Distinct stimuli-induced flowering: characterizing the hormonal and genetic regulation in day-neutral *Fragaria* species

VARINDER SIDHU

Department of Plant Science

McGill University, Montreal, Canada

August 2021

A thesis submitted to the McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Dedicated to my beloved wife, my parents and village farmers.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 3

Chapter 4

Chapter 5

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

LIST OF ABBREVIATIONS

ABA	Abscisic acid
ANOVA	Analysis of variance
AP1	Apetella1
°Bx	Brix
CCA1	Circadian clock associated
CDF	Cycling of factors
cDNA	Complementary deoxyribonucleic acid
CEA	Controlled environment agriculture
CK	Cytokinin
CO	Constans
CRD	Completely randomized design
CRY1	Cryptochrome1
Ct	Cycle threshold
DAP	Days after planting
DE	Day-extension
DLI	Daily light integral
DN	Day-neutral
DNA	Deoxyribonucleic acid
EB	Ever-bearing cultivars
ESI	Electrospray Ionization
EU	Experimental unit
FBI	Flower bud induction

FKF1	Flavin-binding, kelch repeat F-box1
FLC	Flowering locus C
FR	Far-red
FT	Flowering locus T
FUL	Fruitful
GAs	Gibberellins
GI	Gigantea
HPS	High-pressure sodium
IAA	Indole-acetic acid
JB	June-bearing cultivars
K	Potassium
LD	Long-day
LEDs	Light-emitting diodes
LFY	Leafy
LHY	Late hypocotyl elongated
LKP2	Lov-kelch protein
LSD	Least significant difference
MRM	Multiple reaction monitoring
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MSI1	Multicopy suppressor of ira-1
N	Nitrogen
ND	Natural daylight

NH_4^+	Ammonia
NI	Night interruption
NO_3^-	Nitrate
NR	Nitrate reductase
P	Phosphorus
PAR	Photosynthetically active radiation
P_{FR}	Phytochrome far-red
PHYB	Phytochrome-B
P_R	Phytochrome red
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
SAM	Shoot apical meristem
SAS	Statistical analysis software
SD	Short-day
SEM	Standard error of the mean
SFL	Seasonal flowering locus
SOC1	Suppressor of overexpression of constans
TFL1	Terminal flower1
TOC1	Timing of cab expression1
TSF	Twin sister of ft
UPLC	Ultra-performance liquid chromatography
WAT	Weeks after treatment
ZTL	Zeitlupe

ABSTRACT

Strawberry cultivation is an important sector for the Québec horticulture industry in terms of revenue as it represents 47% of Canada's total production. In Québec, strawberry producers face challenges such as shorter production periods and soilborne diseases that severely impact fruit production. To overcome existing challenges, day-neutral (DN) cultivars are increasingly grown in soilless media, which resulted in extended harvesting season and improved fruit yield. Despite the increasing use of DNs in Québec, very little work has been done on DN strawberry production under local conditions, thus, available information is very limited. In this project, we determined the effect of environmental stimuli that can stimulate flower bud induction (FBI) in the widely grown DN cultivar 'Albion' and evaluated the molecular and metabolic mechanism that controls the flowering. The FBI is considered as the most reliable and critical factor for the successful cultivation of the crop as it directly contributes to the qualitative and quantitative characteristics of strawberry fruit. Results demonstrated that low nitrogen (N) supply and long day (LD) photoperiod triggers the FBI process during the later stages in the growing season, while elevated N supply seems to enhance LD-induced effect on flower bud induction in DNs. Flowering in 'Albion' was unaffected under LD photoperiod when incandescent light was used as the predominant light source. Whereas flowering increased considerably under LD photoperiod supplied with light-emitting diodes (LEDs). The combination of two narrow-band light sources of far-red (FR) and blue (B) LEDs at a ratio of 1:5, also referred as dominant blue, significantly stimulated FBI and ensures healthy plant quality during transplant production. The supplementation of dominant blue LEDs coupled with LD photoperiod and night interruption amplifies the impact on flowering.

Flowering in plants involves distinct molecular mechanisms and hormonal signaling. The flowering pathways have been extensively studied in seasonal strawberry. However, it is poorly understood in DN strawberry cultivars. To understand the mechanism for light quality control of flowering in DN cultivars, we have examined the transcript level of floral-related genes and concentration levels of plant hormones during the FBI process. The transcript level of flowering-related genes i.e., FvFT1 and FvTFL1 were recognized at a higher fold change under dominant blue LEDs, suggesting that both genes are involved in flowering in response to light quality, although, flowering seems to occur independently of FvTFL1. In contrast to 'Albion', 'Alexandria' displayed strong flowering inhibition in all the FR and B combinations when supplemented during night interruption. Our findings postulate that FR light-controlled phytochromes may be involved in the floral inhibition response in *F. vesca* through differential regulation of the FvFT1 transcription factor. Further, increased levels of gibberellins and cytokinin in the crown tissue seems to regulate FBI during transplant production in both woodland and cultivated strawberry. Our results highlight that dominant blue LEDs could be a potential light source to improve flowering traits that subsequently can increase fruit production and extend the harvesting season for DN strawberry cultivars.

RÉSUMÉ

La production de la fraises est un secteur important pour l'industrie horticole québécoise en termes de revenus puisqu'elle représente 47% de la production totale du Canada. Au Québec, les producteurs de fraises font face à des défis tels qu'une période de production courte et des maladies telluriques qui affectent gravement la production de fruits. Pour surmonter les défis existants, les cultivars à jour neutre (DN) sont de plus en plus cultivés dans des systèmes hors-sols, ce qui a permis d'allonger la saison de récolte et d'améliorer le rendement en fruits. Malgré l'utilisation croissante des DN au Québec, très peu de travaux ont été réalisés sur la production de fraises DN dans des conditions locales, ainsi, l'information disponible est très limitée. Dans ce projet, nous avons déterminé l'effet des stimuli environnementaux qui peuvent stimuler l'induction des boutons floraux (FBI) chez le cultivar à jour neutre (DN) 'Albion' largement cultivé et comprendre le mécanisme moléculaire et métabolique qui contrôle la floraison. Le FBI est considéré comme le facteur le plus fiable et le plus critique pour la réussite de la culture car il contribue directement aux caractéristiques qualitatives et quantitatives des fraises. Les résultats ont démontré qu'un faible apport en N et une photopériode LD déclenchent le processus d'induction florale au cours des derniers stades de la saison de croissance, alors qu'un apport élevé en N semble augmenter l'effet induit par la LD sur l'induction florale dans les DN. La floraison dans 'Albion' n'était pas affectée par la photopériode LD lorsque la lumière incandescente était utilisée comme source lumineuse prédominante, alors que la floraison augmentait considérablement sous la photopériode LD alimentée par des diodes électroluminescentes (DEL). La combinaison de deux sources lumineuses à bande étroite de DEL le rouge lointain et bleu à un ratio de 1:5, également appelée bleu dominant, a considérablement stimulé le FBI et assure une plante saine et de qualité pendant la production

de transplants. La supplémentation en DEL bleues dominantes couplée à la photopériode LD et à l'interruption nocturne amplifie l'impact sur la floraison.

La floraison chez les plantes implique des mécanismes moléculaires et une signalisation hormonale distincts. Les voies de floraison ont été largement étudiées chez les fraisiers à jour court, mais elles sont mal comprises chez les cultivars DN. Pour comprendre le mécanisme de contrôle de la qualité de la lumière sur la floraison chez les cultivars DN, nous avons examiné le niveau de transcription des gènes liés à la floraison et les niveaux de concentration d'hormones végétales pendant le processus d'induction florale. Le niveau de transcription des gènes liés à la floraison, c'est-à-dire FvFT1 et FvTFL1, a été plus élevé sous les DEL bleues dominantes, suggérant que les deux gènes sont impliqués dans la floraison en réponse à la qualité de la lumière, bien que la floraison semble se produire indépendamment de FvTFL1. Contrairement à 'Albion', 'Alexandria' présentait une forte inhibition de la floraison dans toutes les combinaisons FR et B lorsqu'elle était supplémentée pendant l'interruption nocturne. Nos résultats postulent que les phytochromes FR contrôlés par la lumière peuvent être impliqués dans la réponse d'inhibition florale chez *F. vesca* par le biais d'une régulation différentielle du facteur de transcription FvFT1. De plus, des niveaux accrus de gibbérellines et de cytokinine dans le tissu de la couronne semblent réguler l'induction florale pendant la production de transplants dans les fraisiers de bois et cultivés. Nos résultats mettent en évidence que les DEL bleues dominantes pourraient être une source de lumière potentielle pour améliorer les traits de floraison qui peuvent par la suite augmenter la production de fruits et prolonger la saison de récolte pour les cultivars de fraises DN.

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere appreciation to my principal investigator, Dr. Valerie Gravel, who had given me the opportunity to work on this research project. Throughout this journey, she has guided me in the right direction and provided tremendous support through the tough times. Her expertise knowledge, scientific and statistical skills and her critical guidance have always helped to resolve the existing challenges. Without her persistent support and contribution, the objective of this study would not have been accomplished.

I would also like to express my special thanks to the members of my advisory committee and comprehensive exam committee Dr. Suha Jabaji, Dr. Philippe Seguin, Dr. Mark Lefsrud, and Dr. Stéphane Bayen, McGill University, for their insightful comments and tremendous contribution in this study. They have always provided the constructive criticism and the suggestions that has facilitated in carrying out this project. I would like to thank them for asking the hard questions during the committee meetings, that really helped to broaden my research prospective.

I would also like to thank the department of plant science at McGill University and especially Mr. Guy Rimmer, Ian Ritchie and Serghei Dernovici who have provided the technical assistance during the experiment setups in the greenhouse. I thank my fellow labmates Césarée, Angela, Zahra for maintaining the safe, healthy and easy-going environment in the lab. I appreciate their continuous help to perform the plant dissection, for data collection trips to Québec City and constantly sharing the scientific knowledge. I wish to send out my special thanks to my fellow colleagues Irfan Iqbal, Meha Sharma, Niranjan Hegde, Nancy Soni for actively engaging in scientific discussions, supporting during the hard times and sharing their work and life experiences. I would like to thank Césarée for translating the abstract to French.

I would like to express my deep sense of thankfulness to Dr. Jun Han and their team members at Genome BC Proteomics Centre of University of Victoria, for the phytohormone analysis. I am grateful to Marianne, Valérie and another team members from Ferme Onésime Pouliot (FOP), who have helped with the data collection for two major experiments. My big thanks go to FUNLAB, where I performed my gene expression studies and their members i.e., Dr. Suha Jabaji, Mamta Rani, Meha Sharma, Dina Saleh for sharing the healthy lab environment, consistent knowledge, and few sleepless nights in the lab.

I would like to thank McGill University and the great administrative staff including Susan Gregus and Erin Minnett for providing timely financial aids such as graduate assistantship, teaching assistantship and graduation excellence award. My special appreciation goes to the Plant Science Department for providing the travel grants to attend the conferences and present oral presentation of my research findings. I would also like to acknowledge the financial support provided for this research by Natural Sciences and Engineering Council of Canada (NSERC) and Ferme Onésime Pouliot.

Finally, my biggest thanks go to my roommates, my wife, my parents and the whole Sidhu family for always believing in me. My special thanks go to Jaspreet Virk, Rigve, Rahul, Harpreet and TJ for watering my plants and generously providing me the endless support. I would like to thank my wife for encouraging me during the tough times and always being there for me. I am sincerely thankful to my hardworking father and mother for their love and support. Finally, I would like to express my gratitude to all my friends, family, and colleagues for their tremendous contribution in science and in life. Your support has kept me going and this work would not have been possible without your inputs.

CONTRIBUTION TO ORIGINAL KNOWLEDGE

Preface

The following thesis work is presented in a manuscript-based format according to the "Guidelines Concerning Thesis Preparation" of McGill University. In this research study, I have identified several stimuli that can stimulate flowering particularly flower bud induction (FBI) for widely grown day-neutral cultivar 'Albion'. The light quality or wavelength containing increased blue along with far-red LEDs seems highly effective to stimulate FBI during the transplant production stage. Further, phytohormones and transcription levels of the genes that regulate FBI were studied in DN cultivars. The thesis mainly comprises three individual chapters (Chapter III-V), representing separate research manuscripts. Chapter IV is accepted for the publication in *Canadian Journal of Plant Science*. Detailed information regarding how different chapters are connected is mentioned in the connecting statement before each chapter. The general explanation of the thesis and the contribution of each author are mentioned in the following section.

Contribution to knowledge

The chapter of the present thesis explains the novel and original findings on the flowering response of DN strawberry plant in response to distinct environmental stimuli and listed below:

- Outlined the flower bud induction and development processes during the transplant production and growing season to advance the development of flower stalks that subsequently forecasts the yield potential of day-neutral strawberry plants.
- Defined the optimum management of photoperiod, nitrogen and light quality during transplant production that can trigger morphological processes such as flowering and vegetative growth.

- Far-red and blue LEDs at a ratio of 1:5, also referred to as dominant blue LEDs, were identified to considerably enhances the flower bud induction and development during transplant production for day-neutral plants. Supplementation of dominant blue LEDs coupled with long-day photoperiod and night interruption amplifies the light quality-induced effect on flowering.
- For the first time in the DN cultivar, the transcription of flowering genes i.e., FvFT1, FvSOC1 and FvTFL1 were studied in response to the combination of two narrow-band light sources. The flowering behavior may be associated with the increased transcript levels of these genes.
- The regulation of hormonal levels during flower bud induction was studied in day-neutral cultivars of woodland and cultivated strawberry. Increased levels of gibberellins (GA1 and GA7) and cytokinin (kinetin) in crown tissue seem to enhance flowering in response to light quality.
- Overall, the present study highlights the supplementation of dominant blue LEDs as a potential light source to improve flowering traits, extend the harvesting season and subsequently increase fruit production for day-neutral strawberry.

CONTRIBUTION OF AUTHORS

Varinder Sidhu was the principal researcher for each manuscript. The experimental designs were planned and designed entirely by him under the supervision of Dr. Valerie Gravel. He performed most of the field and laboratory experiments, collected data, analyzed, and wrote manuscripts. Dr. V. Gravel has provided constructive comments for manuscripts, technical assistance and funding for all three chapters.

Chapters III and IV were co-authored by Valérie Bernier-English and Marianne Lamontagne-Drolet, who assisted in setting up the experiments and data collection for the trials conducted at Ferme Onésime Pouliot. Chapter V is co-authored by Dr. Suha Jabaji, Department of Plant Science, McGill University, who has provided the technical facilities to carry out the gene expression study and insightful knowledge on the molecular biology procedures.

CHAPTER I: GENERAL INTRODUCTION

The cultivated strawberry (*Fragaria* x *ananassa*) is a perennial plant that belongs to the *Rosaceae* family. *F. ananassa* is a hybrid of two wild species i.e., *F. virginiana* and *F. chiloensis*. The woodland strawberry (*Fragaria* x *vesca*) was the first strawberry species cultivated in the 17th century. However, nowadays, it is not widely cultivated because of its small fruit size and delicate nature (Stewart and Folta 2010). The cultivated strawberry has replaced *F. vesca* in commercial production. *F. vesca* is considered as a model plant for the genetic study in *Rosaceae* family because its genome is simple and sequenced (Folta and Davis 2006; Shulaev et al. 2011).

The cultivated strawberry is an important fruit crop for the Québec and Canadian horticulture industry in terms of production and revenue. Québec is the third major strawberry producing state in North America, after California and Florida, representing 47% of Canada's total production (26248 metric tons) (Agricultural and Agri-food Canada, 2020). Traditionally, strawberry production has been mainly dependent on seasonal strawberry or short-day cultivars because of the abundant production, winterhardiness, and low cost associated with production (Black et al. 2002). However, the brief production period (4-6 weeks) has been a limiting factor for local growers. The introduction of day-neutral (DN) cultivars has steered strawberry production in Québec towards higher productivity. Unlike seasonal cultivars, DNs can produce flowers and fruits continuously as if conditions are appropriate for the plant growth, this resulting in the extension of the harvesting season.

In open field strawberry production, soilborne diseases severely impact the plant agronomic growth and cause significant fruit losses (Koike et al. 2010). As strawberry production continues to evolve, specialized growing techniques such as the use of umbrella-like rain shelters and soilless substrates are gaining popularity in Québec. The combination of these techniques has significantly

reduced the vulnerability of the crop to diseases and allowed a better distribution of the fruit production throughout the production season. Nowadays, these specialized systems are widely used in combination with DN cultivars to address the existing challenges (Neri et al. 2012). However, very little work has been done on DN strawberry production under these techniques in Québec conditions, despite their increasing use by local growers. Therefore, it is important to understand the physiological development of DN cultivars under these production systems to take full advantage of them.

Under Québec conditions, DN cultivar plants are raised under one set of controlled conditions during transplant production (usually August to November), followed by cold storage (December to May) and then transplants are transferred to umbrella shelters in May and the harvesting season starts in July until October. Consequently, DN cultivars are grown under two different environmental regimes. The production of high-quality transplants is the key pre-requisite to advance flower stalk development and improve the fruit yield (Durner et al. 2002). During the early transplant stage, plant architecture is considered an important indicator that explains the discrete distribution of reproductive and vegetative organs and their developmental stage (Massetani et al. 2011). Thus, it determines the plant quality during plant propagation and predicts strawberry fruit production. Plant architecture can be strongly modified by controlling the growing conditions during transplant production.

Strawberry flowering is divided into four different stages: floral initiation, flower bud induction, differentiation, and development (Durner and Poling 1985). Upon exposure to a stimulus, plant exhibit morphological changes that ultimately result in production of flower buds in the shoot apical meristem, recognized as flower bud induction (Durner and Poling 1985). Several stimuli such as photoperiod, temperature, nutrient availability, exposure to cold conditions and light

quality (wavelength) affect flower bud induction (FBI) in strawberry plants. In fact, multiple studies have been conducted in seasonal cultivars, where transplants are conditioned with inductive SD photoperiod, temperature, nitrogen (N) and distinct light wavelengths to stimulate FBI (Rowley et al. 2010; Durner et al. 2015; Wan et al. 2017). Acquiring knowledge of environmental conditions that modulate physiological processes leading to flower induction is unknown in day-neutrals. Therefore, it is important to understand the morphological responses of DNs under different environmental stimuli to enhance the plant quality and fruit production.

Light is the major environmental factor that controls plant morphogenesis, photosynthesis, and flowering time (Liao et al. 2014). Plants detect light conditions that include photoperiod, light intensity and quality (referring to wavelength) to adapt their growth and development (Kami et al. 2010). In general, light quality and photoperiod are more important factors than light intensity. Photoperiod is a reliable and predictable signal for plants that indicates seasonal changes from year to year. Thus, many plants utilize photoperiod information to forecast upcoming environmental conditions and accordingly, controls phase transition from vegetative to flowering (Thomas and Vince-Prue, 1997). In addition, light quality has been reported to stimulate early flowering in many plant species, however, its effect on flowering varies among plant species (Kim et al. 2008; Rantanen et al. 2014). In general, long-day (LD) plants require long daylight exposure followed by short period exposure to far-red (FR) and blue (B) light to induce flowering whereas short-day plants are less affected by light quality and require longer darkness to stimulate flowering (Thomas and Vince-Prue, 1997).

Most plants synchronize their life cycle according to seasonal changes and initiate flowering to ensure their survival and reproduction. Plants use diverse genetic networking that integrates environmental and endogenous signals to regulate flowering. In LD model plant

Arabidopsis thaliana, genetic pathways of flowering are mainly controlled by photoperiod, light quality, temperature, plant age and endogenous phytohormones such as gibberellins and auxin (Andrés and Coupland 2012; Rantanen et al. 2014; Kinoshita and Richter 2020). The flowering time is mainly governed by mobile proteins called florigen (floral inducer) and anti-florigen (floral repressors) that are produced in leaves and shoot apical meristem respectively under different environmental conditions (Higuchi 2018). In *A. thaliana*, two homologous proteins i.e., FLOWERING LOCUS T (FT) and TERMINAL FLOWER 1 (TFL1) which belong to the phosphatidylethanolamine binding proteins family (PEBP), acts as floral inducer and repressor genes, respectively (Tamaki et al. 2007; Corbesier et al. 2007). The molecular studies have identified FT/TFL1-like protein homologues in chrysanthemum and seasonal strawberry that are considered as the breeding target to control perpetual or continuous flowering (Higuchi et al. 2013; Mouhu et al. 2013). Flowering mechanisms have been extensively elucidated in seasonal cultivars (Rantanen et al. 2014; Yoshida et al. 2016). However, it is still unclear how floral inducer and inhibitor-like genes regulate flowering in DN cultivars.

Under floral-inductive conditions, axillary bud differentiation appears to be facilitated by endogenous growth-promoting hormones such as gibberellins (GAs), auxin (IAA), cytokinins (CK) and abscisic acid (ABA) in cultivated strawberry and sunflower (Eshghi and Tafazoli 2007; Kurepin et al. 2007; Hytönen et al. 2009). The involvement of GAs to control phase transition from vegetative to reproductive growth and to promote floral organ development is well known in LD plants species (Mutasa-Göttgens and Hedden 2009). CK concentration in the shoot apical meristem plays an important role in the flower induction process (Eshghi and Tafazoli 2007). Cytokinin-mediated morphogenesis and flowering is a complex process and seems to involve interactions with other hormones (Sweere et al. 2001). Furthermore, the involvement of IAA and

ABA during the transition from vegetative to reproductive is debatable as stimulating and repressive effects have been reported (Riboni et al. 2016). Multiple studies in the literature have demonstrated hormonal mediated flowering and morphogenesis among many species such as *Arabidopsis thaliana, Helianthus annuus* (Riboni et al. 2016). However, hormonal control of flowering remains elusive in DN strawberry cultivars.

OBJECTIVES AND HYPOTHESES

The main objective of this study is to identify the most effective environmental condition that can optimize the flower bud induction during transplant production and flower stalks development in DN strawberry plants grown in a soilless system. Primarily, we investigated the physiological and morphological changes of the strawberry plants in response to photoperiod, nitrogen, and light quality. Further, we explored the molecular and metabolic mechanisms to understand the FBI process.

OBJECTIVES

1. To evaluate the effect of fertilization that includes nitrogen (N) concentration, N sources and ratios of N and potassium (K) on flower bud induction for day-neutral strawberry grown in soilless systems.

2. To determine the optimum light conditions including photoperiod and light quality to produce day-neutral strawberry transplants that can enhance the development of induced flower stalks.

3. To understand the flowering mechanism of day-neutral strawberry plants by investigating the transcription of flowering-related genes and endogenous hormonal profiling.

HYPOTHESIS

1. Long-day photoperiod during transplant production and limited nitrogen during growing season stimulates flower bud induction and advanced development of flower stalks that can lead to harvesting season extension and increase fruit production.

2. Light quality comprising two narrow band light sources i.e., far-red and blue enhances flower bud induction for day-neutral strawberry plants during transplant production. The supplementation of suitable light wavelength during night interruption and extended photoperiod augment flowering rate.

3. The endogenous phytohormones such as gibberellins, auxin and cytokinin, and FT1/TFL1-like genes are involved in the flower bud induction process in response to environmental conditions for perpetual flowering accessions.

CHAPTER II: REVIEW OF LITERATURE

2.1 Strawberry production in Québec

The strawberry production has been considered as a prominent sector in the Canadian and Québec horticulture industry in terms of production and revenue. Québec is the third major strawberry producing region in North America after California and Florida, producing around 47% of Canada's total strawberries (Agriculture and Agri-food Canada. 2020). Until the 90's, strawberry production relied on seasonal cultivars, which produces fruit for a limited period (June-July). Canada's strawberry industry sector has been less competitive and compromises efficiency primarily due to the lower production from late July until October especially when both market demand and price are at their peak (Sterthem et al. 2013). As a result, Canada imports a large amount of strawberries from California.

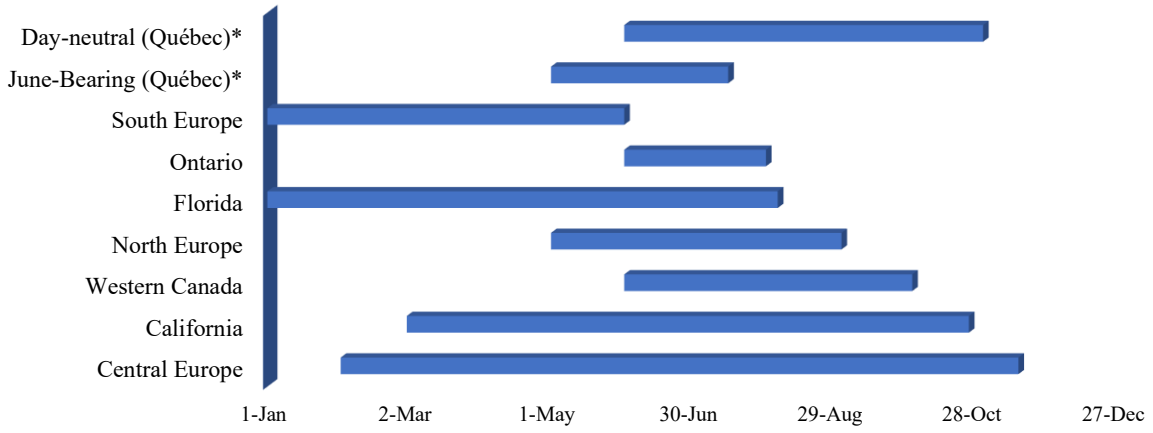


Figure 2.1 Typical strawberry production calendar for different regions. * represents production calendar for cultivars.

The arrival of day-neutral cultivars made breakthroughs for Canadian growers. DNs can produce flowers and fruits regardless of photoperiod throughout the production season if the conditions are appropriate for plant growth. Further, the commercial strawberry cultivation sector continues to evolute and specialized production systems such as umbrella shelter and soilless substrates have been developed. These techniques protect the crop from heavy rainfall, frost protection and soil-

borne diseases and thus, facilitates the extension of harvesting period and improves the fruit yield (Ballington et al. 2008; Claire et al. 2018). New production systems are gaining popularity in Europe (specifically France and the Netherlands) and Québec especially in combination with DN cultivars ((Neri et al. 2012; Claire et al. 2018).

2.1.1 Strawberry transplant production

In Canada, commercial strawberry producers use either bare-root or plug transplants. The bare-root transplant is the traditional propagation technique in which plants are harvested dormant from the soil during the winter season and refrigerated in cold temperature (-1.5°C). Whereas plug transplants have unique growth as they are grown in distinct substrate media with an intact rooting system. The development of plug transplant techniques is leading to the phasing out of bare root for strawberry production worldwide (Durner et al. 2002). The plug transplant exhibits early plant growth and provides advanced flowering in comparison to bare-root transplants (Hochmuth et al. 2006). Although most growers in Québec still use bare-root transplants, however, nowadays, plug plants are gaining popularity because of the benefits associated with their use.

2.1.2 Soilless strawberry production

Open field strawberry production is challenging due to the crop vulnerability to soil-borne diseases and pathogens. The strawberry growers worldwide heavily rely on chemical pesticides to control soil-borne diseases and weeds under open field strawberry production (Cecatto et al. 2013). In 2012, strawberry was characterized as one of the four crops with maximum pesticide contamination (Cecatto et al. 2013). Soilless system eradicates the dependency of chemicals and has the potential to improve the sustainability of strawberry crop (Paranjpe et al. 2008). Strawberry production under modified cultural practices such as soilless media, have been proposed to maximize the yield and improved control against pests and diseases.

2.2 Distinct flowering species and physiology of strawberry

Strawberry genotypes are categorized based on their flowering response to photoperiod such as seasonal cultivars, also known as short-day, long-day, ever-bearing (EB) and day-neutral. The EB and DN cultivars are generally mixed and called interchangeably, however they are quite different from each other. EB cultivars produces the first crop in early summer and the second in late summer over two months gap between. Whereas DNs consistently produces berries throughout the entire summer until late fall if conditions are appropriate (Rubinstein 2015). Seasonal strawberry plants initiate flowering during early spring and late fall when the photoperiod is shorter than 14h and commence yielding fruits during June-July. DNs produce flower buds irrespective of day length starting from May until October (Rubinstein 2015). Because of their repetitive flowering behavior, DNs are known as remontant cultivars (Bradford et al. 2010). Previous studies have suggested that DNs behaves like quantitative long-day plants at intermediate temperatures of 22/18°C (Day/Night T°C) and qualitative long-day plant at higher temperatures of 30/26°C (Day/Night T°C) (Sønsteby and Heide 2007; Nishiyama and Kanahama 2000). Qualitative long day plant means that DN cultivars produce flowers only under LD photoperiod at higher temperature whereas at intermediate temperature, DN cultivars may flower under SD photoperiod. However, more flowers will be produced under LD photoperiod, and therefore they are called quantitative long-day plants (Sønsteby and Heide 2007).

Nowadays, DN cultivars are increasingly grown in Québec. Growers, who produce their own transplants, harvest the runners from the previous crop during July to Aug and provide favorable conditions for the transplant to maximize the FBI during fall (Figure 2.2). During winter season, transplants are kept inside cold storage until April and May depending upon the weather

conditions. Transplants complete the following stages of flowering such as differentiation, development and fruit harvesting from May to Oct.

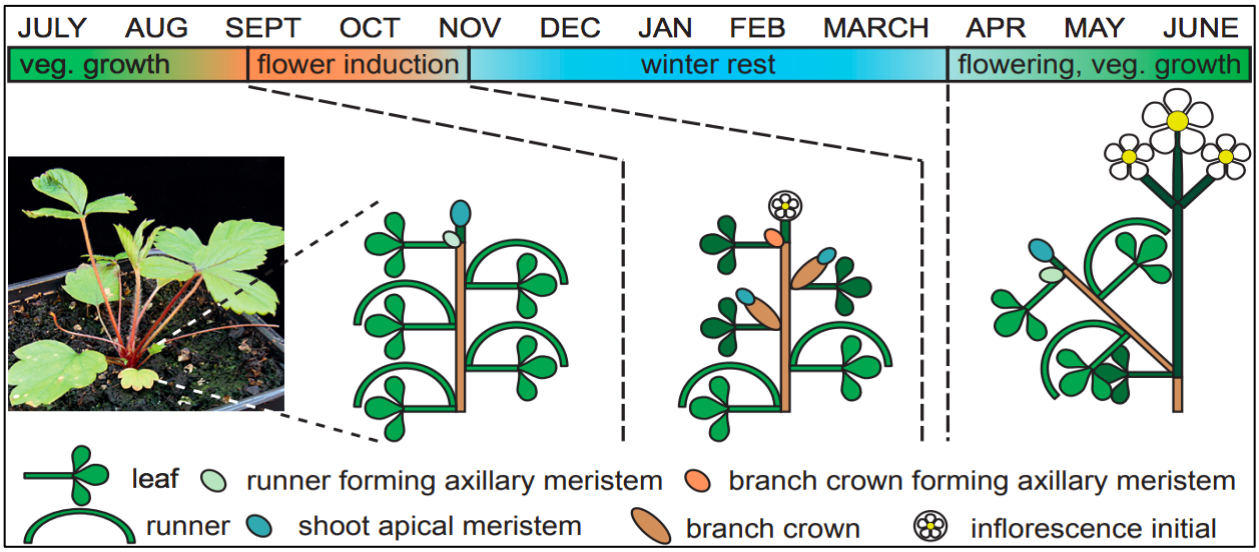


Figure 2.2 Flowering cycle of day-neutral strawberry under Québec conditions (Adapted from Mouhu et al. 2013)

2.2.1 Physiology of strawberry

All strawberry species are herbaceous perennials, produced through both sexual and asexual reproduction via flowering and runners, respectively (Johnson et al. 2014). Strawberries have a thick stem called the crown, which develops very close to the soil and has both aerial and underground parts. During the vegetative phase, the crown apical meristem is composed of internodes, and each node differentiates into one trifoliate leaf and an axillary bud (Figure 2.3). Axillary buds may develop into flower stalks or runners. The fate of the axillary meristem is dependent upon environmental conditions. Runners are the horizontal stems growing above the ground with long internodes, establishing genetically identical daughter plants. In contrast, branch crowns have short internodes, and develop into terminal inflorescence. During LD photoperiod, seasonal strawberry plant promotes vegetative growth and axillary buds differentiate into runners. However, during SD photoperiod (fall or spring), axillary buds develop into branch crowns. In contrast to seasonal cultivars, DNs produces new flowers under longer photoperiod and higher

temperatures (22-26°C), usually experienced during the summertime in Québec. In these conditions, DN plants produce repetitive cycles of flowering until October (Nishiyama and Kanahama 2000; Sønsteby and Heide 2007).

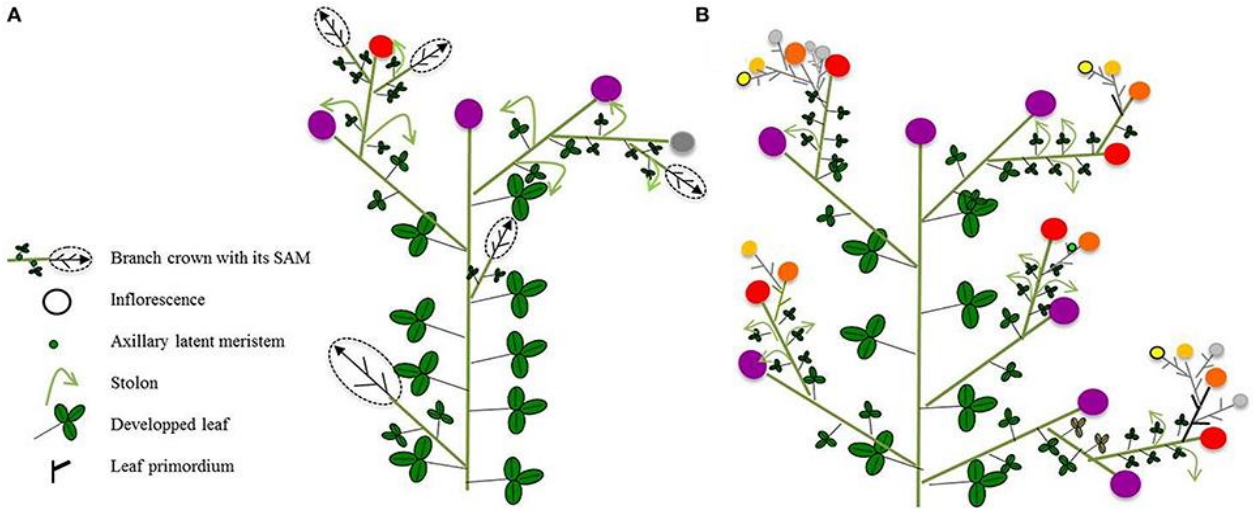


Figure 2.3 Strawberry plant physiology. **A.** For a once flowering genotype, in which axillary meristem develops into branch crown or stolons. **B.** For a continuous flowering genotype, in which axillary meristem develops into new inflorescence (Taken from Costes et al 2014). Colors of inflorescences illustrates the developmental stage; purple when already harvested, red when fruits are mature, dark orange when fruits are developing, light orange or yellow when inflorescences are newly emerged, gray when inflorescences not likely to emerge.

Once the apical meristem of the crown develops into a terminal inflorescence, continued vegetative growth is taken over by the uppermost lateral buds. These dominate the lower lateral buds and appear like a monopodial inflorescence. However, the growth habit of strawberry is characterized as sympodial inflorescence because after the lower axillary meristem differentiation, a new terminal bud may develop into secondary and tertiary flower stalks from the main axis (Heide et al. 2013). The physiological development of flower bud in response to environmental conditions is the prerequisite that determines the fruit potential. During FBI, the transition of shoot apical meristem advances into inflorescence, which overwinters and complete its growth during the following spring. Later, the inflorescence meristem grows into flower buds, and then flower buds differentiate into primary, secondary, or tertiary flowers. Eventually, the central flower bud turns into the largest fruit of the flower stalk while secondary and tertiary buds develop into smaller

fruits (Taylor 2000; Darnell 2003). Plant with one or two side crowns in addition to the main crown is optimal as it accounts for significant yield potential. As described by de Camacaro et al. (2002), a higher number of branch crowns contributes to higher fruit yield for DN strawberry plants, although, excessive growth (6-7 branching crowns per plant) may lead to the significant reduction in fruit size.

Photoperiod, light quality, temperature, and nutrient availability are dominant factors that control flowwering (Sønsteby and Heide 2007; Bradford et al. 2010). Studies showed that crown branching is stimulated by long photoperiod and higher temperature (22-26°C) in DN strawberries. Moreover, it was also recognized that flowering in DN strawberries can be promoted under narrow-band light source of far-red (740 nm) and blue (455 nm) light, as reported by Rantanen et al. (2014) and Nadalini et al. (2017). It has been reported that an increased level of nitrogen (N) delayed the flowering in seasonal strawberry (Wan et al. 2017). Supplemental lighting during the middle of night, called night interruption, stimulates flowering in DNs (Van Delm et al. 2013). Based on previous studies, it is indicated that there might be multiple pathways that regulate flowering in *Fragaria* species. In the present study, we mainly focus on how light quality, photoperiod and nitrogen effect morphological growth of strawberry based on their metabolic changes and gene regulation.

2.3 Environmental control of lowering

2.3.1 Photoperiod and light quality

Light regulated flowering is a well-known mechanism (Suárez-López et al. 2001; Koskela et al. 2012). The environmental control of flowering in strawberry is mainly controlled by photoperiod and light quality (Sønsteby and Heide 2007; Rantanen et al. 2014. Seasonal changes in day length are a reliable signal for the plant that indicates time of the year. Based on daylength and spectrum

perceived by the photoreceptors, plant generates its internal circadian clock rhythm that controls the physiological changes in plants, and this process is called photoperiodism (Garner and Allard, 1920). The internal circadian rhythm can either be gene expression patterns or the physiological changes repeated every 24h cycle and controlled by the internal plant biological clock. Photoperiod and light quality have been extensively used in horticultural crops to control plant growth and flowering, especially in strawberry (Darnell et al. 2003).

Light quality is the composition of light wavelengths, another important factor that contribute to flowering. Visible light known as the photosynthetically active radiation (PAR) ranges from 430 nm to 730 nm and is mainly used for photosynthesis and photomorphogenesis (Terashima et al. 2009). Previous studies reported that, during the plant nursery stage, the application of far-red (FR; 740 nm) and blue (B; 450 nm) LEDs at the end of the natural light promotes flower bud initiation (Rantanen et al. 2014). An alternative study showed similar results as LD exposure of single peak B and FR light advances the flowering in comparison to fluorescent and red light (Yoshida et al. 2016). Several reports show that a combination of different light wavelengths stimulates flowering in *Arabidopsis* (Eskins 1992), *Salvia* plant (Heo et al. 2006) and strawberry (Naznin et al. 2016). In addition, Choi et al. (2013) suggested that strawberries grown under mixed red-blue LEDs produced higher fruit yields and increased the content in fructose.

For each specific spectra of wavelength have distinctive effects, even a small difference (20-40 nm) in single peak wavelength can cause a significant difference in plant growth and development (Goto et al. 2013). Flowering of strawberry in response to single peak lights is extensively studied, however, physiological development of flower bud in response to combination of lights is unknown in strawberries, specifically in DNs. It has been reported that far-red (740 nm) and blue (455 nm) lights produced maximum number of flowers (Rantanen et al. 2014). It would be

interesting to know how the combination of different ratios of FR and B lights affect plant physiology, specifically flower bud induction during the transplant production.

2.3.1.1 Photoreceptors

Plants utilize light as a fundamental energy source via photosynthesis. Besides photosynthesis, light also regulates plant physiological development with the help of photoreceptor. Photoreceptors are light-sensitive proteins that sense wavelengths, quantity and duration of light and control gene expression via protein interaction and regulate physiological development of plant. Phytochrome (the first photoreceptor discovered in plants), cryptochrome and phytotropins are major photoreceptors that have been identified based on the light wavelength they perceive (Møller et al. 2002). Phytochromes perceive R/FR light (600-750 nm) and cryptochrome, phototropin and recognized ZTL-protein families (i.e., ZEITLUPE (ZTL), LOV-KELCH protein (LKP2) and FLAVIN-BINDING, KELCH REPEAT F-BOX_1 (FKF1) protein perceive blue light (320-500 nm) and triggers plant response (Li et al. 2011).

Phytochromes are soluble proteins, synthesized in the cytoplasm, where their subunits (i.e., apoprotein and chromophore) are assemble (Li et al. 2011). Phytochromes exist in two conformational forms: phytochrome red ($P_{R)}$ and phytochrome far-red (P_{FR}). It is expressed in many plant tissues at different developmental stages (Schäfer and Bowler 2002). Both P_R and P_{FR} are sensitive to red and FR light respectively, are interconvertible depending on the daylength and light quality (Rockwell et al. 2006). The photoreceptor senses daylength and light wavelength, leading to phytochromic structural changes (P_R to P_{FR}). These changes regulate the light-mediated development, called photomorphogenesis, includes seed germination, plant architecture, senescence and determines the timing of the transition from the vegetative to the reproductive phase of plant development. For instance, during daytime, sunlight enriched with red light converts

rapidly from P_R to P_{FR} (biologically active form) and as night approaches, FR light or darkness change the protein slowly to biologically inactive form P_R (Rockwell et al. 2006).

Earlier, it was discovered that day length controls flowering (Garner and Allard 1920) and accordingly plants were classified into short-day and long-day plants based on their photoperiodic requirements. Later it was found that night length plays a key role to trigger flowering due P_{FR} abundance accumulated during night length (Hamner and Bonner 1938). Higher accumulation of active form of phytochrome (P_{FR}) stimulates flowering in long-day plants, whereas inhibits in short-day plants (Hamner and Bonner 1938). Further, it was reported that supplemental lighting during the dark period induces the flowering in long-day plants because night interruption (NI) converts inactive form (P_R) back to its active form (P_{FR}) (Hamamoto et al. 2005; Meng and Runkle 2017).

2.3.2 Fertilization

During the plant lifecycle, the transition from vegetative to reproductive state requires optimal environmental conditions such as photoperiod, temperature, water supply and macronutrients, especially nitrogen (N). N has been directly connected with transition and significantly affects flowering time in strawberry (Nestby and Tagliavini 2005; Andriolo et al. 2011; Sønsteby et al. 2013). Day-neutral strawberry are increasingly grown in soilless systems which require new fertilizer standards for adequate plant growth and development. DN cultivars demand more fertilizers compared to the seasonal cultivars because they produce flowers and fruits throughout the season (Hoover et al. 2017). As previously reported, N and K control the vegetative and reproductive equilibrium of the strawberry plant, whereas phosphorus (P) has no significant effect on flowering (Heide et al. 2013; Ijaz et al. 2016).

During flower bud initiation, when the apical meristem growth terminates, the continuous supply of N encourages the lateral meristem growth and increases crown branching (Massetani et al. 2011). N influences the flower bud differentiation depending on the time of application and the concentration used (Savini and Neri 2003). Studies showed that a low amount of nitrogen induces flowering. For instance, Wan et al. (2017) demonstrated that an N application of 0.1 g/plug transplant immediately after an inductive photoperiod provides early flowering and resulted in advanced fruit ripening in seasonal strawberry. Contrastingly, excessive N applications primarily promote the vegetative growth of the plant during the transplanting stage (Kirschbaum et al. 2010). Also, low N concentrations (e.g., 0.05g/plug plant) may delay fruit formation because floral organs require nitrogen during flower differentiation (Yamasaki and Yano 2008). Corresponding results have been reported in *Arabidopsis*, as plants receiving low nitrate flowered in advance compared to those receiving higher nitrate (Castro Marín et al. 2011). The optimal N application largely depends upon the stage of the transplant, i.e., early-stage transplant requires less nitrogen whereas the blooming phase requires the highest amount of nitrogen (Durner et al. 2015). Timely application of N at an early stage can enhance flower bud induction, thus, the yield potential of strawberry plants (Sønsteby et al. 2013).

Nitrogen fertilizer findings in seasonal strawberry have shown variable results based on the concentration used. Optimal N concentrations that control floral bud induction is still unknown for DN strawberry plants despite their increasing use by Québec farmers. Considering the importance of N fertilizer for the onset of flowering in strawberry, no potential N-regulated flowering pathway has been explained yet, perhaps because nitrogen majorly controls the vegetative growth of the plant rather than the reproductive growth. This study focuses on how nitrogen shapes up the transition from vegetative to reproductive growth of strawberry plants.

2.4 Molecular control of flowering

2.4.1 Flowering pathway in *Arabidopsis thaliana*

The flowering pathway has been widely studied in LD model plant *Arabidopsis thaliana* (Suárez-López et al. 2001). Although *Arabidopsis* and strawberry belong to distinct families, they have certain similarities; both are dicots, behave as quantitative LD plant (based on plant type) and exhibits parallel flowering gene network (Suárez-López et al. 2001, Mouhu et al. 2009; Koskela, 2016; Kurokura et al. 2017). It demonstrates that the co-linearity between two plant species might be helpful to understand the flowering mechanisms in *F. vesca.*

The photoperiod regulated flowering and external coincidence model demonstrates how flowering is connected to environmental stimuli (Srikanth and Schmid, 2011). Based on the model, flowering takes place when external factors interact with plants to generate an internal circadian rhythm. Circadian clock regulated genes produce rhythmic expression of CO, the central transcription factor required for promotion of the flowering gene FT under LD (Valverde et al 2004; Kurokura et al. 2017). Photoperiod and light quality primarily affect the stability of the CO protein. Stabilization of the CO protein is controlled by the *CYCLING DOF FACTORs* (CDFs), which bind rapidly onto the CO transcription promotor and repress flowering during the morning (Fornara et al. 2009, Golembeski et al. 2014). The expression of CO is facilitated in the afternoon by circadian clock regulated proteins (i.e., *GIGANTEA* (GI) and *FLAVIN-BINDING KELCH REPEAT F-BOX*$_1$ (FKF$_1$)). The GI-FKF1 complex formed causes the degradation of CDFs and induces flowering through CO-FT regulation (Imaizumi et al. 2005). During LD conditions, CO repressor CDF is released through the GI-FKF1 complex. During SD photoperiod, FKF1 and GI expression stays very low (Fornara et al. 2009, Golembeski et al. 2014). Unlike PHYA, CRY2 and FKF1, PHYB delays flowering by destabilizing the CO protein (Endo et al. 2013). It was reported that red and

blue light antagonistically regulate CO mRNA transcription in *Arabidopsis* (Valverde et al. 2004). For instance, red light delays flowering thru the destabilization of the CO protein by PHYB transcript, while B and FR lights induce flowering as they activate FKF_1 (Valverde et al. 2004).

Beyond its role as a nutrient, N has been characterized as a signaling molecule that controls flowering time. Transcriptomic studies have revealed that nitrate outlines the onset of flowering and acts as signal (Castro Marín et al. 2011). Studies suggests that in *Arabidopsis*, low nitrate induces the expression of central integrators *CONSTANS* (CO), which upregulates floral integrator gene *FLOWERING LOCUS T* (FT), *LEAFY* (LFY), and *APETELLA1* (AP1) (Abe et al. 2005). It represses the MADS-box gene FLC (*FLOWERING LOCUS C*). FLC prevents the transcriptional activation of floral integrator genes FT and *SUPRESSOR OF OVEREXPRESSION OF CONSTANS* (SOC1) and thus, represses flowering (Castro Marín et al. 2011; Liu 2013). There are also reports showing that low N stimulates CRY1 (*CRYPTOCHROME1*) protein abundance, and subsequently affect the expression of central oscillator genes i.e., *LATE HYPOCOTYL* ELONGATED (LHY), *CIRCADIAN CLOCK ASSOCIATED1* (CCA1), *TIMING OF CAB EXPRESSION1* (TOC1) and GIGANTEA (GI)) and leads to a stimulation of flowering through the GI-CO-FT hierarchy (Yuan et al. 2016).

The circadian oscillator genes control the CO protein in vascular tissues of the leaves during the inductive photoperiod and upregulates FT by binding to its promoter (Suárez-López et al. 2001). During the floral-inductive period, FT protein is transported from the leaf to the shoot apical meristem (SAM) where it forms a complex with the *bZIP* transcription factor FD and the FT-FD complex acts as transcriptional activators that targets SOC1 and floral meristem-identity genes *APETALA1*(AP1), LEAFY (LFY) and *FRUITFUL* (FUL) (Abe et al. 2005). As a result, once meristem identity genes are activated, the SAM is conclusively devoted to flowering.

2.4.2 Flowering pathway in strawberry species.

Due to the complex genome of the cultivated strawberry (*F.* x *ananassa*), woodland strawberry (*F. vesca*) has been used to elucidate the molecular control of flowering in *Fragaria* species. Earlier studies showed that seasonal regulation of flowering and runner formation is caused by the *SEASONAL FLOWERING LOCUS* (SFL) gene (Albani et al. 2004). SFL was recognized as an *Arabidopsis* homolog of the *TERMINAL FLOWER 1* (FvTFL1) (Koskela et al. 2012). FvTFL1 is a photo-periodically regulated floral inhibitor. Although LD photoperiod upregulates FvTFL1 expression in shoot tip, thus, inhibiting flowering in seasonal cultivar of *F. vesca*, SD conditions downregulate FvTFL1 (Mouhu et al. 2009). Koskela et al. (2012) demonstrated that two base pair mutations in FvTFL1 prevent LD suppression of flowering and suggested that a single gene modification could reverse the photoperiodic requirement for flowering in seasonal strawberry. Mouhu et al. (2013) stated the functional analysis of the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (FvSOC1) gene in short day strawberry. The study suggested that FvFT1 mediates the photoperiodic regulation of FvSOC1, and it further upregulates the expression of FvTFL1, which suppresses flowering under LD photoperiod (Mouhu et al. 2013). Interestingly, when FvFT1 (RNAi) was silenced, the expression of FvSOC1 was decreased and similarly, FvTFL1 expression was downregulated and triggered photoperiod independent flowering (Mouhu et al. 2013). These observations propose a FvFT1-FvSOC1-FvTFL1 pathway, that is activated under LD photoperiod and represses flowering in short day strawberry (Figure 2.4).

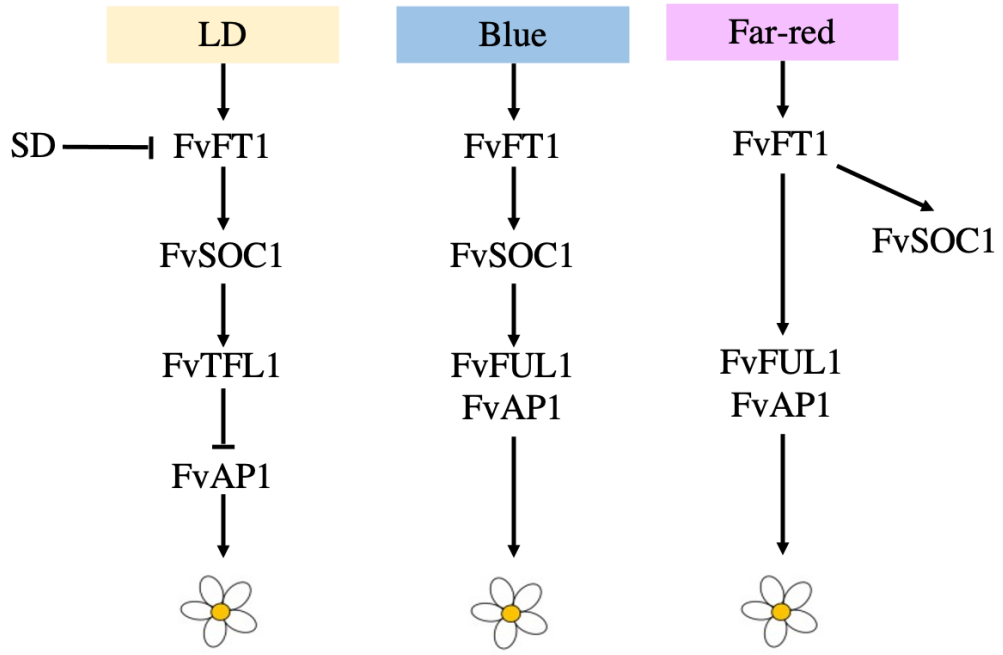


Figure 2.4 Photoperiod and light quality regulation of flowering in *Fragaria vesca*. (Adapted from Mouhu et al. 2013; Rantanen et al. 2014). Arrows indication activation and bars indicate repression. LD and SD represent long-day and short-day photoperiod respectively.

Studies have reported that perpetual flowering accessions have non-functional FvTFL1 protein, causing continuous flowering even under LD photoperiod for seasonal cultivars (Iwata et al. 2012; Koskela et al. 2012). In both seasonal and perpetual flowering accessions, putative floral meristem identity genes FvAP1 and FvFUL1 were upregulated during flowering time (Koskela et al. 2012; Mouhu et al. 2013).

Nakano et al. (2015) and Koskela et al. (2016) revealed the molecular pathways regulating flowering in cultivated strawberry and reported comparisons to the woodland strawberry. Similar to *F. vesca* (FvTFL1), TFL1-RNAi silencing in short-day cultivar "Elsanta" (*F.* x *ananassa*) produced daylength independent perpetual flowering, suggesting that TERMINAL FLOWER1 is a breeding target for perpetual flowering in cultivated strawberry as well (Koskela et al. 2016). As reported in *F. vesca*, the FvFT1-FvSOC1-FvTFL1 pathway is activated under LD photoperiod for *F.* x *ananassa*. In contrast to that, higher expression of FaFT1 and FaSOC1 was observed under LD photoperiod although no linear response of FaTFL1 was recorded in all cultivars and

photoperiods (Koskela et al. 2016). This indicated that FaTFL1 transcription occurs independently of FaFT1-FaSOC1 segment. Like FvFT1, FaFT1 is expressed in leaves, but FaFT3 was reported to have a crucial role in controlling flowering under SD photoperiod and low temperature. Higher FaSOC1 expression was observed in apical meristem although, it decreased with a two week-long SD treatment and remained low and again increased when subjected to LD photoperiod.

2.4.3 Light quality regulation of flowering

In addition to the photoperiodic pathway, flowering time is also regulated by light quality at the molecular level, as reported by Kim et al. (2008). They indicated that plants grown under FR enriched lights have higher accumulation of CO protein, which activates SOC1 through FT1 to promote flowering in *Arabidopsis* (Yoo et al. 2005: Mouhu et al. 2013). In *Arabidopsis*, PHYA and CRY perceives FR light signals and induce flowering, whereas PHYB mediates contrasting signal (red light) that mainly leads to the inhibition of flowering (Mockler et al. 1999; Valverede et al. 2004).

In another study, Rantanen et al. (2014) explained how FvFT1 and FvSOC1 control flowering under different light wavelengths. Plants flowered independently of FvSOC1 under far-red light, whereas FvSOC1 showed a definite role in flowering in the presence of blue light. Furthermore, the expression of FvFT1 and FvSOC1 correlated negatively with flowering due to the higher accumulation of FvTFL1 in the shoot tip in seasonal accession, speculating that FvFT1 acts as anti-florigen in the presence of FvTFL1 (Rantanen et al. 2014).

Rantanen et al. (2014) showed that single narrow-band light source of blue (455 nm) and FR (740 nm) LEDs promote flower induction, however, red (R) light has a contrasting effect in woodland strawberry (*F. vesca*). Yanagi et al. (2016) revealed that FR light induces flowering in *F. chiloensis* under LD photoperiod. For example, a low R/FR ratio promotes flowering in long-day plants such

as *Arabidopsis* (Hori et al. 2011), *Stellaria longipes* (Kurepin et al. 2007) and *Pyrus pyrifolia* (Ito et al. 2014). Studies using combinations of far-red and blue light can affect flower bud induction in strawberry is limited. Consequently, it is important to understand the effect of far-red and blue light on flowering time and the expression level of flowering related genes of DN *F. vesca*.

2.4.4 Hormonal regulation of flowering

Gibberellins (GAs) are large family of tetracyclic diterpenoid plant hormones that regulate many aspects of plant growth and development such as seed germination, cell division and expansion, flower induction and fruit senescence (Goldberg-Moeller et al. 2013; Mutasa-Göttgens and Hedden 2009). Although, 136 diverse GAs have been identified from bacteria, plants and fungi, but, few GAs have biological functions. For instance, GA_1 regulates flowering and GA_4 controls strawberry fruit size and quality (Csukasi et al. 2011). The bioactive form of GAs is derived from diterpene precursor geranylgeranyl diphosphate mainly by three enzymes i.e., GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox), and GA 2-oxidase (GA2ox). GA3ox and GA20ox catalyzes the activation of GAs, whereas GA2ox catalyzes the deactivation of GAs biosynthesis (Mutasa-Göttgens and Hedden 2009). Overall, these enzymes are responsible for the biosynthesis of gibberellins at specific site, where it regulates different biological processes.

The ability of gibberellins to enhance flower development and bolting (production of the flowering stem) under non-floral inductive conditions contributed to the realization that GAs essentially acts as endogenous floral regulator (Mutasa-Göttgens and Hedden 2009). In addition, GAs have been reported to control the phase transition from the vegetative to the reproductive phase in long-day and biennial plants (Mutasa-Göttgens and Hedden 2009).

Studies have reported that gibberellins concentration is regulated by environmental conditions such as photoperiod and light quality (Rieu et al. 2008; Kurepin et al. 2007). For instance, GAs

concentration increased substantially in apical meristem when long day plants were transferred from a SD to a LD photoperiod (Rieu et al. 2008). Further, the transcript levels of GA20ox and GA3ox validated the activation of GAs biosynthesis in the plant (Rieu et al. 2008). Higher levels of GA20ox and GA3ox during the vegetative and flowering stage showed that GAs is the absolute requirement for the flower initiation process (Mutasa-Göttgens and Hedden 2009). Both FR light and gibberellins have significant effects on the transition from the vegetative to the reproductive phase, particularly in long-day plants. For instance, FR light increased GA levels in *Vigna sinensis* (Martínez Garcia et al. 2000) and in *Eustoma grandiflorum* (Takemura et al. 2015). Similarly, *Chrysanthemum* showed reduced levels of active GA_1 and consequently, delayed flowering in FR deficient light (Maki et al. 2002).

In *Fragaria* species, the axillary bud differentiates into either runner or branch crown (Hytönen et al. 2009). Earlier, Guttridge and Thompson (1964) anticipated that a mobile hormone is produced under LD conditions in seasonal *F. vesca* that promotes petiole elongation, runner formation and inhibits flowering. Later, this outcome was mimicked by GA application. Hytönen et al. (2009) suggested that Pro-Ca (prohexadione-calcium), inhibitor of GA biosynthesis, blocks the functioning of the enzyme GA3ox leading to decreased levels of GA_1 and indicating that GA plays an important role in axillary bud differentiation in short-day strawberry as well.

Cytokinins (CK) are the important plant hormones that controls multiple biological process including differentiation of vegetative meristem, regulation of shoot apical meristem (SAM), flower initiation and accelerated inflorescence (Yamasaki and Yamashita 1993; D'Aloia et al. 2011). CK concentration changes significantly during the vegetative to the reproductive stage transition in strawberry (Yamasaki and Yamashita 1993; Eshghi and Tafazoli 2007). Flowering regulation of CK biosynthesis has been reported in *Arabidopsis* where elevated levels of CK in

SAM suggest that it might be responsible for flowering. D'Aloia et al. (2011) demonstrated that CK induce flowering in *Arabidopsis* through the transcriptional activation of TSF (the FT paralogue of TWIN SISTER OF FT), independently of FT. CK biosynthesis is primarily catalyzed by the adenosine phosphate-isopentenyl transferase enzyme (IPT) (AtIPT1 and AtIPT4, gene family reported in *Arabidopsis*) which regulates the expression of CK synthesis (Hirose et al. 2008). In addition to GAs, light quality has been reported to stimulate apical meristem differentiation through increased levels of endogenous CK in *Chrysanthemum* (Dobisova et al. 2017). Light quality dependent regulation of CK and GAs levels in strawberry remains elusive. It is important to investigate how different light wavelengths influence GAs and CK concentration in strawberry and correlate with flowering.

CONNECTING STATEMENT FOR CHAPTER III

As reviewed in Chapter II, the strawberry production sector continues to evolute and in recent years, Québec has implemented new production technologies such soilless system and umbrella shelters especially for the cultivation of day-neutral cultivars to maximize the crop productivity. The challenges remain to control the growing conditions under these production systems. Chapter III contains information about the potential growing conditions for day-neutral cultivars under local conditions that can considerably increase strawberry production. It explains how photoperiod and nitrogen fertilization practices can be manipulated in order to maximize the flower bud induction, that leads to the advanced development of flower stalks and increases fruit production for widely grown day-neutral cultivar. This study describes how different nitrogen forms and nitrogen: potassium ratios control the flower bud induction during transplant production for 'Albion' cultivar.

CHAPTER III

Optimization of photoperiod and nitrogen conditioning to enhance flowering and fruit production for strawberry grown under an umbrella shelter

Varinder Sidhu[A], *Valérie Bernier-English*[B], *Marianne Lamontagne-Drolet*[B] *and Valérie Gravel*[A]

[A]Department of Plant Science, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec, Canada

[B]Ferme Onésime Pouliot Inc., Saint-Jean-de-l'Île-d'Orléans, Québec, Canada

3.1 Abstract

Photoperiod and nitrogen (N) are important factors that control flowering and fruit production in cultivated strawberry (*Fragaria* x *ananassa* Duchesne). Under Québec conditions, day-neutral (DN) cultivars are increasingly grown in soilless media under umbrella shelters because they protect crop from heavy rainfall, chilling stress, and soil-borne diseases, which results in longer harvesting period and improved fruit production. However, systematic evaluation of photoperiod and N conditioning for DN cultivars under these specialized techniques remains limited. Over 2-years, three individual experiments were conducted to optimize the photoperiod, N concentrations, different N forms (ammonia: nitrate; NH_4^+: NO_3^- ratios) and N interaction with potassium (K) to improve the overall performance of the 'Albion' cultivar. Results showed that plants exposed to long-day (LD) conditions such as extended photoperiod and night interruption showed no effect on flower development, however, enhanced flower bud induction (FBI) during the later stage of the growing season. Limited N supply advances the FBI, however, once the plants have commenced FBI, N levels should be augmented to ensure the successful differentiation. Like long-day cultivars, flowering behavior for 'Albion' cultivar can be manipulated with photoperiod and

N conditioning and lead to extended harvesting season. 20:80 ratio (NH_4^+: NO_3^-) in the nutrient solution produced the best results to maximize early flower bud induction. Early flowering remained unaffected in transplants supplied with different N:K ratios. Taken together, our study highlights the important factors such as photoperiod and different N forms that can manage flowering for DN cultivar grown in soilless system under umbrella shelter.

Keywords

Day-neutral, Nitrogen, Flower bud induction, Soilless culture, Incandescent light.

3.2 Introduction

Québec is the highest strawberry producing province in Canada (26248 metric tons), representing 47% of the entire commercial production for Canada (Agriculture and Agri-food Canada. 2020). Short-day or seasonal cultivars are mainly known for their winter hardiness and low establishment cost (Black et al. 2002). However, their brief production period (4-6 weeks) has been a limiting factor for local growers. Introduction of day-neutral (DN) cultivars in recent years has steered the strawberry cultivation in Québec towards higher productivity. Unlike seasonal cultivars, DNs can produce flowers and fruits continuously if conditions are appropriate for plant growth. Under local conditions, this resulted in the extension of the harvesting season and in uninterrupted fruit supply until the first frost.

As the commercial strawberry cultivation sector continues to evolve, specialized production systems have been developed to extend the harvesting period, to provide frost protection and improve the fruit yields. New production systems such as umbrella shelters and soilless substrates are gaining popularity in Europe (specifically France and the Netherlands) and Québec especially in combination with DNs cultivars (Neri et al. 2012; Claire et al. 2018). Umbrella shelters are structures that protect the crop from heavy rainfall, frost or chilling stress. Most importantly, it

facilitates harvest period extension by 3-4 weeks when market value of the crop is at its premium (Claire et al. 2018). These structures are economically sustainable and creates favorable microclimates concerning relative humidity and ventilation compared to greenhouses and tunnels (Xu et al. 2013; Inada et al. 2005). Umbrella shelters seems to be a practical alternative for the cultivation of berry crops in North America where heavy rains occur during the production season. In open field systems, strawberry plants are generally grown on raised beds. The major challenge associated with production systems is excessive use of pesticides being used to eradicate the soilborne disease that severely impacts the plant agronomic growth and causes significant crop losses (Koike et al. 2010). Until imposed restriction on chemicals, growers heavily relied on pesticides to control soilborne diseases, citing strawberry as one of the four crops with the maximum pesticide contamination in 2012 (Cecatto et al. 2013). Strict regulatory constraints on chemicals have dramatically increased use of soilless culture in Europe and North America (Paranjpe et al. 2008). Studies have shown that soilless media is a prevailing alternative for strawberry productions as it significantly reduces the usage of chemicals and produces higher yield with improved quality (Kempler 2002; Nafiye and Gubbuk 2015). However, there is still a need to understand the physiological development of plants under these production conditions to further expand it.

Under specialized cultivation systems in Québec, DN strawberry transplants are grown under one set of environmental conditions during nursery stage, followed by cold storage, thereafter, being transferred to umbrella shelter for the production season. The production of high-quality transplants during the nursery period is critical for high-yielding production season. Strawberry plug plants are increasingly used in North America for high quality plant production (Ballington et al. 2008). Plugs conditioned with appropriate environmental conditions offers earlier flowering

and higher fruit productivity (Rowley et al. 2010). Photoperiod and nitrogen (N) are well recognized factors that control flower bud induction and fruit productivity for short-day and long-day cultivars (Durner et al. 2015; Wan et al. 2017). Supplemental lighting during end-of-day (day extension) and night interruption (NI) are successful alternative to supply LD photoperiod conditions that advances flowering and prevent transplants from entering early dormancy (Hidaka et al. 2013). Perpetual flowering cultivars, including DNs, behave as quantitative long-day plants and triggers flowering response under extended daylength (Sønsteby and Heide 2007). Elevated nitrogen (N) application followed by inductive photoperiod stimulates floral initiation. However, if applied before flower initiation, N may inhibit flowering and result in reduced yield (Sønsteby et al. 2009).

The combination of ammonium (NH_4^+) and nitrate (NO_3^-) N forms determines the total N uptake of a plant and can have various effects. Nutrient solution containing both N forms in specific ratio led to optimum plant growth and increase crop productivity compared to a single N source for various species such as apple (*Malus domestica* Borkhausen) (Babalar et al. 2015) and tomato (*Solanum lycopersicum* Linnaeus) (Horchani et al. 2010). Sole application of NO_3^- stimulates floral induction in *Arabiodpsis* (Castro Marín et al. 2011), whereas dominant NH_4^+ supply leads to impaired photosynthesis and causes plant toxicity in beans (*Phaseolus vulgaris* Linnaeus) (Guo et al. 2002). Differential preferences of plants for N source can be increased by environmental factors (Wada et al. 2015). Sufficient supply of potassium (K) in nutrient solution improves ammonia uptake when a mix of nitrate and ammonia is supplied (Hoopen et al. 2010). The interaction between nitrogen and potassium controls the balance between reproductive and vegetative growth and ensures high quality fruit formation (Preciado-Rangel et al. 2020). Little is known regarding

how different forms of nitrogen and N:K ratio may affect FBI and flower development in DN strawberry.

The major objective of the present study is to evaluate the optimal photoperiod conditions for the transplant production and nitrogen fertilization for the growing season to achieve higher fruit productivity for day-neutral cultivar 'Albion' grown in soilless system under umbrella shelters. This present defines the best management practices for the day-neutral strawberry grown. It provides the fundamental knowledge related to the growing conditions of transplants and for the entire production cycle of strawberry plant that can lead to improved FBI and increased fruit productivity.

3.3 Materials and methods

3.3.1 Photoperiod and N conditioning effect on flowering and fruit production

3.3.1.1 Plant material and conditioning

Both experiments related to photoperiod and nitrogen conditioning were conducted at Ferme Onésime Pouliot Inc. (45°54'50.584" N, 70°57'6.8" W; Saint-Jean-de-l'Île-d'Orléans, QC, Canada) over 2-years. Young runners of strawberry plants cultivar 'Albion' (*Fragaria* x *ananassa*) were harvested from a previous crop and rooted in 12-cell mini-trays (250ml per cell) containing coconut fiber (Dutch Plantin, Coimbatore, India) for transplant production. After rooting, plants remained in the nursery area under natural daylight and ambient temperature (as there was no temperature control) for 21 days until they had developed three to four unfolded leaves. For the first-year trial (2016-17), transplants were grown under the two following light treatments at low intensity ($15 \mu molm^{-2}s^{-1}$) beginning from 30th Aug: T1 (natural daylight) with only natural daylight varying in daylength from 13h in Aug to 10h in Oct and T2 (extended photoperiod) additional end-of-day low intensity incandescent electrical lighting to natural daylight to maintain a 15h

continuous photoperiod. Mini trays were kept in the nursery area until Nov 1st. Then, transplants were removed from the trays, wrapped in a perforated plastic bag, and stored in a cardboard box in a freezer for the winter storage (temperatures maintained between -1 to -4°C).

In 2017-18, transplants were subjected to the following three light conditioning starting from Sept 1st until Nov 9th before being transferred to cold storage for the winter: T1 (Natural daylight) natural daylight varying in daylength from 13h in Sept to 9h in Nov 2017; T2 (Extended photoperiod) additional end-of-day low intensity incandescent lighting to natural daylight to maintain a 15h continuous photoperiod and T3 (Night interruption; NI) consisting of natural daylight followed by a low intensity ($15\mu molm^{-2}s^{-1}$) light supplied during a night interruption ranging between 1 to 3h, depending upon the duration of natural daylight (T1 + NI=15h).

In both trials, developed flowers and runners were removed from the plants during propagation to direct plant energy towards crown development. Transplants were fertilized for six weeks with an over-head sprinkler system supplying, per m^2 containing 114 plants, 1800mg nitrogen (N), 400mg phosphorus (P), and 1700mg potassium (K) for the first three weeks, followed by 1010mg N, 200mg P, and 800mg K during 4th and 5th week, and 3100mg N, 20mg P, and 2900mg K for the 6th week Above mentioned fertilizer compositions were mixed in two barrels (A & B) and subsequently, fertilizer solutions were injected in the irrigation system at about 1% at maintained EC and pH at 1.2dS/m and 5.8 to 6.2 respectively. 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, allowed us to compare the effect of the main treatment.

3.3.1.2 Nitrogen conditioning during the growing season

The following season, transplants were moved out of the cold room and kept in the greenhouse for 2 weeks at a temperature between 16-20°C to acclimatize with outside conditions and lift dormancy. Transplants were rooted in rectangle plastic container (8L; 50cm x 18cm x 16cm; Bato Plastics, Zevenbergen, Netherlands) containing coconut fiber at a density of four plants per container under umbrella shelters during the last week of May 2017 and 2018. The experiment was established using a split-plot design, where photoperiod treatments represented the main plots and N concentrations represented the sub plots, providing two levels of randomization. This experimental was designed into 6 blocks and 54 experimental units (EU). Each block consisted in 9 experimental units (EU) and each EU contained 90 plug transplants. The treatments were randomly assigned to the main plots and the sub-plots. Photoperiod and nitrogen treatments were determined based on standard cultural practices being used for DN cultivars under local Québec conditions with some modifications. For the first-year trial, plants were fertilized through a drip irrigation system with different N concentrations i.e., 25N, 50N and 100N supplying 0.60, 1.17 and 2.34mg N per plug per week respectively (Table 3.1). Here, 100N represents the control treatment because this is the standard fertilizer practice during growing season. Whereas other concentrations such as 25N and 50N means 25% and 50% of the total concentration of N that is applied to strawberry plants. Nutrient solutions were supplied through a drip irrigation system twice a week. For the remaining week, transplants were irrigated 2-3 times with drip irrigation system depending upon on the temperature. In 2017-18, plants were fertilized with different N concentrations, i.e., 75N, 100N, 125N supplying 1.77, 2.34 and 2.91mg N per plug per week respectively (Table 3.1). In this case, 75N and 125N represents that N concentrations is applied to the plants at a 75% and 125% rate of total N concentration normally applied and 100N is

considered as control. For both the years, natural daylight followed by 100N treatment was considered as control. In addition to N, fertilizer solutions contained a constant concentration of phosphorus (0.45mg of P per plug per week), of potassium (2.78mg of K per plug per week) and micronutrients.

Table 3.1 Fertilizer concentrations used for the nitrogen conditioning experiments that was conducted for DN 'Albion' cultivar during 2016-17, 2017-18 and 2018.

Fertilizer	2016-2017			2017-2018			2018		
	25N[s]	50N[t]	100N[v]	75N[u]	100N[v]	125N[w]	3:4[x]	1:1[y]	4:3[z]
Calcium Nitrate (kg)	0.70	4.10	4.72	4.70	4.72	4.70	0.53	0.53	0.53
Ammonium Nitrate (kg)	0	0	0	0	0	0.91	0.03	0.06	0.24
Magnesium Nitrate (kg)	2.00	0	2.32	0	2.32	2.30	0	0.26	0.26
Iron-EDTA (kg)	0.14	0.14	0.14	0.14	0.14	0.14	0.01	0.01	0.01
Monopotassium Phosphate (kg)	1.04	1.04	1.04	1.04	1.04	1.04	0.12	0.12	0.12
Potassium Sulphate (kg)	2.80	2.80	0.96	1.21	0.96	1.00	0.14	0.11	0.11
Manganese Sulphate (kg)	0.30	2.20	0	2.10	0	0	0.24	0	0
Potassium Nitrate (kg)	0	0	2.08	1.70	2.08	2.10	0.19	0.23	0.24
Manganese Chelate (g)	61.0	61.0	61.0	61.0	61.0	61.0	6.85	6.85	6.85
Zinc Sulphate (g)	23.9	23.9	23.9	23.9	23.9	23.9	2.69	2.69	2.69
Borax (g)	7.83	7.83	7.83	7.83	7.83	7.83	0.88	0.88	0.88
Copper Sulphate (g)	1.14	1.14	1.14	1.14	1.14	1.14	0.13	0.13	0.13
Molybdate (g)	0.69	0.69	0.69	0.69	0.69	0.69	0.08	0.08	0.08

Above mentioned concentrations were mixed in the three barrels (A, B and C) containing 80L of water. Transplants were fertilized using drip irrigation system at a maintained electrical conductivity (EC) = 1.2dS/m and pH = 5.8 to 6.2 during the production season. Calcium carbonate and citric acid was used to adjust the pH.

[s] Nitrogen treatments of 25N supplied 0.60mg N per plug per week.

[t] 50N supplied 1.17mg N per plug per week.

[u] 75N supplied 1.77mg N per plug per week.

[v] 100N supplied 2.34mg N per plug per week.

[w] 125N supplied 2.91mg N per plug per week.

[x] Nitrogen treatments of 3:4 supplied 2.05mg N and 2.75mg K per plug per week.

[y] 1:1 supplied 2.80mg N and 2.80mg K

[z] 4:3 supplied 3.82mg N and 2.86mg K.

3.3.1.3 Plant growth parameters

Plant morphology, FBI, marketable and non-marketable fruit yield and dry biomass were evaluated. Morphology data includes periodic growth of leave, developed flower stalks and runners. For each treatment, four plants per experimental unit (in total 72 plants) were randomly tagged at the beginning of the season to monitor the morphological development. Newly developed flower stalks were tagged with tape and runners were detached after counting. New leaves were not calculated during the production season 2017-18. Similarly, four transplants were dissected

once a month 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). Fully ripened fruits were harvested and classified into marketable or non-marketable yield. Fruit yield (g/plant) was determined by dividing the total fruit harvested by the number of healthy plants. For 2016-17, fruit yield data is presented on monthly basis from July to October. For the production season 2017-18, fruit yield data was divided into five periods: 1-15 Jul, 16-31 Jul, 1-15 Aug, 16-31 Aug, and 1-15 Sept, based on harvesting dates. Fruits that are very small in size, underdeveloped and contains radical cracking, punctures and pathogen infections were considered as non-marketable. Brix (°Bx) (Sper-scientific refractometer 300051, Cole-Parmer Canada company, Montreal, Canada) determines the pure sugar content in water through 1 degree brix = 1g of sucrose/100g of solution. Brix was measured on fresh strawberry juice collected from nine fruit samples that has uniform appearances. The fresh strawberry juice was obtained after the homogenization of nine fruits with a kitchen blender and filtered with muslin cloth before the measurement. The strawberry juice drops were placed on refractometer to determine the brix. After the harvesting, nine plants were sampled per block for each treatment, then oven dried at 70°C for 5 days to determine dry biomass.

3.3.2 Nitrogen forms effect on floral bud induction during transplant production for 'Albion'

This experiment was 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). Strawberry plugs of cultivar 'Albion' were obtained from Ferme Onésime Pouliot Inc. (45°54'50.584" N, 70°57'6.8" W; Saint-Jean-de-l'Île-d'Orléans, QC, Canada) and planted in 12-cell mini-trays containing coconut fiber (Teris, Laval, QC) on Jun 26th, 2018. Throughout the experiment, plants were grown in the greenhouse under high-pressure sodium lamps (HPS; P.L.

Light System, Beamsville, ON, Canada) with a 14h daylength and temperature were maintained at 24±1°C/18±1°C (day/night). Plants with similar physical appearance were hand fertigated weekly with nutrient solutions containing four different NH_4^+: NO_3^- ratios (0:100, 20:80, 40:60 and 50:50) (Table 3.2). This experiment was arranged in a randomized complete block design with four treatments and three replicates. Each experimental unit contained 12 transplants assigned to a specific treatment, for total of 144 transplants. Three plants per EU were randomly labelled at the beginning to observe weekly morphological data of the plant from 13 days after planting (DAP) to 49 DAP. Three transplants per EU were assigned for dissection every two weeks to observe floral bud induction under a stereomicroscope. The nutrient status of the plant was measured through chlorophyll content in the leaves using a portable meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co., Japan). Chlorophyll concentration measurements were taken every week starting 15 DAP, on one leaf per plant, the 3rd fully-expanded leaf from the top downward of plants (Nhut et al. 2003). Three different readings per leaf were collected and the average value was recorded.

Table 3.2 Composition of different fertilizer in the nutrient solution used to investigate the effect of NH_4^+:$NO3^-$ ratios on the flowering characteristics of strawberry transplants (cv 'Albion').

Fertilizers	Formulation	NH_4^+:NO_3^- ratios (g/L)			
		0:100	20:80	40:60	50:50
Calcium Nitrate	$Ca(NO_3)_2$	7.92	5.72	3.16	0
Magnesium Nitrate	$Mg(NO_3)_2$	3.62	3.62	3.62	2.37
Iron	Fe-EDTA	0.18	0.18	0.18	0.18
Ammonium Sulphate	$(NH_4)_2 SO_4$	0	1.62	3.25	6.50
Monopotassium Phosphate	KH_2PO_4	1.60	1.60	1.60	1.60
Potassium Sulphate	K_2SO_4	1.87	1.87	1.87	1.87
Potassium Nitrate	KNO_3	0.62	0.62	0.62	0.62
Manganese Sulphate	$Mn(SO_4)_2$	0.11	0.11	0.11	0.11
Zinc Sulphate	$ZnSO_4$	0.03	0.03	0.03	0.03
Copper Sulphate	$CuSO_4$	0.01	0.01	0.01	0.01
Molybdate	Mo	0.01	0.01	0.01	0.01

Above mentioned concentrations were mixed with water in 20L container. Transplants were hand-fertigated at a maintained electrical conductivity (EC)=1.2dS/m and pH=5.8 to 6.2. Calcium carbonate and citric acid was used to adjust the pH. For each treatment, the total amount of nitrogen was supplied at a rate of 20mg/plant weekly in all the treatments. N sources were replaced with ammonium and nitrate according to the treatment in the nutrient solution.

3.3.3 Interactive effect of nitrogen and potassium on floral bud induction for 'Albion'

Three-week-old strawberry transplants were produced from field-grown runners (Ferme Onésime Pouliot Inc.) during the second week of May 2018. This experiment was also conducted in the Plant Science Research Greenhouse Facility using similar growing condition as mentioned previously. Transplants were treated every week with different ratios of nitrogen and potassium i.e., 4:3, 3:4 and 1:1 (Table 3.1). For each ratio, K concentration remained constant and N concentration were adjusted to obtain the desired fraction based on the fertilizer rates commonly used by commercial growers. In addition to nitrogen and potassium, phosphorus (0.45mg per plug per week) and other micronutrients were supplied. Each transplant was supplied with 120ml of nutrient solutions containing different fertilizer ratios to maintain uniformity. The EC and pH of the nutrient solutions were maintained at 1.2dS/m and between 5.8 to 6.2 respectively. This experiment was established using a randomized complete block design with 3 replicates. Each experiment unit consisted in 20 transplants for total of 180 plants. Three plants per EU were allocated to record the weekly morphological growth from 10 to 49 DAP. Similarly, three plants per replicate were randomly selected for dissection every two weeks to evaluate floral bud architecture.

3.3.4 Statistical methods

Statistical analysis was performed through Analysis of Variance (ANOVA) using a statistical analysis software (SAS 9.4 version, Analytics Software and Solutions, North Carolina, USA) for all the data obtained from the different experiments. The main effects of factors and their interaction were determined using two-way or three-way ANOVAs, based on the experimental 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 results for each treatment were expressed as mean values.

3.4 Results

3.4.1 Photoperiod and N conditioning effect on flowering and fruit production

3.4.1.1 Production season 2016-17

Based on the three-way ANOVA, there is no significant interaction between extended photoperiod and nitrogen with respect to time for FBI and flower stalk development (Table 3.3). The two-way interaction between nitrogen and time showed significant interaction, where low N concentration triggered the FBI during the later stages in the growing season only (Table 3.3). Transplants treated with 25N produced significantly higher number of flower buds inside the crown (11 per plant) compared to 50N (6 per plant) and 100N (7 per plant) during the month of September (Figure 3.1A). Like nitrogen, main effect of extended photoperiod showed no significant effect on FBI (p-value:0.38) and flower development (p-value:0.49) throughout the season. Plants grown under extended photoperiod (T2) followed by 25N and 100N application exhibited maximum flower stalk count, and dry biomass compared to 50N, however, it was statistically non-significant (Table 3.4). Extended photoperiod produced on average 8 runners per plant and 30 new leaves throughout the season compared to natural daylight, where transplants exhibited 6 runners and 27 leaves per plant (Table 3.4). The three-way interaction between photoperiod and N with regards to time for marketable fruit yield was not significant. However, the main effect of N showed significant results on average marketable yield data (Table 3.3). When average marketable fruit yield was compared for each month, transplants treated with 25N and 100N produced higher marketable yield compared to 50N in August (Figure 3.1B). Overall, 25N produced significantly higher marketable compared to 50N (136.09 g/plant). Irrespective to specific treatments, the new flower stalk growth

was significantly higher during the month of June compared to July, August, and September. A significantly higher marketable yield (230 g/plant) was produced during the month of September (Figure 3.1B). Overall, T2 light treatment followed by 100N demonstrated the most effective outcomes, 9% higher total marketable fruit yield (632.48 g/plant) than average (580.7 g/plant) under umbrella shelter. For the fruit quality, non-marketable fruit percentage were recorded between 8.1-10.5% (p-value:0.88) and sugar content (°Bx) ranged between 7.39-7.67 g of sucrose per 100 g of solution irrespective of treatments (p-value:0.78).

Table 3.3 p-values for the main effects of photoperiod and nitrogen treatments with respect to time and their interactive effects on marketable fruit yield, flower bud induction and flower stalk development for 'Albion' cultivar during the 2016-17 production season.

Source of variation	Marketable fruit yield	Flower bud induction	Flower stalk development
Nitrogen	**0.0484**	0.4684	0.2086
Photoperiod	0.4826	0.3871	0.4986
Time	**0.0001**	0.8190	**0.0001**
Nitrogen*Photoperiod	0.9711	0.6240	0.9581
Nitrogen*Time	0.3121	**0.0317**	0.4722
Photoperiod*Time	0.7858	0.5028	0.6573
Photoperiod*N*Time	0.9791	0.8155	0.5348

Table 3.4 Total marketable fruit yield, dry biomass and number of flower stalks, runners, and new leaves per plant in response to photoperiod and Nitrogen conditioning in 2016-17 at Ferme Onésime Pouliot. Table data represents the interaction effect between photoperiod and fertilizer on different growth parameters for 'Albion' cultivar.

Photoperiod[z]	Nitrogen[y]	Marketable fruit yield (g/plant)	Dry biomass (g/plant)	Flower stalks (number/plant)	Runners (n/plant)	Leaves (n/plant)
T2	25N	573.4	51.7	16	8	30
	50N	558.6	41.2	13	8	30
	100N	602.3	48.0	16	8	30
T1	25N	561.2	46.6	15	6	29
	50N	548.8	43.6	13	7	26
	100N	586.6	48.9	13	6	27

[z] T2: 15h continuous extended photoperiod and T1: natural daylight.

[y] 25N: 0.60 mg nitrogen (N) per plug per week, 50N: (1.17 mgN) and 100N: (2.34 mgN).

Data presented in the table is the mean value for each growth parameter. Standard error of the mean is 42.9 for marketable fruit yield, 6.8 for dry biomass, 2.2 for flower stalks, 1.2 for runner and 3.4 for leaves, calculated using Fisher's LSD test. Means value are calculated from six replicates with (n=540) for fruit yield, and (n=24) for dry biomass, flower stalk, runner and leaves.

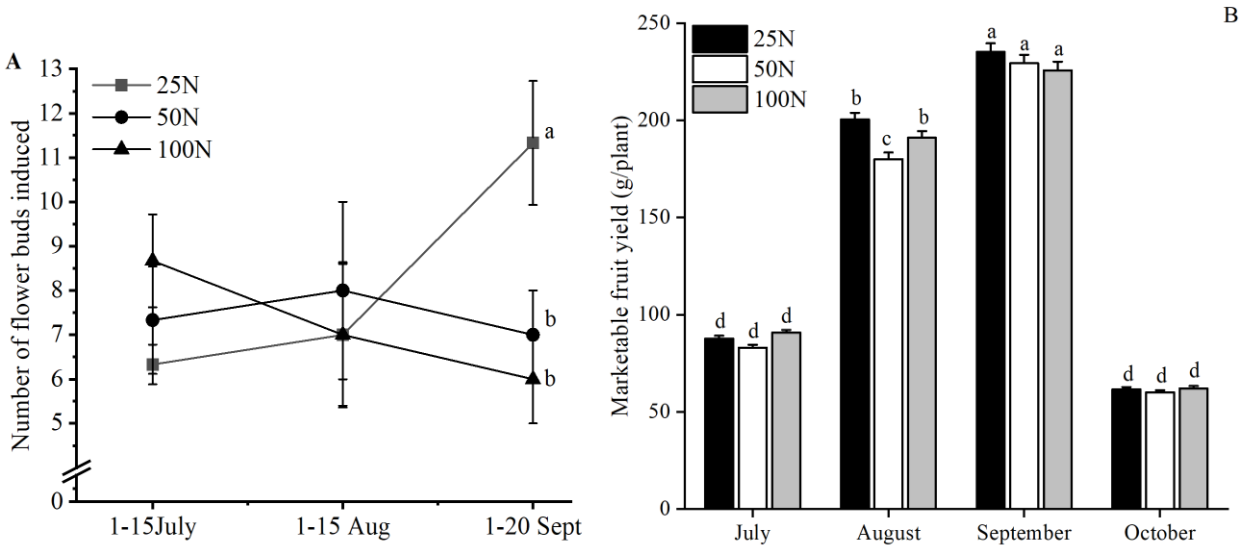


Figure 3.2 Effect of nitrogen concentrations on flower bud induction and marketable yield for 'Albion' cultivar. (A) Effect of nitrogen concentration on flower bud induction for the cultivar 'Albion'. (B) Effect of different N concentration on marketable fruit yield for each month during production season 2016-17. Data presented in the figure is the mean value ± SEM calculated with Fisher's LSD test from three replicates. Mean values with the different lowercase are significantly different among each group at p-value=0.05. 25N supplied 0.60 mg N per plug per week, 50N (1.17 mgN) and 100N (2.34 mgN).

3.4.1.2 Production season 2017-18

The three-way ANOVA showed no significant interaction between photoperiod, N conditioning and time for flower bud induction, flower stalk development and marketable fruit yield during the production season (Table 3.5). The two-way interaction between photoperiod and time showed significant (p-value:0.04) results for marketable fruit yield (Table 3.5). Where extended photoperiod showed the highest marketable fruit yield (92.70 g/plant) compared to T1 (83.38 g/plant) and T3 (82.45 g/plant) during 1-15 Aug (Figure 3.2A). For FBI, the main effect of photoperiod displayed significant results (Figure 3.2B). Transplants supplemented with night interruption and extended photoperiod produced significantly higher number of cumulative flower buds inside the crown compared to natural daylight. Whereas the main effect of N showed no significant effect on FBI. Transplants subjected to night interruption followed by 75N produced 21 flower stalks per plant, the highest among photoperiod and nitrogen treatment (Table 3.6). while natural daylight conditioned transplants followed by 125N exhibited the lowest number of flower

stalks with 16 flower stalks. Runner and dry biomass accumulation were unaffected by photoperiod and nitrogen. Overall, natural daylight photoperiod followed by 75N exhibited highest total marketable fruit yield (340.5 g/plant).

It is important to mention that early frost have restricted plant growth and considerably affected fruit yield and plant dry biomass at the end of the season of the 2017-18. For the control (natural daylight photoperiod followed by 100N) treatment, the marketable fruit yield was recorded as 605.1 g/plant in 2016-17, whereas control treatment yield was reduced by almost 45% with only 327.7 g/plant in the 2017-18 production season. Similarly, plant dry biomass was decreased by 20% from 48.9 g/plant in 2016-17 to 38.8 g/plant in the 2017-18 production season for the control treatment. For the fruit quality, the non-marketable fruit percentage was recorded between 13-15% (p-value:0.78) and the sugar content (°Bx) ranged from 7.76-7.91 g of sucrose per 100 g of liquid (p-value:0.84), irrespective of treatments.

Table 3.5 p-values for the main effects of photoperiod and nitrogen treatments with respect to time and their interactive effects on marketable fruit yield, flower bud induction and flower stalk development for 'Albion' cultivar during the 2017-18 production season.

Source of variation	Marketable fruit yield	Flower bud induction	Flower stalk development
Nitrogen	0.7913	0.6664	0.4879
Photoperiod	0.5872	**0.0201**	0.7885
Time	**0.0001**	**0.0001**	**0.0001**
Nitrogen*Photoperiod	0.0767	0.9664	0.8143
Nitrogen*Time	0.9943	0.2908	0.9445
Photoperiod*Time	**0.0441**	0.6710	0.8167
Photoperiod*N*Time	0.9606	0.4755	0.9987

Table 3.6 Total marketable fruit yield, dry biomass and number of flower stalks and runners per plant in response to photoperiod and Nitrogen conditioning in 2017-18 at Ferme Onésime Pouliot. Represents the interaction effect between photoperiod and fertilizer. Table data represents the interaction effect between photoperiod and fertilizer on different growth parameters for 'Albion' cultivar.

Photoperiod[z]	Nitrogen[y]	Marketable fruit yield (g/plant)	Dry biomass (g/plant)	Flower stalks (number/plant)	Runners (number/plant)
T3	75N	320.1	37.3	21	7
	100N	330.9	36.8	18	7
	125N	328.4	36.8	18	8
T2	75N	320.2	29.7	17	7
	100N	336.5	36.0	18	7
	125N	337.3	35.6	18	7
T1	75N	340.5	30.1	19	7
	100N	322.1	38.8	19	8
	125N	308.4	34.7	16	7

[z] T3: Night interruption supply (ND+NI=15h), T2: 15h continuous extended photoperiod and T1: natural daylight.
[y] 75N: 1.77 mg nitrogen (N) per plug, 100N: 2.34 mgN per plug and 125N: 2.91 mgN per plug. Data presented in the table is the mean value for each growth parameter. Standard error of the mean is 20.8 for marketable fruit yield, 4.6 for dry biomass, 2.4 for flower stalks and 1.6 for runner, calculated using Fisher's LSD test. Means value are calculated from six replicates with (n=540) for fruit yield, and (n=24) for dry biomass, flower stalk, runner and leaves.

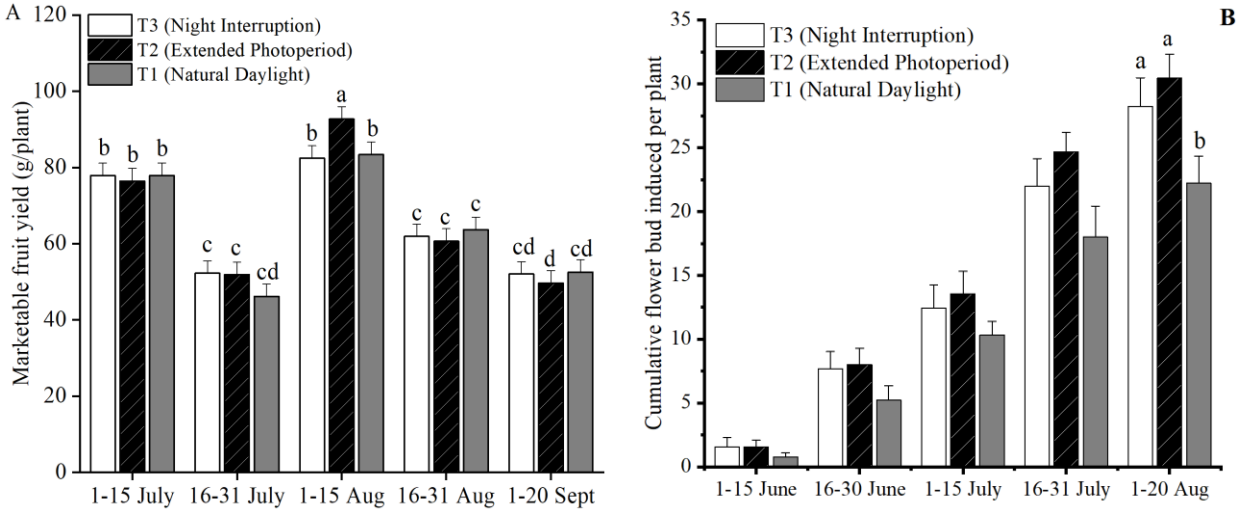


Figure 3.2 Effect of different photoperiods on marketable fruit yield and flower bud induction during production season 2017-18. (A) Effect of different photoperiods on marketable fruit yield 'Albion' cultivar (B) Cumulative number of flower bud induced per plant in response photoperiod. Data presented in the figure is the mean value ± SEM calculated with Fisher's LSD test from three replicates with n=18. Mean values with the different lowercase are significantly different among each group at p-value=0.05. T3: Night interruption (ND+NI=15h), T2: 15h continuous extended photoperiod and T1: natural daylight.

3.4.2 Nitrogen forms effect on flower bud induction during transplant production

Two-way interaction between time and ammonium:nitrate ratios showed no significant effect on FBI for 'Albion' cultivar (p-value:0.68). Overall, 20:80 (NH_4^+: NO_3^-) treatment produced

maximum flower buds, however, non-significant (p-value:0.10). The significant effect of NH_4^+: NO_3^- ratios was detected when one-way ANOVA was performed only on the cumulative FBI data collected 5 weeks after the treatment (WAT) began (Figure 3.3). Transplants conditioned with 20:80 (NH_4^+: NO_3^-) produced 25 cumulative flower buds per plant compared to 50:50 (19), 0:100 (17) and 40:60 (17) 5WAT. For the morphology data, NH_4^+: NO_3^- ratios showed no significant results for flower stalk development, leave and runner growth among the treatments. N ratio with 20% and 40% ammonia concentration in the nutrient solution produced 9 and 8 flower stalks per plant respectively, whereas 50% ammonia resulted in the development of 6 flower stalks. Plant that received 100% nitrate produced 7 flower stalks and 8 new leaves. All the transplant treated with different ammonia and nitrate levels showed no sign of toxicity and deficiency. Regardless of the nitrogen forms supplied, the plants chlorophyll content remained between 31 and 43 SPAD units.

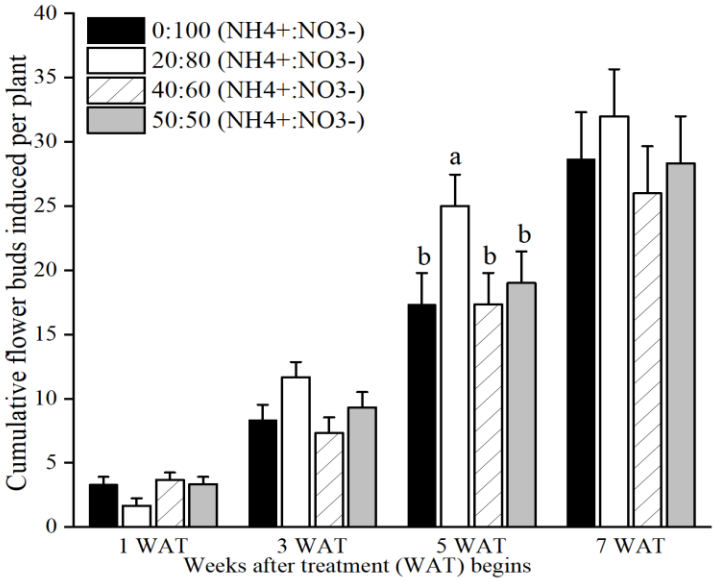


Figure 3.3 Effect of different ($NH4^+$:$NO3^-$) ratios on flower bud induction for the strawberry cultivar 'Albion' during transplant production. Data presented in the figure is the mean value ± SEM calculated with Fisher's LSD test from three replicates with n=18. Mean values with the different lowercase are significantly different among each group at p-value=0.05.

3.4.3 Interactive effects of nitrogen and potassium on flower bud induction in 'Albion'

Two-way interaction between nitrogen:potassium ratios (N: K) and time showed no significant effect on FBI (p-value:0.39) and flower development (p-value:0.21) data for 'Albion' cultivar (Figure 3.4). Transplants conditioned with 3:4 and 4:3 ratio exhibited on average 10 developed flowers per plant, while a ratio of 1:1 developed 8 per plant. Similarly, FBI data indicated no significant difference among the treatments. Treatment with a ratio of 3:4 induced on average five flower buds inside the crown while ratios of 1:1 and 4:3 induced four. Transplants supplied with different N: K ratios showed no sign of toxicity and deficiency. Like for the flowering data, plant dry biomass (p-value:0.69) and runner growth (p-value:0.55) data showed no significant difference among treatments.

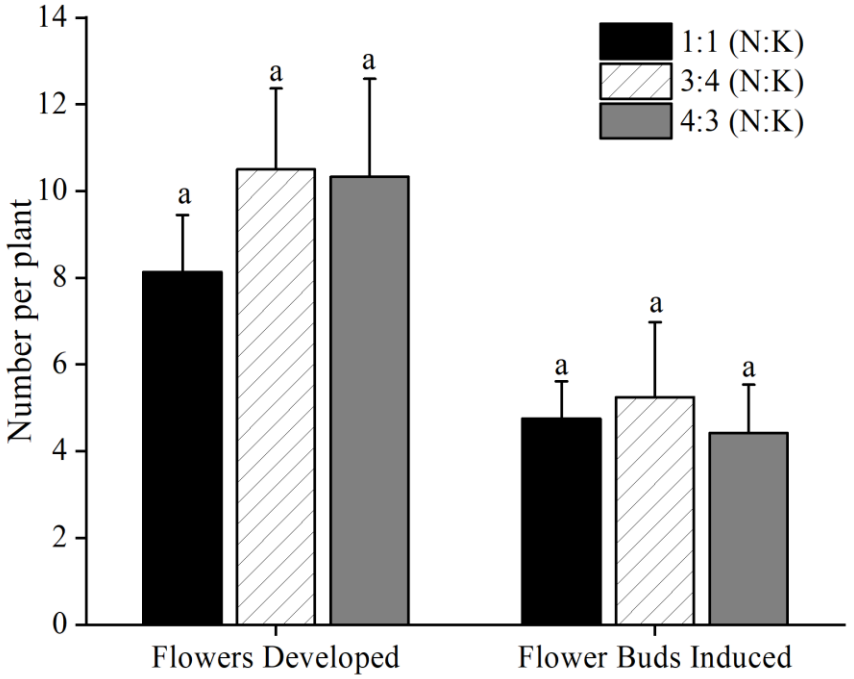


Figure 3.4 Effect of nitrogen: potassium ratio on flower bud induction and flower development for the strawberry cultivar 'Albion' during transplant production. Data presented in the figure is the mean value ± SEM calculated with Fisher's LSD test from three replicates with n=18. Mean values with the different lowercase are significantly different among each group at p-value=0.05.

3.5 Discussion

Previous studies have revealed that each flowering stage such as floral initiation, induction, differentiation, and development must be evaluated individually, because each respond differently to photoperiod conditions in strawberry plants (Durner 2015). Everbearing and modern DN cultivars behave like quantitative and qualitative long-day plants depending upon the temperature, thus, promotes flowering under LD photoperiod (Sønsteby and Heide 2007). In the present study, each flowering stage of 'Albion' cultivar was evaluated separately under different photoperiod and nitrogen conditions. LD photoperiod showed no significant effect on flower development and fruit production for DN 'Albion' cultivar grown under umbrella shelter. However, it appears that LD photoperiod stimulates the FBI during the later stage in the production season 2017-18. It is important to emphasize that during 2017-18 production season, photoperiod treatments were applied during the transplant production stage (September to November 2017) and their photoperiodic effect on FBI was observed in Aug 2018. It is most likely that plants have already begun FBI upon exposure to LD conditions either during transplant production stage or early during the growing season. Studies have shown that elevated nitrogen supply following induction would likely to promote flowering (Durner 2017). In the present study, the FBI increase in August is not entirely related to photoperiod treatments; it is possible that elevated N supply have enhanced the LD-induced effect on flowering. As supported by previous studies, Durner (2017) indicated that increased N supply enhances the LD-induced effect that resulted in higher flower production and enhanced fruiting in open field production. Similar findings have been observed in the present study, as FBI was significantly stimulated under extended photoperiod and night interruption during the 2017-2018 production season when each transplant received significantly higher amount of nitrogen compared to 2016-17. Average N concentration received per plug transplant

for 2016-17 (1.37 mgN per plug) was relatively lower compared to 2017-18 (2.34 mgN per plug) trial. Under umbrella shelter, the elevated N supply did not promote flowering in 'Albion' individually, however it may have enhanced the LD-induced consequence on rate of flowering as previously reported in LD cultivars 'Elan', 'Tarpan' and 'Gasana' (Durner 2016, 2017).

According to Sønsteby and Heide (2007), flowering response of LD cultivars becomes more apparent when grown for at least 4 weeks under inductive photoperiod. Both years, transplants conditioned with LD photoperiod for 9 weeks showed no difference for FBI and flower development during the early growing season, perhaps due to the light source. Previous studies have showed that supplementation of 16h photoperiod with blue (455 nm) LEDs instead of incandescent light advances FBI in 4-6 weeks (Nadalini et al. 2017). Photoperiod (Sønsteby and Heide (2007), light intensity (Rezazadeh et al. 2018), and light source (Nadalini et al. 2017) are the important factors that controls flowering in strawberry. Augmented FBI and development can be easily accomplished by blue LEDs alone or in combination with other light sources during a 6-week induction period (Nadalini et al. 2017; Sidhu et al. 2021).

LD photoperiod conditions is often delivered either by daylength extension (DE) or night interruption (NI). Generally, both DE and NI creates long-day photoperiodic response for various ornamental plants and enhances flowering. However, when compared, the efficacy of NI is slightly stronger than DE using the same source Meng and Runkle (2017). Based on the present study, based on the data from the 2017-18 production season, it appears that DE is more effective than NI to increase marketable fruit production during the month of August.

Elevated N supply immediately after the FBI promotes flowering, whereas excessive N supply before, at the beginning of induction restricts flowering (Sønsteby et al. 2013). In the present study, low N concentration (25N), prepared mainly from nitrate source, triggered the FBI during the later

stage in the growing season, which could potentially extend the harvesting season. Similar results have been reported by Wan et al. (2017) and Lin and Tsay (2017), who indicated that low nitrate supply accelerate floral induction and differentiation for strawberry and *Arabidopsis,* respectively. In accordance with previous findings, the present study suggests that limited supply of N can be applied to advance the FBI. However, once the plants have commenced induction process, N levels should be augmented to ensure the successful differentiation. Enhanced N supply before induction may significantly delay flowering. Besides FBI, low N (25N) and 100N supply significantly improved the average plant marketable fruit yield compared to 50N, suggest that N concentration can be manipulated during the production season in order target specific flowering response. Overall, low N concentration can be used to enhance flower bud induction and elevated N for the advancement of the flower development that subsequently contributes to fruit production.

The average marketable fruit yield for the control treatment in 2017-18 was reduced almost 45% compared to 2016-17. This significant decline in marketable yield in the 2017-2018 season was most likely related with a sharp decline in temperature and early frost that has restricted the fruit yield considerably by the end of the season. The last harvesting date in 2017 season was October 12, whereas plants turned brown and froze around 18 Sept in 2018. Average mean daily temperature recorded for Sept and Oct 2017 was 15.4 °C and 10.5 °C respectively. Whereas Sept and Oct 2018 recorded cold temperatures of 14.2 °C and 4 °C respectively.

In the present study, both experiments were conducted at production site where environmental conditions demonstrated high relevancy to local conditions however, not controlled. Since, most of the significant results were observed during later stage of the production season, environmental conditions could have influenced the outcome of the experiment. The production site experiments offered advantages over protected conditions such as more replications and high relevancy to local

conditions. However, extreme weather conditions as encountered in production season 2017-18, and less precise evaluation of larger populations remains the biggest disadvantages. In contrast, the protected or controlled conditions trials manage optimal growing conditions throughout the crop life cycle and offers precise assessments of the treatments.

Maximizing FBI during the nursery stage is a prerequisite to enhance fruit production in strawberry. Studies have shown that 40:60 (NH_4^+:NO_3^-) ratio allows strawberry plants to maintain balanced vegetative and reproductive growth (Latigui et al. 2011). In general, optimal ammonium and nitrate ratios can differ with physiological stage of cultivar (Marschner, 1995). In our study, a 20:80 (NH_4^+:NO_3^-) supply of two nitrogen forms significantly stimulated the FBI 5 weeks after the application of treatment. Our results are in agreement with previous reports by Marschner (1995) and Claussen (2002), who demonstrated that 20 to 30% ammonium is the best possible N form to maximize flowering and to improve the overall plant growth and fruit yield. Britto and Kronzucker (2002) revealed that the appropriate supply of both ammonium and nitrate in the growing media improves plant strength and reduces leaf chlorosis. Increasing ammonium concentration more than nitrate during the flowering stage restricts root activity due to rhizosphere acidification caused by excess H^+ (Britto and Kronzucker, 2002). Thereafter, it inhibits nitrate reductase activity (NR), an enzyme responsible for N assimilation (Taghavi et al. 2004). Therefore, in our study we provided the maximum ammonium treatment up to 50%. Moderate amount of ammonium leads to higher photosynthetic rate, increase NR activity, and subsequently leads to an overall enhanced growth in strawberry plants (Tagahvi et al. 2004). Regardless of the combination of N form supplied in our study, the plants chlorophyll contents were similar and remained above 31 SPAD units, in agreement with Guler et al. (2006), who found that the leaf chlorophyll in strawberry during flowering and fruiting stages should be maintained above a SPAD value of 31.

Our results indicate that DN ‘Albion’ can benefit from a 20:80 (NH_4^+: NO_3^-) ratio of these two forms of nitrogen in the fertilization plan, however, it greatly depends on the growth stage.

Nitrogen and potassium are essential macronutrients that regulate flowering, fruit yield and quality in strawberry (Marschner 1995; Ijaz et al. 2016). Our results indicate that different N:K ratios showed no significant effect on FBI and flower development in DN ‘Albion’ cultivar grown in soilless media. Nitrogen and potassium interactions predominantly affects fruit quality (i.e., phenolic and antioxidant capacity) as previously reported in grapes (Boonterm et al. 2010) and wild blueberries (Percival and Sanderson 2004). Similar results have been reported by Preciado-Rangel et al. (2020) in strawberry where an increased NO_3^- concentration enhanced fruit yield and reduced antioxidant capacity, while higher K^+ improved nutraceutical capacity of strawberry with a reduced yield. In the present trial, N and K ratios supplied during the transplant production stage and plants were kept for 8 weeks to observe their effect on FBI and development, which in the end was not significant. Based on the previous studies, it appears that different N and K ratios may have significant effect on strawberry fruit yield and fruit quality, however, FBI and development was unaffected.

3.6 Conclusions

Photoperiod and N conditioning demonstrated distinct consequences on flower bud induction, flower development and marketable fruit yield for DN ‘Albion’ cultivar grown under umbrella shelters. The present study findings suggested that limited N supply advances the FBI, however, once the plants have commenced induction process, N levels should be augmented to ensure the successful differentiation. Like N, photoperiod showed no substantial effect on flower development, although elevated N seemed to enhance the LD-induced effect on flower bud induction during the later stage in the production season 2017-18. Based on the results, it can be

suggested that N and photoperiod can be manipulated to manage flowering response and to extend the harvesting as both regulate FBI during the later stage of the season for DN cultivars grown under umbrella shelter. These findings have practical advantages for increasingly grown DN 'Albion' cultivar in Québec regions. Furthermore, 20:80 (NH_4^+: NO_3^-) induced the maximum number of floral buds for 'Albion' cultivar during the transplant production stage. 'Albion' transplants remained unaffected under different nitrogen and potassium combination supplied during transplant production. The adaptation of present findings to commercial strawberry production requires further investigations for other day-neutral cultivars.

CONNECTING STATEMENT FOR CHAPTER IV

In Chapter III, we mainly investigated the effect of photoperiod and nitrogen on flowering attributes and fruit production for day-neutral cultivars during the production season. Results from the previous chapter demonstrated that LD photoperiod mainly augments the flower bud induction (FBI) during the later stages, which could result in season extension. However, our main objective was to enhances the early flower bud induction, especially during transplant production.

Flower bud induction is the most reliable indicator that leads to development of flower stalks and predicts the harvesting time and yield. In general, the strawberry plugs are commonly conditioned with different stimuli to enhance the FBI during transplant production. Based on the literature mentioned in Chapter II, photoperiod and light quality are the important factors that stimulates the FBI in seasonal strawberries. Therefore, in Chapter IV, we investigated the photoperiodic control of flowering in widely grown day-neutral cultivar 'Albion'.

We hypothesized that light source may contribute toward flowering stimulation. Thus, we conducted the subsequent experiments, where we tested the effect of different light qualities supplied through LEDs.

Chapter IV, entitled "Effect of light quality and extended photoperiod on flower bud induction during transplant production of day-neutral strawberry cultivars" authored by Varinder Sidhu, Valérie Bernier-English, Marianne Lamontagne-Drolet and Valérie Gravel has been accepted for publication in Canadian Journal of Plant Science. The contribution of author and co-authors towards this manuscript is mentioned in "contributions of authors" section.

CHAPTER IV

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

Varinder Sidhu[A], *Valérie Bernier-English*[B], *Marianne Lamontagne-Drolet*[A] *and Valérie Gravel*[A].

[A]Department of Plant Science, Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue, Québec, Canada.

[B]Ferme Onésime Pouliot Inc., Saint-Jean-de-l'Île-d'Orléans, Québec, Canada

4.1 Abstract

Day-neutral (DN) strawberry cultivars are increasingly grown in Canada because they produce flowers and fruits continuously until October. Appropriate lighting conditions during preparation of high-quality transplants is critical. Unfortunately, systematic evaluation of appropriate 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; 24h), short-day (SD; 10h) photoperiods and during a night interruption (NI) for 2h. '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 8

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 FBI and floral development to subsequently increase fruit production.

Keywords

Photoperiod, LEDs, Light quality, Night interruption, Flower bud induction.

4.2 Introduction

Québec strawberry (*Fragaria* x *ananassa* Duchesne) 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 or seasonal (June-July) cultivars; however, the arrival and cultivation of day-neutrals (DN; perpetual flowering) caused a substantial increase in the fresh market. DN strawberries continue producing new flowers and fruits until October, depending upon weather conditions. This 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 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 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 exhibiting floral bud 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* Linnaeus (Hori et al. 2011), *Pyrus pyrifolia* Nakai (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 (ex: 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., Cockleburs (*Xanthium strumarium*) and Pharbitis (*Ipomoea purpurea*) (Thomas and Vince-Prue 1997) and long-day 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 long-day plant when grown at intermediate temperature (22/18°C; day/night) and qualitative long-day 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 long-day flowering response when supplemented with low intensity (7$\mu molm^{-2}s^{-1}$) 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, electrical 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 flower bud induciton during transplant production could significantly increase strawberry fruit production. It is important to determine the optimum light conditions to maximize 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.

4.3 Materials and methods

4.3.1 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* x *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 weeks.

4.3.2 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 two weeks 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 72h in an oven (Isotemp Incubator, Fisher Scientific, Hampton, NH) for dry biomass measurements.

4.3.3 Light Treatments

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

Runner tips of 'Albion' were rooted for 2 weeks 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 Aug 24th to Oct 16th, then the transplants were transferred in the fridge for the winter (-1°C). For the

24h light treatment (24LD), transplants were exposed to natural daylight until end-of-day. Low intensity incandescent lighting was provided 30 minutes 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 24h lighting. Similarly, incandescent lightings were adjusted in addition to natural daylight to provide total 18h (18LD) light treatment. In the control treatment (ND), transplants were exposed to natural daylight, where daylength varied from 13h in Sept to 10h in Oct 2019. Light intensity during the extended lighting period was kept very low (between 10-15$\mu molm^{-2}s^{-1}$) 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 two weeks 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 six weeks with an over-head sprinkler system supplying, per m^2 containing 114 plants, 1800mg nitrogen (N), 400mg phosphorus (P), and 1700mg potassium (K) for each of the first three weeks, followed by 1010mg N, 200mg P, and 800mg K during the 4^{th} and 5^{th} week, and 3100mg N, 20mg P, and 2900mg K for the 6^{th} week. Above mentioned fertilizer compositions were mixed in two barrels (A & 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, allowed us to compare the effect of the main treatment.

4.3.3.2 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 16-20°C and 14h 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 x 32 cm x 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 50-60 $\mu molm^{-2}s^{-1}$ for 45 days supplying consistent DLI between 2.8 to 3.2 $molm^{-2}d^{-1}$. Spectral output and light intensity were determined using a spectroradiometer (Apogee Instruments Inc., model PS-300, MN, USA) and light meter (LI-COR LI-250A, 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 1500mg N, 200mg P, and 2200mg K per m^2 (comprises 114 plants) with maintained EC:1.2dS/m, pH: 5.8 to 6.2 for light quality experiments.

4.3.3.3 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; 24h) and short-day (SD; 10h). Different light regimes were established amid constant light intensity of 45-50 $\mu molm^{-2}s^{-1}$. SD was achieved with 10h of distinct light ratios delivering low DLI $1.62molm^{-2}d^{-1}$ whereas LD was achieved with 24h of continuous light spectrum giving a high DLI $3.8molm^{-2}d^{-1}$. Transplants were exposed from 6:00 to 16:00 in SD, and 24h 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.

4.3.3.4 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 6:00-20:00 (14h), followed by a night interruption (NI) at midnight for 2h (00:00 to 2:00) using far-red and blue LEDs ratios of 1:5, 5:1 or 1:1. Treatments were initiated on Oct 23rd until Dec 10th. Uninterrupted night treatment was considered as the control. During the daytime, light intensity was maintained between 100-120$\mu molm^{-2}s^{-1}$ and low intensity (50 $\mu molm^{-2}s^{-1}$) was supplemented

during the night interruption. Temperature was maintained at 24°C/18°C (day/night) throughout the experiment for 48 days. 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 days 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).

4.3.4 Statistical methods

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

4.4 Results

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

Transplants grown under different photoperiod showed no significant difference on flower bud induction and development. Transplants treated with 24LD and ND produced on average three terminal inflorescences bearing axillary buds, while 18LD developed two inflorescences (Table 4.1). 18LD-conditioned transplants exhibited the first emerging flower stalk outside the crown 10 days after treatment (DAT) commenced. Comparatively, ND transplants developed their first stalk after 20 days, and 24LD after 30 days. Overall, 24LD treated plants produced an average of five flowers compared to four for 18LD and ND but differences were non-significant (Table 4.1). 'Albion' transplants grown under long-day (24LD and 18LD) photoperiods produced 9 new leaves per plant compared to 7 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.

Table 4.1 Effect of photoperiodic conditioning on flowering and phenology growth for 'Albion' during nursery stage.

Photoperiod[z]	Flower stalks[x]	Flowers[x]	New leaves[x]	Induced buds[x]	Inflorescences[x]	Biomass partitioning[y]
24LD	2	5	9	6	3	36:45:7:13
18LD	1	4	9	6	2	34:48:8:10
ND	1	4	7	6	3	39:46:6:10
p-value	0.3823	0.2286	0.0584	0.7962	0.4584	0.3674[w]

[z] Photoperiodic treatment of 24-hour (24LD), 18-hour continuous light (18LD) and natural daylight (ND). [y] Percentage of roots : leaves : crown : stalks biomass. [W] Represents p-value for crown. [x] Represents data in number per plant. Data presented is the mean value from three replicates (n=18), n represents the sample size. Standard error of the mean is 0.30 for flower stalks, 0.68 for flowers, 0.64 for new leaves, 1.30 for induced buds and 0.44 for inflorescences, calculated using Fisher's LSD test.

4.4.2 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 to transplants conditioned with dominant FR (5:1) and 1:1 (Figure 4.1A). Transplants in the dominant blue light regime commenced flowering in 8-14 days compared to dominant FR and 1:1 that exhibited a delayed

anthesis at around 18-24 days and 20-30 days, respectively. Dominant FR light significantly promoted the growth of new leaves compared to the dominant blue light (Figure 4.1A; p-value:0.04). However, results showed no significant difference in runner production for 'Albion' between the light quality treatment (Figure 4.1A; 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 to other treatments, although the difference was not statistically significant (Figure 4.1B; p-value:0.36).

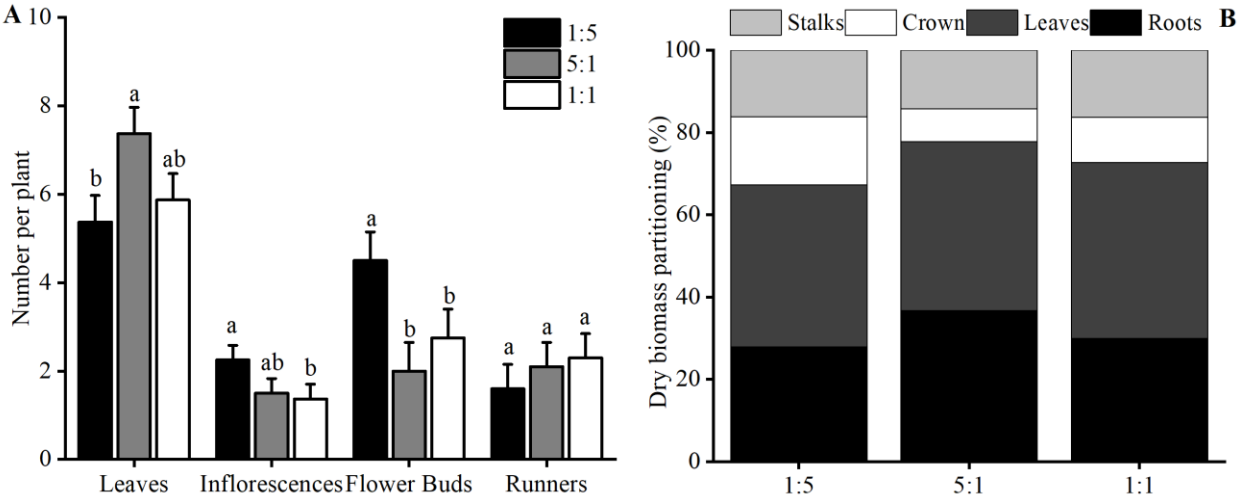


Figure 4.1 Light quality effect on flower bud induction, phenological growth and dry biomass partitioning for 'Albion' cultivar. (A) Average number of new leaves, inflorescences, flower buds and runners (per plant) in response to light quality. (B) Dry biomass partitioning (stalks, crown, leaves and 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.

4.4.3 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 four weeks, transplants grown under dominant blue light (1:5) for 24h substantially advanced flowering and produced five fully opened flowers per plant compared to two for 1:1 and 5:1 (Figure 4.2A). However, 5:1 and 1:1 hastened flowering in the last two weeks and produced similar number of flowers by the end of the 6-week experiment

(Figure 4.2A). LD photoperiod significantly promoted the growth of flower stalks, flowers and new leaves compared to SD, regardless of light quality (Figure 4.2B).

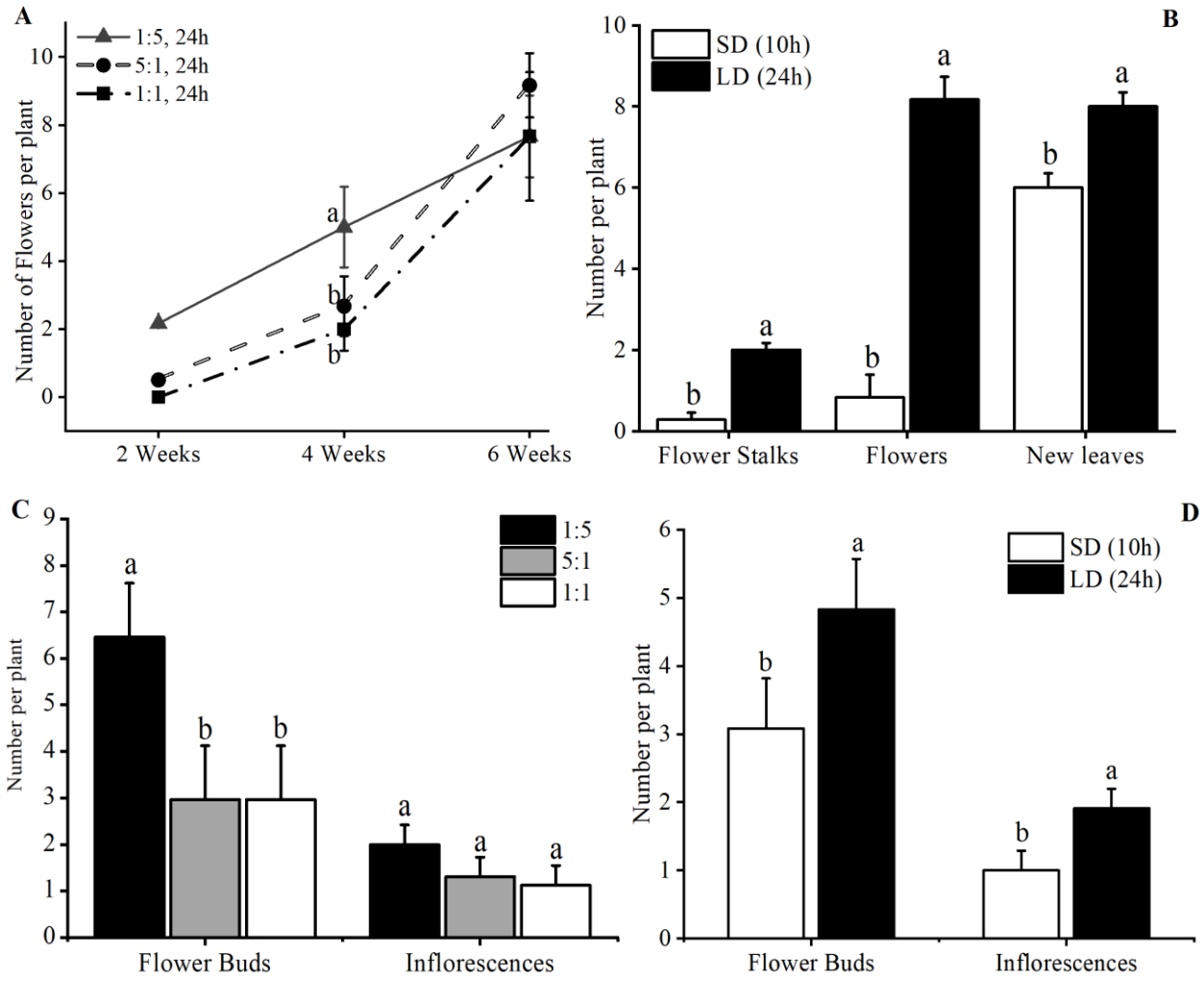


Figure 4.2 Light quality, photoperiod and their interactive effect on flower bud induction, flower development for 'Albion' cultivar. (A) Effect of light quality on flowering of the 'Albion' cultivar during six weeks of treatment. (B) Average number of flower stalks, flowers and new leaves per plant 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 (10h) and LD (24h) 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.

During the dissection of the 'Albion' transplants, no significant interaction effect between light quality and photoperiod on FBI was observed (Table 4.2). However, light quality and photoperiod stimulated FBI independently. Dominant blue LEDs (1:5) produced significantly more flower buds (six) inside the crown compared to 5:1 (three) and 1:1 (3) but showed no effect on the number of

inflorescences (as it was observed in the second trial) (Figure 4.2C). LD photoperiod exposure of diverse light combinations significantly promoted the inflorescences and flower buds inside the crown (Figure 4.2D). Transplants grown under LD photoperiod demonstrated significantly greater dry biomass accumulation compared to SD (Figure 4.3A) whereas light ratios showed no statistical differences on dry biomass (Figure 4.3B).

Table 4.2 P-values for light quality and photoperiod conditioning main effects and their interactive effects on flowers, flower buds inside the crown, new leaves growth and dry biomass for 'Albion' cultivar.

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

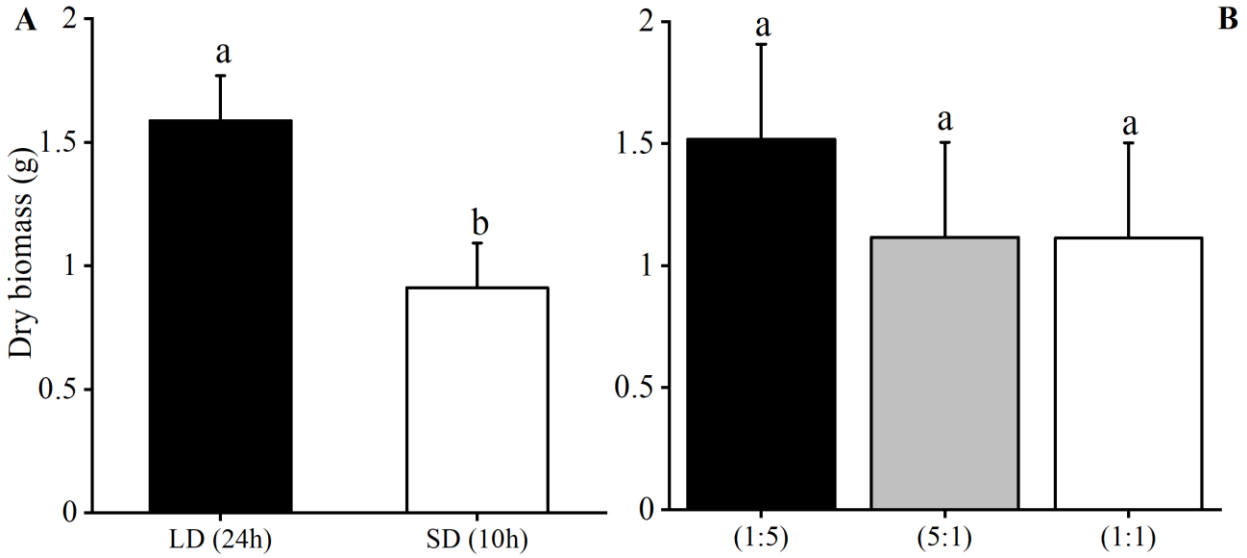


Figure 4.3 Light quality and photoperiod effect on dry biomass accumulation during transplant production for 'Albion'. (A) Dry biomass in response to photoperiod and (B) light quality. SD (10h) and LD (24h) continuous light exposure. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm):Blue (455 nm). 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.

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

'Albion' showed a significant increase in FBI and flower production outside the crown when subjected to different light qualities during night interruption. Transplants grown under dominant blue lights (1:5) produced eight flower buds inside the crown (Figure 4.4A) within 48 days and simultaneously exhibited five opened flowers per plant (Figure 4.4B), 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 days and 1:1 or HPS in 38 days. Dominant blue conditioned plants triggered FBI and displayed linear growth that yielded a significantly higher number of flower buds (Figure 4.5A). 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 (Figure 4.5B). Very few runners were observed during the experiment. The architectural mapping of plants conditioned with distinct light quality is presented in Figure 4.6. Plant dissection revealed that the dominant blue treatment stimulated secondary and tertiary branching as well as resulted in additional inflorescences.

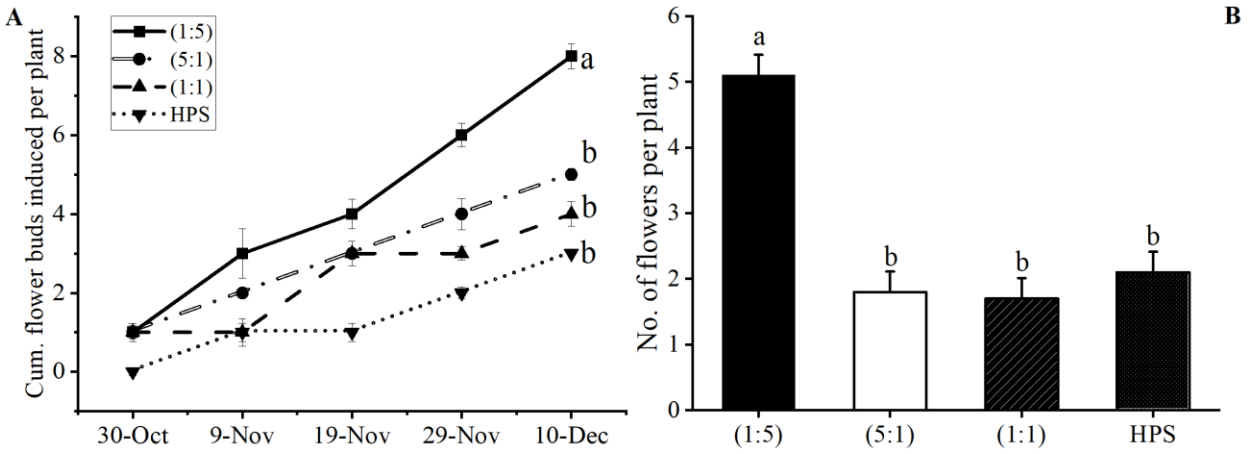


Figure 4.4 Effect of light quality supplemented during night interruption on flower bud induction and flower development during transplant production for 'Albion'. (A) Effect of light quality supplemented during night interruption on flower bud induction for the 'Albion' cultivar. (B) Average number 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 2h 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.

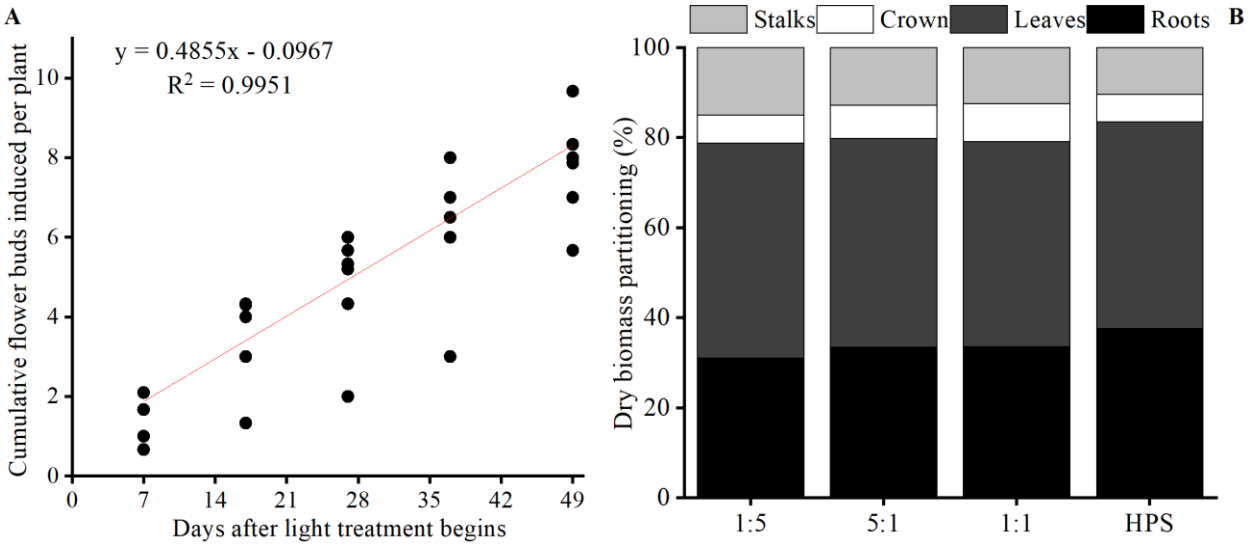


Figure 4.5 Scatter plot for flower bud induction and dry biomass accumulation during transplant production for 'Albion' under different light quality. (A) Scatter plot for dominant blue light FR:B (1:5) presenting linear growth among replicates for flower bud induction (B) Dry biomass partitioning (stalks, 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 2h 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.

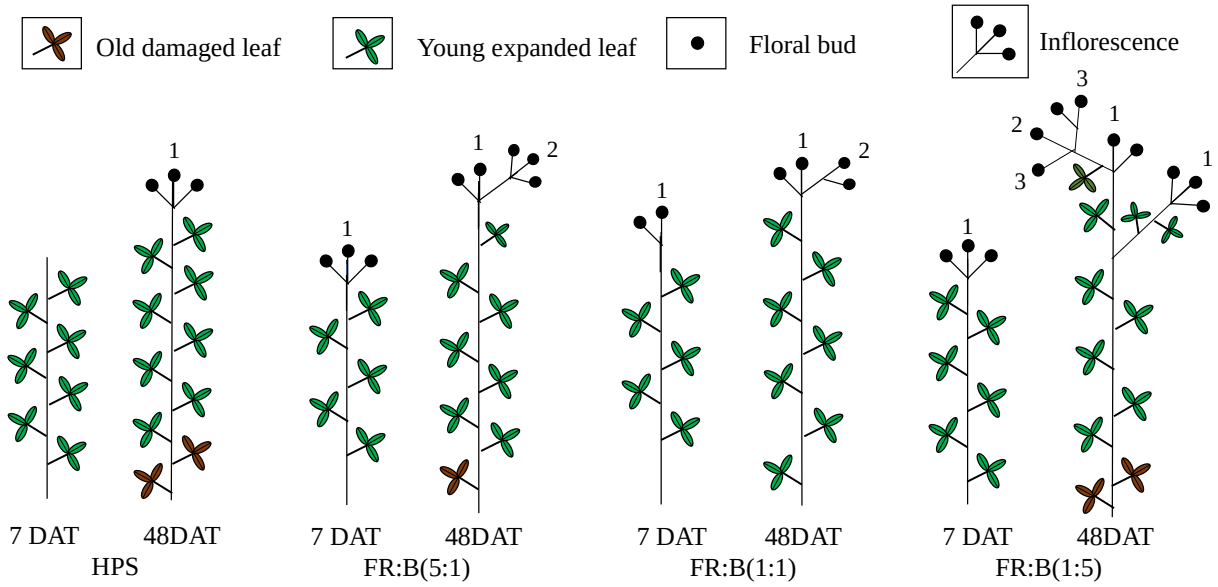


Figure 4.6 Strawberry plant architecture in response to different light quality supplemented during night interruption at 7 and 48 days after treatment (DAT) affect for 'Albion' cultivar. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm): Blue (455 nm). Numbers represents order of inflorescences. Data presented in the figure is collected from three replicates (n=9), n represents the sample size.

4.5 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 long-day plant when grown at intermediate temperature (18°C) and qualitative long-day 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 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 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 long-day 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 the present study, overall dry mass 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 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 long-day 'HS138' strawberries 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 FBI 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 FBI, while FR light preserves leafy growth of the plant. Advanced FBI during transplant production could result in earliness of harvesting time by 10-15 days 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 4.2). Whereas LD photoperiod supplied with incandescent light showed no significant effect on flowering during transplant production (Table 4.1). Here, we suggest that light source plays an important role while determining photoperiodic control of flowering. A prompt increase in FBI 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 (*Hibiscus rosasinesis*) (Warner and Erwin 2003), begonia (*Begonia rex*), marigold (*Tagetes erecta*), and petunia (*Petunia atkinsiana*) (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 (24h) of dominant blue light combination within 4 weeks of conditioning and then relapsed. Remarkably, the dominant FR LED (5:1) recuperated in the last two weeks 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 electrical 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 to 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 FBI and produced average 6 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.

4.6 Conclusions

Electrical 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 four weeks 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.

4.7 Acknowledgement

This research was financially supported through a Natural Science and Engineering Research Council (NSERC) of Canada – Collaborative Research and Development grant in collaboration with the Ferme Onésime Pouliot Inc. The authors would also like to thank Ferme Onésime Pouliot Inc. for the plant material and the space provided to perform the research.

CONNECTING STATEMENT FOR CHAPTER V

In chapter IV, we showed that the combination of far-red and blue narrow-band light sources at a ratio of 1:5 stimulate flowering, which includes flower bud induction and flower development in 'Albion' cultivar grown in soilless media. Previous studies have reported that *F. vesca* homologue of *FT1/TFL1*-like genes facilitates flowering under far-red and blue LEDs in seasonal strawberry (Rantanen et al. 2014). We hypothesized that strawberry homologue of floral inducer and floral repressor gene i.e., *FvFT1* and *FvTFL1* respectively, may regulate flowering in day-neutral cultivars as well in response to dominant blue LEDs. Therefore, we evaluated the plant morphological response and the transcript levels of flowering related genes in *Fragaria vesca* cv 'Alexandria'. The interaction between light conditions and plant hormones plays important role during flowering process (Yan et al. 2019). The hormones that considerably affect flowering are gibberellin, cytokinin, auxin and abscisic acid and their role vary between plants. Thus, in the present study, for the first time, role of these endogenous hormones was studied during the flower bud induction process for both day-neutral cultivars 'Albion' and 'Alexandria' in response to light quality.

CHAPTER V

Role of plant hormones and regulatory genes in controlling flowering in response to light quality in day-neutral *Fragaria* species

Varinder Sidhu, Suha Jabaji and Valérie Gravel

Department of Plant Science, Macdonald Campus, McGill University, Sainte-Anne-de-Bellevue, Québec, Canada

5.1 Abstract

Flowering is the most important phase for the strawberry fruit production, which involves distinct molecular mechanisms and hormonal signaling. The flowering pathways have been extensively studied in seasonal accessions of the woodland strawberry, *Fragaria vesca* Linnaeus, under different environmental cues. However, the flowering mechanisms are poorly understood in day-neutral (DN) cultivars. In the present study, we determined the transcript levels of floral related genes *FT1/TFL1*-like genes in response to the combination of two narrow-band light sources in *Fragaria vesca* cv 'Alexandria'. Further, we determined the differences in levels of endogenous hormones that are involved in flowering process in response to light quality for 'Albion' and 'Alexandria' cultivars of cultivated and woodland strawberry respectively. We show that the combination of far-red and blue at a ratio of 1:5 activates the expression of *FvFT1* and *FvTFL1* in DN strawberry cultivar, however, flowering seems to occur independently of *FvTFL1*. Further, increased levels of gibberellins and cytokinin in the crown tissue seems to regulate flower bud induction during transplant production in both woodland and cultivated strawberry. The model strawberry species and cultivated strawberry follows the similar morphological changes i.e., flowering when light quality is supplied during daytime. In contrast to cultivated, woodland strawberry plants displayed strong flowering inhibition in all the FR and B combinations when

supplemented during night interruption. Our findings postulates that the FR light-controlled phytochromes may be involved in the floral inhibition response in woodland strawberry through differential regulation of the *FvFT1* transcription factor.

Keywords

Light quality, Flowering pathways, Transcription factor, Hormones, Molecular mechanism.

5.2 Introduction

Light is a major environmental signal that imposes significant effect on plant morphogenesis, photosynthesis, and flowering time (Liao et al. 2014). Plants monitor light environment, which includes light intensity, duration, spectrum, and direction, through photoreceptors or light sensitive protein, to adapt their growth and development (Kami et al. 2010). Phytochromes, cryptochromes, phototropins and the ZEITLUPE (ZTL/FKF1/LKP2) family proteins are important photoreceptors that collectively controls photomorphogenesis over a wide range of light spectrum (Thomas, 2006). Phytochromes are sensitive to far-red (FR) and red light (Strasser et al. 2010), whereas cryptochromes and phototropins mediate blue (B) and UV light respectively (Mockler et al. 2003). Most plant rely on seasonal change in daylength to control the important physiological processes including the transition from vegetative to flowering. Furthermore, light quality or spectrum also have been reported to stimulate early flowering in many plant species. For instance, LD exposure of single peak B (450 nm) and FR (740 nm) light regulates flowering in *Arabidopsis*, *Chrysanthemum* and *Fragaria vesca* (Wollenburg et al. 2008; Higuchi et al. 2012; Rantanen et al. 2014), while red light governs vegetative growth (Yoshida et al. 2016). The light quality control of flowering varies with plant species. In general, long-day plants require long daylight exposure followed by short period exposure to FR and B light to induce flowering, whereas short-day plants

are less affected by the light quality and require longer darkness period to stimulate flowering (Thomas and Vince-Prue, 1997).

The cultivated strawberry (*Fragaria* x *ananassa* Duchesne) is an important crop species grown under a wide range of environmental conditions across North America. Cultivated strawberry is an octoploid species, originated from *F. virginiana* Miller and *F. chiloensis* Linnaeus through accidental hybridization. Strawberry cultivars can be characterized into two groups based on their flowering habits i.e., seasonal and perpetual flowering (Heide et al. 2013). Flowering in seasonal cultivars can be enhanced under SD photoperiod, mainly during the late fall and early summer seasons (Sønsteby and Heide, 2007), whereas flowering in perpetual cultivar occurs continuously if conditions are favorable for plant growth. In short-day plants, flowering is inhibited when long nights are interrupted with short exposure of light and this inhibition can be reversed by successive exposure of FR light in chrysanthemum (Cathey and Borthwick 1957), which indicates the role of phytochromes in flowering response. In a recent study, combined light wavelengths (B and FR LEDs) supplied during daytime and night interruption led to significant increase in FBI and flower development in perpetual cultivars (Sidhu et al. 2021).

Generally, flowering time is mainly controlled by floral inducer and floral repressor gene that adapt to different endogenous and environmental cues. Photoperiodic flowering pathways have been extensively studied in the model plant *Arabidopsis thaliana* (Suárez-López et al. 2001; Valverde et al. 2004). Under inductive photoperiod, photoreceptors mediate light input into circadian clock rhythm and modulate the stability of the CONSTANS (CO) protein that acts as a transcription factor for the floral inducer gene *FLOWERING LOCUS T* (*FT*) (Valverde et al. 2004). The FT gene moves from the leaf phloem, where it is produced, to the shoot apical meristem (SAM) where it binds to the *FLOWERING LOCUS D* (*FD*) and develop a FT-FD complex (Taoka

et al. 2011). Further, this complex upregulates the gene expression of floral meristem identity genes *APETALA1* (*AP1*) and *FRUITFUL* (*FUL*), and as a result induces flowering (Wigge et al. 2005). Conversely, the floral repressor gene *TERMINAL FLOWER1* (*TFL1*) is mainly involved in maintaining vegetative growth by inhibiting the expression of floral meristem identity genes that causes flowering (Hanano and Goto 2011; Koskela et al. 2016). The floral inducer and repressor (FT/TFL1-like protein) homologues have been identified in chrysanthemum (*Chrysanthemum grandiflorum*) and woodland strawberry (*Fragaria vesca*) and considered as the breeding target to control their flowering response (Higuchi et al. 2013; Mouhu et al. 2013). Rantanen et al. (2014) revealed that end of the day FR and B light induces the expression of *FvFT1* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*FvSOC1*). In *Fragaria vesca* Linnaeus, *FvFT1* and *FvSOC1* seem to facilitate flowering in response to photoperiod and light quality through *FvTFL1* (Mouhu et al. 2013; Rantanen et al. 2014). Under inductive conditions, *FvFT1* stimulates the expression of *FvSOC1*, which upregulates the transcript levels of *FvTFL1* (Mouhu et al. 2013). These genes appear to form a linear pathway *FvFT1-FvSOC1-FvTFL1*, that is activated under LD photoperiod and strongly represses flowering in seasonal strawberry (Koskela et al. 2016). The mechanisms involved in light quality control of flowering have been elucidated extensively in seasonal cultivars (Rantanen et al. 2014; Yoshida et al. 2016). However, it is still unclear how floral inducer and inhibitor-like genes respond under combined light wavelengths for DN cultivars. Plants subjected to different light quality exhibit altered flowering behavior and morphological growth, which are mainly regulated by endogenous phytohormones i.e., gibberellins (GAs), auxin (IAA), cytokinins (CK) and abscisic acid (ABA) (Kurepin et al. 2012). Generally, there are several forms of bioactive gibberellins i.e., GA1, GA3, GA4 and GA7 that control multiple aspects of plant growth and development (MacMillan 2001). GA1 is a major endogenous floral regulator that

controls the phase transition and mediates flowering in response to light quality (Kurepin et al. 2007). For instance, low R/FR ratio enhances concentration of growth active GAs in sunflower internodes triggering stem elongation, whereas reduced levels of active GA_1 caused delayed flowering in chrysanthemum under FR deficient light condition (Kurepin et al. 2007). The crosstalk between light and cytokinin-mediated morphogenesis has been long known (Sweere et al. 2001). CK concentration in the SAM plays an important role in the flower induction process (Eshghi and Tafazoli 2007). Furthermore, the involvement of IAA and ABA during the transition from vegetative to reproductive is debatable as positive and negative effects have been reported (Riboni et al. 2016). In response to light quality, ABA is considered as a negative regulator of axillary bud growth under low R/FR, whereas IAA stimulates bud growth, flowering, and stem elongation (Kurepin et al. 2007; Yao and Finlayson 2015).

Light quality dependent regulation of phytohormones and flowering gene in DN strawberry remains elusive. Here, the effects of light quality on the expression of flowering related genes and concentration of different phytohormones were studies to determine the flowering mechanism in day-neutral cultivars. In *F. ananassa* and *F. vesca*, both have seasonal and perpetual genotypes, and their flowering response is mainly dependent on floral-related genes under different environmental conditions (Nakano et al. 2015; Koskela et al. 2016). Flowering genes such as *FT1/TFL1*-like homologues have been identified in both species and controls flowering (Nakano et al. 2015). Due to the genetic complexity of octoploid cultivated strawberry, in the present study, diploid *Fragaria vesca* has been used to elucidate the expression of flowering gene (*FT1/TFL1*-like) in response to light quality in perpetual cultivars.

5.3 Materials and methods

5.3.1 Plant material and growing conditions

The perpetual cultivars ‘Alexandria’ (*Fragaria vesca* L.) and ‘Albion’ (*Fragaria* x *anannassa* Duchesne) were used for the experiment. Plants of ‘Alexandria’ were propagated through seeds (Johnny’s Selected Seeds, Winslow, ME, USA), sown in 288-cell trays containing coconut fiber (Teris, Laval, QC). ‘Albion’ transplants were derived from runner tips of mother plants obtained from Ferme Onésime Pouliot Inc. (45°54’50.584” N, 70°57’6.8” W; Saint-Jean-de-l’Île-d’Orléans, QC, Canada) and rooted in 12-cell mini-trays (250ml per cell) containing coconut fiber. During the establishment phase, ‘Albion’ transplants and ‘Alexandria’ seedlings were grown for 28 days under a 16h photoperiod supplied with high-pressure sodium lamps (HPS) (P.L. Light System, Beamsville, ON, Canada) at maintained temperatures of 22±1°C/18±1°C (Day/Night). Earlier studies have demonstrated that plant age substantially impacts the light quality control of flowering (Verheul et al. 2006). To compare different plant age sensitivity to light quality, 4-week and 10-week-old ‘Alexandria’ seedlings were subjected to the light quality treatments in a greenhouse. Ten-week-old seedlings were kept under 16h photoperiod supplied with HPS lamps at a 50-60$\mu molm^{-2}s^{-1}$ light intensity in the greenhouse. After rooting, transplants were transplanted in 12-cell trays (250-ml cells) for the remainder of the experiment. Light treatments were started once plants had developed three to four unfolded leaves. ‘Albion’ and ‘Alexandria’ plants were hand-fertigated uniformly every week using a nutrient solution supplying, per m^2 containing 114 plants, 1500mg N, 200mg P, and 2200mg K at maintained EC:1.2dS/m, pH: 5.8 to 6.2.

5.3.2 Lighting treatments

Light emitting diodes (LED) (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 B (1:5), dominant FR light (5:1), and/or an equal ratio (1:1) were used for lighting treatment. The LEDs light array prototypes (1.20 m x 32 cm x 8 cm) contained 288 FR plus 1440 small B lamps to obtain dominant B (1:5) ratio designed in four strips. Similarly, 1440 small FR lamps were combined with 288 B to make a dominant FR prototype and 864 lamps of B and FR each were used to achieve a 1:1 light ratio. Each light treatment was isolated using double-layered black perforated cloth that allowed air circulation but no light to go through. Spectral distribution and light intensity were determined using a spectroradiometer (Apogee Instruments Inc., Model PS-300, MN, USA) and quantum light meter (LICOR LI-250A, LICOR, Lincoln, NE, USA) respectively. Instruments were calibrated initially using the manufacturer's guidelines.

5.3.2.1 Light quality control of flowering in DN 'Alexandria'

Once the seedlings reached the 4-week and 10-week stage, they were illuminated with different LEDs (1:5, 5:1 and 1:1) continuously for 16h (06:00 to 22:00) at a light intensity of 50- 60μmolm^{-2}s^{-1} for 45 days. In this experiment, 108 transplants were assigned to a specific light treatment, each experimental unit contained 36 plants and were replicated three times. The morphology data, time to anthesis, tissue collection for gene expression analyses and phytohormonal analysis were carried out. For this trial, morphological data represents the number of flower stalks, developed flowers, new leaf growth and runners. Time to anthesis observations were carried 2-3 times per week to observe the date of appearance of the first fully opened flower. For gene expression, plant tissues were collected separately from young transplants three times throughout the experiment i.e., 7, 28 and 42 days after the treatment began. Tissue sampling was performed in the morning between 10:00 to 11:00 and immediately kept in liquid nitrogen and thereafter stored in -80ºC freezer until further analysis. The crown tissue of 4-week-old seedlings of 'Alexandria' were

collected for phytohormone analysis at two stages i.e., 1 week and 6 weeks after treatment (WAT) began, and immediately stored in -80°C as described previously.

5.3.2.2 Light quality control of flowering in DN 'Albion'

'Albion' transplants were conditioned with different LEDs (1:5, 5:1 and 1:1) continuously for 16h (06:00 to 22:00) at a light intensity of 50-60μmol$m^{-2}s^{-1}$ for 45 days in the greenhouse with consistent DLI between 2.8 to 3.2mol$m^{-2}d^{-1}$. The experiment was established using completely randomized design with three replicates. For each experimental unit, 24 transplants were allocated for a specific light treatment per replication. Three plants were randomly selected for dissection, three for biomass analysis biweekly, and six to measure weekly morphology progress for six consecutive weeks. Crown tissue for 'Albion' cultivar were collected for phytohormone analysis at two stages i.e., 1 week and 6 weeks after treatment (WAT) began. Plant samples were collected from three separate plants per replicate for each treatment and immediately stored in -80°C until the analyses began.

5.3.2.3 Effect of light quality during night interruption on flowering in 'Alexandria' and 'Albion'

Four-week-old 'Alexandria' seedlings and 'Albion' transplants were grown in the greenhouse under continuous high-pressure sodium lamps (HPS) light supplied from 6:00-20:00 (14h), followed by a night interruption (NI) at midnight for 2h (00:00 to 2:00) using FR and B LEDs ratios of 1:5, 5:1 or 1:1. During the night interruption, light intensity was maintained at 50μmol$m^{-2}s^{-1}$. Uninterrupted night treatment where HPS were kept off, was considered as the control. 'Albion' transplants were conditioned under light treatment for 7 weeks, while 'Alexandria' plants were kept for a total of 11 weeks because flowering did not commence within the first 7 weeks of treatment. After 7 weeks, 'Alexandria' plants were transferred to floral-inductive conditions,

where transplants were grown under HPS lamps for 16h-continuous (06:00-22:00) for another 4 weeks to observe whether flowering inhibition is reversible or irreversible. The experiment was arranged in a completely randomized design with three replicates. For each experimental unit, 24 transplants of 'Alexandria' were randomly assigned (as in previous trials) for morphology data, anthesis and tissue collection for gene expression analyses. Tissue samples were collected separately from young transplants five times throughout the experiment i.e., 1, 3, 5, 7 and 11 weeks after treatment began. For 'Albion' cultivar, each experimental unit contained 24 transplants, allocated to specific light treatment and used to collect data for time to anthesis, morphology data and dissection analysis (as previously stated in 'Albion' trial). For this trial, morphological data presented as number of flower stalks, developed flowers, new leaf growth and runners. For the dissection data, results are presented as induced flower bud and inflorescence inside the main crown.

5.3.3 Real-time (RT) quantitative PCR analysis

The gene expression quantification was performed using RT-qPCR in leaf and shoot apex samples of DN *Fragaria vesca* 'Alexandria'. For leaf samples, middle leaflets of the youngest completely opened leaves were pooled from several individuals. For crown samples, upper region of SAM containing 1-2mm pieces from different plants were pooled. Three biological replicates for individual treatment were collected according to the tissue sampling schedule described previously. DNA extraction was done using the CTAB method (Porebski et al.1997) with the following modifications such as two additional phase separation steps with chloroform and isoamyl alcohol were added to further purify the DNA. Multiple PCR was performed to optimize gene specific primers (shown in Table 5.1). RNA extraction was performed using a Spectrum^{TM} Plant Total RNA kit (Sigma-Aldrich Canada Co., Oakville, ON). For cDNA synthesis, an iScript

gDNA Clear cDNA kit was used (Bio-Rad Laboratories Canada Ltd., Montreal, QC, Canada) which includes an iScript reverse transcription supermix. Quantitative PCR reactions were performed using the SsoAdvanced Universal SYBR Green Supermix kit (Bio-Rad Laboratories Canada Ltd., Montreal, QC, Canada). Quantitative RT-PCR reactions was performed using a Stratagene MxPro3005P instrument (Agilent Technology, Mississauga, ON, Canada). RT-PCR cycling conditions were as follows: 30 sec of denaturation at 95ºC, followed by 40 cycles of 15 sec at 95ºC and 30 sec at 56ºC (annealing temperature of primer). *FvMSI1* gene was used as a housekeeping gene and confirmed that cycle threshold (Ct value) did not differ significantly among leaf samples and shoot apex samples. Three technical and three biological replicates were analyzed in this experiment. Relative expression of the targeted genes was calculated using the ΔΔCt method equation (5.1) with stable *FvMSI1* as a housekeeping gene. For the data calculation, 1:1 (FR:B) is considered as the average control group, therefore gene expression data is presented only for dominant B and FR light.

$$\Delta\mathrm{Ct} = \text{Target gene Ct} - \text{ Housekeeping gene Ct} \qquad 5.1$$

$$\Delta\Delta\mathrm{Ct} = \text{Sample } \Delta\mathrm{Ct} - \text{Average Control group } \Delta\mathrm{Ct}$$

$$\text{Relative gene expression} = 2^{(-\Delta\Delta\mathrm{Ct})}$$

Table 5.1 List of qPCR primers used to evaluate the expression of flowering related gene in response to light quality.

Gene	Forward primer sequence	Reverse primer sequence	Accession	Source[z]
FvMSI1	TCCCCACACCTTTGATTGCCA	ACACCATCAGTCTCCTGCCAAG	XM_004307240	Rantanen et al. 2014
FvFT1	GGCCAAGAGATTGTGTGTTATG	GGCAAAGTCTCTGGTGTTAAAG	NM_001280022	Primer Designed from IDT
FvTFL1	CTGGCACCACAGATGCTACA	AACGGCAGCAACAGGAAC	JN172097	Rantanen et al. 2014
FvSOC1	ACTTGCTGGGTTCATTTTCC	GAGCTTTCCTCTGGGAGAGA	JF806634	Rantanen et al. 2014
FvphyB	GTTCCTTGAGACTAGTGCTGCTTG	CCTTCTCAAGCTCCAAAGAACC	XM_004295029	Primer Designed from IDT

[z] Primers for *FvFT1* and *FvphyB* were designed using the PrimerQuest tool from IDT (Integrated DNA technologies) for qPCR assay. Primer sets for *FvMSI1*, *FvTFL1* and *FvSOC1* were defined by Rantanen et al. (2014).

5.3.4 Endogenous hormone analysis

The phytohormones quantification for ‘Alexandria’ and ‘Albion’ was performed by the UVic-Genome BC Proteomics Centre (University of Victoria, Victoria, BC, Canada).

5.3.4.1 Sample preprocessing

For the analysis, 100mg of frozen shoot apex was grounded into fine powder with a mortar and pestle and kept in 2-ml safe lock Eppendorf tubes (Sigma-Aldrich Canada Co., Oakville, ON, Canada). For each mg of raw tissue, 4µl of 2% formic acid and two 4-mm stainless steel balls were added. The sample was homogenized at a shaking frequency of 30Hz on a MM4000 mixer mill (Verder Scientific Inc., Newton, PA, USA) for 1 min, repeated three times. Methanol (16µl per mg raw tissue) was used as the extraction solvent. The samples were homogenized again for 1 min, repeated three times, followed by sonication in an ice-water bath for 5 min and centrifugal clarification at 21000g and 10ºC for 10min. The clear supernatant was collected for analysis.

5.3.4.2 Analysis of ABA

A stock standard solution of ABA was prepared in 80% methanol at 20 nmol/ml. This solution was serially diluted with the same solvent in a volume ratio of 1 to 4 to have working standard solutions in a range of 0.0001 to 5 µM. For each sample, 50µl aliquots of the supernatant and each standard solution was mixed with 50µl of an internal standard solution (ursodeoxycholic-D4 acid). Each resultant solution of 20µl was injected in an ultrahigh-performance liquid chromatography (UPLC) coupled to multiple-reaction monitoring mass spectrometry (MRM/MS) on an Agilent 1290 UHPLC system coupled to an Agilent 6495B QQQ mass spectrometer (Agilent Technologies Inc. Santa Clara, California, USA), equipped with an electrospray ionization source which was operated in the negative-ion mode. The chromatographic separation was carried out on a Waters BEH C18 column (2.1 x 150 mm,1.8 µm) with 5 mM ammonium acetate buffer (A) – acetonitrile

(B) as the binary solvents for gradient elution at 50 ºC and 0.35 mL/min. The elution gradient was 15% to 100% B in 12.5 min, followed by 4-min column equilibration between injections.

5.3.4.3 Analysis of kinetin, zeatin and auxins

A mixed stock standard solution of kinetin, zeatin and three 3 auxin compounds (indole acetic acid, indole butyric acid and indole propionic acid) was prepared in an internal standard solution of 13C3-cortisol in 80% methanol. This solution was serially diluted with the internal standard solution to have calibration solutions in a range of 0.0001 to 1µM. For each sample, 100µl of the supernatant was mixed with 50µl of the internal standard solution and 900µl of 80% methanol. The mixture was then loaded onto a polymeric reversed-phase Oasis HLB cartridge (30 mg/1mL, Waters Inc. Milford, MA, USA), which was activated with 1 mL of methanol and reconditioned with 1 mL of water before use. Under a positive pressure, the flow-through fraction was collected and dried under a gentle nitrogen gas flow at 30 ºC. The dried residue was reconstituted in 50µl of 10% methanol. 20-µl aliquots of each standard solution and each resultant sample solution were injected in an UPLC-MRM/MS on the same Agilent 1290 UHPLC system coupled to an Agilent 6495B QQQ mass spectrometer but with positive-ion detection. The chromatographic separation was carried out on the same C18 column. The mobile phase was 0.1% formic acid in water (A) and acetonitrile (B) as the binary solvents for gradient elution at 40 ºC and 0.3 mL/min. The elution gradient was 5% to 100% B in 15 min, followed by 4-min column equilibration between injections.

5.3.4.4 Analysis of gibberellins

A mixed stock standard solution of 4 gibberellin compounds (A1, A3, A4 and A7) was prepared in an internal standard solution of 13C3-cortisol in 80% methanol. This solution was serially diluted with the same internal standard solution to have calibration solutions in a range of 0.0001 to 1 nmol/ml. For each calibration solution, 30µl was dried under a gentle nitrogen gas flow at

30ºC. For each sample, 200µl of the supernatant was mixed with 40µl of the internal standard solution. The mixture was then mixed with 900µl of water inside a polymeric reversed-phase Oasis HLB cartridge (30 mg/1ml) on the top of HLB resin, which was activated with 1ml of methanol and reconditioned with 1ml of water before use. Under a positive pressure, the flow-through fraction was discarded. The analytes were eluted with 1ml of methanol and the collected fraction was dried under a gentle nitrogen gas flow at 30ºC. The dried residues of each calibration solution and each sample solution were added with 40µl of 50-mM N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide solution. The mixture was allowed to react at 30ºC for 2h. 20µl of each resultant solution was injected in an UPLC-MRM/MS on an Acquity UPLC system (Milford, MA, USA) coupled to a QTRAP 6500 Plus mass spectrometer (Sciex Inc., Concord, ON, Canada) with positive-ion detection. The chromatographic separation was carried out on the same C18 column (2.1 x 150 mm, 1.8 µm) and with 0.1% formic acid in water (A) and methanol (B) as the binary solvents for gradient elution at 50 ºC and 0.3ml/min. The elution gradient was 20% to 100% B in 15 min, followed by 4-min column equilibration. For the above UPLC-MRM/MS analyses, molar concentrations of individual compounds detected in the samples were calculated by interpolating their respective, constructed linear-regression calibration curves with the analyte-to-internal standard peak area ratios measured from injections of the sample solutions.

5.3.5 Statistical methods

Difference between the light treatments were determined using the Analysis of Variance (ANOVA) using a statistical analysis software (SAS 9.4 version, Analytics Software and Solutions, North Carolina, USA). The effects of individual treatments and their interactions 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.

5.4 Results

5.4.1 Light quality control of flowering in DNs 'Alexandria' and 'Albion'.

5.4.1.1 Dominant blue LEDs promotes flowering in DN 'Alexandria'

The two-way interaction between plant age and light quality showed significant results for flower development (p-value:0.01) and for flower stalk growth data (p-value:0.01). Four-week-old seedlings of 'Alexandria' grown under dominant B (1:5) LEDs developed significantly higher number of flower stalks compared to 5:1 and 1:1 (Figure 5.1A). However, older seedlings (10-week) kept under LEDs for 45 days showed no significant difference in flower stalk growth among the light treatments (Figure 5.1B). The two-way interaction between plant age and light quality showed non-significant results for leaf growth (p-value:0.92) and flower development (p-value:0.08). However, the individual effect of light quality showed significant results for flower development (p-value:0.02), where seedlings produced 14 flowers per plant for dominant B LEDs compared to dominant FR (3 flowers per plant) and 1:1 (5 flowers per plant) by the end of the light treatments. Along with the reproductive boost, dominant B LEDs significantly (p-value:0.03) increased new leaf growth compared to dominant FR and the 1:1 ratio.

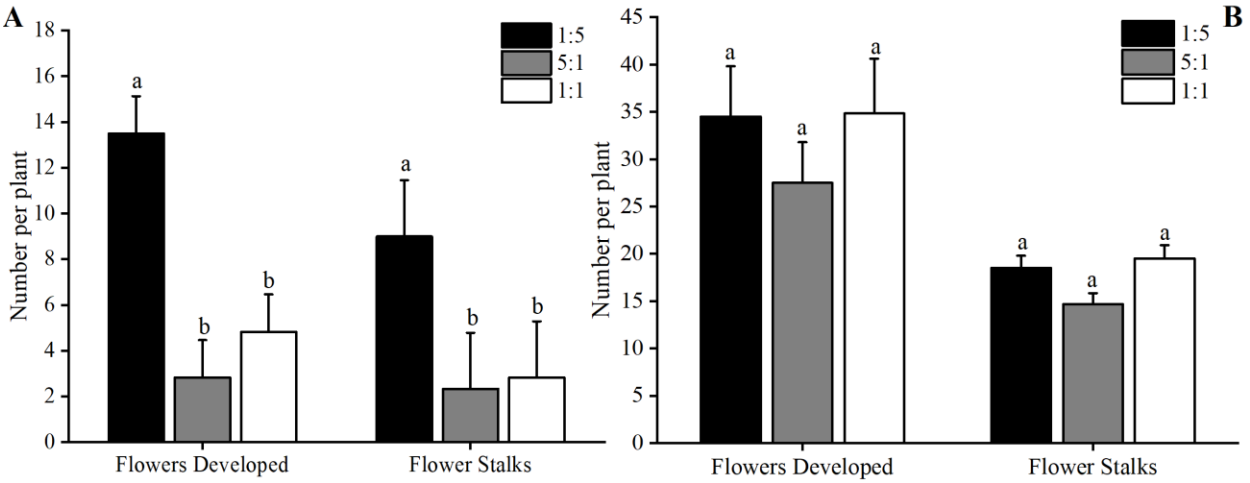


Figure 5.1 The flowering response of 4-week and 10-week-old 'Alexandria' seedlings under different light quality. (A) Average number of flowers developed and flower stalks (per plant) in young (4-week) 'Alexandria' seedlings in response to light quality. (B) Average number of flowers developed and flower stalks (per plant) in old 'Alexandria' (10-week) seedlings in response to light quality. Mean values with the same lowercase are not significantly different among Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm):Blue (455 nm).

5.4.1.2 Dominant blue LEDs promotes flowering in DN 'Albion'

'Albion' transplants grown under dominant B (1:5) LEDs exhibited significantly higher number of FBI (p-value:0.02) inside the crown compared to 5:1 and 1:1 (Figure 5.2A). Dominant B LEDs produced significantly higher number of inflorescences compared to 1:1, while results were statistically not significantly different from 5:1 (Figure 5.2A). Dominant FR LEDs significantly stimulated the growth of new leaves compared to the dominant B (Figure 5.2A; p-value:0.04). Results showed no significant difference in runner production for 'Albion' in response to light quality (Figure 5.2A; p-value:0.24). Similarly, for the dry biomass allocation, transplants showed no significant (Figure 5.2B; p-value:0.36) differences in response to light quality.

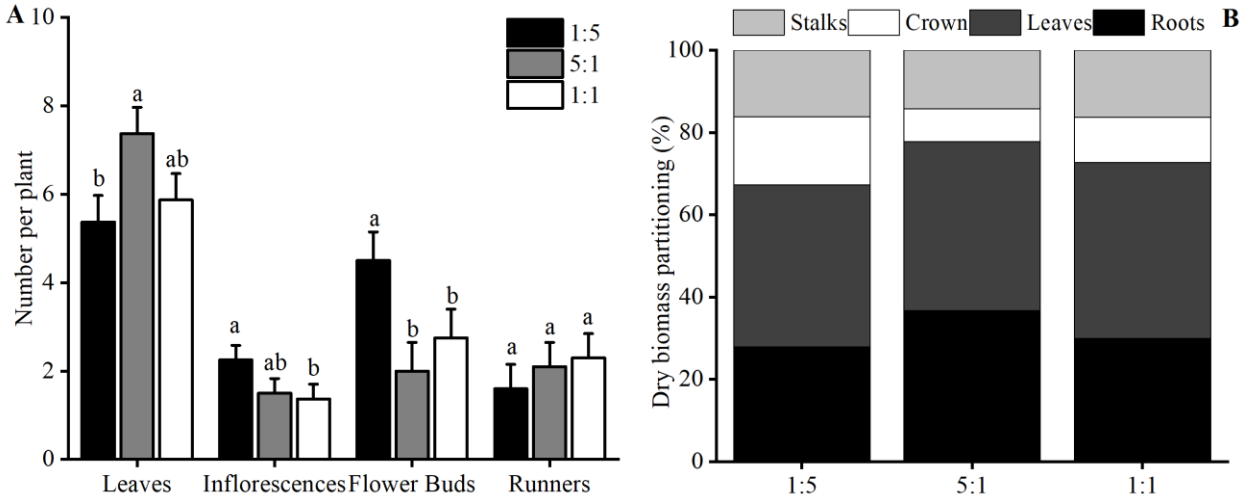


Figure 5.2 Light quality effect on flowering, morphological growth and dry biomass partitioning for 'Albion' cultivar. (A) Average number of new leaves, inflorescences, flower buds and runners (per plant) in 'Albion transplants in response to light quality. (B) Dry biomass partitioning (stalks, crown, leaves and roots) of 'Albion' transplants in response to light quality. Mean values with the same lowercase are not significantly different among Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm):Blue (455 nm).

5.4.1.3 Dominant blue LEDs induces the expression of *FvFT1* and *FvTFL1*

The two-way interaction between light quality and time showed non-significant (p-value:0.62) interaction for relative gene expression of *FvFT1* and *FvTFL1*. However, it is interesting to observe that gene expression levels in young and old seedlings followed the similar trend in response to light quality. For instance, dominant B exhibited higher expression levels for *FvFT1* and *FvTFL1* during the first week, however, dominant FR demonstrated higher expression for both the genes towards the end of light treatment. Like for the flowering response, the transcript levels of the floral inducer gene, *FvFT1*, was upregulated in the leaves of young (4-week) seedlings under dominant B LEDs. One week after the beginning of light treatment, the expression of *FvFT1*, in young seedlings was significantly upregulated (three-folds, p-value:0.03) under dominant B LEDs compared to dominant FR (Figure 5. 3A). Similarly, the expression of the floral repressor gene *FvTFL1* in shoot apex was upregulated (1.5-fold) under dominant B LEDs (Figure 5.3C), although not significantly.

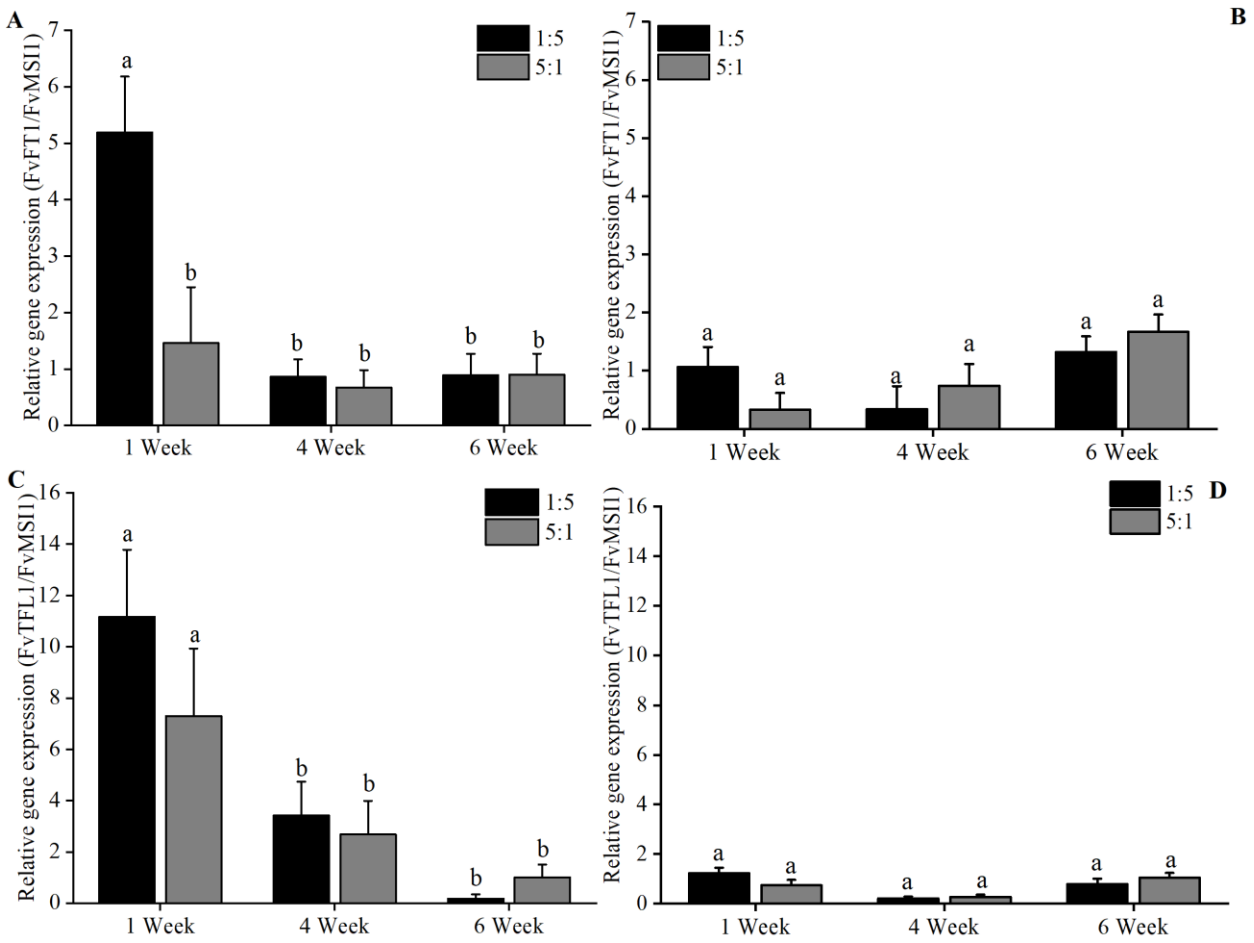


Figure 5.3 RT-qPCR expression study of flowering related genes *FvFT1* and *FvTFL1* under different light quality for 'Alexandria' cultivar. (A) The relative gene expression of *FvFT1* in the leaves of young (4-week) seedlings of 'Alexandria' collected at different time intervals (1, 4 and 6 weeks after the treatment (WAT) began) in response to light treatment. (B) The relative gene expression of *FvFT1* in the leaves of old (10-week) seedlings of 'Alexandria' collected at different time intervals (1, 4 and 6 WAT) in response to light treatment. (C) The expression of *FvTFL1* in the shoot apex of young (4-week) seedlings of 'Alexandria' collected at different time intervals (1, 4 and 6 WAT) in response to light treatment. (D) The expression of *FvTFL1* in the shoot apex of old (10-week) seedlings of 'Alexandria' collected at different time intervals (1, 4 and 6 WAT) in response to light treatment. Mean values with the same lowercase are not significantly different among Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. *FvMSI1* referred as housekeeping gene to determine relative expression. Light ratios of 1:5 and 5:1 of Far-red (725 nm):Blue (455 nm).

The higher level of gene expression was recorded for both flowering related genes during the first week after the start of light treatment. In contrast, very low or no expression was detected for both the genes in the following weeks, regardless of light treatment. The expression of *FvFT1* and *FvTFL1* were recorded even lower in older seedlings (10-week) compared to young seedlings and exhibited non-significant differences among light treatments (Figure 5.3B & D).

Light spectrum inconsistently affects the expression of flowering genes in both tissues i.e., leaves and shoot apex. Here, we detected that the transcription levels of *FvFT1* in shoot apex and *FvTFL1* in leaves. Unlike leave tissue, the expression of *FvFT1* in shoot apex showed non-significant results in response to different light quality. The expression of *FvFT1* was significantly downregulated following 6-weeks exposure of dominant B LEDs in the shoot apex compared to dominant FR. In contrast, the expression of *FvTFL1* in leaves remained unaffected by different light quality (Supplementary 7.2).

5.4.1.4 *FvFT1-FvSOC1-FvTFL1* pathway in day neutral 'Alexandria'

The transcript levels of *FvFT1* and *FvTFL1* upregulated at earlier stage (1-WAT) under dominant blue light (Figure 5.3A & C). Similarly, for 4-weeks old seedlings, the mRNA levels of *FvSOC1* in the shoot apex was upregulated in response to dominant blue LEDs at earlier stage followed by low expression until the end of the treatments (Figure 5.4A). Under dominant B LEDs, the transcription levels of floral related genes i.e., *FvFT1*, *FvSOC1* and *FvTFL1* closely mimicked each other during the first week (1-WAT) in response to light quality. However, the expression of *FvSOC1* in 10-week-old seedlings was significantly (five-folds, p-value:0.02) upregulated, in contrast to *FvFT1* and *FvTFL1*, towards the end of treatments under dominant FR LEDs (Figure 5.4B).

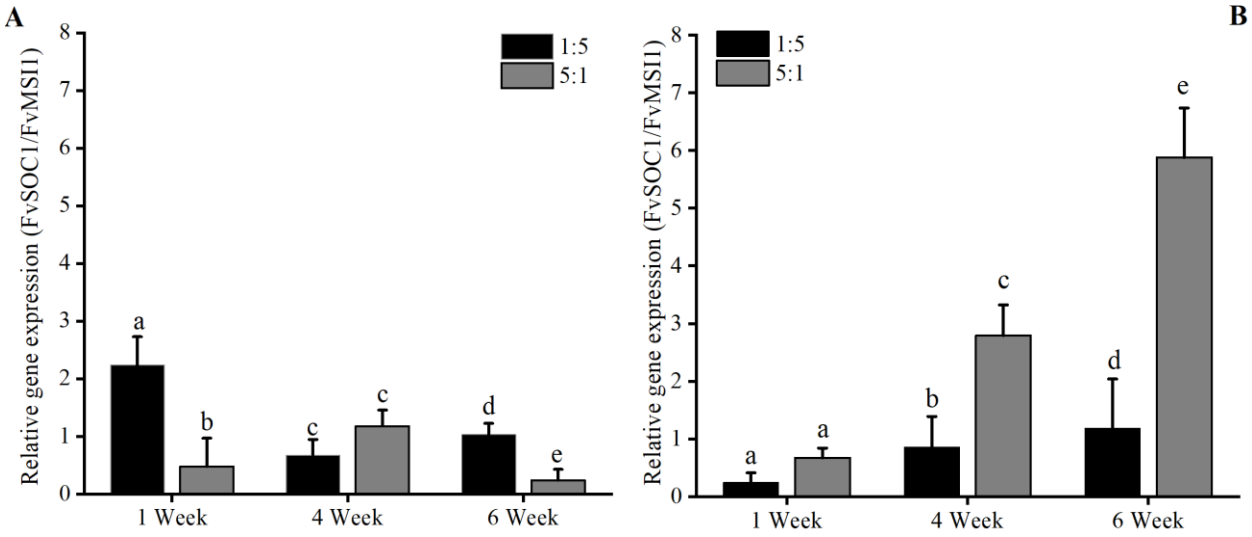


Figure 5.4 RT-qPCR expression study of flowering related genes *FvSOC1* under different light quality for 'Alexandria' cultivar. (A) The relative gene expression of *FvSOC1* in the shoot apex of young (4-week) seedlings of 'Alexandria' collected at different time intervals (1, 4 and 6 WAT) in response to light treatment. (B) The expression of *FvSOC1* in the shoot apex of old (10-week) seedlings of 'Alexandria' collected at different time intervals (1, 4 and 6 WAT) in response to light treatment. Mean values with the same lowercase are not significantly different among Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. *FvMSI1* referred as housekeeping gene to determine relative expression. Light ratios of 1:5 and 5:1 of Far-red (725 nm):Blue (455 nm).

5.4.2 Effect of light quality supplied during NI on flowering in 'Albion' and 'Alexandria'

Dominant B LEDs supplied during night interruption significantly promoted flower bud induction in the crown (p-value:0.001) and flower development (p-value:0.035) in 'Albion' (Figure 5.5A). Transplants grown under dominant B LEDs produced 4 flower buds (per plant) compared to 2 for dominant FR, or 3 for 1:1 and the control. However, transplants showed no significant difference among new leaf growth (p-value:0.53) and plant height (p-value:0.79). In contrast, 'Alexandria' seedlings failed to form flowers when grown under dominant B LEDs, dominant FR and 1:1 supplied during night interruption (Figure 5.5B). Although, growing conditions for 'Alexandria' seedlings were maintained the same as for 'Albion' transplants for 7 weeks, there was no sign of flower induction or development, except in the control treatment (Figure 5.5B). After 7-weeks, the 'Alexandria' seedlings were transferred to 16h continuous exposure to LEDs for another 4-weeks to trigger flowering. However, still there was no sign of flower initiation. It is interesting to observe that exposure of FR and B LEDs ratios (1:1, 1:5 and 5:1) significantly promoted the shoot

extension in ‘Alexandria’ seedlings compared to the control, in contrast to ‘Albion’ (Figure 5.5B). New leave production revealed no significant differences among the treatments.

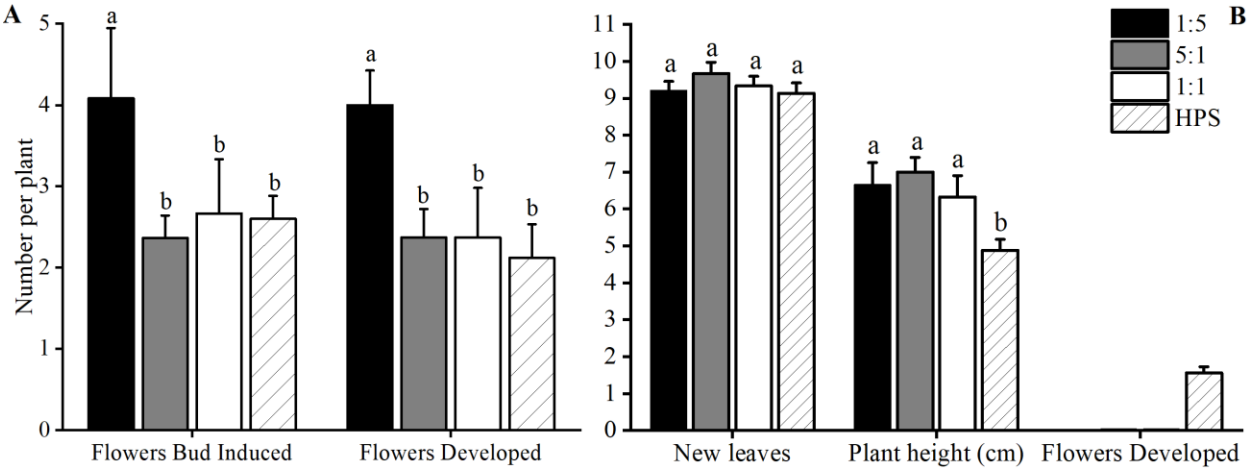


Figure 5.5 The flowering and morphological growth response of ‘Albion’ and ‘Alexandria’ cultivars under different light quality supplied during night interruption. (A) Average number of flowers buds induced, and flowers developed (per plant) in ‘Albion’ transplants in response to light quality during night interruption. (B) Average number of new leaves, plant height (in cm) and flowers developed (per plant) in ‘Alexandria’ seedlings in response to light quality supplied during night interruption. Mean values with the same lowercase are not significantly different Different lowercase shows the significance among each group of light treatment evaluated using Fisher’s LSD significance test. Light ratios of 1:5, 5:1, 1:1 of Far-red (725 nm):Blue (455 nm) and control.

5.4.2.1 Possible involvement of phytochrome (phyB) in flowering inhibition during NI.

The two-way interaction between light quality and time showed no significant results (p-value: 0.69) for the expression of *FvFT1* in young leaves of ‘Alexandria’. Based on RT-qPCR data, the expression of *FvFT1* was peaked in all the treatment 1-week after the light treatment, followed by a substantial drop until the 7th week (Figure 5.6A). After the plants were transferred to 16h photoperiod supplied with HPS for another 4 weeks, the significant surge was observed in the mRNA level of *FvFT1* for all light treatments especially under dominant B LEDs and 1:1 (p-value:0.03). In contrast *to FvFT1*, the main effect of light quality (p-value: 0.32) and time (p-value: 0.20) showed no significant results for *FvphyB* due to high variability among biological replicates. The mRNA levels of *FvphyB* dropped very low in all the light treatments following 4-week 16h photoperiod treatment (Figure 5.6B). Under different light quality treatments, the transcription

level of *FvphyB* was peaked under dominant FR LEDs. After the plants were transferred to 16h photoperiod, the mRNA level of *FvphyB* was recorded very low.

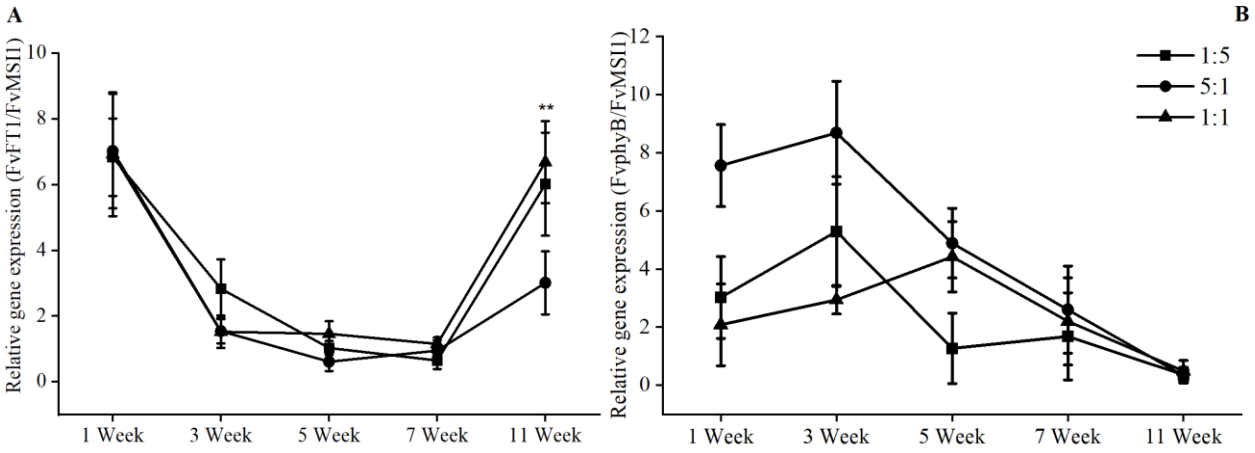


Figure 5.6 RT-qPCR expression study of *FvFT1* and *FvphyB* under different light quality for 'Alexandria' cultivar. (A) The relative gene expression of *FvFT1* in the young leaves of 'Alexandria' seedlings collected at different time intervals (1, 3, 5, 7 and 11 weeks) in response to light quality supplied during night interruption. (B) The relative gene expression of *FvphyB* in the young leaves of 'Alexandria' seedlings collected at different time intervals (1, 3, 5, 7 and 11 weeks) in response to light quality supplied during night interruption. Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. *FvMSI1* was used as the housekeeping gene to determine relative expression. Light ratios of 1:5, 5:1 and 1:1 of Far-red (725 nm):Blue (455 nm).

5.4.3 Light quality effect on phytohormones in 'Alexandria' and 'Albion'

Strong interactions were detected between light quality and time in *Fragaria vesca* cv. 'Alexandria' for the concentrations of kinetin (Figure 5.7 A), GA1 (Figure 5.7 C), ABA (Figure 5.8 A) and GA3 (Figure 5.8 C) hormones. The concentration of GA1, GA3 and Kinetin increased significantly after a 6-week exposure to 1:5 LEDs (Table 5.2). In contrast, the ABA concentration increased significantly after 6-week in 5:1 and 1:1 light treatment, however, there was no significant change observed under 1:5. The two-way interaction between light quality and time showed no significant results for GA7, GA4, IAA and Zeatin (Table 5.3). However, the main effect of light quality demonstrated significant results, where the concentration of GA7, zeatin and IAA increased significantly under 1:5. The concentrations of GA4 showed no significant differences among light treatments (Table 5.3).

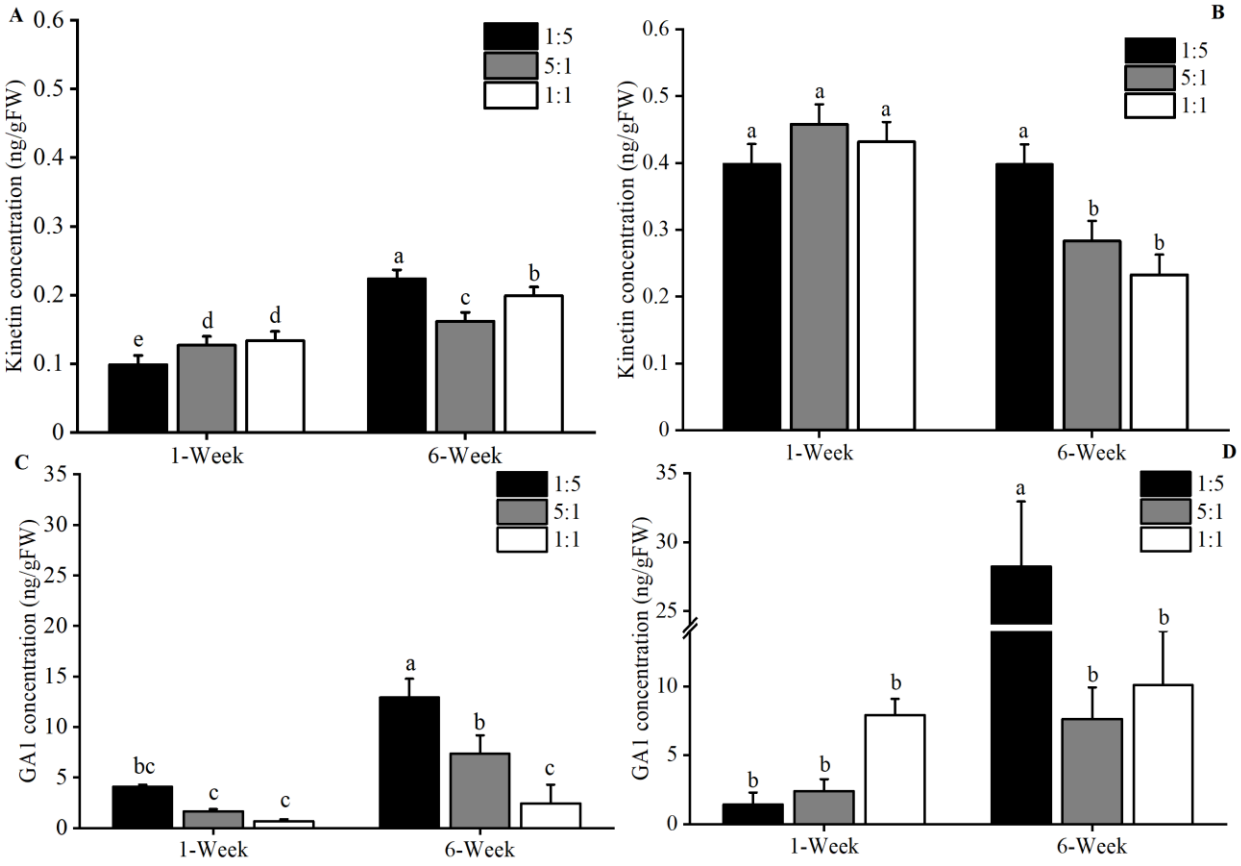


Figure 5.7 Effect of light quality on the concentrations of different phytohormones in the shoot apices of *Fragaria* x *vesca* cv 'Alexandria' and *Fragaria* x *ananassa* cv 'Albion'. (A) The concentration of cytokinin (Kinetin) hormone in the shoot apex of *Fragaria* x *vesca* cv 'Alexandria' at two-time intervals (1-week and 6-week after the light treatment began). (B) The concentration of cytokinin (Kinetin) hormones in the shoot apex of *Fragaria* x *ananassa* cv 'Albion' at two-time intervals (1-week and 6-week after the light treatment began). (C) The concentration of gibberellins (GA1) hormone in the shoot apex of *Fragaria* x *vesca* cv 'Alexandria' at two-time intervals (1-week and 6-week after the light treatment began). (D) The concentration of gibberellins (GA1) hormones in the shoot apex of *Fragaria* x *ananassa* cv 'Albion' at two-time intervals (1-week and 6-week after the light treatment began). Statistical analyses of effect of light quality and time on plant hormones concentrations are presented. Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. Light ratios of 1:5, 5:1 and 1:1 contained of Far-red (725 nm): Blue (455 nm) respectively. FW; Fresh Weight.

Table 5.2 p-values for the main effects of light quality and time and their interactive effects on concentration of plant hormones in the shoot apex for cultivated and woodland strawberry.

Source of variation	*Fragaria ananassa* cv 'Albion'				*Fragaria vesca* cv 'Alexandria'			
	Kinetin[z]	GA1[z]	GA3[z]	ABA[z]	Kinetin[z]	GA1[z]	GA3[z]	ABA[z]
Light Quality	0.0004	0.1513	0.2323	0.0031	0.0041	0.0044	0.0016	0.4414
Time	0.0037	0.0114	0.0078	0.0013	0.0002	0.0016	0.0086	0.0016
Light Quality*Time	0.0053	0.0438	0.1872	0.7692	0.0034	0.0113	0.0045	0.0304

[z] Concentrations of phytohormones is described in nano-gram per gram fresh weight of plant sample.

Like for the 'Alexandria' cultivar, light quality and time demonstrated a strong interaction to regulate the concentration levels of kinetin and GA1 hormones in shoot apices of 'Albion' cultivar following 6-week exposure of dominant B LEDs (Figure 5.7 B & D). In addition, the concentration

of GA7, GA4, IAA and Zeatin showed strong interaction among light quality and time (Table 5.3). Dominant FR LEDs increased GA4 concentration compared to dominant B, whereas 1:1 (FR:B) increased the concentrations of IAA and Zeatin after 6-weeks of light exposure (Table 5.4). Unlike in 'Alexandria', GA3 and ABA concentration declines significantly after light treatment in 'Albion' (Figure 5.8 B & D).

Table 5.3 Effect of light quality on the concentrations of Gibberellins (GA7 and GA4), Auxin (IAA) and Cytokinin (Zeatin) in the shoot apices of *Fragaria* x *vesca* cv 'Alexandria'. p-values demonstrates the main effect of light quality and time and their interactive effects on the concentration of hormones.

Light Quality	GA7 (ng/g^{z})	GA4 (ng/g^{z})	IAA (ng/g^{z})	Zeatin (ng/g^{z})
1:5	0.66 a	0.48 a	11.09 a	1.29 a
5:1	0.45 b	0.51 a	7.12 b	1.31 a
1:1	0.44 b	0.52 a	8.29 b	1.03 b
Light Quality	0.0054	0.9414	0.0078	0.0253
Time	0.0116	0.1973	0.0068	0.0026
Light Quality*Time	0.07339^{ns}	0.1678^{ns}	0.2697^{ns}	0.2704^{ns}

ns; non-significant, * interaction between light quality and time, ^{z} Concentrations of phytohormones is described in nano-gram per gram fresh weight of plant sample. Data presented in the table is the mean value for each hormone concentration. Standard error of the mean is 0.07 for GA7, 0.11 for GA4, 0.81 for IAA and 0.12 for zeatin, calculated using Fisher's LSD test. Means value are calculated from three replicates with sample size (n=9). Light quality ratios of 1:5, 5:1 and 1:1 contained Far-red (725 nm):Blue (455 nm) respectively.

Table 5.4 Effect of light quality on the concentrations of Gibberellins (GA7 and GA4), Auxin (IAA) and Cytokinin (Zeatin) in the shoot apices of *Fragaria* x *ananassa* cv 'Albion' at different time intervals. p-values demonstrates the main effect of light quality and time and their interactive effects on the concentration of hormones.

LQ	Time	GA7 (ng/g^{z})	GA4 (ng/g^{z})	IAA (ng/g^{z})	Zeatin (ng/g^{z})
1:5	1-Week	0.69 a	0.42 c	3.42 c	0.64 b
5:1	1-Week	0.72 a	0.86 b	3.45 c	0.37 c
1:1	1-Week	0.57 ab	0.56 bc	4.71 bc	0.57 b
1:5	6-Week	0.70 a	0.46 c	9.34 b	0.27 c
5:1	6-Week	0.43 b	1.35 a	4.74 bc	0.36 c
1:1	6-Week	0.81 a	0.49 c	19.16 a	0.85 a
p-value	Light	0.0085	0.0024	0.0028	0.0061
	Time	0.1602	0.0326	0.0027	0.2676
	LQ*T	0.0034**	0.0076**	0.0017**	0.0016**

ns; non-significant, * interaction between light quality and time, ^{z} Concentrations of phytohormones is described in nano-gram per gram fresh weight of plant sample. Standard error of the mean is 0.06 for GA7, 0.18 for GA4, 0.87 for IAA and 0.12 for zeatin, calculated using Fisher's LSD test. Means value are calculated from three replicates with sample size (n=9). Light quality ratios of 1:5, 5:1 and 1:1 contained Far-red (725 nm):Blue (455 nm) respectively.

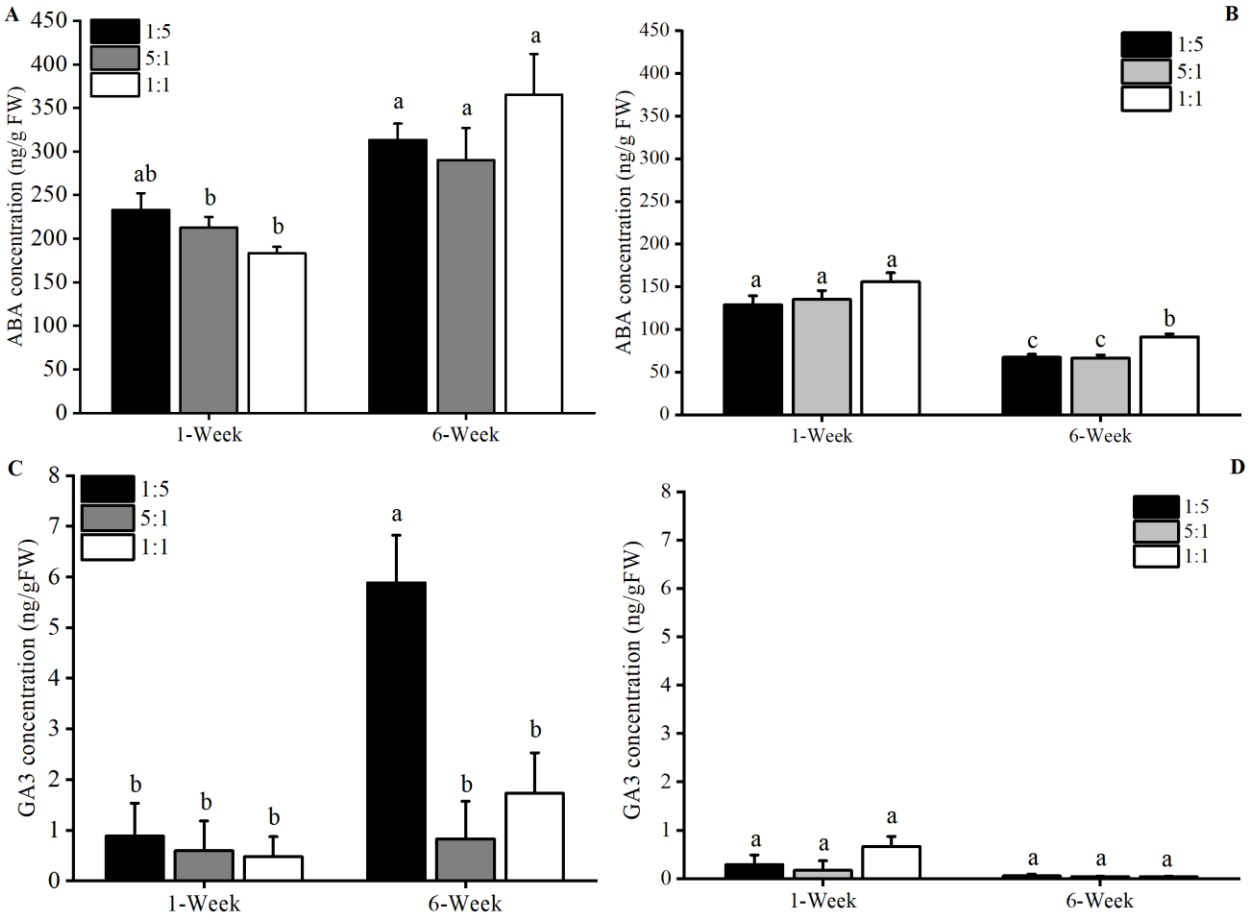


Figure 5.8 Effect of light quality on the concentrations of different phytohormones in the shoot apices of *Fragaria vesca* cv 'Alexandria' and *Fragaria* x *ananassa* cv 'Albion'. (A) The concentration of abscisic acid (ABA) hormone in the shoot apex of *Fragaria* x *vesca* cv 'Alexandria' at two-time intervals (1-week and 6-week after the light treatment began). (B) The concentration of abscisic acid (ABA) hormones in the shoot apex of *Fragaria* x *ananassa* cv 'Albion' at two-time intervals (1-week and 6-week after the light treatment began). (C) The concentration of gibberellins (GA3) hormone in the shoot apex of *Fragaria* x *vesca* cv 'Alexandria' at two-time intervals (1-week and 6-week after the light treatment began). (D) The concentration of gibberellins (GA3) hormones in the shoot apex of *Fragaria* x *ananassa* cv 'Albion' at two-time intervals (1-week and 6-week after the light treatment began). Statistical analyses of effect of light quality and time on plant hormones concentrations are presented. Different lowercase shows the significance among each group of light treatment evaluated using Fisher's LSD significance test. Light ratios of 1:5, 5:1 and 1:1 contained of Far-red (725 nm): Blue (455 nm) respectively. FW; Fresh Weight.

5.5 Discussion

Plants perceive environmental cues such as photoperiod and light quality through photoreceptors and anticipates the functioning of circadian clock that regulates multiple genetic pathways, to ensure appropriate growth and development (Srivastava et al. 2019). Flowering in plants is regulated by a complex network of genetic pathways that integrates environmental conditions and endogenous signaling. Here, we show the light quality control of flowering by evaluating the

transcription of floral-related genes and concentration of phytohormones. In the present study, the flower stalk and flower development were strongly advanced in 4-week-old seedlings of ‘Alexandria’ under dominant B LEDs (1:5) compared to 5:1 and 1:1. Similar flowering response was observed in day neutral ‘Albion’ of *F. ananassa* under dominant B LEDs in combination with FR (1:5) (Sidhu et al. 2021). This is a standard response of several long-day plants to narrow-band light source of blue LEDs as it significantly promotes flowering in *Arabidopsis*, cultivated and woodland strawberry respectively (Mockler et al. 2003; Rantanen et al. 2014; Nadalini et al. 2017). Kim et al. (2005) stated that minimum amount of blue light is necessary for normal growth and development of the plant. Blue light plays a significant and superior role in controlling flowering compared to FR light, thus, enriched blue light in combination with FR upregulates flowering in ‘Alexandria’. Studies have shown that light spectrum requirement is likely to changes with plant age for normal plant growth and development (Cope and Bugbee 2013). For instance, younger plant requires higher amount of blue light to maximize light interception and prevent excessive stem elongation (Cope and Bugbee 2013). In the present study, we observed that 4-week-old seedlings are more responsive to dominant B compared to 10-week-old seedlings, which facilitate flowering in younger seedlings. It is possible that plant age plays an important role to control light quality-based flowering in day-neutral ‘Alexandria’.

Light supplied during NI mimics the LD conditions and advances the phase transition from vegetative to reproductive development in long-day and day-neutral plants (Park et al. 2017; Rashidi et al. 2018 Sidhu et al. 2021). In agreement with previous studies, the present study demonstrates that dominant B LEDs supplemented during NI considerably advances the flower bud induction and development in ‘Albion’. Whereas, in contrast to ‘Albion’, all the FR and B combinations supplied during NI strongly inhibited the flowering and enhanced shoot extension

in ‘Alexandria’. Similar flowering inhibition and shoot extension was previously reported in chrysanthemum when plants were subjected to single-color B and FR lights during night break, which implies the possible involvement of phytochromes in flowering inhibition (Higuchi et al. 2012).

In *Arabidopsis*, *FT* and *TFL1* genes have been extensively studied to understand the photoperiodic control of flowering (Suárez-López et al. 2001 and Koskela et al. 2012). Similalry, *FT1* and *TFL1*-like genes have been identified in *Fragaria vesca* and considered as breeding target to control flowering under different environmental conditions (Mouhu et al. 2013; Koskela et al. 2016). Rantanen et al. (2014) have revealed that *FvFT1* and *FvTFL1* genes regulate flowering in response to the light quality. The upregulation of *FvFT1* in response to single-color B and FR LEDs is responsible to advance flowering in seasonal and perpetual accessions. Consistent with Rantanen et al. (2014), here, we found that expression of *FvFT1* strongly upregulated (three-folds) 1-week after the light treatment comprised of two narrow-band light sources of FR and B at a ratio of 1:5, whereas very low expression was observed in 5:1.

The photoperiodic flowering pathways are well known in *Arabidopsis* and *F. vesca* (Yoo et al. 2005; Mouhu et al. 2013). Studies revealed that *FT1* and *SOC1*-like genes may facilitate the photoperiod control of flowering through *TFL1* (Mouhu et al. 2013). In *F. vesca*, the upregulated expression of *FvFT1* in leaves activates the expression of *FvSOC1* in the shoot apex under inductive LD photoperiod. Further, increased transcript level of *FvSOC1* regulates the mRNA levels of *FvTFL1* in shoot apex that acts as floral repressor in seasonal strawberry (Koskela et al. 2012). Several studies have supported the presence of the *FvFT1-FvSOC1-FvTFL1* regulated pathway in short-day and long-day accession of strawberry (Koskela et al. 2012; Mouhu et al. 2013; Rantanen et al. 2014). In the present study, the transcript levels of *FvFT1* in leaves and

mRNA levels of *FvSOC1* and *FvTFL1* in shoot apex was recorded significantly higher under dominant B during the first week. Further, the expression levels of *FvFT1*, *FvSOC1* and *FvTFL1* declines significantly in the following weeks in all light treatments. Results indicates that these genes formulate the linear pathway in response to a combination of FR and B lights, suggesting that the *FvFT1-FvSOC1-FvTFL1* mechanism is possibly be present in day-neutral *F. vesca* 'Alexandria'. Since, the flowering response of day-neutral *F. ananassa* cv 'Albion' (Sidhu et al. 2021) is similar to *F. vesca* cv 'Alexandria' under different light quality, suggesting that orthologs of *FvFT1-FvSOC1-FvTFL1* may be present in cultivated strawberry, although this is not confirmed yet (Koskela et al. 2016).

FvTFL1 is considered as a strong floral repressor that determines the reverse the flowering response in seasonal genotype (Koskela et al. 2012). Under LD photoperiod, the expression of *FvTFL1* gets activated and over-dominates the expression of *FvFT1*, thus, represses flowering in seasonal cultivars. In contrast to short-day, long-day genotype have non-functional *FvTFL1* (Mouhu et al. 2009; Mouhu et al. 2013; Koskela et al. 2012). In the present study, we observed that the transcript level of floral repressor *FvTFL*1 closely followed the expression of *FvFT1* and *FvSOC1* especially during first week of light treatment. Although, the expression of *FvTFL1* is higher under dominant B, however, there was no inhibition of flowering. In accordance with previous studies, the present study suggests that the presence of non-functional *FvTFL1* is possible in the continuous flowering cultivar 'Alexandria' (Koskela et al. 2012; Mouhu et al. 2013). Although, our results are in agreement with previous studies, however, functional analyses using transgenic lines are required to confirm the light quality control flowering.

Phytochromes (phyB) perceives far-red, red and blue light signals and mediate physiological responses in plants, along with cryptochromes (Usami et al. 2004; Franklin and Whitelam, 2005).

Phytochromes plays an important role to regulate night break response on flowering in short-day plants (Ishikawa et al. 2005). For instance, single night break with blue light restricts flowering in rice via phyB that controls the expression of *Hd3a* (ortholog of *FT*), whereas flowering was reversed in phyB-1 mutant (Ishikawa et al. 2009). In *Arabidopsis*, both phytochromes (phyA and phyB) gets activated under enriched FR light and controls physiological responses (Lim et al. 2018). Here, we determine that the transcript level of FvphyB gene was significantly higher especially under dominant FR LEDs during first three weeks. The *FvFT1* expression was downregulated considerably, whereas *FvphyB* expression was quite higher under dominant FR, indicating that magnitude of FR radiance in light combination may controls the expression of both genes. Cerdán and Chory (2003) previously reported that *phyB* downregulates the transcription of *FT* gene and restricts flowering in *Arabidopsis* in response to red and FR light. In the present study, when the plants were transferred to floral inductive conditions, the *FvFT1* was upregulated significantly coupled with a reduced mRNA level of *FvphyB*. This indicates that the phytochromes may have contradictory role to regulate flowering by differentially controlling the *FvFT1* expression. The flowering inhibition in rice during night break occur due to the suppression of floral inducer gene (Ishikawa et al. 2005). However, the inhibition was reversible after the plants were moved back to inductive photoperiod. Similarly, night breaks-induced inhibition of flowering response in wheat was reversed under inductive conditions (Shaw et al. 2013), indicating that flowering in long-day and short-day plants can be reversed. However, flowering was irreversible for DN 'Alexandria'.

Involvement of plant hormones, such as gibberellins (GAs), auxins (IAA) and cytokinin (CK) in light-regulated morphogenesis and flowering has been known for decades (Neff et al. 2006). GAs plays an important role in one of the flowering pathways, regulates stem extension, seed

development and floral transition (MacMillan 2001). Among different bioactive forms of GAs, GA4 acts as a floral inducer that stimulates flower initiation and stem extension in *Arabidopsis* (Eriksson et al. 2006), whereas *L. temulentum* uses GA5 and GA6 to control flowering (King et al. 2006). In strawberry, GA1 controls the fate of axillary bud into flower or runner (Hytönen et al. 2009), and GA3 and GA4 determines fruit size expansion and ripening (Csukasi et al. 2011). For the first time, the present study unveiled a strong association between light quality and the concentration of gibberellins and cytokinin in DN cultivars of woodland and cultivated strawberry. The significant increase in flowering under dominant B is accompanied by a significant increase in the concentrations of floral inducing hormones i.e., GAs and CK. Light spectrum affects the endogenous levels of plant hormones by regulating their secondary metabolism as previously shown in the Norway spruce (OuYang et al. 2015). Kurepin et al. (2006) and Yang et al. (2018) demonstrated that light quality (low R/FR ratio) causes significant increase in the concentration of bioactive GA1 that induces morphological changes in sunflower and soybeans. The biosynthesis and production site of gibberellins is mainly controlled by three enzymes *gibberellin-20-oxidase* (GA20ox), *gibberellin-3-oxidase* (GA3ox) and *gibberellin-2-oxidase* (GA2ox) (Mutasa-Göttgens and Hedden 2009). Blue light has been reported to upregulate the mRNA levels of the GA20ox and GA3ox genes that is required for the synthesis of bioactive GA1 in *Arabidopsis* (Reid et al. 2002; Zhao et al. 2007). The GAs signaling pathway gene, GA20ox, determines the concentration of GA in the shoot apical meristem that promotes the transcript level of FT gene under LD in *Arabidopsis thaliana* (Hisamatsu et al. 2008). Similarly, it is possible that transcript levels of GA20ox have induced FvFT1 expression in crown tissue and induced flowering in response to dominant blue (1:5) light.

Auxin (IAA) is another important plant hormone that regulates many aspects of plant growth and development. In *Arabidopsis*, auxin specifies the site of floral initiation and further contributes towards the development of flowering organs (Krizek 2011). However, the role of IAA during flowering in response to light quality seems unclear in both *Fragaria* species. In *Fragaria vesca* cv 'Alexandria', the concentration of IAA increased significantly under dominant blue light (1:5), whereas, in 'Albion', IAA concentration increased significantly under 1:1 ratio following 6-week exposure. Auxin have been reported to regulate the concentration of GA1 under different environmental conditions (Wolbang and Ross 2001; Yang et al. 2018). For instance, lower levels of IAA caused significant reduction in internode elongation in pea and tobacco (Ross et al. 2000; Wolbang and Ross 2001). Similarly, in 'Alexandria', increased levels of IAA under 1:5 is coupled with increased GA1 concentration that enhanced flowering. It is possible that, in response to light quality, IAA regulates the concentration of GA1 in woodland strawberry as well. Overall, the involvement of IAA during flowering phase is debatable as positive and negative effects have been reported in fruit crops (Riboni et al. 2016; Wan et al. 2017). In accordance with previous studies, our results implies that light quality-dependent changes in plant hormonal levels may be limited to specific tissue and species (Zhao et al. 2007).

Cytokinin (CK) is the primary and important target to light that regulates bud outgrowth in rose (*Rosa grandiflora* Walters) (Roman et al. 2016) and mediates light-controlled stem elongation in *Arabidopsis* (Su and Howell 1995). In the present, we demonstrate the strong interaction between light quality and increased concentration of cytokinin (kinetin) in the shoot apex of both cultivated and woodland strawberry. In 'Alexandria' and 'Albion', the concentration of kinetin increased significantly under 1:5 light during FBI. In complete agreement with previous studies in apple tree (Cao et al. 2003), seasonal strawberry (Eshghi and Tafazoli 2007) and olives (Ulger et al. 2004),

our results indicates that increased levels of cytokinin (kinetin) is likely promotes flowering in day-neutral strawberry in response to light quality. The different effects of CK are mainly dependent on light source and developmental stage of the plant (Zubo et al. 2008). Molecular studies suggested that cytokinin promotes flowering in *Arabidopsis* through the transcriptional activation of the floral inducer gene (FT) (D'Aloia et al. 2011). Similar association between ortholog of FT (FvFT1) and the cytokinin concentration in shoot apex tissue was detected in DN strawberry as well.

Abscisic acid (ABA) is a major stress key regulator in plants (Negin et al. 2019). Under stressed conditions, ABA concentration increases significantly in apple (Cao et al. 2000), loquat (Liu et al. 2007) and seasonal strawberry (Wan et al. 2017) during flower bud differentiation. In contrast, studies have reported that increased level of ABA inhibits flowering in *Arabidopsis* (Lefebvre et al. 2006) and *Polianthes tuberosa* (Su et al. 2002). In the present study, we observed that ABA concentration in shoot apex of 'Alexandria' increased substantially towards the flowering stage irrespective of light treatments, indicating that high ABA may be associated with flowering in woodland strawberry. In contrast, ABA concentration decreases significantly in 'Albion' cultivar in all light conditions during the flowering stage. The ABA concentration varied significantly in both species; it is most likely that ABA effect on flowering could be species dependent. Further transcriptomic analysis of genes that are responsible for the biosynthesis of the studied hormones, is required to provide strong validation of the interplay between light spectrum, flowering and hormonal concentration to better understand the underlying mechanisms.

5.6 Conclusions

In the present study, we have shown that the combination of far-red and blue at a ratio of 1:5, also called dominant blue, promotes flowering in DN cultivars i.e., 'Albion' and 'Alexandria' of

Fragaria species. Molecular analysis of model plant (*Fragaria vesca*) suggested that dominant blue LEDs activates the transcription of *FvFT1* and *FvTFL1* gene during the first week of supplementation, however, flowering seemed to occur independently *of FvTFL1*. Further, in contrast to 'Albion', 'Alexandria' displayed a strong flowering inhibition in all combination of far-red and blue LEDs when applied during night interruptions. It appears that phytochromes (phyB) mediates floral inhibition in *F. vesca* through differential regulation the *FvFT1* transcription. Here, we also provide the evidence that concentration of gibberellins (GA1 and GA7) and cytokinin (kinetin) mediates light quality-controlled flowering in both woodland and cultivated strawberry. Whereas the effect of abscisic acid and auxin hormones on flowering in response light quality is still unclear in DN cultivars. Although, our data aligns with literature, however, further analyses using transgenic and gene knockout lines are required to validate the functioning of floral genes in response to combined light source.

CHAPTER VI: GENERAL DISCUSSIONS, SUMMARY AND SUGGESTIONS FOR FUTURE RESESRCH

Cultivated strawberry (*Fragaria* x *ananassa*) is an important horticultural crop with an annual production of 9.4 million tons worldwide (FAO 2019). Between 2008 and 2018, global strawberry production has increased up to 40%, particularly in China and Mexico. The strawberry production of world leading producer, China, has increased considerably in the last decade from 1.9 to 3.2 million tons between 2011-2019 (FAO 2019). While strawberry production in Canada have shown slight surge from 22,950 to 27,252 metric tons (FAO 2019). In 2019, during the pandemic, Canada imported 127,233 metric tons of fresh strawberries mainly from United States and Chile (Agriculture and Agri-food Canada. 2020). In Canada, Québec is the highest strawberry producing province that represents 47% of the entire commercial production (Agriculture and Agri-food Canada. 2020). In past few decades, new production systems such as umbrella shelters, soilless systems have been incorporated in Québec for the cultivation on day-neutral cultivar. These production techniques protect the crop from heavy rainfalls, soil-borne diseases and have the potential to extend the harvesting season that can substantially benefits the strawberry fruit production. However, challenges linked to precise the growing conditions under these production systems remain elusive. The present thesis illustrates the potential growing conditions for day-neutral cultivars that can considerably increase the strawberry production.

The present thesis was written with two major objectives in mind; to identify the best possible environmental factor that can stimulate FBI in widely grown day-neutral (DN) cultivar ‘Albion’ and to understand the molecular and metabolic mechanism that controls the flowering. This study provides in-depth evidence for light conditions that regulate FBI in DN cultivars of *Fragaria* species. The FBI is considered as the most reliable and critical factor for the successful cultivation

of the crop as it directly contributes to the qualitative and quantitative characteristics of strawberry fruit. Each flower bud that is induced during transplant production leads to the development of flower stalk on which fruits are borne during growing season. Therefore, flower bud induction is considered as the primary target for the success of the crop and producers.

The photoperiod and nitrogen (N) are recognized as the important factors that regulates early flower bud induction and subsequently increase fruit productivity in seasonal and long-day strawberry (Durner 2016; 2017; 2018; Wan et al. 2017). In fact, local growers in Québec use photoperiod and N conditioning in order to manipulate FBI process especially in new production systems i.e., umbrella shelter and soilless system for the cultivation of DN cultivars. However, reports on photoperiod and nitrogen treatment effect during FBI for DN cultivars is limited. Therefore, in chapter III, we determined the effect of photoperiod and N conditioning on flowering and fruit production for 'Albion' cultivar grown in soilless substrate under umbrella shelter at production site (Ferme Onésime Pouliot). Results demonstrated that low N supply and LD photoperiod triggers the FBI during the later stages in the growing season. In general, flowering response of long-day cultivars become more apparent if grown for 4-weeks under LD photoperiod (Sønsteby and Heide 2007). However, in the present study, LD photoperiod applied during the transplant production stage (September to November 2017), showed their effect on FBI in Aug 2018. It is most likely that plants have begun induction upon LD exposure but following elevated N supply during the production season 2017-18 may have enhanced the LD-induced effect on FBI as previously reported in long-day cultivars (Durner 2016; 2017; 2018). In addition to FBI, low N and LD photoperiod supplied through day extension appeared to benefit the fruit production.

Enhanced FBI during the later stage in the growing season can lead to the extension of harvesting season only if the weather conditions remain appropriate for the plant growth. However, in north-

climate such as Québec, where early frost remains the biggest threat, thus, later induction of flower buds during harvesting season does not necessarily contributes toward the fruit yield. Therefore, it is essential to maximize the flower bud induction either during the transplant production or early during the growing season (June-July). Further, it is important to highlight that above mentioned experiments were conducted at production site where environmental conditions are highly relevant to local conditions but, have less stringent control. Since most of the significant results were observed during later stage of the production season, environmental conditions could have influenced the outcome of the experiments. Thus, all the following experiments were conducted under controlled environment.

In addition to photoperiod and nitrogen, light quality, referring to wavelength, is another major environmental factor that controls physiological and morphological changes throughout the entire life cycle of plants (Paradiso and Proietti 2021). Plants mainly rely on harvesting specific light spectrum to flourish, which are not provided by traditional light sources such as incandescent light. Modern agriculture has steered towards the adoption of LEDs for better cultivation of horticultural crops especially under protected conditions (Neri et al. 2012; Choi et al. 2015). Whereas local growers in Québec predominantly use high-pressure sodium lamps (HPS) or incandescent light under transplant production conditions. Among photosynthetic active radiations, narrow-band light source of far-red and blue LEDs are described as flowering stimulant wavelengths in short-day and long-day strawberry cultivars (Rantanen et al. 2014; Yoshida et al. 2016). Studies suggested 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). Therefore, in chapter IV, for the first time, we demonstrate that combination of two distinct light wavelengths supplied with LEDs source could be potentially

used to enhance and accelerate the FBI in widely grown ‘Albion’ cultivar grown. In the present study, the combination of two narrow-band light sources of far-red and blue LEDs at a ratio of 1:5, also referred as dominant blue, significantly stimulated FBI and ensures healthy plant quality during transplant production. Plant architecture indicated that floral stimulation is mainly due to secondary and tertiary branching that could resulted in additional terminal inflorescences.

In ‘Albion’ cultivar, the FBI was unaffected when LD (24h) photoperiod was supplied with incandescent light. Whereas FBI accelerated considerably under 24h exposure of dominant blue LEDs, indicates that light spectrum regulates photoperiod-induced effect on flowering. Under local conditions, strawberry plants are conditioned for 2 months during transplant production to enhance FBI, however, ‘Albion’ transplants induced significantly higher number of floral buds within 6-weeks after the commencement of treatment, which implies that faster production rate can be achieved with light quality. Compared to traditional light sources, LEDs are most energy-efficient and sustainable light sources that can essentially supply tailored light spectrum depending upon certain crop or growth stages requirements (Paradiso and Proietti 2021). It is important to point out that upfront cost of LEDs maybe higher compared to incandescent light source, however, they use 75% less electricity and last about 25 times longer (Saskatchewan Research Council 2018).

Previous studies have shown that the combination of enriched blue light with far-red suppresses the physiological disorder such as intumescence injury in greenhouse grown horticultural crops (Eguchi et al. 2016). Likewise, no disease or pest problems were observed for ‘Albion’ cultivar grown under different light quality. Further, narrow-band of blue LEDs alone or in combination with other lights, not only increases the fruit yield but also improves the fruit quality traits such as greater accumulation of organic acids, phenolic compounds, and total soluble solids in strawberry fruits (Choi et al. 2015; Nadalini et al. 2017).

Controlled environment agriculture (CEA) is gaining popularity in urban areas. It is essentially an indoor technology-based production system such as plant factories, greenhouses, vertical farming and hydroponics, where crops are grown under highly controlled conditions. Strawberry is considered as the candidate crop for the CEA in the future due to the smaller plant size, easy propagation and require less space (Yoshida et al. 2016). The full spectrum LEDs lighting has the potential to bring sunlight in indoor facilities and provide complete control of plant development. The present research findings will allow the smart use of LEDs lighting in indoor facilities that can lead to programmed production systems in near future and open new avenues for year-round supply of fresh strawberry.

The flowering is a complex process that involves multiple genetic pathways and hormonal signaling. To understand the mechanism for light quality control of flowering in DN cultivars, we have examined the transcript level of floral-related genes and concentration levels of plant hormones during FBI process. Due to the genetic complexity of octoploid strawberry (*Fragaria* x *ananassa*), the model strawberry species, diploid strawberry (*Fragaria* x *vesca*), was used to determine the molecular mechanism. 'Alexandria' seedlings of *F. vesca* established similar morphological response as *Fragaria* x *ananassa* cv 'Albion' when grown under dominant blue LEDs. The transcript level of flowering-related genes i.e., FvFT1 and FvTFL1 were recognized at a higher fold change under dominant blue LEDs, suggesting that both the genes are involved in flowering in response to light quality, although, flowering seems to occur independently of FvTFL1.

Woodland and cultivated strawberry established similar flowering response when light quality is supplied during the daytime. In contrast to *F. ananassa*, *F. vesca* plants displayed strong flowering inhibition in all the FR and B combinations when supplemented during night interruption. Our

findings postulates that the FR light-controlled phytochromes may be involved in the floral inhibition response in *F. vesca* through differential regulation of the FvFT1 transcription factor. The near-complete genome assembly for cultivated strawberry (*Fragaria* x *ananassa*) have been recently published (Edger et al. 2019). Completely sequenced genome will assist scientists in further explanation of regulation of flowering in cultivated strawberry in near future. Molecular studies in combination with physiological understanding of the crop is tremendously useful to develop more precise growing conditions for horticultural crops.

The plant hormones play an important role in regulating major physiological changes such as seed development, shoot elongation and flowering, in the life cycle of plant. To further understand the FBI process, we investigated the levels of different phytohormones such as gibberellins, auxin, abscisic acid and cytokinin. In response to dominant blue LEDs, increased levels of gibberellins (GA1 and GA7) and cytokinin (kinetin) in the crown tissue was observed that appears to be involved in FBI process in both woodland and cultivated strawberry. Whereas the concentration of abscisic acid and auxin varied significantly in both species; suggesting that light quality-dependent changes in plant hormonal levels may be limited to specific tissue and species. Overall, our results strongly suggests that initial FBI process can be easily managed by light quality and have practical application for the cultivation of DN strawberry under controlled conditions. The molecular and metabolic characterizations have allowed us to gain insight on the flowering mechanism in response to light quality. The genetic findings of present research offer novel understandings into the importance of light quality control of flowering and will assist to pave the way for breeding programs of strawberry.

Suggested future studies

- The supplementation of dominant blue LEDs during transplant production can be implemented at large scale (production site) to validate the results. In addition, the economic assessment of LEDs use in strawberry production would allow growers to understand and facilitate the adoption of these practices at commercial scale.
- This study has proved the different combination of far-red and blue can promote the flowering traits in DN 'Albion'. To further understand the light quality effect in DN cultivars, it is essential to understand the flowering response of day-neutral cultivars such as Seascape and San Andreas under light quality, to confirm whether all the DN cultivars behaves similarly.
- Determine how different concentration of nitrogen and photoperiod regulates flowering mechanism in DN cultivars of *Fragaria* x *ananassa* through molecular and metabolic studies.
- The flowering related genes i.e., FT1/TFL1-like genes can be studied in knockout, overexpression transgenic lines in *Fragaria* x *ananassa*, to understand the flowering response to light quality of cultivated strawberry at molecular level.
- The transcript levels of candidate genes i.e., GA3ox, GA20ox and GA2ox that are responsible for the biosynthesis of gibberellins can be identified in day-neutral *Fragaria* species in response to light quality, their expression can be correlated with the involvement of GAs in flowering. Further studies are required to elucidate the interaction between phytohormone signaling and floral related genes that coordinately controls the physiological changes in DN cultivars in response to different stimuli.

REFERENCES

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K. and Araki, T. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Sci. 309: 1052-1056.

Adams, S.R., and Langton, F.A. 2005. Photoperiod and plant growth: a review. J. Hort. Sci. Biotech. 80: 2-10.

Agriculture and Agri-food Canada. 2020. Statistical overview of the Canadian fruit industry. Available online at: https://www.agr.gc.ca/eng/horticulture/horticulture-sector-reports/statistical-overview-of-the-canadian-fruit-industry-2019/?id=1564485377504).

Albani M. C., Battey N. H., and Wilkinson M. J. 2004. The development of ISSR-derived SCAR markers around the SEASONAL FLOWERING LOCUS (SFL) in *Fragaria vesca*. Theo. App. Gen. 109: 571–579.

An, H. R., Kim, Y. J., Park, Y. J., and Kim, K. S. 2015. Night interruption improves subsequent cut flower quality in *Cymbidium* 'Red Fire'. Hort. Env. Biotech. 56: 455-461.

Andriolo, J. L., Erpen, L., Cardoso, F. L., Cocco, C., Casagrande, G. S., and Jänisch, D. I. 2011. Nitrogen levels in the cultivation of strawberries in soilless culture. Hort. Bra. 29: 516-519.

Andrés, F. and Coupland, G. 2012. The genetic basis of flowering responses to seasonal cues. Nat. Rev. Gen. 13: 627-639.

Babalar, M., Sokri, S.M., Lesani, H., Asgari, M.A., and Barker, A.V. 2015. Effects of nitrate: ammonium ratios on vegetative growth and mineral element composition in leaves of apple. J. Pl. Nutr., 38: 2247–2258.

Ballington, J. R., Poling, B., and Olive, K. 2008. Day-neutral strawberry production for season extension in the midsouth. Hort. Sci. 43: 1982-1986.

Black, B. L., Enns, J. M., and Hokanson, S. C. 2002. A comparison of temperate-climate strawberry production systems using eastern genotypes. Hort. Tech. 12:670-675.

Boonterm, V. Silapapun, A. and Boonkerd, N. 2010. Effects of nitrogen, potassium fertilizers and clusters per vine on yield and anthocyanin content in Cabernet Sauvignon grape. Sur. J. Sci. Tech. 17: 155–163.

Bradford, E., Hancock, J. F., and Warner, R. M. 2010. Interactions of temperature and photoperiod determine expression of repeat flowering in strawberry. J. Amer. Soc. Hort. Sci. 135: 102-107.

Britto, D. T., and Kronzucker, H. J 2002. NH_4^+ toxicity in higher plants: a critical review. J. Pl. Phys. 159: 567-584.

Cao, S., Zhang, J. and Wei, L. 2000. Studies on the changes of endogenous hormones in the differentiation period of flower bud in apple trees. J. Fruit Sci. 17: 244-248.

Cao, S. Y., Zhang, Q. M. and Wu, S. 2003. Advances in research on the mechanism of flower-bud differentiation of fruit trees. J. Fruit Sci. 20: 345-350.

Castro Marín, I., Loef, I., Bartetzko, L., Searle, I., Coupland, G., Stitt, M., and Osuna, D. 2011. Nitrate regulates floral induction in *Arabidopsis*, acting independently of light, gibberellin and autonomous pathways. Pl. 233: 539-552.

Cathey, H. M. and Borthwick, H. A. 1957. Photo-reversibility of floral initiation in *chrysanthemum.* Bot. Gaz. 119: 71-76.

Cecatto, A. P., Calvete, E. O., Nienow, A. A., Costa, R. C. D., Mendonça, H. F. C., and Pazzinato, A. C. 2013. Culture systems in the production and quality of strawberry cultivars. Act. Sci. Agro. 35: 471-478.

Cerdán, P. D. and Chory, J., 2003. Regulation of flowering time by light quality. Nature. 423: 881-885.

Choi, H. G., Kwon, J. K., Moon, B. Y., Kang, N. J., Park, K. S., Cho, M. W., and Kim, Y. C. 2013. Effect of different light emitting diode (LED) lights on the growth characteristics and the phytochemical production of strawberry fruits during cultivation. K. J. Hort. Sci. Tech. 31: 56-64.

Choi, H. G., Moon, B. Y., and Kang, and N. J. 2015. Effects of LED light on the production of strawberry during cultivation in a plastic greenhouse and in a growth chamber. Sci. Hort. 189: 22-31.

Claire, D., Watters, N., Gendron, L., Boily, C., Pépin, and S. Caron, J. 2018. High productivity of soilless strawberry cultivation under rain shelters. Sci. Hort. 232:127-138.

Claussen, W. 2002. Growth, water use efficiency, and proline content of hydroponically grown tomato plants as affected by nitrogen source and nutrient concentration. Pl. Soil. 247: 199–209.

Cope, K. R. and Bugbee, B. 2013. Spectral effects of three types of white light-emitting diodes on plant growth and development: absolute versus relative amounts of blue light. Hort Sci. 48: 504-509.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, and C. Coupland, G. 2007. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis.* Sci. 316: 1030-1033.

Costes, E., Crespel, L., Denoyes, B., Morel, P., Demene, M. N., Lauri, P. E., and Wenden, B. 2014. Bud structure, position and fate generate various branching patterns along shoots of closely related *Rosaceae* species: a review. Fr. Pl. Sci. 666: 1-11.

Csukasi, F., Osorio, S., Gutierrez, J. R., Kitamura, J., Giavalisco, P., Nakajima, M., Fernie, A. R., Rathjen, J. P., Botella, M. A., Valpuesta, V. and Medina-Escobar, N. 2011. Gibberellin biosynthesis and signalling during development of the strawberry receptacle. New Phyto. 191: 376-390.

D'Aloia, M., Bonhomme, D., Bouché, F., Tamseddak, K., Ormenese, S., Torti, S., Coupland, G. and Périlleux, C. 2011. Cytokinin promotes flowering of *Arabidopsis* via transcriptional activation of the FT paralogue TSF. The Plant J. 65: 972-979.

D'Aloia, M., Bonhomme, D., Bouché, F., Tamseddak, K., Ormenese, S., Torti, S., and Périlleux, C. 2011. Cytokinin promotes flowering of *Arabidopsis* via transcriptional activation of the FT paralogue TSF. Pl. J. 65: 972-979.

Darnell, R. 2003. Strawberry growth and development. In: The strawberry, a book for growers, Gainesville, FL: Institute of Food and Agricultural Sciences, Horticultural Sciences Department 1: 3-10.

Darnell, R. L., Cantliffe, D. J., Kirschbaum, D. S., and Chandler, C. K. 2003. The physiology of flowering in strawberry. Hort. Rev. 28: 325-349.

Dasti, A. A., Fatima, K. and Malik, S. A. 2002. How photoperiod affects growth rate and biomass allocation pattern: a comparative study on three genotypes of *Arabidopsis thaliana*. Asi. J. Pl. Sci. 1: 176-179.

de Camacaro, M. P., Camacaro, G. J., Hadley, P., Battey, N. H., and Carew, J. G. 2002. Pattern of growth and development of the strawberry cultivars Elsanta, Bolero, and Everest. J. Amer. Soc. Hort. Sci. 127: 901-907.

Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., and Vian, A. 2016. Plant responses to red and far-red lights, applications in horticulture. Env. Exp. Bot. 121: 4-21.

Dobisova, T., Hrdinova, V., Cuesta, C., Michlickova, S., Urbankova, I., Hejatkova, R., and Hejatko, J. 2017. Light-regulated expression of sensor histidine kinase CKI1 controls cytokinin-related development. Pl. Physio. 1: 177-196.

Durner, E. F., Poling, E. B., and Maas, J. L. 2002. Recent advances in strawberry plug transplant technology. Hort. Tech.12: 545-550.

Durner, E. F. 2015. Photoperiod affects floral ontogeny in strawberry (*Fragaria* × *ananassa* Duch.) plug plants. Sci. Hort. 194: 154-159.

Durner, E. F. 2016. Enhanced flowering of the F1 long day strawberry cultivar 'Elan' via nitrogen and daylength manipulation. AIMS Agri. Food, 1: 4–19.

Durner, E. F. 2017. Enhanced flowering of the F1 long day strawberry cultivars "Tarpan" and "Gasana" with nitrogen and daylength management. AIMS Agri. Food. 2: 1–15.

Durner, E. F. 2018. Plant architecture of Albion strawberry (*Fragaria*× *ananassa Duch.*) is not influenced by light source during conditioning. Agri. Food. 3: 246-265.

Durner, E. F. 2018. Long-day and nitrogen conditioning of 'Albion' strawberry (*Fragaria* X *ananassa* Duch.) enhances off-season field production. J. Hort. Sci. Biotech. 93: 296-305.

Durner, E. F., and Poling, E. B. 1985. Comparison of three methods for determining the floral or vegetative status of strawberry plants. J. Amer. Soc. Hort. Sci. 110: 808-811.

Edger, P. P., Poorten, T. J., VanBuren, R., Hardigan, M. A., Colle, M., McKain, M. R., Smith, R. D., Teresi, S. J., Nelson, A. D., Wai, C. M. and Alger, E. I. 2019. Origin and evolution of the octoploid strawberry genome. Nat. Gen. 51: 541-547.

Eguchi, T., Hernández, R. and Kubota, C. 2016. Far-red and blue light synergistically mitigate intumescence injury of tomato plants grown under UV-deficit light environment. Hort Sci. 51: 712-719.

Endo, M., Tanigawa, Y., Murakami, T., Araki, T., and Nagatani, A. 2013. PHYTOCHROME-DEPENDENT LATE-FLOWERING accelerates flowering through physical interactions with phytochrome B and CONSTANS. Proc. Nat. Aca. Sci. 110(44), 18017-18022.

Eriksson, S., Böhlenius, H., Moritz, T. and Nilsson, O. 2006. GA4 is the active gibberellin in the regulation of LEAFY transcription and *Arabidopsis* floral initiation. The Pl. Cell. 18: 2172-2181.

Eshghi, S., and Tafazoli, E. 2007. Possible role of cytokinins in flower induction in strawberry. Am J Plant Physio. 2:167–174.

Eskins, K. 1992. Light-quality effects on *Arabidopsis* development. Red, blue and far-red regulation of flowering and morphology. Physio. Plant. 86: 439-444.

Faust, J. E., Holcombe, V., Rajapakse, N. C., and Layne, D. R. 2005. The effect of daily light integral on bedding plant growth and flowering. HortScience. 40: 645-649.

F A O 2019. Food and Agriculture Organization of the United Nations: Rome

Folta, K. M., and Davis, T. M. 2006. Strawberry genes and genomics. Crit. Rev. Plant Sci. 25: 399–415.

Fornara, F., Panigrahi, K. C., Gissot, L., Sauerbrunn, N., Rühl, M., Jarillo, J. A. and Coupland, G., 2009. *Arabidopsis* DOF transcription factors act redundantly to reduce CONSTANS expression and are essential for a photoperiodic flowering response. Dev. cell. 17: 75-86.

Franklin, K. A. and Whitelam, G. C. 2005. Phytochromes and shade-avoidance responses in plants. Ann. of Bot., 96: 169-175.

Garner, W. W., and Allard, H. A. 1920. Effect of the relative length of day and night and other factors of the environment of growth and reproduction in plants. J. Agri. Res. 18: 553–603.

Goldberg-Moeller, R., Shalom, L., Shlizerman, L., Samuels, S., Zur, N., Ophir, R., and Sadka, A. 2013. Effects of gibberellin treatment during flowering induction period on global gene expression and the transcription of flowering-control genes in Citrus buds. Pl. Sci. 198: 46-57.

Golembeski, G. S., Kinmonth-Schultz, H. A., Song, Y. H., and Imaizumi, T. 2014. Photoperiodic flowering regulation in *Arabidopsis thaliana*. In Adv. Bot. Res. 72: 1-28.

Goto, E., Matsumoto, H., Ishigami, Y., Hikosaka, S., Fujiwara, K., and Yano, A. 2013. Measurements of the photosynthetic rates in vegetables under various qualities of light from light-emitting diodes. Acta Hort. 1037: 261-268.

Guler, S., Macit, I., Koç, A. and Ibrikci, H. 2006. Leaf nitrogen status of strawberry by using chlorophyll meter reading. J. Bio. Sci. 6: 1011-1016.

Guo S., Bruck H., and Sattelmacher B. 2002. Effects of supplied nitrogen form on growth and water uptake of French bean (Phaseolus vulgaris L.) plants. Pl. Soil 239: 267-275.

Guttridge, C.G. and Thompson, P.A. 1964. The effect of gibberellins on growth and flowering of *Fragaria and Duchesna.* J. Expt. Bot. 15: 631-646.

Hamamoto H., Hideo S., and Tadahisa H. 2005. Budding response of horticultural crops to night break with red light on alternate days. Env. Cont. Bio. 43:21-27.

Hamner, K. C., and Bonner, J. 1938. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100: 388-431.

Hanano, S., and Goto, K. 2011. *Arabidopsis* TERMINAL FLOWER1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression. Pl. Cell 23: 3172–3184.

Heide, O. M., Stavang, J. A. and Sønsteby, A. 2013. Physiology and genetics of flowering in cultivated and wild strawberries–a review. J. Hort. Sci. Biotech. 88: 1-18.

Heo, J. W., Lee, C. W., and Paek, K. Y. 2006. Influence of mixed LED radiation on the growth of annual plants. J. Pl. Bio. 49: 286-290.

Hidaka, K., Dan, K., Imamura, H., Miyoshi, Y., Takayama, T., Sameshima, K., Kitano, M. and Okimura, M. 2013. Effect of supplemental lighting from different light sources on growth and yield of strawberry. Env. Con. Bio. 51: 41-47.

Hidaka, K., Dan, K., Imamura, H., Takayama, T., Sameshima, K., and Okimura, M. 2015. Variety comparison of effect of supplemental lighting with LED on growth and yield in forcing culture of strawberry. Env. Con. Bio. 53: 135-143.

Higuchi, Y. 2018. Florigen and anti-florigen: Flowering regulation in horticultural crops. Bre. Sci. 68: 109-118.

Higuchi, Y., Narumi, T., Oda, A., Nakano, Y., Sumitomo, K., Fukai, S. and Hisamatsu, T. 2013. The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemum. Pro. Nat. Aca. Sci. 110: 17137-17142.

Higuchi, Y., Sumitomo, K., Oda, A., Shimizu, H. and Hisamatsu, T. 2012. Day light quality affects the night-break response in the short-day plant chrysanthemum, suggesting differential phytochrome-mediated regulation of flowering. J. Pl. Physio. 169: 1789-1796.

Hirose, N., Takei, K., Kuroha, T., Kamada-Nobusada, T., Hayashi, H., and Sakakibara, H. 2007. Regulation of cytokinin biosynthesis, compartmentalization and translocation. J. Exp. Bot. 59: 75-83.

Hisamatsu, T. and King, R.W. 2008. The nature of floral signals in *Arabidopsis*. II. Roles for FLOWERING LOCUS T (FT) and gibberellin. J. Exp. Bot. 59: 3821-3829.

Hochmuth, G., Cantliffe, D., Chandler, C., Stanley, C., Bish, E., Waldo, E., and Duval, J. 2006. Containerized strawberry transplants reduce establishment-period water use and enhance early growth and flowering compared with bare-root plants. Hort. Tech. 16: 46-54.

Hoopen, F. T., Cuin, T. A., Pedas, P., Hegelund, J. N., Shabala, S., Schjoerring, J. K. and Jahn, T. P. 2010. Competition between uptake of ammonium and potassium in barley and *Arabidopsis* roots: molecular mechanisms and physiological consequences. J. Exp. Bot. 61: 2303-2315.

Hoover, E., Rosen, C., Luby, J. and Burkness, S.W. 2017. Day neutral strawberry production in Minnesota. Fact sheet. Department of Horticulture Science. University of Minnesota.

Horchani, F., Hajri, R., and Aschismiti, S. 2010. Effect of ammonium or nitrate nutrition on photosynthesis, growth, and nitrogen assimilation in tomato plants. J. Pl. Nutr. Soil Sci. 173: 610–617.

Hori, Y., Nishidate, K., Nishiyama, M., Kanahama, K., and Kanayama, Y. 2011. Flowering and expression of flowering-related genes under long-day conditions with light-emitting diodes. Planta. 234: 321-330.

Hytönen, T., Elomaa, P., Moritz, T., and Junttila, O. 2009. Gibberellin mediates day length-controlled differentiation of vegetative meristems in strawberry *(Fragaria× ananassa* Duch). BMC Pl. Bio. 9: 1-12.

Ijaz, F., Sohail, A. A, and Khan, R. 2016. The effect of potassium on growth and yield of strawberry (*Fragaria ananassa* (Duchesne ex Weston) Duchesne ex Rozier). Pak. J. Bot. 48: 1407-1413.

Imaizumi, T., Schultz, T. F., Harmon, F. G., Ho, L. A., and Kay, S. A. 2005. FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in *Arabidopsis*. Sci. 7: 293-309.

Inada, M., Yamaguchi, J. and Furuta, A. 2005. Control of strawberry [*Fragaria ananassa*] anthracnose (*Glomerella cingulata*) by rain shelter and systematic application of fungicides. Ky. Pl. Pro. Res. 51: 15-20.

Ishikawa, R., Shinomura, T., Takano, M., and Shimamoto, K. 2009. Phytochrome dependent quantitative control of Hd3a transcription is the basis of the night break effect in rice flowering. Gen. and Gen. Syst. 84: 179-184.

Ishikawa, R., Tamaki, S., Yokoi, S., Inagaki, N., Shinomura, T., Takano, M. and Shimamoto, K. 2005. Suppression of the floral activator Hd3a is the principal cause of the night break effect in rice. The Plant Cell. 17: 3326-3336.

Ito, A., Saito, T., Nishijima, T., and Moriguchi, T. 2014. Effect of extending the photoperiod with low-intensity red or far-red light on the timing of shoot elongation and flower-bud formation of 1-year-old Japanese pear (*Pyrus pyrifolia*). Tree Physiol. 34: 534-546.

Iwata, H., Gaston, A., Remay, A., Thouroude, T., Jeauffre, J., Kawamura, K., Oyant, L. H., Araki T., Denoyes B., and Foucher F. 2012. The TFL1 homologue KSN is a regulator of continuous flowering in rose and strawberry. Pl. J 69: 116–125.

Johnson, A. L., Govindarajulu, R., and Ashman, T. L. 2014. Bioclimatic evaluation of geographical range in *Fragaria* (*Rosaceae*): consequences of variation in breeding system, ploidy and species age. Bot. J. Lin. Soc. 176: 99-114.

Jones, M. A. 2018. Using light to improve commercial value. Hort. Res. 5: 1-13.

Kami, C., Lorrain, S., Hornitschek, P. and Fankhauser, C. 2010. Light-regulated plant growth and development. Curr. Topics in Dev. Bio. 91: 29-66.

Kempler, C. 2002, August. 'Out-of-season' greenhouse production of raspberry and strawberry. XXVI Int. Hort. Cong. Pro. Cult. 633: 459-465.

Kim, H. H., Wheeler, R. M., Sager, J. C., Yorio, N. C. and Goins, G. D. 2005. Light-emitting diodes as an illumination source for plants: A review of research at Kennedy Space Center. Hab. 10: 71-78.

Kim, S. Y., Yu, X., and Michaels, S. D. 2008. Regulation of CONSTANS and FLOWERING LOCUS T expression in response to changing light quality. Pl. Physio. 148: 269-279.

King, R. W., Moritz, T., Evans, L. T., Martin, J., Andersen, C. H., Blundell, C., Kardailsky, I. and Chandler, P. M. 2006. Regulation of flowering in the long-day grass *Lolium temulentum* by gibberellins and the FLOWERING LOCUS T gene. Pl. Physio. 141: 498-507.

Kinoshita, A., and Richter, R. 2020. Genetic and molecular basis of floral induction in *Arabidopsis thaliana*. J. Exp. Bot. 71: 2490-2504.

Kirschbaum, D. S., Larson, K. D.; Weinbaum, S. A., and DeJong, T. M. 2010. Late-season nitrogen applications in high-latitude strawberry nurseries improve transplant production pattern in warm regions. Afr. J. Biotech. 9:1001-1007.

Koike, S. T., Gordon, T., Ajwa, H., Daugovish, O., Bolda, M., and Legard, D. 2010. Disease Management Studies on Strawberry Plant Collapse Problems in California. Cal. Str. Comm. Ann. Prod. Res. Rep. 2008-09: 41–51.

Koskela, E. A., Mouhu, K., Albani, M. C., Kurokura, T., Rantanen, M., Sargent, D. J., and Hytönen, T. 2012. Mutation in TERMINAL FLOWER1 reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. Pl. Physio. 159: 1043-1054.

Koskela, E. A., Sønsteby, A., Flachowsky, H., Heide, O. M., Hanke, M. V., Elomaa, P. and Hytönen, T. 2016. TERMINAL FLOWER 1 is a breeding target for a novel everbearing trait and tailored flowering responses in cultivated strawberry (*Fragaria×ananassa* Duch.). Pl. Biotech. J. 14: 1852-1861.

Krizek, B. A. 2011. Auxin regulation of *Arabidopsis* flower development involves members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) family. J. Exp. Bot. 62: 3311-3319.

Kurepin, L. V., Emery, R. J. N., Pharis, R. P., and Reid, D. M. 2007. Uncoupling light quality from light irradiance effects in *Helianthus annuus* shoots: putative roles for plant hormones in leaf and internode growth. J. Exp. Bot. 58: 2145–2157.

Kurepin, L. V., Walton, L. J., Hayward, A., Emery, R. N., Pharis, R. P. and Reid, D. M. 2012. Interactions between plant hormones and light quality signaling in regulating the shoot growth of *Arabidopsis thaliana* seedlings. Bot. 90: 237-246.

Kurepin, L. V., Walton, L. J., Reid, D. M., Pharis, R. P. and Chinnappa, C. C. 2006. Growth and ethylene evolution by shade and sun ecotypes of *Stellaria longipes* in response to varied light quality and irradiance. Pl. Cell Env. 29: 647-652.

Kurokura, T., Samad, S., Koskela, E., Mouhu, K., and Hytönen, T. 2017. *Fragaria vesca* CONSTANS controls photoperiodic flowering and vegetative development. J. Exp. Bot. 68: 4839-4850.

Latigui, A., Choi, J. M. and Lee, C. W. 2011. Growth and nutrient uptake responses of 'Seolhyang' strawberry to various ratios of ammonium to nitrate nitrogen in nutrient solution culture using inert media. Afr. J. Biotech. 10: 12567-12574.

Lefebvre, V., North, H., Frey, A., Sotta, B., Seo, M., Okamoto, M., Nambara, E. and Marion-Poll, A. 2006. Functional analysis of *Arabidopsis* NCED6 and NCED9 genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. The Pl. J. 45: 309-319.

Li, J., Li, G., Wang, H., and Deng, X.W. 2011. Phytochrome signaling mechanisms. The *Arabidopsis* Book. Amer. Soc. Pl. Bio. 9.

Liao, Y., Suzuki, K., Yu, W., Zhuang, D., Takai, Y., Ogasawara, R., Shimazu, T. and Fukui, H. 2014. Night-break effect of LED light with different wavelengths on shoot elongation of *Chrysanthemum morifolium* Ramat 'Jimba'and 'Iwa no hakusen'. Env. Con. in Bio. 52: 51-55.

Lim, J., Park, J. H., Jung, S., Hwang, D., Nam, H. G. and Hong, S. 2018. Antagonistic roles of phyA and phyB in far-red light-dependent leaf senescence in *Arabidopsis thaliana*. Pl. Cell Physio. 59: 1753-1764.

Lin, Y. L., and Tsay, Y. F. 2017. Influence of differing nitrate and nitrogen availability on flowering control in *Arabidopsis*. J. Exp. Bot. 68: 2603-2609.

Liu T. 2013 Nitrate or NaCl regulates floral induction in *Arabidopsis thaliana*. Bio. 68: 215–222.

Liu, Z. L., Lin, S. Q. and Chen, H. B. 2007. Time course changes of endogenous hormone levels during the floral and vegetative buds formation in loquat (*Eriobotrya japonica Lind1*.). Acta. Hort. Sin. 34: 339-344.

MacMillan, J. 2001. Occurrence of gibberellins in vascular plants, fungi, and bacteria. J. Plant Gro. Regu. 20: 387–442.

Maki, S. L., Rajapakse, S., Ballard, R. E. and Rajapakse, N. C. 2002. Role of gibberellins in *chrysanthemum* growth under far red light-deficient greenhouse environments. Journal of the Amer. Soc. Hort. Sci. 127: 639-643.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd. Edn. Aca. Pres. Available at: https://academic.oup.com/aob/article/78/4/527/2587551.

Martínez-García, J. F., Santes, C. M., and García-Martínez, J. L. 2000. The end-of-day far-red irradiation increases gibberellin A1 content in cowpea (*Vigna sinensis*) epicotyls by reducing its inactivation. Physio. Pl. 108: 426-434.

Massetani, F., Gangatharan, R. and Neri, D. 2011. Plant architecture of strawberry in relation to abiotic stress, nutrient application and type of propagation system. Gen. Gen. Gen. 5:12-23.

Meng, Q. and Runkle, E. S. 2017. Moderate-intensity blue radiation can regulate flowering, but not extension growth, of several photoperiodic ornamental crops. Env. Exp. Bot. 134:12-20.

Mockler, T., Guo, H., Yang, H., Duong, H., and Lin, C. 1999. Antagonistic actions of *Arabidopsis* cryptochromes and phytochrome B in the regulation of floral induction. Dev. 126: 2073-2082.

Mockler, T., Yang, H., Yu, X., Parikh, D., Cheng, Y. C., Dolan, S. and Lin, C. 2003. Regulation of photoperiodic flowering by *Arabidopsis* photoreceptors. Pro. Nat. Aca. Sci. 100: 2140-2145.

Møller, S. G., Ingles, P. J., and Whitelam, G. C. 2002. The cell biology of phytochrome signalling. New Phy. 154: 553-590.

Mouhu, K., Hytönen, T., Folta, K., Rantanen, M., Paulin, L., Auvinen, P. and Elomaa, P. 2009. Identification of flowering genes in strawberry, a perennial SD plant. BMC Pl. Bio. 9: 1-16.

Mouhu, K., Kurokura, T., Koskela, E. A., Albert, V. A., Elomaa, P. and Hytönen, T. 2013. The *Fragaria vesca* homolog of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 represses flowering and promotes vegetative growth. The Pl. Cell. 25: 3296-3310.

Mutasa-Göttgens, E., and Hedden, P. 2009. Gibberellin as a factor in floral regulatory networks. J. Exp. Bot. 60: 1979-1989.

Nadalini, S., Zucchi, P., and Andreotti, C. 2017. Effects of blue and red LED lights on soilless cultivated strawberry growth performances and fruit quality. Eur. J. Hort. Sci. 82: 12-20.

Nafiye, A. D. A. K., and Gubbuk, H. 2015. Effect of planting systems and growing media on earliness, yield and quality of strawberry cultivation under soilless culture. Not. Bot. Hort. Agro. Cl-Napoca, 43: 204-209.

Nakano, Y., Higuchi, Y., Yoshida, Y. and Hisamatsu, T. 2015. Environmental responses of the FT/TFL1 gene family and their involvement in flower induction in *Fragaria*× *ananassa*. J. Pl. Physio. 177: 60-66.

Naznin, M. T., Lefsrud, M., Gravel, V., and Hao, X. 2016. Using different ratios of red and blue LEDs to improve the growth of strawberry plants. In *VIII* Int. Sym. L. Hort. 1134: 125-130.

Neff, M. M., Street, I. H., Turk, E. M. and Ward, J. M. 2006. Interaction of light and hormone signalling to mediate photomorphogenesis. In Photomor. in Pl. and Bac. Springer: 439-473.

Negin, B., Yaaran, A., Kelly, G., Zait, Y. and Moshelion, M. 2019. Mesophyll abscisic acid restrains early growth and flowering but does not directly suppress photosynthesis. Pl. Physio. 180: 910-925.

Neri, D., Baruzzi, G., Massetani, F., and Walther, F. 2012. Strawberry production in forced and protected culture in Europe as a response to climate change. Can. J. Plant Sci. 92: 1021–1036.

Nestby, R., and Tagliavini, M. 2005. Foliar uptake and partitioning of urea-N by strawberry plants as affected by timing of supply and plant N status. J. Hort. Sci. Biotech. 80: 272-275.

Nhut, D. T., Takamura, T., Watanabe, H., Okamoto, K. and Tanaka, M. 2003. Responses of strawberry plantlets cultured in vitro under superbright red and blue light-emitting diodes (LEDs). Pl. Cell. 73:43-52.

Nishiyama, M., and Kanahama, K. 2000. Effects of temperature and photoperiod on flower bud initiation of day neutral and everbearing strawberries. Acta Hort. 567: 253-255.

O'Carrigan, A., Hinde, E., Lu, N., Xu, X. Q., Duan, H., Huang, G., Mak, M., Bellotti, B., and Chen, Z. H. 2014. Effects of light irradiance on stomatal regulation and growth of tomato. Env. Exp. Bot. 98: 65–73.

OuYang, F., Mao, J. F., Wang, J., Zhang, S., and Li, Y. 2015. Transcriptome analysis reveals that red and blue light regulate growth and phytohormone metabolism in Norway spruce [*Picea abies* (L.) Karst]. PLoS One. 10: p.e0127896.

Ouzounis, T., Fretté, X., Rosenqvist, E., and Ottosen, C.O. 2014. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums and campanulas. J. Plant Physiol. 171: 1491-1499.Paradiso, R. and Proietti, S. 2021. Light-quality manipulation to control

plant growth and photomorphogenesis in greenhouse horticulture. The state of the art and the opportunities of modern LED systems. J. Pl. Gro. Reg. 1-39.

Paranjpe, A. V., Cantliffe, D. J., Stoffella, P. J., Lamb, E. M., and Powell, C. A. 2008. Relationship of plant density to fruit yield of Sweet Charlie strawberry grown in a pine bark soilless medium in a high-roof passively ventilated greenhouse. Sci. Hort. 115: 117–123.

Park, Y., and Runkle, E. 2019. LEDs: far red and light intensity interaction. [Online]. Available: https://www.growertalks.com/Article/?articleid=24033 [3 Jan 2019].

Park, Y. G., Muneer, S., Soundararajan, P., Manivnnan, A. and Jeong, B. R. 2016. Light quality during night interruption affects morphogenesis and flowering in *Petunia hybrida*, a qualitative long-day plant. Hort. Env. Biotech. 57: 371-377.

Park, Y. G., Muneer, S., Soundararajan, P., Manivnnan, A., and Jeong, B. R. 2017. Light quality during night interruption affects morphogenesis and flowering in *geranium*. Hort. Env. Biotech. 58: 212-217.

Percival, D., and Sanderson, K. 2004. Main and interactive effects of vegetative-year applications of nitrogen, phosphorus, and potassium fertilizers on the wild blueberry. Sm. Fr. Rev. 3: 105–121.

Poling, B. E. 2012. Strawberry plant structure and growth habit. NY St. Berry Growers Association, Berry EXPO.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193: 30-50.

Porebski, S., Bailey, L. G. and Baum, B. R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Pl. Mole. Bio Rep. 15: 8-15.

Preciado-Rangel, P., Troyo-Diéguez, E., Valdez-Aguilar, L. A., García-Hernández, J. L. and Luna-Ortega, J. G. 2020. Interactive Effects of the Potassium and Nitrogen Relationship on Yield and Quality of Strawberry Grown Under Soilless Conditions. Pl. 9: 441-451.

Rantanen, M., Kurokura, T., Mouhu, K., Pinho, P., Tetri, E., Halonen, L., Palonen, P., Elomaa, P. and Hytönen, T. 2014. Light quality regulates flowering in FvFT1/FvTFL1 dependent manner in the woodland strawberry *Fragaria vesca*. Front. in Pl. Sci. 271: 1-11.

Rashidi, A., Tehranifar, A., and Nemati, H. 2018. Effect of light combination and timing of supplemental lighting on growth characteristics and flowering of pansy (*Viola X wittrockiana* Rose). J. Orna. Plant 8: 227-240.

Reid, J. B., Botwright, N. A., Smith, J. J., O'Neill, D. P. and Kerckhoffs, L. H. J. 2002. Control of gibberellin levels and gene expression during de-etiolation in pea. Pl. Physio. 128: 734-741.

Rezazadeh, A., Harkess, R. L., and Telmadarrehei, T. 2018. The effect of light intensity and temperature on flowering and morphology of potted red firespike. Hort. 36:1-7.

Riboni, M., Robustelli Test, A., Galbiati, M., Tonelli, C. and Conti, L. 2016. ABA-dependent control of GIGANTEA signalling enables drought escape via up-regulation of FLOWERING LOCUS T in *Arabidopsis thaliana*. J. Exp. Bot. 67: 6309-6322.

Rockwell, N. C., Su, Y. S., and Lagarias, J. C. 2006. Phytochrome structure and signaling mechanisms. Ann. Rev. Pl. Bio. 57:837-858.

Roman, H., Girault, T., Barbier, F., Péron, T., Brouard, N., Pěnčík, A., Novák, O., Vian, A., Sakr, S., Lothier, J. and Le Gourrierec, J. 2016. Cytokinins are initial targets of light in the control of bud outgrowth. Pl. Physio.172: 489-509.

Ross, J. J., Ó Neill, D. P., Smith, J. J., Kerckhoffs, L. H., and Elliott, R. C. 2000. Evidence that auxin promotes gibberellin A1 biosynthesis in pea. Pl. J. Cell Mol. Biol. 21: 547–552.

Rowley, D., Black, B., and Drost, D. 2010. Strawberry plug plant production. Utah State University Extension.

Rubinstein, J. 2015. *Fragaria* x *ananassa*: Past, Present and Future Production of the Modern Strawberry. Master thesis. University of Minnesota.

Runkle, E. S. and Heins, R. D., 2001. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J. Am. Soc. Hort. Sci. 126: 275-282.

Saskatchewan Research Council. 2018. Why are the LED light bulbs are the best. Find out. Available online: https://www.src.sk.ca/blog/why-are-led-light-bulbs-best-find-out.

Savini, G. and Neri, D. 2003. Strawberry architectural model. Eur. Ber. Sym. Work. 649:169-176.

Schäfer, E., and Bowler, C. 2002. Phytochrome-mediated photo perception and signal transduction in higher plants. EMBO reports. 3: 1042-1048.

Shaw, L. M., Turner, A. S., Herry, L., Griffiths, S. and Laurie, D. A. 2013. Mutant alleles of Photoperiod-1 in wheat (*Triticum aestivum* L.) that confer a late flowering phenotype in long days. PLoS One, 8: p.e79459.

Shibuya, T., and Kanayama, Y. 2014. Flowering response to blue light and its molecular mechanisms in *Arabidopsis* and horticultural plants. Adv. Hort. Sci. 28: 179-183.

Shulaev, V., Sargent, D. J., Crowhurst, R. N., Mockler, T. C., Folkerts, O., Delcher, A. L., and Burns, P. 2011. The genome of woodland strawberry (*Fragaria vesca*). Nat. gen. 43: 109-116.

Sidhu, V., Bernier-English, V., Lamontagne-Drolet, M., and Gravel, V. 2021. Effect of light quality and extended photoperiod on flower bud induction during transplant production of day-neutral strawberry cultivars. Can. J. Pl. Sci.

Sønsteby, A., and Heide O. M. 2007. Long-day control of flowering in everbearing strawberries. J. Hort. Sci. Biotech. 82: 875–884.

Sønsteby, A., Opstad, N. and Heide, O. M. 2013. Environmental manipulation for establishing high yield potential of strawberry forcing plants. Sci. Hort. 157: 65-73.

Sønsteby, A., Opstad, N., Myrheim, U. and Heide, O. M. 2009. Interaction of short day and timing of nitrogen fertilization on growth and flowering of 'Korona'strawberry (*Fragaria* × *ananassa* Duch.). Sci. Hort., 123: 204-209.

Srikanth, A., and Schmid, M. 2011. Regulation of flowering time: all roads lead to Rome. Cell Mol. Sci. 68: 2013–2037.

Srivastava, D., Shamim, M., Kumar, M., Mishra, A., Maurya, R., Sharma, D., Pandey, P. and Singh, K. N. 2019. Role of circadian rhythm in plant system: An update from development to stress response. Env. Exp. Bot. 162: 256-271.

Statistics Canada. 2016. Crop profile for strawberry in Canada, 2016. Retrieved from: http://publications.gc.ca/collections/collection_2019/aac-aafc/A118-10-17-2016-eng.pdf.

Sterthem, A. 2013. Influence des minis tunnels sur le développement et la productivité des fraisiers aphotopériodiques. *Doc. Diss.* University of Laval.

Stewart, P. J., and Folta, K. M. 2010. A review of photoperiodic flowering research in strawberry (*Fragaria* spp.). Cri. Rev. Pl. Sci. 29: 1-13.

Strasser, B., Sánchez-Lamas, M., Yanovsky, M. J., Casal, J. J. and Cerdán, P. D. 2010. *Arabidopsis thaliana* life without phytochromes. Pro. Nat. Aca. Sci. 107: 4776-4781.

Su W., and Howell S. H. 1995. The effects of cytokinin and light on hypocotyl elongation are independent and additive. Pl. Physio. 108: 1423–1430.

Su, W. R., Huang, K. L., Shen, R. S. and Chen, W. S. 2002. Abscisic acid affects floral initiation in *Polianthes tuberosa*. J. Pl. Physio. 159: 557-559.

Suárez-López, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. and Coupland, G. 2001. CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature. 410:1116-1120.

Sweere, U., Eichenberg, K., Lohrmann, J., Mira-Rodado, V., Bäurle, I., Kudla, J., Nagy, F., Schäfer, E. and Harter, K. 2001. Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling. Sci. 294: 1108-1111.

Taghavi, T. S., Babalar, M., Ebadi, A., Ebrahimzadeh, H. and Asgari, M. A. 2004. Effects of nitrate to ammonium ratio on yield and nitrogen metabolism of strawberry (*Fragaria x ananassa* cv. Selva). Int. J. Agri. Bio. 6: 994-997.

Takemura, Y., Kuroki, K., Katou, M., Kishimoto, M., Tsuji, W., Nishihara, E., and Tamura, F. 2015. Gene expression changes triggered by end-of-day far-red light treatment on early developmental stages of *Eustoma grandiflorum* (Raf.) Shin. Sci. Rep. 5, 1-11.

Tamaki, S., Matsuo, S., Wong, H. L., Yokoi, S., and Shimamoto, K. 2007. Hd3a protein is a mobile flowering signal in rice. Sci. 316: 1033-1036.

Taoka, K. I., Ohki, I., Tsuji, H., Furuita, K., Hayashi, K., Yanase, T., Yamaguchi, M., Nakashima, C., Purwestri, Y.A., Tamaki, S. and Ogaki, Y. 2011. 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature. 476: 332-335.

Taylor, D. R. 2000. The physiology of flowering in strawberry. Acta Hort. 567: 245-251.

Taylor, D. R., Atkey, P. T., Wickenden, M. F., and Crisp, C. M. 1997. A morphological study of flower initiation and development in strawberry (*Fragaria* x *ananassa*) using cryo scanning electron microscopy. Ann. Appl. Biol. 130: 141-152.

Terashima, I., Fujita, T., Inoue, T., Chow, W. S., and Oguchi, R. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Pl. Cell Physio. 50: 684-697.

Thomas B, and Vince-Prue D. 1997. Photoperiodism in plants, 2nd edition. Academic Press, San Diego.

Thomas B., 2006. Light signals flowering. J Exp. Bot. 57:3387–3393.

Ulger, S., Sonmez, S., Karkacier, M., Ertoy, N., Akdesir, O. and Aksu, M. 2004. Determination of endogenous hormones, sugars and mineral nutrition levels during the induction, initiation and differentiation stage and their effects on flower formation in olive. Pl. Gro. Reg. 42: 89-95.

Usami, T., Mochizuki, N., Kondo, M., Nishimura, M. and Nagatani, A. 2004. Cryptochromes and phytochromes synergistically regulate *Arabidopsis* root greening under blue light. Pl. and Cell Phy. 45: 1798-1808.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A. and Coupland, G. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Sci. 303: 1003-1006.

Van Delm, T., Melis, P., Stoffels, K., and Baets, W. 2013. Pre-harvest night-interruption on everbearing cultivars in out-of-soil strawberry cultivation in Belgium. Int. J. F. Sci. 13 :217-226.

Van Sterthem, A., Desjardins, Y., Gauthier, L., Medina, and Y. Gosselin, A. 2016. Use of low tunnels to improve the productivity of day-neutral strawberry plants under the Québec climatic conditions. In VIII Int. Straw. Sym.1156: 555-562.

Verheul, M. J., Sønsteby, A. and Grimstad, S. O. 2006. Interactions of photoperiod, temperature, duration of short-day treatment and plant age on flowering of *Fragaria* x *ananassa* Duch. cv. Korona. Sci. Hort. 107: 164-170.

Wada, S. Niedz, R. P. and Reed, B. M. 2015. Determining nitrate and ammonium requirements for optimal in vitro response of diverse pear species. Cell. Dev. Biol. Pl. 51: 19–27.

Wan, C., Mi, L., Chen, B., Li, J., Huo, H., Xu, J., and Chen, X. 2017. Effects of nitrogen during nursery stage on flower bud differentiation and early harvest after transplanting in strawberry. Braz. J. Bot. 41: 1-10.

Warner, R. M., and Erwin, J. E. 2003. Effect of photoperiod and daily light integral on flowering of five *Hibiscus* sp. Sci. Hort. 97: 341-351.

Wigge, P. A., Kim, M. C., Jaeger, K. E., Busch, W., Schmid, M., Lohmann, J. U. and Weigel, D. 2005. Integration of spatial and temporal information during floral induction in *Arabidopsis*. Sci. 309: 1056-1059.

Wolbang, C. M., and Ross, J. J. 2001. Auxin promotes gibberellin biosynthesis in decapitated tobacco plants. Pl. 214: 153–157.

Wollenberg, A. C., Strasser, B., Cerdán, P. D. and Amasino, R. M. 2008. Acceleration of flowering during shade avoidance in *Arabidopsis* alters the balance between FLOWERING LOCUS C-mediated repression and photoperiodic induction of flowering. Pl. Physio. 148: 1681-1694.

Xu, Q., Gosselin, A., Desjardins, Y., Medina, Y., and Gauthier, L. 2013. October. Red raspberries production under high tunnel, umbrella-like structure and open field under northern Canadian climate. Int. Sym. New Tech. Env. Con. 1037: 771-776.

Yamada, A., Tanigawa, T., Suyama, T., Matsuno, T., and Kunitake, T. 2009. Effects of red: far-red light ratio of night-break treatments on growth and flowering of *Eustoma grandiflorum* (Raf.) Shinn. Acta Hort. 907: 313-317.

Yamasaki, A. and Yamashita, A. 1993. Changes in endogenous cytokinins during flower induction of strawberry. Acta. Hort. 345:93-99.

Yamasaki, A., and Yano, T. 2008. Effect of supplemental application of fertilizers on flower bud induction and development of strawberry and possible role of Nitrogen. In VI Int. Str. Sym. 842: 765-768.

Yan, B., Hou, J., Cui, J., He, C., Li, W., Chen, X., Li, M. and Wang, W. 2019. The effects of endogenous hormones on the flowering and fruiting of *Glycyrrhiza uralensis*. Pl. 519: 1-12.

Yanagi, T., Okuda, N. and Okamoto, K. 2016. Effects of light quality and quantity on flower initiation of *Fragaria chiloensis* L. CHI-24-1 grown under 24 h day-length. Sci. Hort. 202: 150-155.

Yang, F., Fan, Y., Wu, X., Cheng, Y., Liu, Q., Feng, L., Chen, J., Wang, Z., Wang, X., Yong, T. and Liu, W. 2018. Auxin-to-gibberellin ratio as a signal for light intensity and quality in regulating soybean growth and matter partitioning. Front. in Pl. Sci. 56: 1-13.

Yao, C. and Finlayson, S. A. 2015. Abscisic acid is a general negative regulator of *Arabidopsis* axillary bud growth. Pl. Physio. 169: 611-626.

Yoo, S. K., Chung, K. S., Kim, J., Lee, J. H., Hong, S. M., Yoo, S. J., Yoo, S. Y., Lee, J. S. and Ahn, J. H. 2005. Constans activates suppressor of overexpression of constans 1 through Flowering Locus T to promote flowering in *Arabidopsis*. Pl. Physio. 139: 770-778.

Yoshida, H., Mizuta, D., Fukuda, N., Hikosaka, S., and Goto, E. 2016. Effects of varying light quality from single-peak blue and red light-emitting diodes during nursery period on flowering, photosynthesis, growth, and fruit yield of ever-bearing strawberry. Plant Biotech. 33: 267-276.

Yuan, S., Zhang, Z. W., Zheng, C., Zhao, Z. Y., Wang, Y., Feng, L. Y., and Xu, F. 2016. *Arabidopsis* cryptochrome 1 functions in nitrogen regulation of flowering. Pro. Nat. Aca. Sci. 113: 7661-7666.

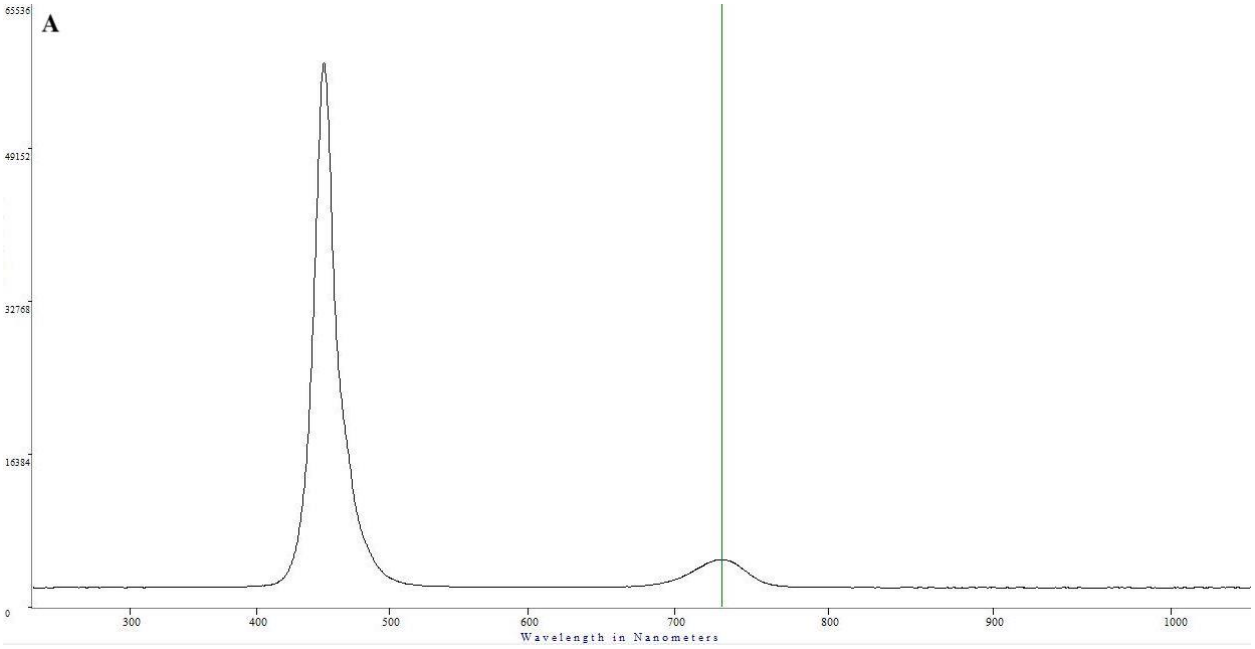
Zahedi, S. M., and Sarikhani, H. 2016. Effect of far-red light, temperature, and plant age on morphological changes and induction of flowering of a ‘June-bearing’ strawberry. Hort. Env. Biotech. 57: 340-347.

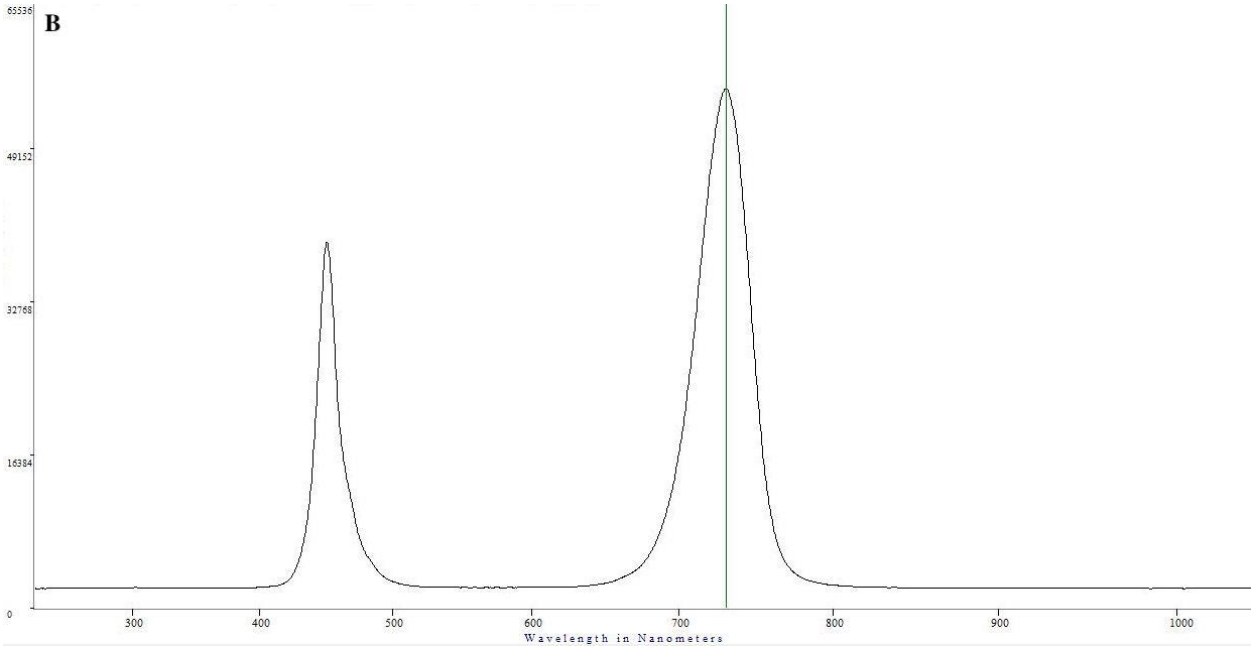
Zhao, C., Fan, X., Hou, X., Zhu, Y., Yue, Y., and Wu, J. 2017. Extended light exposure increases stem digestibility and biomass production of switchgrass. PloS One. 12: e0188349.

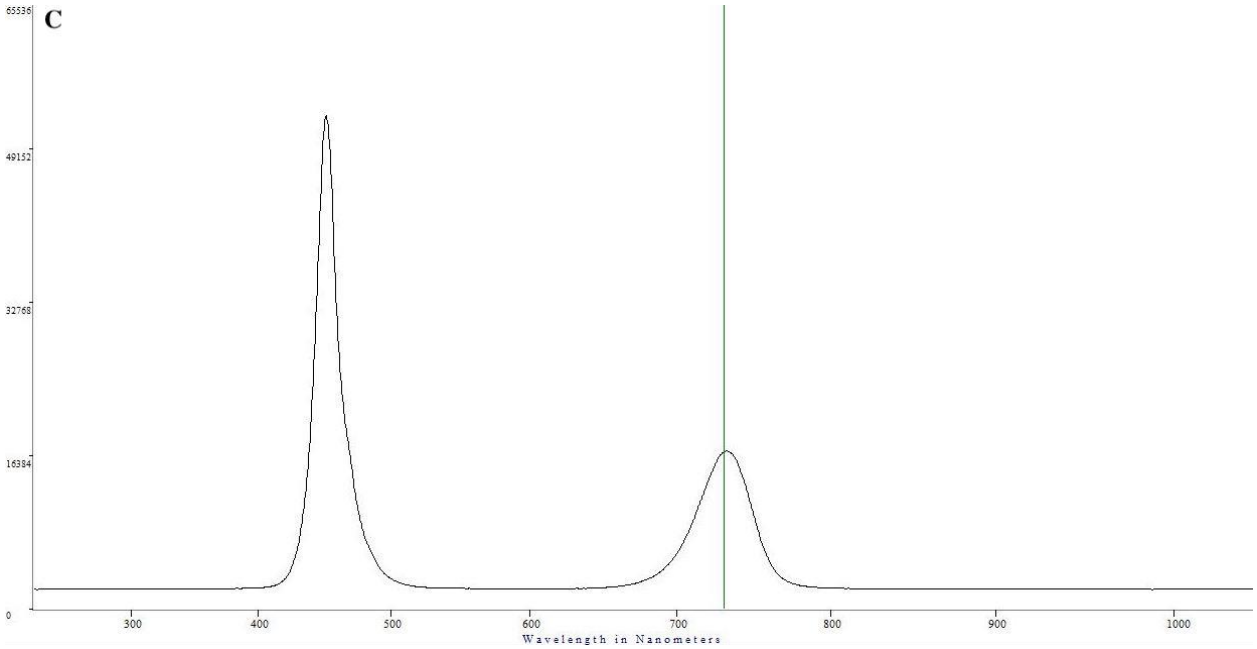
Zhao, X., Yu, X., Foo, E., Symons, G.M., Lopez, J., Bendehakkalu, K. T., Xiang, J., Weller, J. L., Liu, X., Reid, J. B. and Lin, C. 2007. A study of gibberellin homeostasis and cryptochrome-mediated blue light inhibition of hypocotyl elongation. Plant Physio. 145: 106-118.

Zubo, Y. O., Yamburenko, M. V., Selivankina, S. Y., Shakirova, F. M., Avalbaev, A. M., Kudryakova, N. V., Zubkova, N. K., Liere, K., Kulaeva, O. N., Kusnetsov, V. V. and Börner, T. 2008. Cytokinin stimulates chloroplast transcription in detached barley leaves. Pl. Physio. 148: 1082-1093

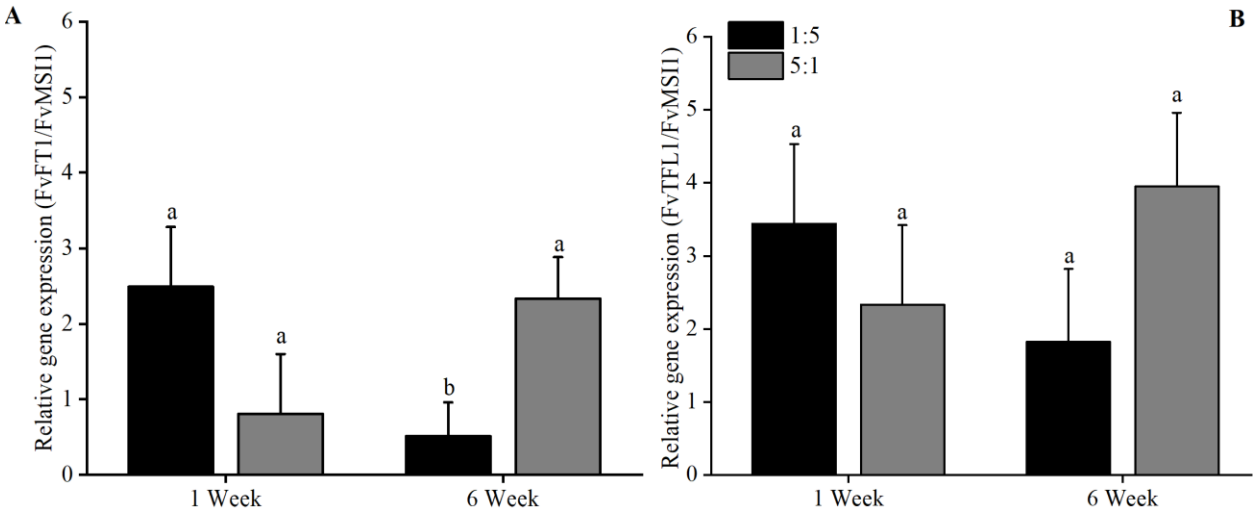
APPENDIX


A
65536
49152
32768
16384
0
300
400
500
600
700
800
900
1000
Wavelength in Nanometers


B
65536
49152
32768
16384
0
300
400
500
600
700
800
900
1000
Wavelength in Nanometers



Supplementary 7.1 Spectral output were measured using a spectroradiometer (Apogee Instruments Inc. model PS-300, MN, USA). (A) Dominant blue LEDs; far-red (peaked at 725 nm) and blue (peaked at 455 nm) at ratio of 1:5. (B) Dominant far-red; far-red (peaked at 725 nm) and blue (455 nm) light at ratio of 5:1. (C) FR:B (1:1), Far-red (peaked at 725 nm) and blue (peaked at 455 nm) light at ratio of 1:1. Graphs provides information related to spectral distribution only.



Supplementary 7.2 (A) The relative gene expression of FvFT1 in the shoot apex of young (4-week) seedlings of 'Alexandria' collected at different time intervals (1 and 6 weeks) in response to light treatment. (B) The expression of FvTFL1 in the leaves of young (4-week) seedlings of 'Alexandria' collected at different time intervals (1 and 6 weeks) in response to light treatment. Mean values with the same lowercase are not significantly different among each treatment group. FvMSI1 referred as housekeeping gene to determine relative expression. Light ratios of 1:5 and 5:1 of Far-red (725 nm):Blue (455 nm).

Supplementary 7.3 Photos of strawberry production under umbrella shelter experiment conducted at Ferme Onésime Pouliot Inc.

Supplementary 7.4 Photos of 'Albion' transplants collected 45 days after light quality and photoperiod treatments conducted in the greenhouse at Macdonald Campus of McGill University.