenced the absorption of essential nutrients including nitrogen which is essential for the production of chlorophyll [24]. Further, it provided an environment that was conducive to the synthesis of chlorophyll [25], subsequently, increasing the amount of chlorophyll content in leaves consistently through the plant growth period (Fig. 5).

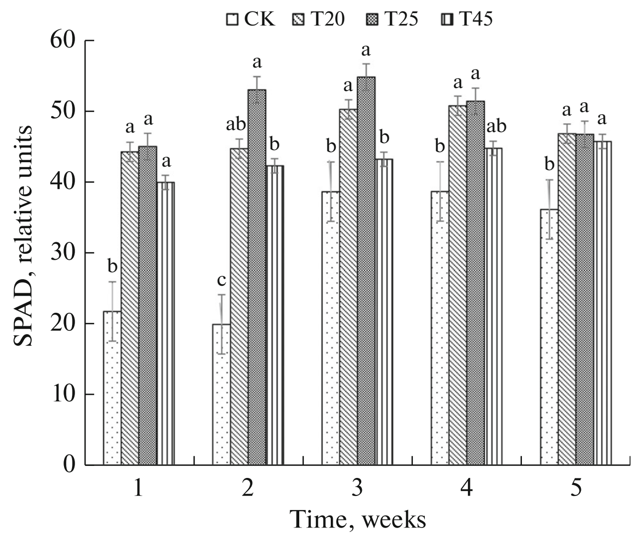

Fig. 5. Variation of relative chlorophyll content over time of pepper plants under different root zone temperatures. Data are Means 2 SE. Means with different letters above the bars are statistically different at $p < 0.05$ as determined by Tukey's test.

CONCLUSIONS

The growth of pepper plants in an unheated greenhouse in the Yangtze River Delta area of Southern China during winter was adversely affected by the cold stress. The plants in the CK group showed poor growth, poorly developed root system and photo-inhibition. The benefits of root zone heating to RZT values of 20 and 25°C was observed with enhanced plant growth, appreciation of F_v/F_m values, development of a well-established root system and overall plant health. RZT of 25°C would be the most suitable for cultivation of pepper plants.

In addition, root zone heating provided a simple, affordable and cost-effective method of growing greenhouse crops under cold stress in the Yangtze River Delta area of Southern China, offering the possibility of low energy consumption with high crop productivity.

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