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ARTICLE

Effect of light quality and extended photoperiod on flower bud induction during transplant production of day-neutral strawberry cultivars

Varinder Sidhu, Valérie Bernier-English, Marianne Lamontagne-Drolet, and Valérie Gravel

Abstract: Day-neutral (DN) strawberry cultivars are increasingly grown in Canada because they produce flowers and fruits continuously until October. Appropriate artificial lighting conditions during preparation of high-quality transplants is critical. Unfortunately, systematic evaluation of appropriate artificial lighting conditions during transplant production is limited. The objective of this study was to determine how an extended photoperiod supplemented with different light quality affects the vegetative and reproductive growth of a day-neutral cultivar during transplant production. In the first trial, we investigated the photoperiodic nature of the DN cultivar 'Albion' under low intensity incandescent light. Transplants were grown under three light combinations with different far-red : blue ratios (1:5, 5:1 and 1:1), supplemented for long day (LD; 24 h), short day (SD; 10 h) photoperiods and during a night interruption (NI) for 2 h. 'Albion' cultivar exhibited similar degree of flowering sensitivity regardless of photoperiod duration when incandescent light was used as predominant light source. In case of light emitting diodes (LEDs), dominant blue (1:5) LEDs prompted a significant increase in flower bud induction (FBI), more explicitly under the LD photoperiod. Furthermore, transplants grown under dominant blue light (1:5) supplied during NI produced eight flower buds per plant, the highest among all treatments, and promoted flower development outside the crown. Based on the results, it appears that lower wavelengths advance flowering and higher wavelengths contribute towards the morphological traits especially during transplant production. Results suggest that combination of far-red and blue LEDs at 1:5 ratio could be a potential light source to improve flower bud induction and floral development to subsequently increase fruit production.

Key words: photoperiod, LEDs, light quality, night interruption, flower bud induction.

Résumé : On cultive de plus en plus de cultivars de fraisier insensibles à la photopériode (IP) au Canada, car ces variétés produisent sans arrêt des fleurs et des fruits jusqu'en octobre. Un éclairage artificiel approprié est crucial à la préparation de plants de qualité à repiquer. Malheureusement, on évalue de façon peu méthodique ce qui constitue un tel éclairage lors de la production de transplants. Les auteurs voulaient établir comment une photopériode plus longue accompagnée d'un éclairage de qualité variable affecte la croissance végétative et reproductive d'un cultivar IP au repiquage. Dans le cadre d'un premier essai, ils ont étudié le photopériodisme du cultivar IP Albion sous un éclairage à incandescence de faible intensité. Les plants repiqués ont été cultivés sous trois éclairages dont le rapport rouge lointain-bleu différait (1:5, 5:1 et 1:1), avec une photopériode longue (24 h) ou courte (10 h) et une interruption nocturne de 2 h. Quand la principale source de lumière est un éclairage à incandescence, Albion exprime une floraison similaire, peu importe la longueur de la photopériode. Quand on recourt à un éclairage à diodes électroluminescentes (DEL), la lumière bleue dominante (1:5) accroissent de façon significative l'induction des bourgeons floraux, surtout sous une longue photopériode. Par ailleurs, les plants cultivés sous un éclairage avec une dominance bleue (1:5), avec une interruption nocturne, produisent huit bourgeons floraux par plant, soit le nombre le plus élevé pour les différents traitements. Ils développent de surcroît des fleurs hors du collet. Selon ces résultats, il semble que les longueurs d'onde plus courtes accélèrent la floraison alors que les plus longues favorisent les caractères morphologiques, surtout lors de la production des plants à repiquer.

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Toujours selon ces résultats, un éclairage DEL combinant le rouge lointain et le bleu dans un rapport 1:5 pourrait améliorer l'induction des bourgeons floraux et le développement de fleurs, menant potentiellement à une hausse de la production fruitière. [Traduit par la Rédaction]

Mots-clés : photopériode, DEL, qualité de l'éclairage, interruption nocturne, induction des bourgeons floraux.

Introduction

Québec strawberry cultivation is an important sector in terms of production and revenue. The province is the third major producer in North America, after California and Florida, as it yields around 47% of Canada's total production (Statistics Canada 2016). Strawberry genotypes are characterised as seasonal and perpetual flowering based on their flowering habits. Québec strawberry production mostly relied on short-day (SD) or seasonal (June–July) cultivars; however, the arrival and cultivation of day-neutrals (DN; perpetual flowering) caused a substantial increase in the fresh market. Unlike SD, DN strawberries continue producing new flowers and fruits until October, depending upon weather conditions. This therefore facilitates off-season production caused either by forcing early summer crops or extending the late harvest season (Ballington et al. 2008). The preparation of high-quality transplants is important to increase fruit production during the growing season. In Japan, plant factories have improved commercial strawberry transplant production using controlled artificial lighting that favorably influences plant health, rate of transplant establishment and triggers early flowering (Yoshida et al. 2016). Despite these benefits, the use of controlled artificial lighting for transplant production is still lacking in Québec and Canada.

Strawberry flowering is commonly divided into four stages: floral initiation, induction, differentiation, and development (Durner and Poling 1985). Upon inductive stimuli exposure, leaves produce a systemic signal, known as florigen, that translocate from the leaves to the shoot apical meristem (SAM), where it initiates morphological changes that prompt floral initiation (Durner and Poling 1985). Floral bud induction refers to the production of flower buds at the terminal end of the meristem. Floral differentiation is the specific enlargement of floral organs on inflorescence and macroscopic production of floral buds (Durner and Poling 1985). A better understanding of morphological and physiological changes in transplants following light conditioning is crucial to program the flowering based on a growers' requirements. Inflorescence, floral count, and flower mapping are frequently used parameters to determine the stimuli effect on flowering during transplant production (Durner 2018). Floral architecture mapping describes the position of floral buds and fate of differentiation of axillary buds into runner or floral buds, whereas inflorescence and floral count are labelled as useful growth scales. Mapping has the advantage over floral growth of providing a comprehensive evaluation of meristem response to stimuli (Savini and Neri 2003).

Plants perceive light signals through light-sensitive proteins, called photoreceptors, which generates their internal circadian rhythm and subsequently regulates physiological response (Shibuya and Kanayama 2014). Light quality and photoperiod controls photomorphogenesis and flowering behaviour in plants as previously reported in *Arabidopsis thaliana* (Hori et al. 2011), *Pyrus pyrifolia* (Ito et al. 2014), and strawberry (Yoshida et al. 2016). Photoperiod and light quality have been widely studied in seasonal strawberries and the flowering pathways in *F. vesca* (woodland strawberry) and *F. ananassa* (cultivated strawberry) are quite similar to *Arabidopsis thaliana* (Koskela et al. 2012; Rantanen et al. 2014; Nakano et al. 2015). Among photosynthetic active radiations (PAR), narrow-band light source of far-red (FR; 740 nm) and blue (455 nm) light-emitting diodes (LEDs) were described as flowering stimulant wavelengths in the seasonal and perpetual flowering cultivars (Rantanen et al. 2014; Yoshida et al. 2016). Even small differences (20–40 nm) in wavelength alter plant response considerably (Goto et al. 2013), which means that the combination of distinct wavelengths may activate unique mechanisms or gene expression, leading to either a positive or negative effect on plant growth and development (O'Carrigan et al. 2014). Photoperiodic conditioning is frequently delivered either by supplementing end-of-day light (day-extension) or during the middle of the night, also called night interruption (NI) (Sønsteby and Heide 2007; Park et al. 2016). Studies have recognized that flowering in photoperiodic plants is determined mainly by night length, and concise pulse of light (e.g., several seconds to hours) during the middle of the night divides the long night into short dark periods, resulted in stimulation of flowering in plants (i.e., *Xanthium* and Pharbitis) (Thomas and Vince-Prue 1997) and LD ornamentals (Meng and Runkle 2017).

According to the classification suggested by Nishiyama and Kanahama (2000) and Sønsteby and Heide (2007), DN cultivars are considered as quantitative LD plant when grown at intermediate temperature (22/18 °C; day/night) and qualitative LD plant at higher temperature (30/26 °C; day/night), due to the perpetual flowering under the LD photoperiod. Sønstesby and Heide (2007) suggested that generally everbearing cultivars display LD flowering response when supplemented with low intensity (7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) incandescent lighting. Based on the variability of the response of DN cultivars, we hypothesized that flowering in those

cultivars might be dependent upon light sources in addition to photoperiod and temperature.

In strawberry production, artificial lighting is widely used to advance phase transition from vegetative to reproductive growth under protected environment (Yoshida et al. 2016). Along with photoperiod, specific light wavelengths have been reported to regulate flower differentiation and fruit production in seasonal strawberry (Yanagi et al. 2016; Nadalini et al. 2017). Studies have highlighted that the narrow-band lights (i.e., blue and far-red regulates flowering and enhances the crop yield while maintaining the fruit quality) (Rantanen et al. 2014; Nadalini et al. 2017). Based on the literature, it appears that our understanding of the application of combined wavelengths is limited in DN strawberry. In the present study, we therefore aimed to determine the response of different proportions of blue and far-red LEDs on flowering for DN strawberry during the nursery stage under protected environment. The manipulation of floral induction during transplant production could significantly increase strawberry fruit production. It is important to determine the optimum light conditions to maximize flower bud induction (FBI) during transplant production. Suitable light combination and their interaction with photoperiod and night interruption to stimulate flowering were also investigated to develop an effective production system.

Materials and Methods

Plant material and experimental design

Four different experiments were conducted using completely randomized designs (CRD). An initial trial, to evaluate the photoperiodic control of flowering, was conducted in a greenhouse at Ferme Onésime Pouliot Inc. (45° 54′50.584″ N, 70° 57′6.8″ W; Saint-Jean-de-l'Île-d'Orléans, QC, Canada). The remaining three experiments, related to light quality, were performed in the Plant Science Research Greenhouse Facility located at the Macdonald Campus of McGill University (45° 24′27″ N, 73° 56′18″ W; Sainte-Anne-de-Bellevue, QC, Canada). Runner tips of a day-neutral cultivar (*Fragaria* × *ananassa* cv. 'Albion') were collected from field-grown stock plants (Ferme Onésime Pouliot Inc.) and rooted in coconut fiber (Teris, Laval, QC) in 12-cell trays (250 mL cells) for 2–3 wk.

Plant morphology and flowering analysis

For each experiment, runners and developed flower stalks were removed to maintain the uniformity among transplants at the beginning of each experiment. Plants with three to four fully expanded trifoliate leaves were labeled and randomly selected to measure the phenological growth of the plant. Phenology data included the number of flower stalks, opened flowers developed on each stalk, runners, and new fully expanded leaves. Phenology data was recorded every week and flower stalks were tagged with tape and runners were removed from all the transplants to direct the energy into establishing high quality crown growth. Plants were randomly selected for dissection to visualize the axillary bud development on each inflorescence inside the main crown under a stereomicroscope (Boreal2, VWR, Ontario) and development stages were identified according to Taylor et al. (1997). Transplants were collected randomly every 2 wk to determine dry biomass distribution among roots, shoots, leaves and crown. Transplants were collected and separated into different plant parts and dried at 70 °C for 72 h in an oven (Isotemp Incubator, Fisher Scientific, Hampton, NH) for dry biomass measurements.

Light treatments

Photoperiodic control of flowering in day-neutral 'Albion' strawberry

Runner tips of 'Albion' were rooted for 2 wk starting from 9th Aug to 24th Aug. During the rooting phase, transplants were exposed to natural daylight and ambient temperature (as there was no temperature control in the greenhouse). Light conditioning began once plants had developed three to four unfolded leaves. The different photoperiods were applied in the greenhouse from 24th Aug. to 16th Oct., then the transplants were transferred in the fridge for the winter (−1 °C). For the 24 h light treatment (24LD), transplants were exposed to natural daylight until end-of-day. Low intensity incandescent lighting was provided 30 min before the sunset. Since the sunset and sunrise hours changes over time during fall, we adjusted the hours at which lights were turned on and off each week to obtain 24 h lighting. Similarly, incandescent lightings were adjusted in addition to natural daylight to provide total 18 h (18LD) light treatment. In the control treatment (ND), transplants were exposed to natural daylight, where daylength varied from 13 h in Sept. to 10 h in Oct. 2019. Light intensity during the extended lighting period was kept very low (between 10 and 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to dispense a comparable daily light integral (DLI) under all three treatments. The calculated monthly mean temperature recorded in the greenhouse varied between 20.1 °C in Aug., 16.7 °C in Sept., and 13.5 °C in Oct. In this trial, 144 transplants were assigned to a specific light treatment, and each experimental unit (EU) contained 48 plants replicated thrice. For each EU, three and six plants were randomly collected every 2 wk for dissection and dry biomass measurement respectively. Similarly, six transplants were randomly selected to measure the weekly phenological progress. The initial flush of flowers and runners were removed from all the transplants except those sampled for phenology data, to establish high quality crown growth. Transplants were fertilized for 6 wk with an over-head sprinkler system supplying, per m^2 containing 114 plants, 1800 mg nitrogen (N), 400 mg phosphorus (P), and 1700 mg potassium (K)

for each of the first 3 wk, followed by 1010 mg N, 200 mg P, and 800 mg K during the 4th and 5th week, and 3100 mg N, 20 mg P, and 2900 mg K for the 6th week. Above mentioned fertilizer compositions were mixed in two barrels (A and B) and subsequently, fertilizer solutions were injected in the irrigation system at a rate of 1%. Nitrogen concentrations were changed over the course of the experiment to stimulate floral initiation. Since all the transplants, regardless of photoperiod treatment, received the same amount of N, this allowed us to compare the effect of the main treatment.

Light quality control of flowering in day-neutral 'Albion' strawberry

The experiment was conducted in the greenhouse to determine the effect of differential light quality on flowering of the 'Albion' cultivar. Transplants were kept in the greenhouse at temperature ranging between 16 °C and 20 °C and 14 h photoperiod until the light conditioning initiated. LEDs (U Technology Corporation, Calgary, Alberta, Canada) featuring the combination of far-red (peaked at 725 nm) and blue (peaked at 455 nm) wavelengths at ratios of FR:B with dominant blue light (1:5), dominant far-red light (5:1), and (or) an equal ratio (1:1) were installed 70 cm above the plant canopy. The FR:B ratios are based on number of LED lights used in each prototype. For example, LED light array fixtures (1.20 m × 32 cm × 8 cm) that contained 288 far-red plus 1440 small blue LED lights designed in four strips, is considered as dominant blue (1:5). Similarly, 1440 small far-red were combined with 288 blue LED lights to make a dominant far-red (5:1) fixture. For the 1:1 light ratio, an equal number of blue and far-red LED lights (864 for each) were used. Each light treatment was isolated using double-layered black perforated cloth that allowed air circulation but no light to go through. Plug transplants were grown in a greenhouse where light intensity in each treatment was maintained between 50 and 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 45 days supplying consistent DLI between 2.8 to 3.2 $mol \cdot m^{-2}\, d^{-1}$. Spectral output and light intensity were determined using a spectroradiometer (Apogee Instruments Inc., model PS-300, MN, USA) and light meter (LI-COR LI-250 A, LI-COR, Lincoln, NE, USA) equipped with a spherical underwater quantum sensor (LI-193, LI-COR). Instruments were calibrated initially using the manufacturer's guidelines. In this experiment, 24 transplants (in triplicate) were allocated in each EU for specific light treatment. For each EU, three plants were randomly selected for dissection, three for biomass analysis biweekly, and six to measure weekly phenology progress for six consecutive weeks. All transplants were manually fertilized every week using a nutrient solution containing 1500 mg N, 200 mg P, and 2200 mg K per m^2 (comprises 114 plants) with maintained EC:1.2 dS/m, pH: 5.8 to 6.2 for light quality experiments.

Light quality interaction with photoperiod and the effect on flowering

Three-week-old 'Albion' transplants were produced from field-grown runners (Ferme Onésime Pouliot Inc.), beginning in first week of Sept. 2019. This experiment was conducted in a greenhouse using a similar setup as mentioned in the previous trial. Sets of transplants were conditioned with 1:5, 5:1 and 1:1 (FR:B) light ratios under two photoperiods: long-day (LD; 24 h) and short-day (SD; 10 h). Different light regimes were established amid constant light intensity of 45–50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. SD was achieved with 10 h of distinct light ratios delivering low DLI 1.62 $mol \cdot m^{-2} \cdot d^{-1}$ whereas LD was achieved with 24 h of continuous light spectrum giving a high DLI 3.8 $mol \cdot m^{-2} \cdot d^{-1}$. Transplants were exposed from 6:00 to 16:00 in SD, and 24 h continuous lighting was maintained in LD for each experimental unit. In this trial, 144 transplants were randomly assigned (as in previous trials) for dissection, phenological growth, and dry biomass analysis for each experiment unit where specific light regimes were kept.

Light quality during night interruption controls flowering in DN 'Albion'

Three-week old plug transplants were grown in a greenhouse under continuous light provided by high-pressure sodium lamps (HPS) (P.L. Light System, Beamsville, ON, Canada) from 0600 to 2000 (14 h), followed by a night interruption (NI) at midnight for 2 h (00:00 to 2:00) using far-red and blue LEDs ratios of 1:5, 5:1 or 1:1. Treatments were initiated on 23rd Oct. until 10th Dec. Uninterrupted night treatment was considered as the control. During the daytime, light intensity was maintained between 100 and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and low intensity (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was supplemented during the night interruption. Temperature was maintained at 24 °C/18 °C (day/night) throughout the experiment for 48 d. Seventy-two transplants were randomly selected to measure weekly growth parameters including phenology growth, dissection, and dry biomass. Seventy-two transplants were assigned to a specific light treatment, where each experimental unit was comprised of 24 plants, replicated three times. For each EU, three transplants were randomly assigned to measure the weekly phenological progress. Similarly, three plants were randomly collected every 10 d starting from 30th Oct. to 10th Dec. for dissection and dry biomass. Plants were dissected using stereomicroscope to evaluate floral architecture. The floral mapping provides a visual illustration of the number of inflorescences, and the position of primary, secondary, and tertiary floral buds on each inflorescence to evaluate their stimuli sensitivity (Durner 2018).

Statistical methods

The growth parameter data followed a normal distribution assumption except for the new leaf growth. Leaf

Table 1. Effect of photoperiodic conditioning on flowering and phenology growth for 'Albion' during nursery stage.

Photoperiod[a]	Flower stalks[b]	Flowers[b]	New leaves[b]	Induced buds[b]	Inflorescences[b]	Biomass partitioning[c]
24LD	2 ± 0.30	5 ± 0.68	9 ± 0.64	6 ± 1.30	3 ± 0.44	36:45:7:13
18LD	1 ± 0.30	4 ± 0.68	9 ± 0.64	6 ± 1.30	2 ± 0.44	34:48:8:10
ND	1 ± 0.30	4 ± 0.68	7 ± 0.64	6 ± 1.30	3 ± 0.44	39:46:6:10
p value	0.3823	0.2286	0.0584	0.7962	0.4584	0.3674[d]

[a]Photoperiodic treatment of 24-h (24LD), 18-h continuous light (18LD) and natural daylight (ND).
[b]Represents data in number per plant. Data presented is the mean value ± SEM calculated using Fisher's LSD test, from three replicates (n = 18), n represents the sample size.
[c]Percentage of roots: leaves: crown: stalks biomass.
[d]Represents p value for crown.

growth data were transformed using a log transformation, which improved the homoscedasticity of variance and subsequently, analysis of variance (ANOVA) was performed. ANOVA was conducted using a statistical analysis software (SAS 9.4 version, Analytics Software and Solutions, North Carolina, USA) for all the experiments. The main effects of individual factors and their interaction were determined using two-way or three-way ANOVAs based on the experiment design. Standard error of the mean (SEM) for all the growth parameters was determined using Fisher's least significant differences (LSD) test at a 5% level. The correlation between cumulative flower bud induction and light quality treatment was analyzed by a linear fitting method.

Results

Photoperiodic control of flowering in day-neutral 'Albion' strawberry

Transplants grown under 24LD and ND produced on average three terminal inflorescences bearing axillary buds, while 18LD developed two inflorescences (Table 1). Likewise, flower buds inside the crown data showed no significant difference among the treatments. 18LD-conditioned transplants exhibited the first emerging flower stalk outside the crown 10 d after treatment (DAT) commenced. Comparatively, ND transplants developed their first stalk after 20 d, and 24LD after 30 d. Overall, 24LD treated plants produced an average of five flowers compared with four for 18LD and ND but differences were non-significant (Table 1). 'Albion' transplants grown under long-day (24LD and 18LD) photoperiods produced nine new leaves per plant compared with seven for ND, although the difference was not significant. Transplants produced very few runners regardless of photoperiod. Photoperiod conditioning showed no significant impact on dry biomass distribution of roots, leaves, crown and stalks.

Light quality control of flowering in day-neutral 'Albion' strawberry

Transplants conditioned with a blue-dominant combination of light (1:5) exhibited a significantly higher number of flower buds (p value: 0.02) inside the crown compared with transplants conditioned with dominant FR (5:1) and 1:1 (Fig. 1A). Transplants in the dominant blue light regime commenced flowering in 8–14 d compared with dominant FR and 1:1 that exhibited a delayed anthesis at around 18–24 d and 20–30 d, respectively. Dominant FR light significantly promoted the growth of new leaves compared with the dominant blue light (Fig. 1A; p value: 0.04). However, results showed no significant difference in runner production for 'Albion' between the light quality treatment (Fig. 1A; p value: 0.24). Regardless, it is important to observe that flowering seemed to exhibit an antagonistic effect on runner emergence from the axillary buds in dominant blue conditioned plants. Transplants conditioned with the dominant blue light combination exhibited an increased crown biomass partitioning compared with other treatments, although the difference was not statistically significant (Fig. 1B; p value: 0.36).

Light quality interaction with photoperiod and their effect on flowering

Light quality, photoperiod and their interaction effect showed statistically significant differences on flowering with respect to time. After 4 wk, transplants grown under dominant blue light (1:5) for 24 h substantially advanced flowering and produced five fully opened flowers per plant compared with two for 1:1 and 5:1 (Fig. 2A). However, 5:1 and 1:1 hastened flowering in the last 2 wk and produced similar number of flowers by the end of the 6-wk experiment (Fig. 2A). LD photoperiod significantly promoted the growth of flower stalks, flowers and new leaves compared with SD, regardless of light quality (Fig. 2B).

During the dissection of the 'Albion' transplants, no significant interaction effect between light quality and photoperiod on flower bud induction was observed (Table 2). However, light quality and photoperiod stimulated flower bud induction independently. Dominant blue LEDs (1:5) produced significantly more flower buds (six) inside the crown compared with 5:1 (three) and 1:1 (3) but showed no effect on the number of inflorescences

Fig. 1. (A) Average number of new leaves, inflorescences, flower buds, and runners (per plant) in response to light quality. (B) Dry biomass partitioning (stalks, crown, leaves, and roots) in response to light quality. Mean values with the same lowercase are not significantly different among each group. Light ratios of 1:5, 5:1 and 1:1 of far-red (725 nm): Blue (455 nm). Data presented in the figure is mean value ± SEM calculated using Fisher's LSD test, from three replicates (n = 18), n represents the total sample size.

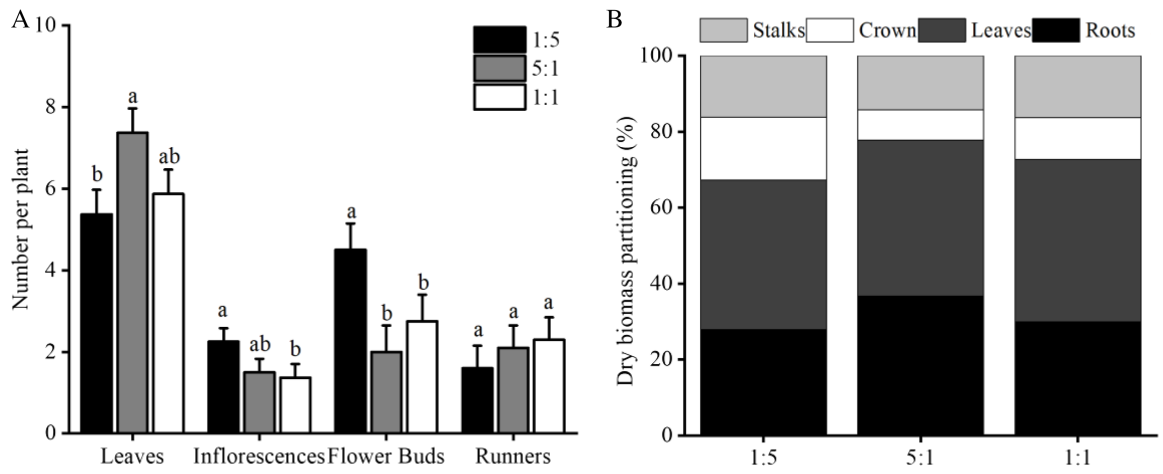


Fig. 2. (A) Effect of light quality on flowering of the 'Albion' cultivar during 6 wk of treatment. (B) Average number of flower stalks, flowers, and new leaves per plant in response to photoperiod (SD and LD). (C) Average number of flower buds and inflorescences per plant inside the crown in response to light quality. (D) Average number of flower buds and inflorescences per plant inside the crown in response to photoperiod. Mean values with the same lowercase letter are not significantly different among groups. SD (10 h) and LD (24 h) continuous light exposure to three light ratios of 1:5, 5:1 and 1:1 of far-red (725 nm): Blue (455 nm). Data presented in the figure is mean value ± SEM calculated using Fisher's LSD test. The phenology data and dissection is collected from three replicates with sample size n = 18 and n = 36, respectively.

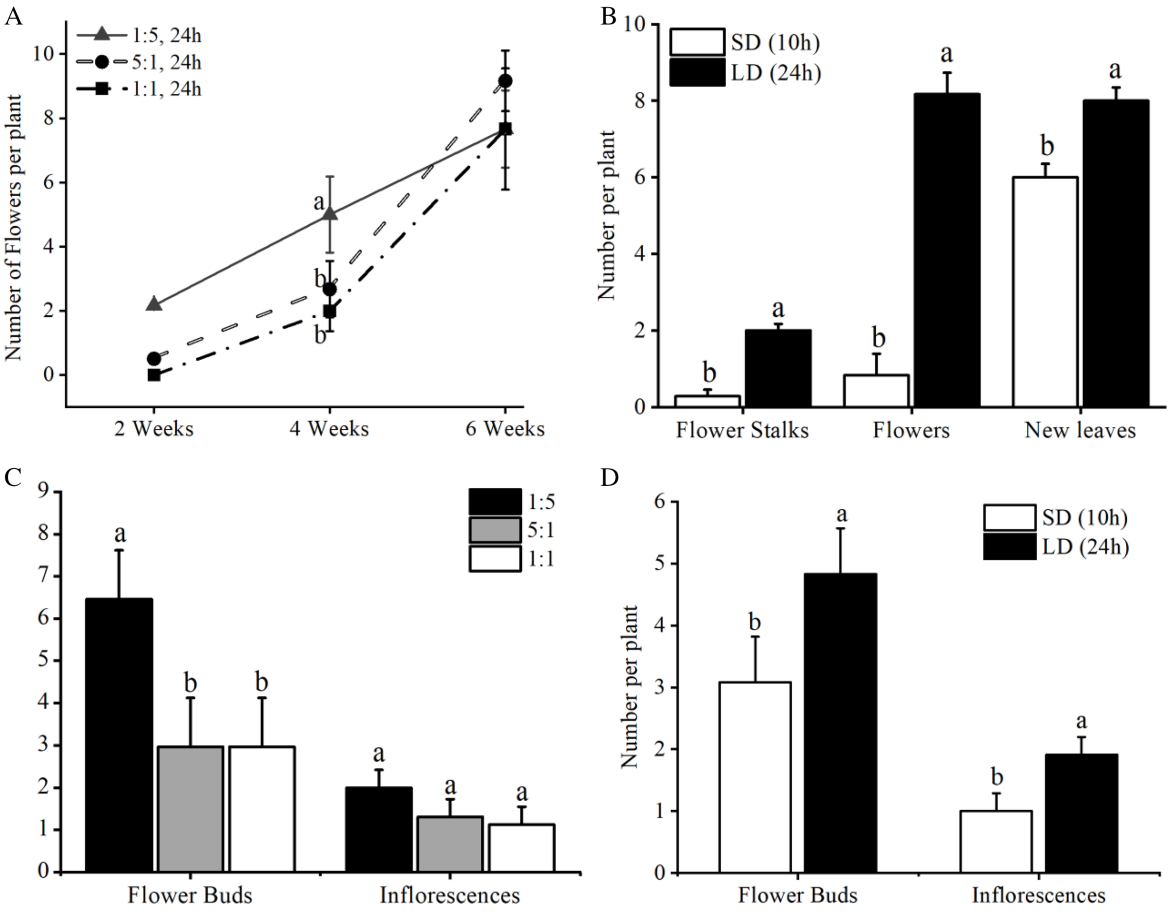
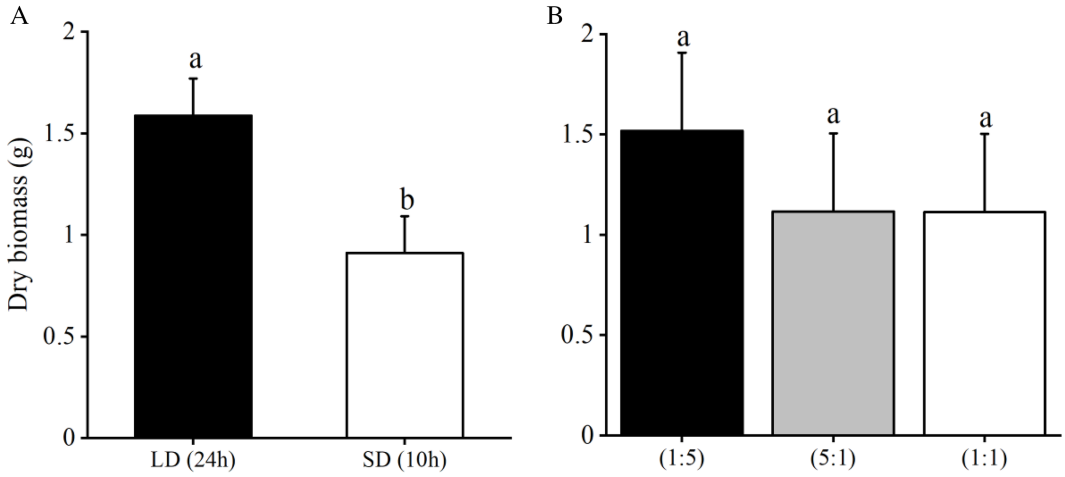


Table 2. *P* values for light quality and photoperiod conditioning main effects and their interactive effects on flowers, flower buds inside the crown, new leaf growth, and dry biomass for 'Albion'.

Source of variation	Flowers	Flower buds	New leaves	Dry biomass
Light quality	0.3743	0.0323	0.2439	0.7624
Photoperiod	0.0232	0.0213	0.0443	0.0384
Light quality × Photoperiod	0.5636	0.9362	0.9865	0.4637

Fig. 3. (A) Dry biomass (g) in response to photoperiod and (B) light quality. SD (10 h) and LD (24 h) continuous light exposure. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm): Blue (455 nm). Data presented in the figure is the mean value ± SEM calculated using Fisher's LSD test, from three replicates (n = 18), n represents the sample size.



(as it was observed in the second trial) (Fig. 2C). LD photoperiod exposure of diverse light combinations significantly promoted the inflorescences and flower buds inside the crown (Fig. 2D). Transplants grown under LD photoperiod demonstrated significantly greater dry biomass accumulation compared with SD (Fig. 3A) whereas light ratios showed no statistical differences on dry biomass (Fig. 3B).

Light quality during night interruption controls flowering in DN 'Albion'

'Albion' showed a significant increase in flower bud induction and flowering when subjected to different light qualities during night interruption. Transplants grown under dominant blue lights (1:5) produced eight flower buds inside the crown (Fig. 4A) within 48 d and simultaneously exhibited five opened flowers per plant (Fig. 4B), the highest among all treatments. 1:5 supplemented plants showed comparatively advanced flowering, and emerged flowers outside the crown within 10 DAT, whereas 5:1 reached that in 24 d and 1:1 or HPS in 38 d. Dominant blue conditioned plants triggered floral induction and displayed linear growth that yielded a significantly higher number of flower buds (Fig. 5A). Nonetheless, leaf growth (p value: 0.56), inflorescence development (p value: 0.15), and dry biomass (p value: 0.52) results showed no significant difference among treatments (Fig. 5B). Very few runners were observed during the experiment. The architectural mapping of plants conditioned with distinct light quality is presented in Fig. 6. Plant dissection revealed that the dominant blue treatment stimulated secondary and tertiary branching as well as resulted in additional inflorescences.

Discussion

The control of flower bud induction and plant morphology is a complex process involving the manipulation of multiple environmental factors (Eskins 1992). Photoperiod and light quality are essential elements that control flowering for seasonal and day-neutral strawberry cultivars during the nursery stage (Hidaka et al. 2015). According to the general classification provided by Sønsteby and Heide (2007), most DN cultivars are considered as quantitative LD plant when grown at intermediate temperature (18 °C) and qualitative LD plant at high temperature (27 °C). However, photoperiod-based flowering could be different and should be determined separately for each individual cultivar (Heide et al. 2013). In this study, 'Albion' transplants grown under distinctive (24LD, 18LD and ND) photoperiods produced

Fig. 4. (A) Effect of light quality supplemented during night interruption on flower bud induction for the 'Albion' cultivar. (B) Average number of flower development outside the crown per plant in response to light quality. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm): Blue (455 nm). These light qualities were applied separately during 2 h night interruption from 00:00 to 02:00. Data presented in the figure is mean value ± SEM calculated using Fisher's LSD test. The phenology data and dissection are collected from three replicates with sample size $n = 18$ and $n = 36$ respectively.

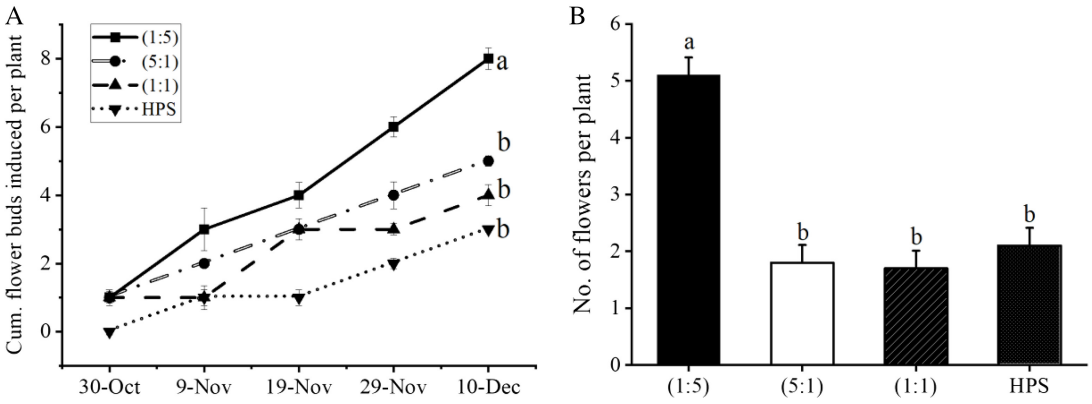
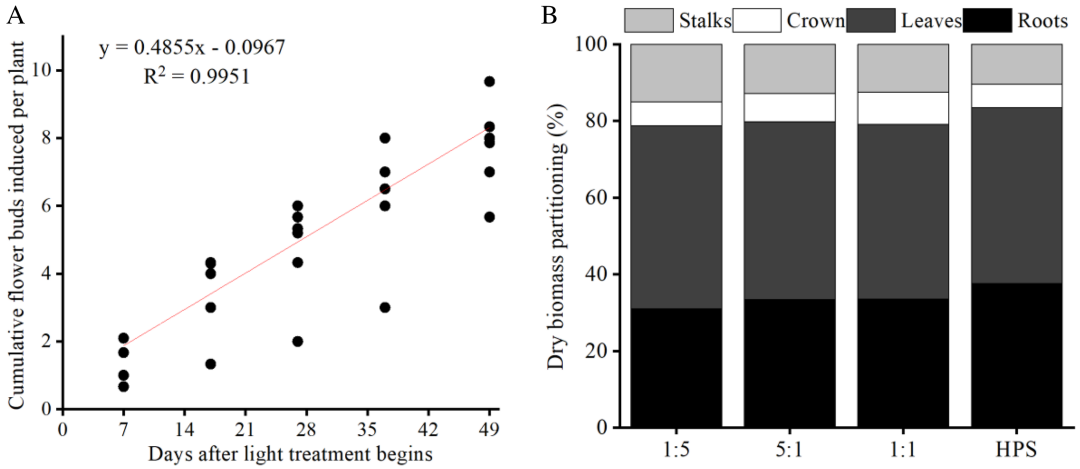


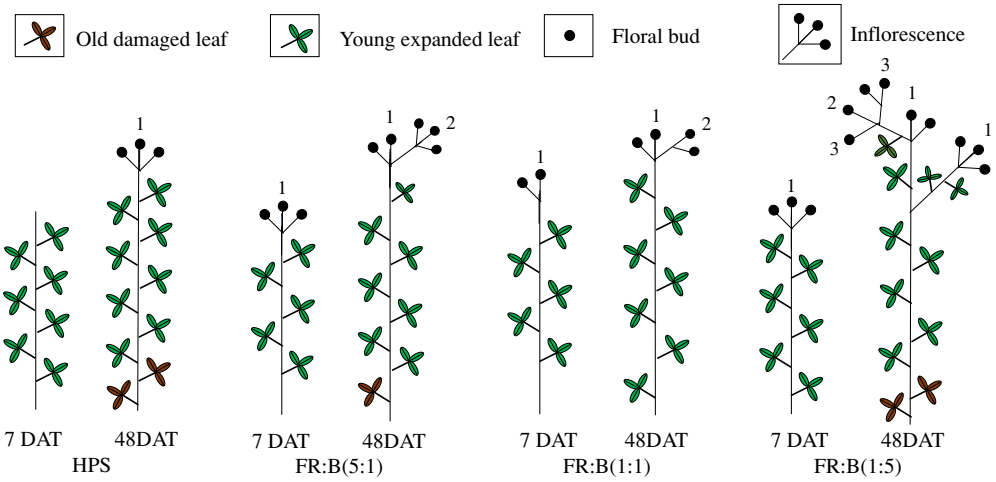
Fig. 5. (A) Scatter plot for dominant blue light FR:B (1:5) presenting linear growth among replicates for flower bud induction (B) Dry biomass partitioning (stalks, crown, leaves, and roots) in response to light quality. Light ratios of 1:5, 5:1 and 1:1 of far-red (725 nm): Blue (455 nm). These light qualities were applied during 2 h night interruption from 00:00 to 02:00. Data presented in the figure is mean value ± SEM calculated using Fisher's LSD test, from three replicates ($n = 18$), n represents the sample size. [Colour online.]



similar floral characteristics including terminal inflorescences, induced flower buds and flower stalks bearing fully opened flowers. Results indicated that the flowering response for 'Albion' established a similar degree of sensitivity to photoperiod while using incandescent light as the predominant light source. Our study validates that floral initiation and differentiation in 'Albion' transplants is regulated at the same frequency irrespective of photoperiod. In agreement with Durner (2015), the present study suggests that the general classification of strawberry cultivars in response to photoperiod does not apply to all the everbearing or day-neutral cultivars, as it could vary with specific cultivars. It is also important to evaluate each stage of floral development (flower bud induction and differentiation) separately as they are independently affected by photoperiod (Durner 2015). LD photoperiod increasing dry biomass is considered as a common plant response (Adam and Langton 2005). In LD plant *Arabidopsis thaliana*, LD photoperiod allocates biomass to stem growth especially during reproductive phase, whereas SD photoperiod invest towards new leave development (Dasti et al. 2002). However, in present study, overall dry weight and biomass allocated to different plant parts showed no significant difference regardless of photoperiod. Biomass allocation response in DN is quite similar to flowering behavior, which implies the true day-neutral nature of the cultivar.

A single-light source of FR (Zahedi and Sarikhani 2016) and blue (Yoshida et al. 2016) primarily accelerates flowering in June-bearing 'Paros' and LD 'HS138' strawberries

Fig. 6. Strawberry plant architecture in response to different light quality supplemented during night interruption at 7 and 48 d after treatment (DAT) affect for 'Albion' cultivar. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm): Blue (455 nm). Numbers represents order of inflorescences. Data presented in the figure is collected from n = 9 from three replicates, n represents the sample size. [Colour online.]



respectively. However, studies reported that a narrow-band light source is not satisfactory to regulate normal plant growth and development, especially in horticultural crops (Ouzounis et al. 2014). Blue and FR lights control flowering through the activation of photoreceptors, i.e., cryptochrome and phytochrome (Jones 2018), and therefore, can be promoted or inhibited depending on the synergetic interaction between the photoreceptors. Our results affirm that the combination of FR and blue directed transplants to function efficiently and consequently, a dominant fraction of blue light (1:5) showed a significant increase in flower bud induction for the DN cultivar 'Albion'. In contrast, the dominant FR (5:1) combination resulted in a significant increase in new leaf growth, perhaps because the inclusion of FR light potentially upturns leaf growth and leaf size expansion in floriculture crops during the flowering process (Park and Runkle 2019). Results suggested that blue light plays a superior role in mediating flower bud induction, while FR light preserves leafy growth of the plant. Advanced floral induction during transplant production could result in earliness of harvesting time by 10–15 d during the production season (Yoshida et al. 2016).

Flower bud induction is significantly advanced under dominant blue light for the 'Albion' cultivar, more explicitly under the LD photoperiod. Here we show that LD photoperiod supplied with different far-red and blue ratios enhanced flower stalks, flowers, new leaf growth, flower buds and inflorescences (Table 2). Whereas LD photoperiod supplied with incandescent light showed no significant effect on flowering during transplant production (Table 1). Here, we suggest that light source plays an important role while determining photoperiodic control of flowering. A prompt increase in flower bud induction under LD photoperiod supplied with dominant blue LED can be explained by two distinct factors. First, increased daily light integral hastened the flower initiation process as previously reported in several plant species including Hibiscus (Warner and Erwin 2003), begonia, marigold, and petunia (Faust et al. 2005). Secondly, blue light plays an equal or greater role than far-red to stimulate flowering for day-neutral cultivars (Runkle and Heins 2001). Similar results were reported in seasonal cultivars 'Daewang' (Choi et al. 2015) and 'Elsanta' (Nadalini et al. 2017), suggesting that blue light is a potential lighting tool that can be used alone or in combination with light sources to enhance flowering and fruit yield.

It is further important to comprehend that the dominant blue light combination not only promoted FBI, but also prompted floral development outside the crown. Transplants exhibited augmented floral development outside the crown under the LD photoperiod (24 h) of dominant blue light combination within 4 wk of conditioning and then relapsed. Remarkably, the dominant FR LED (5:1) recuperated in the last 2 wk and produced comparable flowers eventually. Floral development outside the crown is not ideal for transplant production since growers detach developed flowers before cold storage during the winter. It therefore does not contribute to fruit yield. However, these results may be useful to implement artificial lighting during growing season and enhance flower stalk development, especially in early spring.

Significant interaction between light quality and photoperiod with respect to time implies that blue light accelerates flowering in early days and FR regulates delayed flowering. According to Demotes-Mainard et al. (2016), plants grown under FR tend to elongate to avoid shady environments, a phenomenon called shade

avoidance response. If plants grow for a longer period under abundant FR, they perceive this shady environment as a stress and start reproducing quickly to deliver their genetics to offspring. This explains how dominant FR (5:1) promoted flowers at a later stage, corresponding with previous studies in wheat, cucumber, tomato, and *Eustoma grandiflorum* (Yamada et al. 2009).

Night interruption (NI) supplemented with differential light quality during the middle of the night could be an alternative to deliver long-day conditions that regulate flowering (Park et al. 2017). Specific light quality during NI promotes flowering and increases flower number and stalk length in herbaceous plants including *Eustoma grandiflorum* (Yamada et al. 2009), petunia, *Cymbidium* (An et al. 2015) and other horticultural crops (Park et al. 2017). Similar results were observed in the 'Albion' cultivar during the nursery stage. Plants grown under dominant blue light during NI produced a considerably increased number of flower buds and enhanced flower development outside the crown compared with day-time application. Plants produce flower buds at linear progression until the end of the supplemental lighting period. Flowering outside the crown substantially increased under dominant blue light possibly because NI is more effective than a daylength extension approach to control the vegetative and reproductive growth of the plant (Rashidi et al. 2018). Comparatively, blue light essentially subsidized the obligatory time required to induce flowering and revealed a significant association between supplementation time and light spectrum to control flowering in 'Albion'.

Our results confirmed that dominant blue supplementation for the LD photoperiod accelerates floral induction and produced average six flower buds by the end of conditioning. Similarly, when subjecting the transplants to dominant blue during night interruption, they produced an average of eight flower buds and led to the shortest flowering time. It is important to note that both the experiments were conducted independently, but growing conditions were identical except for the time of light conditioning. This indicates that scheduling of combined light spectra as supplemental lighting is crucial to control the flowering traits of transplants (Rashidi et al. 2018).

Runner and inflorescence are considered to have an antagonistic effect during flower initiation (Bradford et al. 2010). Runner production is an undesirable trait during transplant production. Results showed that transplants treated with the distinct light regimes used in these trials displayed minimized runner production, indicating that conditions were not promoting their growth.

Plants develop a unique distribution approach to outgrow for better light harvesting. Light quality can be a major trigger that controls vegetative and reproductive structures that subsequently alter biomass allocation (Poorter et al. 2012). To quantify biomass allocation in response to light quality, we measured biomass partitioning among the leaf, root, crown, and stalk. Our study demonstrated that photoperiod may have a more pronounced effect on dry biomass allocation for 'Albion' than light quality. These results agree with Zhao et al. (2017) who suggested that extended daylength significantly enhances biomass accumulation.

Conclusions

Artificial lighting has been extensively used in horticultural crops to stimulate flowering under protected conditions. Recently, studies have stated the prospective benefits of single-color LEDs such as blue and far-red that enhances flowering and fruit production. The present study demonstrates the combination of far-red and blue LEDs at 1:5 ratio, stimulates flower bud induction and development in day neutral strawberry. In accordance with literature, blue light plays a superior role to enhance flowering and morphological traits for the 'Albion' cultivar during transplant production. Supplementation of dominant blue LEDs coupled with LD photoperiod and night interruption amplifies the impact on flowering. Based on the results, it appears that light supplementation should be restricted to the first 4 wk during transplant production as the benefits decline dramatically after this period. Furthermore, dominant blue LEDs significantly enhanced floral growth outside the crown as well, suggesting that it could be supplemented during the growing season to advance stalk development and extend the harvesting season even further.

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