

Article

Night Temperature Affects the Growth, Metabolism, and Photosynthetic Gene Expression in *Astragalus membranaceus* and *Codonopsis lanceolata* Plug Seedlings

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Abstract: *Astragalus membranaceus* and *Codonopsis lanceolata* are two important medical herbs used in traditional Oriental medicine for preventing cancer, obesity, and inflammation. Night temperature is an important factor that influences the plug seedling quality. However, little research has focused on how the night temperature affects the growth and development of plug seedlings of these two medicinal species. In this study, uniform plug seedlings were cultivated in three environmentally controlled chambers for four weeks under three sets of day/night temperatures (25/10 °C, 25/15 °C, or 25/20 °C), the same relative humidity (75%), photoperiod (12 h), and light intensity (150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) provided by white LEDs. The results showed that night temperature had a marked influence on the growth and development of both species. The night temperature of 15 °C notably enhanced the quality of plug seedlings evidenced by the increased shoot, root, and leaf dry weights, stem diameter, and Dickson's quality index. Moreover, a night temperature of 15 °C also stimulated and increased contents of primary and secondary metabolites, including soluble sugar, starch, total phenols and flavonoids. Furthermore, the 15 °C night temperature increased the chlorophyll content and stomatal conductance and decreased the hydrogen peroxide content. Analysis of the gene expression showed that *granule-bound starch synthase (GBSS)*, *ribulose biphosphate carboxylase large chain (RBCL)*, and *ferredoxin (FDX)* were up-regulated when the night temperature was 15 °C. Taken together, the results suggested that 15 °C is the optimal night temperature for the growth and development of plug seedlings of *A. membranaceus* and *C. lanceolata*.

Keywords: GBSS; growth; FDX; medicinal plant; night temperature; stomata; RBCL; total phenols and flavonoids

1. Introduction

As two important medicinal herbs in Oriental medicine, *Astragalus membranaceus* and *Codonopsis lanceolata* are mainly distributed in East Asia, especially in Korea, China, and Japan [1,2]. It has been widely reported that the roots of these two species can be used for preventing diseases including inflammation, obesity, and cancer [3–5]. In addition, as a high-class vegetable, *C. lanceolata* has been consumed as food for several centuries, especially in ancient Korea [6,7]. Nowadays, new medicinal compounds continue to be reported from these two species, and their medicinal mechanisms have been demonstrating [3,8,9]. Focused research on the separation, extraction, and characteristics of the

phytochemicals of these two species are ongoing [8,10]. However, there has been little work done on the cultivation of these two species in an environmentally controlled conditions for obtaining high quality plug seedlings.

Temperature is an important abiotic factor that has a great influence on the growth, yield, and quality of crop plants and shapes the morphology of seedlings [11–13]. High and low temperature extremes usually contribute to physiological disorders and dysfunction, resulting in a loss of yield and a degradation of quality [14]. Previous studies widely report on such effects in a variety of species, including rice [15], wheat [16], tomato [17], strawberry [18], and lettuce [19]. In recent years, more and more researchers have paid attention to the effects of the night temperature on crops [20–23]. It seemed that low and high night temperatures caused a reduction in the biomass with different patterns. For example, Jing et al. [24] found that the effects of a high night temperature were primarily related to biomass allocation to seeds, while the effects of a low night temperature were more connected with the total biomass. The results from Loka and Oosterhuis [25] showed that two high temperature regimes (27 °C and 30 °C) caused a significant increase in the respiration rate (by 49% and 56% respectively), compared to the control (24 °C). However, an opposite result has also been reported. Kanno et al. [26] found that the final biomass of rice (*Oryza sativa* L.) was the greatest in plants grown at the highest night temperature (27 °C) rather than in those grown with lower night temperatures (17 °C and 22 °C). Moreover, there is little research regarding the effects of the night temperature on the growth and development of plug seedlings. Therefore, further research is still needed to clarify how the night temperature influences the growth, biomass, and quality, especially in plug seedlings of medicinal plants.

Photosynthesis is an important physiological process that is strictly regulated by a series of enzymes [27]. In light-dependent reactions, ferredoxin, coded by the *FDX* gene, is an important iron-sulfur protein that accepts electrons produced from sunlight-excited chlorophyll and mediates them to NADP⁺ in the electron transport chain [28]. In dark reactions, ribulose biphosphate carboxylase (Rubisco, coded by the *RBC* gene) is a crucial enzyme in the initial step of the Calvin cycle in plants, since it catalyzes the primary CO₂ fixation step and controls the photosynthesis rate [29]. Additionally, granule-bound starch synthase, coded by the *GBSS* gene, is indispensable for the synthesis of linear glucan (amylose) in starch and also takes part in building the final structure of amylopectin [30–32]. Previous studies have proven that those enzymes are sensitive to the temperature. The expression of the *FDX*, *GBSS*, and *RBC* genes are suppressed by high or low day temperatures, resulting in a reduction of crop yield [33–35]. However, few studies have focused on how those genes respond to the night temperature.

In the current study, it was hypothesized that a suitable night temperature (15 °C) could enhance the plug seedling quality and too high or low temperatures would have a negative effect on the growth and development of *A. membranaceus* and *C. lanceolata* plug seedlings. In order to test the above hypothesis, uniform plug seedlings were cultivated at night temperatures of 10 °C, 15 °C, and 20 °C for four weeks. Characteristics of the growth, development and morphology of *A. membranaceus* and *C. lanceolata* plug seedlings grown with different night temperatures were evaluated. The contents of the primary and secondary metabolites such as soluble sugar, starch, total phenols, and flavonoids, were further determined. Moreover, photosynthesis-related parameters including the chlorophyll content and stomatal conductance were measured. In addition, the expression of *FDX*, *RBC*, and *GBSS* genes were assayed. These data could provide a theoretical and practical basis not only for enhancing the quality of plug seedlings by manipulating the night temperature, but also for guarding the management of other medicinal plants.

2. Results

2.1. Growth, Development and Morphology

In this study, the night temperature had a remarkable influence on the growth, development, and morphology of plug seedlings. As shown in Figure 1, a diverse morphology of plug seedlings of *A. membranaceus* and *C. lanceolata* were shaped by different night temperatures. Compared with 15 °C and 20 °C, a night temperature of 10 °C markedly inhibited the growth and development of both species with dwarfed shoots, curved stems, and sparse roots. Plug seedlings grew better with a night temperature of 15 °C than with 20 °C, evidenced by the slightly higher biomass, stem diameter, and Dickson's quality index (DQI), although differences in the growth parameters of seedlings between these two treatments were not significant (Tables 1 and 2).

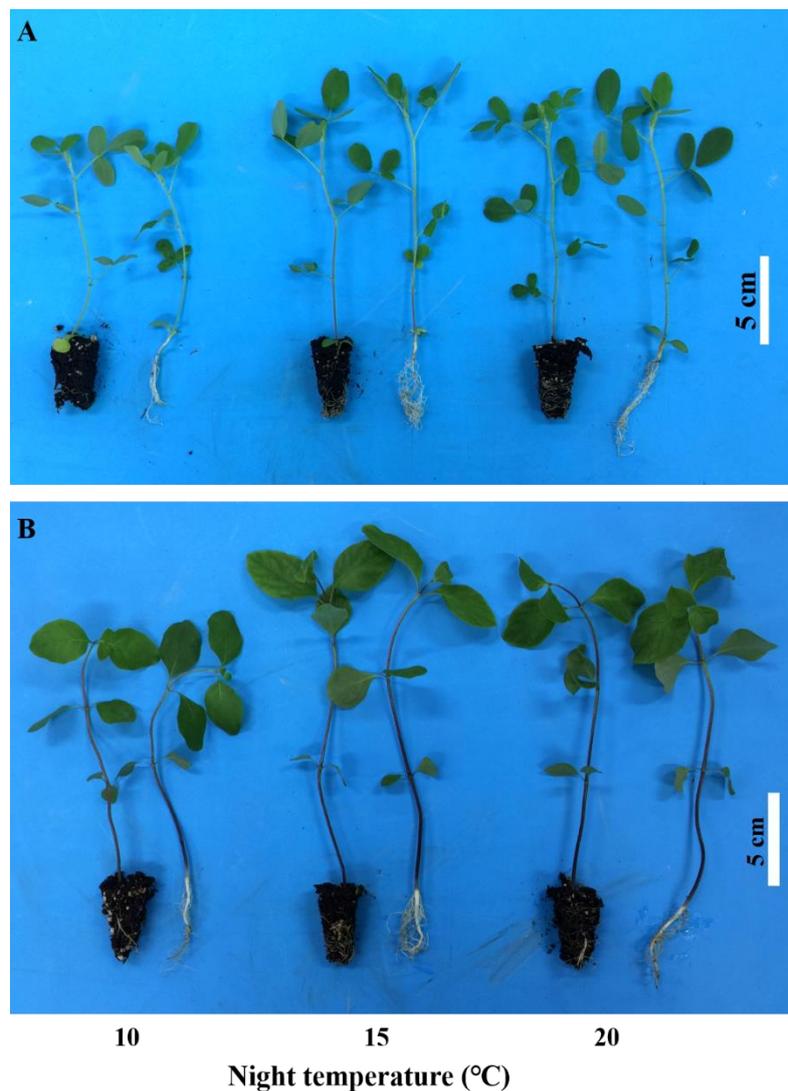


Figure 1. Morphological characteristics of plug seedlings of *Astragalus membranaceus* (A) and *Codonopsis lanceolata* (B) as affected by the night temperature. Bar, 5 cm.

Table 1. Effect of night temperature on the growth parameters of *A. membranaceus* plug seedlings.

Night Temperature (°C)	Length (cm)		Dry Weight (mg)		Leaf Area (cm ⁻²)	Stem Diameter (mm)	Root DW: Shoot DW Ratio	Shoot Dry Weight per Shoot Length (g·m ⁻¹)	Root Dry Weight Per Root Length (g·m ⁻¹)	Dickson's Quality Index (×10 ⁻⁴)
	Shoot	Root	Shoot	Root						
10	10.8 ± 0.4 b ^z	4.1 ± 0.3 b	54.9 ± 7.8 b	8.7 ± 1.2 b	4.3 ± 0.2 a	0.83 ± 0.03 b	0.16 ± 0.01 b	0.51 ± 0.07	0.21 ± 0.03 b	4.6 ± 0.6 b
15	14.0 ± 0.4 a	4.5 ± 0.5 b	78.2 ± 4.3 a	17.8 ± 1.7 a	4.8 ± 0.2 a	1.06 ± 0.04 a	0.23 ± 0.02 a	0.56 ± 0.03	0.42 ± 0.06 a	7.1 ± 0.4 a
20	13.4 ± 0.4 a	5.9 ± 0.2 a	72.8 ± 5.0 a	14.8 ± 1.3 a	3.5 ± 0.2 b	0.98 ± 0.03 a	0.20 ± 0.01 a	0.54 ± 0.03	0.25 ± 0.03 b	6.2 ± 0.5 a
F-test	***	**	*	**	**	**	**	NS	**	*

^z Different letters (a and b) indicate the significant separation within columns by Duncan's multiple range test at a 0.05 level. NS, *, **, and ***, represent no significant or significant difference at $p = 0.05, 0.01, \text{ or } 0.001$, respectively. Data were presented as the mean ± standard error (n = 6).

Table 2. Effect of night temperature on the growth parameters of *C. lanceolata* plug seedlings.

Night Temperature (°C)	Length (cm)		Dry Weight (mg)			Leaf Area (cm ⁻²)	Stem Diameter (mm)	Root DW: Shoot DW Ratio	Shoot Dry Weight per Shoot Length (g·m ⁻¹)	Root Dry Weight per Root Length (g·m ⁻¹)	Dickson's Quality Index (×10 ⁻⁴)
	Shoot	Root	Shoot	Root	Leaf						
10	15.1 ± 0.6 b ^z	3.3 ± 0.3 b	33.4 ± 2.6 b	4.1 ± 0.4 b	7.1 ± 0.9 b	6.6 ± 0.6	1.46 ± 0.05 b	0.19 ± 0.02 b	0.22 ± 0.02	0.13 ± 0.02 b	3.4 ± 0.3 b
15	17.5 ± 0.7 a	3.9 ± 0.1 b	57.6 ± 3.9 a	9.0 ± 0.6 a	11.7 ± 0.8 a	7.7 ± 0.6	1.81 ± 0.05 a	0.29 ± 0.02 a	0.34 ± 0.03	0.23 ± 0.02 a	6.5 ± 0.5 a
20	17.8 ± 0.7 a	4.9 ± 0.3 a	51.0 ± 5.6 a	6.9 ± 1.3 a	9.4 ± 1.0 ab	8.0 ± 0.5	1.80 ± 0.04 a	0.23 ± 0.01 b	0.29 ± 0.04	0.15 ± 0.03 b	5.5 ± 0.8 a
F-test	*	**	**	***	**	NS	***	**	NS	**	**

^z Different letters (a and b) indicate significant separation within columns by Duncan's multiple range test at a 0.05 level. NS, *, **, and ***, represent no significant or significant difference at $p = 0.05, 0.01, \text{ or } 0.001$, respectively. Data were presented as the mean ± standard error (n = 6).

2.2. Contents of Soluble Sugar and Starch

In this research, the night temperature had an impact on the primary metabolism in *A. membranaceus* and *C. lanceolata* plug seedlings (Figure 2A,B). The same feature was found in both species where the contents of soluble sugar and starch were increased first and then decreased as the night temperature was raised from 10 °C to 20 °C. The maximum contents of soluble sugar and starch were 12.4 ± 0.2 and 27.0 ± 0.1 mg·g⁻¹ FW for *A. membranaceus*, and 11.0 ± 0.4 and 41.3 ± 0.3 mg·g⁻¹ FW for *C. lanceolata*, respectively.

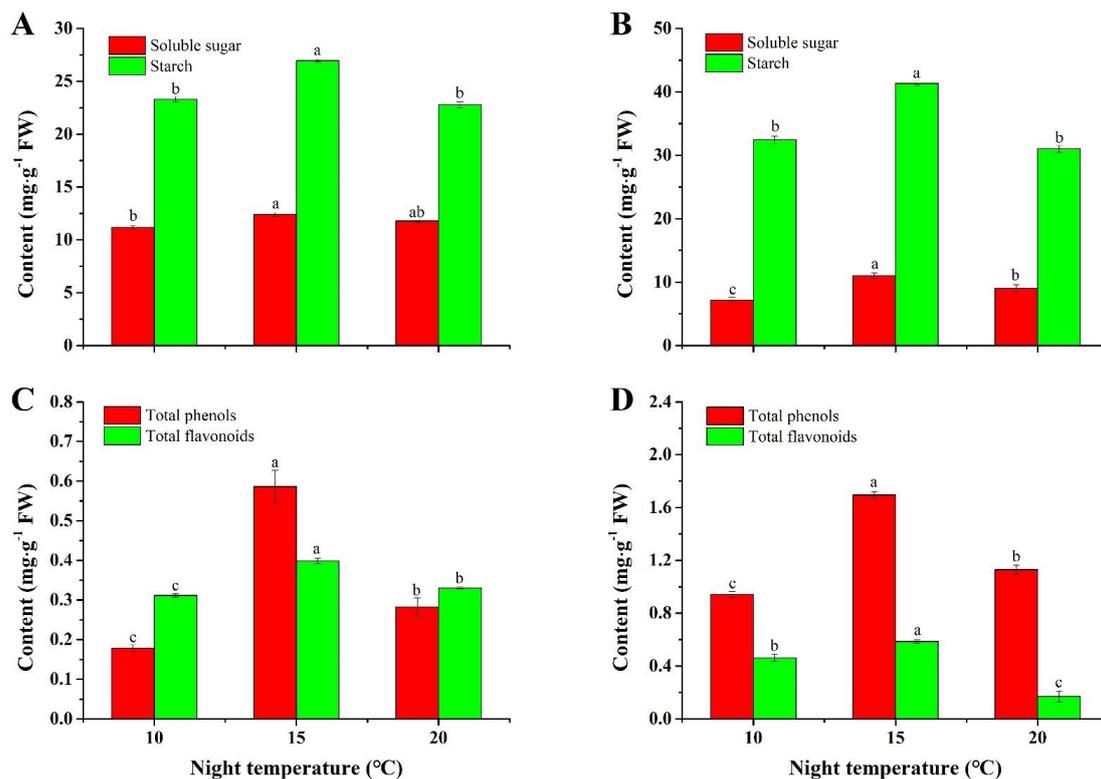


Figure 2. Contents of soluble sugar, starch, total phenols, and flavonoids in *A. membranaceus* (A,C) and *C. lanceolata* (B,D) plug seedlings as affected by night temperature. Data are presented as the mean \pm standard error (n = 3). Different letters (a, b, and c) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

2.3. Contents of Total Phenols and Flavonoids

The data showed that secondary metabolites were markedly influenced by different night temperatures (Figure 2C,D). In *A. membranaceus*, maximum contents of both total phenols and flavonoids were observed when grown at 15 °C, where they were respectively 0.59 ± 0.04 and 0.40 ± 0.01 mg·g⁻¹ FW. The highest contents of total phenol and flavonoid were observed at 15 °C, followed by those at 20 °C and 10 °C. In *C. lanceolata*, the contents of total phenols and flavonoids were 1.70 ± 0.03 and 0.59 ± 0.01 mg·g⁻¹ FW at 15 °C, significantly greater than those grown with the other two night temperatures.

2.4. Hydrogen Peroxide Content

As shown in Figure 3, the lowest H₂O₂ content in *A. membranaceus* and *C. lanceolata* was found at 15 °C, which were respectively 0.198 ± 0.004 and 0.244 ± 0.004 mg·g⁻¹ FW. A lower or higher night temperature (10 °C and 20 °C) significantly increased the H₂O₂ content.

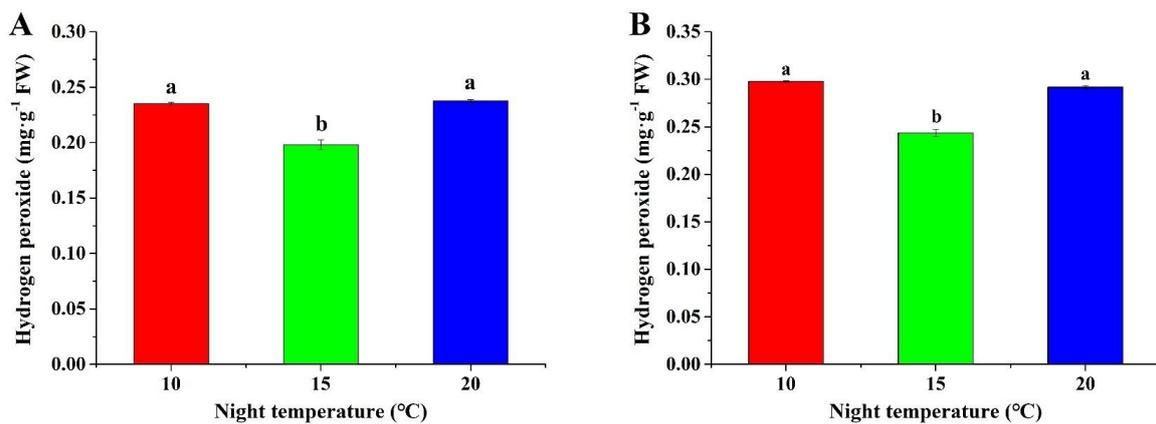


Figure 3. Effect of night temperature on the hydrogen peroxide content in *A. membranaceus* (A) and *C. lanceolata* (B) plug seedlings. Data are presented as the mean \pm standard error (n = 3). Different letters (a and b) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

2.5. Chlorophyll Content and Stomatal Conductance

In this study, an elevated night temperature (15 °C) increased the chlorophyll content in plug seedlings of both *A. membranaceus* and *C. lanceolata* (Figure 4A,B). Slightly higher chlorophyll content was observed in seedlings grown with a 15 °C night temperature, compared to those grown with a 20 °C night temperature, but the difference was not significant.

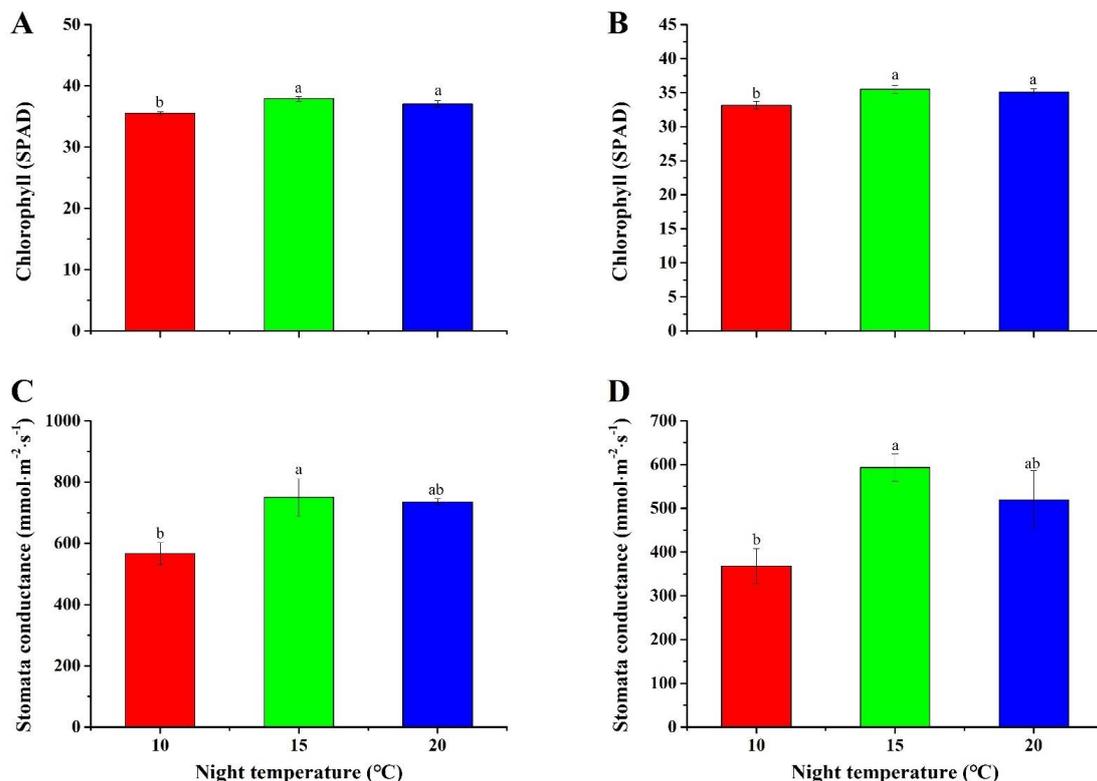


Figure 4. Effect of night temperature on the chlorophyll content and stomatal conductance in plug seedlings of *A. membranaceus* (A,C) and *C. lanceolata* (B,D). Data are presented as the mean \pm standard error (n = 3). Different letters (a and b) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

In the current study, the stomatal conductance significantly increased with the increase of the night temperature from 10 °C to 15 °C, and then decreased slowly (Figure 4C,D). The peak values

of the stomatal conductance were 749.7 ± 61.6 and 593.3 ± 31.0 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in plug seedling of *A. membranaceus* and *C. lanceolata*, respectively.

2.6. Gene Expression Analysis

The data showed that expression of the photosynthetic genes was influenced by the night temperature (Figure 5). In *A. membranaceus*, a night temperature of 15 °C significantly increased the expression of *granule-bound starch synthase* (GBSS), and *ribulose bisphosphate carboxylase large chain* (RBCL), more than those at a night temperature of 10 °C. Although there were no significant differences between those at 15 °C and 20 °C, the expression of these two genes was higher at 15 °C than at 20 °C. Similarly, the highest expression of *ferredoxin* (FDX) was also at 15 °C in *C. lanceolata*. The expression of RBCL in *C. lanceolata* was remarkably greater at 15 °C and 20 °C than that at 10 °C. However, the RBCL expression at night temperatures of 15 °C and 20 °C was not significantly different.

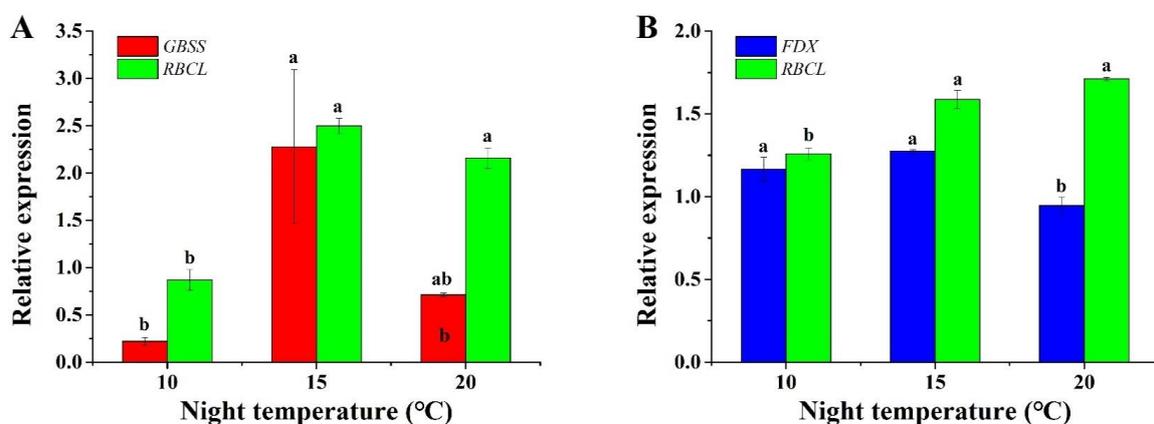


Figure 5. Effect of night temperature on the gene expression in *A. membranaceus* (A) and *C. lanceolata* (B) plug seedlings. GBSS: *granule-bound starch synthase*, RBCL: *ribulose bisphosphate carboxylase large chain*, and FDX: *ferredoxin*. The 18S and β -Actin were used as the housekeeping genes in *A. membranaceus* and *C. lanceolata*, respectively. Data are presented as the mean \pm standard error ($n = 3$). Different letters (a and b) indicate significant differences among treatments by Duncan's multiple range test at a 0.05 level.

3. Discussion

Temperature is one of crucial abiotic factors that greatly decides the quality and yield of crops [15,36]. Recently, an increasing number of studies have shown that the night temperature has a considerable influence on the growth, development, and morphology of plants [37–39]. In this study, a night temperature at 15 °C markedly improved the quality of *A. membranaceus* and *C. lanceolata* plug seedlings, evidenced by the higher biomass, stem diameter, and DQI. These results are supported by other studies. For instance, Jing et al. [24] found that high and low night temperatures both had a negative effect on the plant yield by collecting and analyzing data from 112 papers. Morita et al. [40] reported that the rate of grain growth in rice (*O. sativa* L.) was lower with a low night temperature than with a high night temperature. In addition, Cheng et al. [41] found that a high night temperature not only decreased the rice yield but also reduced the stimulatory effect of elevated CO₂ levels on the rice production. However, an opposite conclusion in rice had been reported from Kanno et al. [26], who found that a high night temperature stimulated leaf photosynthesis and increased biomass production by promoting the growth of leaf blades. More research is needed to clarify this discrepancy and the underlying mechanisms regarding the same species.

The night temperature had an influence on the primary and secondary metabolism [20,42]. In the current study, a night temperature of 15 °C had a positive effect on the biosynthesis and accumulation of soluble sugar and starch in both species. Similar results have been reported by Loka and Oosterhuis [43],

who found that high night temperatures significantly decreased carbohydrate concentrations such as hexose, sucrose, and starch in cotton (*Gossypium hirsutum* L.). The reason is that a high night temperature contributed to a boosted respiration, leading to an increased consumption of carbohydrates [25,44,45]. Another reason is due to the increased mobilisation and faster metabolic use of soluble sugar and starch in plug seedlings grown at 15 °C night temperature. Moreover, higher metabolic rates at 15 °C than 10 °C during the night will lead to a better preparation for the coming day, which partly explained the differences in quality of plug seedlings. Further study should be carried out to demonstrate how night temperature influences carbohydrates allocation, partition, and metabolic use. Besides, the data in this study showed that contents of total phenols and flavonoids were greatly increased by the 15 °C night temperature in *A. membranaceus* and *C. lanceolata*. Similarly, Ryu et al. [46] also found that the anthocyanin biosynthesis in apple was inhibited by a high night temperature. Li et al. [47] reported that phenolic compound accumulation in pitaya fruits increased with elevated temperature from 5 °C to 15 °C. However, Gaiotti et al. [22] found that the capacity of anthocyanin biosynthesis was increased by cool nights (10 °C–11 °C) during veraison of *Vitis vinifera*. As antioxidants in nature, these two phytochemicals are beneficial to humans in preventing diseases, such as cancer, inflammation, and stroke [9]. A high level of total phenol and flavonoids in seedlings grown with a night temperature of 15 °C not only indicates a high value for humans, but also suggests benefits for the growth and development of plug seedlings to face abiotic and biotic stresses.

Photosynthesis is an important physiological process for biomass production in plants [48], and is regulated by temperature. The data showed that a night temperature of 15 °C up-regulated photosynthesis-related genes, including *GBSS*, *RBCL*, and *FDX*. In the photosynthetic chain, *FDX* codes the ferredoxin protein, which accepts and transfers electrons to the next receptor, NADP⁺ [28]. A higher expression of *FDX* with a night temperature of 15 °C implies an enhanced photosynthesis, resulting in higher biomass. In CO₂ assimilation, *RBCL* plays a key role in CO₂ fixation and regulates the photosynthesis rate. Up-regulated expression of *RBCL* suggested that a 15 °C night temperature promoted CO₂ assimilation and synthesized more carbohydrates, which is consistent with the higher contents of soluble sugar and starch observed. In addition, expression of *GBSS* was positively modulated by the 15 °C night temperature, indicated by increased biomass and accumulation of starch. The lower expression of *GBSS*, *RBCL*, and *FDX* at night temperatures of 10 °C and 20 °C was possibly related to metabolic master regulators such as SnRKs which have specialized roles in controlling growth, stress responses, and photosynthesis program in plants [49,50]. More research is needed to clarify how SnRKs modulate photosynthesis-related gene expression, growth, and metabolism of plug seedlings under abiotic stress condition [50,51]. Another reason is perhaps due to H₂O₂ (Figure 3), since H₂O₂ has a negative effect on nucleic acids and proteins, leading to a degradation of photosynthetic genes and proteins [52]. Those results are in accordance with those of previous studies. For instance, Bukhov et al. [35] reported that a high temperature inhibited the ferredoxin-dependent electron transport in barley (*Hordeum vulgare* L.) leaves. Jin et al. [53] found that a high temperature decreased the activity of Rubisco and the quantum yield of photosystem (PS) II in *Euonymus japonicus* seedlings. Phan et al. [33] reported that a high temperature strongly inhibited the expression of *Granule-bound starch synthase* in grains during the early grain filling in rice (*O. sativa* L.).

The night temperature had an influence on the chlorophyll content. In this study, an elevated night temperature (15 °C) increased the chlorophyll content in both *A. membranaceus* and *C. lanceolata* plug seedlings (Figure 4A,B). The results were similar with others. Bertamini et al. [54] reported that a low night temperature significantly decreased the contents of photosynthetic pigments in grapevine “Lagrein”. In addition, the contents of chlorophyll and carotenoids were decreased with a low night temperature in the grapevine [55]. Moreover, an enhanced night temperature (6 °C higher than the control) decreased the chlorophyll content, quantum yield of PSII, and photosynthesis in *Sorghum bicolor* [56]. Lesjak and Calderini [23] also found a significant difference in the SPAD values between plants grown with an increased night temperature and the control. As a core pigment in the photosystem, chlorophyll is an essential component of light-harvesting complex for capturing photons

and transferring energy to the reaction center of photosystems. Any abiotic or biotic stresses such as heat, chilling, and drought will degrade the chlorophyll content [57–59]. A greater content of chlorophyll in seedlings grown with a night temperature of 15 °C implies a higher photosynthetic efficiency, since a positive relationship between chlorophyll and photosynthesis had been proven [60]. This also partly explains the higher biomass and metabolites in seedlings grown with a night temperature of 15 °C.

Stomatal conductance was affected by the night temperature. In this study, the maximum stomatal conductance was found at 15 °C in both *A. membranaceus* and *C. lanceolata* plug seedlings. Similarly, Machado et al. [61] reported that the stomatal and mesophyll conductance in the leaves of orange plants decreased after diminishing the night temperature to 8 °C. Additionally, under a cool-night condition (20 °C), the stomatal conductance and the net CO₂ uptake rate in *Phalaenopsis aphrodite* were significantly greater than those in the control (28 °C) [62]. In the current study, a greater stomatal conductance at a night temperature of 15 °C implies a higher photosynthesis rate, which resulted in more primary and secondary metabolites such as soluble sugar, starch, total phenols, and flavonoids [63,64] (Figure 2).

4. Materials and Methods

4.1. Plant Materials and Treatments

A. membranaceus and *C. lanceolata* seeds were sown in 200-cell plug trays filled with the BioPlug Medium (FarmHannong Co. Ltd, Seoul, South Korea). After germination, uniform plug seedlings were selected and cultivated in three environmentally controlled chambers under the same day temperature (25 °C) and different night temperatures: 10 °C, 15 °C, or 20 °C. Each treatment was set up with the same relative humidity (75%), photoperiod (12 h), and light intensity (150 μmol·m⁻²·s⁻¹ PPFD) provided by white LEDs. Before the experiment, the light intensity in all treatments was adjusted and calibrated using a photoradiometer (HD2102.1, Delta OHM, Padova, Italy). The plug seedlings were subirrigated once every two days with a multipurpose greenhouse nutrient solution (in mg·L⁻¹ Ca(NO₃)₂·4H₂O 737.0, KNO₃ 343.4, KH₂PO₄ 163.2, K₂SO₄ 43.5, MgSO₄·H₂O 246.0, NH₄NO₃ 80.0, Fe-EDTA 15.0, H₃BO₃ 1.40, NaMoO₄·2H₂O 0.12, MnSO₄·4H₂O 2.10, and ZnSO₄·7H₂O 0.44 (pH 6.5 and electrical conductivity 1.5 mS·cm⁻¹)) throughout the experiment. After four weeks of cultivation, some plug seedlings were harvested at 10:00 during the daytime for the measurement of the growth parameters, and others were frozen immediately in liquid nitrogen for further analyses. The Dickson's quality index was calculated according to a previous formula [65]. The formula is

$$\text{Dickson's quality index} = \text{Total DW} / ((\text{shoot length} / \text{stem diameter}) + (\text{shoot DW} / \text{root DW})) \quad (1)$$

where the DW means dry weight.

4.2. Contents of Soluble Sugar and Starch

For extracting the soluble sugar and starch, frozen leaves (0.2 g) were ground and then mixed with distilled water (14 mL) for 30 min at 100 °C. After centrifugation at 3000 rpm for 15 min, the supernatant was transferred into new tubes for the assay of soluble sugar. The residue was re-extracted in distilled water mixed with perchloric acid (2 mL, 52%) for the measurement of starch.

Contents of soluble sugar and starch were determined by the anthrone colorimetric method [66]. In summary, the supernatant (0.5 mL) was added to distilled water (1.9 mL) combined with anthrone (0.5 mL, 2%), and concentrated sulfuric acid (5 mL, 98%), followed by 15 min of incubation at 100 °C. The absorbance at 630 nm and 485 nm was recorded using a UV-spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK), respectively. The calibration curves of soluble sugar and starch were made with standard solutions.

4.3. Contents of Total Phenols and Flavonoids

For extracting total phenols and flavonoids from frozen leaves, 80% methanol was prepared and used. The contents of total phenols and flavonoids were measured by the methods described by Manivannan et al. [67]. In brief, the extract solution (50 µL) was mixed with distilled water (900 µL), phenol reagent (500 µL, 1:1 water), and sodium carbonate (2.5%, 1 mL), followed by incubation for 40 min in dark. The absorbance at 765 nm was recorded. For the assay of total flavonoids, the extract solution (50 µL) was mixed with methyl alcohol (80%, 900 µL) and aluminium chloride (2%, 1 mL) and incubated for 30 min. The absorbance at 415 nm was recorded using a UV-spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK). The total phenols and flavonoids were calculated from the standard gallic acid and quercetin calibration curve, respectively.

4.4. Hydrogen Peroxide Content

The hydrogen peroxide (H₂O₂) content in frozen leaves was assayed as previously done [66]. In detail, samples (100 mg) were ground and homogenized with the TCA solution (0.1%, 1.0 mL). After centrifugation for 15 min at 12,000 rpm, the supernatant was collected and transferred into a new tube. The supernatant (0.3 mL) was mixed with a sodium phosphate buffer (50 mM, 0.5 mL), and potassium iodide (1 M, 0.5 mL), followed by incubation for 30 min in the dark. The absorbance of the mixture at 395 nm was recorded using a UV spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK).

4.5. Assessments of Chlorophyll Content and Stomatal Conductance

The chlorophyll content was determined before harvesting using the Plus Chlorophyll Meter (SPAD 502, Spectrum technologies, Korea). The stomatal conductance was measured on leaves at 10 am before harvesting using a Decagon Leaf Porometer SC-1 (Decagon Device Inc., Pullman, WA, USA), according to the introduction of this instrument.

4.6. Analysis of the Gene Expression by Quantitative Real-Time PCR

Leaf tissues of *A. membranaceus* and *C. lanceolata* were ground and homogenized in lysis buffer under an RNase-free condition. For RNA extraction and cDNA synthesis, a total RNA extraction kit (iNtRON Biotechnology, Seoul, Republic of Korea) and the PrimeScript RT Reagent Kit (Takara, Shiga, Japan) were used, according to manufacturer's protocols. Total RNA was reverse transcribed to cDNA using the GoScript Reverse Transcription System (Promega, Madison, WI, USA). The gene expression was assayed using the Rotor-Gene Q detection system (Qiagen, Hilden, Germany), and was calculated using the 2^{-ΔΔCt} method [68]. All primers used in this study were designed by using Premier 5.0 (Premier Biosoft Inc., Palo Alto, CA, USA) and were shown in Table 3.

Table 3. The primer sequence used in this study to measure the gene expression using quantitative real-time PCR.

Gene	Forward Primer	Reverse Primer
18S	5'-CTCAACCATAAA CGATGCCGACC-3'	5'-AGTTTCAGCCTTGGCACCATACTCC-3'
<i>AmGBSS</i>	5'-ATAACATAGCGTATCAGGG-3'	5'-CTCGGTCAGATTCTAACACT-3'
<i>AmRBCL</i>	5'-TGGCTGTTCTATCGTCA-3'	5'-AAGTAATCTCCCTTTCTCCT-3'
<i>β-Actin</i>	5'-CGAGAAGAGCTACGAGCTACCCGATGG-3'	5'-CTCGGTGCTAGGGCAGTGATCTCTTTGCT-3'
<i>ClFDX</i>	5'-CTTCGGCGTTTCTTCGT-3'	5'-CTGCCAAACCCCTTGATAACT-3'
<i>ClRBCL</i>	5'-GCTTACCCATTAGACCTTT-3'	5'-GGGACGACCATACTTGTT-3'

Am, *Astragalus membranaceus*; *GBSS*, granule-bound starch synthase; *RBCL*, ribulose biphosphate carboxylase large chain; *Cl*, *Codonopsis lanceolata*; and *FDX*, ferredoxin. 18S and *β-Actin* were used as the housekeeping genes in *A. membranaceus* and *C. lanceolata*, respectively.

4.7. Data Collection and Analysis

The data were collected with three individual biological repeats and presented as the mean \pm standard error. One-way analysis of variance (One-way ANOVA) was performed to estimate the differences among treatments, followed by Duncan's multiple range test ($p < 0.05$) using SPSS (Statistical Package for the Social Sciences, version 21). All figures in this study were prepared using the OriginPro software (version 9.0).

5. Conclusions

In this study, the effects of the night temperature on the growth and development of plug seedling of *A. membranaceus* and *C. lanceolata* were demonstrated. A night temperature of 15 °C greatly upgraded the quality of plugs seedlings in both species, with a higher dry weight (shoot, root, and leaf), stem diameter, and DQI. Moreover, high and low night temperatures had a negative effect on primary and secondary metabolites, and a night temperature of 15 °C significantly increased the contents of soluble sugar, starch, total phenols, and flavonoids in *A. membranaceus* and *C. lanceolata*. Furthermore, the stomatal conductance and chlorophyll content were improved by a night temperature of 15 °C and decreased with night temperatures of 10 °C and 20 °C. An analysis of the gene expression showed that a 15 °C night temperature enhanced photosynthesis by up-regulating the expression of the *GBSS*, *RBCL*, and *FDX* genes. In conclusion, the results suggested that a night temperature of 15 °C is recommended for producing high quality plug seedlings of *A. membranaceus* and *C. lanceolata*. Many further studies should be carried out in the future, such as how night temperature affects carbohydrates allocation and partition from source to sink, how night temperature influences the expression of genes related to secondary metabolite biosynthesis and degradation, and how night temperature impacts the absorption and transport of mineral elements.

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References

- Kim, J.H.; Hong, J.Y.; Shin, S.R.; Yoon, K.Y. Comparison of antioxidant activity in wild plant (*Adenophora triphylla*) leaves and roots as a potential source of functional foods. *Int. J. Food Sci. Nutr.* **2009**, *60*, 150–161. [[CrossRef](#)] [[PubMed](#)]
- Park, Y.J.; Thwe, A.A.; Li, X.H.; Kim, Y.J.; Kim, J.K.; Arasu, M.V.; Al-Dhabi, N.A.; Park, S.U. Triterpene and flavonoid biosynthesis and metabolic profiling of hairy roots, adventitious roots, and seedling roots of *Astragalus membranaceus*. *J. Agric. Food Chem.* **2015**, *63*, 8862–8869. [[CrossRef](#)] [[PubMed](#)]
- Yang, J.; Jia, M.M.; Zhang, X.J.; Wang, P.L. Calycosin attenuates MPTP-induced Parkinson's disease by suppressing the activation of TLR/NF-kappa B and MARK pathways. *Phytother. Res.* **2019**, *33*, 309–318. [[CrossRef](#)] [[PubMed](#)]
- Han, A.Y.; Lee, Y.S.; Kwon, S.; Lee, H.S.; Lee, K.W.; Seol, G.H. *Codonopsis lanceolata* extract prevents hypertension in rats. *Phytomedicine* **2018**, *39*, 119–124. [[CrossRef](#)] [[PubMed](#)]
- Chun, J.; Kang, M.; Kim, Y.S. A triterpenoid saponin from *Adenophora triphylla* var. *Japonica* suppresses the growth of human gastric cancer cells via regulation of apoptosis and autophagy. *Tumor Biol.* **2014**, *35*, 12021–12030. [[CrossRef](#)] [[PubMed](#)]
- Boo, H.O.; Kim, H.H.; Barasch, D.; Nemirovski, A.; Lee, M.S.; Gorinstein, S.; Ku, Y.G. *Codonopsis lanceolata* and *Nelumbo nucifera* Gaertn. root extracts for functional food: Metabolic profiling by MS, FTIR and fluorescence and evaluation of cytotoxicity and anti-obesity properties on 3T3-L1 cell line. *Eur. Food Res. Technol.* **2017**, *243*, 689–700. [[CrossRef](#)]

7. Moon, K.G.; Um, I.S.; Jeon, S.H.; Cho, Y.S.; Kim, Y.G.; Rho, I.R. Effect of organic fertilizer application on growth characteristics and saponin content in *Codonopsis lanceolata*. *Hortic. Environ. Biotechnol.* **2018**, *59*, 125–130. [[CrossRef](#)]
8. Ghimire, B.K.; Seong, E.S.; Yu, C.Y.; Kim, S.H.; Chung, I.M. Evaluation of phenolic compounds and antimicrobial activities in transgenic *Codonopsis lanceolata* plants via overexpression of the gamma-tocopherol methyltransferase (gamma-tmt) gene. *S. Afr. J. Bot.* **2017**, *109*, 25–33. [[CrossRef](#)]
9. Gao, J.Q.; Liu, Z.J.; Chen, T.; Zhao, D.Q. Pharmaceutical properties of calycosin, the major bioactive isoflavonoid in the dry root extract of *Radix astragali*. *Pharm. Biol.* **2014**, *52*, 1217–1222. [[CrossRef](#)]
10. Li, Y.; Guo, S.; Zhu, Y.; Yan, H.; Qian, D.W.; Wang, H.Q.; Yu, J.Q.; Duan, J.A. Flowers of *Astragalus membranaceus* var. *mongholicus* as a novel high potential by-product: Phytochemical characterization and antioxidant activity. *Molecules* **2019**, *24*, 434. [[CrossRef](#)]
11. McClung, C.R.; Lou, P.; Hermand, V.; Kim, J.A. The importance of ambient temperature to growth and the induction of flowering. *Front. Plant Sci.* **2016**, *7*, 1266. [[CrossRef](#)]
12. Rondanini, D.; Mantese, A.; Savin, R.; Hall, A.J. Responses of sunflower yield and grain quality to alternating day/night high temperature regimes during grain filling: Effects of timing, duration and intensity of exposure to stress. *Field Crop. Res.* **2006**, *96*, 48–62. [[CrossRef](#)]
13. Liu, K.; Wang, X.Y.; Lu, B.L.; Tian, X.H.; Punzalan, B.; Laza, R.C.; Zhang, Y.B. Effects of high night temperature on grain quality under field chamber system condition. *Philipp. J. Crop. Sci.* **2017**, *42*, 56–62.
14. Coast, O.; Ellis, R.H.; Murdoch, A.J.; Quinones, C.; Jagadish, K.S.V. High night temperature induces contrasting responses for spikelet fertility, spikelet tissue temperature, flowering characteristics and grain quality in rice. *Funct. Plant Biol.* **2015**, *42*, 149–161. [[CrossRef](#)]
15. Shi, W.; Yin, X.; Struik, P.C.; Xie, F.; Schmidt, R.C.; Jagadish, K.S.V. Grain yield and quality responses of tropical hybrid rice to high night-time temperature. *Field Crop. Res.* **2016**, *190*, 18–25. [[CrossRef](#)]
16. Lobell, D.B.; Ortiz-Monasterio, J.I. Impacts of day versus night temperatures on spring wheat yields: A comparison of empirical and CERES model predictions in three locations. *Agron. J.* **2007**, *99*, 469–477. [[CrossRef](#)]
17. Li, L.; Li, J.; Gao, Q.; Chen, J.X. Effects of day and night temperature difference on growth, development, yield and fruit quality of tomatoes. *Chin. J. Appl. Ecol.* **2015**, *26*, 2700–2706.
18. Kadir, S.; Sidhu, G.; Al-Khatib, K. Strawberry (*Fragaria xananassa* Duch.) growth and productivity as affected by temperature. *HortScience* **2006**, *41*, 1423–1430. [[CrossRef](#)]
19. Boo, H.O.; Heo, B.G.; Gorinstein, S.; Chon, S.U. Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. *Plant Sci.* **2011**, *181*, 479–484. [[CrossRef](#)]
20. Tombesi, S.; Cincera, I.; Frioni, T.; Ughini, V.; Gatti, M.; Palliotti, A.; Poni, S. Relationship among night temperature, carbohydrate translocation and inhibition of grapevine leaf photosynthesis. *Environ. Exp. Bot.* **2019**, *157*, 293–298. [[CrossRef](#)]
21. Zhou, L.H.; Yan, T.; Chen, X.; Li, Z.L.; Wu, D.Z.; Hua, S.J.; Jiang, L.X. Effect of high night temperature on storage lipids and transcriptome changes in developing seeds of oilseed rape. *J. Exp. Bot.* **2018**, *69*, 1721–1733. [[CrossRef](#)] [[PubMed](#)]
22. Gaiotti, F.; Pastore, C.; Filippetti, I.; Lovat, L.; Belfiore, N.; Tomasi, D. Low night temperature at veraison enhances the accumulation of anthocyanins in corvina grapes (*Vitis vinifera* L.). *Sci. Rep.* **2018**, *8*, 8719. [[CrossRef](#)] [[PubMed](#)]
23. Lesjak, J.; Calderini, D.F. Increased night temperature negatively affects grain yield, biomass and grain number in Chilean quinoa. *Front. Plant Sci.* **2017**, *8*, 352. [[CrossRef](#)] [[PubMed](#)]
24. Jing, P.; Wang, D.; Zhu, C.; Chen, J. Plant physiological, morphological and yield-related responses to night temperature changes across different species and plant functional types. *Front. Plant Sci.* **2016**, *7*, 1774. [[CrossRef](#)] [[PubMed](#)]
25. Loka, D.A.; Oosterhuis, D.M. Effect of high night temperatures on cotton respiration, ATP levels and carbohydrate content. *Environ. Exp. Bot.* **2010**, *68*, 258–263. [[CrossRef](#)]
26. Kanno, K.; Mae, T.; Makino, A. High night temperature stimulates photosynthesis, biomass production and growth during the vegetative stage of rice plants. *Soil Sci. Plant Nutr.* **2009**, *55*, 124–131. [[CrossRef](#)]

27. Losada, M.; Arnon, D.I. Enzyme Systems in Photosynthesis. In *Modern Methods of Plant Analysis/Moderne Methoden Der Pflanzenanalyse*; Springer: Berlin/Heidelberg, Germany, 1964; pp. 569–615.
28. Schuermann, P.; Buchanan, B.B. The ferredoxin/thioredoxin system of oxygenic photosynthesis. *Antioxid. Redox Signal.* **2008**, *10*, 1235–1273. [[CrossRef](#)]
29. Sage, R.F.; Kubien, D.S. The temperature response of C-3 and C-4 photosynthesis. *Plant Cell Environ.* **2007**, *30*, 1086–1106. [[CrossRef](#)]
30. Merida, A.; Rodriguez-Galan, J.M.; Vincent, C.; Romero, J.M. Expression of the granule-bound starch synthase I (Waxy) gene from snapdragon is developmentally and circadian clock regulated. *Plant Physiol.* **1999**, *120*, 401–410. [[CrossRef](#)]
31. Visser, R.G.F.; Stolte, A.; Jacobsen, E. Expression of a chimeric granule-bound starch synthase-gus gene in transgenic potato plants. *Plant Mol. Biol.* **1991**, *17*, 691–699. [[CrossRef](#)]
32. Umemoto, T.; Nakamura, Y.; Ishikura, N. Activity of starch synthase and the amylose content in rice endosperm. *Phytochemistry* **1995**, *40*, 1613–1616. [[CrossRef](#)]
33. Phan, T.T.T.; Ishibashi, Y.; Miyazaki, M.; Tran, H.T.; Okamura, K.; Tanaka, S.; Nakamura, J.; Yuasa, T.; Iwaya-Inoue, M. High temperature-induced repression of the rice sucrose transporter (OsSUT1) and starch synthesis-related genes in sink and source organs at milky ripening stage causes chalky grains. *J. Agron. Crop. Sci.* **2013**, *199*, 178–188. [[CrossRef](#)]
34. Zhao, H.; Dai, T.; Jiang, D.; Cao, W. Effects of high temperature on key enzymes involved in starch and protein formation in grains of two wheat cultivars. *J. Agron. Crop. Sci.* **2008**, *194*, 47–54. [[CrossRef](#)]
35. Bukhov, N.G.; Dzhibladze, T.G.; Egorova, E.A. Elevated temperatures inhibit ferredoxin-dependent cyclic electron flow around photosystem I. *Russ. J. Plant Physiol.* **2005**, *52*, 578–583. [[CrossRef](#)]
36. Shi, W.J.; Muthurajan, R.; Rahman, H.; Selvam, J.; Peng, S.B.; Zou, Y.B.; Jagadish, K.S.V. Source-sink dynamics and proteomic reprogramming under elevated night temperature and their impact on rice yield and grain quality. *New Phytol.* **2013**, *197*, 825–837. [[CrossRef](#)] [[PubMed](#)]
37. Mortensen, L.M.; Gislerod, H.R. Night temperature drop affects the diurnal photosynthetic rhythm of *Kalanchoe blossfeldiana*. *Eur. J. Hortic. Sci.* **2018**, *83*, 160–165. [[CrossRef](#)]
38. Liu, W.F.; Qin, Z.W.; Xin, M.; Zhou, X.Y.; Yang, J.; Wang, C.H. Analysis of cspap-fib regulation of cucumber female differentiation in response to low night temperature conditions. *Sci. Hortic.* **2018**, *240*, 81–88. [[CrossRef](#)]
39. Annunziata, M.G.; Apelt, F.; Carillo, P.; Krause, U.; Feil, R.; Koehl, K.; Lunn, J.E.; Stitt, M. Response of arabidopsis primary metabolism and circadian clock to low night temperature in a natural light environment. *J. Exp. Bot.* **2018**, *69*, 4881–4895. [[CrossRef](#)] [[PubMed](#)]
40. Morita, S.; Yonemaru, J.; Takanashi, J. Grain growth and endosperm cell size under high night temperatures in rice (*Oryza sativa* L.). *Ann. Bot.* **2005**, *95*, 695–701. [[CrossRef](#)] [[PubMed](#)]
41. Cheng, W.; Sakai, H.; Yagi, K.; Hasegawa, T. Interactions of elevated CO₂ and night temperature on rice growth and yield. *Agric. For. Meteorol.* **2009**, *149*, 51–58. [[CrossRef](#)]
42. Glaubitz, U.; Li, X.; Schaedel, S.; Erban, A.; Sulpice, R.; Kopka, J.; Hinch, D.K.; Zuther, E. Integrated analysis of rice transcriptomic and metabolomic responses to elevated night temperatures identifies sensitivity- and tolerance-related profiles. *Plant Cell Environ.* **2017**, *40*, 121–137. [[CrossRef](#)]
43. Loka, D.A.; Oosterhuis, D.M. Increased night temperatures during cotton's early reproductive stage affect leaf physiology and flower bud carbohydrate content decreasing flower bud retention. *J. Agron. Crop. Sci.* **2016**, *202*, 518–529. [[CrossRef](#)]
44. Peraudeau, S.; Lafarge, T.; Roques, S.; Quinones, C.O.; Clement-Vidal, A.; Ouwerkerk, P.B.F.; Van Rie, J.; Fabre, D.; Jagadish, K.S.V.; Dingkuhn, M. Effect of carbohydrates and night temperature on night respiration in rice. *J. Exp. Bot.* **2015**, *66*, 3931–3944. [[CrossRef](#)] [[PubMed](#)]
45. Mohammed, A.R.; Cothren, J.T.; Tarpley, L. High night temperature and abscisic acid affect rice productivity through altered photosynthesis, respiration and spikelet fertility. *Crop. Sci.* **2013**, *53*, 2603–2612. [[CrossRef](#)]
46. Ryu, S.; Han, H.H.; Jeong, J.H.; Kwon, Y.; Han, J.H.; Do, G.R.; Choi, I.M.; Lee, H.J. Night temperatures affect fruit coloration and expressions of anthocyanin biosynthetic genes in 'hongro' apple fruit skins. *Eur. J. Hortic. Sci.* **2017**, *82*, 232–238. [[CrossRef](#)]

47. Li, X.A.; Li, M.L.; Han, C.; Jin, P.; Zheng, Y.H. Increased temperature elicits higher phenolic accumulation in fresh-cut pitaya fruit. *Postharvest Biol. Technol.* **2017**, *129*, 90–96. [[CrossRef](#)]
48. Yamori, W.; Kondo, E.; Sugiura, D.; Terashima, I.; Suzuki, Y.; Makino, A. Enhanced leaf photosynthesis as a target to increase grain yield: Insights from transgenic rice lines with variable rhesus protein content in the cytochrome b6/f complex. *Plant Cell Environ.* **2016**, *39*, 80–87. [[CrossRef](#)]
49. Jamsheer, K.M.; Jindal, S.; Laxmi, A. Evolution of TOR-SnRK dynamics in green plants and its integration with phytohormone signaling networks. *J. Exp. Bot.* **2019**, *70*, 2239–2259. [[CrossRef](#)]
50. Wang, X.; Peng, F.; Li, M.; Yang, L.; Li, G. Expression of a heterologous SnRK1 in tomato increases carbon assimilation, nitrogen uptake and modifies fruit development. *J. Plant Physiol.* **2012**, *169*, 1173–1182. [[CrossRef](#)]
51. Zhang, Y.; Primavesi, L.F.; Jhurrea, D.; Andralojc, P.J.; Mitchell, R.A.C.; Powers, S.J.; Schlupe, H.; Delatte, T.; Wingler, A.; Paul, M.J. Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiol.* **2009**, *149*, 1860–1871. [[CrossRef](#)]
52. Szymanska, R.; Slesak, I.; Orzechowska, A.; Kruk, J. Physiological and biochemical responses to high light and temperature stress in plants. *Environ. Exp. Bot.* **2017**, *139*, 165–177. [[CrossRef](#)]
53. Jin, S.H.; Li, X.Q.; Zheng, B.S.; Wang, J.G. Response of the photosynthesis and antioxidant systems to high-temperature stress in *Euonymus japonicus* seedlings. *Science* **2010**, *56*, 172–180.
54. Bertamini, M.; Zulini, L.; Muthuchelian, K.; Nedunchezian, N. Low night temperature effects on photosynthetic performance on two grapevine genotypes. *Biol. Plant.* **2007**, *51*, 381–385. [[CrossRef](#)]
55. Bertamini, M.; Muthuchelian, K.; Rubinigg, M.; Zorer, R.; Nedunchezian, N. Low-night temperature (LNT) induced changes of photosynthesis in grapevine (*Vitis vinifera* L.) plants. *Plant Physiol. Biochem.* **2005**, *43*, 693–699. [[CrossRef](#)]
56. Prasad, P.V.V.; Djanaguiraman, M. High night temperature decreases leaf photosynthesis and pollen function in grain sorghum. *Funct. Plant Biol.* **2011**, *38*, 993–1003. [[CrossRef](#)]
57. Yadav, S.K.; Tiwari, Y.K.; Singh, V.; Patil, A.A.; Shanker, A.K.; Jyothi Lakshmi, N.; Vanaja, M.; Maheswari, M. Physiological and biochemical basis of extended and sudden heat stress tolerance in maize. *Proc. Nat. Acad. Sci. India Sect. B Biol. Sci.* **2018**, *88*, 249–263. [[CrossRef](#)]
58. Li, S.; Yang, Y.; Zhang, Q.; Liu, N.F.; Xu, Q.G.; Hu, L.X. Differential physiological and metabolic response to low temperature in two zoysiagrass genotypes native to high and low latitude. *PLoS ONE* **2018**, *13*, e0198885. [[CrossRef](#)]
59. Liu, J.L.; Wang, W.X.; Wang, L.Y.; Sun, Y. Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regul.* **2015**, *77*, 317–326. [[CrossRef](#)]
60. Van der Tol, C.; Verhoef, W.; Rosema, A. A model for chlorophyll fluorescence and photosynthesis at leaf scale. *Agric. For. Meteorol.* **2009**, *149*, 96–105. [[CrossRef](#)]
61. Machado, D.F.S.; Ribeiro, R.V.; da Silveira, J.A.G.; Magalhaes, J.R.; Machado, E.C. Rootstocks induce contrasting photosynthetic responses of orange plants to low night temperature without affecting the antioxidant metabolism. *Theor. Exp. Plant Phys.* **2013**, *25*, 26–35. [[CrossRef](#)]
62. Chen, W.H.; Tseng, Y.C.; Liu, Y.C.; Chuo, C.M.; Chen, P.T.; Tseng, K.M.; Yeh, Y.C.; Ger, M.J.; Wang, H.L. Cool-night temperature induces spike emergence and affects photosynthetic efficiency and metabolizable carbohydrate and organic acid pools in *Phalaenopsis aphrodite*. *Plant Cell Rep.* **2008**, *27*, 1667–1675. [[CrossRef](#)] [[PubMed](#)]
63. Ball, J.T.; Woodrow, I.E.; Berry, J.A. A Model Predicting Stomatal Conductance and its Contribution to the Control of Photosynthesis Under Different Environmental Conditions. In *Progress in Photosynthesis Research: Volume 4 Proceedings of the VIIIth International Congress on Photosynthesis, Providence, RI, USA, 10–15 August 1986*; Biggins, J., Ed.; Springer: Dordrecht, The Netherlands, 1987; pp. 221–224.
64. Gago, J.; Daloso, D.D.M.; Figueroa, C.M.; Flexas, J.; Fernie, A.R.; Nikoloski, Z. Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: A multispecies meta-analysis approach. *Plant Physiol.* **2016**, *171*, 265. [[CrossRef](#)] [[PubMed](#)]
65. Dickson, A.; Leaf, A.L.; Hosner, J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. *For. Chron.* **1960**, *36*, 10–13. [[CrossRef](#)]
66. Liu, Y.; Ren, X.; Jeong, H.; Wei, H.; Jeong, B. Growth and physiological responses of *Adenophora triphylla* (Thunb.) A.DC. plug seedlings to day and night temperature regimes. *Agronomy* **2018**, *8*, 173. [[CrossRef](#)]

67. Manivannan, A.; Soundararajan, P.; Park, Y.G.; Jeong, B.R. In vitro propagation, phytochemical analysis, and evaluation of free radical scavenging property of scrophularia kakudensis franch tissue extracts. *Biomed Res. Int.* **2015**, *2015*. [[CrossRef](#)]
68. Ren, X.; Liu, Y.; Jeong, H.; Jeong, B. Supplementary light source affects the growth and development of codonopsis lanceolata seedlings. *Int. J. Mol. Sci.* **2018**, *19*, 3074. [[CrossRef](#)]



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