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Effects of a weak supply of light at night on the growth and quality components of tea plants

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ABSTRACT

Supplying artificial light is widely used in crop cultivation to improve yield and quality. In this study, we investigated the effects of a weak supply of light (WSL) on the growth and quality components of tea plants. Starting from mid-winter (20 January 2021), the purple tea (*Camellia sinensis*) cultivar 'Ziyan' was exposed to four different spectra at an intensity of 2 μmol · m^{-2} · s^{-1} or 5 μmol · m^{-2} · s^{-1} for 3 h or 5 h after sunset. The field observations showed that the sprouting index, which represents the time and speed of bud flush, was significantly higher in most of the WSL treatments than in the control (CK, $p < 0.01$). The total content of catechin in the harvested leaves of 3 WSL treatments was 11.51%–18.94% higher than that of the CK, but the content of anthocyanin of 4 WSL treatments unexpectedly decreased by 6.77%–11.69% ($p < 0.05$). The differences in yield, free amino acids and caffeine contents between the WSL treatments and CK were not significant. We concluded that the WSL treatments during the early spring night had positive impacts on the growth and some quality components of tea plants.

Keywords: anthocyanin, *Camellia sinensis*, catechin, weak supply of light

INTRODUCTION

Artificial supplementary light technology is considered an effective method to prolong the day, which can regulate secondary metabolites and the time to market crops (Alrifai et al., 2019; Lee et al., 2021). Therefore, it has been applied in seedling cultivation and field management of crops in agricultural facilities (Massa et al., 2008; Zhang et al., 2020b). In these applications, weak light (or low light) that is lower than the plant light compensation point is often used because of the lower cost of electricity. Although weak light has a limited effect on photosynthesis, it can introduce physiological regulation through photoreceptors and bioclock oscillators, thereby regulating the bud flush, growth and accumulation of metabolites in plants (Xiong et al., 2020; Singh et al., 2021).

Camellia sinensis (L.). O. Kuntze is one of the vital economic crops in the world. It contains various physiologically active metabolites, including catechins, anthocyanins, free amino acids and caffeine. Previous studies have shown that light has significant effects on the growth and metabolites of tea plants. Barua (1969) reported that using artificial light to extend the photoperiod to 13 h · day^{-1} in tea plants during winter induced early bud burst and largely increased the winter yield. Zheng et al. (2019) found that additional night-time light (light intensity >400 μmol · m^{-2} · s^{-1}) had a

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positive effect on the growth and new shoot yellowing of yellowish tea plants.

The most successful application of light control in tea plant cultivation is shading in summer, which can significantly increase the content of free amino acids, while decreasing that of the catechins (Ji et al., 2018). Extending the light period or adding UV light could also significantly increase the content of anthocyanins in the young leaves of purple tea plants (Sun et al., 2016; Li et al., 2020). Wang et al. (2020) identified 48 significantly changed metabolites in tea plants under three different intensities of blue light. Lin et al. (2021) found that filtering the UV-B of the natural light in the field decreased bitter- and astringent-tasting flavonol glycosides and increased the non-galloylated catechins of tea.

In spring, the overwintering buds of tea plants will flush as the day length and temperature increase, then the best quality spring tea is harvested. However, few studies have investigated the effects of treatments of a weak supply of light (WSL) on the growth and metabolites of tea plants. In this study, we investigated the effects of six different WSL treatments from 20 January to 4 April on tea plants. We used the late-bud flush and anthocyanin-rich tea cultivar 'Ziyan' as the plant material (Lai et al., 2016) with the expectations that speeding up its bud flush in the early spring and increasing the content of anthocyanins or other metabolites of tea, which are related to quality.

MATERIALS AND METHODS

Plant materials and experimental design

Five-year-old *Camellia sinensis* (L.). O. Kuntze 'Ziyan' tea plants were grown in the experimental site located in Muchuan County, Sichuan Province, China (28°59' N, 103°53' E, altitude 493 m). This site is characterised by a subtropical monsoon humid climate with an annual mean temperature of 17.3 °C. A randomised block experimental design was used with three biological replicates. A 1-m × 1-m plot was randomly selected to perform each treatment (Figure 1A).

Four types of LED lights with different spectra were used in this study (Figure 1B). The light intensities were regulated to 5 ± 1 μmol · m^{-2} · s^{-1} or 2 ± 1 μmol · m^{-2} · s^{-1} at the plucking surface of the tea plants and two durations during which light was supplied (3 h and 5 h start from 18:30 every day) were established for the WSL treatments

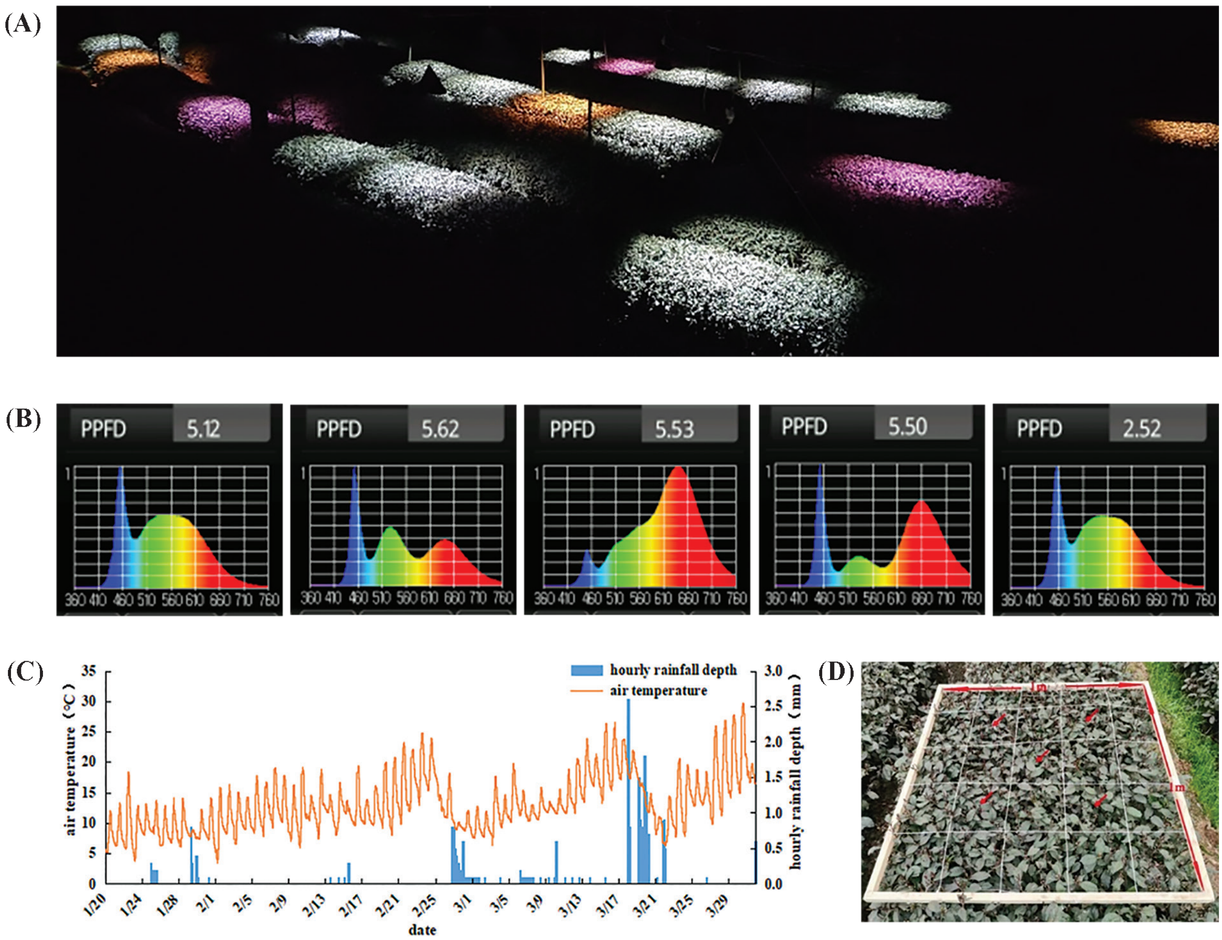

Figure 1. Field experiments of this study; (A) a picture showing the treatments that supply light; (B) spectrum of different WSL treatments; (C) air temperature and rainfall during the experimental period; (D) five points for recording the SPI. PPFD, photosynthetic photon flux density; SPI, sprouting index; WSL, weak supply of light.

Table 1. WSL treatment in this study.

Experimental design	Light quality	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Duration (h)	Light mass ratio		
				Blue	Green	Red
Control check (CK)	/	<0.01	0			
Treatment 1 (TM1)	Light I	5 ± 1	5	1.36	2.35	1.29
Treatment 2 (TM2)	Light II	5 ± 1	5	1.35	1.78	1.87
Treatment 3 (TM3)	Light III	5 ± 1	5	0.40	1.54	3.06
Treatment 4 (TM4)	Light IV	5 ± 1	5	1.07	0.92	3.01
Treatment 5 (TM5)	Light I	5 ± 1	3	1.36	2.35	1.29
Treatment 6 (TM6)	Light I	2 ± 1	5	1.36	2.35	1.29

PPFD: photosynthetic photon flux density; WSL, weak supply of light.

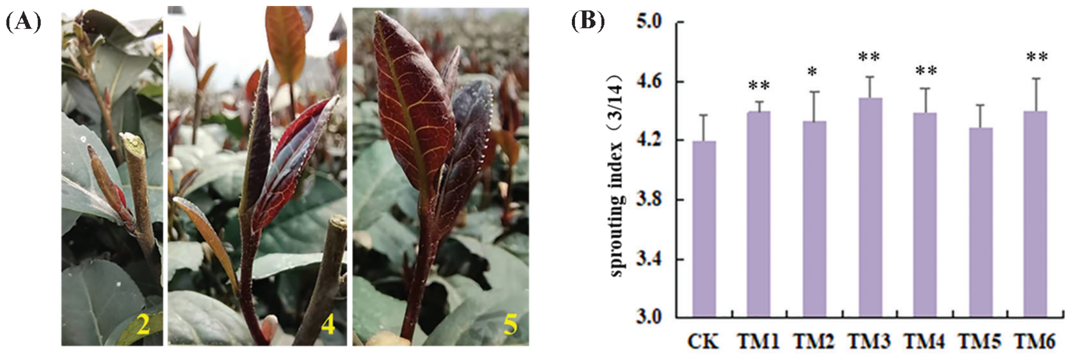

Figure 2. Effects of WSL treatments on the bud flush of tea plants; (A) representative pictures of tea buds with different SPIs, which are shown in yellow numbers; (B) SPIs of different treatments on 14 March 2021. *$p < 0.05$ and **$p < 0.01$ indicate significant differences compared with those of the CK. CK, control; SPI, sprouting index; TM, treatment; WSL, weak supply of light.

(TM1 to TM6, Table 1). The lights were turned on and off automatically by a programmed controller from 20 January to 4 April 2021. The photosynthetic photon flux density (PPFD) and light mass ratio (red/green/blue) were measured by a hand-held spectrometer (Taiwan HiPoint Corporation, Taiwan, China). Air temperature and rainfall were obtained from a weather station 100 m away from the experimental site, and the results are shown in Figure 1C. The fields were irrigated with 50 mm of water on 22 February and 30 February 2021 to replenish the soil moisture.

Determination of the photosynthetic parameters

A portable photosynthetic measurer (Li-6400, Li-COR, Lincoln, NE, USA) was used to measure net photosynthetic parameters (NPRs) in the mature leaves of different treatments. The measurements were performed during 14:00–16:00 and 19:30–21:30 on 2 February 2021. For each treatment, nine mature leaves exposed to light were selected, and each leaf was measured three times.

Measurement of the status of bud flush and yield-related traits

The sprouting index (SPI) was used to quantify the bud flush status of the tea plants. For each plot, five points were selected (Figure 1D), and all the overwintered apical axillary buds within the sampling points were recorded for the SPI. The SPI was recorded with a number, where 0 = dormant, 1 = start to flush, 2 = scales open, 3 = fish leaf open, 4 = one bud and one leaf, 5 = one bud and two leaves, and 6 = one bud and three leaves (Figure 2A). The measurements were performed on 7 March 2021 and 14 March 2021, and the average SPI of >90 buds in each plot was calculated.

Starting from March 16, ‘1 bud and 2 leaves’ (1B2L) were plucked when the bud flush status met the standards. Multiple batches of spring tea were harvested before the Ching-ming festival (4 April). The sum of the fresh weight of all the batches of each treatment was calculated. In addition, the fresh weight of 20 1B2Ls and the average length of them were measured.

Measurement of biochemical components

The harvested fresh leaves were fixed by inactivating the enzymes in a microwave oven (Midea Group, Beijiaozhan, China) for 2 min, followed by drying in an electric oven (Qixin Industrial Limited, Zhengzhou, China) at 80 °C for 4 h. The composition and content of anthocyanin were measured using a high-performance liquid chromatography (HPLC Agilent 1260; Agilent

Technologies, Santa Clara, CA, USA), as described by Li et al. (2020). A ZORBAX SB-C18 column (Agilent Technologies), mobile phase A (water/acetonitrile/formic acid = 87:3:10, v/v/v) and mobile phase B (100% acetonitrile) were used to separate anthocyanins. The chromatographic conditions were as follows: 0 min, 0% B; 5 min, 5% B; 10 min, 14% B and 30 min, 30% B, column temperature of 35 °C and a flow rate of 1 mL $\cdot$ min^{-1}. The signal was detected by using an Agilent VWD at 520 nm.

The contents of catechins and caffeine were determined by HPLC (Agilent 1260), as described by Tan et al. (2020). A Phenomenex (Torrance, CA, USA) phenyl–hexyl column was used. Six catechins, namely, epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), gallic catechin gallate (GCG), epicatechin gallate (ECG) and catechin gallate (CG), were measured. The chemical standards were purchased from Sigma-Aldrich (Gillingham, UK). The HPLC conditions were as follows: a flow rate of 1 mL $\cdot$ min^{-1}, a column temperature of 35 °C and a detection wavelength of 278 nm. The content of free amino acid was determined using the ninhydrin colorimetric method according to national standards (GB/T 8314-2013).

Statistical analysis

An analysis of variance (ANOVA) was performed by SPASS.22 using the general linear model. The least significant range method was applied for multiple comparisons. All data were presented as means ± standard deviation (n = 3).

RESULTS AND DISCUSSION

The WSL treatments have little effect on photosynthesis

To evaluate the effects of the WSL treatments on photosynthesis, we measured the NPRs of the tea plants during both day and night. The NPRs of the mature leaves during 14:00–16:00 varied from 3.38 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ to 7.36 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, while at night (~20:00), it decreased to –0.60 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in the CK. The NPRs of TM1, 4 and 6 were not significantly higher than those of the CK when lights were turned on at night. The NPRs of TM2, 3 and 5 varied from –0.03 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ to 0.12 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ and were significantly higher than those of the CK ($p < 0.05$). However, there was little effect on the photosynthesis of WSL treatments.

The WSL treatments accelerated bud flush in the spring

On 7 March, the mean SPI of CK was 3.07, while that of the WSL treatments varied from 3.25 to 3.35. One week later (14 March), the SPI of CK increased to 4.20 (fish leaf just open), while the SPI increased by 1.03–1.18 for the WSL treatments. The ANOVA showed that all the WSL treatments, except TM5, were significantly higher than that of CK ($p < 0.05$ or 0.01) (Figure 2B). Among the treatments, TM3 had the highest SPI, which was 0.29 higher than that of the CK. Considering that it requires approximately 7 days to open one leaf in the early spring, the harvesting time of 1B2L was 2 days earlier in TM3 than in the CK.

The results are consistent with those of the study of Barua (1969), who found that supplying weak light in spring could accelerate the bud flush of tea plants. Although the effects were not striking when compared with the genetic variation on the bud flush time of tea plants (Tan et al., 2022), production at 2 days earlier could contribute to the economic value of this particular cultivar for the spring tea.

Interestingly, the SPI of TM5 (light I, 5 μmol $\cdot m^{-2} \cdot s^{-1}$ for 3 h) was not significantly higher than that of the CK, but the SPI of TM6 (light I, 2 μmol $\cdot m^{-2} \cdot s^{-1}$ for 5 h) suggested that the duration of supply of light was important. Ohno and Yamawo (2021) reported that both day interruption (total time of light period is the same as short day length) and long day treatments on the twigs of Japanese beech (*Fagus crenata*) could significantly accelerate the timing of bud flush. Combined with the results in this study, it is possible to accelerate the bud flush in spring by regulating the light period without significantly changing the amount of photosynthesis. The effect of intermittently supplying weak light at night on tea plants also merits further study.

The WSL treatments had a limited impact on yield

Compared with the CK, the WSL treatments showed no significant increase in the bud weight or total yield, although their averages were higher (Table 2). The results suggest that the WSL treatments had no or a limited positive impact on the yield. Considering the minimal contribution to photosynthesis and up to 2-day acceleration in bud flushing of the WSL treatments on the tea plants, the limited impact on yield was not surprising. Wang et al. (2019) reported that the yields of tea plants were significantly improved by a stronger supply of light during the bud flushing period. Thus, the light intensity is critical for the yield of fresh leaves.

Impacts on the important biochemical components

Catechin is an important component for tea flavour and largely contributes to the health benefits of tea (Xu et al., 2018; Prasanth et al., 2019). As shown in Table 3, the total content of catechins in TM1, TM3 and TM5 increased by 11.51%–18.94% compared with that of the CK, and the differences were significant ($p < 0.05$) in the ANOVA. It appears that the quality and intensity of light and its duration will affect the content of catechins. The accumulation of catechins decreased in the light shade experiments (Ye et al., 2021). Research by Xiang et al. (2021) also showed that the content of catechins

Table 2. Bud weight, length and total yield of the treatments.

Treatment	CK	TM1	TM2	TM3	TM4	TM5	TM6
Weight (g)	5.08 ± 0.40	5.49 ± 0.15	5.12 ± 0.50	5.17 ± 0.31	5.43 ± 0.63	5.39 ± 0.25	5.42 ± 0.11
Length (cm)	5.22 ± 0.25	5.57 ± 0.32	5.17 ± 0.43	5.39 ± 0.30	5.18 ± 0.41	5.40 ± 0.21	5.33 ± 0.23
Yield ($g \cdot m^{-2}$)	243.56 ± 32.16	266.17 ± 29.80	259.73 ± 72.24	274.43 ± 48.72	278.69 ± 61.07	282.39 ± 68.51	279.41 ± 54.60

**p* < 0.05 and ***p* < 0.01 indicate significant differences compared with those of the CK. CK, control; TM, treatment.

Table 3. Catechin content in the treatments ($mg \cdot g^{-1}$).

Treatment	CK	TM1	TM2	TM3	TM4	TM5	TM6
EGC	13.76 ± 1.86	15.13 ± 1.08	14.45 ± 1.88	15.41 ± 1.50	14.90 ± 1.35	14.02 ± 0.15	14.04 ± 0.56
EC	7.50 ± 1.11	8.79 ± 0.52	7.79 ± 1.16	8.44 ± 0.81	8.83 ± 0.37	7.65 ± 0.18	7.90 ± 0.96
EGCG	49.92 ± 6.54	61.35 ± 1.74	53.60 ± 6.96	56.19 ± 6.41	56.37 ± 9.30	58.30 ± 7.86	56.54 ± 8.88
GCG	3.34 ± 0.25	3.53 ± 0.43	3.31 ± 0.38	3.33 ± 0.31	3.27 ± 0.42	3.57 ± 0.19	3.37 ± 0.31
ECG	7.69 ± 0.98	9.82 ± 0.47	8.36 ± 0.97	8.96 ± 1.07	8.92 ± 1.77	9.04 ± 1.36	9.01 ± 1.67
CG	3.45 ± 0.38	3.26 ± 0.09	3.28 ± 0.39	3.20 ± 0.21	3.17 ± 0.16	3.50 ± 0.29	3.23 ± 0.07
Total catechins	85.67 ± 9.97	101.90 ± 2.46**	90.79 ± 8.79	95.53 ± 9.20*	95.47 ± 11.38	96.08 ± 9.48*	94.09 ± 11.69

**p* < 0.05 and ***p* < 0.01 indicate significant differences compared with those of the CK.
CG, catechin gallate; CK, control; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GCG, gallic catechin gallate; TM, treatment.

Table 4. Content of the anthocyanins ($mg \cdot 100\ g^{-1}$ DW), caffeine and free amino acids ($mg \cdot g^{-1}$ DW).

Treatment	CK	TM1	TM2	TM3	TM4	TM5	TM6
Delphinidin	251.37 ± 9.49	234.03 ± 7.10	235.82 ± 8.98	218.41 ± 17.50	225.59 ± 9.57	235.13 ± 7.04	224.25 ± 12.13
Cyanidin	95.22 ± 16.57	94.71 ± 8.92	91.66 ± 13.71	94.14 ± 10.44	96.18 ± 26.72	87.86 ± 7.55	81.70 ± 3.52
Pelargonidin	13.59 ± 0.66	12.71 ± 0.20	13.30 ± 0.77	12.19 ± 0.67	12.39 ± 0.36	12.80 ± 0.84	12.12 ± 1.02
Anthocyanins	360.18 ± 16.31	341.45 ± 11.95	340.78 ± 5.54	324.75 ± 17.96**	334.16 ± 30.68*	335.79 ± 4.40*	318.07 ± 15.00**
Caffeine	39.75 ± 2.25	40.83 ± 0.58	40.16 ± 2.39	40.68 ± 1.22	39.96 ± 1.74	41.23 ± 2.01	40.25 ± 1.54
Free amino acids	51.35 ± 4.99	52.10 ± 4.60	52.00 ± 3.07	53.97 ± 2.07	53.86 ± 4.21	49.47 ± 2.38	51.94 ± 3.92

**p* < 0.05 and ***p* < 0.01 indicate significant differences compared with those of the CK.
CK, control; DW, dry weight; TM, treatment.

increased under appropriately high light intensities. Therefore, the effect of light on the accumulation of catechins is likely to be additive to some extent.

‘Ziyan’ is a tea cultivar that is rich in anthocyanins (Lai et al., 2016). Previous studies have shown that adding day length, light intensity or blue light radiation increased the accumulation of anthocyanins in tea plants (Sun et al., 2016; Zheng et al., 2019; Zhang et al., 2020a). However, the effect of prolonging the photoperiod by weak light on the accumulation of anthocyanin was not investigated. We measured anthocyanins in 1B2L of the WSL treatments. The total contents of the three anthocyanidins detected varied from 318.07 $mg \cdot 100\ g^{-1}$ to 367.18 $mg \cdot 100\ g^{-1}$, with delphinidin accounting for over 60% (Table 4). Unexpectedly, the highest content was observed in the CK, which was even significantly higher than those of TM3, TM4, TM5 and TM6 ($p < 0.05$ or 0.01). The lowest content of anthocyanins was observed in TM6, which decreased by 11.69%, compared with that of the CK. TM6 had a lower light intensity (2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared with the other WSL treatments. Therefore, the results showed that prolonging the photoperiod by weak light, such as 2–5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, in the night has a negative effect on the accumulation of anthocyanin, and this effect could be related to the light intensity.

We speculate that the total illumination radiation, instead of light period, is important for anthocyanin accumulation. The increase in catechins may also lead to the decrease in anthocyanins as they share the upstream biosynthetic pathway (Tan et al., 2020). However, we failed to find previous research that indicated that applying extra weak light during the night decreased the content of anthocyanins. Therefore, the opposite response mechanism of the accumulation of anthocyanin to extra weak and strong light merits further study.

Free amino acids and caffeine are important for the umami flavour and refreshing function of tea. The contents of free amino acids and caffeine did not change significantly in all the WSL treatments compared with those in the CK (Table 4). The results indicate that the biosynthesis of free amino acids and caffeine is less sensitive to weak light than that of catechins and anthocyanins in tea plants.

CONCLUSIONS

In this study, we investigated the impacts of WSL treatments on tea plants during early spring nights. The results showed that the treatments could significantly increase the bud flush and content of catechins but reduce the accumulation of anthocyanins. It is promising to apply artificial light on tea plants during the early spring to obtain beneficial effects. More studies with different light intensities, quality or intermittent light supplies should be performed in the future. In addition, the response mechanism of the accumulation of anthocyanins to additional weak light periods merits further study.

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AUTHOR CONTRIBUTIONS

Q. L., S. D., X. H. and Y. Y. performed the experiments and analysed the data. L. T. and Q. T. designed the experiments. Q. L. and L. T. wrote and revised the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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