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Optimization of Photosynthetic Photon Flux Density and Root-Zone Temperature for Enhancing Secondary Metabolite Accumulation and Production of Coriander in Plant Factory

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Abstract: Coriander is an important aromatic plant, and contains abundant secondary metabolites that are considered to be beneficial for health. The demand for high-quality and fresh coriander in large cities has been growing rapidly. Plant factories are advanced indoor cultivation systems that can produce high-quality plants inside cities with a high productivity. This study aimed to maximize plant growth and the secondary metabolites production of coriander, by regulating photosynthetic photon flux density (PPFD) and root-zone temperature (RZT). Three PPFDs (100, 200, and 300 μmol m^{-2} s^{-1}) and three RZTs (20, 25, and 30 °C) were applied on coriander plants grown hydroponically in a plant factory. The plant biomass and water content of leaf and stem were highest under RZT of 25 °C with a PPFD of 300 μmol m^{-2} s^{-1}. However, chlorogenic acid, rutin, *trans*-2-decenal, total phenolic concentrations and the antioxidant capacity of the coriander plant were greatest under the combination of PPFD (300 μmol m^{-2} s^{-1}) and RZT (30 °C). Chlorogenic acid in leaves responded more sensitively to PPFD and RZT than rutin. Controlling PPFD and RZT is effective in optimizing the yield and quality of coriander plants. The findings are expected to be applied to commercial plant production in plant factories.

Keywords: antioxidant capacity; chlorogenic acid; *Coriandrum sativum* L., rutin; *trans*-2-Decenal; total phenolic content

1. Introduction

Coriander (*Coriandrum sativum* L.) is an annual herbaceous plant, and its fresh leaves are widely used in food flavoring in daily cuisines. All parts of this herb are used as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations [1]. Coriander leaves are rich in minerals and vitamins, such as vitamin C up to 1.35 mg g^{-1}, vitamin B$_2$ of 0.6 mg g^{-1} and vitamin A at 104.6 IU g^{-1} [2]. They are also well-known for their antioxidant properties, such as volatile components, flavonoids, linolenic acid, β-carotene and phenolic compounds [3–6]. The concentrations of those secondary metabolic compounds in herbs are important indices for their quality assessment.

The demand for high-quality and freshly consumed vegetables, including corianders, has been growing rapidly in large cities (e.g., Tokyo). A plant factory with artificial light is a facility that enables food production inside cities with a high productivity. It can satisfy specific demands for qualities in plant growth and secondary metabolite accumulation by controlling the environmental factors [7].

In coriander plants, the chlorogenic acid (CA) and rutin (quercetin-3-rutinoside; QR) are the predominant phenolic compounds in both leaves and stems [5,8]. CA is well-known as a compound with strong anti-diabetic and anti-lipidemic effects [9]. QR demonstrates a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective effects [10]. Another important compound is *trans*-2-decenal (DC), which is one of the major volatile compounds identified in coriander leaves and stems, and is an essential oil that produces the oily, sweet or grassy odor of the plant [2,4,11]. Thus, understanding the effects of the cultivation environment upon the content of CA, QR, DC and total phenolic compounds in coriander plants is important for its production and culinary and clinical applications.

Plant growth and secondary metabolite accumulation are largely affected by various environmental factors, including light, air temperature, carbon dioxide concentration, and the root-zone environment, etc. In a plant factory, the cost for electrical energy required for artificial lighting can be about 28% of the total operation costs [7]. Thus, optimizing photosynthetic photon flux density (PPFD) is the first urgent task to maximize the economic output of plant production. It is reported that menthol mint plants grown under low PPFD (137 μmol m^{-2} s^{-1}) had the lowest biomass, but produced an essential oil with the best commercial profile (high level of neomenthol, menthol and menthyl acetate), whereas plants under high PPFD (543 μmol m^{-2} s^{-1}) had a high biomass and essential oil content, but lower levels of menthol [12].

In addition, plant growth is affected by a combination of various, rather than one, environmental factors. Root-zone temperature (RZT) also has been found to influence physiological processes in roots and plants, such as the uptake of water and mineral nutrients, plant growth, nutrient and secondary metabolite accumulation, and the activity of various enzymes [13–15]. Islam et al. [16] found that red romaine lettuce grown under RZT of 10 °C contained higher anthocyanin content, whereas total chlorophyll content was lower than RZT of 15 °C.

Thus, PPFD and RZT are two factors that can affect growth, as well as the biologically active compound contents in plants. To improve the economic value and health benefits of coriander, the optimal PPFD and RZT that yield both good growth and a high content of SM compounds both need to be determined. However, the references that investigate yield and quality control technologies in coriander in a plant factory are very limited. Therefore, this study aims to determine the optimal combination of PPFD and RZT for coriander plant cultivation. The findings would benefit both fundamental research and any commercial application of food production in a plant factory.

2. Materials and Methods

2.1. Plant Material

2.1.1. Germination and Plant Seedling

Coriander seeds (*Coriandrum sativum* L.; Tokita Seed Co., Ltd., Saitama, Japan) were sown in sponge cubes (2.3 × 2.3 × 2.8 cm, 14.8 cm^3) in a cultivation room. Germinated seeds were placed under PPFD of 150 μmol m^{-2} s^{-1} with a photoperiod of 16 h per day, using cool white fluorescent lamps (FHF32 EX-N-H, Panasonic, Co., Ltd., Japan), and the seedlings were irrigated with a nutrient solution (N 21%, P_2O_5 8%, K_2O 27%, MgO 4%, CaO 23%, Fe 0.18%, Cu 0.002%, Zn 0.006%, Mo 0.002%, MnO 0.1%, B_2O_3 0.1%) (Otsuka hydroponic composition, OAT Agrio Co., Ltd., Tokyo, Japan), starting at 10 days after sowing. The EC and pH of the nutrient solution were adjusted to 0.6 dS·m^{-1} and 6.0, respectively. Air temperature, relative humidity, and CO_2 concentration were set to 25/22 °C (light/dark periods), 60%–80%, and 400 ppm, respectively.

2.1.2. Growth Condition and Treatments with Regulators

At 18 days after sowing, seedlings were transplanted to the deep flow technique hydroponic system in a walk-in type plant factory (2.9 m × 2.0 m × 2.3 m in LWH), and subjected to three PPFD

levels, 100, 200, and 300 µmol m^{-2} s^{-1}, with a photoperiod of 16 h per day, using light emitting diode (LED) lamps (GreenPower Production module, DR/W/FR, NL, Philips Co., Ltd., Poland) and three RZTs, 20, 25, and 30 °C, for 19 days. The PPFD was measured at 5 cm above the surface of the culture panels using a light meter (LI 250A, LI-190R; Li-Cor Inc., Lincoln, NE, USA), before planting the plants. The experiment was carried out in three cultivation beds with three RZTs, and each cultivation bed was split into three plots with three different PPFD levels. Each treatment plot contained 26 plants (178 plants m^{-2} in plant density). RZTs of 25 ± 0.5 °C and 30 ± 0.5 °C were maintained by heating the nutrient solution using an IC auto heater (Type 100; Nisso, Marukan Ltd., Japan). RZT of 20 ± 0.5 °C was maintained by cooling the nutrient solution using a cool water circulator (ZR mini, Zensui Co. Ltd., Japan). RZT was recorded by using a thermos recorder (TR-71wf, T&D Corp., Japan). The EC and pH of the nutrient solution were adjusted to 1.2 dS m^{-1} and 6.0. New nutrient solution (1 L) was added to each cultivation bed every day, and all of the nutrient solution was renewed once a week. Air temperature, relative humidity, and CO$_2$ concentration were set at 23/20 °C (light/dark periods), 60%–80%, and 1000 ppm, respectively.

2.2. Measurement

2.2.1. Growth Parameters

The plants were harvested 19 days after transplanting. Leaf, stem and root fresh weights (FWs) were determined at room temperature (25 °C). The leaf, stem and root samples were placed in an 80 °C oven for 1 week to determine their dry weights (DWs).

The water content (WC) of leaf and stem (%) was calculated as:

$$WC = \frac{FW - DW}{FW} \times 100 \tag{1}$$

where FW and DW of leaf and stem were used.

2.2.2. 1,1-Diphenyl-2-picrylhydrazyl Radical-Scavenging Activity

A 1 g of frozen shoot segment was weighed accurately and homogenized with 5 mL of 80% (*v/v*) methanol for 1 minute. The sample was centrifuged at 10,000 g for 30 min at 4 °C. The supernatant was filtered using a filter paper, transferred to a 10 mL graduated cylinder, made up to 6 mL total with 80% methanol and then stored at −30 °C until analyzed.

The antioxidant activity of the sample, based on the scavenging activity of stable 1,1-Diphenyl-2-picrylhydrazyl (DPPH), was determined using spectrophotometric analysis by the method described in literature [17,18] with some modifications. An aliquot of 50 µl of test sample or Trolox solution (0, 200, 400, 600, 800, and 1000 µM) was added to 2 mL of a DPPH solution (80 µM) in methanol and mixed thoroughly at room temperature. Absorbance at 517 nm was determined after 30 min in darkness using a spectrophotometer (ASV11D, As One, Corp. Osaka, Japan). The results were expressed as milligram Trolox equivalents per gram fresh weight (mg TE g^{-1} FW).

2.2.3. Total Phenolic Content

Total phenolic content (TPC) of the sample described in 2.2.2 was measured using the Folin–Ciocalteu colorimetric assay [17–19] with gallic acid as calibration standard, using a spectrophotometer (ASV11D, As One, Corp. Osaka, Japan). An aliquot of 0.25 mL of test sample or gallic acid solution (0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg mL^{-1}) was added to 1.25 mL of 10% Folin–Ciocalteu reagent, followed by 1 mL of 7.5% sodium carbonate solution, and then this was mixed thoroughly at room temperature. Absorbance at 765 nm was determined after 1 hour. The results were expressed as milligram gallic acid equivalents per gram fresh weight (mg GAE g^{-1} FW).

2.2.4. *Trans*-2-Decenal Content

Analysis of DC content was conducted using high-performance liquid chromatography (HPLC) according to the method described in the literature [5,20] with modifications. The stored sample solution described in DPPH radical-scavenging activity was used for the analysis after filtration. The HPLC system was used as described previously [21]. The HPLC conditions were as follows: analysis was carried out with an InertSustain C18 column (5 μm, 4.6 × 150 mm) (GL Sciences, Tokyo, Japan); temperature, 30 °C; flow rate, 0.8 mL min^{-1}; detector wavelength, 220 nm; injection volume, 10 μL. The elution was as follows: a 5 min linear gradient was set from 70/30% to 35/65% of a water/acetonitrile mixture (*v/v*); then isocratic elution with 35/65% of water/acetonitrile was used until 20 min; from 20 to 30 min, the initial composition (70/30%, water/acetonitrile (*v/v*)) was used to re-equilibrate the column. DC content per shoot fresh weight (μg g^{-1} FW) was estimated by dividing the DC content in samples by the sample weight.

2.2.5. Chlorogenic Acid and Rutin Content

CA and QR content were determined using liquid chromatography/mass spectrometry (LC-MS) based on the methods described in literature [6,8] with modifications. All leaves and stems were dried at 30 °C for 4 weeks. The dried materials were cut into powder and filtered through a sieve. A 15.00–15.50 mg sample was weighed accurately, transferred to a 1.5 mL tube, mixed in 1 mL methanol for 10 min at 2000 rpm and 15 °C using an Eppendorf ThermoMixer C and centrifuged for 5 min. The pellet was extracted two more times in a similar manner. The supernatants (about 3 mL) were combined, transferred to a 5 mL volumetric flask and diluted with methanol to a 5 mL total volume. The solution was filtered before injection to HPLC. A Shimadzu LCMS–2020 mass spectrometer equipped with an electrospray ionization source operating in negative mode was used. The HPLC conditions were as follows: a XBridge BEH C18 column (3.5 μm, 2.1 × 150 mm, Waters, MA, USA) at 35 °C; flow rate, 0.2 mL min^{-1}; injection volume, 1 μL; mobile phase, solvent A (0.1% formic acid, *v/v*) and solvent B (100% acetonitrile). The elution was as follows: 0–5 min, linear gradient 90–80% A; 5–10 min, isocratic, 80% A; 10–20 min, isocratic, 90% A. The MS conditions were used as described previously [21]. For detection, ion monitoring mode was selected with m/z 353 for a molecular ion [M–H]− of CA and with m/z 609 for a molecular ion [M–H]− of QR. CA and QR content per leaf/stem dry weight (μg g^{-1} DW) was estimated by dividing the content of CA and QR in samples by the weight of samples.

2.3. *Statistical Analysis*

All experiments were repeated twice, with nine replications for each treatment. Fifteen plants were sampled from each treatment to evaluate the overall growth parameters, and six to eight plants were sampled from each treatment to determine the antioxidant capacity and total phenolic, DC, CA and QR content. The data were subjected to analysis of variance, and the means were compared between treatments using Tukey's test in SPSS statistical software (IBM SPSS Statistics, Version 25.0. Armonk, NY, USA: IBM Corp.). A *p*-value < 0.05 was considered significant.

3. Results

3.1. *Plant Growth*

The leaf, stem FWs and DWs increased with stronger PPFD under the same RZTs (Figures 1 and 2). Under the same PPFD, these parameters were increased by RZT of 25 °C but decreased by RZT of 30 °C, compared with those under RZT of 20 °C. As a result, the highest leaf and stem FWs (8.55 g and 10.7 g, respectively) were obtained under the condition of 300 μmol m^{-2} s^{-1} in PPFD and 25 °C in RZT. Under a PPFD of 300 μmol m^{-2} s^{-1} and RZT at 20 or 25 °C, coriander plants developed better root system (0.48 g and 0.4 g in root DW, respectively) than that under a RZT of 30 °C (0.34 g in root DW) (Table S1 in Supplementary material). WCs of leaf and stem tended to decrease with increases

in PPFD under RZT of 20 or 30 °C but were not significantly affected by PPFD under RZT of 25 °C (Table S1). Root WCs were not significantly different between RZTs of 20 and 25 °C regardless of PPFDs, however, they were decreased significantly when RZT was up to 30 °C (Table S1). The lowest WCs of leaf, stem and root were observed in the plants grown under PPFD of 300 µmol m^{-2} s^{-1} with RZT of 30 °C, with the significant differences compared with those under other treatments (Figure 3). The effects of the interaction between PPFD and RZT were significant for all plant growth parameters except for leaf WC (Table S2).
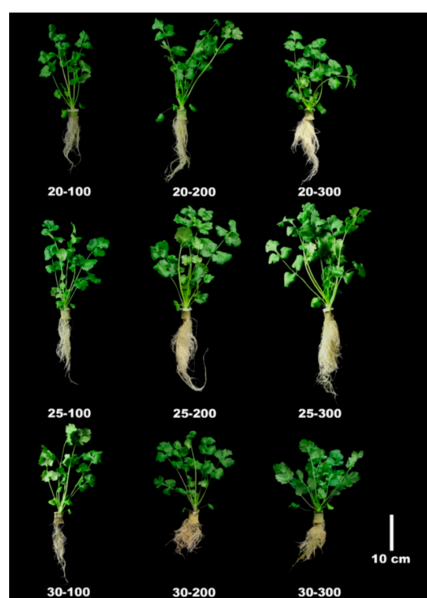
Figure 1. Coriander plants grown under nine different treatments with a combination of photosynthetic photon flux density and root-zone temperature for 19 days after transplanting. The codes below the plants show each treatment: 20, 25 or 30 for 20, 25 or 30 °C in root-zone temperature, respectively; 100, 200 or 300 for 100, 200 or 300 µmol m^{-2} s^{-1} in photosynthetic photon flux density, respectively.

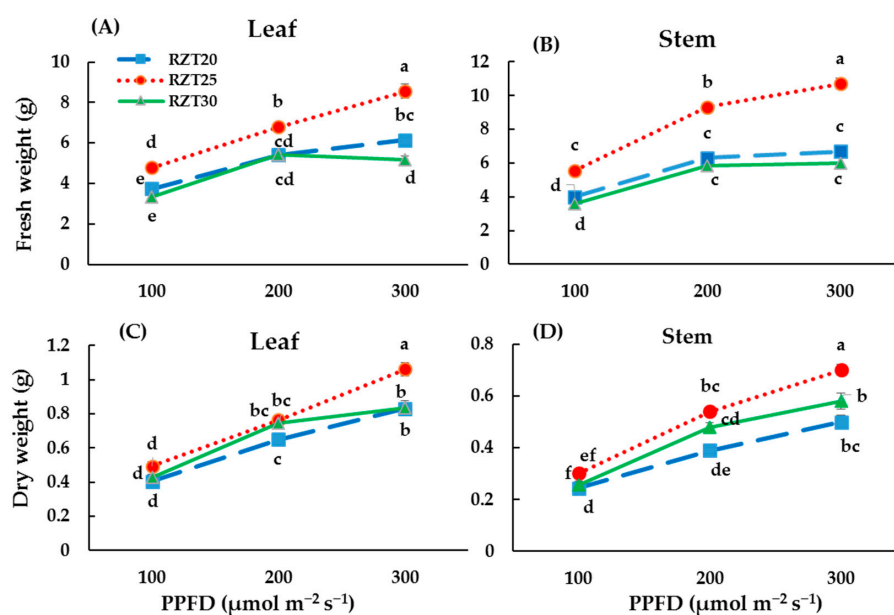**Figure 2.** Leaf (**A**) and stem (**B**) fresh weight and leaf (**C**) and stem (**D**) dry weight of coriander plants after 19 days of cultivation under different photosynthetic photon flux density and root-zone temperatures. Data are shown as the mean ± SE, *n* = 15. Different letters indicate significant differences between treatments (*p* < 0.05, Tukey's test).
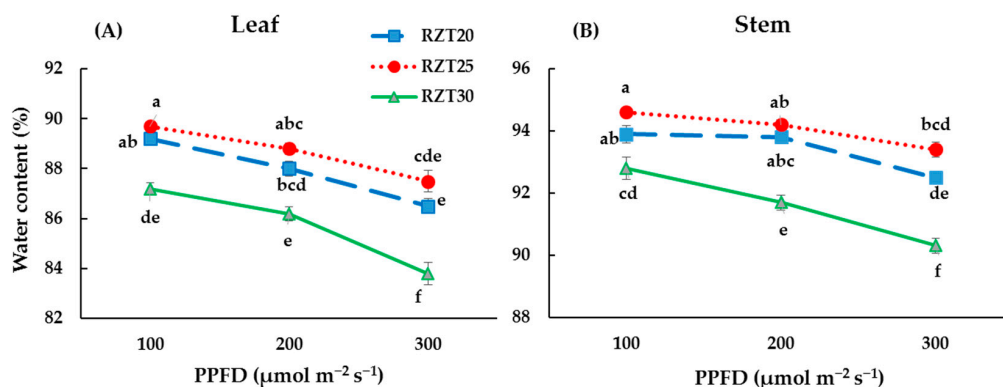
Figure 3. Water content of leaf (**A**) and stem (**B**) in coriander plants after 19 days of cultivation under different photosynthetic photon flux density and root-zone temperatures. Data are shown as the mean ± SE, *n* = 15. Different letters indicate significant differences between treatments ($p < 0.05$, Tukey's test).

3.2. DPPH Radical-Scavenging Activity and Total Phenolic Content

Both TPC and antioxidant activity increased with increases in PPFD under the same RZT, and they were significantly enhanced by an PPFD of 300 µmol m^{-2} s^{-1} compared with PPFDs of 100 and 200 µmol m^{-2} s^{-1} under RZTs of 20 and 30 °C (Figure 4A,B). TPC decreased significantly under an RZT of 25 °C compared with RZTs of 20 and 30 °C in a PPFD of 300 µmol m^{-2} s^{-1}. Antioxidant activity decreased significantly under an RZT of 25 °C compared with RZTs of 20 and 30 °C in PPFDs of 100 and 300 µmol m^{-2} s^{-1}. The highest PPFD (300 µmol m^{-2} s^{-1}) combined with the highest RZT (30 °C) resulted in the highest TPC and antioxidant activity in coriander shoot (2.08 mg GAE g^{-1} FW and 2.47 mg TE g^{-1} FW, respectively), whereas the lowest TPC and antioxidant capacity (0.4 mg GAE g^{-1} FW and 0.14 mg TE g^{-1} FW, respectively) were observed at RZT of 25 °C under PPFD of 100 µmol m^{-2} s^{-1}. TPC and antioxidant capacity of coriander shoot were affected by the interaction between PPFD and RZT ($p < 0.001$).
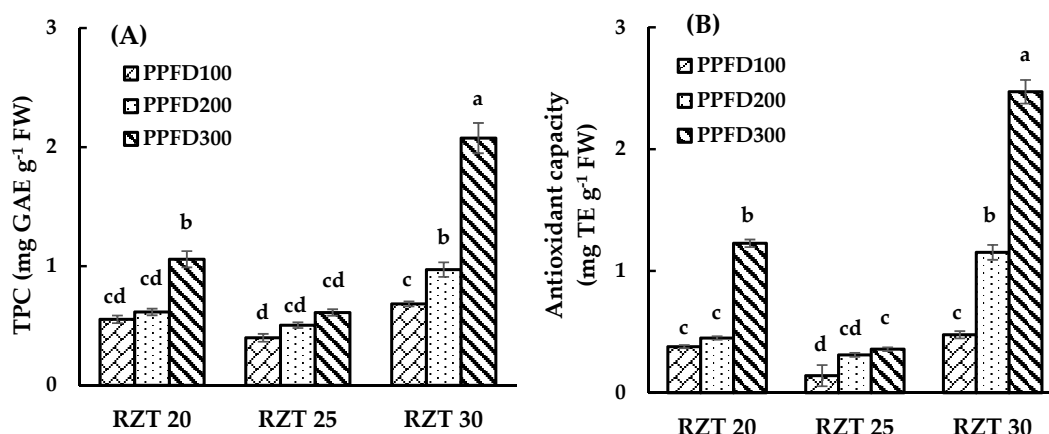

Figure 4. Total phenolic content (TPC) (**A**) and antioxidant capacity (**B**) of coriander shoot (leaf and stem) after 19 days of cultivation under different treatments with photosynthetic photon flux density and root-zone temperature. Data are shown as the mean ± SE, *n* = 8. Different letters indicate significant differences between treatments ($p < 0.05$, Tukey's test).

3.3. DC, CA and QR Content.

DC content per shoot fresh weight was not affected by PPFD and RZT; however, it was significantly increased only by increasing PPFD to 300 µmol m^{-2} s^{-1} and RZT to 30 °C (Figure 5). The highest DC content per plant (368.1 µg plant^{-1}) was obtained in the plants grown under PPFD of 300 µmol m^{-2} s^{-1} combined with RZT of 30 °C, whereas under the same PPFD, lower DC content was observed under RZTs of 20 and 25 °C, at 188 µg plant^{-1} and 113.6 µg plant^{-1}, respectively (Figure S1).
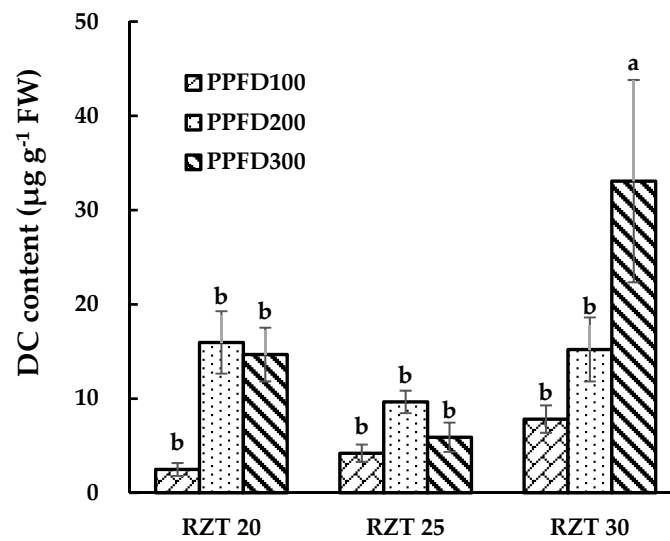
Figure 5. *Trans*-2-Decenal (DC) content per shoot fresh weight in coriander plants after 19 days of cultivation under different photosynthetic photon flux density and root-zone temperatures. Data are shown as the mean ± SE, *n* = 6. Different letters indicate significant differences between treatments (*p* < 0.05, Tukey's test).

CA content in leaves was not significantly affected by RZT, either under PPFD of 100 μmol m^{-2} s^{-1} or 200 μmol m^{-2} s^{-1}, but was affected by RZT when the PPFD increased to 300 μmol m^{-2} s^{-1} (Figure 6A). CA content in leaves increased significantly with an PPFD of 300 μmol m^{-2} s^{-1} under an RZT of 20 and 30 °C compared to those with lower PPFDs. CA content in stems was not significantly affected by PPFDs under RZTs of 20 and 25 °C but increased significantly with an PPFD of 300 μmol m^{-2} s^{-1} compared with 100 and 200 μmol m^{-2} s^{-1} under an RZT of 30 °C (Figure 6B). CA content in leaves was higher (8–114-fold) than that in stems under the same treatments. A combination of the highest PPFD (300 μmol m^{-2} s^{-1}) with the highest RZT (30 °C) significantly promoted CA content in leaves (3488 μg g^{-1} DW) as well as in stems (120.0 μg g^{-1} DW). The accumulation of CA in leaves and stems was influenced by the interaction between PPFD and RZT (*p* < 0.001 for each; Table S3). CA content per plant was not significantly affected by RZT under the PPFD at either 100 μmol m^{-2} s^{-1} or 200 μmol m^{-2} s^{-1} but increased significantly with an RZT of 30 °C under 300 μmol m^{-2} s^{-1} (reached 2546 μg plant^{-1}) (Figure 6C).
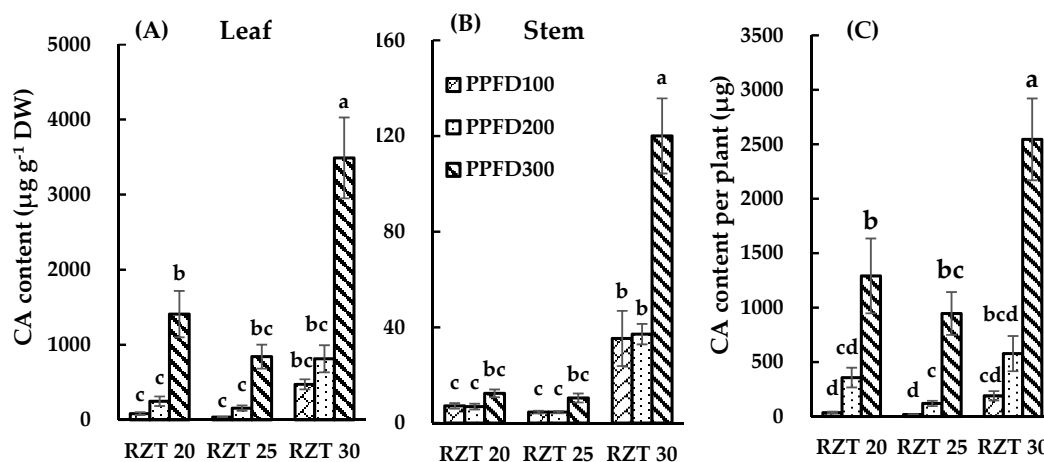

Figure 6. Chlorogenic acid (CA) content per unit of leaf dry weight (DW) (**A**), CA content per unit of stem DW (**B**), and CA content per plant (**C**) in coriander plants after 19 days of cultivation under different photosynthetic photon flux density and root-zone temperatures. Data are shown as the mean ± SE, *n* = 6. Different letters indicate significant differences between treatments (*p* < 0.05, Tukey's test).

A large effect of RZT on QR content in leaves was observed under a PPFD of 300 μmol m^{-2} s^{-1}, in which QR was in the highest with an RZT of 30 °C and in the lowest with an RZT of 25 °C (Figure 7A). QR content in leaves was increased significantly by a PPFD of 300 μmol m^{-2} s^{-1}, compared with 100 μmol m^{-2} s^{-1} and 200 μmol m^{-2} s^{-1} under an RZT of 20 and 30 °C, and compared with 100 μmol m^{-2} s^{-1} under an RZT of 25 °C. QR content in stems was affected neither by PPFD nor by RZT, with the exception of the treatment with PPFD of 300 μmol m^{-2} s^{-1} and RZT of 30 °C (Figure 7B). QR content in leaves was higher (4–20-fold) than that in stems under the same treatments. The highest QR content was obtained in the treatment with the highest PPFD (300 μmol m^{-2} s^{-1}) and the highest RZT (30 °C) in leaves and stems (1644.9 μg g^{-1} DW and 402.6 μg·g^{-1} DW). The accumulation of QR in leaves and stems was influenced by the interaction between PPFD and RZT ($p < 0.001$ for each; Table S3). QR content per plant was not significantly affected by RZT under the PPFD at either 100 μmol m^{-2} s^{-1} or 200 μmol m^{-2} s^{-1} but was affected under the PPFD of 300 μmol m^{-2} s^{-1}. The highest QR content per plant was found at PPFD of 300 μmol m^{-2} s^{-1} with RZT of 30 °C (1330 μg plant^{-1}) (Figure 7C).
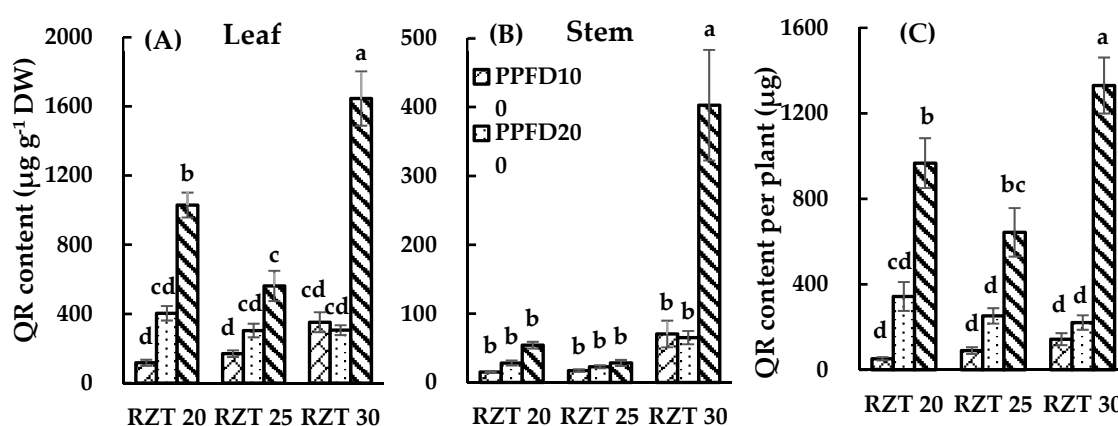

Figure 7. Rutin (QR) content per unit of leaf dry weight (DW) (**A**), QR content per unit of stem DW (**B**), and QR content per plant (**C**) in coriander plants after 19 days of cultivation under different photosynthetic photon flux density and root-zone temperatures. Data are shown as the mean ± SE, $n = 6$. Different letters indicate significant differences between treatments ($p < 0.05$, Tukey's test).

4. Discussion

4.1. Plant Growth

The biomass of coriander plant was significantly enhanced by increasing PPFD from 100 to 300 μmol m^{-2} s^{-1} under all RZT conditions in the present study. Many studies on PPFD have reported that the optimal PPFD for plant growth depends on the plant variety, one of which stated that the PPFD of 290 μmol m^{-2} s^{-1} reached in lettuce resulted in improvement of plant growth and development [22,23]. Based on our results, plant growth and development in coriander were improved with the 300 μmol m^{-2} s^{-1} irradiation regardless of RZT. Moreover, water content of both stems and leaves in coriander were decreased with increases in PPFD. This agreed with the result of Pan and Gou [23] which reported that the water content in *Epimedium* leaf decreased when PPFD was changed from 9 μmol m^{-2} s^{-1} to 127 μmol m^{-2} s^{-1}. Increasing PPFD might foster photosynthesis and produce carbohydrates, thus enhancing dry mass accumulation, which could cause a decrease in the water content in leaf and stem. Meanwhile, RZT is one of various environmental factors that affects plant growth and phytochemical production. It has been found that RZT influenced plant physiological processes, such as uptake of water and minerals, by affecting root growth and initiation [13,14]. RZT of 25 °C has shown the most positive influence on the biomass of coriander plants. The poor development of the root system under 30 °C RZT, as can be seen in Figure 1, resulted in a small uptake of water and minerals through the roots, which might therefore lead to the relatively low water content in the plant leaves and stems. This was in accordance with the results of He et al. [24] who reported that root

and shoot FW as well as mineral content of salad rocket grown under high temperatures (fluctuating 25–38 °C RZT) were lower than those under 20 °C, 25 °C and 30 °C RZT. In the present study, the root biomass under RZT (20 °C) was higher than that under 30 °C RZT regardless of PPFD, although there were no significant differences in leaf and stem biomass between these two RZTs. Therefore, RZT also affected biomass distribution in shoot and root parts of the coriander plant.

Thornley [25] used R/S ratios to examine the optimal environmental condition for root activity and plant growth, and presented that the R/S ratio was lowest at the optimum soil temperature. Awal et al. [26] reported that the R/S ratio in peanut plants was minimum at 25 °C in soil temperature and the R/S ratios increased at soil temperature above or below 25 °C, indicating that the use of the optimal soil temperature could favor the biomass production in plant cultivation. In our study, the lowest R/S DW ratio was observed at the RZT of 25 °C under 300 µmol m^{-2} s^{-1} in coriander plants This condition indeed also achieved the highest biomass production in shoot of coriander plant (Figure S2). As Gosselin and Trudel [27] stated that cucumber transplants achieved maximum shoot growth and total leaf area when the RZT was ranged in 24 °C and 30 °C, our findings imply the optimal RZT on plant growth may vary among species of crops.

Several studies on heating root-zone have shown positive effects on crop production, but crop responses to RZT could also be influenced by other environmental factors such as air temperature, PPFD and photoperiod [28], component and concentration of nutrient solution [29], and CO_2 concentration in root-zone [30]. Agreed with these previous studies, our results also revealed that the growth of coriander plants was affected by interaction between RZT and PPFD and that the combination of mid-RZT (25 °C) and high level of PPFD (300 µmol m^{-2} s^{-1}) gave the greatest plant growth. The similar tendency in tomato plants has been reported by Gosselin and Trudel [28] that plant growth and fruit development of tomato were affected by interaction between RZT and PPFD.

4.2. Total Phenolic Content and DPPH Free Radical Scavenging Activity

Phenolic compounds are the main class of natural antioxidants and predominantly secondary metabolites in plants. In this study, TPC in coriander had significant positive correlation with DPPH free radical scavenging activity ($p \leq 0.01$), exhibiting coefficient $R^2 = 0.902$ (Figure S3). This positive, significant liner relationship between TPC and antioxidant capacity indicates that phenolic compounds are responsible for the antioxidant activity in coriander plants, as the same as reported in other medicinal herbs [31].

The highest amount of TPC was achieved when the highest RZT (30 °C) and the highest PPFD (300 µmol m^{-2} s^{-1}) were applied. This result is in consistence with our hypothesis that the secondary metabolites accumulation could be enhanced by a combination of PPFD and RZT both at the highest level. The effect of PPFD on TPC that shown in coriander plants coincided with the findings in ginger plants. Ghasemzadeh et al. [32] have reported that the TPC in ginger leaves showed a significant positive correlation with increasing PPFD, and the TPC was the highest when plants were grown under the highest PPFD (790 µmol m^{-2} s^{-1}). Hence, we suppose that PPFD was a main factor contributing to the increase of TPC in coriander plants.

Phenolic compounds have aromatic benzene rings with one or more hydroxyl groups in a chemical structure. CA is a caffeate ester of quinic acid and QR is a flavonol glycoside. CA and QR are also classified as phenolic compounds and identified as a major component in coriander. Phenolic compounds are found in the plants mainly as secondary metabolites with a huge chemical diversity, but the reason why such diverse metabolites are needed for the plants remains unclear. Phytochemical researchers have proposed that plants use secondary metabolites for protection or defense against stresses caused by environmental stimuli such as light, temperature, pollution, and infection [33]. In our experiment with coriander plants, the plant biomass decreased with high root temperature; heating the roots at 30 °C in cultivation meant putting the plants under stress, which was enough to decrease the growth of the plants. Thus, the changes that occurred in the content of phenolic compounds, CA and QR from the effects of high RZT could be recognized as a stress response.

Various abiotic and biotic factors are known to induce oxidative stress in plant cells, generating excessive amounts of reactive oxygen species (ROS) including superoxide (O_2·$^-$) and hydrogen peroxide (H_2O_2) [34]. Because plants use antioxidative metabolites, such as phenolic compounds, to prevent the formation of ROS and cope with oxidative stress, the environmental stimuli inducing oxidative stress to coriander plants may promote the production and/or accumulation of phenolic compounds in plant tissues. Many studies have shown that high temperature enhanced the production of ROS in plant cells [35], and heating the plant beyond its optimal temperature might damage various cell functions [34]. As described earlier, we showed that phenolic compounds, including CA and QR, were dominant antioxidant agents in coriander plants, and heating the roots of coriander induced the increase in those antioxidative metabolites that have a main role in scavenging ROS in coriander plants in order to protect the plant cells from damage due to oxidative stress. Therefore, we supposed that RZT is a main factor contributing to the increase in TPC in coriander plants owing to its potency in causing oxidative stress to the plant cells.

Furthermore, we observed a drought-stress-like reduction in leaf and stem water content under environments with strong irradiation and/or high RZT. Drought stress triggers the expression of genes involved in the phenylpropanoid biosynthetic pathway in grape berries [36] and in basil leaves [37]. Therefore, the results that appeared here with accumulation of CA, QR and phenolic compounds of coriander plants are likely the response to drought stress, although in this experiment we did not intend to apply drought conditions to the plants as a treatment. Indeed, there are examples showing that drought stress caused by a deficiency of water supply to the roots has induced the accumulation of total phenolic content in *Hypericum polyanthemum* [38], chlorogenic acid in *Crataegus* spp. [39], and rutin in *Hypericum brasiliense* [40]. In our study, secondary metabolite accumulations in coriander grown at RZT of 20 and 30 °C were almost always higher than at 25 °C. This is probably because RZT of 20 and 30 °C are stressful RZTs for coriander in the present experimental conditions. Moreover, the growth was reduced at these conditions compared to RZT of 25 °C, also indicating that these RZTs were stressful for coriander plant.

4.3. Content of DC, CA and QR

Upon analyzing the content of DC, CA and QR, it was revealed that accumulation of these important secondary metabolites of coriander plants was influenced by the effects of RZT, PPFD and the interaction between RZT and PPFD. As we have discussed, it is clear that RZT and PPFD are important environmental factors contributing to the growth of coriander plants. In addition, these two growth factors could regulate the production of DC, CA and QR in coriander plants.

Currently, there is little information available on the effect of either RZT or PPFD on the accumulation of flavor compounds in coriander. DC is one of the most important flavoring agents in fresh coriander shoot, because with its distinct odor, it can determine the flavor of coriander as a major component of its essential oil. Our results indicate that DC concentration in shoots was not different among treatments, so the main flavor of fresh coriander as derived from DC would not vary from plant to plant, even in a plant that had grown faster and at a higher yield (Figure 1; Tr 25-300). Interestingly, a notable exception in DC concentration was in the example of RZT at 30 °C combined with PPFD of 300 μmol m^{-2} s^{-1}, showing the highest DC concentration with significant differences from that of any other treatment. This suggests that coriander plants responded to the environment with a combination of the highest RZT and the highest PPFD in this experiment and had increased accumulation of DC in cells. It appears that there are limits to these levels, at less than 30 °C of RZT and less than 300 μmol m^{-2} s^{-1} in PPFD, for coriander to not react to these as harmful conditions and to maintain the general accumulation of DC.

Our results reveal that the amount of accumulation of CA in coriander leaves was higher than that of QR under RZT of 30 °C or PPFD of 300 μmol m^{-2} s^{-1}, and the ratio of CA and QR content in coriander leaves differed among treatments. In addition, the content of CA in leaves showed 7 to 23-fold increases over the levels of PPFD at the same RZT, while the content of QR in leaves showed 4 to 8-fold

increases. The content of CA in leaves showed 4 to 13-fold increases over the levels of the RZT at the same PPFD, while the content of QR in leaves showed 1.3–3-fold increases. Therefore, the CA content in leaves differed more widely with treatments than did QR content in leaves. Statistical analysis shows that the interaction between the two factors, PPFD and RZT, made a significant difference to the content of both CA and QR in leaves, so the effect of the interaction of these two factors was the main factor in this trend. These outcomes indicate that CA metabolism in coriander leaves is more sensitive to PPFD and RZT than QR metabolism. In other words, we supposed that the biosynthesis of CA in coriander leaves can be optimally activated by applying such environmental stimuli.

In stems, the opposite metabolic response to the environmental changes was observed with accumulation of CA and QR. The amount of accumulation of QR in stems was always higher than that of CA, regardless of environmental conditions. The content of QR in stems showed 2 to 6-fold increases over the levels of the PPFD at the same RZT, while the content of CA in stems showed 2 to 3-fold increases. The content of QR in stems showed 3 to 14-fold increases over the levels of RZT at the same PPFD, while the content of CA in stems showed 8 to 12-fold increases. The QR content in stems varied more widely than the CA content in stems. The interaction between the two factors was estimated to contribute to this trend in stems. It seems likely that stems need to store and consume much more QR than CA.

Both CA and QR content in leaves of coriander were higher than those in stems. This might indicate that the enzymes involved in the biosynthetic pathways of QR and CA would be induced and expressed more sensitively with the effects of PPFD and RZT in leaf cells than in stem cells.

5. Conclusions

This study focused on investigating the effects of PPFD, RZT and the interaction between PPFD and RZT on the growth of and secondary metabolite accumulation in coriander. Our results showed that both plant growth and secondary metabolite production were influenced by the interaction of two environmental factors, and they significantly increased in coriander plants with an increase in PPFD under the same RZT. Growth parameters including leaf, stem and root biomass and water content of leaf and stem were highest when the nutrient solution was maintained at a mid-range RZT (25 °C) with high light irradiation (300 μmol m^{-2} s^{-1}). In contrast, secondary metabolites including *trans*-2-decenal, rutin, chlorogenic acid and total phenolic concentrations as well as the antioxidant capacity of coriander plant were enhanced with a combination of high RZT (30 °C) and high PPFD (300 μmol m^{-2} s^{-1}). The optimized conditions revealed in this study can be applied in the production of coriander in plant factories to achieve the plant's intended usage purposes. Different regulating strategies can be chosen to produce different features of coriander as cooking materials, raw materials or medicinal materials. Further investigation into the effects of drought stress on plant growth and accumulation of secondary metabolites in coriander is underway.

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