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Effects of root zone temperature and light intensity on plant growth, flowering and fruit quality of plant factory 'festival' strawberry (*Fragaria* × *ananassa* Duch.)

[1,*]Munirah, M., [1]Abdullah, S.A., [1]Kassim, R., [1]Masnira, M.Y., [1]Sakinah, I., [1]Ahmad, M.A. and [2]Tajidin, N.E.

[1]*Horticulture Research Centre, Malaysian Agricultural Research and Development Institute, 43400 Serdang Selangor, Malaysia*
[2]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

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Abstract

The growth and production of strawberries in a plant factory are highly affected by environmental factors such as light and temperature. Recent interest in plant factory production of strawberries in soilless substrates has raised concerns about the potential effects of root zone temperature (RZT) and light intensity on plant growth and fruit yields. The hypothesis suggests that RZT and light intensity can improve plant growth and affect flower production. This study aimed to investigate the effects of RZT and light intensity on the growth and reproductive performance of strawberries. The root zone of the 'Festival' strawberry plants was subjected to a temperature of 15°C during the night for root zone cooling (RZC) treatment meanwhile without RZT as the control, under two different light intensities: 200 mmolm^{-2}s^{-1} and 300 mmolm^{-2}s^{-1}. From the result, the root and crown temperatures in the RZC treatment were maintained at 15-21°C. The number of leaves was significantly affected by the interaction between RZT and light intensity. The crown diameter in the RZC treatment group (13.27 cm) was higher than in the control group (12.06 cm). Higher light intensity (356.9 mmolm^{-2}s^{-1}) significantly increased the chlorophyll content. Flower production in the RZC treatment was 52% higher than in the control group. The enumeration of growth organs and flowers increased in the RZC treatment, suggesting the induction of reproductive growth by the low root zone temperature. However, the light intensity received by the plants and the interaction between RZC and light intensity did not have any effect on flower production. Fruit size, fruit pH, fruit firmness and total soluble solids: titratable acidity were not affected by RZT, light intensity, or the interaction between RZT and light intensity. The increase in flower production can be attributed to the lower plant temperature, specifically at the crown, which was at 10-12°C. In conclusion, RZC can induce growth and reproductive performance, ultimately increasing the fruit yield of hydroponically cultivated strawberries in a plant factory.

1. Introduction

Strawberries are a popular horticultural crop under fruit vegetables and functional food due to their bioactive compounds that could prevent lifestyle diseases such as Alzheimer's, cancer and atherosclerosis. Recently, in tropical and subtropical countries, there has been growing interest in adopting controlled environment agriculture (CEA) such as greenhouses and plant factories (PF) for strawberry production. Plant factory production can help avoid the adverse climate change that occurs in open-field cultivation and produces high quality fruits (Malhi *et al*., 2021). In addition, strawberry production in the lowland can reduce supply chain issues of strawberries (Lee *et al*., 2023). Strawberries are very sensitive to their cultivation environment such as temperature, carbon dioxide, nutrients and light which can affect plant growth, quality and yield of strawberries (Gonzalez-Fuentes *et al*., 2016; Cervantes *et al*., 2019).

In a plant factory, by adopting hydroponic technology, it is possible to optimize the root zone environment (Samno *et al*., 2019). Thus, the crops can grow to their maximum potential. In hydroponic

*Corresponding author.
Email: *muni@mardi.gov.my*

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systems, the nutrient solution is different from ambient temperature (Sun *et al*., 2016). Temperature is one of the most important factors in root zone environments affecting nutrient uptake and water absorption of plants by promoting root development (Xu and Huang, 2000). Alternatively, Root Zone Temperature (RZT) is one cultivation microclimate that can be more economically controlled by optimizing energy consumption and easier management by specific targets and smaller space involved (Kawasaki *et al*., 2013). Optimization of RZT in hydroponic cultivation could lead to the improvement of plant growth and increased flowering in plants.

LEDs (Light-Emitting Diodes) in a plant factory govern plant growth by affecting the physiological response of the plant (Muneer *et al*., 2014). The use of LED as artificial light affects not only photosynthesis but also has other advantages such as efficient plant production for plant growth and sexual reproduction (Dueck *et al*., 2016). The most challenging part of LED lights is to supply sufficient or optimum quantity and quality of light to the plants (Samuolienė *et al*., 2013; Dong *et al*., 2014). The effects of different light compositions on strawberry growth, yield and fruit quality have been studied by several researchers (Piovene *et al*., 2015; Choi *et al*., 2015; Naznin *et al*., 2016). However, the inconsistency of the reports might be due to different sources of light that often influence plant growth and flower production of strawberry plants. Thus, it is important to know the optimum light intensity that can increase plant growth, flowering and fruit quality of Festival strawberries.

Strawberry production in a plant factory is relatively new in Malaysia. Therefore, there is limited information available on strawberry production in a plant factory with regard to RZT and light intensity. This study aimed to investigate plant response towards RZT and light intensity for plant growth and inducing inflorescence of ‘Festival’ strawberries.

2. Materials and methods

2.1 Plant materials

The experiment was conducted in a Plant Factory at the Malaysia Agriculture Research Development Institute located (N2°59’ 51.4932”, E101°41’ 26.2284”). The strawberry cultivar used in this research was ‘Festival’ and the runner was propagated from another plant that originated from a commercial farm in Cameron Highlands. The Runner was propagated in media containing peat moss: cocopeat: perlite (2:1:1) in controlled environment nurseries at 25°C. The media was drenched completely with a nutrient solution with electric conductivity 1.0 dS.m^{-1}, adjusted to pH 5.8 at 3-day intervals. Uniform runners with 2 to 3 leaves/plant were selected and cut from mother plants as seedlings.

2.2 Growth conditions

Strawberry seedlings were transferred to the Nutrient Film Techniques (NFT) hydroponic system. The cultivation conditions are as follows, photoperiod. 12 hrs; CO_2 500 µmol·mol^{-1}; relative humidity, 70-75% and temperature 25°C. The nutrient solution was (in mmol L^{-1} concentrations of NH_4^+ 1.0, NO_3^- 10.6, $H_2PO_4^-$ 1.2, K^+ 5.1, Ca^{2+} 1.0, and SO_4^{2-} 0.3, and in n µmol·L^{-1} concentrations of Fe^{3+} 43.6, $BO^{3-}{}_3$ 22.6, Mn^{2+} 9.4, Zn^{2+} 1.5, and MoO_4^- 0.5) with electric conductivity 1.6-2.0 dS.m^{-1}, adjusted to pH 5. The water flows were kept continuously with a flow rate of 50 L min^{-1}. A pump (Atman At-107, Zhongshan Co., Ltd., China) was used to supply nutrient solutions to each treatment. Axillary bud, runner and flowers were removed from the crown during the acclimatation period.

2.3 Root zone temperature

After 40 days of cultivation, an average of 10-12 main leaves/plant were fully unfolded, and root zone temperature treatments were initiated. 15°C root zone temperature was applied with a chiller (Hailea, HS66A, 1/4HP Guangdao Co. Ltd, China). Low root Zone temperature (15°C) was maintained by cooling the nutrient solution using cool water. Continued aeration enabled the circulation of nutrient solution, resulting in uniform temperature distribution to the root zone. The temperature for RZC was set at 15±2°C and without RZC for Control treatment at 20±2°C.

2.4 Light intensity

Two different light intensities were applied to strawberry plants at 200 mmolm^{-2}s^{-1} and 300 mmolm^{-2}s^{-1}. Light tubes were located 25 cm above the surface of each tier. The photoperiod for this treatment was at 12 hrs. Spectral distribution of the LED lights was measured with LI-1800 (LI-COR Biosciences, Lincoln, USA) as shown in Figure 1. Pollination was done manually by using a small paintbrush.

(A) (B)

Figure 1. The spectral photon distribution of purple LED light with (A) 200 mmolm^{-2}s^{-1} and (B) 300 mmolm^{-2}s^{-1}.

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2.5 Measurement of plant growth, flower production and fruit quality

Examination of plant growth variables (leaf number, crown diameter and SPAD value) were collected at day 75 (reproductive period). Leaf size and leaf number were taken from fully expanded young leaves. After 2 months of experimentation, flower numbers were recorded during the reproductive period. For the measurement of fruit, data were measured as follows; weight (g), total soluble solid (%TSS), pH and titratable acidity (%TTA), acid ascorbic content (AA mg/100 FWg) and firmness (N). Powdered fruit samples (10 mg) were combined with 1 mL of 5% metaphosphoric acid (w/v). Samples were mixed well by vortexing for 1 min and centrifuged. Ascorbic acid was measured using a spectrophotometer (UV-3101P, Labomed Inc., California, USA). Ascorbic acid was expressed as AA mg/100 g

2.6 Statistical analysis

This experiment was designed as a Completely Randomized Design (CRD) which included two factors; root zone temperature (RZC at 15oC and without RZC as Control) in combination with two different light intensities (200 mmolm^{-2}s^{-1} and 300 mmolm^{-2}s^{-1}). Each treatment consisted of 15 plants divided into 3 replications. The data obtained for each variable was analysed using Statistical Analysis Software (SAS Institute, Cary, NC, USA). Differences among treatments were determined by one-way analysis of variance (ANOVA). Mean comparisons were conducted using the LSD comparison test at $p<0.05$.

3. Results and discussion

3.1 Root zone temperature conditions

The experiment was conducted in the plant factory under fully controlled environmental conditions and the climate conditions were not affected by outdoor light and air temperature. As shown in Figure 2 (B) RZC maintained the root zone temperature at 15±2°C at night and clearly chilled the crown at 20±2°C and the aerial part at 22±2°C. In contrast, for Control (without RZT), the average temperature for root, crown and leaves were at 21±2°C, 24±2°C and 25±2°C respectively.

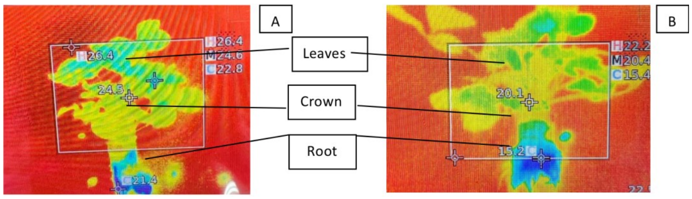

Figure 2. Thermo imager rootzone for (A) control at 21.4°C meanwhile for (B) Root Zone Cooling (RZC) at 15±2°C.

3.2 Plant growth

To evaluate growth during treatment, the changes in the leaf numbers were analysed. At an early stage, each plant was maintained to have 4 leaves/plant. There was a significant interaction effect between RZC and light intensity of new leaf production during plant growth and reproduction stage (Table 1). From Figure 3, the emergence of new leaves was significantly higher in 300 mmolm^{-2}s^{-1} and RZC as compared to other treatment combinations. The Chlorophyll content in the strawberry leaves was monitored throughout the entire growing period and expressed as SPAD index (Table 1).

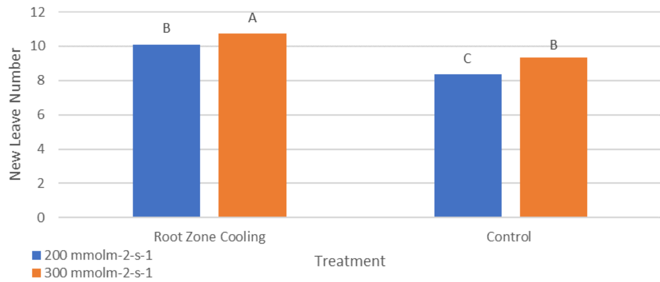

Figure 3. Root zone temperature on new leave number of Festival strawberry at different light intensity. Bars with different notations at statistically significantly different using LSD test ($p<0.05$).

Table 1. Main and interaction effects of root zone temperature and light intensity on plant growth and reproductive measurement of Festival Strawberry.

	New Leaf number	Chlorophyll SPAD	Crown Diameter (cm)	Flower number/plant	Fruit number/ plant	Weight/fuit (g)	Total Fruit Weight (g)
Root Zone Temperature							
RZC (15°C)	10.42^{a}	43.19^{a}	13.27^{a}	9.31^{a}	9.00^{a}	17.95^{a}	161.5^{a}
Control	8.83^{b}	42.16^{a}	12.06^{b}	5.51^{b}	5.00^{b}	16.45^{a}	82.3^{b}
Light intensity							
200 mmolm^{-2}s^{-1}	10.25^{a}	43.73^{a}	12.9^{a}	7.37^{a}	7.00^{a}	16.31^{a}	114.1^{a}
300 mmolm^{-2}s^{-1}	9.00^{b}	41.62^{b}	12.4^{a}	7.45^{a}	7.00^{a}	18.09^{a}	126.6^{a}
Interaction							
RZT × Light Intensity	**	ns	ns	ns	ns	ns	ns

Values with different superscripts within the same row are statistically significantly different according to LSD ($p<0.05$). **significant, ns: not significant.

The SPAD value was significant for light intensity at 300 mmolm^{-2}s^{-1} as compared to 200 mmolm^{-2}s^{-1} by 5%. The strawberry crown is an important plant growth organ that affects plant vigour and fruit yield since it is a source of carbohydrate for fruit growth (Macías-Rodríguez *et al*., 2002; Morgan, 2006; Torres *et al*., 2015). In this experiment, RZC plants showed a larger crown diameter of 1.21 cm on average as compared to controls (without RZC). Clarification on the effects of RZC on the flower production performances was also identified in this experiment. 70% More flowers were recorded in RZC as compared to control. RZC clearly enhanced flower production and it was noted that the production happened 10 days earlier in RZC as compared to control. Other than that, the weight per fruit was also identified, based on marketable yield. There was no significant difference in fruit size among treatments applied. However, the total fruit yield harvested/plant was 9 fruit/plant for RZC, 70% more than of the control plants, only 5/plant was produced. From the observation, RZC fruit was harvested 3 days earlier as compared to the control treatment. In agreement with research done by Niam and Suhardiyanto, (2019), plants grown in a hydroponic system with cooling zones produce higher fruit weight per plant than plants without cooling treatment. Several studies have shown that there is a close relationship between the leaf number and fruit quality or maturity of fruit crops (Usenik *et al*., 2008; Torri *et al*., 2009).

This result indicates that the RZC of strawberries grown in an NFT system effectively chilled the crown in addition to the root zone. When RZC was performed together with high light intensity, the ability of the RZC alone enhanced the yield performance by increasing the flower number and number of fruits per plant. These results have forced strawberry plants to produce a higher yield. In Previous research done by Kinoshita *et al*. (2012), pot grown nursery using cold water in a polyvinyl chloride pipe, did not keep the root zone temperature under 25°C. The NFT system in this study, however, was able to maintain lower root zone temperature, leading to better plant growth and reproductive performance. Some researchers have proven that local temperature techniques can prevent stagnation during extreme temperatures to induce plant growth, accelerate flower production and increase the total yield (Shigeno *et al*., 2001; Kim *et al*., 2009).

Environmental factors induce flower bud differentiation of strawberries and depend on the relationship between day length and air temperature. Festival strawberries have been classified into Short Day (SD) cultivars that initiate flower buds only under 13 hrs of day length. There were no significant effects of light intensity on plant growth and fruit production. Similar results were recorded in 'Albion' strawberries as the light intensity was increased from 200 to 300 mmolm^{-2}s^{-1} (Park *et al*., 2023). Zheng *et al*. (2019) found that the best light intensity range are from 90 to 270 mmolm^{-2}s^{-1} and there is no further improvement at 300 mmolm^{-2}s^{-1} and above. However, Strawberry is regarded as a cold-tolerant and heat-sensitive plant, since it is grown at temperatures (15 to 25°C). The climate determines the flowering habit of octoploid garden strawberry (*F. ananassa*) which is controlled by a key floral gene repressor TERMINAL FLOWER 1 (TFL1) in the shoot apical meristem but no details are available on temperature regulation of this gene (Hytönen and Kurokura, 2020). From this study, the ability to chill the crown and lower the temperature of the shoot meristem induces flower bud differentiation.

3.3 Fruit quality

The main postharvest literature on quality parameters for hydroponic cultivated strawberries in a plant factory for festival cultivars is very limited. Fruit quality traits such as firmness, pH, total soluble solid (TSS), titratable acidity (TA), SSC to TA ratio, and ascorbic acid as shown in Table 2. Temperature and light stress are known to influence the quantities of plant organic compounds (Kaplan *et al*., 2004; Akula and Ravishankar, 2011). No differences were detected in firmness and pH for root zone temperature and light intensity treatments and interaction between both treatments (Table 2). In addition, total soluble solids (% TSS), titratable acidity (TA) and TSS: TA were also not affected by light intensity. However, RZC showed higher TTS and TA values as compared to Control by 26-27%. Consequently, the ratio SSC to TA was almost similar. Applying high temperatures to the herb *Panax quinquefolius* retarded the photosynthesis processes and plant biomass and increased root secondary biomass level (Jochum *et al*., 2007).

Ascorbic acid is a water-soluble vitamin that is essential for the immune system and as a cellular reactive oxygen species scavenger. Generally, ascorbic acid content in fruit is regulated by cultural practices and environmental factors (Dorais *et al*., 2008). Ascorbic acid content was significantly affected by light intensity but not by RZT (Table 2). From the result, acid ascorbic content in 300 mmolm^{-2}s^{-1} was higher than in 200 mmolm^{-2}s^{-1} treatments. Similarly result of Fenech *et al*. (2019), low light intensity causes a reduction in acid ascorbic content. Higher light intensity induces the formation of ROS by an increase in photoreduction and photorespiration. This phenomenon accelerates acid ascorbic formation to detoxify the ROS. Mutual reaction has been discussed from this study showed that light

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Table 2. Main and interaction effects of root zone temperature and light intensity on fruit quality of Festival Strawberry.

	Firmness (N)	pH	Total Soluble Solid (%)	Titratable Acidity (%)	SSC:TA	Ascorbic Acid (AA mg/100 g)
Root Zone Temperature						
RZC (15°C)	0.70^a	3.55^a	10.49^a	0.94^a	11.1^a	2.50^a
Control	0.76^a	3.63^a	8.3^b	0.74^b	11.2^a	2.46^a
Light intensity						
200 mmolm^{-2}s^{-1}	0.78^a	3.73^a	9.25^a	0.88^a	10.5^a	2.06^b
300 mmolm^{-2}s^{-1}	0.68^a	3.62^a	9.00^a	0.80^a	11.2^a	2.90^a
Interaction						
RZT × Light Intensity	ns	ns	ns	ns	ns	ns

Values with different superscripts within the same row are statistically significantly different according to LSD ($p<0.05$). ns: not significant.

increased at 300 was the highest in acid ascorbic content. Similarly, previous studies have shown that the ascorbic acid and sugar contents in strawberry fruits increased in plants grown under high intensities between 300 to 600 mmolm^{-2}s^{-1} (Wang and Camp, 2000). However, RZT did not significantly affect these attributes in this experiment. Therefore, suggests that the light intensity is more practical to enhance acid ascorbic than RZT treatment.

4. Conclusion

In the present study, the findings suggest that RZC in combination with light intensity at 200 mmolm^{-2}s^{-1} provided the best conditions for plant growth and reproduction. Light intensity at 300 mmolm^{-2}s^{-1} contributes to the production of acid ascorbic content enhancement. Understanding the light and temperature responses to plant growth and flowering could help to set the specific environmental conditions to accelerate yield to optimum in the plant factory.

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