

Article

Effects of Different Photoperiods on the Growth and Nutritional Characteristics of Two Celery Cultivars in Plant Factory

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Abstract: Three different photoperiod treatments (8 h/16 h, 12 h/12 h, and 16 h/8 h of light/dark) were implemented to investigate the impact of growth, biomass, and phytochemical accumulation in two celery cultivars, namely ‘Zhangqiubaoqin’ (BQ) and ‘Hongchenghongqin’ (HQ), within a plant factory setting. The findings demonstrated that the 12 h/12 h photoperiod stimulated the growth of both celery varieties, while the 16 h/8 h photoperiod hindered their growth. Notably, the root length, shoot fresh and dry weight, plant height, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content of both celery cultivars exhibited the highest values under the 12 h/12 h light and dark photoperiod. Furthermore, the effective quantum yields of the electron transfer ratio (ETR) and the photochemical quenching coefficient (qP) displayed superior value under the 12 h/12 h light and dark photoperiod. With the exception of cellulose, the levels of soluble sugar, soluble protein, total phenol, and vitamin C were also highest under the 12 h/12 h photoperiod for both cultivars. BQ demonstrated the highest total apigenin content under the 12 h/12 h (light and dark) photoperiod, whereas HQ exhibited the highest content under the 16 h/8 h (light and dark) photoperiod. In summary, celery plants exhibited optimal performance and biomass production when subjected to the 12 h/12 h (light and dark) photoperiod treatment.

Keywords: plant factory; photoperiods; celery; quality



Citation: Chu, Q.; Qin, Y.; Li, C.; Cheng, S.; Su, L.; He, Z.; Zhou, X.; Shao, D.; Guo, X. Effects of Different Photoperiods on the Growth and Nutritional Characteristics of Two Celery Cultivars in Plant Factory.

Agronomy **2023**, *13*, 3039.

<https://doi.org/10.3390/agronomy13123039>

agronomy13123039

Academic Editor: Junfei Gu

Received: 24 October 2023

Revised: 2 December 2023

Accepted: 6 December 2023

Published: 12 December 2023



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1. Introduction

The nutritional quality of vegetables has potential benefits for human health, as vegetables produce secondary metabolites that not only enhance antioxidant capacity but also improve antioxidant capacity [1]. Maintaining produce stability is challenging due to unpredictable outdoor conditions and increasing demand for high-quality vegetables. To address these issues, attention has shifted towards plant factories using artificial lighting. Plant factories employ various artificial light sources to accurately control the environment [2]. Among them, LED is the most commonly used artificial light source. LED offers advantages such as long life and high light efficiency. However, the cost of operating plant factories remains primarily attributed to artificial light sources. As a result, determining the appropriate photoperiod for plant growth is crucial for enhancing the economic benefits of products [3,4]. Light is a crucial factor that greatly influences the nutritional quality of vegetables in plant factories. Its impact varies depending on different light conditions, which can be controlled through light quality, light intensity, and photoperiod [5]. Photoperiod refers to the duration of light exposure in a 24 h period, and different plants have varying requirements for this duration [6]. The photoperiod plays a vital role in

regulating numerous physiological processes in plants, ultimately affecting the yield and quality of vegetables [7]. It exerts diverse regulatory effects on seed germination [8], the elongation of the hypocotyl, and flowering [9]. Moreover, changes in photoperiod can also influence the morphology, anatomical structure, and metabolites of plants by coordinating essential physiological processes [10,11]. Studies conducted by Appolloni et al. have shown that the most commonly used indoor photoperiods in plant production range from 12 to 16 h [12]. Palmer et al. [13] investigated the effects of various photoperiods (ranging from 12 to 24 h) on the growth and photosynthetic characteristics of lettuce. Surprisingly, their findings revealed that prolonged photoperiods did not contribute to an increase in leaf number or dry matter accumulation under light conditions exceeding 18 h. Different outcomes have been observed in studies involving other plant species. For example, when the photoperiod exceeded 12 h, the yield of dandelion increased, and the accumulation of antioxidant metabolites in stevia rebaudiana gradually rose [14,15]. In cruciferous plants, it was found that there was no significant difference in polyphenol and flavonoid content between different photoperiods (12, 14, 16, 18, and 20 h) treatments for kale, and the total mustard oil content was the lowest in cabbage with a 12 h photoperiod and Chinese kale with a 16 h photoperiod [16].

Celery, a member of the apiaceae family, is a significant plant known for its distinct flavor and abundant fiber, nutrients, and health-related phytochemicals. It is extensively cultivated worldwide. Furthermore, celery has gained popularity among consumers in the market due to its low-calorie nature, offering a mere 200 kcal per kilogram of food, thus possessing great economic value [17,18]. Consequently, the objective of this research was to explore the impact of photoperiod on the growth and phytochemical composition of celery. The findings of this study aim to provide valuable insights for the production of top-quality leafy green vegetables in plant factories equipped with artificial lighting systems.

2. Materials and Methods

2.1. Plant Material and Growing Condition

Two cultivars of celery, ‘Zhangqiubaoqin (BQ)’ and ‘Hongchenghongqin (HQ)’, were used in this study. The celery cultivars were purchased from Shouguang Guanhe Agriculture in Shandong, China. ‘Zhangqiubaoqin (BQ)’ is predominantly grown in the eastern and southern regions of China and is known for its unique flavor and quality. On the other hand, ‘Hongchenghongqin (HQ)’ is a distinct variety known for its rich nutritional value. The cultivation of both varieties took place in the plant factory of Sichuan Agricultural University, located at coordinates 30°71′ N, 103°87′ E. The plant factory maintained temperatures of 23–25 °C during the day and 15–18 °C at night. When the seedlings reached a normal growth stage with their true leaves after approximately 45 days, they were transplanted into plastic containers containing 40 L of hoagland solution. The electrical conductivity (EC) of the solution was adjusted to $2 \pm 0.5 \text{ mS cm}^{-1}$, with a pH of 6.5 ± 0.5 .

2.2. Experimental Design

Three different photoperiod treatments were applied as follows: three light/dark cycles of 8 h/16 h, 12/12 h, and 16/8 h. And the photosynthetic photon flux density (PPFD) was $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ based on the mixed light-emitting diode (LED); this LED lamp is full spectrum, power 18 W, luminous flux of 1600 lm. The experiment was conducted in a completely randomized design with three replicates for each treatment (each treatment contained 24 seedlings in one plastic container), and the nutrient solution was changed every 15 days. Plant petioles were collected at 45 days under three different photoperiodic conditions, immediately frozen in liquid nitrogen, and stored at -80 °C for further analysis.

2.3. Determination of Growth Characteristics

After 45 days of different photoperiod treatment, three strains were randomly selected from the two varieties for growth index determination. The fresh weight of aboveground and underground parts was measured by analytical balance, plant height and root length

were measured by ruler, and dry weight of aboveground and underground parts was determined after 72 h of drying at 70 °C.

2.4. Assay of Chlorophyll and Carotenoids

The content of chlorophyll was obtained according to ethanol test [19]. The contents of chlorophyll and carotenoids were determined after 45 days of different photoperiod treatment.

2.5. Determination of Photosynthetic Parameter and Chlorophyll Fluorescence

To examine the physiological responses of treated plants at different photoperiods, the following features were evaluated on day 45 after transplantation. The following gas exchange parameters were evaluated: the photosynthetic rate (Pn), stomatal conductance (Gs), internal concentration (Ci), and transpiration rate (Tr). They were measured using a portable photosynthesis system (LI-6800, LICOR Inc., Lincoln, NE, USA) connected to a standard 2 cm² chamber.

The maximum photochemical efficiency (Fv/Fm), non-photochemical quenching (NPQ), coefficients of photochemical quenching (qP), and electron transfer rate (ETR) of the third functional leaf PSII from the bottom of the main stem up were determined using a portable modulated chlorophyll fluorescence spectrometer (PAM-2500, WALZ manufacturer, Nuremberg, Germany).

2.6. Phytochemical Measurement

The total phenol was determined based on the folinol method. Briefly, 0.125 mL of extract, assuming 0.25 mL of 2% Na₂CO₃ sodium carbonate, was reacted for 5 min with 0.125 mL of folinol reagent for 30 min at room temperature, and the absorbance was measured at 655 nm [20].

The content of total flavonoids was determined by the aluminum trichloride colorimetric method [21]. Briefly, 0.03 mL of each extract, 0.09 mL of 95% ethanol, 0.006 mL of 2% AlCl₃, 0.006 mL of 1 mol/L potassium acetate, and 0.168 mL of water were mixed for 5 min. The reaction mixture was kept at room temperature for 40 min, and the absorbance was measured at 415 nm.

The method for the determination of the soluble sugar content was to take 0.5 g of the fresh sample, boil it in 10 mL of distilled water for 30 min, then add 0.1 mL of ethyl anthrone acetate, 1 mL of sulfuric acid, and 0.3 mL of distilled water into 0.1 mL of extract solution, and measure the absorbance of the sample at 630 nm [22].

The measurement of nitrate content was based the Wu et al. method [23]. Briefly, 0.5 g of a sample was crushed in 10 mL of deionized water and extracted in a boiling water bath for 20 min. Then, a 5% salicylic acid–sulfuric acid mixture was added to the 0.1 mL of the extract. Then, 9.5 mL of a 8% sodium hydroxide solution was added after 20 min. The absorbance monitored at 410 nm was used to calculate the nitrate concentration.

The measurement of soluble protein was based on the brilliant blue colorimetry method [24]. Exactly 0.5 g of the sample was mixed with 10 mL of distilled water, ground into a homogenate, soaked in a boiling water bath for 10 min, and then centrifuged at 4 degrees for 10 min. 0.1 mL of the protein extract was taken, then 5 mL of Coomassie Bright blue was added. The absorbance was measured at 655 nm after two minutes.

The content of vitamin C was determined by molybdenum blue colorimetry. Three samples of fresh tissue (0.2 g) from each treatment were homogenized with 5 mL of oxalic acid-EDTA solution. The assay mixture contained 1 mL of supernatant, 5 mL of oxalic acid EDTA, 0.5 mL of metaphosphoric acid–acetic acid, 3.5% (w/v) H₂SO₄, and 5% ammonium molybdate. Then, the absorbance value was determined at 760 nm, and the content was calculated by a standard curve [25].

The determination method of cellulose was to take 0.5 g of the dry sample, add 100 mL of 60% H₂SO₄, take 2 mL of the supernatant, and add 0.5 mL of 2% anthrone and 5 mL of concentrated H₂SO₄ along the tube wall, let stand for 12 min, and then measure the absorbance at 620 nm wavelength [26].

The apigenin content was determined as follows: the plant material was cleaned, dried in an oven at 60 °C, each sample was mixed with 40 mL extraction solution (20% hydrochloric acid and 60% methanol), they were hydrolyzed for 1 h in a water bath at 90 °C, 60% methanol solution was added to adjust the volume to 50 mL, and the extract was filtered using a 0.22 µm microporous filter membrane before being injected into the high-performance liquid chromatography (HPLC) instrument. The apigenin was determined using an Agilent LC-1100 series automated HPLC machine (Agilent Technologies, Inc., Waldbronn, Germany). The mobile phase was methanol/water (50:50 *v/v*, containing phosphoric acid, pH 2.5) at a flow rate of 0.8 mL/min. In order to quantitatively determine apigenin in celery, a curve was developed based on the standard sample of apigenin [27].

2.7. Data Analysis

The statistical differences between treatments were analyzed using one-way ANOVA in 2019 IBM SPSS Statistics software and determined using the LSD test. The lowercase letters in the diagram indicate the significant difference at the 0.05 significance level between different treatments of the same species. A Heatmap was created and PCA (principal component analysis) and was performed via 2022 R package.

3. Result

3.1. Celery Growth Analysis

The morphological characteristics of BQ and HQ are illustrated in Figure 1. Figure 2A–F demonstrates that under 12 h/12 h light conditions, the plant height, root length, underground dry weight, and fresh weight of the two celery varieties were higher compared to other photoperiod treatments (8 h/16 h and 16 h/8 h). When compared to the 8 h/16 h and 16 h/8 h photoperiods, the aboveground fresh weight of BQ increased by 38.18% and 53.95%, respectively, under the 12/12 h photoperiod treatment. HQ exhibited similar results to BQ, with an increase of 27.04% and 24.77%, respectively (Figure 2A).



Figure 1. Celery morphology 45 days after treatments. (A) 'BQ' and (B) 'HQ' grown under different photoperiod treatments.

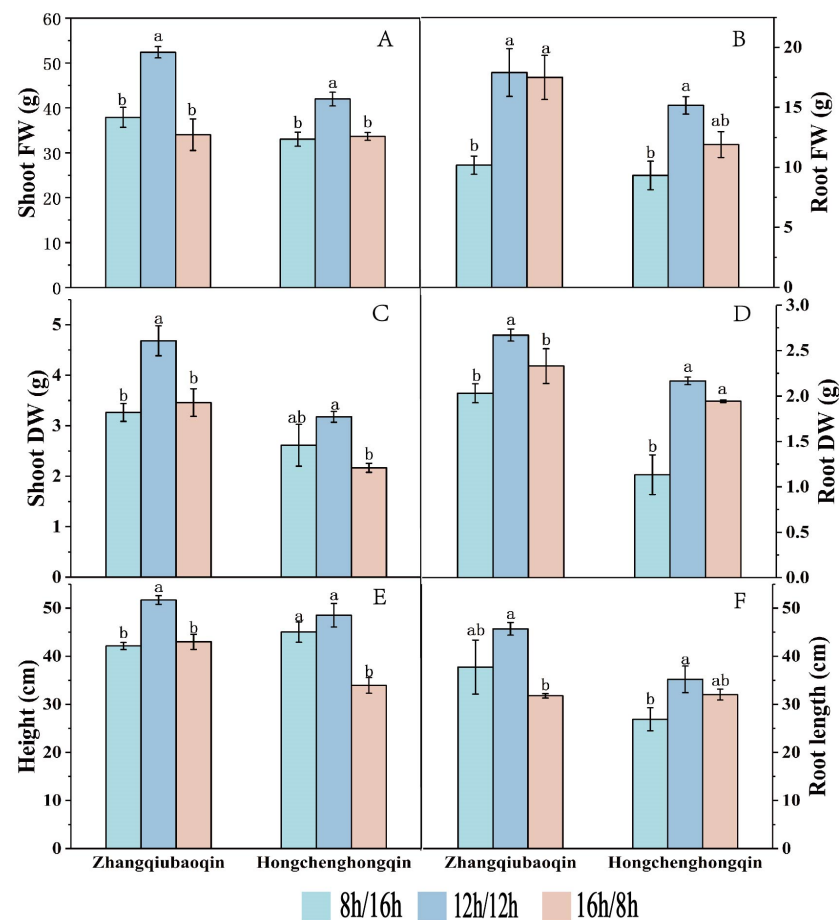


Figure 2. Biomass of celery under different photoperiods. Shoot fresh weight (A), Root fresh weight (B), Shoot dry weight (C), Root dry weight (D), Height (E), Root length (F). Different letters on the top of the columns indicate significant differences at $p < 0.05$ according to one-way ANOVA and Tukey's honestly significant difference tests.

3.2. Chlorophyll Content, Fluorescence, and Photosynthetic Parameters of the Two Cultivars

Changes in the photoperiod had significant effects on the photosynthetic pigment content of two types of celery, as shown in Figure 3. Apart from carotenoids in HQ, the highest contents of chlorophyll a, chlorophyll b, and chlorophyll a + b were observed in the two cultivars under the 12 h/12 h treatment compared to other photoperiod treatments (8 h/16 h and 16 h/8 h) (Figure 3A–D).

The photosynthetic parameters are used as indicators to measure the strength of crop photosynthesis. As shown in Table 1, the photosynthetic parameters (Gs, Ci, and Tr) of two cultivars exhibit an initial increase followed by a decline. Ci, Gs, and Tr reach their maximum values after a 12 h photoperiod treatment. The photoperiodic changes had significant effects on chlorophyll fluorescence parameters of the two kinds of celery. Table 1 also showed that from a comparison of the 8 h/16 h and 16 h/8 h photoperiods with the 12 h/12 h photoperiod, it was found that both celery cultivars showed a significant increase in Fv/Fm and ETR parameters under the 12 h/12 h photoperiod.

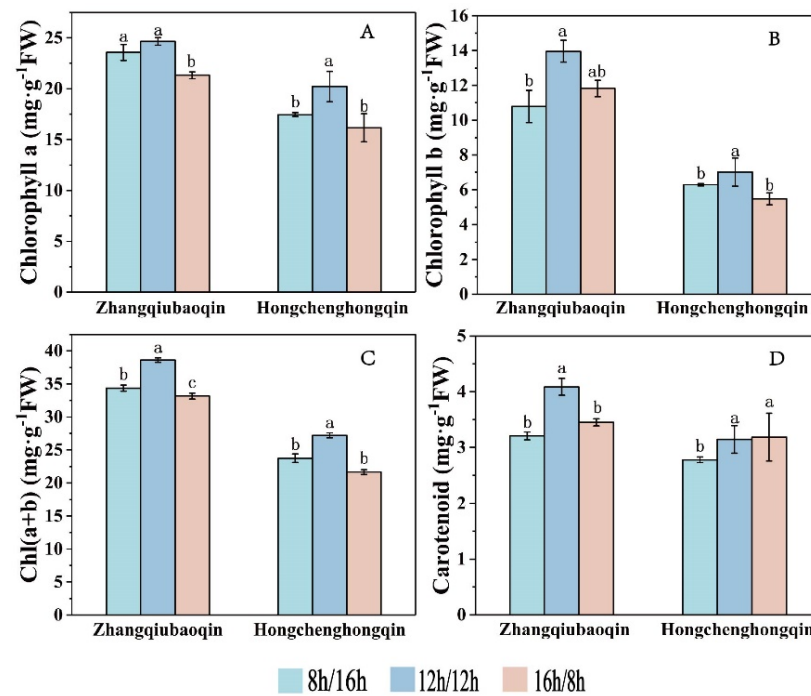


Figure 3. The content of chlorophyll a (A), chlorophyll b (B), chlorophyll a + b (C), carotenoid (D) of two varieties under different photoperiods. Different letters on the top of the columns indicate significant differences at $p < 0.05$ according to one-way ANOVA and Tukey's honestly significant difference tests.

Table 1. Effects of different photoperiod on photosynthesis and fluorescence parameters of two varieties.

Cultivars	Treatment	Pn mol m ⁻² s ⁻¹	Gs μmol m ⁻² s ⁻¹	Ci μmol mol ⁻¹	Tr mmol m ⁻² s ⁻¹	Fv/Fm	NPQ	qP	ETR
BQ	8 h/16 h	15.85 ± 0.09 b	0.39 ± 0.008 b	294.50 ± 1.13 b	4.67 ± 0.008 b	0.70 ± 0.002 b	0.20 ± 0.015 b	0.70 ± 0.006 a	51.26 ± 0.24 b
	12 h/12 h	16.81 ± 0.17 a	0.42 ± 0.002 a	300.48 ± 0.26 a	5.59 ± 0.03 a	0.81 ± 0.001 a	0.21 ± 0.007 b	0.66 ± 0.004 b	52.5 ± 0.08 a
	16 h/8 h	14.41 ± 0.06 c	0.33 ± 0.003 c	297.68 ± 1.20 ab	4.59 ± 0.01 c	0.69 ± 0.002 b	0.26 ± 0.005 a	0.64 ± 0.001 b	49.3 ± 0.20 c
HQ	8 h/16 h	10.70 ± 0.04 a	0.12 ± 0.05 b	223.79 ± 0.02 b	2.27 ± 0.02 b	0.77 ± 0.001 b	0.20 ± 0.018 a	0.67 ± 0.002 b	50.26 ± 0.08 b
	12 h/12 h	10.98 ± 0.12 a	0.40 ± 0.07 a	334.88 ± 0.02 a	6.06 ± 0.02 a	0.80 ± 0.003 a	0.21 ± 0.008 a	0.73 ± 0.002 a	53.3 ± 0.32 a
	16 h/8 h	7.65 ± 0.11 b	0.08 ± 0.09 b	213.29 ± 0.27 b	1.60 ± 0.27 c	0.74 ± 0.008 b	0.23 ± 0.005 a	0.70 ± 0.012 b	48.36 ± 0.09 c

BQ (Zhangqiubaoqin), HQ (Hongchenghongqin), Pn, photosynthetic rate; Gs, stomatal conductance; Tr, transpiration rate; Ci, intercellular CO₂ concentration; Fv/Fm, maximum efficiency of photosystem II photochemistry; ETR, the relative PSII electron transport rate; qP, coefficients of photochemical quenching; NPQ, non-photochemical quenching values. Different letters on the top of the columns indicate significant differences at $p < 0.05$ according to one-way ANOVA and Tukey's honestly significant difference tests.

Different photoperiod treatments resulted in varying levels of phytochemicals. The contents of Vc, SS, SP, and TP in the two celery varieties increased initially and then declined during longer illumination periods. The highest levels of these phytochemicals were observed under the 12 h/12 h photoperiod treatment (Figure 4A,C,D,H). Additionally, a longer photoperiod of 16 h/8 h increased the production of total flavonoids in BQ but reduced it in HQ (Figure 4B). Furthermore, the apigenin content of HQ significantly increased by 49.6% and 148%, respectively, when exposed to the longer photoperiod compared to the other two photoperiods (8 h/16 h and 12 h/12 h). In contrast, the apigenin content of BQ increased significantly at 12 h/12 h, by 173.7% and 25.6%, respectively, compared to the other two photoperiods (8 h/16 h and 16 h/8 h) (Figure 4E). The cellulose content of both celery cultivars decreased by 28.23% and 47.61%, respectively, under the 12 h/12 h photoperiod treatment compared to the 8 h/16 h photoperiod treatment (Figure 4F). Moreover, the nitrate content of BQ and HQ decreased by 26.28% and 16.47%, respectively, under the 18 h/6 h photoperiod treatment compared to the 12 h/12 h photoperiod treatment (Figure 4G).

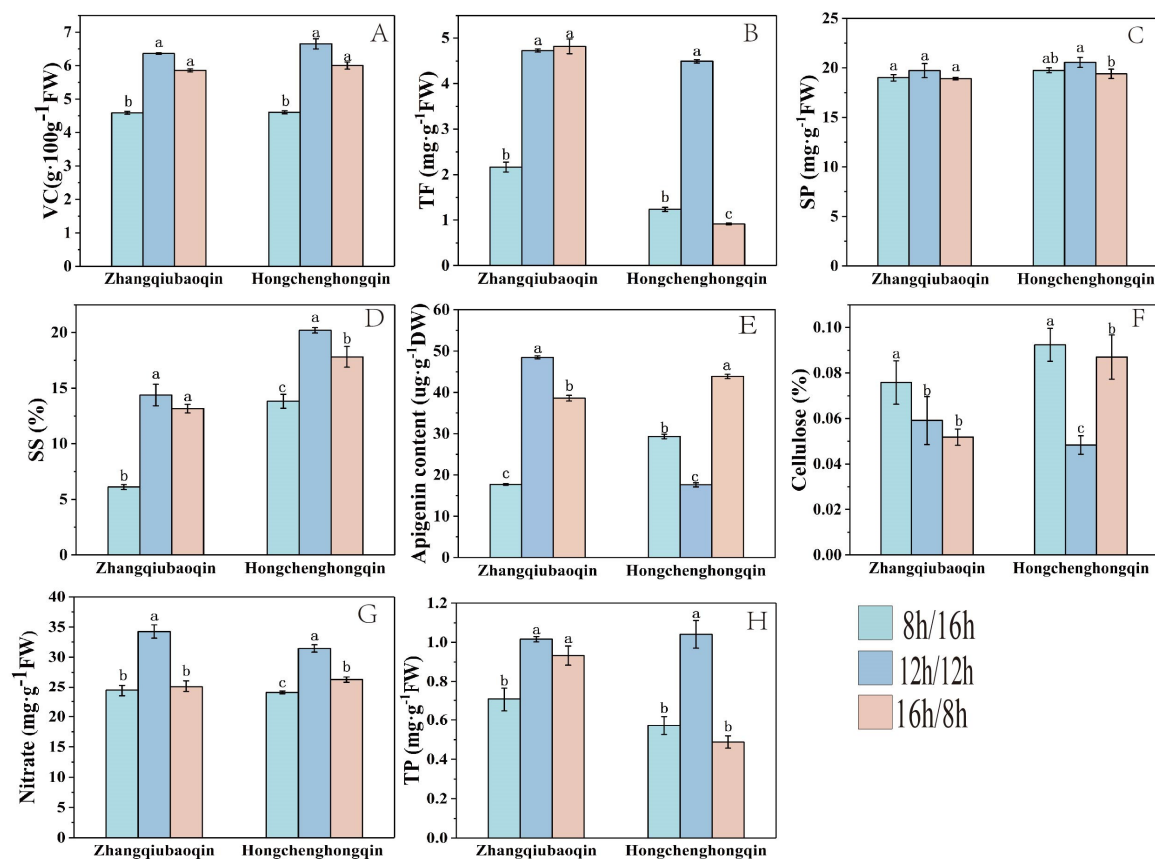


Figure 4. The contents of VC (A), TF (B), SP (C), SS (D), apigenin (E), cellulose (F), nitrate (G), TP (H) in two kinds of celery under different photoperiods treatments. Different letters on the top of the columns indicate significant differences at $p < 0.05$ according to one-way ANOVA and Tukey's honestly significant difference tests. VC = vitamin C, TF = total flavonoids, SP = soluble protein, SS = soluble sugar, TP = total phenols.

For the two celery cultivars, different photoperiod treatments were divided into different quadrants in the principal component analysis, indicating that there were significant differences between different photoperiod treatments and significantly different response patterns. In addition, the principal component analysis method was used to evaluate the changes in quality indexes of two celery cultivars under photoperiod treatment. All variables could be distinguished by the first principal component (PC1) and the second principal component (PC2), which explained 81.3% and 85.6% of the total data variance of BQ and HQ. The treatments were divided into three clear groups in the PCA scatter plot, with the 8 h/16 h, 12 h/12 h, and 16 h/8 h treatments distributed in distinct quadrants. BQ and HQ plants in the upper quadrants (12 h/12 h treatments) were characterized by a higher agronomic performance and nutritional composition (Figure 5A,B).

These parameters can be divided into three clusters on the heat map, corresponding to the photoperiod treatment, with regard to BQ, in terms of the parameters measured, higher phytochemical and lower biomass contents were observed in the 8 h cluster. Compared to 8 h, the 16 h cluster is characterized by a lower biomass and higher phytochemical content. The 12 h cluster was separated from other clusters due to its higher total flavonoid, apigenin, and vitamin C contents. For HQ, the 16 h cluster showed highly different response patterns, as 16 h reduced plant height, aerial dry fresh weight, and led to an increase in nitrate, cellulose, apigenin content compared to 8 h and 12 h (Figure 5C,D).

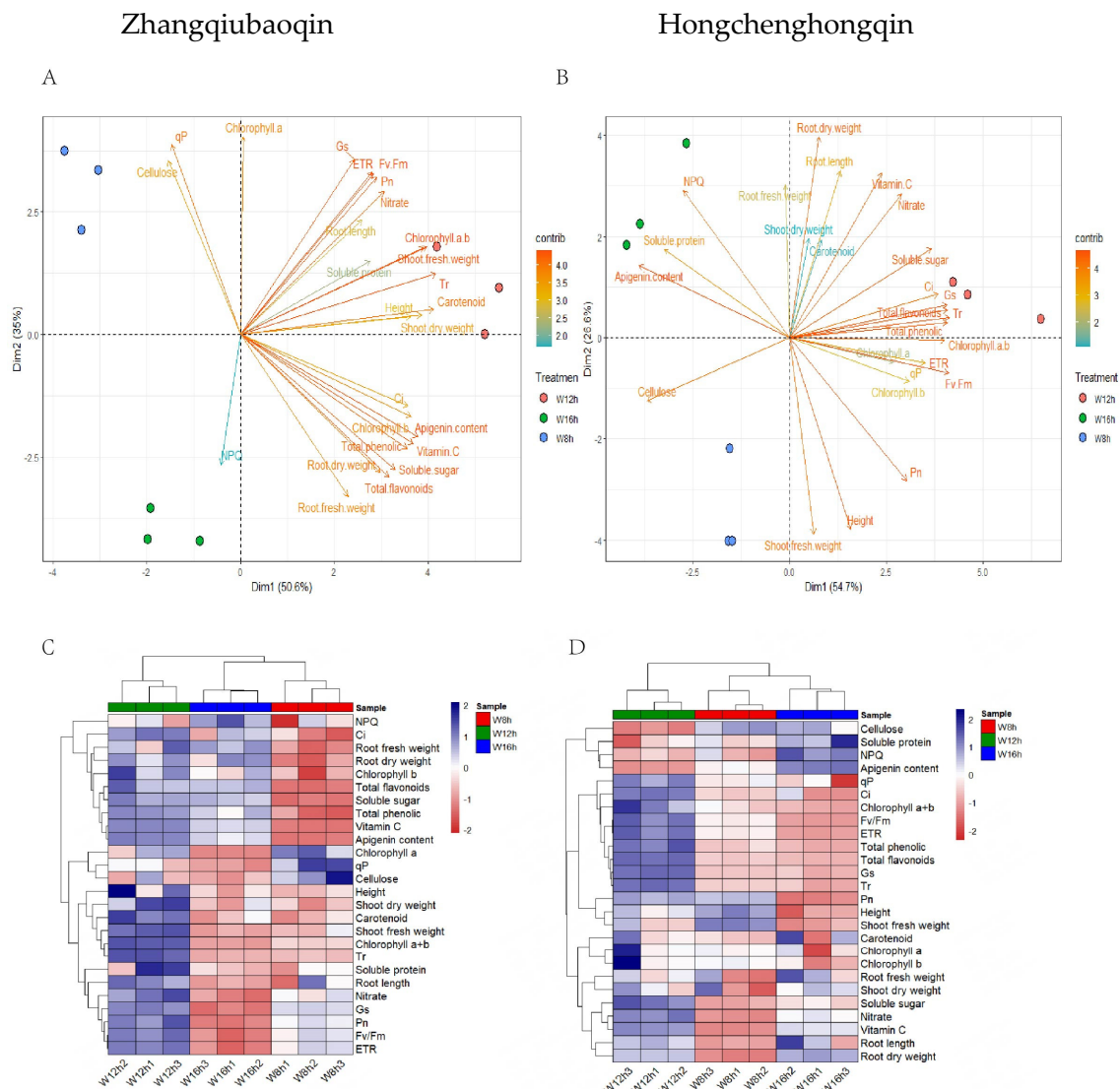


Figure 5. Principal component (A,B) and heat map (C,D) analyses showing differences and correlations in the investigated parameters in ‘BQ’ (A,C) and ‘HQ’ plants (B,D) under different photoperiods. Results are visualized using a false color scale, with blue and pale red indicating an increase and decrease, W8 h = 8 h/16 h (day/night), W12 h = 12 h/12 h (day/night), W16 h = 16 h/8 h (day/night).

4. Discussion

A photoperiod is the response of plants to changes in the light environment, which is crucial to the growth and development of plants [28]. The effects of the photoperiod on vegetables such as lettuce, pepper, garlic, and cucumber have been extensively studied [29]. In this work, it is possible to observe the direct effects of the photoperiod on the primary and secondary metabolism of two celery species.

Generally speaking, different photoperiods have different effects on the growth of different plants, and previous studies have shown that a longer photoperiod contributes to the growth and biomass of plants, that the higher growth performance is founded in the increase in dry weight and leaf area of shoots and roots, and that the photosynthetic rate is also significantly increased [30]. Viridi et al. [31] reported that the height of *agropyron cristatum*, mung bean, chickpea, and lentil increased to varying degrees under longer photoperiods, indicating that longer photoperiods resulted in increased photosynthesis, leading to enhanced meristem activity and energy utilization. However, our results demonstrated that longer photoperiods were not beneficial for the growth of celery, while the 12 h/12 h photoperiod treatment was found to be suitable for its growth and biomass. This

finding aligns with previous studies on pepper and *Stevia*, where *Stevia* plants grown under 12 h/12 h photoperiod exhibited greater height compared to those grown under 15/9 h and 16/8 h photoperiods. Additionally, increasing the illumination time from 12 h to 18 h significantly decreased the aboveground dry weight of pepper plants with prolonged photoperiods [14,32]. This may be attributed to the fact that while prolonging the photoperiod benefits plant growth, excessively long photoperiods can lead to an overabundance of light energy, potentially disrupting the photosynthetic mechanism of chloroplasts and adversely affecting the growth and development of leafy vegetables [33]. Different photoperiods also had significant effects on photosynthetic pigment accumulation in beet, with cabbage exhibiting the highest content of chlorophyll a and chlorophyll b under 12 h photoperiod, and beet producing more chlorophyll under 12 h compared to the 16 h photoperiod [34,35]. These findings are consistent with the results of our experiment, where celery showed the highest levels of chlorophyll a, chlorophyll b, and carotenoids under a 12 h/12 h photoperiod. Previous studies have indicated that extending the illumination time can promote pigment accumulation in leaves, but there is likely a threshold beyond which further extension may reduce pigment content [36]. In this study, the maximum light time is 16 h. Further exploration is needed to determine if extending the photoperiod beyond this limit would result in reduced chlorophyll content.

Changes in the photoperiod have a strong impact on the accumulation of primary and secondary metabolites [37]. Previous studies have reported that as the duration of light increases, the content of vitamin C, soluble sugar, and protein in lettuce and kale also increase, reaching their peak at 16 h, this suggests that extending the photoperiod enhances photosynthesis time and consequently increases carbon assimilation [38,39]. However, our results differ from these findings as the vitamin C, soluble sugar, and protein contents of both celery varieties decreased under a longer photoperiod of 16 h/8 h. This discrepancy may be attributed to variations in the effects of photoperiod on plant metabolism, which can vary across species and growth stages. Additionally, longer photoperiods (16 h/8 h) have been shown to decrease nitrate content [40], which aligns with our results. However, at a 12 h /12 h photoperiod, the growth rate of nitrate suddenly increased. This may be due to insufficient energy provided by the leaves for nitrate reduction. Furthermore, compared to the 8 h photoperiod treatment, the cellulose content of both celery cultivars significantly decreased. It is worth noting that other studies have shown an increase in cellulose content in cucumber seedlings when the light duration was extended from 16 h to 22 h [41]. This discrepancy may be attributed to the differential effects of photoperiod on the metabolism of different plant parts. Therefore, a lower photoperiod of 12 h/12 h may be beneficial in improving the nutritional quality of celery.

Some phytochemicals, such as apigenins [42], polyphenols, and flavonoids, are crucial for human health. In plant factories, optimizing the production of plant chemicals can be achieved by designing light conditions in a rational manner to enhance the quality of production [43]. Previous research has demonstrated that a 12 h/12 h photoperiod contributes to increased antioxidant activity, total phenol content, and betaine content in red beet, red spinach, and red amaranth [44]. This finding aligns with the observed changes in bioactive compounds like total phenols and total flavonoids. Moreover, longer photoperiods have been found to promote anthocyanin synthesis, while there is a metabolic competition between apigenin synthesis and anthocyanin synthesis [45], this competition leads to significantly higher apigenin content in BQ compared to HQ after 12 h of treatment. Although some studies have investigated the impact of photoperiodic changes on the production of secondary metabolites [46,47], little is known about their effects on plant apigenin synthesis, thus further investigation is warranted.

5. Conclusions

In summary, 12 h/12 h photoperiod treatment can significantly improve the yield of two kinds of celery, but also improve the total phenol, vitamin C, soluble protein and sugar content of two kinds of celery, but also reduce the content of cellulose, so the 12 h/12 h photoperiod treatment is the best condition to improve the nutritional quality of celery. Overall, this 12 h/12 h photoperiod is likely to be more suitable for the production of high-quality vegetables. The effects of the photoperiod on the growth and phytochemical composition of celery were studied in order to provide reference for the production of high-quality green leafy vegetables by plant factories utilizing artificial lighting.

Author Contributions: Data analysis, writing—original draft preparation, review, and editing: Q.C. and Y.Q.; research design, Z.H.; conception and supervision of the research, X.Z.; collected samples and processed the data, L.S., S.C., D.S., C.L. and X.G. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Second Tibetan Plateau Scientific Expedition and Research (2019QZKK0303) and Sichuan Luzhou expert workstation (2322339013).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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