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FACULTY OF
BIOSCIENCE ENGINEERING

Light Quality Effects on Anatomy and Physiology of Ornamentals Differing in Their Photosynthetic Pathways

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List of abbreviations

AGR: absolute growth rate

ATP: adenosine triphosphate

CAM: crassulacean acid metabolism

Chl: chlorophyll

CRY: cryptochrome

DLI: daily light integral

DW: dry weight

EGTA: ethylene glycol tetra-acetic acid

ETR: electron transport rate

FAA: Formalin- Acetic Acid- Alcohol

FeCH: ferrochelatase

FMN: flavin mononucleotide

F_v/F_m: maximum potential quantum yield of Photosystem II

FW: fresh weight

GluTR: glutamyl-tRNA reductase

g_s: stomatal conductance

H_2O_2: hydrogen peroxide

HPFM: high pressure flowmeter method

HPS: high pressure sodium

K_{leaf}: leaf hydraulic conductance

LEDs: light emitting diodes

LHC: light-harvesting complex

LMA: leaf mass per area

MgCH: magnesium chelatase

$NADP^+$: nicotinamide adenine dinucleotide phosphate (reduced form)

NADPH: nicotinamide adenine dinucleotide phosphate

NPQ: non-photochemical quenching

PAR: photosynthetically active radiation

PEPC: phosphoenolpyruvate carboxylase

PGA: 3-phosphoglyceric acid

phot: phototropin

PHY: phytochrome

P_{fr}/P_{total}: phytochrome photostationary state

PMSF: phenylmethylsulfonyl fluoride

PPFD: photosynthetic photon flux density

PSI: Photosystem I

PSII: Photosystem II

PVPP: polyvinylpolypyrrolidone

qP: photochemical quenching

RLC: rapid light curve

Rubisco: Ribulose-1,5-bisphosphate carboxylase/oxygenase

ROS: reactive oxygen species

SD: stomatal density

SI: stomatal index

UV: ultraviolet

Φ_{PSII}: quantum yield of Photosystem II electron transport

Φ_{NPQ}: quantum yield of non-photochemical quenching

Φ_{NO}: non-regulated energy dissipation including fluorescence emission

Summary

Artificial lighting has been widely used in horticultural production in northern latitudes. Artificial lighting is applied to increase the light intensity as supplemental lighting in greenhouses during the low irradiance season. More recently, it is used as a sole light source in vertical farming systems. Light emitting diodes (LEDs) are attracting much attention as an alternative light source due to their high photoelectric conversion efficiency, low thermal output, narrowband spectral distribution and adjustable light intensity.

Light quality critically affects plant development and growth. Development of LEDs enables the use of selective narrow band red and/or blue wavelengths that meet the absorption peak of the photosynthetic pigments with adjustable intensities. Their application opens the possibility to regulate not only plant growth but also photo-morphogenetic responses for a targeted crop.

In this thesis, effects of distinct red (R), blue (B), a combination of red with blue (RB) and white (W) light sources were studied in different ornamental species varying in their photosynthetic pathway (C3 and CAM), leaf traits and sun/shade adaptive properties.

In a first explorative study, we set two light intensities, namely low (40 µmol m^{-2} s^{-1}) and control (100 µmol m^{-2} s^{-1}) irradiance with the four light qualities to study their effect on leaf anatomy, photosynthetic efficiency and pigments in *Chrysanthemum*. When comparing both light levels, leaf thickness decreased under the lower irradiation for B, RB and multispectral W but not for the red light treatment. Pigments accumulated irrespective of the light quality while biomass was reduced for the low irradiance. Favorable effects of blue light were observed with respect to the anatomical development of the leaves and biomass accumulation under higher light intensity (100 µmol m^{-2} s^{-1}). Both light intensity and quality affected the stomatal development. Low light decreased the stomatal index and density but increased stomatal aperture area for RB and W. Light quality influenced the photosynthetic efficiency, monochromatic red inhibited Photosystem II for both light intensities, resulting in a decline in maximum quantum yield (F_v/F_m) and quantum efficiency (Φ_{PSII}).

The influence of light quality on leaf morphology, mesophyll anatomy and stomatal development and their relation with light absorption, gas and hydraulic conductance

and photosynthetic capacity were investigated in three pot plants with differing leaf characteristics. We selected *Cordyline australis* (monocot), *Ficus benjamina* (dicot, evergreen leaves) and *Sinningia speciosa* (dicot, deciduous leaves); this for four light qualities at 100 µmol m^{-2} s^{-1}. Blue light increased the leaf thickness and palisade parenchyma of *F. benjamina*. Also in *S. speciosa*, an increase in palisade parenchyma was found under B and RB, though total leaf thickness was unaffected. Palisade parenchyma thickness correlated to the leaf photosynthetic quantum efficiency (Φ_{PSII}). B and RB resulted in a greater maximum quantum yield (F_v/F_m) and quantum efficiency (Φ_{PSII}) in all species compared to R and W. B increased the stomatal conductance compared with R, which was correlated to increasing stomatal index and/or stomatal density but not with the stomatal aperture area. Blue light addition in the spectrum was essential for the normal anatomical leaf development, which also affects the photosynthetic efficiency in the three studied species.

Secondary metabolism is another important aspect that is influenced by light quality. In Chapter 4, the intraspecific responses to light quality in eight *Chrysanthemum* cultivars were investigated. As expected, we saw genotype dependent variations. Overall, red light significantly decreased the leaf area while the thinnest leaves were observed for W. Chlorophyll content and Chl *a/b* ratio was highest for W and lowest under R. B and RB resulted in the highest maximum quantum yield (F_v/F_m) and quantum efficiency (Φ_{PSII}) which is similar to the observations in Chapter 3. Blue light induced the highest hydrogen peroxide content, which is a proxy for total ROS generation. The anti-oxidative response was not always correlated with hydrogen peroxide content and depended on the light quality treatment. Blue light enhanced the proline levels, while carotenoids, total flavonoid and phenolic compounds were higher under W. Intraspecific variation in the responses were observed for most parameters with exception of leaf thickness; this intraspecific variation was most pronounced for total phenolics and flavonoid compounds.

Crassulacean acid metabolism (CAM) is a specialized photosynthetic pathway present in many epiphytic orchids. We tested the effect of light quality on the CAM cycle and monitored how long-term duration affected the cycle and the global photosynthetic performance in *Phalaenopsis*. Plants grown under monochromatic R significantly decreased their quantum efficiency (Φ_{PSII}) and maximum quantum yield (F_v/F_m) after respectively five days and ten days of treatment. A long-term treatment with different

light qualities showed that the total 24h CO_2 exchange was highest under monochromatic blue and full spectrum light. Blue light addition to red (RB) enhanced the daily CO_2 uptake by 18%. CAM and its metabolism were affected by the applied light quality, a longer phase II duration for blue light and an earlier CO_2 uptake in Phase IV for B and RB was observed. The nocturnal malate accumulation was reduced under red light compared to the other light treatments. During the daytime, the basal levels of malate were reached faster under blue and RB. Starch showed an inverse diel pattern with malate content, greater starch breakdown was recorded for RB and W compared with red and blue.

Leaf anatomy and development of plants are highly plastic to light quality, as described in the above studies. Leaves with different morphology and physiology could affect its acclimation to high intensity full light environment as found during summer greenhouses. In Chapter 6, we investigated the acclimation to greenhouse conditions of *Chrysanthemum* (sun species) and *Spathiphyllum* (shade species) after a pre-cultivation time of 4 weeks under four different light qualities (as above). Leaves that developed under monochromatic R and B showed an inhibition of photosynthesis after the light quality treatment. After 1 week B leaves could acclimate to the full light spectrum and their photosynthetic capacity was similar to the levels of leaves with pretreatments of RB and W. However, this was not observed for leaves that developed under R, R limited the leaf structural development and this lead to a lower dry mass assimilation compared to the other light quality treatments still visible after 1-month growth in the greenhouse. Also in *Spathiphyllum*, monochromatic light limited the leaf development and resulted in lower leaf mass per area compared to multispectral light. However, as a shade species, *Spathiphyllum* leaves showed increase in Φ_{NPQ} (quantum yield of non-photochemical quenching) and decrease in the ETR_{max} after one-week acclimation in the greenhouse. In addition, no full recovery for R was found in *Spathiphyllum*.

This study showed that there are species and cultivar depended responses to light quality. Generally, monochromatic red light showed adverse effects on most the species studied. Blue light is beneficial in certain metabolic and physiological responses, thus it should be present in the applied LED spectrum.

Samenvatting

Assimilatiebelichting is een veel gebruikte toepassing in de tuinbouwproductie in het noordelijke halfrond. Assimilatiebelichting wordt toegepast om de lichtintensiteit te verhogen en als aanvullende lichtbron in kassen wanneer natuurlijke lichtcondities ontoereikend zijn voor de beoogde productie en/of plantkwaliteit. Meer recent is het gebruik van kunstlicht als enige lichtbron in verticale tuinbouwsystemen. Light emitting diodes (LED) wekken veel belangstelling op als alternatieve lichtbron omwille van hun hoge foto-elektrische conversie-efficiëntie, lage thermische output, specifieke spectrale distributie en regelbare lichtintensiteit.

Lichtkwaliteit beïnvloedt de groei en ontwikkeling van planten fundamenteel. Het gebruik van ledverlichting maakt het mogelijk om selectief, nauwe banden in het rode en/of blauwe golflengtegebied te gebruiken die corresponderen met de absorptiepiek van de fotosynthetische pigmenten, en dit met nauwkeurig instelbare intensiteiten. Toepassing ervan biedt niet alleen de mogelijkheid om de plantengroei te regelen, maar kan ook foto-morfogenetische reacties van een gewas sturen.

In dit proefschrift werden effecten van afzonderlijk rode (R), blauwe (B), een combinatie van rode en blauwe (RB) en witte (W) lichtbronnen bestudeerd bij verschillende siergewassen met een verschillend fotosynthese proces (C3 en CAM), bladkenmerken en zon/schaduw- adaptieve eigenschappen.

In een eerste exploratieve studie bij *Chrysanthemum* werden de vier lichtkwaliteiten telkens in twee lichtintensiteiten aangewend, een lage (40 µmol m^{-2} s^{-1}) en de controle (100 µmol m^{-2} s^{-1}) instraling om hun effect op bladanatomie, fotosynthetische efficiëntie en pigmenten te bestuderen. Als reactie op lage lichtintensiteit nam de bladdikte af voor B, RB en multispectraal W, maar niet voor de rode lichtbehandeling. Pigmenten accumuleerden ongeacht de lichtkwaliteit, terwijl de biomassa daalde. Gunstige effecten van blauw licht werden waargenomen met betrekking tot de anatomische ontwikkeling van de bladeren en biomassa-accumulatie onder hogere lichtintensiteit (100 µmol m^{-2} s^{-1}). Zowel de lichtintensiteit als de kwaliteit beïnvloedden de ontwikkeling van de stomata. Lage lichtintensiteit verminderde de stomatale index en de dichtheid, maar vergrootte de stomatale openingsgraad voor RB en W. Lichtkwaliteit beïnvloedde de fotosynthetische efficiëntie: monochromatisch rood licht onderdrukte

Fotosysteem II voor beide lichtintensiteiten, wat resulteerde in een daling van de maximale kwantumopbrengst (F_v/F_m) en de kwantumefficiëntie (Φ_{PSII}).

De invloed van lichtkwaliteit op bladmorfologie, mesofylanatomie en stomatale ontwikkeling en hun relatie met lichtabsorptie, gas- en hydraulische geleidbaarheid en fotosynthetische capaciteit werden onderzocht bij drie potplanten met verschillende bladkenmerken, namelijk *Cordyline australis* (monocotyl), *Ficus benjamina* (dicotyl, groenblijvend) en *Sinningia speciosa* (dicotyl, bladverliezend); dit voor vier lichtkwaliteiten bij 100 µmol m^{-2} s^{-1}. Blauw licht verhoogde de bladdikte en het palisadeparenchym van *F. benjamina*. Ook in *S. speciosa* werd een toename in palisadeparenchym vastgesteld onder B en RB, hoewel de totale bladdikte bij deze soort onaangetast bleef. Palisadeparenchymdikte correleerde met de fotosynthetische kwantumefficiëntie (Φ_{PSII}) van het blad. B en RB resulteerden in een grotere maximale kwantumopbrengst (F_v/F_m) en kwantumefficiëntie (Φ_{PSII}) in vergelijking met R en W bij alle soorten. In tegenstelling tot R verhoogde B de stomatale geleidbaarheid, dit was gecorreleerd met een toenemende stomatale index en/of stomatale dichtheid, maar niet met de stomatale openingsgraad. De aanwezigheid van blauw licht in het spectrum was essentieel voor de normale anatomische bladontwikkeling, die ook invloed heeft op de fotosynthetische efficiëntie bij de drie bestudeerde soorten.

Het secundair metabolisme is een ander belangrijk aspect dat beïnvloed wordt door de lichtkwaliteit. In hoofdstuk 4 werden de intraspecifieke reacties op lichtkwaliteit bij acht *Chrysanthemum* species onderzocht. Zoals verwacht konden genotype-afhankelijke variaties vastgesteld worden. Over het algemeen verminderde rood licht de bladoppervlakte significant, terwijl de dunste bladeren waargenomen werden voor W. Chlorofylinhoud en de chlorofyl *a/b* ratio was het hoogste voor W en het laagst onder R. B en RB resulteerden in de hoogste maximale kwantumopbrengst (F_v/F_m) en kwantumefficiëntie (Φ_{PSII}), wat vergelijkbaar is met de waarnemingen in hoofdstuk 3. Blauw licht veroorzaakte het hoogste waterstofperoxidegehalte, wat een indicator is voor de totale ROS generatie. De anti-oxidatieve reactie was niet altijd gecorreleerd met waterstofgehalte en varieerde in functie van de lichtkwaliteit. Blauw licht verhoogde de prolineconcentratie, terwijl de concentraties carotenoïden, totale flavonoïden en fenolische verbindingen hoger waren onder W. Intraspecifieke variatie in de reacties werd waargenomen voor de meeste parameters met uitzondering van bladdikte; deze

intraspecifieke variatie was het meest uitgesproken voor totale fenolische en flavonoïde verbindingen.

'*Crassulacean acid metabolism'* (CAM) is een gespecialiseerd fotosynthese-mechanisme aanwezig bij veel epifytische orchideeën. Het effect van lichtkwaliteit op de CAM-cyclus werd onderzocht en tevens werd gecontroleerd hoe langdurige blootstelling de cyclus en de globale fotosynthetische prestatie in *Phalaenopsis* beïnvloedt. Planten, geteeld onder monochromatische R, hadden een significant lagere kwantumefficiëntie (Φ_{PSII}) en maximale kwantumopbrengst (F_v/F_m) na respectievelijk vijf en tien dagen behandeling. Een langdurige behandeling met verschillende lichtkwaliteiten toonde aan dat de totale CO_2-uitwisseling per etmaal het hoogst was onder monochromatisch blauw en volledig spectrum licht. Aanvullend blauw licht bij rood (RB) verhoogde de dagelijkse CO_2-opname met 18%. De CAM-cyclus werd beïnvloed door de toegepaste lichtkwaliteit: een langere fase II voor blauw licht en een vroegere CO_2-opname in fase IV voor B en RB werd waargenomen. De nachtelijke malaataccumulatie werd gereduceerd onder rood licht in vergelijking met de andere lichtkwaliteiten. Overdag werden de basale malaatconcentraties sneller bereikt onder blauw en RB. Zetmeel vertoonde een invers 24h patroon ten opzichte van malaat: een hogere zetmeelafbraak werd genoteerd voor RB en W in vergelijking met rood en blauw.

Bladanatomie en de ontwikkeling van planten kunnen sterk beïnvloed worden door lichtkwaliteit, zoals hierboven beschreven. Verschillen in bladmorfologie en -fysiologie kunnen invloed hebben op de acclimatisatie tijdens omstandigheden met hoge lichtintensiteiten, zoals dit voorkomt in serres tijdens de zomermaanden. In hoofdstuk 6 hebben we de acclimatisatie van *Chrysanthemum* (zonsoorten) en *Spathiphyllum* (schaduwsoorten) in serre-omgeving onderzocht na een voorafgaandelijke teeltduur van 4 weken onder vier verschillende lichtkwaliteiten (zie eerder). Bij *Chrysanthemum* werd de fotosynthese geremd bij de bladeren die ontwikkelden onder monochromatische R en B. Na 1 week konden de bladeren ontwikkeld onder B acclimatiseren in het volledige lichtspectrum en was hun fotosynthetische capaciteit op vergelijkbaar niveau met voorbehandelingen van RB en W. Dit kon echter niet waargenomen worden bij bladeren die zich onder R ontwikkelden. De negatieve effecten van R op bladstructuurontwikkeling en fotosynthese leidde tot een lagere droge massa-assimilatie, die nog steeds zichtbaar was na 1 maand groei in de kas.

Ook bij *Spathiphyllum* reduceerde monochromatisch licht de bladontwikkeling met een lagere bladmassa per oppervlakte-eenheid als gevolg. Bij *Spathiphyllum* (schaduwplant), vertoonden de bladeren een toename van Φ_{NPQ} en daling van ETR_{max} na een week acclimatisatie in de kas. Daarenboven werd bij *Spathiphyllum* geen volledig herstel voor R verkregen.

Uit deze studie bleek dat soorten en cultivars een afwijkende respons kunnen hebben op lichtkwaliteit. Over het algemeen oefende monochromatisch rood licht een negatieve invloed uit bij de meeste onderzochte soorten. Blauw licht is daarentegen bevorderlijk bij bepaalde metabolische en fysiologische reacties, en zou dus aanwezig moeten zijn in het toegepaste led-spectrum.

Chapter 1

Introduction

Supplemental lighting is used in ornamental greenhouse production to increase crop production and quality during times with low levels of solar radiation. Supplemental lighting is an energy consuming production factor and energy is second only to labor as the most expensive indirect cost of greenhouse production. Supplying light using the advanced light emitting diodes (LED) technology opens the possibility in both energy conservation and plant physiology regulation by modifying light quality. This PhD study aims to gain a greater understanding about the underlying anatomical and physiological responses of several C3 and CAM ornamental plants to different lighting mixes using red and blue LEDs.

1.1 The ornamental sector

Plants with ornamental value have been gathered and domesticated for thousands of years, they play a fundamental role in humans interaction and are grown for decorative purposes (Chandler and Sanchez, 2012). By gathering plants from around the world, cross breeding and mutation breeding, breeders cultivated wide diversity of ornamental plants. Nowadays, thousands of varieties of cut flowers, pot plants, hanging plants, bedding plants, shrubs, and ornamental trees are available to the public.

Cut flowers and ornamental young plants are important export products for several developing countries in East Africa and South and Central America. In 2015, the floricultural production in EU countries was 28% of the world production and the EU is still a net exporter of pot plants (European Commission). The traditional markets for export are located in Western Europe, North America, and Japan but there is a rising consumption in emerging markets like Eastern Europe, China, India and East Asia. With the increasing levels of flower production and cultivation of ornamental plants, the EU is now one of the world's highest densities of flower production (34.3% of world flower and pot-plant production) according to International Association of Horticultural Producers (AIPH) in 2014. The total turnover for all aspects of ornamental plant production is estimated to be more than 250–400 billion USD (Chandler and Sanchez, 2012).

1.2 Ornamental species in this study

***Chrysanthemum morifolium*:** Chrysanthemums are herbaceous perennial plants with alternating lobed leaves, which belong to the Asteraceae family and are classified as quantitative short day plants. Originating from East Asia, chrysanthemum has a longstanding history of ornamental and pharmaceutical purposes in China, Korea and Japan. Commercial chrysanthemum is an important cut flower and pot plant species, it is globally the second economically most important floricultural crop following rose (Teixeira Da Silva, 2004).

***Cordyline australis*:** *C. australis* spp. is a distinctive monocot tree endemic to New Zealand. It is placed in the family Asparagaceae and many species are cultivated as ornamentals. Among the cultivars of *C. australis*, 'Red Star', which was used in this study, is the most valuable decorative pot plant with dark purple foliage.

Ficus benjamina: *F. benjamina*, which belong to Moraceae family, is a hemi-epiphytic tree species native to tropical Southeast Asia, with a large, graceful and broad-headed evergreen canopy, it is one of the most widely grown indoor ornamental plant species.

***Phalaenopsis*:** The genus *Phalaenopsis* belongs to the Orchidaceae family and it contains more than 50 species. *Phalaenopsis* is an epiphytic orchid exhibiting crassulacean acid metabolism (CAM) photosynthesis (Mc Williams, 1970). *Phalaenopsis* is native to tropical and subtropical areas of the South Pacific Islands and Southeast Asia where it grows on tree trunks and limbs that are shaded by the dense forest canopy. *Phalaenopsis* is a popular flowering plant due to its lasting flower with a variety of sizes and colors.

Spathiphyllum wallisii: *S. wallisii*, commonly known as Peace lily, is a very popular indoor plant of the family of Araceae. It is a tropical herbaceous evergreen perennial that is native to Central America. *S. wallisii* cultivars are attractive shade tolerant pot plants with pure white flowers in the typical aroid structure.

Sinningia speciosa: *S. speciosa* is cultivated as a popular pot plant. It is commonly known as gloxinia and widely cultivated throughout the world as an ornamental crop

due to its large, oval leaves and velvety, bell-shaped flowers. It is a perennial fleshy herb of the Gesneriaceae family and found in South America.

Figure 1.1 The ornamental plants used for the experimental work of this study.

1.3 LED lighting in ornamental horticulture

Horticultural production in controlled and closed environments is one of the most energy-intensive cultivation systems in agriculture (Tähkämö and Dillon, 2014). Artificial lighting is an important part of this energy consumption (for instance, the energy consumption of the Dutch greenhouse sector was 37% electricity and 63% heat in 2013, Dieleman et al., 2016), though it allows an all-year-round production

independent of weather conditions and geographic location. As light is one of the most important environmental factors that affects the plant development and regulates many physiological processes (Lepetit and Dietzel, 2015), it is no surprise that supplementary lighting is a standard cultural technique in regions with latitudes higher than 50 degrees where natural light is limited during the winter months.

Conventional lighting systems with broad spectrum light such as fluorescent tubes and high pressure sodium (HPS) lamps were widely utilized in the greenhouse production because of their relatively high efficiency in converting energy into photosynthetic light and their application is still economically affordable (Riikonen et al., 2016; Terfa et al., 2013). However, lamps like HPS emit radiation mainly in the orange-red region between 550 and 650 nm and hardly in the blue spectrum between 400 and 500 nm (Islam et al., 2012; Kim et al., 2005) despite blue light is also strongly absorbed by the photosynthetic pigments. HPS lamps also produce much heat (25% of the electrical energy input is converted to infrared radiation, Nelson and Bugbee, 2014) which can help in the heating requirements of the greenhouse. Yet, this heat production limits the possibility to supply light close to the plants such as in inter-lighting strategies (Olle and Viršile, 2013). Furthermore, the HPS lamps do not provide the possibility for spectral manipulation of the lighting spectrum which could trigger potential benefits for the plants by steering plant growth and architecture (Massa et al., 2008). Therefore, HPS lamps are neither spectrally nor energetically optimal.

Differing from these traditional lamps, a potentially more efficient light emitting diode (LED) lighting source was introduced to plant cultivation in the 2000s (Piovene et al., 2015). Application of LEDs opens the possibility to adjust the spectral composition to the photosynthetic demands of plants (Morrow, 2008), and plant architecture and flowering of photoperiodic crops can be modulated.

Table 1.1 Comparing the properties of LEDs to the commonly used lighting technologies. Adapted from D'Souza et al. (2015).

Properties	LEDs	Light emitting plasma lamps	Fluorescent lamps	HPS lamps	References
Spectral composition	Monochromatic. UV, Infrared (IR) and White LEDs available.	Broad spectrum, Radiation in UV and IR range present	Broad spectrum, Radiation in UV and IR range present.	Broad spectrum, Radiation in UV and IR range present.	Denbaars et al. (2013); Mitchell et al. (2012)
Size and compactness	Small and compact chips, assemble for different formations, shapes, and fixtures.	Bulky	Bulky	Bulky	Mitchell et al. (2012); U.S. Dept. of Energy (2016)
Luminous efficiency	Color-mixed white LEDs: 100 to 180 lm/W	80 to 100 lm/W	45 to 80 lm/W	65 to 150 lm/W	U.S. Dept. of Energy (2016); Pattison et al. (2016)
Photon efficiency	0.89 up to 2.40 μmol J^{-1}	1.00-1.30 μmol J^{-1}	0.95 μmol J^{-1}	1.30 to 1.70 μmol J^{-1}	Nelson and Bugbee (2014); van Iersel and Gianino (2017)
Life expectancy	50000 h	50000 h	10000 to 17000 h	10000 to 17000 h	Nelson and Bugbee (2014); Gupta and Jatothu (2013)
Durability	Not affected by mechanical force.	Brittle components in bulb and fixtures	Brittle components in bulb and fixtures.	Brittle components in bulb and fixtures.	U.S. Dept. of Energy (2016)

LEDs are nowadays widely used in plant factories as a more efficient light source and are expected to reduce the electricity costs of lighting and cooling (Goto, 2012). Light-emitting diodes have a variety of advantages over traditional forms of horticultural lighting (D'Souza et al., 2015) (Table 1.1). Their small size, low power requirement, durability, long lifetime, cool emitting temperature, and the option to select specific wavelengths for a targeted plant response make LEDs more suitable for plant-based uses than many other light sources. Indeed, research to develop tailor-made light strategies especially for horticultural production in controlled environment, has strongly increased in recent years.

1.4 Light absorption and photosynthesis

Chlorophylls, carotenoids, and anthocyanins are three major light absorbing pigments in plants. Plants are able to use spectral wavelengths within the range from 400 to 700 nm for photosynthesis (Davis and Burns, 2016), which is often referred as PAR (photosynthetically active radiation). Light energy is transferred to the reaction center of Photosystem I (PSI) and Photosystem II (PSII) by the photosynthetic pigments chlorophyll and carotenoids (Bonet et al., 2016; Hogewoning et al., 2012). McCree (1971) quantified the spectral absorption of several species and indicated that red wavelengths (600 to 700 nm) are efficiently absorbed by chlorophyll, which is in line with the early developed red LEDs. Yet, chlorophylls absorb also in the blue wavelengths (400 to 500 nm) of the visible spectrum. Chlorophyll a has its absorption peaks at 430 and 665 nm, while chlorophyll b has its absorption peaks at 453 nm and 642 nm (Sager and McFarlane, 1997). The carotenoid pigments lutein and b-carotene absorb strongly in the blue region (maximum absorption at 448 and 452 nm, respectively) (Wright and Shearer, 1984) (Figure 1.2). Anthocyanins prevent photoinhibition and photodamage through the absorption of excessive solar radiation that would otherwise be absorbed by chloroplast pigments and absorb blue, blue-green, and green light.

Therefore, the use of blue and red LEDs is widely accepted since plant pigments efficiently absorb both these wavelengths. The effects of light quality and intensity on horticultural traits and the increased availability of narrow-band width light sources present an opportunity to exploit our knowledge of light-sensory circuitry to custom light regimens that best drive plant responses to match grower's desires.

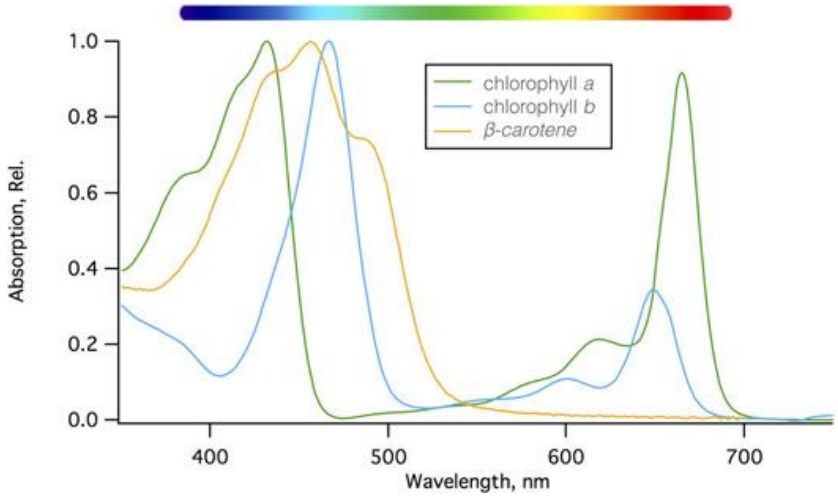

Figure 1.2 Absorption spectra of the major chlorophyll and carotenoid pigments in plants (Johnson, 2016). Chlorophylls absorb light energy mainly in the red and blue part of the visible spectrum, whereas carotenoids absorb blue and green wavelengths.

In the photosynthesis of higher plants, light energy absorbed by the light harvesting pigments is transferred to the reaction centers of two different Photosystems: Photosystem I (PSI) and Photosystem II (PSII). A Photosystem consists of numerous light-harvesting complexes (LHCs) that form an antenna of hundreds of pigment molecules. A light-harvesting complex (LHC) consists of chlorophylls and carotenoids attached to membrane-embedded proteins. The two Photosystems cooperate in the photosynthetic electron chain transfer from H_2O to $NADP^+$, which is commonly described as the Z-scheme (Figure 1.3). PSII is a chlorophyll-containing supramolecular complex embedded in the thylakoid membrane, known as P680 due to their 680 nm absorption peak in the spectrum. The core of this membrane protein is formed by two subunits D1 and D2. For PSI, the chlorophyll-protein complex is known as P700 because of its absorption peak at 700 nm. This protein has two main components forming its core, psaA and psaB.

The light-driven electron transfer reactions of photosynthesis occur in the thylakoid membrane and begin with the splitting of water by Photosystem II (PSII). PSII uses light energy to oxidize two molecules of water into one molecule of molecular oxygen. The four electrons removed from the water molecules are transferred by an electron transport chain and in this process, the primary electron acceptor plastoquinone is reduced to plastoquinol. Plastoquinol then carries the electrons derived from water to another thylakoid-embedded protein complex cytochrome *b*6*f* (cyt*b*6*f*). Cyt*b*6*f*

oxidizes plastoquinol to plastoquinone and reduces a small water-soluble electron carrier protein plastocyanin, which resides in the lumen. The released protons (H^+) from water-splitting reactions at PSII and plastoquinol oxidation at cyt*b*6*f* go into the lumen and build up a proton gradient between the two sides of the membrane. The proton concentration gradient from the lumen to the stroma is utilized by ATP synthase to drive the energy requiring synthesis of ATP from ADP and inorganic phosphate (Pi). The final stage of the light reactions is catalyzed by Photosystem I (PSI). PSI oxidizes plastocyanin and reduces another soluble electron carrier protein ferredoxin that resides in the stroma. Ferredoxin can then be used by the ferredoxin-$NADP^+$ reductase (FNR) enzyme to reduce $NADP^+$ to NADPH (Haehnel, 1984; Johnson, 2016).

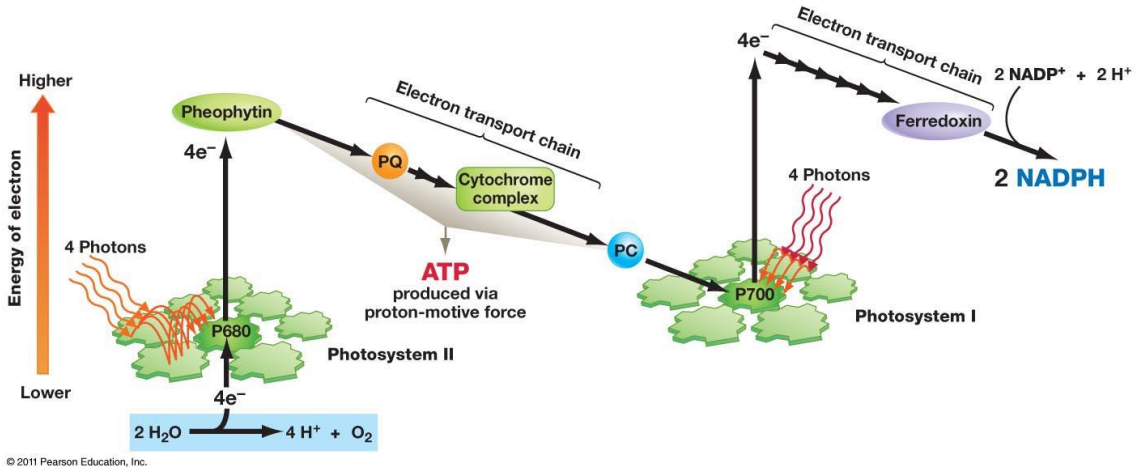

Figure 1.3 The "Z-scheme" of photosynthetic electron transfer (Pearson Education, Inc.). The main components of the linear electron transfer pathway are shown on scale of redox potential to illustrate how two separate inputs of light energy at PSI and PSII result in the transfer of electrons from water to $NADP^+$.

During the Calvin–Benson cycle, which is the "dark reaction", CO_2 is fixed into carbohydrate by consuming the ATP and NADPH produced during the light reaction (Figure 1.4). There are three distinct biochemical types of photosynthesis based on the mechanism that plants employ to form carbohydrates from CO_2 namely C3 photosynthesis, C4 photosynthesis, and CAM photosynthesis. Most of the ornamental crops are C3 and crassulacean acid metabolism (CAM), only in outdoor

production, a limited number of ornamental grasses with a C4 photosynthesis is produced.

Plants with C3 photosynthesis begin the process of energy conversion, known as the Calvin cycle, by producing a three-carbon compound called 3-phosphoglyceric acid (usually referred to as PGA), hence the name C3 photosynthesis. It is generally assumed that C3 is the oldest photosynthetic pathway among higher plants. The carbon fixation step (i.e. the incorporation of CO_2 into carbohydrate) is carried out by a single enzyme, Rubisco. Rubisco is a large soluble protein complex found in the chloroplast stroma and consists of eight large (56 kDa) subunits, which contain both catalytic and regulatory domains, and eight small subunits (14 kDa).

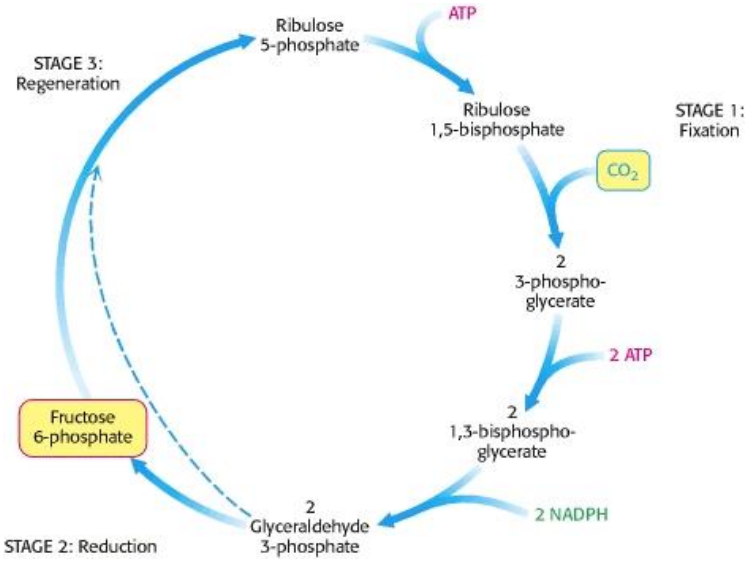

Figure 1.4 Schematic figure of the Calvin cycle (Berg et al., 2002). The Calvin cycle consists of three stages: Stage 1 is the fixation of carbon by the carboxylation of ribulose 1,5-bisphosphate (RuBP); Stage 2 is the reduction of the fixed carbon to begin the synthesis of hexose. Stage 3 is the regeneration of the starting compound, RuBP.

Plants with CAM metabolism operate by sequentially absorbing CO_2 during the night and reducing CO_2 into carbohydrates through the Calvin cycle during the day. CAM plants close their stomata during the daytime to reduce water loss and open them at night for CO_2 uptake and fixation. It is in this way that plants in unfavorable environments are able to withstand these conditions. *Mesembryanthemum crystallinum*, a facultative CAM plant, assimilates CO_2 via the C3 pathway when

water supply is sufficient, but reverts to the CAM pathway under water limited conditions (Tallman et al., 1997). CO_2 is fixed into oxaloacetate by phosphoenolpyruvate carboxylase (PEPC) at night when stomata are open. The oxaloacetate is reduced to malate via NAD-malate dehydrogenase and pumped into the vacuoles. During the day phase, when the stomata are closed, malate is decarboxylated into CO_2; increasing the intercellular CO_2 concentration and the resulting CO_2 is subsequently fixed by Rubisco in the same way as for C3 plants (Figure 1.5).

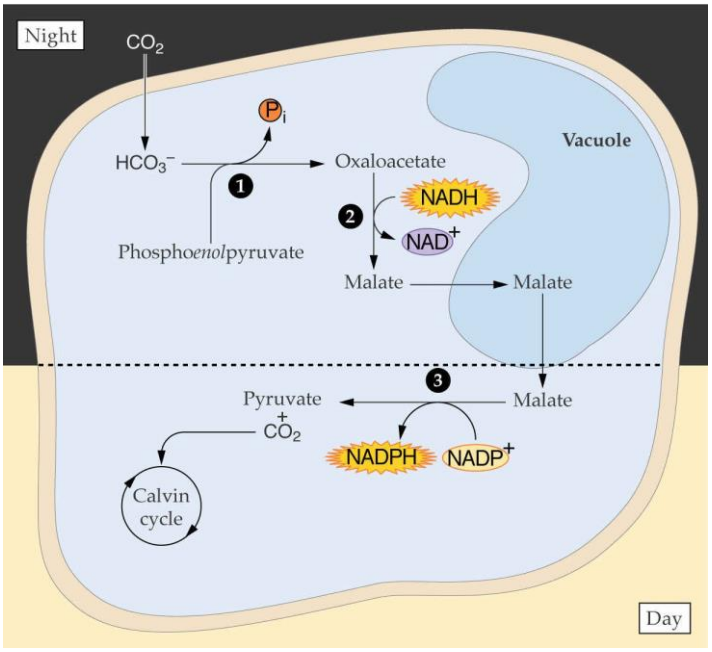

Figure 1.5 Schematic representation of the crassulacean acid metabolism (CAM) pathway (Buchanan et al., 2015). PEP carboxylase (1) incorporates CO_2 (as HCO_3^-) into the organic acid oxaloacetate, which is then reduced to malate by malate dehydrogenase (2); the malate is stored in the vacuole. The stored malate is decarboxylated by $NADP^+$-malic enzyme (3); and the resulting CO_2 is converted to carbohydrate via the Calvin cycle.

Generally, CO_2 uptake of CAM photosynthesis is characterized by four phases (Figure 1.6). Phase I includes the nighttime period when the stomata are open for uptake of CO_2 used for malic acid accumulation. Phase II occurs in the early morning (dawn) when the stomata remain open for a continued uptake of atmospheric CO_2 used in malic acid synthesis and/or the Calvin cycle. Phase III includes most of the daytime when the stomata are closed and storage malic acid is decarboxylated to

supply carbohydrate production by Rubisco in the Calvin cycle. Phase IV happens in late afternoon (dusk) when malic acid storage is exhausted, the stomata are open and atmospheric CO_2 uptake is immediately used in the Calvin cycle (Osmond, 1978). Based upon the major carbohydrate reservoirs used in their daily cycle, CAM plants are divided into two groups: starch-formers and extrachloroplastic carbohydrate-formers. In starch-former CAM plants, malic acid is decarboxylated by NAD(P)-ME and generates pyruvate with CO_2, while in extrachloroplastic carbohydrate-forming CAM plants, oxaloacetic acid produced from malic acid is decarboxylated by PEP carboxykinase, and generates PEP with CO_2 (Chen et al., 2002). Carbonic anhydrase is an ubiquitous enzyme among living organisms that catalyzes the reversible inter-conversion of HCO_3^- and CO_2: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. It represents 1-20 % of total soluble proteins in leaves and its abundance is next only to Rubisco, facilitating CO_2 supply to phosphoenolpyruvate carboxylase in C4 and CAM plants and Rubisco in C3 plants.

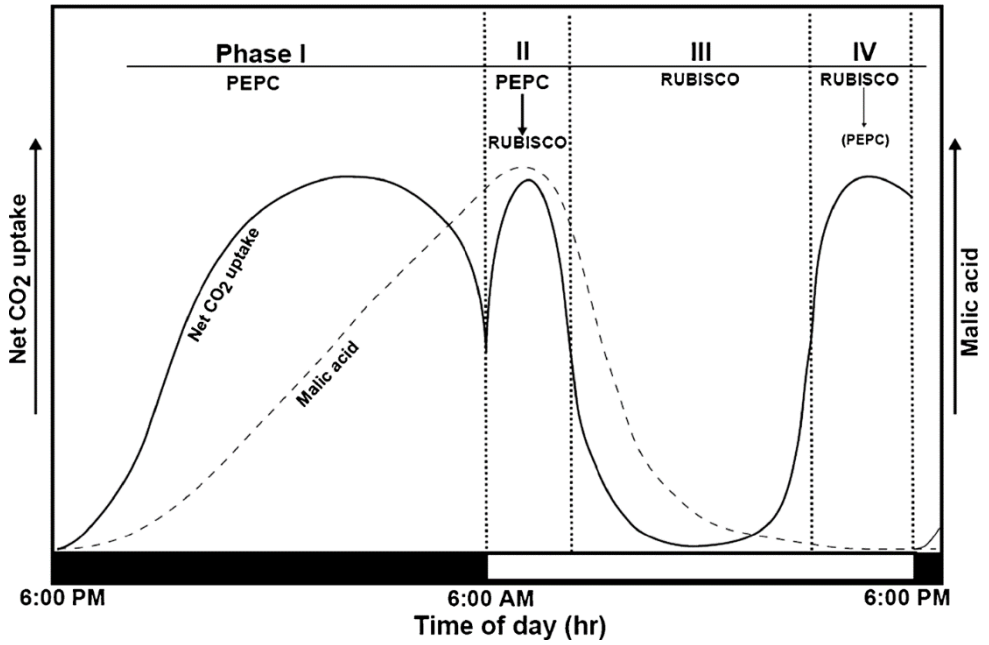

Figure 1.6 The four phases of CAM: net carbon uptake (solid line) displayed with malic acid storage (dashed line) (Bartlett et al., 2014; Osmond, 1978).

1.5 Chlorophyll fluorescence

Light energy absorbed by chlorophyll molecules not only drives photochemistry (photosynthesis), but it can also be lost as heat (thermal dissipation), or re-emitted as light (chlorophyll fluorescence) (Figure 1.7). These three processes occur in competition, such that any increase in the rate of one process will result in a decrease of the other two (Maxwell and Johnson 2000, Murchie and Lawson 2013). Thus, the yield of chlorophyll fluorescence emission gives valuable information about the quantum efficiency of photochemistry and heat dissipation (Baker, 2008).

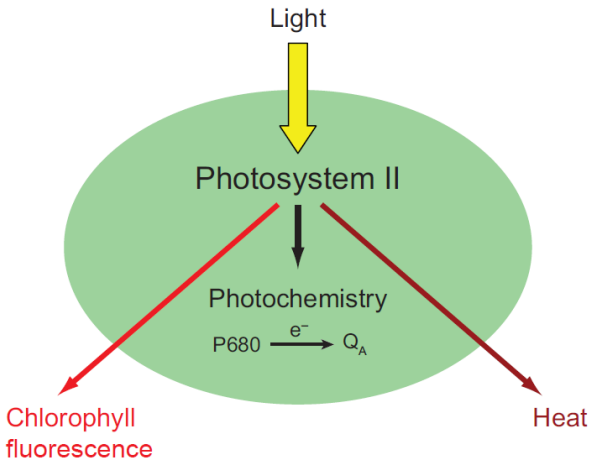

Figure 1.7 A simple model of the possible fate of light energy absorbed by Photosystem II (PSII) (Baker, 2008).

Since the first experiments with chlorophyll fluorescence that were carried out by Kautsky and Hirsch (1931) chlorophyll fluorescence became a rapid, non-destructive and convenient technique that is widely used in the evaluation of higher plant photosynthetic activity (Murchie and Lawson, 2013). It is useful in understanding the physiological performance of plants, and is an indicator of plant responses to ambient environment and stress condition (Murchie and Lawson, 2013; van Kooten and Snel, 1990).

Analyses of the chlorophyll fluorescence quenching kinetics induced in photosynthetic systems by exposure to light have provided considerable qualitative information of the photosynthetic apparatus (Genty et al., 1989) (Figure 1.8). Plenty of fluorescence parameters are calculated which give information about changes in

the efficiency of photochemistry and heat dissipation (Maxwell and Johnson 2000, Murchie and Lawson 2013).

Specifically, the F_v/F_m, where F_v is the difference between F_m (maximal fluorescence in the dark) and F_0 (minimal fluorescence in the dark), provides an estimate of the maximum photochemical efficiency of PSII. This parameter is widely used as a stress indicator when plants are exposed to different and/or stressful conditions. qP (photochemical quenching) is another widely used fluorescence parameter, which gives an indication of the proportion of opening of PSII reaction centers: $F_v/F_m = \Phi_{PSII}/qP$. NPQ (Non-photochemical quenching) is calculated from $(F_m-F_m')/F_m'$, it measures a change in the efficiency of heat dissipation relative to the dark-adapted state. Regarding to the fractions of fluorescence quantum yield, Φ_{PSII} indicates for the quantum yield of Photosystem II, it measures the proportion of light absorbed by chlorophyll associated with PSII that is used for photochemistry. Φ_{NPQ} (the quantum yield of non-photochemical quenching) and Φ_{NO} (the yield of non-regulated energy dissipation) reflect the regulated thermal energy dissipation related to non-photochemical quenching (NPQ) and the non-light induced quenching processes, respectively (Kramer et al. 2004), The sum of all yields for dissipative processes for the energy absorbed by PSII is unity: $\Phi_{PSII}+\Phi_{NPQ}+\Phi_{NO}=1$.

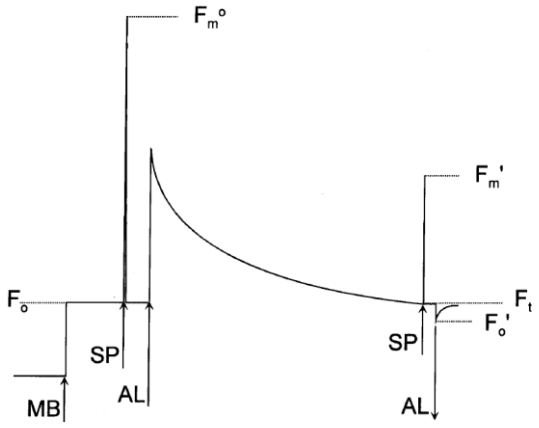

Figure 1.8 Fluorescence quenching analysis using modulated fluorescence (Maxwell and Johnson, 2000). Dark-adapted leaf is exposed to various light treatments. A measuring light (MB) is switched on to measure the zero fluorescence level (F_0). Then, a saturating flash of light (SP) is applied to obtain the maximum fluorescence (F_m). Actinic light (AL) is then applied followed by another saturating light flash (SP) after a period of time to allow the measurement of the maximum fluorescence in light (F_m'). The fluorescence level immediately before the saturating flash is termed F_t. The actinic light (AL) is turned off, typically in the presence of far-red light, to allow the estimation of the zero level fluorescence in light (F_0').

1.6 Photoreceptors

Light not only acts as an energy source for photosynthesis, but also affects virtually all aspects of plant growth and development from germination to aspects of vegetative morphology, reproductive growth and floral initiation, entrainment of circadian rhythms and phototropism (Ahmad, 1999). These responses are initiated by photoreceptors that are sensitive to specific wavelengths. It is through these photoreceptors that plants sense the light quality, intensity, direction, and duration (Barnes et al., 1996; Fankhauser and Chory, 1997) and further generate different responses. The most important photoreceptors identified so far include the phytochromes (phy) which absorb primarily in the red/far-red (600-800 nm wavelength) region of the spectrum (Furuya and Schäfer, 1996; Rockwell et al., 2006), the specific blue/UV-A light absorbing photoreceptors (350-500 nm) are the cryptochromes (cry) and phototropins (phot) (Briggs and Christie, 2002; Cashmore et al., 1999), and the UV-B absorbing photoreceptor UVR8 (Rizzini et al., 2011) (Figure 1.9).

In response to the complex light environment, plants employ multiple photoreceptor systems in monitoring light signaling, and regulating plant behavior. For example, phytochrome and UVR8 cooperate to optimize plant growth and defense in patchy canopies (Mazza and Ballaré, 2015). phyA or phyB are also involved in some responses to blue light in coordination with cryptochrome (Shinomura et al., 1996). The repression of hypocotyl gravitropism in response to very low irradiance blue light (0.1–0.7 μmol m^{-2} s^{-1}) is under the control of phyA in *Arabidopsis* (Lariguet and Fankhauser, 2004). Also phyA irreversibly triggers the seed germination upon irradiation under extremely low irradiance UV-A and blue light while phyB controls the photoreversible effects of low fluency (Shinomura et al., 1996).

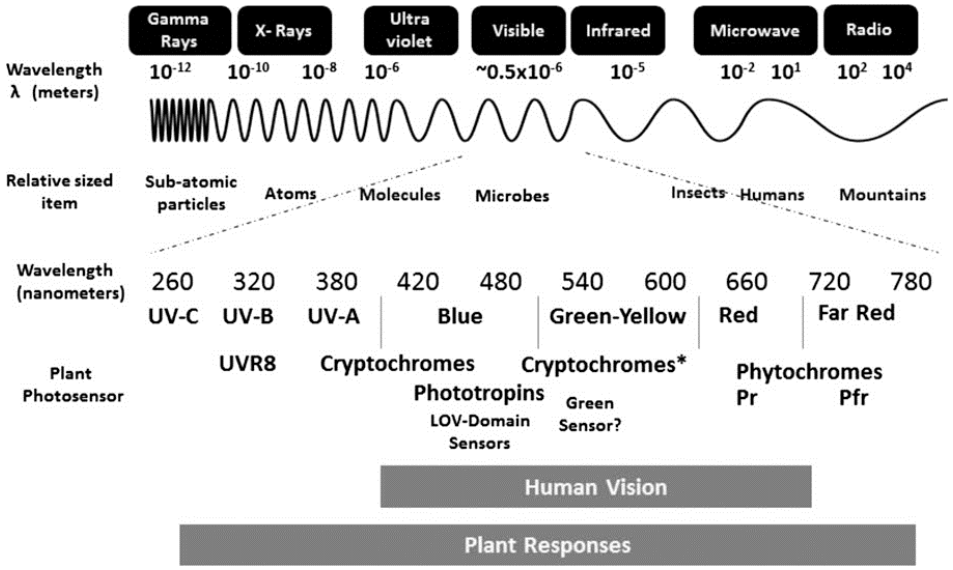

Figure 1.9 The relative distribution of electromagnetic energies and the wavelengths that discretely interact with plant photoreceptors (Folta and Carvalho, 2015).

1.6.1 Phytochrome

Phytochromes were the first identified light-sensing molecules and by far the most studied photoreceptors in plants. Phytochromes are encoded by the *PHYA-PHYE* small gene family in most plant species (Quail, 1997; Rockwell et al., 2006) and control processes during the entire plant life cycle (Kendrick and Kronenberg, 1994). There are five types of phytochromes (phyA, phyB, phyC, phyD, and phyE) currently identified in the dicot model plant *Arabidopsis* (Fankhauser and Chory, 1997) and three types (phyA, phyB and phyC) in the monocot model plant rice (Gu et al., 2011). Phytochromes can be classified into two groups based on their stability: type I (light-labile) phytochrome degrades rapidly on exposure to red or white light which includes phyA, and type II (light-stable) phytochrome that does not degrade rapidly which includes phyB to phyE (Quail, 1997). All plant phytochromes contain two domains: a N-terminal domain and a C-terminal domain. The N-terminal domain can be artificially divided into four subdomains P1, P2, P3 (also known as GAF) and P4 (also known as PHY); and the C-terminal domain can be divided into PAS-A, PAS-B and HKRD subdomains (Bae and Choi, 2008).

Phytochromes influence plant developmental performance including responses as gravitropism, phototropism and the shade-avoidance response (Brouwer et al., 2014; Smith, 2000). There are two phytochrome forms: the red light absorbing form (P_r) which is inactive and the far-red light absorbing form (P_{fr}) which is active. Photo-transformation between these two forms happens when exposed to either red or far red light, illuminating dark-grown tissues with red light converts phytochrome from P_r form to P_{fr} form, reversibly, with far red light illumination restoring P_r (Holmes and Smith, 1975; Quail, 1997), which is associated with a structural conformational change as well as corresponding changes in the absorption peaks between 666 nm (P_r) and 730 nm (P_{fr}) (Sullivan and Deng, 2003) (Figure 1.10). Photo-transformation between the P_r and P_{fr} forms is efficiently achieved by red light, but also by other wavelengths ranging from UV (300 nm) and blue to far-red (800 nm) even though it is far less efficient (Shinomura et al., 1996).

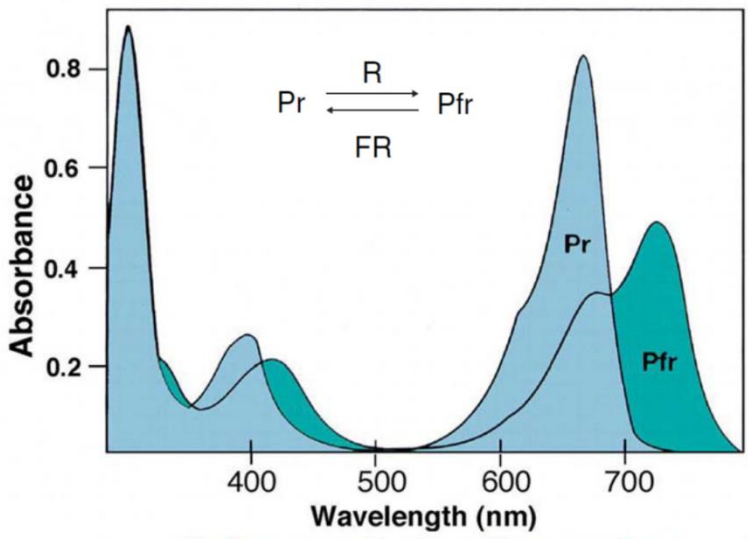

Figure 1.10 Absorption spectra of the two forms (P_r and P_{fr}) of phytochromes, adapted from Wang (2005). The P_r form absorbs maximally at 660 nm, while the P_{fr} form absorbs maximally at 730 nm.

1.6.2 Cryptochrome

It has long been known that plants show biological blue light responses (Cashmore et al., 1999; Lin, 2000) before the gene coding cryptochromes were isolated (Ahmad and Cashmore, 1996; Gressel, 1979). The first sequence of a blue-light receptor,

Cryptochrome1 (CRY1) was published in 1993 (Ahmad and Cashmore, 1993). Cryptochromes are flavin-type photoreceptors that perceive UV-A and blue light with two wavelengths optima (370 and 450 nm). Cryptochromes in *Arabidopsis* genome have three subfamilies: CRY1, CRY2 and CRY3 (Kleine et al., 2003; Lin and Shalitin, 2003). Perrotta et al. (2000) identified two CRY1 members (LeCRY1a and LeCRY1b) and one CRY2 member (LeCRY2) in tomato. Monocot rice possesses four CRY genes, OsCRY1a, OsCRY1b, OsCRY2 and OsCRY-DASH (Hirose et al., 2006). Most plant cryptochromes have two domains, an N-terminal photolyase related (PHR) domain that shares sequence homology to DNA photolyase, and a C-terminal extension that is unrelated to photolyase. The PHR domain of cryptochrome is the chromophore-binding domain, whereas the C-terminal extension is important for the nuclear/cytosol trafficking and protein-protein interactions (Lin and Shalitin, 2003).

Cryptochromes regulate many physiological and developmental processes such as photomorphogenesis (plant height and apical dominance), flowering-time control, circadian clock regulation, guard cell development, chlorophyll biosynthesis, programmed cell death, the high-irradiance stress response and seed dormancy (Sullivan and Deng, 2003; Wang et al., 2014; Yu et al., 2011). Upon absorption of photons, cryptochromes are believed to be photo-excited by a mechanism involving electron transfer and flavin reduction (Chaves et al., 2011; Liu et al., 2010). It is generally clear that cryptochromes mediate light-dependent physiological responses by modulating gene expression through interactions with signal proteins. In *Arabidopsis*, approximately 5–25% of genes change their expression in response to blue light and most of these changes are mediated by CRY1 and CRY2 (Liu et al., 2012; Ohgishi et al., 2004) and also the recent finding of CRY3 (Kleine et al., 2003). CRYs mediate blue light control of gene expression via at least two mechanisms: light-dependent modulation of transcription (e.g., the CRY-CIBs pathway) and light-dependent suppression of protein degradation (the CRY-SPA1/COP1 pathway) (Liu et al., 2012).

1.6.3 Phototropin

Another distinct class of photoreceptors that mediates the effects of UV-A/blue light (320-500 nm) are the phototropins. The phototropin protein is likely to be ubiquitous in higher plants, ranging from 114 to 130 kDa, depending upon the species (Briggs

and Huala, 1999). Phototropins mediate phototropic responses to blue light, UV-A or even green light (Wang et al., 2013). *Arabidopsis* has two phototropins designated phot1 and phot2 (Briggs and Christie, 2002). Phototropin contains two LOV domains (LOV1 and LOV2), which are found in proteins regulating responses to light, oxygen, or voltage. Each of the LOV domains binds a flavin mononucleotide (FMN) as a chromophore to make the holoprotein. Both FMN molecules undergo a photocycle: light activation leads to the formation of a cysteinyl adduct with the FMN, an adduct that breaks down on a time scale of minutes in subsequent darkness (Briggs, 2001).

Phototropins control a wide range of plant responses such as stomatal opening, phototropism (bending toward light), chloroplast movement (Briggs and Christie, 2002), leaf flattening (de Carbonnel et al., 2010), and de-etiolation of the hypocotyl (Casal, 2000).

1.6.4 UVR8

UVR8 is a seven-bladed β-propeller protein originally identified in a screen for *Arabidopsis* mutants hypersensitive to UV-B light (Kliebenstein et al., 2002; Rizzini et al., 2011). The UVR8 protein is localized in both the cytoplasm and the nucleus. Its abundance is unaffected by UV-B or other light qualities (Heijde and Ulm, 2012), UV-B irradiation promotes its accumulation in the nucleus (Brown et al., 2005; Kaiserli and Jenkins, 2007), which is due to redistribution of UVR8 in the cell but not to increased abundance (Kaiserli and Jenkins, 2007). Nuclear accumulation of UVR8 occurs very rapidly (within minutes) and at low fluence rates (Kaiserli and Jenkins, 2007). In the nucleus, UVR8 was shown to associate with the chromatin of UV-B-responsive genes, such as the promoter region of ELONGATED HYPOCOTYL 5 (HY5), suggesting that UVR8 may be directly involved in the transcriptional regulation of its target genes (Brown et al., 2005; Li et al., 2013b). Transcriptome analysis revealed that UVR8 regulates a range of genes with important roles in UV protection and the repair of UV damage (Brown et al., 2005).

1.7 Impact of light quality on plants

Plant productivity not only depends on light quantity through photosynthetic activity from which carbohydrates and oxygen are synthesized from carbon dioxide and water using the energy of light. The qualitative characteristics of light also strongly

influence many aspects of the plant physiology, including growth, morphology, physiology and phytochemical composition.

1.7.1 The influence of light quality on plant growth

LED lighting systems are able to provide multiple light spectra for horticultural production. As described above, red in combination with blue light are being implemented in horticultural production. The benefits of additional blue photons in plant growth have been demonstrated in numerous studies. Goins et al. (1997) found that although wheat plants could complete their life cycle under solo red light, additional blue light induced larger plants with a greater number of seeds and more dry matter. In the production of leafy vegetables such as lettuce, radish, and spinach the combined red and blue light was beneficial for producing more biomass (Yorio et al., 2001). In fruit production, Samuolienė et al. (2010) reported that blue with red light resulted in bigger fruits with higher sugar contents in strawberries while red light alone inhibited the strawberry flowering (Yoshida et al., 2012). Though the necessity of blue light is commonly accepted, there is less consensus regarding the optimal red and blue ratio. There are much species and genotype depend reactions to the ratio of red and blue light. For example, in lettuce, the leaf photosynthetic capacity and photosynthetic rate increases with decreasing R/B ratio which was associated with increasing stomatal conductance, along with increase in stomatal density and shoot dry weight (Wang et al., 2016). However, Son and Oh (2013) reported a decrease of growth rate in lettuce cultivars with an increase of blue and UV-A light, which might be due to a difference in genotype. In leafy and fruit crops (sweet basil and strawberry), the most suitable spectra was found to be a R/B ratio of 0.7 based on a range of analyses (morphological, physiological and biochemical elements) (Piovene et al., 2015). Rapeseed growth rate increases with a higher blue light percentage this in the range from 0% to 75% (Li et al., 2013a). Folta and Childers (2008) observed the greatest growth of strawberry plants under 34% blue light. Furthermore, another disagreement is whether monochromatic blue light exposure is positive or negative for plants despite the necessity for normal development. At short time interval blue wavelengths are less efficient in driving photosynthesis than red wavelengths (Sager and McFarlane, 1997), because blue light is also absorbed by flavonoids in vacuoles and/or non-photosynthetic pigments, such as anthocyanins, in chloroplasts (Terashima et al., 2009). Certain reports described lower photosynthetic rates and

biomass accumulation under monochromatic blue than under a R/B combination or under broad spectrum light (Wang et al., 2009, 2016; Yu and Ong, 2003). Other reports claimed that monochromatic blue induced the greatest biomass accumulation in *Platycodon grandiflorum* (Liu et al., 2014).

1.7.2 Improve crop morphology in ornamental plants

Spectral manipulation could maximize the biomass production; however, in ornamental production compact plants can be desired. Indeed, morphological quality might be negatively influenced if one only focuses on biomass accumulation. There are several ways to regulate plant morphology, including irrigation and electrical conductivity of irrigation solutions, altering temperature profiles as well as plant growth regulators (Davis and Burns, 2016). Nevertheless, the ability to control the light spectrum with LEDs provides the possibility to optimize the plant morphology without chemical intervention (Folta and Childers, 2008).

Research reports on the photomorphogenic responses of blue light are ample. Blue light is known to inhibit stem elongation in many species, such as *Chrysanthemum* and *Tripterospermum*. Stem elongation decreases as the proportion of blue light increases (Heung et al., 2006; Zhiyu et al., 2007), and thus blue light might be used in plant cultivation instead of growth retarding chemicals (Shimizu et al., 2006). Referring to the previous investigations, *Poinsettias* grown under 80% red: 20% blue supplemental LED lighting were 20-34% shorter than those grown under HPS (5% blue) lamps (Islam et al., 2012). The addition of red light in the spectrum provided the greatest effect on reducing plant height of roses and *Chrysanthemum*s (Ouzounis et al. 2014), which is mediated by changing the R/FR ratio. Reducing far-red light with spectral filters could have a similar influence on plant morphology. Differences between plant morphological responses to red/far-red and blue light are associated with differences in the relative contributions of phytochromes and blue-sensitive photoreceptors (cryptochromes and phototropins) to the inhibition of stem extension. The R/B ratio, yet important, is not solely sufficient to control plant morphology. Light intensity is also crucial; the absolute blue light intensity rather than the percentage of blue light controlled hypocotyl length and stem extension in tomato (Nanya et al., 2012).

The quality of light also has a strong influence on leaf morphology, with light treatments causing leaves to become curled in many reports (Fukuda et al., 2008; Higuchi et al., 2012; Hughes, 2013; Ouzounis et al., 2014). This was mainly studied in vegetables and not in ornamentals. In tomato, leaf lamina thickness was significantly reduced in R:B leaves, whereas in the oriental plane leaf lamina thickness was significantly higher in R:B than in control leaves (Arena et al., 2016).

1.7.3 Stomatal morphology and stomatal conductance

There is a common agreement that blue/UV-A light triggers the movement of guard cells through cryptochrome and phototropin thus promoting the opening of stomata and generating a higher stomatal conductance. The stomatal opening under red light is mainly caused by the decreased intercellular CO_2 concentration which is the result of red light driven mesophyll photosynthetic activity (Shimazaki et al., 2007), hence the red light response of stomata requires a high light intensity. However, recent reports suggest that PHYB plays an essential and direct role in inducing the stomatal opening in response to red light, and PHYA might also participate in this regulation (Wang et al., 2010a).

Light also affects the stomatal development, in general, an increase in light intensity results in an increase in stomatal index (Lake et al., 2001). Stomatal development can also be influenced by UV-B light, soybean plant produced fewer stomata after UV-B exposure, which improves drought tolerance and photosynthetic performance (Gitz et al., 2005). Although stomata routinely open and close in response to light to regulate water use and CO_2 uptake, any influence of light quality on the development and density of stomata during leaf growth will have long-term impacts on stomatal conductance, photosynthetic performance, and water use efficiency (Yu et al., 2011).

1.7.4 Photosynthesis

The role of the photon flux density on photosynthesis has been studied in an array of plant species, resulting in light dependent photosynthetic response curves both at leaf and plant level. The extent of which light quality effects photosynthesis is less studied though it will have consequences due to the specific absorption spectrum of photosynthetic pigments (see 1.2) or the higher or lower absorption of micronutrients

essential to the photosynthetic electron transport chain (Korbee et al., 2005; O'Carrigan et al., 2014b).

The short term response to light quality in photosynthetic CO_2 fixation is wavelength dependent and changes rapidly (Evans, 1987). If one considers the wavelength dependent quantum yields then red wavelengths always result in the highest yields (Evans, 1987; McCree, 1971), while there is a reduction for blue wavelengths due to the partial absorption of these wavelengths by non-photosynthetic pigments (Terashima et al., 2009). However long term (hours to days) application of red wavelengths could result in imbalances between the two Photosystems and would in turn reduce the quantum yield. Thus red wavelengths associate with the highest quantum yield in a short term scale but not in a higher plant production yield. Indeed monochromatic wavelengths are unnatural light conditions for plants and reduce the photosynthetic activity in comparison with white light (Abidi et al., 2013; O'Carrigan et al., 2014b). Therefore, a combination of dual wavelengths is generally used/proposed in plant production systems (see 1.4.1). When exposing plants to supplementary blue light in a background of natural light, the photosynthetic activity increases with the increasing blue photon proportion (Hogewoning et al., 2010b; Wang et al., 2016; Yorio et al., 2001).

Beyond the direct photosynthetic activity, light quality regulates many other physiological aspects that will in turn affect the photosynthetic efficiency. For example, blue light was suggested to have a higher efficiency than red light in inducing stomatal opening in C3 plants (O'Carrigan et al., 2014b) which is mediated by cryptochrome (Shimazaki et al., 2007) (also in 1.4.3). Blue spectra also affect the relocation of chloroplasts within the cells, which influences the light capture (Suetsugu and Wada, 2007). Chloroplasts accumulate at the cell surface to maximize light capture and their photosynthetic ability in response to low fluence blue light. In contrast under higher fluence blue, chloroplasts move to the opposite side to avoid photodamage (Kami et al., 2010; Kasahara et al., 2002). This movement is mediated by the phototropins (Kong et al., 2013; Takemiya et al., 2005).

Long-term exposure to a specific light composition could modify the leaf anatomy and orientation and chloroplast characteristics, thus indirectly also affecting photosynthesis. Blue light was reported to be beneficial for chlorophyll accumulation

as well as increasing the Chl a/b ratio (Kurilčik et al., 2008; Matsuda et al., 2008; Poudel et al., 2008; Tanaka and Tanaka, 2006; Yorio et al., 2001). Otherwise there are also reports that monochromatic blue light decreased chlorophyll content while in certain species no effect was found (Abidi et al., 2013; Wang et al., 2015). At molecular level blue light upregulates the gene expression of MgCH, GluTR and FeCH, enzymes involved in the chlorophyll biosynthesis (Wang et al., 2009) and hence promotes chlorophyll accumulation (Kurilčik et al., 2008; Poudel et al., 2008). In contrast, red light is not conducive to the formation of chlorophyll, because of the reduction in tetrapyrrole precursor 5-aminolevulinic acid (Sood et al., 2005; Tanaka and Tanaka, 2006). Leaf thickness, stomatal density and palisade tissue cell length are increased under blue light as compared to plants grown under red or green light (Korbee et al., 2005; Wang et al., 2015). The epidermal cell area of birch leaves is larger and the functional area of chloroplasts (starch-free part of the chloroplast) is greater in plantlets grown under blue light than in plantlets grown under white or red light (Sæbø et al., 1995). In cucumber grown under low radiations, chloroplasts under blue light have a higher number of grana lamellae and more stacked thylakoid membranes than under white or red light (Wang et al., 2014). Through effects on leaf area, leaf orientation and branching, light quality composition can influence light capitation and thus indirectly affect photosynthesis at whole plant level.

1.7.5 Pigmentation and secondary metabolites

The primary metabolites are directly involved in growth, development, and reproduction. Yet, plants produce many other compounds, known as secondary metabolites, which act to improve the fitness of an organism and help it acclimate to changeable environments (Lambers et al., 2008). The production of secondary metabolites is influenced by many environmental factors including light (Shohael et al., 2006).

Ornamentals with different colored leaves or flowers are distinctive and desirable, thus maximizing pigmentation is important during cultivation. The coloration of leaf, flower or fruit is mainly provided by the accumulation of flavonoids (including anthocyanidins), carotenoids and betalains (Mol et al., 1998). Flavonoid synthesis is sensitive to light quality, shorter wavelength, in the range of blue and UV light show the most prominent effect in accumulation of flavonoids by upregulating the

expression of its pathway genes (Zoratti et al., 2014). Blue light via the cryptochromes and phototropins (Kadomura-Ishikawa et al., 2013; Ninu et al., 1999) drives the synthesis of anthocyanin. Supplementary blue light increases the anthocyanin and carotenoid concentration while supplemental far-red decreased anthocyanins, carotenoids and chlorophyll concentration compared to those in the white light control of lettuce (Li and Kubota, 2009). Carotenoid concentration was found to be greater in buckwheat seedlings grown under white light compared to those grown with 100% blue or red light (Tuan et al., 2013). The chlorophyll pigments mainly contribute to the green leaf color. Light quality effects the biosynthesis of chlorophyll, blue light is known to promote the accumulation of chlorophyll (see above 1.4.4).

Photosynthesis inevitably generates reactive oxygen species (ROS), from the electron transport activities of chloroplasts though also electron transport in mitochondrial respiration induces ROS. Environmental stress will enhance this ROS production. Secondary compounds, such as carotenoids, phenolic compounds, tocopherols, ascorbate, and glutathione are active in scavenging the redox stress (Nisar et al., 2015). Phenolic acids and flavonoids are among the most ubiquitous groups of secondary metabolites in the plant kingdom and represent an example of metabolic plasticity enabling plants to adapt to biotic and abiotic environmental changes (Cheynier et al., 2013). They are hypothesized to function as direct antioxidants (Cheynier et al., 2013), most flavonoids outperform well-known antioxidants, such as ascorbate and a-tocopherol (Hernández et al., 2009). Jeong et al. (2012) investigated the influence of LEDs on polyphenol biosynthesis in the leaves of *Chrysanthemum* and characterized nine polyphenols. They were either highest when supplemented with green or red light, while blue and white was inefficient for polyphenol production.

Secondary metabolites can also be important as nutraceutical compounds. Blue light was found to increase the oil content of basil leaves compared to white light treatments (Amaki et al., 2011). Also light intensity influences the biosynthesis of the secondary metabolites. Manukyan (2013) indicated that increasing PAR led to an increase in production of secondary metabolites. It is therefore important to provide plants with sufficient light to drive photosynthesis as this provides the metabolic

building blocks for the various biosynthetic pathways as well as stimulates the biosynthetic pathways to maximize production of desirable compounds.

1.8 Scope and outline

In the ornamental sector, there is a growing interest in the use of LED lighting as supplementary lighting. Furthermore, vertical farming systems, applying only artificial light, might be interesting for the production of seedlings (bedding plant industry), rooted cuttings (*Chrysanthemum*, pelargonium, poinsettia, azalea, woody ornamentals) or young plants (acclimation phase after the micropropagation of many pot plants) as these systems allow a more efficient use of space.

The overall objective of this thesis is to obtain insight in morphological and physiological responses to different light spectra and especially to the blue and red light responses in ornamental species. To understand these responses, we selected several species differing in their photosynthetic pathway (C3 and CAM), leaf morphology (deciduous, evergreen) and belonging to the two groups of angiosperms, namely monocots and dicots. We compared the plant reactions to monochromatic or dichromatic wavelengths in comparison to their reaction to multispectral wavelengths.

Chapter 1 summarizes the background of the application of light quality in ornamental cultivation and gives an overview of the light quality effects from morphological to physiological responses related to photosynthesis.

Chapter 2 examines the effect of light quality at two light intensities on leaf anatomy and morphology, photosynthetic efficiency and pigmentation of *Chrysanthemum* leaves. The selected light intensities were based on the lower and upper range of applied supplementary lighting in ornamental production (40 µmol m^{-2} s^{-1} and 100 µmol m^{-2} s^{-1}). We combined these two light levels with four light quality regimes, namely monochromatic red, monochromatic blue, dichromatic red+blue and a multispectral light source.

Chapter 3 studies the long-term plant effects under different wavelengths and evaluates the influence of narrow-band R, B and RB on leaf anatomy, stomatal traits and stomatal conductance, leaf hydraulic conductance (K_{leaf}) and photosynthetic efficiency and their potential relation in three ornamental pot plants, namely *Cordyline*

australis (monocot), *Ficus benjamina* (dicot, evergreen leaves) and *Sinningia speciosa* (dicot, deciduous leaves).

Chapter 4 investigates the effect of light quality on leaf morphology, photosynthetic efficiency and antioxidant capacity of leaves that fully developed under a specific spectrum in *Chrysanthemum* cultivars. We investigated if light quality affected ROS generation and as a result differentially induced non-enzymatic antioxidants by determining carotenoids, proline, total polyphenols and flavonoids. As responses to light quality differ greatly between species but inter-species effects are hardly studied we evaluated 8 cultivars with a cushion type *Chrysanthemum* phenotype to obtain information of potential intraspecific variation.

Chapter 5 focuses on how light quality might affect CAM metabolism. We chose *Phalaenopsis* as experimental plant, which is an obligate CAM plant. Both short time and long-term effects of different light spectra on the diel rhythm of the CO_2 uptake and malate content, carbohydrate content as well as the chlorophyll fluorescence diel changes are investigated.

Chapter 6 investigated the greenhouse acclimation of ornamental young plants (*Chrysanthemum*, a sun species and *Spathiphyllum*, a shade species) that developed for four weeks under a specific light spectrum at 100 µmol m^{-2} s^{-1}. The change of low light intensity under narrow spectral treatments to the dynamic greenhouse environment with high light intensities in summer will result in a light stress. We investigated if certain light spectra were more beneficial to support this light stress. We approached this mainly with a chlorophyll fluorescence quenching analysis and determination of effects of light quality and subsequent acclimation on the characteristics of the chlorophyll fluorescence rapid light curve. In addition, the long-term effect on biomass was evaluated.

Chapter 7 gives a general discussion of the experimental chapters and includes some future prospects and recommendations.

Chapter 2

Effects of different irradiation levels of light quality on *Chrysanthemum*

This chapter is based on:

Zheng L. and Van Labeke M.C. Effects of different irradiation levels of light quality on *chrysanthemum*. Submitted to Scientia Horticulturae. Under review

Author contribution:

LZ and MCVL conceived and designed the experiment. LZ performed the experiments, analyzed the data and drafted the manuscript, MCVL critically revised the manuscript.

Abstract

The effect of light quality at two light intensities on leaf anatomy, photosynthetic efficiency and pigmentation were investigated in *Chrysanthemum*. Four light qualities were applied at two light intensities of 40 µmol m^{-2} s^{-1} and 100 µmol m^{-2} s^{-1} and with a photoperiod of 14 hours using light-emitting diodes, which were 100% red (R), 100% blue (B), 75% red with 25% blue (RB) and white (W), respectively.

Leaf anatomy responses to light intensity were observed, under 40 µmol m^{-2} s^{-1} leaf thickness decreased for blue, red+blue and multispectral white light in comparison to 100 µmol m^{-2} s^{-1}. At higher light intensity, we also observed a favorable effect of blue light on the anatomical development of the leaves. Both light intensity and quality affected the stomatal development. Low light decreased the stomatal index and stomatal density but increased in the stomatal area for red+blue and multispectral white light. Light intensity affected the pigment accumulation but no quality effects were present. For the lowest light level, an enhanced pigment concentration was observed in *Chrysanthemum* this as well for Chl a, Chl b and total carotenoids. Light quality influenced the photosynthetic efficiency as observed by chlorophyll fluorescence. Monochromatic red resulted in negative effect on Photosystem II, this at both light intensities, resulting in a decline in maximum quantum yield (F_v/F_m) and quantum efficiency (Φ_{PSII}). Light intensity significantly influenced biomass accumulation, higher light intensity increased plant dry weight. At a light intensity of 100 µmol m^{-2} s^{-1}, blue light positively influenced the biomass compared to monochromatic red.

2.1 Introduction

Bedding plants and pot *Chrysanthemum* are typically propagated when natural light intensities are low, namely during winter and early spring. Supplementary lighting is often applied to enhance the quality of the rooted cuttings and seedlings. Both daylength extension as well as supplementary lighting in a background of natural light might be applied. Typically, high-pressure sodium lamps are used in the horticultural sector though there is an increasing interest to apply LED-lighting. Furthermore, there is a growing interest in vertical farming systems as these allow a more efficient use of space in young plant production. In vertical farming or multilayer production, initially fluorescent lamps were applied but sole-source LED lighting offers many possibilities to control plant morphology and architecture. Light emitting diodes (LEDs) attracted much attention as an alternative light source due to its high photoelectric conversion efficiency, narrowband spectral distribution, low thermal output and adjustable light intensity. Another potential advantage of LEDs is the ability to select light qualities and intensities that have beneficial effects on plant growth and photomorphogenesis for a targeted plant response (Goto, 2012; Tennessen et al., 1994).

Plants capture light not only as an energy source for photosynthesis and the building of carbon-based material but also as an environmental signal, with responses to light intensity, wavelength, duration and direction. Light is perceived by photoreceptors such as the red/far-red light-absorbing phytochromes and the UV-A/blue absorbing cryptochromes and phototropins. Plants generate a wide range of specific physiological responses through these photoreceptors (Vollsnes et al., 2012). Plants are able to adjust their anatomy and morphology as well as their physiological and biochemical responses to variations in the ambient light environment (Abreu et al., 2014; Causin et al., 2006; Kamiya et al., 1983; Tallman and Zeiger, 1988; Zheng and Van Labeke, 2017a). This is well known in natural environments. Shade is a common phenomenon where lower light intensity goes together with higher far red/red ratios. In response to these changes in light availability, shade leaves adapt to lower photosynthetic capacity (light-saturated rate of photosynthesis on a leaf area basis), smaller leaf thickness and nitrogen content than sun leaves (Murchie and Horton, 1997). Plants have thus developed sophisticated mechanisms to adapt to the light

environment, ranging from diverse aspects of morphology and physiology to anatomy, developmental and reproductive timing and offspring developmental patterns (Muneer et al., 2014; Sultan, 2000). Various plant characteristics, such as leaf area, number of branches and water content (fresh and dry weight difference) are influenced by light, which were documented in numerous species with respect to various light environments (Hogewoning et al., 2010b; Jeon et al., 2005; Pan and Guo, 2016).

To optimize the ornamental young-plant production in artificial light environments, it is important to understand the responses of a specific species to light quality at a given light intensity. In the past, light quality research was often performed in a background of low natural light intensities thereby modulating the R/FR or the B/R ratio (Li and Kubota, 2009; Ouzounis et al., 2015b; Schuerger et al., 1997). In *Tagetes*, an often-used bedding plant, the stem length was higher under monochromatic blue light compared with fluorescence lamps, while for *Salvia*, plants supplemented with far-red increased their stem length while it was significantly inhibited under red light (Heo et al., 2002).

However, a limited number of studies have been investigating the effects of narrow band spectral light qualities on anatomical responses and photosynthetic performance of ornamental young plants. Although LEDs represent an innovative artificial lighting source for vertical farming, the applied photon fluency will still be low in comparison to natural light. Photoreceptors such as phytochrome, phototropin and cryptochrome are not only important for plants sensing the light environment but also vital signaling pathways regulating many plant processes from germination, stem elongation, branching to flowering and fruit maturation (Sullivan and Deng, 2003). Low light intensities should saturate the reaction of photoreceptors but will not saturate the light conditions for photosynthesis. We selected two light intensities, namely low (40 µmol m^{-2} s^{-1}) and a control (100 µmol m^{-2} s^{-1}) irradiance. These light intensities are based on the lower and upper light levels applied in commercial ornamental productions during the winter months to extend the photoperiod. For both light intensities, we investigated the effect of red, blue, red+blue and multispectral white light. We selected *Chrysanthemum* as model plant and investigated the leaf anatomical adaptations to these light qualities with respect to the applied light

intensity. Next, we investigated how these anatomical adaptations influenced the photosynthetic capacity and biomass.

2.2 Materials and Methods

2.2.1 Plant materials and experimental set-up

The experiment was performed in a climate chamber at the Faculty of Bioscience Engineering, Ghent University. Rooted *Chrysanthemum* cuttings (*Chrysanthemum morifolium* 'Staviski'; Gediflora nv, Belgium) were planted in 0.3 L black plastic pots filled with peat-based substrate (Van Israel nv, Belgium). 16 replicates each treatment were randomly distributed to the treatment sections in the climate chamber. Air temperature was maintained at 22-24 ℃. Plants were irrigated and fertilized with water soluble fertilizer (N: P: K=4:1:2, EC=1.5 dS m^{-1}) twice a week.

Light treatments were two light intensity levels (40 and 100 µmol m^{-2} s^{-1}) with four different light qualities (R, B, RB and W) (Table 2.1). Plants received a photoperiod of 14 h. Light intensity and light spectrum distribution at canopy level was measured by a spectrometer (JAZ-ULM-200, Ocean Optics, USA) (Figure 2.1). Plants grew under the light treatments for 4 weeks, which is the equivalent time period of the rooting phase of 3 weeks followed by 1 extra growth week. All analyses were performed on the third and fourth fully expanded leaves with four biological replicates. Leaves at the same position on different branches of an individual plant were collected as one sample.

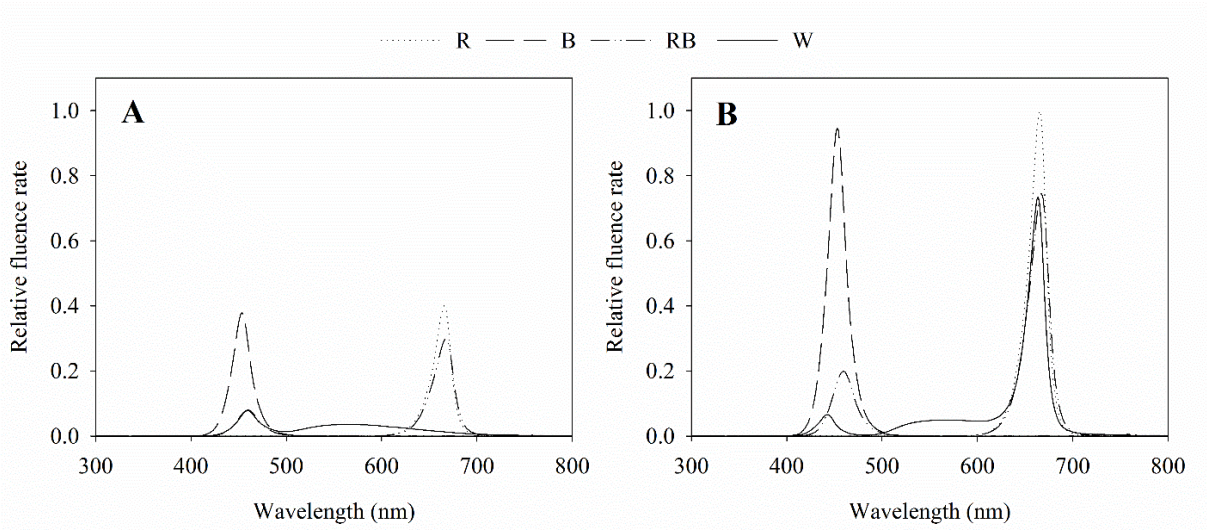

Figure 2.1 Relative fluence rate of the treatments at 40 µmol m^{-2} s^{-1} (A) and 100 µmol m^{-2} s^{-1} (B); R: red, B: blue, RB: red with blue and W: white. Spectrum was measured at canopy level with a JAZ spectrometer (Ocean optic, FL, USA).

Table 2.1 Overview of the light quality and intensity treatments in this experiment.

Light treatment	Wavelength	Light source	
		40 µmol m^{-2} s^{-1}	100 µmol m^{-2} s^{-1}
R	660 nm	Philips Affinium LED string, Philips, Eindhoven, The Netherlands	GreenPower LED production module, Philips, Eindhoven, The Netherlands
B	460 nm	Philips Affinium LED string, Philips, Eindhoven, The Netherlands	GreenPower LED research module, Philips, Eindhoven, The Netherlands
RB 75%/25%	460 nm + 660 nm	Philips Affinium LED string, Philips, Eindhoven, The Netherlands	CI-800 programmable LED system (CID Bio-Science, WA, USA)
W	400-800 nm	Philips Affinium LED string, Philips, Eindhoven, The Netherlands, Sole white LEDs	GreenPower LED production module (white with extra red LEDs), Philips, Eindhoven, The Netherlands
		%B = 30%	%B = 7%
		abs B = 12 µmol m^{-2} s^{-1}	abs B = 7 µmol m^{-2} s^{-1}

2.2.2 Leaf anatomy

The third fully expanded leaves were fixed with formalin-acetic acid-alcohol (FAA) [70% ethanol: formalin: acetic acid, 90:5:5 (v/v/v)], dehydrated using gradient ethanol and embedded with paraffin. After that, the paraffin embedded leaf samples were sectioned with a microtome (R. Jung AG, Heidelberg, Germany) at a thickness of 12 µm. The sections were deparaffinized with xylene and rehydrated with gradient ethanol, then stained with safranin for 30 min and fast green for 30 s and sealed with Canadian Balsam. Images of the section were taken with a bright-field microscope (IX81, Olympus Inc., Tokyo, Japan). Leaf thickness, palisade, spongy parenchyma thickness and epidermis thickness were analyzed with ImageJ software (ImageJ 1.48v, NIH, USA).

2.2.3 Stomatal characteristics and stomatal conductance

Stomatal characteristics were determined using a nail polish print method on the abaxial side of the third fully expanded leaf as described by Mott (1991). The nail polish layer was removed with a transparent tape and pasted on a glass slide, the slide was then observed with a bright field microscopy (IX81, Olympus, Tokyo, Japan) and stomatal density was calculated based on stomatal counts of 12 microscopic fields per leaf, ensuring a 95% confidence level of the results, as the number of stomata per mm^2. The stomatal index was calculated as number of stomata/(number of epidermal cells + number of stomata) × 100 (Kubinova, 1994). The stomatal aperture, width and length was defined as (Chen et al., 2012) and stomatal pore area was calculated by assuming an oval pore shape.

Stomatal conductance (g_s) was measured using a leaf porometer (AP4 porometer, Delta-T Devices, Cambridge, UK) on the third fully developed leaf. Four positions on the abaxial side of each leaf were measured and the average result was used as the stomatal conductance of this leaf.

2.2.4 Chlorophyll fluorescence

Leaf chlorophyll fluorescence was measured with a PAM-2500 portable fluorometer (Walz, Effeltrich, Germany). The third fully expanded leaf was dark adapted with a leaf clip for 20 min, then a 0.6s saturating light pulse (3,450 µmol m^{-2} s^{-1}) was given to obtain the minimal and maximal fluorescence yield (F_0 and F_m). Then, the leaf was

illuminated for 5 min with continuous actinic light (similar to the applied light intensity) with saturating pulse every 25 s, the maximum light adapted fluorescence (F_m') and steady state fluorescence (F_s) were recorded. After that, the actinic light was turned off and a far-red pulse was applied to obtain minimal fluorescence after the PSI excitation (F_0'). The maximum photochemical efficiency of PSII (F_v/F_m) was calculated using $F_v/F_m = (F_m - F_0)/F_m$; PSII operating efficiency (Φ_{PSII}) was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ according to Genty et al. (1989), the photochemical quenching (qP) was calculated as $qP = (F_m' - F_s)/(F_m' - F_0)$. The electron transport rate (ETR) was calculated as $ETR = \Phi_{PSII} \times PAR \times 0.84 \times 0.5$, where the absorbed photon energy (PAR) is assumed to be equally distributed between PSI and PSII and 0.84 is the assumed light absorbance of the leaf. Non-photochemical dissipation of absorbed energy (NPQ) was estimated as $NPQ = (F_m - F_m')/F_m'$ (Baker, 2008; van Kooten and Snel, 1990).

2.2.5 Pigments Content

Leaf chlorophyll content was determined according to Lichtenthaler (2001). 150 mg fresh leaf was grinded using liquid nitrogen and extracted in 80 % acetone overnight at -20 °C. Absorbance at 470 nm (A_{470}), 647 nm (A_{647}) and 663 nm (A_{663}) was measured with a spectrophotometer (Infinite 200, Tecan Group Ltd, Switzerland). The pigment content was calculated as Chl *a* = $12.25 \times A_{663} - 2.79 \times A_{647}$, Chl *b* = $21.50 \times A_{647} - 5.10 \times A_{663}$ and Carotenoids = $(1000 \times A_{470} - 1.82 \times$ Chl *a* $- 85.02 \times$ Chl *b*$)/198$.

2.2.6 Dry weight determination

Four plants per treatment were randomly sampled for the aerial biomass determination. Aboveground shoots were cut to determine its fresh weight (FW); oven dried at 85 ℃ for 72 h until a constant mass was reached and then dry weight (DW) was determined.

2.2.7 Statistical analysis

Data are reported as means ± SE. Results were analyzed using SPSS statistical software Version 24 (SPSS Inc., Chicago, USA), figures were made using SigmaPlot 13.0 (Systat Software, Inc, USA). Homogeneity of variance was verified with

Levene's test, analyses were carried out using 1-way and 2-way ANOVA and significant differences were separated with Tukey's HSD test (p=0.05).

2.3 Results

2.3.1 Leaf anatomy

Light intensity significantly affected leaf thickness of *Chrysanthemum* with low light intensity resulting in overall thinner leaves (P=0.002) (Figure 2.2; Table 2.2). Light quality also significantly affected leaf thickness this at both low and control light intensity (Figure 2.2). Red light decreased the leaf thickness compared to the other light quality treatments.

Light intensity did not affect the thickness of the upper (adaxial) and lower (abaxial) epidermal layer. The epidermal layers were significantly influenced by light quality (Table 2.2 and Figure 2.2). The thickness of the adaxial epidermal cells was the greatest under W for both light intensities in comparison with the other treatments. Only in the low light intensity, RB equaled the W treatment. The thickness of the abaxial epidermal cells was lowest under B this for both light intensities. Yet, some more variation for the other treatments was found. At 100 µmol m^{-2} s^{-1}, abaxial epidermal cells were thickest under W and were intermediate for R and RB. At 40 µmol m^{-2} s^{-1}, the abaxial epidermal cells were thicker under RB and W but decreased significantly under B and R. Overall there was a significant light effect on the palisade parenchyma layer, especially through the reaction to low and high intensities of B and RB. The palisade parenchyma layer was thicker under B and RB at 100 µmol m^{-2} s^{-1}, while it was the thickest under W followed by R and RB and significantly thinner under B at 40 µmol m^{-2} s^{-1}. The spongy parenchyma tissue was unaffected by the light intensity. Light quality, however, and especially R decreased the spongy layer compared to the other light qualities at 100 µmol m^{-2} s^{-1}, while no effects were present at 40 µmol m^{-2} s^{-1}.

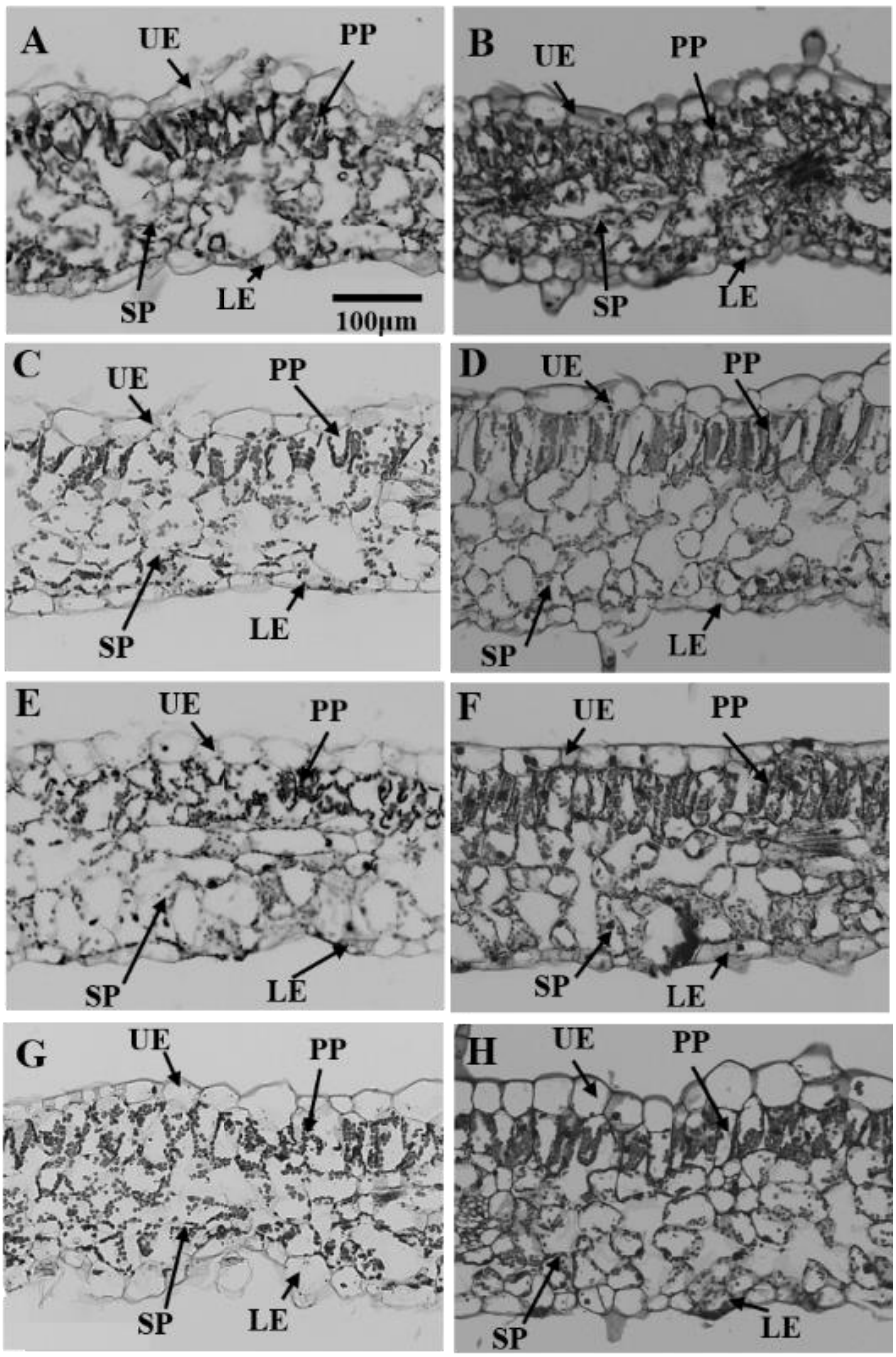

Figure 2.2 The leaf anatomy of *Chrysanthemum* under light intensity and light quality treatments (A, C, E and G: R, B, RB, W at 40 μmol m^{-2} s^{-1}; B, D, F and H: R, B, RB, W at 100 μmol m^{-2} s^{-1}). UE: upper epidermal; LE: lower epidermal; PP: palisade parenchyma; SP: spongy parenchyma. Scale bar is 100 μm.

Table 2.2 Effects of light quality and light intensity on the leaf anatomy of *Chrysanthemum*.

Light intensity	Light quality	Leaf thickness (μm)	Adaxial epidermis (μm)	Abaxial epidermis (μm)	Palisade parenchyma (μm)	Spongy parenchyma (μm)
40 μmol m^{-2} s^{-1}	R	196.69 ± 5.15 b	23.40 ± 0.40 b	17.68 ± 1.65 b	50.26 ± 3.93 ab	105.35 ± 4.74 a
	B	210.03 ± 4.25 ab	23.84 ± 1.45 b	17.15 ± 1.13 b	46.47 ± 1.29 b	122.56 ± 4.58 a
	RB	226.65 ± 3.12 a	32.22 ± 1.39 a	21.64 ± 2.23 a	50.26 ± 2.18 ab	122.48 ± 5.37 a
	W	224.87 ± 4.61 a	33.58 ± 2.15 a	22.71 ± 1.79 a	57.38 ± 2.24 a	111.20 ± 2.96 a
100 μmol m^{-2} s^{-1}	R	187.80 ± 6.43 b	23.32 ± 0.96 b	22.01 ± 2.33 ab	52.36 ± 1.07 b	90.02 ± 8.75 b
	B	247.04 ± 5.34 a	24.97 ± 2.07 b	16.89 ± 1.17 b	64.33 ± 0.64 a	140.85 ± 8.14 a
	RB	250.84 ± 5.12 a	25.65 ± 0.59 b	19.29 ± 0.57 ab	62.87 ± 1.21 a	143.02 ± 3.89 a
	W	236.05 ± 8.59 a	32.09 ± 1.21 a	24.83 ± 1.78 a	55.89 ± 1.22 b	123.24 ± 8.50 a
Light intensity effect		**	n.s.	n.s.	***	n.s.
Light quality effect		***	***	***	n.s.	***

Data are means ± SE (n=4). Means followed by the same letter in each column means no significant difference between light qualities by Tukey's HSD test at P<0.05. n.s.: not significant. ** and *** indicate significance at P<0.01 and P<0.001, respectively.

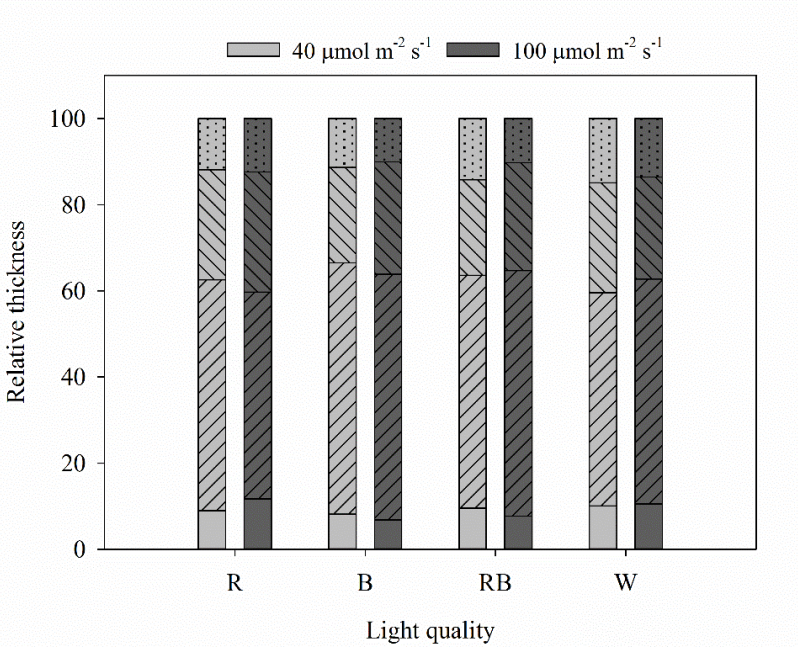

Figure 2.3 The relative thickness of leaf anatomy of *Chrysanthemum* developed under different light intensity and quality treatments. From the uppermost to the lowest: abaxial epidermis (with dots), palisade parenchyma (with backslashes), spongy parenchyma (with slashes) and adaxial epidermis (with blank fill).

2.3.2 Stomatal traits and stomatal conductance

Light quality and intensity affected the stomatal traits of *Chrysanthemum* (Table 2.3, Figure 2.4). There was no overall light quality effects for the different aperture parameters, light quantity showed influences. At a PPFD level of 100 μmol m^{-2} s^{-1}, the stomatal aperture length was smallest under RB and highest under R, though no significant effects were found for aperture width and area. Aperture width/length was the greatest under R followed by W and RB while it significantly declined under B. Under a light intensity of 40 μmol m^{-2} s^{-1}, the stomatal aperture length, width and area were all greater under RB and W while they significantly decreased under R and B. The aperture length/width was unaffected by light quality.

The stomatal index and density were influenced by both light intensity and quality. Higher light intensity increased both parameters. At 100 μmol m^{-2} s^{-1} the stomatal index was the greatest under R, followed by B and W and significantly lower under RB, the stomatal density was unaffected by light quality. At 40 μmol m^{-2} s^{-1} the stomatal index and density were the highest under RB, while they were significantly lower for the other light quality treatments. Higher light intensities tended to result in

higher stomatal conductance (P=0.052) but there were no significant effects of light quality (Figure 2.5).

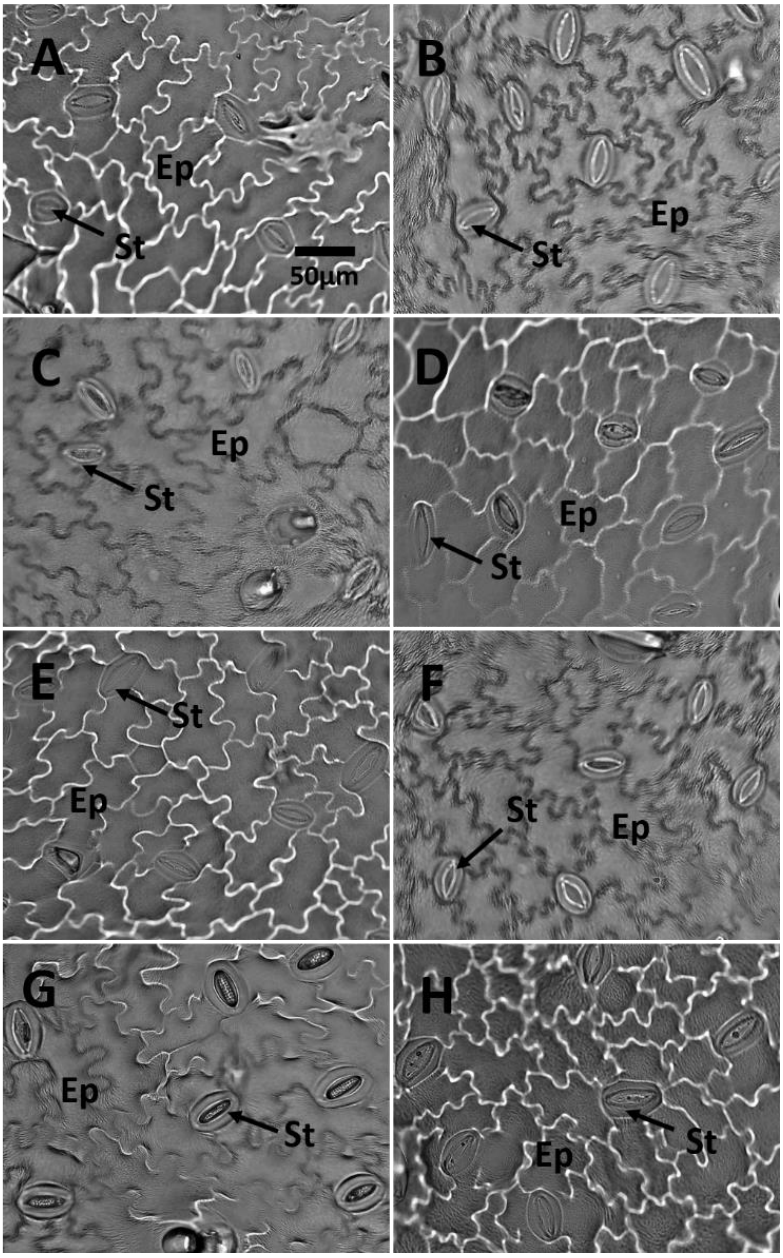

Figure 2.2 The abaxial side stomata and epidermis of *Chrysanthemum* leaves developed under different light intensity and light quality treatments (A, C, E and G: R, B, RB and W at 40 μmol m^{-2} s^{-1}; B, D, F and H: R, B, RB and W at 100 μmol m^{-2} s^{-1}). Ep: epidermal cells; St: stomata. Scale bar is 50 μm.

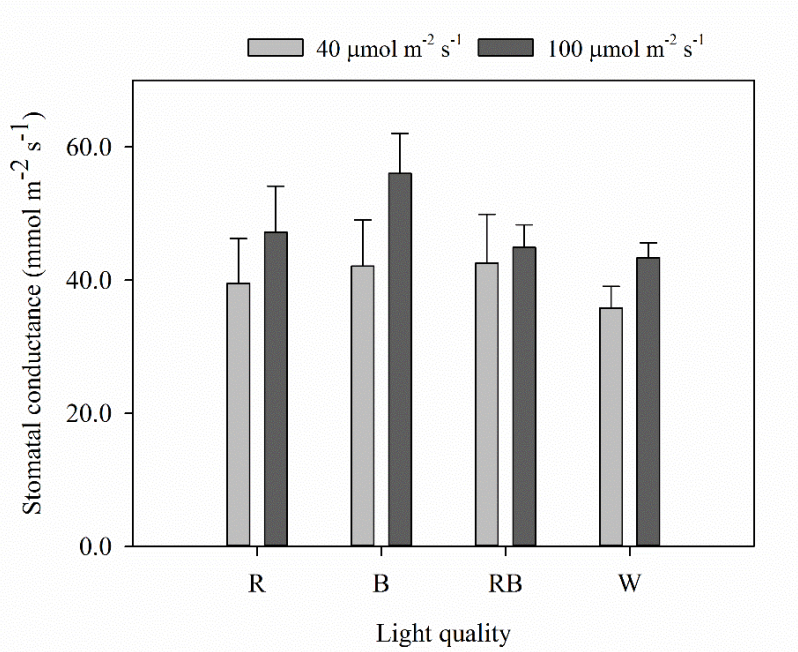

Figure 2.3 Effects of light intensity and light quality on the stomatal conductance of *Chrysanthemum* leaf. Data present in mean ± SE with vertical error bar (n=4), no significant differences between different light intensity.

2.3.3 Pigments content

Light intensity significantly affected the leaf chlorophyll ($P < 0.001$) and carotenoids content ($P < 0.001$), lower light intensities enhanced the pigment content. At 100 µmol m^{-2} s^{-1} the total Chl content ranged from 0.306 ± 0.003 (R) to 0.383 ± 0.002 (B) mg g^{-1} FW, while it ranged between 0.388 ± 0.027 (B) and 0.552 ± 0.058 (R) mg g^{-1} FW at light intensity of 40 µmol m^{-2} s^{-1}. Chl a/b ratios ranged between 2.07 ± 0.01 (R) and 2.25 ± 0.02 (B) at light intensity of 100 µmol m^{-2} s^{-1}, while it ranged between 2.29 ± 0.11 (B) and 2.54 ± 0.10 (RB) at light intensity of 40 µmol m^{-2} s^{-1}. Light quality did not affect the pigment content irrespective of the light intensity (Figure 2.6). Overall there was a significant effect of light quality on carotenoids ($P = 0.042$), though this was not present for the individual light intensities (Figure 2.6).

Table 2.3 Effects of light quality and light intensity on the stomatal traits of *Chrysanthemum* leaves.

Light intensity	Light quality	Aperture length (µm)	Aperture width (µm)	Aperture area (μm^2)	Aperture width/length	Aperture area per leaf area ($cm^2\ m^{-2}$)	Stomatal index (%)	Stomatal density (N mm^{-2})
40 µmol $m^{-2}\ s^{-1}$	R	26.4 ± 0.9 b	8.8 ± 0.5 ab	184.6 ± 15.2 b	3.1 ± 0.1 a	97.1 ± 5.7 b	12.7 ± 0.8 b	53.3 ± 3.3 ab
	B	27.4 ± 1.0 b	7.9 ± 0.3 b	167.5 ± 3.8 b	3.6 ± 0.2 a	86.9 ± 5.7 b	13.1 ± 0.5 b	51.8 ± 3.0 b
	RB	31.0 ± 0.8 a	9.8 ± 0.4 a	238.8 ± 12.9 a	3.3 ± 0.2 a	157.1 ± 11.9 a	16.6 ± 0.7 a	66.3 ± 4.1 a
	W	31.1 ± 0.7 a	9.9 ± 0.6 a	241.0 ± 12.9 a	3.3 ± 0.2 a	108.2 ± 9.8 ab	13.1 ± 0.7 b	44.3 ± 2.6 b
100 µmol $m^{-2}\ s^{-1}$	R	29.9 ± 1.2 a	8.0 ± 0.5 a	190.8 ± 17.5 a	3.8 ± 0.2 a	155.5 ± 17.7 a	19.9 ± 0.8 a	80.7 ± 2.8 a
	B	28.6 ± 0.7 ab	8.9 ± 0.3 a	199.5 ± 9.0 a	3.3 ± 0.2 b	151.8 ± 6.0 ab	18.3 ± 0.4 ab	76.8 ± 2.3 a
	RB	26.2 ± 0.9 b	7.9 ± 0.3 a	164.6 ± 11.5 a	3.4 ± 0.1 ab	117.4 ± 7.7 b	17.4 ± 0.4 b	72.8 ± 3.8 a
	W	28.3 ± 0.7 ab	7.9 ± 0.2 a	177.1 ± 7.3 a	3.6 ± 0.1 ab	131.7 ±7.5 ab	18.0 ± 0.7 ab	74.6 ± 3.2 a
Factorial analysis								
Light intensity		n.s.	**	**	n.s.	*	***	***
Light quality		n.s.	n.s.	n.s.	n.s.	n.s.	*	*

Data are means ± SE (n=4). Different letters indicate significant differences between values for each parameter between light qualities according to Tueky's HSD test at P = 0.05. n.s.: not significant. *, ** and *** indicates significance at P<0.05, P<0.01 and P<0.001, respectively.

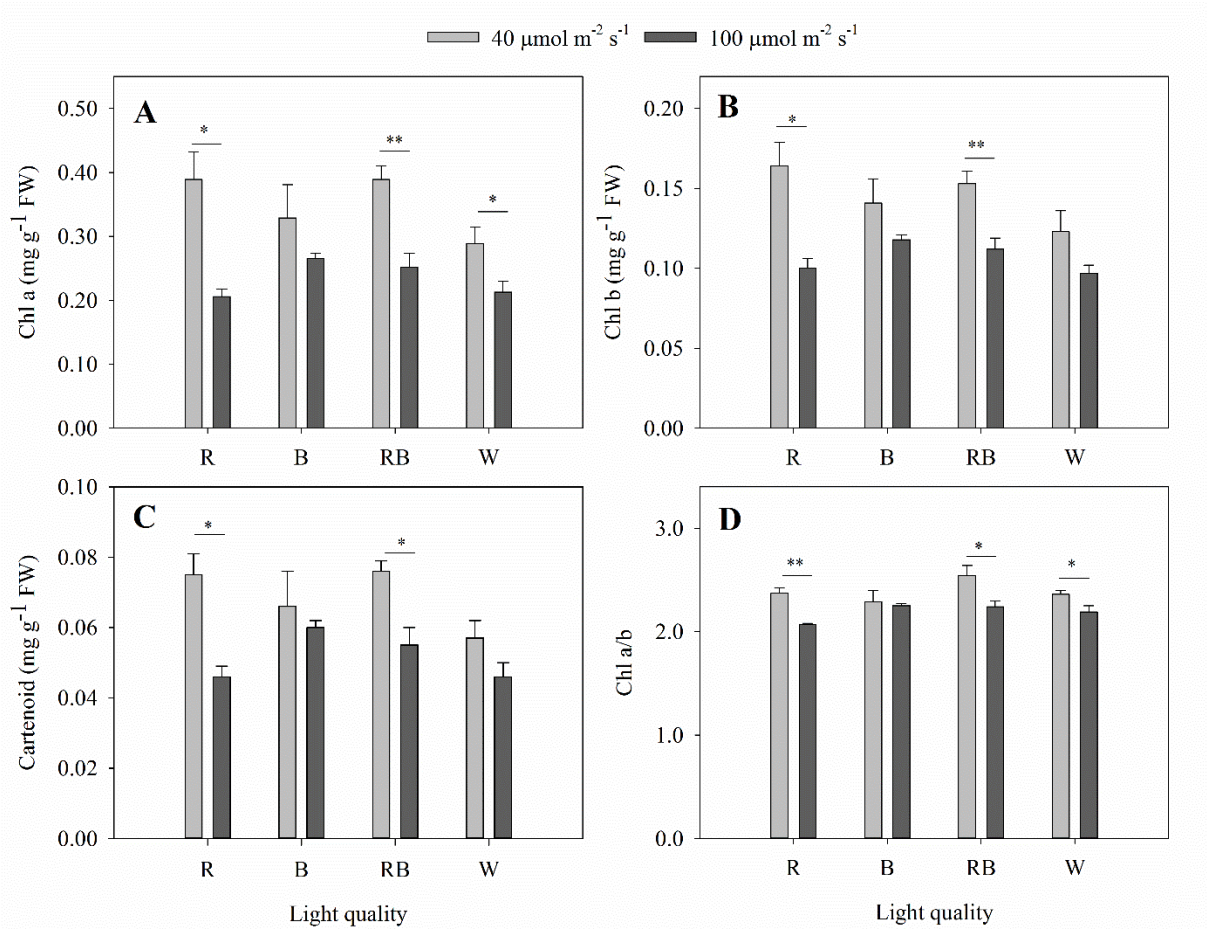

Figure 2.4 Effects of light intensity and light quality on the pigments content of *Chrysanthemum* leaves. Data present in mean ± SE with vertical error bar (n=4), * and ** indicating significant difference according to Tukey's HSD test (P<0.05 and P<0.01).

Table 2.4 Two-way ANOVA analysis of the effects of light quality and light intensity on biomass and pigments content.

Parameter	FW	DW	Chl a	Chl b	Carotenoids	Total Chl	Chl a/b
Light intensity	***	***	***	***	**	***	***
Light quality	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.

* ** ***: significant by two-way ANOVA at P < 0.05, 0.01 and 0.001 respectively; n.s.: not significant.

2.3.4 Chlorophyll fluorescence

Light intensity did not affect the chlorophyll fluorescence parameters in *Chrysanthemum* (Table 2.5). Light quality significantly affected all the studied parameters, both at 40 and 100 µmol m^{-2} s^{-1}. F_v/F_m was highest under B and significantly lower under R while RB and W had intermediate values, this for both light intensities. Φ_{PSII} was significantly lower under R in comparison with the other treatments, this for both light intensities. qP and ETR showed a similar trend under two light intensities, it was the greatest under B and W followed by RB and significantly lower under R. NPQ was not affected by the light quality at 40 µmol m^{-2} s^{-1}, while at 100 µmol m^{-2} s^{-1} significant lower values were found under W while for R and RB an increase was noted.

2.3.5 Biomass

Light intensity strongly influenced the fresh and dry biomass (Figure 2.7), while the overall effect of light quality was not significant (P=0.07 and 0.15, for 40 and 100 µmol m^{-2} s^{-1}, respectively). Fresh weight of *Chrysanthemum* was enhanced under blue light at 100 µmol m^{-2} s^{-1} followed by RB and W and was significantly smaller under R. Dry weight tended to be greater under B compared to the other treatments (P=0.055). At 40 µmol m^{-2} s^{-1}, no significant effect of light quality was noted (Figure 2.7).

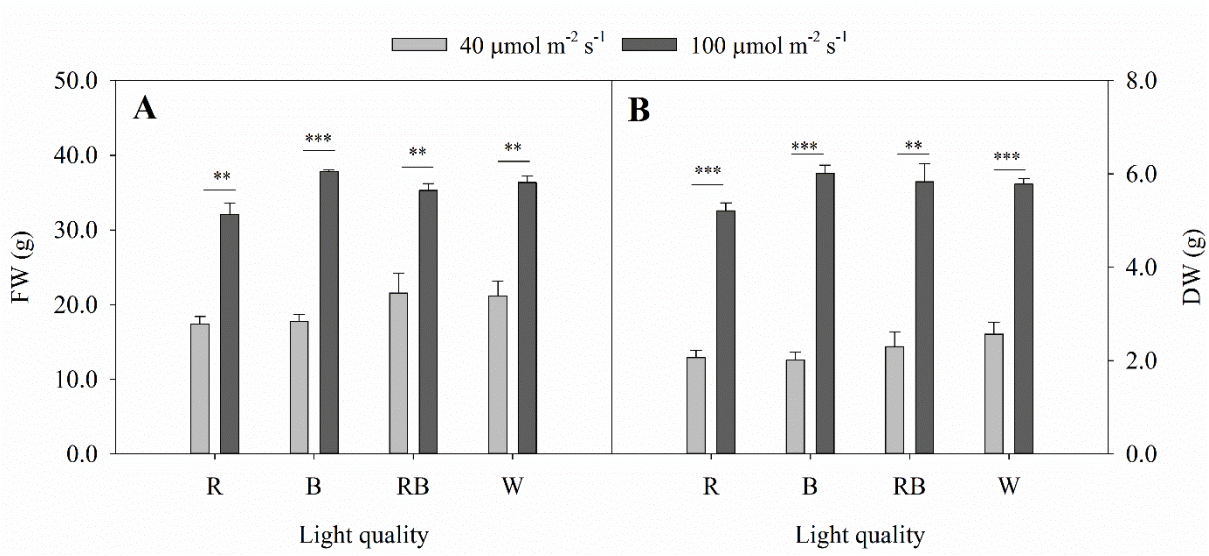

Figure 2.5 Effects of light intensity and light quality on the fresh and dry weight of *Chrysanthemum*. Data present in mean ± SE with vertical error bar (n=4), ** and *** indicating significant difference according to Tukey's HSD test (P<0.01 and P<0.001).

Table 2.5 Chlorophyll fluorescence parameters of *Chrysanthemum* grown under different light qualities and intensities.

Light intensity	Light quality	Φ_{PSII}	NPQ	qP	ETR	F_v/F_m
40 µmol m^{-2} s^{-1}	R	0.549 ± 0.024 c	0.352 ± 0.027 a	0.867 ± 0.026 b	21.2 ± 0.9 b	0.722 ± 0.004 c
	B	0.651 ± 0.002 a	0.255 ± 0.013 a	0.953 ± 0.002 a	25.0 ± 0.0 a	0.758 ± 0.002 a
	RB	0.607 ± 0.006 a	0.323 ± 0.063 a	0.917 ± 0.006 ab	23.2 ± 0.3 ab	0.745 ± 0.005 b
	W	0.614 ± 0.008 a	0.337 ± 0.025 a	0.930 ± 0.006 a	23.4 ± 0.4 a	0.752 ± 0.001 ab
100 µmol m^{-2} s^{-1}	R	0.583 ± 0.005 b	0.390 ± 0.035 a	0.890 ± 0.005 b	22.2 ± 0.2 c	0.729 ± 0.002 c
	B	0.650 ± 0.006 a	0.305 ± 0.009 ab	0.939 ± 0.010 a	24.8 ± 0.2 a	0.764 ± 0.003 a
	RB	0.624 ± 0.016 a	0.395 ± 0.072 a	0.927 ± 0.012 a	24.0 ± 0.6 ab	0.752 ± 0.007 ab
	W	0.624 ± 0.008 a	0.249 ± 0.022 b	0.943 ± 0.003 a	23.6 ± 0.3 b	0.745 ± 0.003 bc
Light Intensity effect		n.s.	n.s.	n.s.	n.s.	n.s.
Light Quality effect		***	*	***	***	***

Data are means ± SE (n=4). Means followed by the same letter in each column means no significant difference between light qualities by Tukey's HSD test at P<0.05. n.s.: not significant. *, ** and *** indicates significance at P<0.05, P<0.01 and P<0.001, respectively.

2.4 Discussion

Leaf sectioning anatomy

Leaves are the main organ for plant photosynthesis and transpiration. The structural characteristics of leaves reflect the impacts of environmental factors on plants or the adaptability of plants to the environment (Ou et al., 2015). Although both 40 and 100 µmol m^{-2} s^{-1} are already low light intensities plants further adapt by thinner leaves for the lowest light intensity with exception of the red light treatment. In most of the species, including the studied *Chrysanthemum*, only one layer of palisade parenchyma is present. Light intensity mainly affected palisade cells and to a lesser extent the spongy parenchyma. Thicker leaves as found under 100 µmol m^{-2} s^{-1} were the result of an increment in the size of palisade cells and also due to a major number of spongy parenchyma layers (Figure 2.2). Thinner leaves are considered as a way to optimize the light penetration into the leaf and thus to increase the light absorption for chloroplasts (Brodersen and Vogelmann, 2010; Terashima and Saeki, 1983). It is therefore considered a common adaptation to low irradiances (Marler et al., 1994) and is present in many other species such as *Quercus, Mahonia bodinieri* and *Schefflera arboricola* (Ashton and Berlyn, 1994; Kong et al., 2016; Kubatsch et al., 2007).

Light quality also significantly influenced the total leaf thickness as well as the palisade and spongy parenchyma. Leaf thickness decreased under red light and it was mainly due to a decrease of the spongy parenchyma, which represented 47.9% of the total leaf thickness (Figure 2.3). Macedo et al. (2011) found that the boundary of the palisade and spongy mesophyll tissues of *Alternanthera brasiliana* leaves grown under R was not clear, which is consistent with our result (Figure 2.2). This might explain why we did not see light intensity responses for leaves that developed under the applied R fluencies, monochromatic red was insufficient for the development of the palisade layer. The W treatment at 100 µmol m^{-2} s^{-1}, lead to a thinner palisade parenchyma compared to RB and B. This might explained by its relative low blue proportion (7%) which was much higher for the 40 µmol m^{-2} s^{-1} W (Table 2.1). Light quality highly effects leaf palisade/spongy parenchyma development and thus leaf thickness as already shown for *Arabidopsis* (Weston et al., 2000) and *Alternanthera brasiliana* (Macedo et al., 2011). Schuerger et al. (1997)

found that supplementary blue light correlated with an increase of palisade and spongy mesophyll thickness in pepper plants. Shengxin et al. (2016) found that when the blue ratio increased above 25% in rapeseed leaves, two cell-layers in the palisade tissue appeared and it indicated the decisive role of blue light for the development of the palisade tissues.

Stomatal conductance and stomatal traits

Stomata are important channels for the exchange of water and CO_2 with the environment. Stomatal initiation is most active early in the development of the leaf and effects of light on initiation are greatest at this early stage (Gay and Hurd, 1975). Our measurements took place on leaves that fully developed under the light treatment, thus including this early stage. Light intensity significantly influenced the formation of stomatal cells in the lower epidermis of *Chrysanthemum* resulting in a lower stomatal density and stomatal index at low light intensities (40 µmol m^{-2} s^{-1}). The reduction of the stomatal density is considered a common adaptation of plants to low light conditions and it was found in many species both in natural and controlled conditions (Gay and Hurd, 1975; Marler et al., 1994). Tomato leaves developed at 40 µmol m^{-2} s^{-1} averaged stomatal densities of 35 mm^{-2} (Gay and Hurd, 1975), this is even lower than our observations in *Chrysanthemum*, which averaged 54 mm^{-2}. However at 160 µmol m^{-2} s^{-1} the stomatal density rose to 200 mm^{-2} which is a much higher increase than we found for *Chrysanthemum* (76 mm^{-2} at 100 µmol m^{-2} s^{-1}) indicating that plasticity for light intensity is lower in *Chrysanthemum*.

Both red and blue light regulated the stomatal development at 100 µmol m^{-2} s^{-1}. Stomatal density was not affected while stomatal index was greatest under R indicating that *Chrysanthemum* developed smaller epidermal cells under red (Figure 2.4). This coincides with observations on *Pelargonium* where the blue spectrum enhanced the elongation of abaxial epidermal cell by 7-13% compared to monochromatic red (Fukuda et al., 2008). At lower light intensities (40 µmol m^{-2} s^{-1}) both stomatal density and stomatal index decreased though this was least pronounced under RB (Table 2.3). This suggests that dichromatic RB was beneficial for initiation of the stomata in *Chrysanthemum* under low light intensities.

Stomatal opening is influenced by both light intensity level and light quality. Under lower light intensity, the aperture area was smaller under R and B compared to

dichromatic RB and full spectrum W, which was due to smaller aperture length and width. As no differences in length/width ratio were observed, light quality showed no significant effect on the opening of the stomata at 40 µmol m^{-2} s^{-1}. It is suggested that a high irradiance response in the blue light fraction is present with a certain threshold to induce stomatal opening (> 3.8 µmol m^{-2} s^{-1}) and higher light intensities result in a linear opening response until fully open (Habermann, 1973). Our applied blue light intensity in W is beyond the threshold (3-fold), which might induced the non-significant opening with B. Habermann (1973) recorded that the stomatal opening under low intensity monochromatic blue and red light of exposed sunflower and tobacco leaf discs was not affected. If light intensities are too low, stomata hardly open and light quality has no effect.

Pigments

The light environment influenced chlorophyll biosynthesis. Lower light intensity resulted in higher chlorophyll content in *Chrysanthemum*. These results are similar to the increased chlorophyll content observed in plants that acclimate to low light/shade environments (Evans, 1988; Sarijeva et al., 2007). According to Lichtenthaler et al (1982), plants exposed to high light conditions develop chloroplasts with a higher proportion of PSI units, a higher level of electron carriers and high rates of photosynthetic quantum conversion. In contrast, during chloroplast development in leaves under low light conditions, large pigment antennae are formed with a high proportion of light harvesting chlorophyll proteins, resulting in a much higher thylakoid density per chloroplast. These chloroplasts thus possess a high capacity to absorb light. This adaptation maximizes light interception and increased carbon gain in low light conditions, through a more efficient investment in photosynthetic machinery (Evans and Poorter, 2001).

Under 100 µmol m^{-2} s^{-1}, total chlorophyll content tended to be higher under B and RB which is consistent with previous observations that a blue spectrum enhances the biosynthesis of chlorophyll (Sood et al., 2005). The Chl a/b ratio is typically the value for shade leaves, this in both light conditions. Under R a decrease in Chl a/b ratio was recorded, this could explain partially the lower chlorophyll fluorescence performance under R. The Chl a/b ratio is related to the capacity for electron

transport and increases the Calvin cycle enzymes on a chlorophyll basis (Evans, 1988).

Chlorophyll fluorescence and growth

The relative quantum efficiency of R is higher than that of B because fractions of the blue spectrum are absorbed by flavonoids in vacuoles and/or anthocyanins without a function for photosynthesis in chloroplasts (McCree, 1971). Despite this short-time effect of red light, prolonged cultivation under red light resulted in less vigorous plants compared to full spectrum light at the same light intensity in several plant species, including lettuce (Wang et al., 2016; Yorio et al., 2001), wheat (Goins et al., 1997) and spinach (Ohashi-Kaneko et al., 2007; Yorio et al., 2001). Also in this study, monochromatic R was adverse for *Chrysanthemum* development. A lower F_v/F_m and Φ_{PSII} under R, irrespective of the light intensity, indicated malfunctioning in PSII, based on suboptimal activity of both Photosystems due to an inhibited electron transport from PSII to PSI. Additional blue to red light improved the photosynthetic rate, increase shoot dry weight, leaf area and leaf number with increasing R/B ratio in lettuce (Wang et al., 2016) and cucumber (Hogewoning et al., 2010b). These results underline the importance of blue light for photosynthesis and subsequent biomass production and should be combined in artificial lighting systems for plant production (Goto, 2012; Piovene et al., 2015).

Light intensity showed no significant effect on the chlorophyll fluorescence parameters; this indicates that the lower light intensity we applied did not limit the efficiency of PSII. The *Chrysanthemum* plants could acclimated to these low light intensities (difference in pigments and anatomy) and develop into a fully functional leaf. Shade-adapted carambola leaflets even resulted in a high photosynthetic capacity during a short-term exposure to high light (Marler et al., 1994).

Plant growth is defined as an increase in plant size, which is a function of biomass production driven by photosynthetic activity (Gerovac et al., 2016). Therefore, the biomass response is a result of additional light energy provided for photosynthesis activity. As both light intensities are far below the light saturation for *Chrysanthemum* (300-400 µmol m^{-2} s^{-1} at leaf level, 20°C) (Weerakkody and Suriyagoda, 2015) the biomass increase to higher light levels is strong. Also, the higher stomatal density and aperture area per leaf area under the higher light intensity affects gas exchanges

and are positively correlated with photosynthetic rate (Kundu and Tigerstedt, 1999; Tanaka et al., 2013).

The reaction to light quality in *Chrysanthemum* was also dependent on the light intensity (Figure 2.7). Under lower irradiation (40 µmol m^{-2} s^{-1}), monochromatic R and B tended to develop a smaller biomass than polychromatic RB and full spectrum W. Under low light conditions, the combined effect of blue and red is more efficient for a good efficient photosynthetic activity (Hogewoning et al., 2010b), under light intensities of 100 µmol m^{-2} s^{-1}, only plants grown under R showed a negative effect on biomass production.

2.5 Conclusion

The present study provides a better understanding of the responses of growth, photosynthesis, anatomical development in *Chrysanthemum* young plants exposed to various light quality under different light intensity. Both light intensity and light quality influenced the *Chrysanthemum* leaf development. Blue photons were necessary for the development of a well-established leaf anatomical structure, while red light resulted in thinner leaves and shorter palisade cells. Blue light was also favorable for the development of stomata. Lower light intensities increased the photosynthetic pigment content, while higher light intensity is beneficial for biomass accumulation. Light quality effects on the photosynthetic performance but not the light intensity, additional blue light improves the development from leaf level anatomy, stomatal development and movement to biomass accumulation.

Chapter 3

Long-term effects of red- and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants

This chapter is based on:

L. Zheng and M. C. Van Labeke., 2017. Long-term effects of red- and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. Frontiers in Plant Science. doi: 10.3389/fpls.2017.00917

Author contribution:

LZ and MCVL conceived and designed the experiments. LZ performed the experiments, analyzed the data and drafted the manuscript, MCVL critically revised the manuscript.

Abstract

Light quality critically affects plant development and growth. Development of light-emitting diodes (LEDs) enables the use of narrow band red and/or blue wavelengths as supplementary lighting in ornamental production. Yet, long periods under these wavelengths will affect leaf morphology and physiology. Leaf anatomy, stomatal traits and stomatal conductance, leaf hydraulic conductance (K_{leaf}) and photosynthetic efficiency were investigated in three ornamental pot plants, namely *Cordyline australis* (monocot), *Ficus benjamina* (dicot, evergreen leaves) and *Sinningia speciosa* (dicot, deciduous leaves) after eight weeks under LED light. Four light treatments were applied at 100 µmol m^{-2} s^{-1} and a photoperiod of 16 hours using 100% red (R), 100% blue (B), 75% red with 25% blue (RB) and broad-spectrum white light (W), respectively. B and RB resulted in a greater maximum quantum yield (F_v/F_m) and quantum efficiency (Φ_{PSII}) in all species compared to R and W and this correlated with a lower biomass under R. B increased the stomatal conductance compared with R. This increase was linked to an increasing stomatal index and/or stomatal density but the stomatal aperture area was unaffected by the applied light quality. Leaf hydraulic conductance (K_{leaf}) was not significantly affected by the applied light qualities. Blue light increased the leaf thickness of *F. benjamina*, and a relative higher increase in palisade parenchyma was observed. Also in *S. speciosa*, increase in palisade parenchyma was found under B and RB, though total leaf thickness was not affected. Palisade parenchyma tissue thickness was correlated to the leaf photosynthetic quantum efficiency (Φ_{PSII}). In conclusion, the role of blue light addition in the spectrum is essential for the normal anatomical leaf development which also impacts the photosynthetic efficiency in the three studied species.

3.1 Introduction

Light strongly influences plant growth and development. Light, as an energy source, affects photosynthesis and its related parameters. Light quality is one of the main factors of light signaling and affects numerous processes from seed germination, leaf formation to flower development (Demotes-Mainard et al., 2016; Hogewoning et al., 2010a; Johkan et al., 2012; Wang et al., 2010c). Artificial lighting has been used to extend the photoperiod and to increase the light intensity in horticultural production. Development of light-emitting diodes (LEDs) enables the application of narrow spectrum band red or blue wavelengths in the cultivation of horticultural crops at the exact absorption peaks of chlorophyll (Dutta Gupta and Jatothu, 2013) which in short-term results in the highest photosynthetic efficiencies per leaf unit area (McCree, 1971). Yet, long periods under monochromatic or dichromatic wavelengths with low natural light fluencies might lead to many morphological and physiological changes in response to the ambient light environment thus affecting plant development (Brodersen and Vogelmann, 2010; Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016; Terashima and Saeki, 1983).

Various traits affecting photosynthesis are influenced by light quality including both red and blue light responses. Leaf anatomy may directly influence light capture by its leaf thickness as well as by the differentiation of palisade and spongy mesophyll. Schuerger et al. (1997) reported that leaf thickness increased when red light was supplemented with blue light. Light absorption will also be dependent on chlorophyll concentration. Wang et al. (2009) reported that blue light enhanced the expression of different enzymes such as MgCH (magnesium chelatase), GluTR (glutamyl-tRNA reductase) and FeCH (ferrochelatase) which regulate the synthesis of chlorophyll. Red light is less conducive for the chlorophyll biosynthesis, because of its reduction of the tetrapyrrole precursor 5-aminolevulinic acid (Fan et al., 2013; Sood et al., 2005). Stomatal density and conductance are other traits that will influence the CO_2 uptake and thus photosynthesis. Effects of blue light on stomatal opening are well documented (Talbott, 2002; Tallman and Zeiger, 1988). Monochromatic red light has been reported to reduce the photosynthetic efficiency and it leads to photo-damage (photoinhibition of Photosystems) for cucumber leaves that developed under monochromatic red light after three weeks (Trouwborst et al., 2016). In contrast, blue

light which is sensed by cryptochrome and phototropin optimizes photosynthesis by improving the efficiency of light capture, reducing photo-damage, and regulating gas exchange between leaves and atmosphere (Takemiya et al., 2005).

Light quality not only affects the gas exchange but also the water transportation within leaves (Lee et al., 2007; Savvides et al., 2012; Sharkey and Raschke, 1981). Leaf hydraulic conductance (K_{leaf}) affects different aspects of plant functioning such as respiration, evaporation and photosynthetic carbon fixation (Prado and Maurel, 2013). Leaf hydraulic conductance reflects the water flow through the leaf veins, across the mesophyll tissue and to the stomatal aperture. The extra-veinal phase of water flow is influenced by the leaf mesophyll spongy/palisade anatomy and thickness and the stomatal aperture characteristics (Nardini et al., 2003; Sack et al., 2004; Sack and Holbrook, 2006). Despite the great importance of leaf hydraulic conductance in plant water relations, knowledge of the relationships between hydraulic conductance and light quality is limited. Savvides et al (2012) reported that blue in the light spectrum drives both K_{leaf} and g_s towards higher values in cucumber. In bur oak, hydraulic conductance was enhanced in response to blue and green light (Voicu et al., 2008).

In ornamental horticulture, the commercial value depends on the visual quality, which mainly results from architectural traits such as stem elongation, compactness, branching and flowering. The management of light quality opens the way to improved control of the ornamental value. Control of the light quality by LED lights could also focus on a specific production phase namely the ornamental young plants where LED could be the sole-source light in multilayer production units. However, this phase under monochromatic or dichromatic narrow band LED lights might not only influence the architectural traits but also anatomical traits of leaves developing under this light treatment.

The objective of this study was to evaluate how narrow-band R, B and RB would modulate leaf morphology, mesophyll anatomy and stomatal formation, which could in consequence influence the light absorption and hydraulic conductance of leaves. To assess the impact on photosynthetic performance, chlorophyll fluorescence parameters were quantified as well as the biomass. For this study we selected three commonly produced ornamentals with different leaf traits namely *Cordyline australis*

(monocot), *Ficus benjamina* (dicot, evergreen leaves) and *Sinningia speciosa* (dicot, deciduous leaves).

3.2 Materials and Methods

3.2.1 Plant material and growth conditions

The experiment was conducted in a growth chamber at Ghent University, Belgium. Three ornamental species were selected: *Cordyline australis* 'Red Star' (monocot), *Ficus benjamina* 'Exotica' (dicot, evergreen leaves) and *Sinningia speciosa* 'Sonata Red' (dicot, deciduous leaves). Young plants were obtained from a commercial plant producer and transplanted into 0.3 L pots filled with peat-based potting soil (Van Israel nv, Belgium). The plants were acclimated for 1 week in broad spectrum light (100 µmol m^{-2} s^{-1}) provided by SON-T high-pressure sodium lamps (Philips Inc. Eindhoven, the Netherlands). Then for each species, 12 replicates per treatment were randomly allocated to four spectral light treatments. Air temperature of the growth chamber was set at 22 ± 2 ℃ and plants received a photoperiod of 16 h. Irrigation and fertilization with a water-soluble fertilizer (N:P:K = 4:1:2, EC 1.5 ds m^{-1}, pH = 6.5) was applied once every two days.

3.2.2 Light treatment

Light intensity at the canopy level was set at 100 µmol m^{-2} s^{-1} by adjusting the distance of the light source and a photoperiod of 16 h per day was given. Light treatment sections were separated with curtains, four treatments were applied using different light qualities equipped with LED lighting, which were B (100% blue, peak at 460 nm), R (100% red, 660 nm), and W [white, 7 % blue (400-500 nm), 16% green (500-600 nm), 75% red (600-700 nm) and 2% far red (700-800 nm)] (Philips Inc., Eindhoven, The Netherlands) as well as RB (75% R and 25% B, peak at 460 nm and 660 nm) by a CID-800 programmable LED lighting system (CID Bio-Science, USA), respectively. Light distribution was recorded using JAZ-ULM-200 spectrometer (Ocean Optics, FL, USA) and converted with Spectrasuite software (Ocean Optics) to µmol m^{-2} s^{-1} (Figure 3.1) and uniformity was verified by measuring the light intensity at five points of each light treatment at the canopy level (Table 3.1).

The plants were grown for 8 weeks and then the second or third leaf counting from the apex (fully expanded leaves that developed entirely under the given light quality) were selected for the measurements. All measurements were performed in 4 replications per treatment and per plant species.

Table 3.1 Overview of the characteristics of the light treatments: average PPFD per treatment, phytochrome photostationary state (P_{fr}/P_{total}) and blue light proportion.

Parameter	R	B	RB	W
PPFD (400-700 nm) (µmol m^{-2} s^{-1})[a]	97.4 ± 4.2	100.1 ± 1.2	100.3 ± 3.6	97.6 ± 4.7
%B	0	100 %	25 %	7 %

[a] Mean ± standard deviation, n = 5

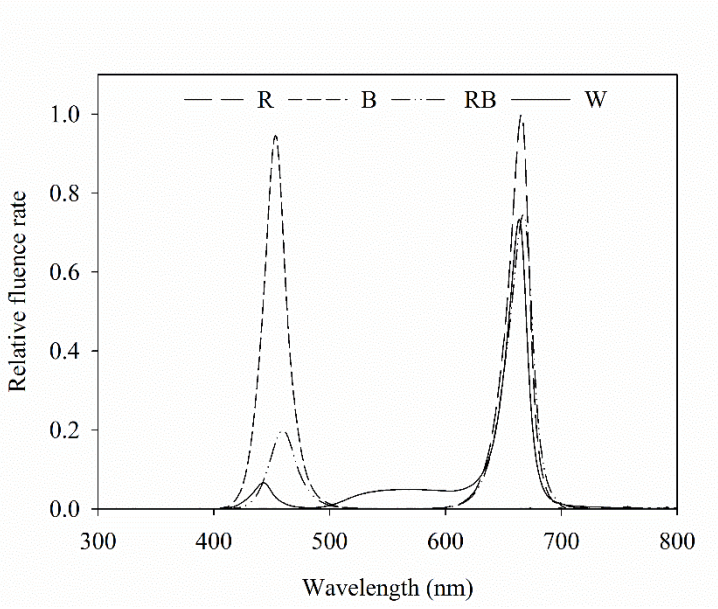

Figure 3.1 Relative fluence rate of the light treatments used in this experiment: R: red, B: blue, RB: red/blue (3:1) and W: white. Spectrum was measured at the plant canopy level with a JAZ spectrometer (Ocean Optics, FL, US).

3.2.3 Leaf anatomy

Leaf segments of 2×2 cm of the central leaf blade next to main vein were excised and fixed for at least 24 hours in a formaldehyde-based fixative (FAA). Then, leaf segments were dehydrated using a series graded concentration ethanol, embedded

in paraffin and sectioned at thickness of 12 µm with a microtome (R. Jung AG, Heidelberg, Germany). The sections were deparaffinized with xylene and rehydrated with graded ethanol, stained with safranin for 30 min and fast green for 30 s. Stained sections were sealed with Canadian balsam and examined with a bright field microscopy (IX81, Olympus, Japan) at magnification 400 x. Images of the cross sections were taken and measured for widths of whole-leaf, palisade mesophyll, spongy mesophyll and abaxial and adaxial epidermal tissues with ImageJ (ImageJ 1.48v, NIH, USA).

3.2.4 Leaf hydraulic conductance

The hydraulic conductance of whole leaves (K_{leaf}) was performed according to Sack et al. (2002) with slight modifications. The second or lower fully expanded leaf (depending on the species) was cut next to the petiole stem insertion and immediately placed in a water bath. The petiole was cut under water with a sharp blade to 1 cm length, then wrapped with parafilm (to ensure good seal between petiole and tubing) and inserted into the silicon tube which was connected to the HPFM hydraulic measurement system as described by Tyree (Tyree et al., 2005). Briefly high pressure water was pressed into the leaf vein, leaves were perfused at 0.3 MPa with distilled water for around 60 min until steady-state conditions (±5%), the flow rate was recorded and used to calculate the leaf hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1}). Leaf area was measured afterward with a leaf area meter (Li-Cor 3000, LiCor, USA) to normalize hydraulic measurements by leaf area.

3.2.5 Stomatal characteristics and stomatal conductance

Stomatal traits were analyzed using a nail polish print method on the leaf abaxial side as describe by Mott (1991). The total stomatal aperture area per unit leaf area (cm^2 m^{-2}) was calculated as stomatal average density × stomatal aperture area. For detail see 2.2.3.

Stomatal conductance (g_s) was measured using a leaf porometer (AP4 porometer, Delta-T Devices, Cambridge, UK). The second/third fully developed leaf (different according to the plant species) was chosen for measurements. Four positions on each leaf were measured and the average result was used as the stomatal conductance of this leaf. *C. australis* is characterized by narrow leaves, which did not

allow a correct measurement of g_s by porometry, therefore g_s was estimated based on stomatal characteristics as described by (Franks and Farquhar, 2001):

$$g_s = \frac{SDDa'}{V\left(l + \frac{\pi}{4}\sqrt{\frac{a'}{\pi}}\right)}$$

Where SD = stomatal density (N m^{-2}), D = diffusivity of water in air (22℃, 24.5 × 10^{-6} m^2 s^{-1}), a' = stomatal aperture area (m^{-2}), V= molar volume of air (m^3 mol^{-1}), l = depth of stomatal pore (m, 12 × 10^{-6} m for *C. australis*, mean of 10 replicates).

3.2.6 Chlorophyll a fluorescence

The leaf chlorophyll fluorescence measurement was conducted using a PAM-2500 portable chlorophyll fluorometer (Walz, Effeltrich, Germany). The second fully expanded leaf of *S. speciosa* and the third leaf for *C. australis* and *F. benjamina* were selected for this measurement. Leaves were dark adapted with a leaf clip for 20 min, then a 0.6 s saturating light pulse (3,450 µmol m^{-2} s^{-1}) was given to obtain the F_m and F_0. After that, the leaf was light adapted with 5 min continuous actinic light (100 µmol m^{-2} s^{-1}, similar as the growing condition) with saturating pulses every 25 s, after that, the maximum light adapted fluorescence (F_m') and steady state fluorescence (F_s) were recorded. For the calculation of F_v/F_m, Φ_{PSII}, qP, NPQ and ETR see 2.2.4.

3.2.7 Pigments content

Leaf chlorophyll content was determined according to Lichtenthaler (2001). For details see 2.2.5.

3.2.8 Plant growth measurements

The second fully expanded leaf area counting from the apex was measured using a leaf area meter (Li-Cor 3000, Li-Cor, USA) this in four replicates. Four plants per treatment and cultivar were used for the biomass measurements. After aerial fresh weight (FW) determination plants were oven-dried at 85 ℃ for 3 days until a constant mass was reached to determine dry weight (DW).

3.2.9 Statistical analysis

Data are presented as means ± SE. Data were analyzed for light quality for each species by one-way analysis of variance (ANOVA), after verifying homoscedasticity by Levene's test. Tukey's HSD test was used to compare means at $p < 0.05$. Correlations between traits were tested using Pearson's correlation coefficients. A regression testing K_{leaf} as function of leaf thickness and stomatal conductance was performed. All statistical analyses were conducted using SPSS Statistics 22 (IBM Software, Chicago, USA).

3.3 Results

3.3.1 Biomass and leaf characteristics

In *C. australis*, total aboveground fresh weight was the greatest under W, followed by B and RB and significantly decreased under R, similar the dry weight was greatest under W and declined under R (Figure 3.2). Biomass (both FW and DW) of *F. benjamina* and *S. speciosa* were significantly lower under R, while no significant difference between the other light qualities were found.

The three species had very different leaf morphologies (Figure 3.2, Table 3.2). *C. australis* and *F. benjamina* had relative small leaves, while *S. speciosa* developed large leaves. B enhanced the leaf area of *F. benjamina* followed by RB and W while it significantly decreased under R. B tended to increase the individual leaf area in both *C. australis* and *S. speciosa* though this was not significant (P=0.070 and 0.183, respectively).

Leaf thickness in *C. australis* was highest under W followed by RB and B while the thinnest leaves were found under R (Table 3.2, Figure 3.3). As *C. australis* is a monocot, the leaf anatomy is isobilateral and the mesophyll is hardly differentiated into palisade and spongy parenchyma cells. Therefore only the adaxial and abaxial epidermal thickness was measured which contribute respectively 6.1 ± 0.23% and 6.7 ± 0.26% of the total leaf thickness. Abaxial epidermis was not affected by light quality while the thinnest adaxial epidermis was found under R while B and RB had the thickest epidermal cells.

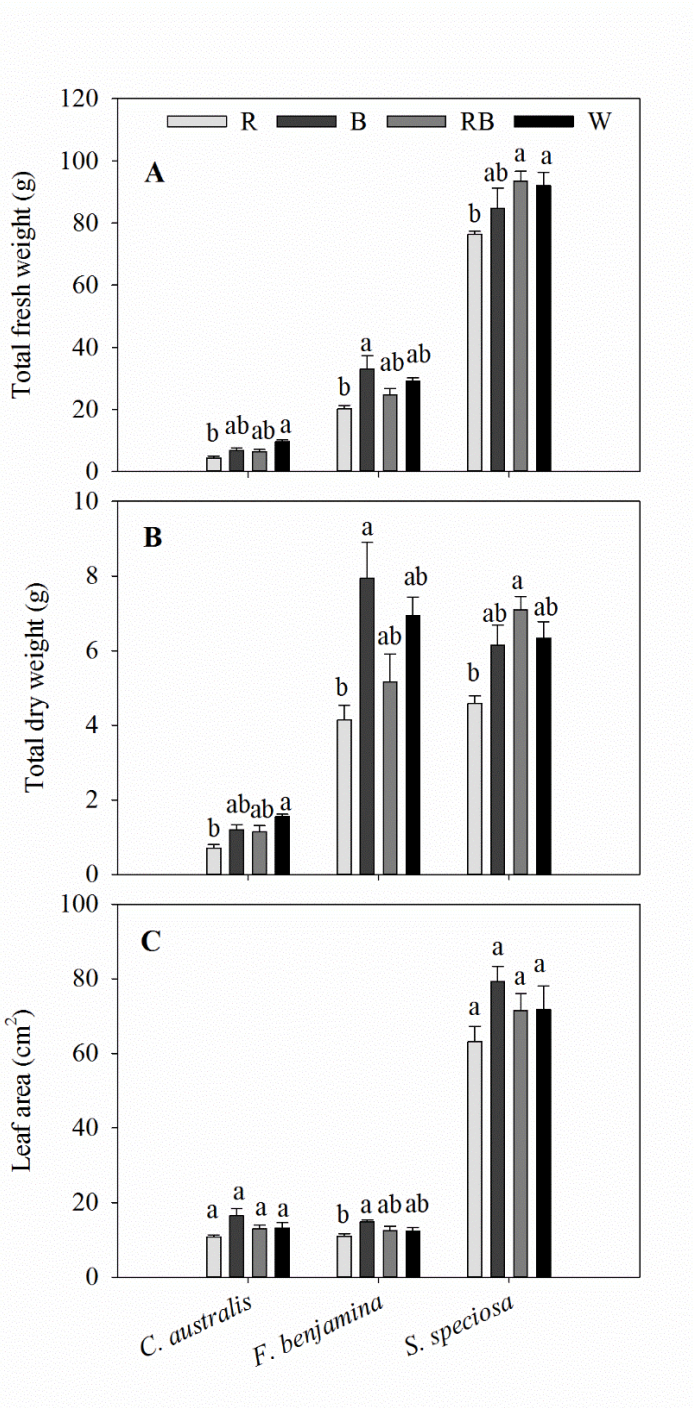

Figure 3.2 Effects of light quality on total aboveground fresh weight (A), total dry weight (B) and individual leaf area (C) of *C. australis, F. benjamina* and *S. speciosa*. Data are presented as means ± standard error (n = 4). Different letters indicate significant differences between values by Tukey's HSD test at P=0.05.

Leaf thickness in *F. benjamina* was greatest under B, lower under RB and W while it was significantly thinner under R (Table 3.2). *F. benjamina* has evergreen glossy leaves and the adaxial and abaxial epidermis contribute respectively 21.8 ± 1.0% and

10.6 ± 0.5% to the leaf thickness. Especially the adaxial epidermis is strongly reduced under R followed by W. The effect on the abaxial epidermis is not as strong though also here the thinnest cell layers are under R and W. The leaf thickness difference is strongly influenced by the mesophyll. In absolute value, the palisade parenchyma is highest under B although it represents only 15.5% of the total leaf thickness while the palisade layer is respectively 26% under RB and 24% under W. B also strongly enhances the spongy parenchyma while it is not affected by the other light qualities. In *S. speciosa*, leaf thickness was not affected by the different light qualities (Table 3.2). *S. speciosa* has velvety hairy leaves and the adaxial and abaxial epidermis contribute respectively 10.2 ± 0.5% and 6.5 ± 0.4% to the leaf thickness. Adaxial epidermal thickness was found thinnest under R while it tended to be thicker under B though not significantly differing from RB and W. Abaxial epidermis was thickest under B. Palisade parenchyma thickness was found lower under R and W and significantly greater under B and RB while no effect were found for the spongy parenchyma.

3.3.2 Leaf hydraulic conductance

Light quality tended to influence the leaf hydraulic conductance of the selected ornamentals though effects were not significant (Figure 3.4). In *C. australis* K_{leaf} was lowest under B and slightly increased under R, RB and W. In *F. benjamina* and *S. speciosa* K_{leaf} was lowest under R and highest under B. On average K_{leaf} was highest in *C. australis*, followed by F. benjamina and quite low in *S. speciosa*.

Correlation study between K_{leaf} and other leaf characteristics showed positive correlations with leaf thickness and stomatal conductance in *F. benjamina* and *S. speciosa* (Figure 3.5). However, for the monocot *C. australis*, a negative trend with stomatal conductance was found and no correlation with leaf thickness.

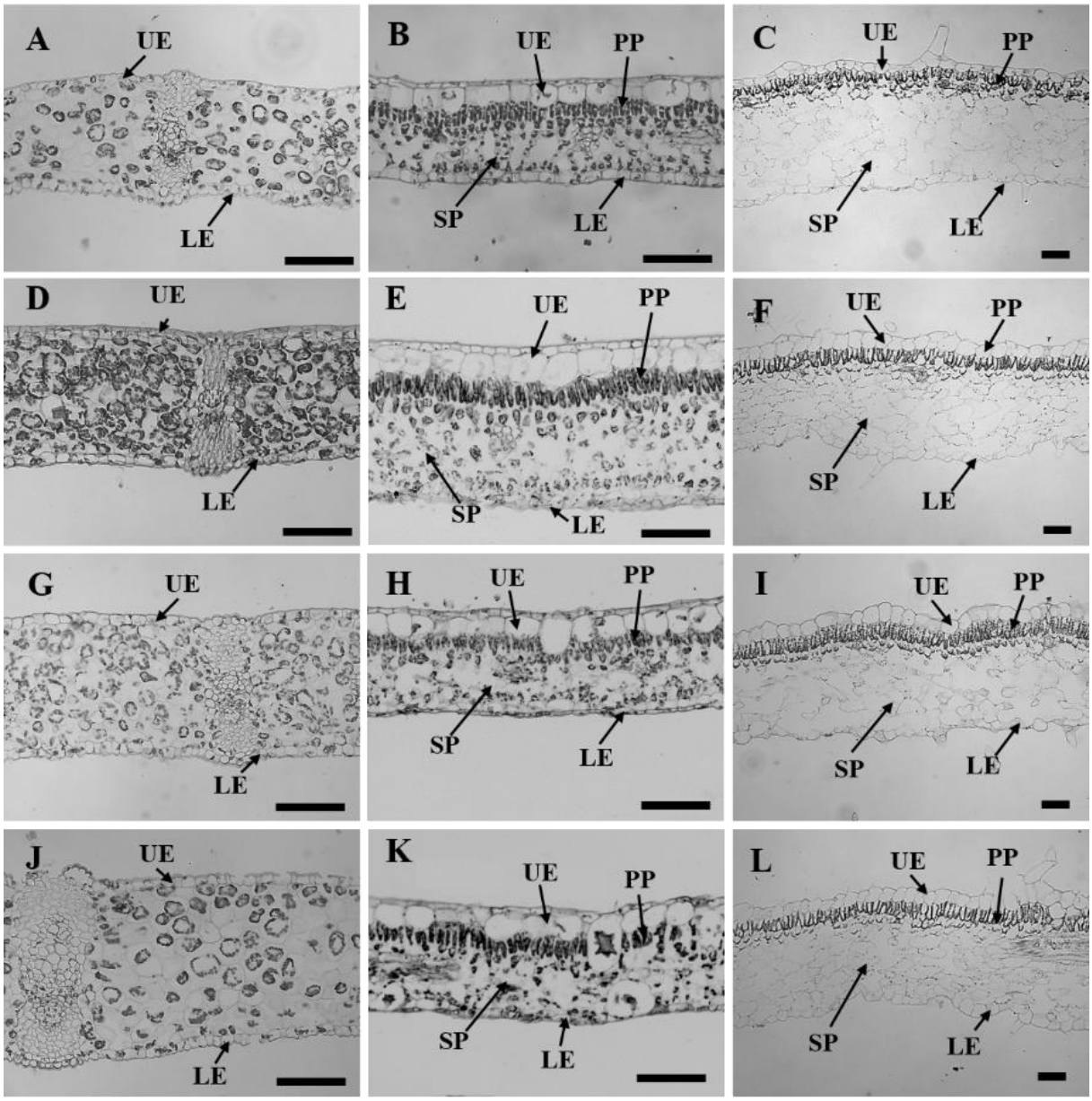

Figure 3.3 Leaf sectioning anatomy of *C. australis* (left panel), *F. benjamina* (middle panel) and *S. speciosa* (right panel) developed under Red light (A, B and C), Blue light (D, E and F), Red with Blue (G, H and I) and White (J, K and L). UE: upper epidermal; LE: lower epidermal; PP: palisade parenchyma; SP: spongy parenchyma. Black bar = 100 μm.

Table 3.2 Effects of light quality on the leaf anatomy of leaves of *C. australis*, *F. benjamina* and *S. speciosa*.

Species	Light quality	Adaxial epidermis (µm)	Abaxial epidermis (µm)	Palisade parenchyma (µm)	Spongy parenchyma (µm)	Leaf thickness (µm)
C. australis	R	10.06 ± 0.69 b	12.25 ± 0.55 a	/	/	168.97 ± 3.46 c
	B	12.90 ± 0.56 a	14.81 ± 0.69 a	/	/	196.29 ± 0.78 b
	RB	13.50 ± 0.39 a	12.22 ± 0.54 a	/	/	205.53 ± 1.42 b
	W	12.63 ± 0.84 ab	14.88 ± 0.75 a	/	/	244.44 ± 3.29 a
F. benjamina	R	28.10 ± 0.59 c	18.58 ± 0.76 ab	20.10 ± 1.41 c	83.40 ± 3.99 b	150.19 ± 3.88 c
	B	45.95 ± 1.08 a	20.97 ± 0.83 a	35.68 ± 0.59 a	127.63 ± 2.75 a	230.28 ± 2.82 a
	RB	43.64 ± 0.72 ab	19.97 ± 0.90 a	23.01 ± 0.63 c	81.81 ± 3.43 b	168.43 ± 4.62 b
	W	40.33 ± 1.14 b	16.69 ± 0.46 b	27.31 ± 0.90 b	95.14 ± 2.98 b	179.46 ± 3.43 b
S. speciosa	R	31.33 ± 0.92 b	21.61 ± 1.81 b	45.43 ± 2.16 b	282.18 ± 17.64 a	380.54 ± 18.65 a
	B	46.85 ± 1.14 a	32.94 ± 2.35 a	53.70 ± 1.05 a	264.56 ± 13.91 a	398.05 ± 17.83 a
	RB	42.12 ± 1.85 a	25.03 ± 0.94 b	57.13 ± 1.11 a	280.44 ± 4.17 a	404.71 ± 6.69 a
	W	43.11 ± 0.99 a	24.33 ± 1.55 b	45.12 ± 1.49 b	301.09 ± 9.54 a	413.64 ± 8.95 a

Data given as means ± SE (n = 5). Different letters indicate significant differences between values ($p < 0.05$) for each parameter.

3.3.3 Stomatal characteristics and stomatal conductance

The effects of light quality on the stomatal characteristics are given in Table 3.3. The aperture length was not affected by light quality, this for the three species. An increase of aperture area was found in *C. australis* under B, while no effects were found in *F. benjamina* and *S. speciosa.* The width/length ratio was not affected by light quality (data not shown). Total aperture area per unit leaf area was not affected by light quality though it tended to be lower under R for *F. benjamina* and *S. speciosa*. Stomatal index and density were significantly affected by the light quality treatments. In *C. australis* stomatal index decreased under R though density was not affected. *C. australis* also showed the highest stomatal density of the studied ornamentals, as it ranged between 274.75 N° mm^{-2} under B up to 325.10 N° mm^{-2} under R. Likewise a high density of epidermal cells per unit leaf area was present (Table 3.3). In *F. benjamina*, both R and B gave the lowest stomatal index while the highest index was found under W; the stomatal density was lowest under R and highest under W. In *S. speciosa* both the highest stomatal density and index were found under B and W and the lowest under R.

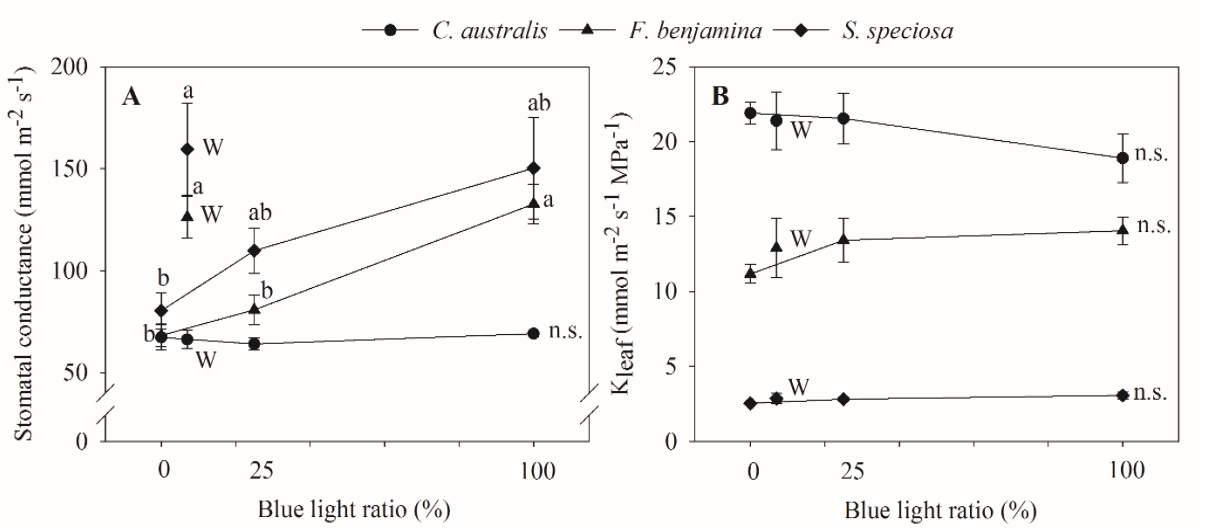

Figure 3.4 Effects of blue light ratio on stomatal conductance (A) and leaf hydraulic conductance (B) of *C. australis, F. benjamina* and *S. speciosa*. Data are presented as means ± standard error (n = 4). Different letters indicate significant differences between values ($p < 0.05$) according to Tukey's HSD test and n.s. indicates no significant differences. W indicates the multispectral white treatment.

Table 3.3 Effects of light quality on the stomatal characteristics of leaves of *C. australis*, *F. benjamina* and *S. speciosa.*

Species	Light quality	Aperture length (µm)	Aperture width (µm)	Aperture area (μm^2)	Total aperture area/leaf area ($cm^2\ m^{-2}$)	Stomatal index (%)	Stomatal density ($N\ mm^{-2}$)	Epidermal cell density ($N\ mm^{-2}$)
C. australis	R	10.4 ± 0.7 a	3.2 ± 0.2 ab	26.3 ± 0.8 b	88.3 ± 8.5 a	18.4 ± 1.3 b	325.1 ± 5.2 a	1502.8 ± 33.9 a
	B	11.4 ± 0.3 a	3.8 ± 0.1 a	33.7 ± 0.8 a	92.5 ± 1.8 a	23.6 ± 1.3 a	274.6 ± 4.2 a	1174.4 ± 44.0 b
	RB	10.8 ± 0.2 a	3.1 ± 0.1 b	26.4 ± 1.2 b	84.0 ± 4.0 a	24.6 ± 1.5 a	320.3 ± 17.9 a	1315.5 ± 59.5 ab
	W	11.1 ± 0.4 a	3.3 ± 0.1 ab	28.8 ± 1.5 ab	87.5 ± 6.0 a	24.1 ± 1.0 a	304.4 ± 9.6 a	1264.6 ± 38.4 b
F. benjamina	R	12.6 ± 0.3 a	5.2 ± 0.2 ab	51.2 ± 2.1 a	67.0 ± 7.5 a	13.9 ± 0.7 b	130.1 ± 10.2 b	935.4 ± 33.4 a
	B	13.1 ± 0.8 a	5.7 ± 0.2 a	58.8 ± 4.9 a	84.0 ± 7.1 a	15.4 ± 0.8 b	143.6 ± 8.1 ab	935.8 ± 17.8 a
	RB	13.2 ± 0.3 a	5.0 ± 0.2 ab	52.0 ± 2.1 a	86.2 ± 4.0 a	19.0 ± 1.0 ab	165.8 ± 5.0 ab	877.9 ± 25.0 ab
	W	12.7 ± 0.4 a	4.8 ± 0.1 b	48.2 ± 2.3 a	83.7 ± 3.6 a	22.0 ± 2.0 a	175.5 ± 6.5 a	799.1 ± 15.6 b
S. speciosa	R	15.8 ± 0.8 a	5.5 ± 0.2 a	68.42 ± 3.8 a	25.8 ± 3.0 a	17.4 ± 0.7 b	37.7 ± 1.9 b	872.4 ± 27.4 a
	B	18.6 ± 0.5 a	6.2 ± 0.2 a	91.51 ± 3.8 a	46.9 ± 3.0 a	25.8 ± 1.3 a	51.2 ± 1.9 a	805.6 ± 11.2 a
	RB	18.2 ± 0.7 a	6.9 ± 0.6 a	99.83 ± 11.2 a	43.0 ± 6.5 a	21.1 ± 1.6 ab	42.4 ± 2.6 ab	815.2 ± 32.8 a
	W	16.1 ± 1.2 a	5.9 ± 0.3 a	76.10 ± 9. a	40.6 ± 7.1 a	24.6 ± 1.6 a	52.9 ± 4.1 a	866.3 ± 40.2 a

Data given as means ± SE (n = 5). Different letters indicate significant differences between values ($p < 0.05$) for each parameter.

The stomatal conductance of the ornamentals was differentially affected by the different light qualities (Figure 3.4). For *C. australis*, no effects were noted on the stomatal conductance with respect to increasing B. For both *F. benjamina* and *S. speciosa* stomatal conductance increased with increasing B when comparing R, RB and B. However, multispectral W yielded the highest stomatal conductance in both species. A strong correlation of stomatal density (r=0.979) with g_s and stomatal index (r=0.995) with g_s was found *in S. speciosa*.

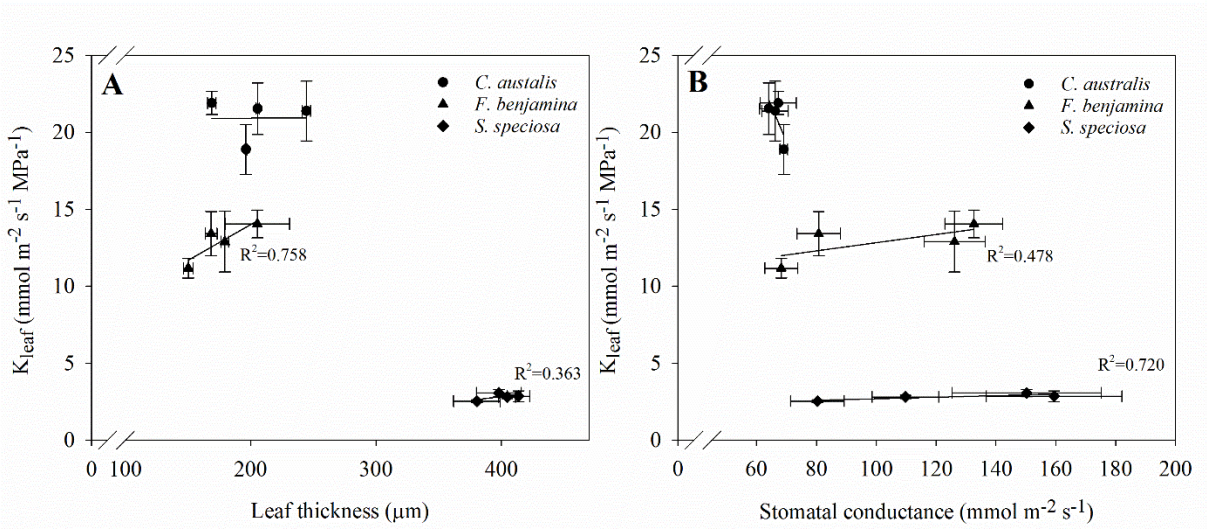

Figure 3.5 Correlation analysis between K_{leaf} and leaf thickness and stomatal conductance of *C. australis*, *F. benjamina* and *S. speciosa* under different light quality. Values presented the mean of four replicates with standard errors (n=4).

3.3.4 Chlorophyll a fluorescence

Effects of light quality on chlorophyll fluorescence parameters of the studied ornamentals are given in Table 3.4. The maximum quantum efficiency F_v/F_m, was influenced by the applied light quality and overall we saw a lower value of F_v/F_m for R (P=0.003). For *C. australis*, the lowest value was observed under R, F_v/F_m increased under W and RB while B gave the highest F_v/F_m value. For *F. benjamina* and *S. speciosa* F_v/F_m declined under R compared to the other spectral qualities.

Φ_{PSII}, qP and ETR showed a similar reaction to the light quality treatments. For both *C. australis* and *S. speciosa* the lowest values for Φ_{PSII} were observed under R. For *F.*

benjamina, Φ_{PSII} was significant higher under B, while R and W gave lower values. For both *C. australis* and *S. speciosa* highest qP were found for RB and W while no effect of light quality was found for *F. benjamina*.

NPQ significantly increased under B followed by RB compared to W and R in *C. australis*, while for *S. speciosa*, it is significantly greater under R and W followed by B compared with RB. However, for *F. benjamina*, no effect of light quality was found on NPQ (P = 0.117), though it tended to be higher under W.

Leaf thickness correlated with Φ_{PSII} in *C. australis* (r=0.855) but this correlation was weaker in *F. benjamina* (r=0.622) while thickness of the palisade parenchyma correlated moderately with Φ_{PSII} in *S. speciosa* (r=0.674).

3.3.5 Leaf pigment contents

The total pigment content was different between the species (Figure 3.6). In *F. benjamina*, the total chlorophyll content ranged from 1.102 to 1.338 mg g^{-1}, while it was 0.395 to 0.668 mg g^{-1} and 0.395 to 0.668 mg g^{-1} for *S. speciosa* and *C. australis*, respectively. The carotenoids were higher in *C. australis* (0.103-0.138 mg g^{-1}) and *F. benjamina* (0.100-0.190 mg g^{-1}) followed by *S. speciosa* (0.050-0.103 mg g^{-1}). Overall the total chlorophyll content was not significantly affected by the light quality (P= 0.468) though there were species differences (Figure 3.56). In *C. australis* the highest Chl a, Chl b and Chl a/b was found under RB and the lowest content was found under R, while no significant effect on carotenoid content was present. In *F. benjamina*, no significant effects of light quality on chlorophyll and carotenoid content were observed. Blue light yielded the highest Chl a, Chl a/b and carotenoid content in *S. speciosa* leaves followed by R. The lowest Chl a, Chl a/b and carotenoid content were found for W, this treatment lead to a decrease of 55% and 51% for Chl a and carotenoids compared to B.

Table 3.4 Effect of light quality on chlorophyll fluorescence parameters: F_v/F_m, Φ_{PSII}, qP, NPQ and ETR for *C. australis*, *F. benjamina* and *S. speciosa.*

Species	Light quality	F_v/F_m	Φ_{PSII}	qP	NPQ	ETR
C. australis	R	0.536 ± 0.040 c	0.349 ± 0.034 b	0.791 ± 0.030 b	0.303 ± 0.027 c	13.75 ± 1.25 b
	B	0.738 ± 0.009 a	0.427± 0.019 ab	0.814 ± 0.023 ab	0.934 ± 0.091 a	16.60 ± 0.75 ab
	RB	0.702 ± 0.008 ab	0.477 ± 0.014 a	0.873 ± 0.007 a	0.692 ± 0.048 b	18.40 ± 0.60 a
	W	0.654 ± 0.014 b	0.479 ± 0.012 a	0.887 ± 0.003 a	0.440 ± 0.051 c	18.60 ± 0.60 a
F. benjamina	R	0.745 ± 0.005 b	0.603 ± 0.024 bc	0.898 ± 0.023 a	0.270 ± 0.066 a	15.80 ± 0.58 bc
	B	0.792 ± 0.003 a	0.677 ± 0.003 a	0.941 ± 0.007 a	0.272 ± 0.025 a	17.80 ± 0.20 a
	RB	0.785 ± 0.007 a	0.662 ± 0.010 ab	0.937 ± 0.011 a	0.252 ± 0.011 a	17.17 ± 0.31 ab
	W	0.772 ± 0.006 a	0.598 ± 0.011 c	0.890 ± 0.006 a	0.447 ± 0.109 a	15.25 ± 0.48 c
S. speciosa	R	0.628 ± 0.021 b	0.358 ± 0.031 b	0.786 ± 0.025 b	0.666 ± 0.049 a	13.60 ± 1.21 b
	B	0.733 ± 0.011 a	0.490 ± 0.041 a	0.862 ± 0.023 ab	0.582 ± 0.088 ab	18.80 ± 1.66 a
	RB	0.745 ± 0.010 a	0.598 ± 0.007 a	0.940 ± 0.007 a	0.364 ± 0.028 b	23.00 ± 0.32 a
	W	0.749 ± 0.005 a	0.520 ± 0.021 a	0.877 ± 0.022 a	0.605 ± 0.054 a	19.80 ± 0.86 a

Data given as means ± SE (n = 5). Different letters indicate significant differences between values ($p < 0.05$) for each parameter.

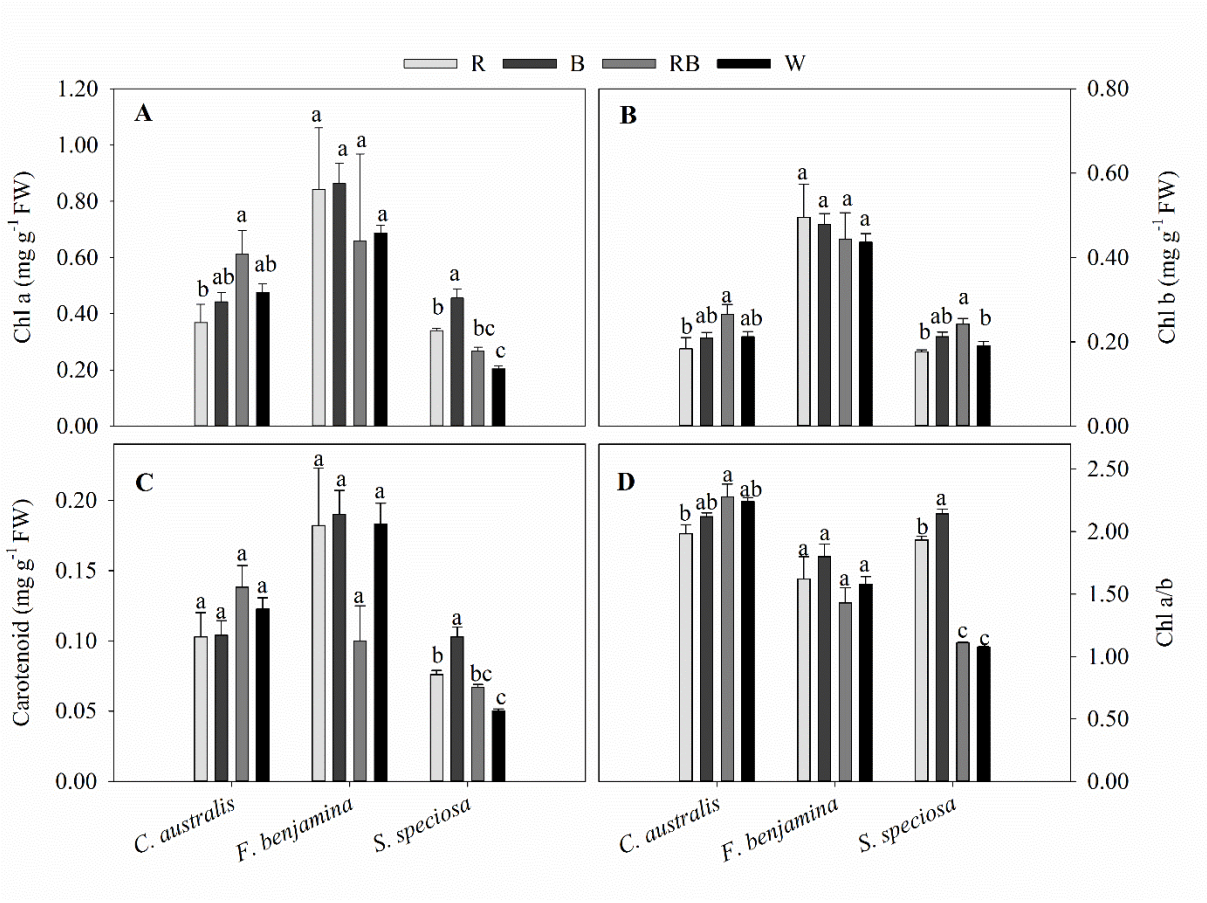

Figure 3.6 Effects of light quality on chlorophyll a and b (A, B) and carotenoid (C) content and Chl a/b ratio (D) of *C. australis*, *F. benjamina* and *S. speciosa*. Different letters indicate significant differences between values according to Tukey's HSD test (P = 0.05).

3.4 Discussion

Leaf photosynthesis requires the interception of light. Light inside the leaf is influenced by the wavelength, the light level and the angle of the incident light (Brodersen and Vogelmann, 2010) as well as by the leaf anatomy. Light is absorbed by chloroplasts while passing through the palisade and spongy mesophyll. The vertically elongated palisade cells minimize light scattering, allowing a deeper penetration, while spongy tissue enhances the light capture by scattering light (Evans, 1999). *F. benjamina* and *S. speciosa* are both dicots with palisade and spongy mesophyll. *F. benjamina* reacted strongly to B not only in total leaf thickness but also by an increasing effect on all anatomical structures. Reduction or absence of blue light decreased leaf thickness and respective anatomical structures and this was

most pronounced for monochromatic R. This reaction reflects the observations on pepper (Schuerger et al., 1997) and wheat (Goins et al., 1997) where increased levels of B to R increased the palisade and spongy mesophyll thickness. In *S. speciosa,* however, total leaf thickness was not affected but a reorganization of the mesophyll resulting in a higher percentage of palisade parenchyma (16%) was observed for B and RB while for W and R the palisade parenchyma averaged 13% of the total mesophyll. The greater cell surface area per unit of mesophyll volume makes palisade tissue a more efficient structure in term of photosynthesis than spongy mesophyll (Evans, 1999). For the monocot *C. australis*, the full spectrum W resulted in the thickest leaves though comparing R with RB and B also indicated the favorable effect of B on leaf thickness.

Schuerger et al. (1997) also reported an effect of blue light on secondary xylem formation in peppers suggesting an effect of light quality on water translocation. Buckley et al. (2015) suggested that greater leaf thickness should contribute to a higher leaf conductance (K_{leaf}) given the greater number of parallel pathways for horizontal transport to the sites of evaporation, if those sites are distributed throughout the leaf. More specifically the maximal K_{leaf} correlated with palisade thickness, and palisade/spongy mesophyll ratio for tropical rainforest tree species (Sack and Frole, 2006). K_{leaf} of bur oak enhanced under blue and green light compared to other wavelengths (Voicu et al., 2008). However, in bur oak one focused mainly on short term responses to light quality while this study was conducted on leaves that were formed under a given spectral light quality. Therefore, effects on K_{leaf} can be attributed to differences in the development of leaf mesophyll and veins. K_{leaf} varied strongly between the studied species and was much greater in *C. australis* than in *S. speciosa,* while *F. benjamina* was intermediate (Figure 3.4). This variation in K_{leaf} among species is reported by several authors and can fluctuate up to 65-fold across plant species (Brodribb et al., 2012; Buckley, 2015; Sack and Holbrook, 2006). Under B, K_{leaf} of the dicots *F. benjamina* and *S. speciosa* tended to be higher. This is in agreement with Savvides et al. (2012), who were the first to report that cucumber leaves that developed under B and RB had a higher K_{leaf}. Furthermore K_{leaf} correlated with thickness of leaf (r=0.79) and palisade parenchyma (r=0.78) in *F. benjamina* as well as in *S. speciosa* (r =0.46 and r =0.50 respectively) (Figure 6). In contrast, we found quite different results in the monocot *C australis*,

where K_{leaf} was independent of leaf thickness. The leaf anatomical structure of monocots makes that water in the major vein exits into surrounding tissue of bundle sheath cells instead of the minor veins (Xiong et al., 2015). We did not quantify leaf venation in this study although it might influence the leaf hydraulic conductance (Nardini et al., 2003). However, it is more likely that the small variations in both K_{leaf} and leaf thickness explain the absence of a relation in *C. australis*. K_{leaf} and g_s correlated positively (r= 0.48 and 0.72 respectively) in both *F. benjamina* and *S. speciosa* which agrees with previous observations (Augé et al., 2008; Brodribb et al., 2012; Savvides et al., 2012).

Stomatal development is influenced by light quality, which in turn will influence the conductance (g_s) of air through the leaf mesophyll and stomata. Blue light increased the stomatal density of *Chrysanthemum* (Kim et al., 2004; Table 2.3) and this was also observed in *F. benjamina* and *S. speciosa*. Moreover, additional blue light increased the stomatal index in all the studied species and both parameters (stomatal index and stomatal density) were highly correlated in *F. benjamina* and *S. speciosa* (r=0.99 and 0.97, respectively). These results reflect the effect of blue light on the development of stomata, which is mediated through the additive function of CRY1 and CRY2 (Pillitteri and Torii, 2012). Stomatal density and index are not correlated in *C. australis*, which is due to the lower stomatal density under blue (Table 3.3). In *C. australis* the total number of epidermal cells per unit of area was also reduced under B in comparison with monochromatic R, indicating larger epidermal cells under B. Likewise in *Pelargonium* leaves the positive effect of blue light on the elongation of epidermal cells was shown (Fukuda et al., 2008).

However, not only the stomatal density but also the additive effect of the stomatal aperture influences the stomatal conductance. It is well known that blue light affects stomatal opening through the photoreceptors phototropin and cryptochrome (Boccalandro et al., 2012; Liscum et al., 2003; Shimazaki et al., 2007). Because of this blue light signaling, increased stomatal conductance if blue is added to red might be expected. Indeed, we found a positive effect if B was added to the R spectrum on the stomatal conductance in *F. benjamina* and *S. speciosa* (Figure 3.4). Likewise, blue light or addition of B to the spectrum enhanced the total aperture area per unit of leaf area in both *F. benjamina* and *S. speciosa* (Table 3.3) even though the correlations with g_s were not significant (r=0.61 and 0.79, respectively). In cucumber,

the decline of stomatal conductance under monochromatic green, yellow and red light correlated also with reduced photosynthesis (Wang et al., 2009). However, we did not find significant correlations between g_s and Φ_{PSII} in *F. benjamina* and *S. speciosa*. The lower light intensities in this study (100 µmol m^{-2} s^{-1} compared to 350 µmol m^{-2} s^{-1} in cucumber) may indicate that we were still below the threshold of g_s to limit photosynthesis.

Chlorophyll content directly influences the photosynthetic potential as well as the primary production (Curran et al., 1990; Gitelson et al., 2003). Also the chlorophyll content is affected by the light quality and several studies showed the beneficial effect of blue in the light spectrum (Hoffmann et al., 2015b; Sæbø et al., 1995). Long-term exposure of leaves to blue light enhances the 5-aminolevulinic acid synthesizing activity (Kamiya et al., 1983) which in turn mediates the biosynthesis of all tetrapyrroles such as hemes and chlorophylls. Also in our study, B or RB was favorable for chlorophyll content in *S. speciosa* and *C. australis* though this effect was not very strong. For *F. benjamina* no effects on chlorophyll content were found. This differential response might be due to species effects as also Lin and Hsu (2004) found no effect on pigment content in lettuce leaves.

Different wavelengths penetrate differently into the leaf, blue and red are efficiently absorbed close to the surface, whereas green light contributes more to photosynthesis in deeper leaf layers (Brodersen and Vogelmann, 2010; Sun et al., 1998). In spinach leaves blue light was almost completely absorbed at 300 µm leaf depth while red tailored to 400 µm and green light to 600 µm leaf depth (Evans, 1999). This reflects the more effective absorption of blue light by chlorophyll (Terashima et al., 2009). Thicker leaves and thicker palisade parenchyma may thus lead an increased light absorption and will therefore benefit the photosynthetic yield at leaf level (Haliapas et al., 2008; Hanba et al., 2002; Shengxin et al., 2016). The decrease in leaf mesophyll thickness by red light led to a lower photosynthetic yield and photochemical quenching (Table 3.2 and 3.4), so leaf thickness did contribute to the higher photosynthetic performance under B and RB in this study. The reduced Φ_{PSII} in *F. benjamina* under W (leaf thickness=179.46 µm) compared to B (leaf thickness=230.28 µm) might be explained by the partial absorbance of the green wavelengths which were not captured by the photosynthetic pigments (Fankhauser and Chory, 1997) though we did not observe this in the monocot species, *C. australis*.

Irrespective of the penetration depths of light, the applied light quality strongly influenced the photosynthetic efficiency (F_v/F_m, Φ_{PSII}) and R had a significant negative effect in the three species. This negative effect of monochromatic R was already reported in cucumber (Savvides et al., 2012; Wang et al., 2009), despite the fact that R coincides with the absorbance peak of chlorophyll and is known for its higher relative quantum efficiency than B in the instantaneous photosynthetic response (McCree, 1971). Tennessen et al. (1994), however, showed that long term monochromatic R causes an imbalance of photons available to Photosystem I and Photosystem II. Long term absence of blue light reduces the photosynthetic performance which is known as the 'red light syndrome' (Trouwborst et al., 2016). This leads to photo-damage as shown by the reduced F_v/F_m in this experiment. The energy distribution between PSII and PSI is affected by light through a state transition process. The light-harvesting antenna of the two Photosystems have distinct absorption spectra; excitation of PSI is obtained by far red while PSII is preferentially excited with red light (Walters and Horton, 1994). Energy distribution from light harvesting antenna (LHCII) is regulated by protein phosphorylation. Phosphorylation of LHCII complexes causes them to migrate away from PSII towards PSI, thereby altering the distribution of excitation energy between the two Photosystems. If only red light is provided a strong overall phosphorylation of both the PSII core and LHCII takes place and PSI excitation is strongly favored over PSII, leading to imbalances in Photosystem excitation (Ferroni, 2012; Tikkanen et al., 2010). The effects of additional blue light on photosynthetic performance are integrated in the produced plant biomass, which was lowest under R in the three species while no significant differences in B, RB and W were found.

3.5 Conclusion

We show here for the first time how narrow-band R, B and RB modulates leaf morphology, mesophyll anatomy, stomatal formation and hydraulic conductance of leaves of *Cordyline australis*, *Ficus benjamina* and *Sinningia speciosa* in comparison with broad-spectrum white-light-emitting diodes.

Blue light enhanced leaf thickness in *C. australis* and *F. benjamina* and palisade parenchyma thickness in *S. speciosa*, which suggest a better light absorption for this treatment. Adding blue to red light increased the stomatal index in the three species

and enhanced the total aperture per leaf unit in *F. benjamina* and *S. speciosa*. Although K_{leaf} was not significantly affected by light quality a moderate correlation between K_{leaf} and leaf thickness and K_{leaf} and stomatal conductance was found for both dicot species *F. benjamina* and *S. speciosa* though not for the monocot *C. australis*.

Leaves of the three species that developed solely under red light were characterized by a lower F_v/F_m and Φ_{PSII} indicating a malfunctioning of photosynthesis, which also resulted in a lower dry mass production under red. The chlorophyll fluorescence parameters of the other three light treatments (B, RB and W) were hardly influenced and the dry weight production was not influenced.

Chapter 4

Light quality differentially modifies *Chrysanthemum* morphology, photosynthetic efficiency and antioxidant capacity

This chapter is based on:

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Author contribution:

LZ and MCVL conceived and designed the experiments. LZ performed the experiments, analyzed the data and drafted the manuscript, Mireia Morera Font, master student of Ghent University, conducted the analysis of proline and hydrogen peroxide content. MCVL critically revised the manuscript.

Abstract

The effect of light quality on leaf morphology, photosynthetic efficiency and antioxidant capacity of leaves that fully developed under a specific spectrum was investigated in *Chrysanthemum* cv. Four light treatments were applied at 100 µmol m^{-2} s^{-1} and a photoperiod of 14 hours using light-emitting diodes, which were 100% red (R), 100% blue (B), 75% red with 25% blue (RB) and white (W), respectively. Intraspecific variation was investigated by studying the response of eight cultivars. Overall, red light significantly decreased the leaf area while the thinnest leaves were observed for W. Chlorophyll content and Chl a/b ratio was highest for W and lowest under R. B and RB resulted in the highest maximum quantum yield (F_v/F_m) and quantum efficiency (Φ_{PSII}). A negative correlation between heat dissipation (NPQ) and ΦPSII was found. Blue light induced the highest hydrogen peroxide content, which is a proxy for total ROS generation, followed by W and RB while low contents were found under R. The antioxidative response was not always correlated with hydrogen peroxide content and differed depending on the light quality treatment. Blue light enhanced the proline levels, while carotenoids, total flavonoid and phenolic compounds were higher under W. Intraspecific variation in the responses were observed for most parameters with exception of leaf thickness; this intraspecific variation was most pronounced for total phenolic and flavonoid compounds.

4.1 Introduction

Light is an important environmental factor, which regulates plant growth and development. Plants capture light energy for photosynthesis as well as for light signaling in different regulatory processes (Jiao et al., 2007). Changes in light quality or intensity cause responses at physiological and biochemical level thus influencing plant morphology and functioning (Eskins et al., 1991; Zhiyu et al., 2007). Increasing interest in vertical farming systems with artificial light as the solely light source brings potential for the use of light-emitting diodes (LED). This technology allows the application of monochromatic wavelengths or their combinations to optimize plant growth. It also implies that plants develop for a longer period under these specific spectra.

Growth relies primarily on photosynthesis. Earlier studies reported already that plants grown under blue light were characterized by a higher Chl a/b ratio and yielded higher photosynthetic electron transport rates than plants grown under red light (Eskins et al., 1991; Sharkey and Raschke, 1981). Hogewoning et al. (2010b) studied in detail effects of blue and red combinations on cucumber leaf physiology and described disorders when only red light was supplemented. Lower photosynthetic rates under red light could not be ascribed to lower chlorophyll or nitrogen content or to starch accumulation. It was also suggested that a minimal blue light threshold was needed for synthesis of PSII core proteins. In contrast, 100 % blue light did not lead to a dysfunction in photosynthesis. Chlorophyll quenching analysis indicated that non-regulated energy loss (Φ_{NO}) in Photosystem II in cucumber leaves was more pronounced under monochromatic red than under red+blue combinations (Trouwborst et al., 2016). For *Chrysanthemum*, however, low natural light fluencies supplemented with red or red+blue LED light did not affect photosynthetic rates (Ouzounis et al., 2014).

Spectral light quality not only affects primary metabolism but has also effects on nutraceuticals in vegetables (Ouzounis et al., 2015c; Piovene et al., 2015). These secondary metabolites, which include phenolic and flavonoid compounds are also part of the defense responses of plants to both biotic and abiotic stress (Ventura-Aguilar et al., 2013). Most of the phenolic and flavonoid compounds have free radical scavenging capacities or reduce free radical generation by donating electrons or

hydrogen (Asada, 1999; Smirnoff, 1998). However, the role of light quality in the biosynthesis of secondary metabolites in plant cells is not well established and species-specific differences are reported (Taulavuori et al., 2016). A few studies have been published on ornamental species, such as *Campanula, Kalanchoe pinnata, Prunella vulgaris* and rose (Fazal et al., 2016; Nascimento et al., 2013; Ouzounis et al., 2014), however, knowledge on how light quality might affect upregulation of secondary metabolites and thus contribute to its defense mechanism is still not well developed.

Many studies have investigated the effects of light quality on the morphology of ornamentals (Jeong et al., 2006; Mortensen and Strømme, 1987; Runkle and Heins, 2001). *Chrysanthemum* (*Chrysanthemum* x *morifolium*) is an important ornamental plant and effects of B/R ratio on morphology such as leaf expansion , internodes and bud development are often reported (Jeong et al., 2014; Kim et al., 2004). Also inhibition of stem elongation increases with the increase of blue light proportion (Oyaert et al., 1999; Shimizu et al., 2006). Irrespective of its production as cut flower or pot plant the young plant phase could take place in multilayer systems using solely artificial LED light. Light quality will, however, not only impact plant morphology but also affect other physiological and biochemical characteristics. Flavonoids were favored by increasing the blue light component in *Chrysanthemum* (Jeong et al., 2012; Ouzounis et al., 2014).

*Chrysanthemum*s are complex hybrids, genetic material of multiple species are used during a long period of breeding and selection (Zhang et al., 2014). Classification systems are mainly based on phenotypic traits with flower head type and flower diameter as the principal traits. Interspecific hybridizations in pot *Chrysanthemum* lead to a low growing phenotype with a symmetrical hemisphere; at flowering the outer surface is completely covered with flowers (cushion type). This phenotype has the majority market share in pot *Chrysanthemum*s (Anderson, 2006).

The objective of the present study was to determine effects of different light qualities (R/B ratios) on photosynthetic performance of pot *Chrysanthemum* by studying chlorophyll fluorescence parameters. As it is known that, the antioxidative status of the plants might be affected especially under blue light we hypothesized that this biosynthesis is linked to the magnitude of oxidative stress. As phenotypic plasticity is

an adaptive trait, we investigated if light quality differentially induced non-enzymatic antioxidants by determining carotenoids, proline, total polyphenols and flavonoids. As responses to narrow band light quality differ greatly between species but inter-species effects are hardly studied, we evaluated 8 cultivars with a cushion type phenotype to obtain information of potential intraspecific variation as well.

4.2 Materials and Methods

4.2.1 Plant material and experimental set up

The experiment was conducted in a climate chamber using eight cultivars of *Chrysanthemum morifolium*. All cultivars had a cushion type phenotype and could be divided in three groups with respect to their breeding background (1) 'Marco' a late flowering cultivar (2) 'Orlando' and 'Tappino' fast branching and late flowering cultivars; these two cultivars share one common parent and (3) 'Bolero', 'Lana', 'Loretto', 'Katelijn', 'Orlando' and 'Sunny' which share an ancestral parent, in this group 'Katelijn' and 'Loretto' are early flowering cultivars. Rooted cuttings were obtained from a commercial young plant producer (Dataflor, Belgium) and transplanted into 0.3 L pots with commercial peat-based substrate (Van Israel nv, Belgium). After an acclimation period of 7 days under a full spectrum light provided by high pressure sodium lamps (SON-T, 400 W, Philips, Eindhoven, The Netherlands) at 100 µmol m^{-2} s^{-1}, plants were pinched and 12 replicates per treatment were randomly allocated to four spectral light treatments (Figure 3.1, Table 3.1). The different light quality treatments were provided by light-emitting diodes (LEDs), which were white [W, 7 % blue (400-500 nm), 16% green (500-600 nm), 75% red (600-700 nm) and 2% far red (700-800 nm)] (GreenPower LED production module, Philips, Eindhoven, The Netherlands), blue (B, peak at 460 nm) (GreenPower LED research module, Philips, Eindhoven, The Netherlands), red (R, peak at 660 nm) (GreenPower LED production module), as well as a combination of red with blue (RB, 75%/25%) with a programmable LED experimentation system (CI-800, CID Bio-science, WA, USA), respectively. Light intensity was set at 100 µmol m^{-2} s^{-1} and verified by measuring the light intensity at five points of each light treatment at the canopy level (Table 4.1). Plants received a photoperiod of 14h. The light spectral distribution was measured using a spectrometer (JAZ-ULM-200, Ocean Optics, US) and converted with Spectrasuite (Ocean Optics) to µmol m^{-2} s^{-1} (Figure 3.1). The air temperature

was maintained at 20 ℃. Irrigation and fertilization with a water-soluble fertilizer (NPK 4-1-2, EC = 1.5 dS m^{-1}) was applied twice a week.

Plants grew under the light treatments for 4 weeks, after that, all the analyses were performed on the third and fourth leaf counting from the apex (fully expanded leaves that developed entirely under the given light quality). Leaves at the same position on the different branches of an individual plant were collected as one sample and four biological replicates were used in the analyses.

4.2.2 Leaf morphology

Leaf area was measured with a leaf area meter (Li-2500, Lincoln, Nebraska, USA), the interveinal leaf thickness was measured with a leaf thickness meter with an accuracy of 0.01 mm. Each measurement was conducted in three replications per cultivar and treatment.

4.2.3 Chlorophyll a fluorescence

Chlorophyll a fluorescence measurements were conducted 2 h after the start of the light period, using a portable chlorophyll fluorometer (PAM-2500, Walz, Germany). The third fully expanded leaf was dark adapted for 30 min, after that, a 0.6s saturating light (3450 µmol m^{-2} s^{-1}) was given to obtain the maximal and minimal fluorescence yield (F_m and F_0). Then, leaf was light adapted with 5 min continuous actinic light at 100 µmol m^{-2} s^{-1} and saturating pulses every 25 s, the maximum (F_m') and the steady state fluorescence (F_s) signal were recorded. For the calculation of F_v/F_m Φ_{PSII} and NPQ see 2.2.4.

4.2.4 Pigments

The leaf chlorophyll and carotenoid content was determined according to Lichtenthaler and Buschmann (2001). For details see 2.2.5.

4.2.5 Hydrogen peroxide content

The leaf hydrogen peroxide content was measured following the description of Junglee et al. (2014). Homogenized leaf material (30-40 mg) was extracted in potassium phosphate buffer (pH 5.8) with TCA (1%) and KI (1M) at 4°C, then

centrifuged at 15,000 g. The method is based on KI oxidation by H_2O_2 in acidic medium according to the following equations:

$$2I^- + 2H^+ + H_2O_2 \rightarrow I_2 + 2H_2O$$

$$I_2 + I^- \rightarrow I_3^-$$

The absorbance of the supernatant at 350 nm was measured with a spectrophotometer (Infinite M200 TECAN), and leaf hydrogen peroxide content was expressed as µmol H_2O_2 mg^{-1} fresh weight.

4.2.6 Proline content

Extraction and determination of proline was performed according to Bates et al. (1973). Homogenized fresh leaf material (1 to 1.5 g) was extracted in 10 mL 3% (w/v) sulfosalicylic acid. After filtration, 1 mL ninhydrine acid and 1 mL acetic acid was added to the extracts (1 mL) and this was kept at 95°C for 1 hour when the reaction was stopped in an ice-bath. The formed chromophore was extracted from the acid aqueous solution by means of cold toluene (2 mL) and measured spectrophotometrically at λ=520 nm (Infinite M200, TECAN Group Ltd., Switzerland). Proline concentration was calculated as µmol proline g^{-1} fresh weight.

4.2.7 Total phenolic and flavonoid content

Fresh leaf material (250 mg) was extracted for 30 min in 10 mL 80% methanol. The extract was centrifuged at 5,000 g and its supernatant was used for total flavonoid and total phenolic analysis.

Total phenolic content was determined according to the Folin-Ciocalteu method. Briefly, 200 µL of the supernatant was added to 1.5 mL Folin-Ciocalteu (1:10) reagent. After 4 min, 800 µL of 7.5% Na_2CO_3 was added. The mixture was shaken and reacted for 2 hours at room temperature in the dark. Absorbance was measured at λ=765nm (Infinite M200, TECAN Group Ltd., Switzerland). Total phenolic content were expressed as gallic acid equivalent per gram of fresh weight.

Total flavonoid content was determined colorimetrically following the method of Hong et al. (2008). The supernatant (400 µL) was added sequentially to 600 µL of distilled water, 60 µL of 5% $NaNO_2$ for 5 min, then 60 µL of 10% $Al(NO_3)_3$ was added. After 6

min, 0.4 mL of 1.0 M NaOH and 0.4 mL of distilled water were added. The absorbance at λ=510 nm was measured after 15 min (Infinite M200, TECAN Group Ltd., Switzerland). The content of total flavonoid content was measured and then expressed as rutin equivalent per gram of fresh weight.

4.2.8 Statistical analysis

Data are reported as means ± SE. Results were analyzed using SPSS statistical software Version 24 (SPSS Inc., Chicago, USA), figures were made using Sigmaplot 13.0 (Systat Software, Inc, USA). Homogeneity of variance was verified with Levene's test, analyses were carried out using 1-way and 2-way ANOVA and significant differences were separated with Tukey's HSD test (P=0.05). Correlations were calculated using Pearson's test (P=0.05). Principal Component Analysis (PCA) was performed to classify responses to light treatments and genotypes. Only PCAs with eigenvalues > 1, thus explaining more than a single parameter alone, were extracted. For these principal components a varimax rotation was applied on the obtained factor loadings. A one-way ANOVA to separate effects of light quality on the factor scores of PCA1 and PCA2 was applied.

4.3 Results

4.3.1 Leaf morphology

Overall, red light significantly reduced the leaf area ($P < 0.001$), while no differences between the other spectral qualities were observed (Figure 4.1). However, there was also a clear interaction between light quality and cultivar ($P < 0.01$; Figure 4.1). Four cultivars ('Katelijn', 'Lana', 'Sunny' and 'Tappino') strongly decreased their leaf area under R though B also reduced the leaf area of 'Katelijn' and 'Tappino'. White light resulted in the highest leaf area for six cultivars, but not in 'Tappino' where the RB combination was better. Leaf area was hardly affected by the light treatments for 'Bolero' and 'Loretto' though tended to be smaller under R and higher under W. For 'Marco' narrow beam light qualities (R, B and RB) reduced leaf area compared to the broader white spectrum. Irrespective of the light quality, 'Sunny' had the greatest overall leaf area.

Overall, the thinnest leaves were observed for W ($P < 0.05$, Figure 4.1). However, leaf thickness was not significantly affected by the light treatments in six of the eight studied genotypes (Figure 4.1; 'Bolero', 'Lana', 'Marco', 'Orlando', 'Sunny' and 'Tappino') while in 'Katelijn', leaves were thinnest for W and for 'Loretto', the thinnest leaves were found under RB and thickest under B.

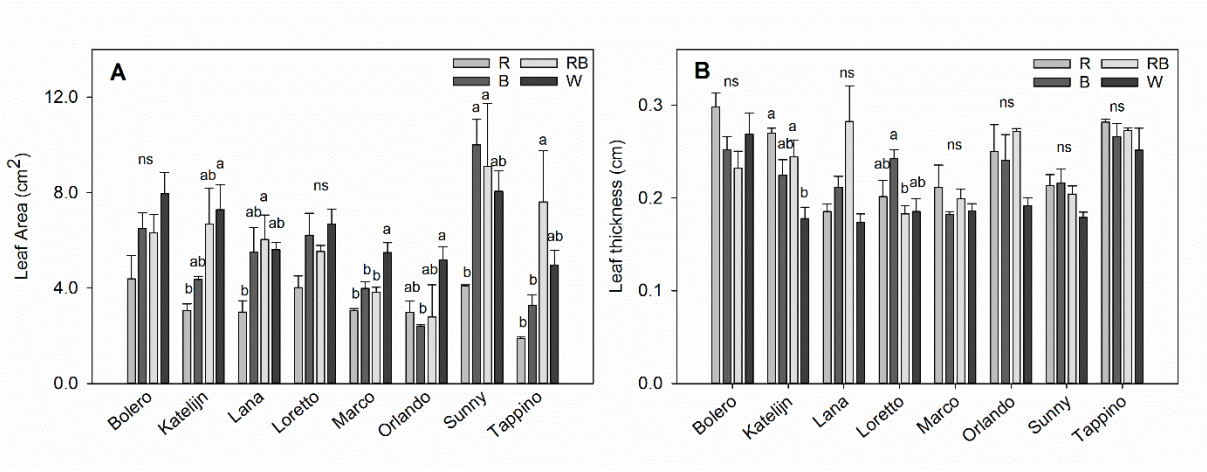


Figure 4.1 Leaf area (A) and leaf thickness (B) of eight *Chrysanthemum* cultivars grown under red (R), blue (B), red + blue (RB) and white (W) LED treatments. Data are mean values (n = 3) ± SE. Different letters within each cultivar indicate significant differences ($P<0.05$) between the light quality treatments.

4.3.2 Chlorophyll a fluorescence and chlorophyll content

The dark-adapted F_v/F_m averaged 0.77; yet overall B and RB had slightly higher F_v/F_m in comparison with R and W light ($P < 0.001$, Table 4.1). Only for 'Bolero' F_v/F_m was not affected by the light treatments (Figure 4.2). For 5 out of 8 cultivars B and RB yielded the highest F_v/F_m ('Katelijn', 'Loretto', 'Marco', 'Sunny' and 'Tappino').

The quantum yield (Φ_{PSII}) was 0.65-0.70 at the applied irradiance for most light qualities. Overall Φ_{PSII} was lowest for the W treatment ($P < 0.001$, Table 4.1) though a significant interaction with the cultivars was found ($P < 0.01$). For 'Bolero', 'Loretto' and 'Tappino' Φ_{PSII} was significant lower under white light. For three cultivars (Lana, Marco and Sunny) no effect of light quality on Φ_{PSII} was found (Figure 4.2) while for 'Katelijn' RB and for 'Orlando' both B and RB resulted in higher Φ_{PSII}. Overall, non-photochemical quenching (NPQ) was lowest under B and RB ($P < 0.001$, Table 4.)

but also here a significant interaction with cultivars was observed ($P < 0.01$, Figure 4.2). No light quality effect on NPQ was found for 'Katelijn', 'Lana' and 'Sunny'. For 'Bolero', 'Loretto', 'Orlando' and 'Tappino', NPQ is significantly greater under W compared to the other treatments. In 'Marco', both R and B had low NPQ values but a significant increase took place under RB and W. Overall, a significant negative correlation ($r= -0.927$; $P<0.01$) between NPQ and Φ_{PSII} was found.

Table 4.1 Effects of different light qualities on leaf area and thickness, chlorophyll fluorescence parameters (F_v/F_m, Φ_{PSII}, NPQ), chlorophyll (Chl) and Chl a/b ratio.

Light quality	Leaf area (cm^2)	Leaf thickness (cm)	F_v/F_m	Φ_{PSII}	NPQ	Total Chl ($mg.g^{-1}$)	Chl a/b
R	3.30 b	0.239 a	0.773 b	0.671 a	0.263 a	1.252 b	2.14 b
B	5.28 a	0.229 a	0.784 a	0.686 a	0.249 b	1.223 b	2.41 a
RB	5.98 a	0.257 a	0.785 a	0.689 a	0.252 b	1.336 ab	2.42 a
W	6.40 a	0.202 b	0.774 b	0.638 b	0.398 a	1.467 a	2.53 a
Light quality	***	*	***	***	***	***	***
Cultivar	***	**	***	***	***	***	***
Light quality x Cultivar	**	n.s.	***	**	**	***	***

Data of 8 cultivars are pooled for a global analysis and main effects of light quality are presented. Data are mean values ± SE (n = 4). Different letters indicate significant differences using Tukey's HSD test (P=0.05). Analysis of the 2-way ANOVA: n.s.: not significant; *, ** and *** indicates significance at $P<0.05$, $P<0.01$ and $P<0.001$ respectively.

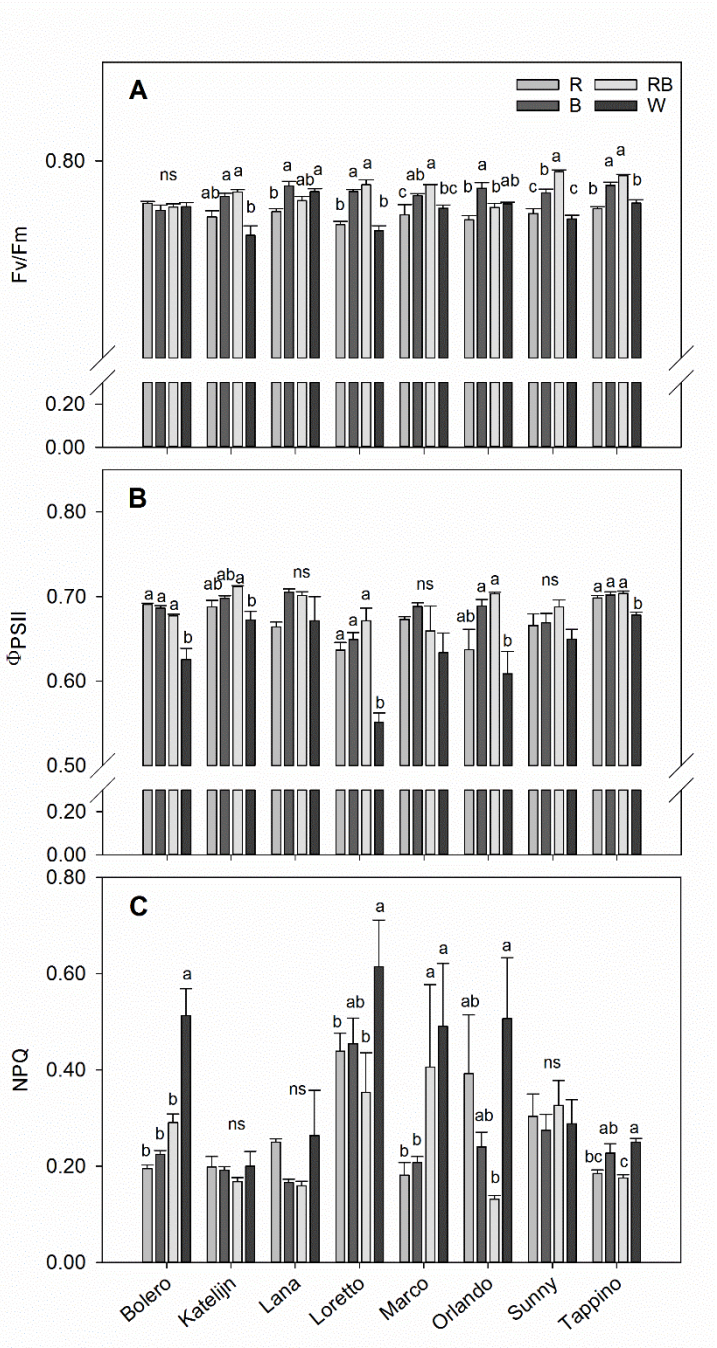


Figure 4.2 Chlorophyll fluorescence parameters: F_v/F_m (A), Φ_{PSII} (B) and NPQ (C) of eight *Chrysanthemum* cultivars grown under red (R), blue (B), red + blue (RB) and white (W) LED treatments. Data are mean values ± SE (n = 4). Different letters within each cultivar indicate significant differences (P < 0.05) between the light quality treatments according to Tukey's HSD test.

Total leaf chlorophyll content under W (= full spectrum light) did not differ for most cultivars and ranged from 1.30-1.55 mg g^{-1} FW; only 'Tappino' had significantly lower contents and 'Marco' significantly higher contents ($P < 0.001$). Overall, total leaf chlorophyll content was highest under W and lowest for B and R while the lowest Chl a/b ratio was found under R (Table 4.1). Significant interactions between light quality and cultivar were present ($P < 0.001$). For 'Bolero' and 'Tappino', R and RB resulted in the highest total chlorophyll content (Figure 4.2) while for 'Katelijn', 'Loretto' and 'Orlando', no significant differences were observed though W tended to yield higher total chlorophyll content. For 'Lana', 'Marco' and 'Sunny' W yielded the highest chlorophyll content.

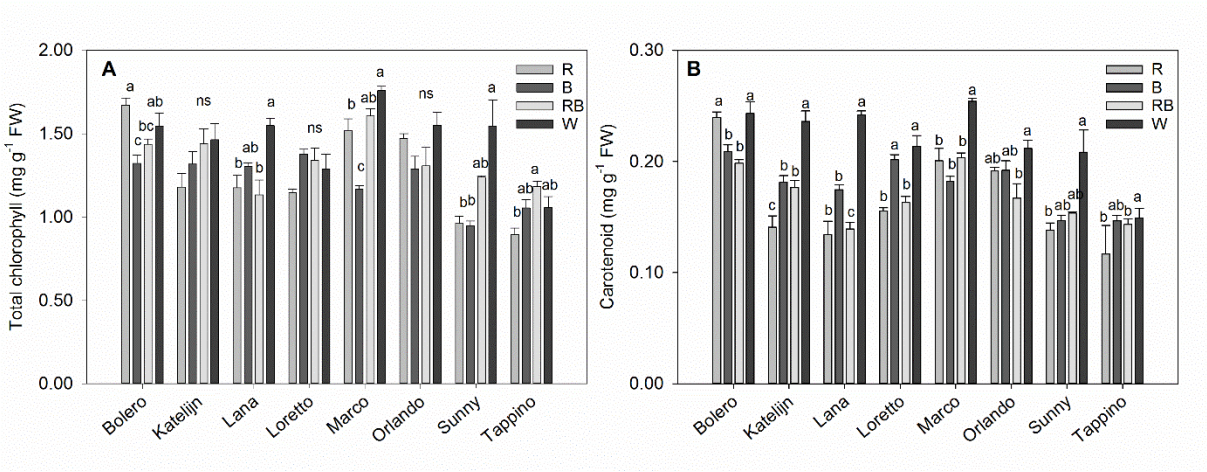


Figure 4.3 Chlorophyll content (A) and carotenoid content (B) of eight *Chrysanthemum* cultivars grown under red (R), blue (B), red + blue (RB) and white (W) LED treatments. Data are mean values ± SE (n = 4). Different letters within each cultivar indicate significant differences ($P < 0.05$) between the light quality treatments according to Tukey's HSD test.

4.3.3 Hydrogen peroxide

Overall, blue light resulted in the highest H_2O_2 accumulation while RB and R had significantly lower contents (Table 4.2). In 4 of 8 cultivars ('Bolero', 'Marco', 'Orlando' and 'Tappino') the greatest H_2O_2 content was observed under B, while for 'Katelijn', the highest H_2O_2 content was found under W (Figure 4.4).

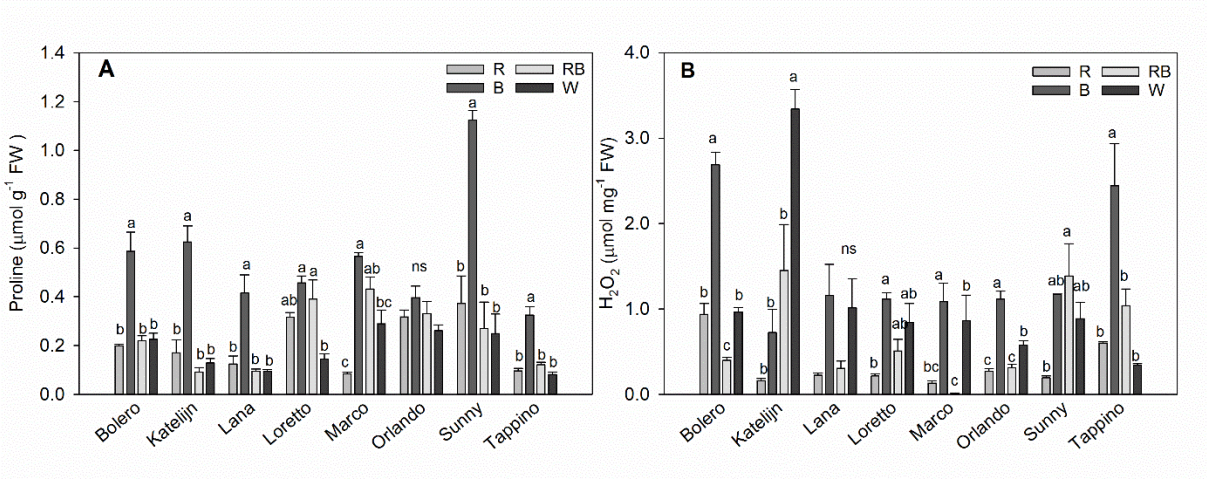


Figure 4.4 Leaf proline content (A) and hydrogen peroxide content (B) of eight *Chrysanthemum* cultivars grown under red (R), blue (B), red + blue (RB) and white (W) LED treatments. Data are mean values ± SE (n = 4). Different letters within each cultivar indicate significant differences (P<0.05) between the light quality treatments according to Tukey's HSD test.

Table 4.2 Effects of different light qualities on carotenoids, total phenolic and flavonoid content, proline and hydrogen peroxide.

Light quality	Carotenoids ($mg.g^{-1}$ FW)	Total phenolic ($mg.g^{-1}$ FW)	Total flavonoid ($mg.g^{-1}$ FW)	Proline ($\mu mol.g^{-1}$ FW)	H_2O_2 ($\mu mol.mg^{-1}$ FW)
R	0.165 b	1.263 a	2.543 ab	0.225 b	0.342 c
B	0.179 b	1.013 a	2.459 ab	0.579 a	1.499 a
RB	0.168 b	0.694 b	2.075 b	0.233 b	0.663 bc
W	0.220 a	1.378 a	3.235 a	0.184 b	1.104 ab
Light quality	***	***	**	***	***
Cultivar	***	***	**	***	***
Light quality x Cultivar	***	***	***	***	***

Data of 8 cultivars are pooled for a global analysis and main effects of light quality are presented. Different letters indicate significant differences using Tukey's HSD test (P=0.05). Analysis of the 2-way ANOVA: ns: not significant; *, ** and *** indicates significance at P<0.05, P<0.01 and P<0.001 respectively.

4.3.4 Antioxidant compounds, carotenoid, flavonoid and phenolic content

Overall, the highest carotenoid content was found under W (Table 4.2) while no significant differences between the other treatments were observed. This was a rather general trend for the cultivars with exception for 3 out of 8 cultivars (Figure 4.3). In 'Bolero' both R and W resulted in higher carotenoid contents while for 'Loretto' both B and W resulted in the highest carotenoid content. For 'Katelijn' carotenoids were highest under W, followed by B and RB while lowest content was found for R.

The total flavonoid and total phenolic contents were highest under W and lowest under RB (Table 4.2). Yet, also for these metabolites an interaction between treatment and cultivar was observed ($P < 0.001$ for both flavonoids and phenolics) (Figure 4.5). For 'Katelijn' and 'Orlando', the flavonoid content declined significantly for both B and RB. In 'Marco', we saw the lowest flavonoid content under R. 'Sunny' reacted quite contrasting with respect to the other cultivars with lowest flavonoid content under R and W. Effects of light quality on phenolic content was cultivar dependent. Low total phenolic contents were found under RB for 'Bolero', 'Katelijn' and 'Loretto', while for Tappino R clearly resulted in the highest phenolic content. No significant effects of light quality were found for 'Lana', 'Marco' and 'Sunny'. No significant correlations between H_2O_2 accumulation and flavonoid or phenolic content were present.

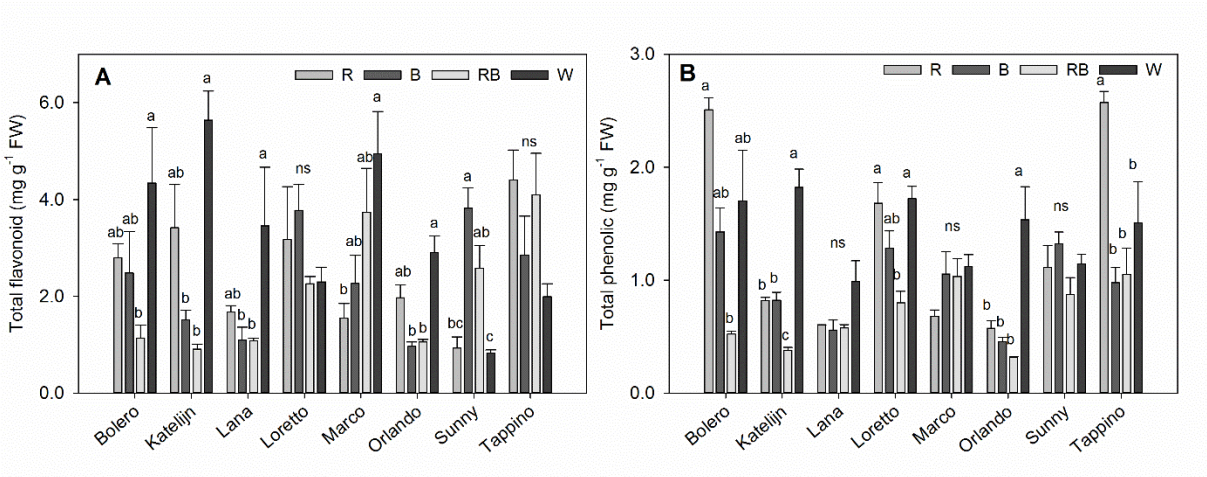


Figure 4.5 Leaf total flavonoid (A) and total phenolic content (B) of eight *Chrysanthemum* cultivars grown under red (R), blue (B), red + blue (RB) and white (W) LED treatments. Data are mean values (n = 4) ± SE. Different letters within each cultivar indicate significant differences (P<0.05) between the light quality treatments.

4.3.5 Proline content

Overall, significantly higher proline levels were found under B compared to the other light qualities (Table 4.4). However, cultivar differences were present ($P < 0.001$). For 'Orlando' no significant effects were found while for 'Loretto' and 'Marco' RB also yielded high proline levels (Figure 4.4). For four cultivars a positive correlation between H_2O_2 accumulation and proline levels was observed, namely for 'Bolero' ($r = 0.904$, $P < 0.01$), for 'Orlando' ($r=0.865$, $P = 0.865$), for 'Tappino' ($r = 0.748$, $P < 0.01$) and for 'Tropical' ($r= 0.654$, $P < 0.05$).

PCA score plots were used to compare the responses of the cultivars to the light quality treatments with respect to H_2O_2, proline, carotenoids, total phenolic and flavonoid content (Figure 4.6). The scores did not separate the type of cultivars but separated the light quality response. PC1 explained 39% of the variability and was mainly explained by total flavonoid and phenolic compounds and by carotenoid contents. This axis separated W from B, R and RB (Tukey HSD test, P=0.05). PC2 captured 21.8% of the variance and was mainly explained by proline thus separating B from the other light treatments (Tukey HSD test, p=0.05).

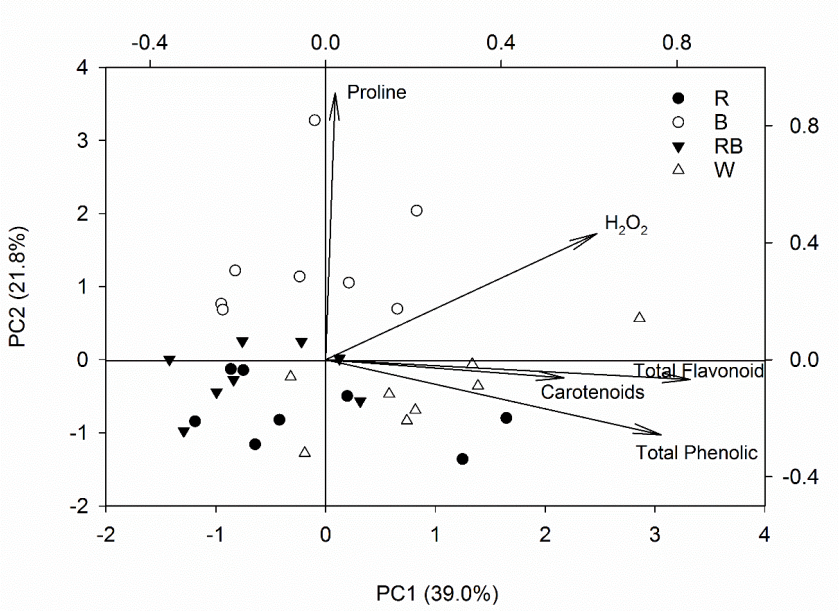


Figure 4.6 A scatter plot of PC1 versus PC2 explaining the main sources of variability between the light treatments with respect to H_2O_2 and antioxidant compounds.

4.4 Discussion

Light quality strongly influences the morphology of various plant species including ornamentals (Fazal et al., 2016; Jeong et al., 2014; Mengxi et al., 2011; Schuerger et al., 1997). Promotion of cotyledon expansion and the inhibition of hypocotyl elongation are regulated primarily by phyB in red light and cry1 in blue light (Neff and Van Volkenburgh, 1994). Furthermore genetic analyses of a variety of photoreceptor mutants showed that both phytochromes and cytochromes are redundantly involved in the control of leaf blade expansion (Kozuka et al., 2005). In this study, leaves that developed under monochromatic R resulted in the smallest leaf area in most *Chrysanthemum* cultivars indicating that blue light is needed in the light spectrum to enhance leaf expansion in this species. This corresponds with previous reports where additional B in the spectrum increased leaf area in *P. grandiflorum* (Liu et al., 2014), in lettuce (Sæbø et al., 1995; Wang et al., 2016), in *Alternanthera* (Macedo et al., 2011) and in *Chrysanthemum* (Kim et al., 2004). Also in our results, RB and W tended to have a higher leaf area than B alone for certain cultivars. 'Katelijn' and 'Tappino' responded favorable to RB while for 'Marco' and 'Orlando' the broader W spectrum resulted in the highest leaf expansion. For an optimal leaf blade expansion of *Chrysanthemum* B seems necessary in the light spectrum. This is, however, not universal as in roses R light was favorable for leaf expansion (Ouzounis et al., 2014).

Leaf area and thickness were reciprocally correlated ($r=-0.207$, $P=0.043$), but this correlation was rather weak. Barreiro et al. (1992) showed that a decrease of R:FR (lower φ) at both 300 and 800 µmol m^{-2} s^{-1} PAR decreased leaf thickness and enhanced leaf area which are typically adaptions of shade leaves. Under monochromatic light leaf thickness in *Brassica napus* L. in vitro plantlets was greater under R than under B (Li et al., 2013a) while Schuerger et al. (1997) reported leaf thickness increased when red light was supplemented with blue light compared to red alone. Especially thickness of palisade parenchyma and upper epidermis are influenced by blue though spongy parenchyma is also affected (Macedo et al., 2011). In our experiment however, we found no differences between R and B or an added effect of B to R on leaf thickness.

Thinner leaves (as found under W and this especially for 'Katelijn') allow an enhanced absorption of the light energy and therefore relate to the capacity in

photosynthetic activity, there is a positive correlation between the leaf thickness and Φ_{PSII} (P=0.13). F_v/F_m provides an estimate of the maximum photochemical efficiency of PSII. In our study, the leaves of most cultivars under RB or monochromatic B yielded a higher F_v/F_m compared to R or W. Tennessen et al. (1994) suggested that monochromatic red light causes an imbalance of light energy distribution available for Photosystems I and II, which induced the inhibition of the photosynthetic performance and subsequent shoot growth. Also Trouwborst et al. (2016) indicated that monochromatic red light could induce a physiological disorder, including the decrease in F_v/F_m, which was defined as the “red light syndrome”. Although we found negative effects of R on F_v/F_m in the *Chrysanthemum* cultivars, white light also negatively affected F_v/F_m. The applied white light is characterized by a high content of R and only 7% B; this might explain the similarities with the R response. Surprisingly Φ_{PSII} decreased only under white light and not under R and this was most pronounced for ‘Loretto’. This decrease was reflected in an increased NPQ indicating that reduced electron transport and a certain oxidative stress was present.

Blue light is important for the synthesis of chlorophyll (Dougher and Bugbee, 1998), though monochromatic R at our applied intensities (100 µmol m^{-2} s^{-1}) will not impair chlorophyll biosynthesis either (Tripathy and Brown, 1995). We indeed observed higher total chlorophyll content in W and RB compared to R and B alone though Chl a/b ratio was lower under red. Abadía et al. (1999) reported that plants with less chlorophyll have a higher absorptance of blue wavelengths than green and red wavelengths indicating these leaves may be more efficient under blue light. Sæbø et al. (1995) found that birch with less chlorophyll content seemed to use it more efficiently than those with excessive chlorophyll. The loss of photosynthetic pigments was also viewed as a protection mechanism as it would decrease the capacity of the leaf to absorb incident radiation and hence reduce the amount of excess excitation energy dissipated by NPQ (Burritt and Mackenzie, 2003). In our case, B grown *Chrysanthemum* leaves resulted in a significantly higher value of F_v/F_m and lower NPQ value compared to W, though its chlorophyll content was lower. Also Hoffmann et al. (2015b) showed that UV stressed pepper plants had higher photosynthetic rates (P_n), higher F_v/F_m ratio and lower non-photochemical quenching (NPQ) when plants were subsequently grown under a high fraction blue light (62%) than under a lower amount of blue light (30%).

Excess excitation energy unavoidably leads to the production of reactive oxygen species (ROS) in chloroplasts but also in other organelles as mitochondria and peroxisomes (Apel and Hirt, 2004). We found that leaf hydrogen peroxide content (H_2O_2) was high under B followed by W and lowest levels were found for R. Also illumination of barley protoplasts with blue or UV-A light resulted in a rapid increase in intracellular H_2O_2 production (Bethke and Jones, 2001). This is however not universal as Wang et al. (Wang et al., 2010b) found higher H_2O_2 contents in cucumber plants exposed to R compared to W.

Carotenoids are not only essential pigments for photosynthesis but also efficient antioxidants, thus protecting more specifically the lipophilic compartments but also through direct scavenging of ROS generated in photo-oxidative processes (Stahl and Sies, 2003). We saw no total carotenoid differences in R, B and RB grown plants, and a significantly lower content in W, which can be explained by the relatively high H_2O_2 content in W and higher NPQ which also indicates oxidative stress at chloroplast level. Proline is another compound that counteracts the inhibitory effects of ROS (Chen and Dickman, 2005), its metabolism is also closely related to ROS formation (Ben Rejeb et al., 2014). Although effects of light quality on proline biosynthesis were cultivar dependent most of the cultivars grown under red light had low proline levels while blue light could result in a significant accumulation of free proline. This was partially correlated with H_2O_2 content in plants, which were highest under B, and lowest under R though this correlation was not found for all the cultivars. Also Kim et al. (2013) found enhanced proline accumulation in *Chrysanthemum* under blue.

Polyphenolic compounds and the subgroup of the flavonoids are another group of metabolites known as antioxidants. Their antioxidant effect is due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 2000). The present study showed that W resulted in the greatest content though dichromatic RB had overall the lowest contents. Looking into the cultivars, we had very specific and contrasting responses. In general blue light enhances phenolic and flavonoids in plants as reported in *Prunella vulgaris* L. (Fazal et al., 2016), in *Kalanchoe pinnata* (Nascimento et al., 2013), in lettuce (Ouzounis et al., 2015b), tomato (Kim et al., 2013) and in *Chrysanthemum* (Ouzounis et al., 2014). Ouzounis et al. (2016) also investigated the responses of nine tomato genotypes (mainly *S. lycopersicon*) and

found that supplementing R light with 12% B increased flavonoids and reduced anthocyanins though the response was genotype dependent. The biosynthesis of flavonoids is initiated by the enzymatic step catalyzed by chalcone synthase (CHS) (Schijlen et al., 2004). Blue light was suggested to induce the CHS expression in *Arabidopsis thaliana*, which involved the cryptochrome (CRY1) photoreceptor (Feinbaum et al., 1991; Kubasek et al., 1992). Under blue light, cryptochromes increase the stability of HY5 (hypocotyl 5) and accumulation of HY5 protein by preventing ubiquitination by COP1 (constitutively photomorphogenic 1), HY5 binds to the promoters of CHS to stimulate gene expression, thus blue light promotes the flavonoids synthesis (Vandenbussche et al., 2007). However, only in 'Sunny' we observed higher flavonoids under B compared to R while in 3 cultivars monochromatic R resulted in higher flavonoids than B. Mutant studies in *Arabidopsis* suggested that phytochrome also participates in the regulation of CHS expression, red light induction of CHS was mediated by phytochrome A and PHYA is not a component of the blue light signaling pathway (Huché-Thélier et al., 2016). Also in *Sinapis alba* phytochrome mediated flavonol accumulation (Beggs et al., 1987). This pathway might explain the higher total flavonoid content under R in the three cultivars. On the other hand, it was suggested that both blue and red light may be needed to regulate the accumulation of phenolics in basil (Taulavuori et al., 2016). This combined effect is indeed observed in most of the *Chrysanthemum* cultivars but only in the broad band W and not under RB. This lower content under RB might correspond to a lower H_2O_2 in *Chrysanthemum* leaf tissue compared to W as well as to the lower NPQ values compared to W. In view of these results, it seems that depending on the applied light quality a trade-off in energy use for biosynthesis of secondary metabolites occurs. Under blue light, the biosynthesis of proline is favored while under R and W phenolic and flavonoid compounds are higher.

The studied *Chrysanthemum* cultivars were characterized by the same plant architecture, but were derived from three different genetic backgrounds in a breeding program. PCA analysis could not separate these three groups when analyzing H_2O_2, carotenoids, polyphenols, flavonoids and proline. Despite differences in the individual cultivar responses, PCA analysis indicated some clustering and especially the responses under B and under W could be differentiated. The broad scattering in

these clusters is most probably due to the inherent genetic differences that are even present in more genetically linked cultivars.

4.5 Conclusion

In this study, we evaluated eight cultivars of pot *Chrysanthemum* under four different light qualities and focused on aspects of photosynthesis and antioxidative status.

Red light reduced leaf area and the thinnest leaves were observed under the full spectrum white light. Chlorophyll content as well as the Chl a/b ratio was highest under white light. Blue and red+blue light yielded the highest F_v/F_m and Φ_{PSII}. Monochromatic blue light induced the highest hydrogen peroxide content followed by white light while low contents were found under monochromatic red light. Monochromatic blue light enhanced the proline biosynthesis while carotenoids, total flavonoid and phenolic compounds were higher under white light.

Within the studied *Chrysanthemum* cultivars we found genotypes that were highly reactive to light quality triggers while others hardly reacted differently to the light environment. Such intraspecific variation clearly seems to be adaptive but also raises the potential for selection to favor genotypes with greater secondary biochemical plasticity that might be favorable for (a)biotic stress tolerance.

Chapter 5

Light quality affects energy dissipation and carbon sequestration during the diel cycle of crassulacean acid metabolism

This chapter is based on:

Zheng L., Ceusters J. and Van Labeke M.C. Light quality affects energy dissipation and carbon sequestration during the diel cycle of crassulacean acid metabolism. In preparation.

Author contribution:

LZ and MCVL conceived and designed the experiments. LZ performed the experiments, analyzed the data and drafted the manuscript, MCVL and JC critically revised the manuscript.

Abstract

Crassulacean acid metabolism (CAM) is a specialized photosynthetic pathway present in many epiphytic orchids. CAM physiology and metabolism is under circadian control and can be sub-divided into four discrete phases during a diel cycle. We evaluated the effect of monochromatic blue and red light as well as its combination on the photosynthetic performance and diel changes of metabolites during the CAM cycle. *Phalaenopsis* was grown under four different light qualities (red, blue, red+blue and full spectrum white light) at a fluence of 100 µmol m^{-2} s^{-1} and a photoperiod of 12 h this for 8 weeks. Plants grown under monochromatic red light showed a significant decline of the quantum efficiency (Φ_{PSII}) after five days and for the maximum quantum yield (F_v/F_m) after ten days under this treatment. This was also reflected in the total diel CO_2 uptake measured after 8 days, which tended to decline under red. After 8 weeks under different light qualities, total 24 h CO_2 exchange was highest under monochromatic blue and full spectrum light. Adding blue to the red spectrum enhanced the daily CO_2 uptake by 18%. CAM phases were also influenced by the light quality; we observed an extended phase II for blue light and an earlier CO_2 uptake in Phase IV for blue and red+blue. Nocturnal malate accumulation was considerably less under red light compared to the other light treatments. During daytime, the basal levels of malate under blue and RB were reached earlier. Starch showed an inverse diel pattern with malate whilst greater starch breakdown was recorded for RB and W compared with red and blue. PEPC was activated at dusk but no significant differences of PEPC activity were noticed with respect to the applied light quality. Blue light is important in regulating an efficient Photosystem II and it influences the diel CAM rhythm. Further investigations on the stomatal behavior explaining the effects of light quality on the regulation of CAM phases are needed.

5.1 Introduction

Crassulacean acid metabolism (CAM) is a specialized photosynthetic pathway that improves atmospheric CO_2 assimilation in water-limited terrestrial and epiphytic habitats and increases water-use efficiency (Yang et al., 2015). CAM species are widely distributed throughout semiarid tropical and subtropical environments, including epiphytes in the humid tropics (Silvera et al., 2010). In CAM plants, a temporal separation of carboxylation (physiological) and decarboxylation (biochemical) events takes place. These temporal events are separated in four discrete phases according to the patterns of gas exchange and stomatal behavior (Osmond, 1978) i.e. nocturnal CO_2 uptake with open stomata in Phase I, early morning CO_2 uptake in Phase II, stomatal closure during the light period in phase III and late afternoon stomatal opening for CO_2 uptake in Phase IV. CAM plants take up atmospheric CO_2 through open stomata mainly via phosphoenolpyruvate carboxylase (PEPC) during the night and store it as malate in cell vacuoles. During the major part of the day (phase III), stomata are closed and malate is remobilized and degraded into CO_2 as source for photosynthetic activity via ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Osmond, 1978). During transition phases II and IV CO_2 can be taken up by PEPC, Rubisco or either a combination of both. Environmental factors such as drought influence the intrinsic CAM activity and thus the employment of the different phases of CAM. For example, phases II and IV are lost under conditions of severe drought stress, and stomata close throughout day and night while respiratory CO_2 is recycled as source for malic acid accumulation (Dodd et al., 2002; Ceusters et al., 2009).

Light, as one of the most important environmental factors, has profound effects on the development and metabolism of plants (Fankhauser and Chory, 1997; Smith, 1982). It is therefore not surprising that light and especially light quality might affect CAM. Effects of light quality have mainly been studied in facultative CAM plants, which are plants that employ C3 or C4 photosynthesis, but under stressful conditions optionally use CAM photosynthesis. In *Kalanchoe blossfeldiana,* CAM is induced by short days through the red-light controlled synthesis of PEPC (Brulfert et al., 1988). In *Clusia minor,* an UV-A/blue light receptor was suggested to mediate the high-light induced C3-photosynthesis/CAM transition (Grams and Thiel, 2002). However,

information of light quality on the endogenous circadian rhythm of CAM hardly exists. Effects of light quality on the CAM diel rhythm (diel gas exchange and metabolite turnover) has only been reported previously by Ceusters et al. (2014) on *Aechmea*, an obligate CAM Bromeliaceous plant. They studied effects on CAM by using low-fluencies (10 µmol m^{-2} s^{-1}) of red, blue and green light, thus minimizing the direct involvement of photosynthetic processes but sufficient to sustain a typical CAM pattern. This study gave the first clear indication that both red and blue light signaling is inevitable to synchronize the diel CAM cycle.

To further increase our understanding about the influences of light quality on CAM photosynthetic performance, high fluence rates of blue, red and a combination of both were provided to *Phalaenopsis* orchids. *Phalaenopsis* orchids are epiphytes exhibiting obligate CAM photosynthesis (Guo and Lee, 2006; Mc Williams, 1970; Pollet et al., 2010; Sayed, 2001) and its hybrids became the most important flowering pot plants worldwide. Despite their horticultural importance, no information of light quality on their CAM cycle is present. The present study aims to discover both relative short and long-term adaptations of the photosynthetic performance using chlorophyll fluorescence analysis. Next, we investigated if these light qualities influenced the carbon balance and diel rhythm at both the physiological (CO_2 balance) and biochemical level (malate content, carbohydrate content, PEPC activity).

5.2 Materials and Methods

5.2.1 Plant material and growth condition

Phalaenopsis 'Exquisite Edessa' young-plants (Microflor, Belgium) were acquired when the second leaf from the apex had an average length of 6.3 ± 0.8 cm (n=8). They were transplanted in 12-cm plastic pots (600 mL) filled with orchid substrate based on pine bark (*Pinus maritima* Lam.). The acclimation and the light treatments were performed in a growth chamber and the growth conditions were 100 µmol m^{-2} s^{-1} PPFD and 28°C day/night temperature with a 12-h day-length (08:00 to 20:00). They acclimated under high-pressure sodium lamps (SON-T, Philips Inc., Eindhoven, The Netherlands) for ten days. Fertigation with water-soluble fertilizer for orchids (N:P:K = 20:20:20, pH = 6.0, EC = 1.0 dS cm^{-1}) was supplied twice a week.

5.2.2 Light treatments

After one week of acclimation, plants were randomly allocated to a light treatment. Light treatment sections were separated with black, plastic curtains in the growth room. Four light treatments were applied namely multispectral white (W, 300-800 nm, light emitting plasma lamp, Gavita BV, The Netherlands), blue (B, peak at 460 nm, Philips, Eindhoven, The Netherlands), red (R, peak at 660 nm, Philips, Eindhoven, The Netherlands) and a combination of red and blue (RB, 60%/40%, CI-800 programmable LED system, CID Bio-Science, WA, USA). Light intensity was 100 µmol m^{-2} s^{-1} and light period was 12 h. Light spectral distribution was recorded using a JAZ-ULM-200 spectrometer (Ocean Optics, FL, USA) and converted with Spectrasuite software (Ocean Optics) to µmol m^{-2} s^{-1} (Figure 5.1) and the uniformity was verified by measuring the light intensity at five points of each light treatment at the canopy level.

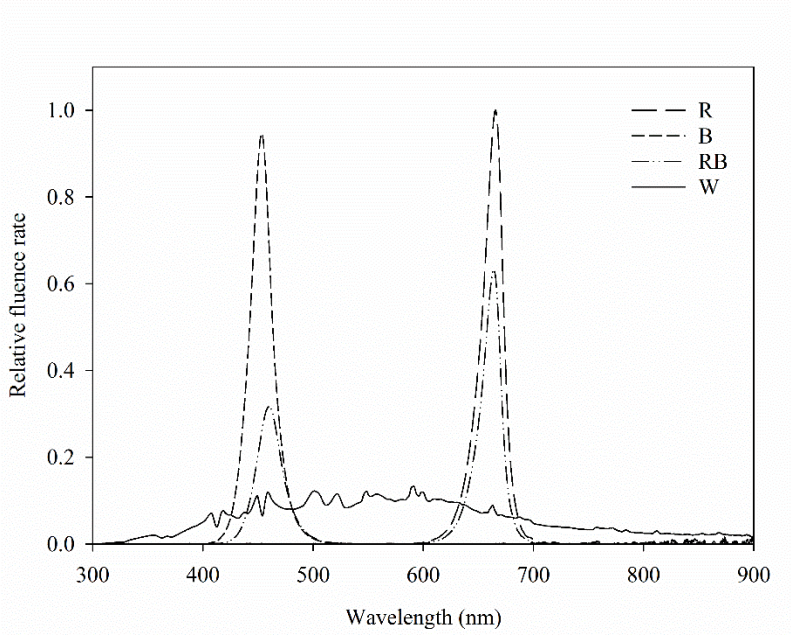


Figure 5.1 Relative fluence rate of the four light treatments. R, red light; B, blue light; RB, red/blue (60%/40%) polychromatic light; W, white light. Spectrum was measured at the plant canopy level with a JAZ spectrometer (Ocean Optics, FL, USA).

5.2.3 Photosynthesis

Net CO_2 uptake was measured using a LI-6400 portable gas exchange system (Li-Cor Biosciences, NE, USA) on the second expanding leaf counting from the apex, this in four replicates per treatment. Measurements were conducted halfway the leaf avoiding the leaf vein (1 cm away from the main vein) for each treatment. All measurements were made under standard conditions (PPFD at 100 µmol m^{-2} s^{-1} during the day-time and at night the light was switched of in the sensor head, CO_2 concentration at 400 µmol mol^{-1}, leaf temperature at 28°C and vapor pressure deficit inside the chamber at 1.4-1.8 kPa.

Measurements were performed every 2 h for a 24 h period to calculate the diel CO_2 uptake respectively one week and eight weeks after the start of the light treatments. For the eight weeks measurement the net absorption/release of CO_2 during the 24-h cycle was divided into the four CAM phases (Griffiths, 1989; Nelson and Sage, 2008) by integration of the gas exchange data and malate dynamics. Briefly phase I (night) started at 20:00 and ended at 08:00, phase II started at 08:00 and ended when the CO_2 uptake was negligible, at which Phase III began, Phase IV began when CO_2 uptake was not negligible and malate levels returned to their baseline and continued until 20:00 which is the onset of dark period.

5.2.4 Chlorophyll a fluorescence

The leaf chlorophyll a fluorescence measurement was conducted on the same leaf as the photosynthesis measurement using a portable PAM 2500 chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The leaf was dark-adapted for 30 min, then 0.6s saturating light (3,450 µmol m^{-2} s^{-1}) was given to obtain the maximal and minimal fluorescence yield (F_m and F_0). Next, the leaf was light-adapted with 5 min continuous actinic light at 100 µmol m^{-2} s^{-1} and saturating pulses were given every 25 s, the maximum (F_m') and the steady state fluorescence (F_s) signal were recorded. The actinic light was turned off and a far-red pulse was applied to obtain the minimal fluorescence after the PSI excitation (F_0'). For the calculation of F_v/Fm, Φ_{PSII}, qP and NPQ see 2.2.4.

The effects of light quality on the chlorophyll fluorescence parameters were monitored each 5 days for the first 20 days and then at 10 days intervals (total period

of 8 weeks), this in five replicates per treatment. The measurements started 2 h after the start of the photoperiod. The diel change of chlorophyll fluorescence parameters were monitored after 8 weeks under the light quality treatments by measuring every 2 h of the day cycle followed by two measurements during the night cycle at 4 h intervals.

5.2.5 Chlorophyll and carotenoids

After 8 weeks of light treatment, pigments were extracted with 80% (v/v) acetone overnight at -20℃. Absorbance at 470 nm (A_{470}), 647 nm (A_{647}) and 663 nm (A_{663}) were quantified spectrophotometrically (Infinite M200, TECAN Group Ltd., Switzerland). For the detail calculation see 2.2.5.

5.2.6 Metabolites and PEPC activity

Leaf samples were taken after 8 weeks of treatment each 2 h for a 24 h cycle. The two upper leaves were sampled, immediately frozen in liquid N_2 and stored in -80 ℃ until further analysis.

Malate was extracted by boiling distilled water for 15 min and quantified by anion-exchange chromatography (Thermo Fisher Scientific, USA) with 17-50 mM NaOH as gradient eluent and electrochemical detection with a Dionex IonPac AS 19 column at 30 ℃.

Carbohydrates (200 mg FW) were extracted by 80% ethanol and quantified by means of high performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD, Waters, USA) using a CarboPac PA-100 column (Dionex). Starch was extracted from the precipitate by 1M HCl at 95 °C for 2 h. Starch content, expressed as glucose equivalents, was determined enzymatically by the reduction of $NADP^+$ (measured at 340 nm, UV-VIS, Biotek Uvikon XL) with a hexokinase/glucose-6-phosphate dehydrogenase assay.

PEPC activity was expressed in terms of specific enzyme activity and protein content was determined according to Bradford (1976). The protein extraction buffer contained 100 mM HEPES-KOH (pH 8.0), 5 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM ethylene glycol tetra-acetic acid (EGTA), 10 mM dithiothreitol (DDT), 1 mM phenylmethylsulfonyl fluoride (PMSF) and polyvinylpolypyrrolidone (PVPP, 1%, w/v). The PEPC activity

was determined according to López-Millán et al. (2000). Briefly, 50 µL of the extraction and 950 µL of enzyme buffer containing 100 mM Bicine [N,N'-bis(2-hydroxyethylglycine)] - HCl (pH 8.5), 5.0 mM $MgCl_2$, 10 mM $NaHCO_3$, 2 mM PEP and 0.2 mM NADH were added. PEPC activity (µmol mg^{-1} protein min^{-1}) was measured by the reduction of the absorbance at 340 nm at 30 °C.

5.2.7 Growth parameters

After 8 weeks under the specific light treatments, four plants per treatment were randomly collected for the biomass determination. Plants were oven-dried at 85 ℃ for 72 h until a constant mass was reached then the dry mass was determined with an analytical balance (Mettler-Toledo, Greifensee, Switzerland). Leaf area was measured with a leaf area meter (Li-Cor 3000, Li-Cor Inc., USA) and the specific leaf area (SLA) was calculated as SLA = leaf area/leaf dry weight.

5.2.8 Data analysis

Data are presented as means ± SE. Results were analyzed using SPSS Statistics Version 24 (SPSS Inc., Chicago, USA). Homogeneity of variance was verified with Levene's test, analyses were carried out using one-way ANOVA and means with significance difference were separated with Tukey's HSD test (P=0.05), figures were made with SigmaPlot 13.0 (Systat Software Inc., USA).

5.3 Results

5.3.1 Temporal effects on chlorophyll fluorescence

Effects of an increasing period under monochromatic or dichromatic light in comparison with a control treatment were assessed by the maximum quantum yield (F_v/F_m) and PSII operating efficiency (Φ_{PSII}) (Figure 5.2). At day one F_v/F_m was 0.74-0.75 for all treatments and Φ_{PSII} ranged between 0.694 and 0.714. After ten days under monochromatic R light a significantly lower F_v/F_m (P=0.001) was found while no significant differences among the other light treatments were observed. F_v/F_m continued to decrease for the R grown plants and stabilized at 0.650 after 30 days. No significant time trend was observed for the other treatments. Similarly, Φ_{PSII} decreased under R compared to the other light treatments. R grown *Phalaenopsis* resulted in a significantly lower Φ_{PSII} after five days (P<0.001). In the following days,

Φ_{PSII} continued to decline and reached a steady lower value (Φ_{PSII} = 0.620) compared with the other treatments after 15 days, No significant differences between B, RB and W were observed and no temporal effect was present.

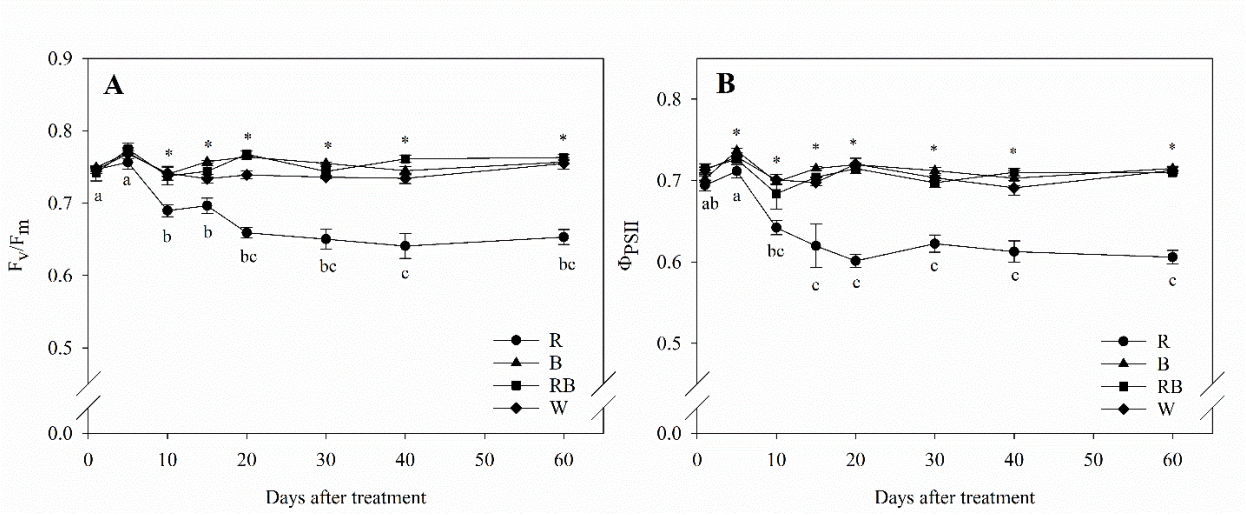


Figure 5.2 Maximum quantum efficiency of Photosystem II (F_v/F_m, A) and quantum yield of Photosystem II (Φ_{PSII}, B) changes after transfer to treatments differing in light quality. Values are the means with standard errors shown by vertical bars (n=4). Asterisk indicates for significant difference (P<0.05) among treatments according to Tukey's HSD test. Time course change for both parameters under R are marked with different letters to indicate significant differences according to Tukey's HSD test (P=0.05).

5.3.2 Diel change of chlorophyll fluorescence parameters

F_v/F_m hardly fluctuated during the 24-h cycle. As already shown in Figure 5.2, F_v/F_m was significantly lower under R. A slight fluctuation was present ranging from 0.654 (at 08:00) to 0.701 (at 14:00) followed by a slow decrease. For the other light treatments, time course fluctuations were hardly observed. Yet small differences between the treatments were present: at 14:00 F_v/F_m was the greatest under B followed by RB and was significantly lower for W while two hours later no significant differences were found between B, RB and W.

The diel changes in the light-adapted chlorophyll fluorescence parameters clearly indicate the CAM cycle (Figure 5.3). The quantum yield of PSII (Φ_{PSII}) and the photochemical quenching (qP) were strongly affected by the light and dark conditions. At the start of the day phase (08:00) Φ_{PSII} and qP were high and these values

remained rather stable for the next 8 h (16:00), then followed a decrease till the start of the night phase (20:00). During the night steady lower levels were maintained. Although the trend was similar for the four light treatments significantly lower values were obtained for R compared to the other light treatments. The diel change of NPQ (Figure 5.3D) excludes the R treatment, because NPQ is comparable when plant exhibiting similar F_m values (Baker, 2008). NPQ fluctuated between 0.085 and 0.221 and was highest under RB when the lights switched on; however, there were no significant differences with the other light quality treatments. During the night period, NPQ dropped to very low values.

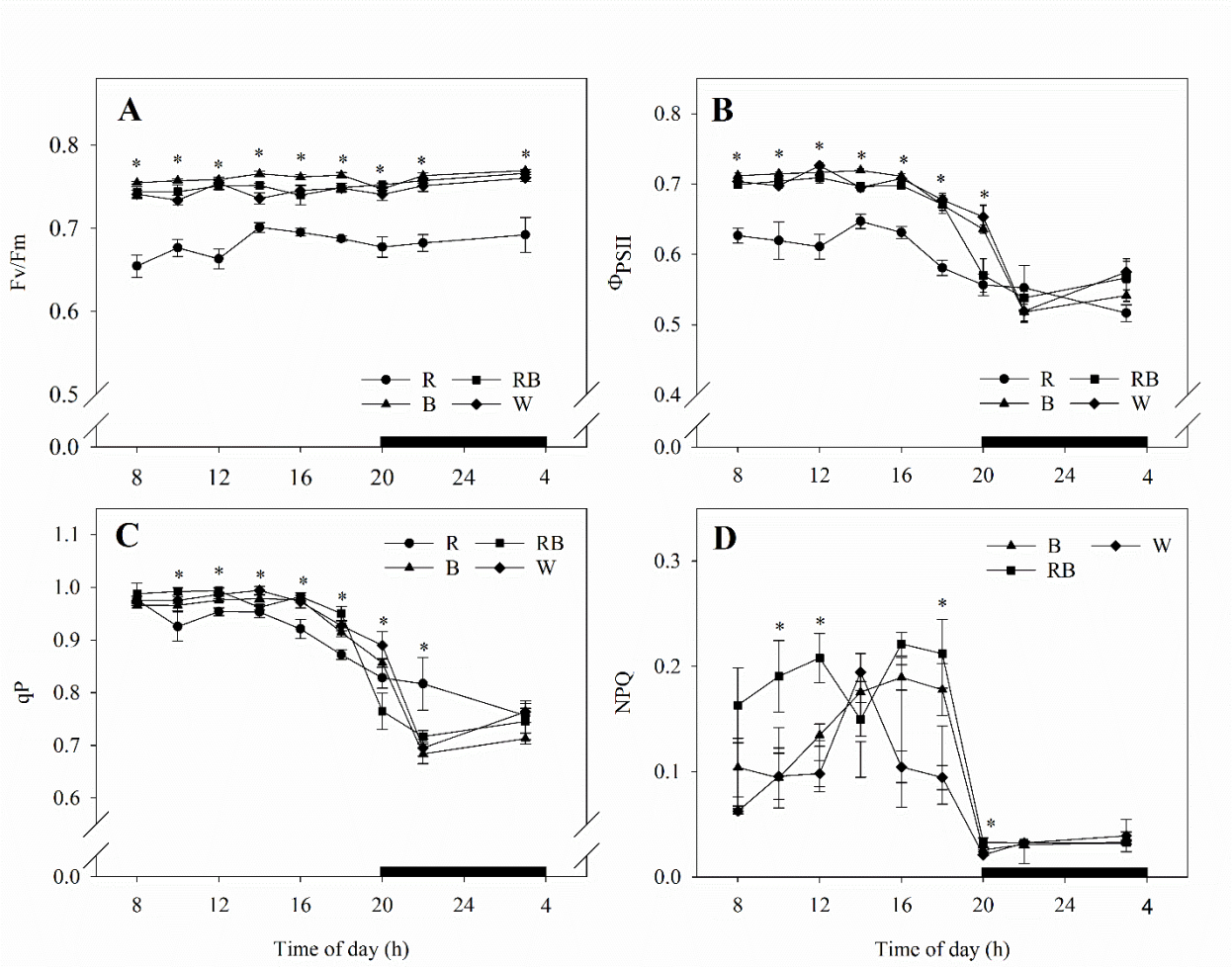


Figure 5.3 Diel change of chlorophyll fluorescence parameters, maximum quantum efficiency of Photosystem II (F_v/F_m, A), quantum yield of Photosystem II (Φ_{PSII}, B), photochemical quenching (qP, C) and non-photochemical quenching (NPQ, D) of *Phalaenopsis* under different light quality. The horizontal black bar indicates the night period. Data represent the mean of four individual plants with standard errors shown by vertical bars (n=4). Asterisk indicates for significant difference (P=0.05) among treatments according to Tukey's HSD test.

5.3.3 Effects on leaf gas exchange

To investigate effects of light quality on photosynthesis, both short time (1 week, Table 5.1) and long term effects (8 weeks, Figure 5.4, Table 5.2) when leaf lengths were already 17.6 ± 0.9 cm (± 3-fold increase compared to the start) were investigated. As expected *Phalaenopsis* showed a CAM pattern of CO_2 uptake, with open stomata to accommodate nocturnal CO_2 fixation and closed stomata during the main part of the light period when the degradation of malate to CO_2 took place in all light treatments. CO_2 uptake rates were integrated for 24 h, and after one week the total diel CO_2 uptake under R tended to decline though this was not significant (Table 5.1).

Table 5.1 Integrated CO_2 uptake of young *Phalaenopsis* leaves (mmol CO_2 m^{-2}) over a 24 h period after 1 week under light quality treatments.

Light quality	Night	Day	Total 24 h
R	44.3 ± 2.5	-7.8 ± 1.1	36.5 ± 3.4
B	49.2 ± 2.5	-7.1 ± 1.9	42.1 ± 1.4
RB	45.1 ± 3.6	-6.7 ± 0.4	38.4 ± 3.3
W	48.2 ± 1.4	-5.3 ± 1.4	42.9 ± 2.8

Data are mean ± SE (n=4). No significant differences at P=0.05 were observed.

After eight weeks, the total diel CO_2 uptake was highest under B followed by W and significantly lower under RB and R (Table 5.2). Taking the data of malate degradation and starch accumulation into account, the four phases of the CAM cycle could clearly be distinguished (Figure 5.4, Table 5.2).

Table 5.2 Integrated CO_2 uptake (mmol CO_2 m^{-2}) of mature leaves and phase duration by phase over a 24 h period for each light treatment after 8 weeks.

Light quality	CO_2 uptake amount integrated by phase (mmol CO_2 m^{-2})				
	Phase I	Phase II	Phase III	Phase IV	Total 24h
R	34.6 ± 1.1 b (92.1%)	0.9 ± 0.1 ab (2.6%)	-4.4 ± 0.6 a	2.1 ± 0.7 a (5.6%)	33.2 ± 1.9 c
B	45.3 ± 1.2 a (91.9%)	1.5 ± 0.2 a (3.0%)	-3.0 ± 1.2 a	3.2 ± 0.6 a (6.3%)	47.7 ± 2.2 a
RB	42.4 ± 1.6 a (94.5%)	1.3 ± 0.2 a (2.9%)	-5.5 ± 0.4 a	1.1 ± 0.5 a (2.5%)	39.4 ± 1.8 bc
W	48.1 ± 1.5 a (96.9%)	0.5 ± 0.1 b (1.1%)	-6.5 ± 1.4 a	1.0 ± 0.4 a (2.0%)	43.1 ± 1.7 ab
Phase duration (h)					
R	12	1.23 ± 0.05 ab	8.83 ± 0.46 ab	1.95 ± 0.23 a	24
B	12	1.76 ± 0.13 a	6.89 ± 0.41 b	3.38 ± 0.23 a	24
RB	12	1.41 ± 0.22 ab	8.32 ± 0.53 ab	2.27 ± 0.67 a	24
W	12	1.05 ± 0.06 b	9.29 ± 0.38 a	1.66 ± 0.41 a	24

Data present in mean ± SE (n=4). Different letters indicate significantly difference between treatment (P=0.05) according to Tukey's HSD test.

CO_2 uptake during the night (phase I) was significantly lower for plants under R light (Table 5.2). During the transition to the light phase, i.e. phase II, integrated CO_2 uptake was significantly greater under B and RB followed by R compared to W (Table 5.2). The duration of phase II was the longest under B followed by RB and R and was significantly shorter under W. In phase III, CO_2 losses tended to be lower under B though no significant effects were present. In phase IV, which was the late afternoon before the night phase started, CO_2 uptake started again in all treatments. This phase started earlier under B followed by RB and R and was the shortest phase under W, however, no significant differences were found.

5.3.4 Diel change of metabolite contents

Malate content: the CAM pattern of nocturnal accumulation and degradation during the light period is clearly shown for all treatments (Figure 5.4). At the end of phase I (08:00) the malate level of the light treatments was: R 54.3 ± 1.6, B 60.4 ± 1.6, RB 66.0 ± 4.5 and W 63.8 ± 2.1 µmol g^{-1} FW, respectively. Lowest levels were found under R (P=0.01). The dusk-dawn malate accumulation was also significantly lower under R compared to the other light treatments (Figure 5.5). The kinetics for malate degradation during phase III were clearly different for the treatments. Under B and RB basal malate levels were obtained at 16.00, a slower decrease was observed for W where basal levels were reached at 18.00. The decrease of malate in R grown *Phalaenopsis* was significantly retarded compared to the other treatments and continued during the first four hours of the night phase. Upon onset of the dark period (20:00), malate levels increased in B, RB and W, whilst this increase was observed only four hours later for R.

Carbohydrate and starch content: Figure 5.4 shows the diel changes of starch and sucrose contents in *Phalaenopsis*; both displayed an inverse diel pattern compared to malate content. As storage sugars, they accumulated during the day period due to the carboxylation of CO_2 released from malate and degraded at night to provide PEP for nocturnal CO_2 fixation. At the time of the start of the light period (08:00), starch content of the four light treatments were low and averaged around 2.2 ± 0.4 µmol D-glucose equivalents g^{-1} FW. During the light period starch values increased to reach their highest levels at the start of the night period (20:00) with averages of 18.3 ± 1.6, 17.9 ± 1.4, 21.5 ± 1.7 and 25.8 ± 0.1 µmol D-glucose equivalents g^{-1} FW for R, B, RB

and W respectively. W resulted in significantly higher values compared to R (Figure 5.4). Starch contents decreased during the night from 22:00 for W and from 24:00 for the other light qualities.

The diel pattern was less pronounced for sucrose. At the start of the light period (08:00) it was respectively 2.8 ± 0.3, 2.2 ± 0.1, 4.2 ± 0.3 and 4.0 ± 0.6 µmol g^{-1} FW for R, B, RB and W. Fluctuations during the light period were observed but only after two hours night phase a significant decrease was recorded for all light treatments. The difference between the start of the photoperiod (08:00) and the start of the nocturnal period (20:00) (Figure 5.4) was 2.7, 1.8, 1.4 and 1.7 µmol g^{-1} FW for R, B, RB and W, respectively.

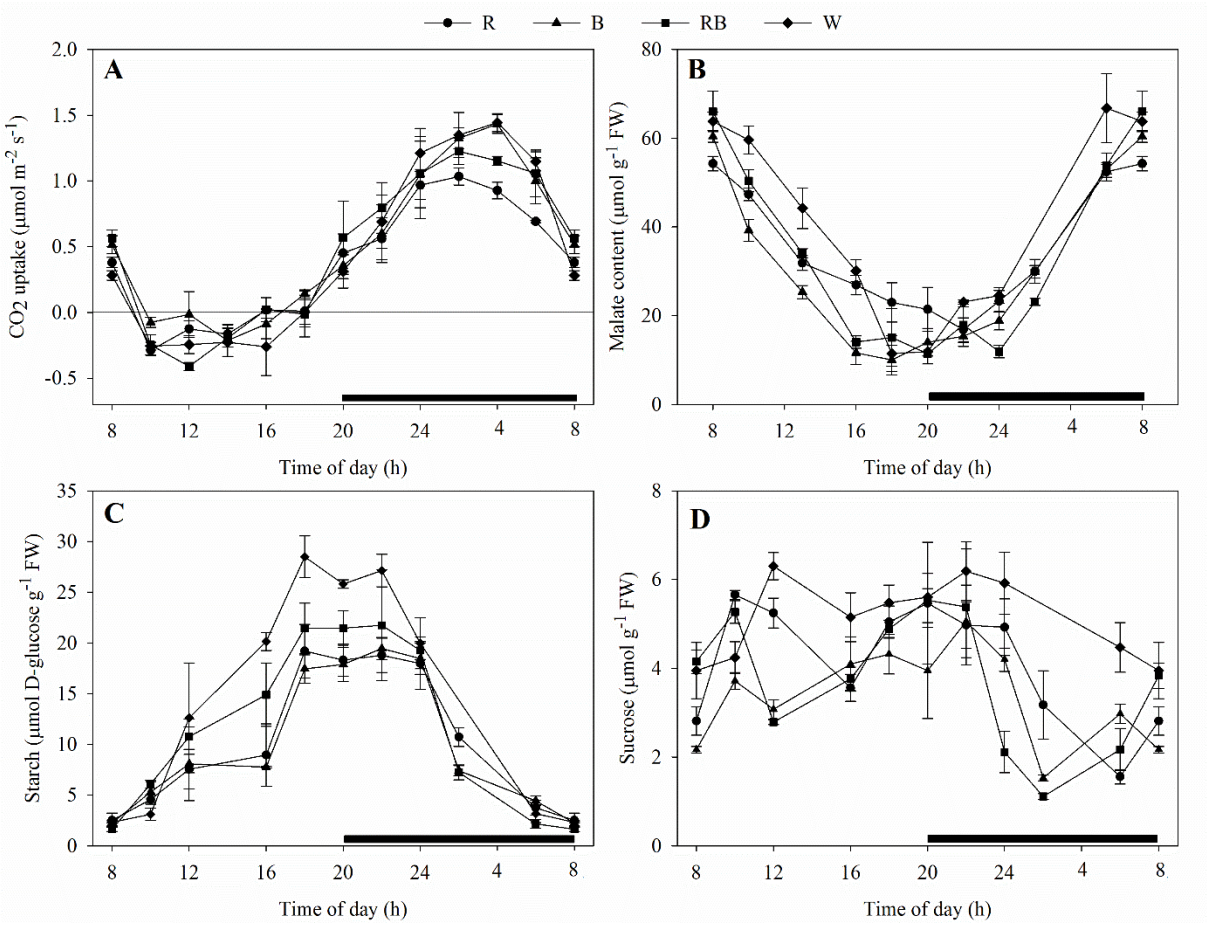


Figure 5.4 Diel change of the CO_2 uptake (A), malate content (B) and storage carbohydrates: starch (C) and sucrose (D) in *Phalaenopsis* leaves under different light quality. The horizontal black bar indicates the night period. Data represent the mean of four replicates with standard errors shown by vertical bars. Asterisk indicates for significant difference (P=0.05) among treatments according to Tukey's HSD test.

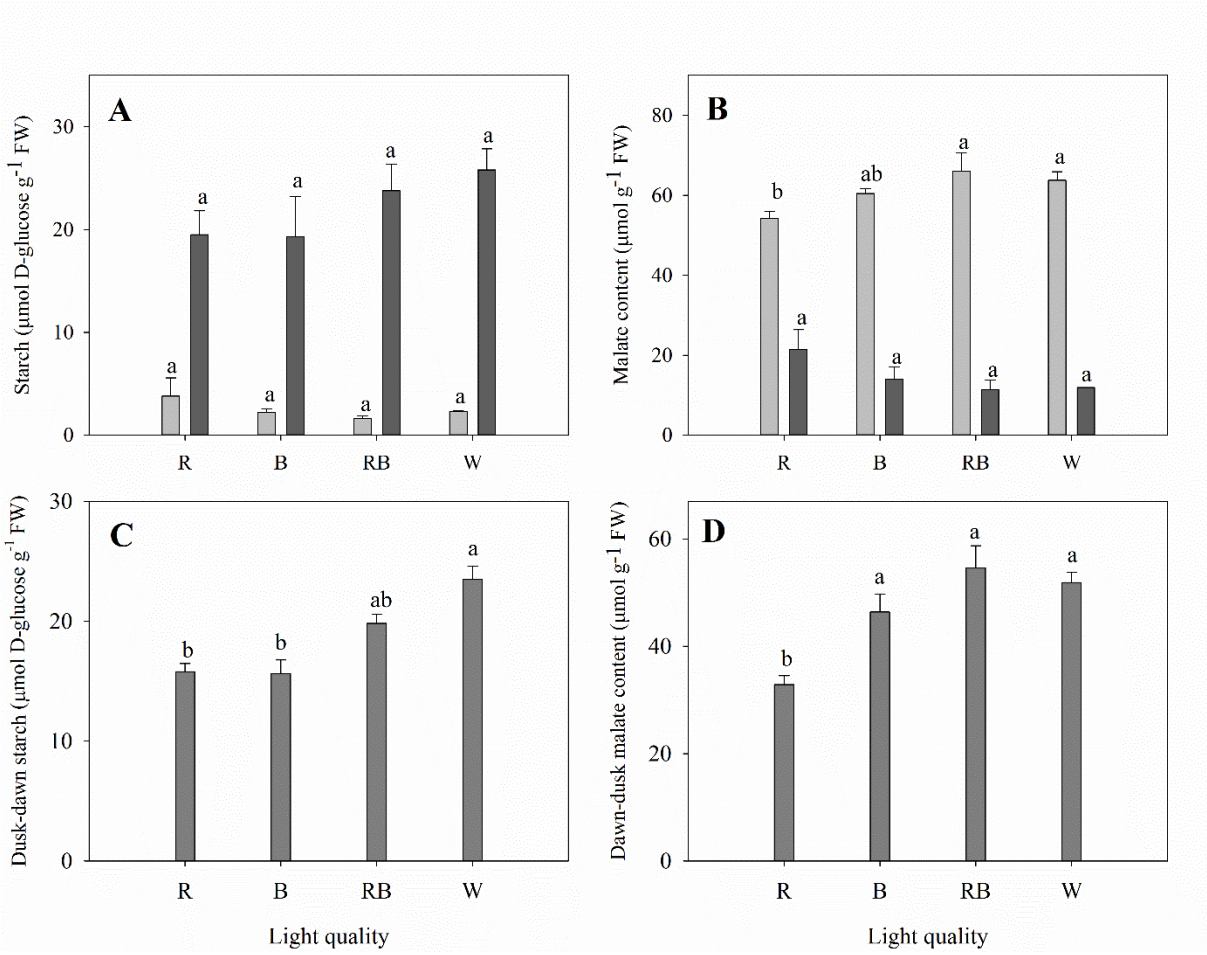


Figure 5.5 Starch content (A) and malate content (B) of *Phalaenopsis* leaves under different light quality at dawn (08:00) and dusk (20:00), and the dawn and dusk difference of starch content (C) and malate content (D). Data are shown as mean ± SE (n=4), different letters indicate for significant differences (P=0.05) at the same time point according to Tukey's HSD test.

PEPC activity: The total protein content was not significantly affected by the light treatment (P > 0.05) though it tended to be lower under R and W. Figure 5.6 shows the PEPC activity at four time points: 00:00, 08:00, 13:00 and 20:00. It is clearly shown that at midnight (00:00), PEPC activity was the greatest, whilst only negligible activity was present at noon (13:00). At midnight, PEPC activity was greater under RB and W compared to R and B though this difference was not significant (P >0.05). As expected, dawn activity was lower than dusk activity (P=0.02), but no significant differences underpinned by light quality were found concerning PEPC activity.

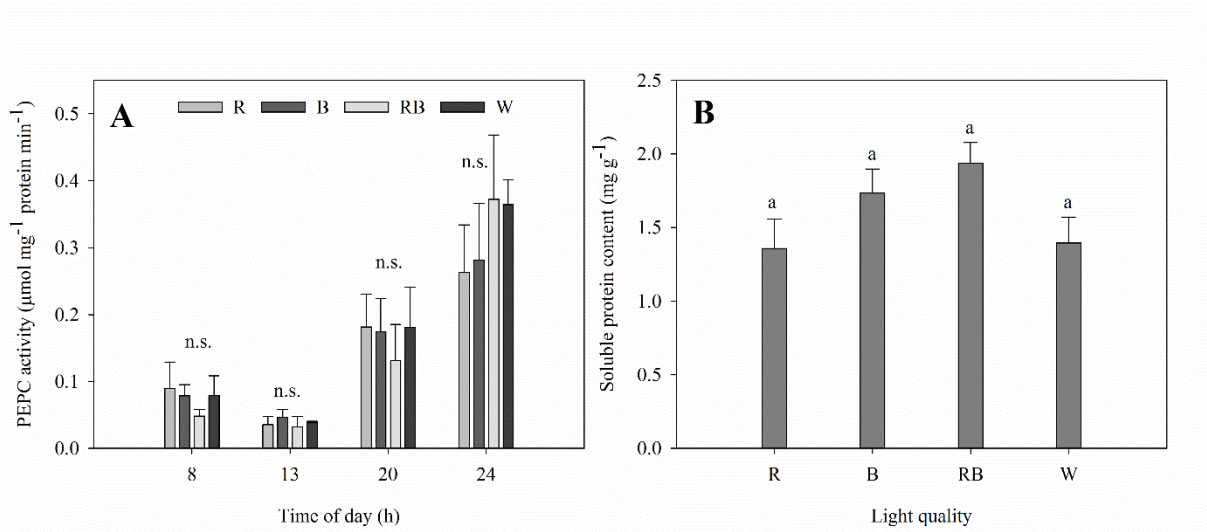


Figure 5.6 PEPC activity and total soluble protein content of *Phalaenopsis* leaves at four time points (08:00, 13:00, 20:00 and 24:00) under different light quality treatments. Data shown as means with standard errors (n=4), different letters indicate for significant differences according to Tukey's HSD test (P=0.05), n.s., not significant.

5.3.5 Growth and pigment contents of *Phalaenopsis*

Table 5.3 presents chlorophyll and carotenoid contents after 8 weeks of growth under the four different light treatments. The highest content of all pigments was found under B. In detail, Chl *a* was significantly higher under B and RB, while it decreased under W and was lowest under R; Chl *b* was significantly higher under B, declined under RB and W while it was lowest under R. Chl *a/b* ratio was unaffected by light quality. Carotenoid content was significantly higher under B, followed by RB and W and the lowest content was found under R.

Table 5.3 Pigments contents of *Phalaenopsis* grown for 8 weeks under different light quality treatments.

Light quality	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Chl a/b
R	15.29 ± 1.51 c	7.62 ± 0.51 d	3.94 ± 0.45 c	22.91 ± 1.96 c	2.0 ± 0.1 a
B	34.14 ± 1.05 a	15.68 ± 0.19 a	8.65 ± 0.11 a	49.82 ± 1.24 a	2.2 ± 0.0 a
RB	30.33 ± 2.44 a	13.62 ± 0.62 b	7.28 ± 0.44 b	43.95 ± 3.06 a	2.2 ± 0.1 a
W	23.73 ± 0.55 b	11.61 ± 0.19 c	6.22 ± 0.08 b	35.33 ± 0.20 b	2.1 ± 0.0 a

Data present in mean ± SE (n=4). Different letters indicate significant differences between treatments (P=0.05) according to Tukey' HSD test.

The total leaf area, specific leaf area and dry biomass are shown in Table 5.4. The highest dry mass was obtained for RB and W compared to R, while B had an intermediate dry mass. No significant effects of light quality on total leaf area and specific leaf area were found though SLA tended to be higher under R compared to W.

Table 5.4 Total dry biomass, leaf area and specific leaf area of the second leaf from the apex (SLA) of *Phalaenopsis* grown for 8 weeks under different light quality treatments.

Light quality	Biomass (g)	Leaf area (cm^2)	SLA ($cm^2\ g^{-1}$)
R	1.58 ± 0.02 b	198.3 ± 2.4 a	125.54 ± 3.3 a
B	1.77 ± 0.07 ab	200.9 ± 7.8 a	112.95 ± 3.3 a
RB	1.79 ± 0.03 a	211.2 ± 3.5 a	113.75 ± 4.3 a
W	1.86 ± 0.04 a	192.6 ± 4.6 a	107.73 ± 8.3 a

Data present in mean ± SE (n=4). Different letters indicate significant differences between treatments (P=0.05) according to Tukey's HSD test.

5.4 Discussion

Chlorophyll fluorescence analysis has widely been used to assess the functioning of Photosystem II under abiotic and biotic stresses (Jones and Hoegh-Guldberg, 2001; van Kooten and Snel, 1990) F_v/F_m of unstressed plants varies typically between 0.75 and 0.85 (Quiles, 2005). At the start of the experiment values for *Phalaenopsis* ranged between 0.75-0.80 which is in agreement with values reported by Ouzounis et al. (2015) and Pollet et al. (2009).

When grown under monochromatic red light a significant decline of both F_v/F_m and Φ_{PSII} appeared after respectively 10 and 5 days (Figure 5.2). A decline in F_v/F_m is correlated with loss of PSII photosynthetic activity of isolated thylakoids (Krause et al., 1990), which indicates photo-damage (Baker, 2008). This decline of the photosynthetic capacity under R continued and reached a stable significantly lower level compared with the other light treatments. These negative effects of R persisted and were also clearly visible in the diel change of F_v/F_m and Φ_{PSII} after 8 weeks of

acclimation (Figure 5.3). Although a short term exposure to red light has been reported previously to result in higher photosynthetic performance (McCree, 1971); it is no surprise that long-term red light exposition abated photosynthetic performance. Previous studies showed that monochromatic red light reduced F_v/F_m in different C3 species, including cucumber (Trouwborst et al., 2016), *Chrysanthemum*, *Cordyline*, *Ficus, Spathiphyllum* (Chapter 2 and 3) and rapeseed (Shengxin et al., 2016). In *Phalaenopsis*, red light in a background of daylight also led to the lowest F_v/F_m (even < 0.6) (Ouzounis et al., 2015a). In this experiment, *Phalaenopsis* takes about 5-10 days to reach this imbalance (as already explained in previous chapters), suggesting a long-term reaction including gene modulation (Ferroni, 2012). If only monochromatic blue is provided no negative effects were observed and addition of blue to red (RB) restored the imbalances as well.

The observed long-term reaction of both F_v/F_m and Φ_{PSII} is also reflected in the pigment concentration. Monochromatic red light significantly reduced Chl *a*, Chl *b*, total Chl and carotenoid content compared to the other light treatments (Table 5.3). As chlorophyll absorbs light both in the red and blue spectrum (Terashima et al., 2009), the reduced chlorophyll content under R will result in a lower photosynthetic efficiency as assessed by F_v/F_m and Φ_{PSII}. These observations clearly indicate that blue light is inevitable to accommodate an efficient Photosystem processing. On the other hand, monochromatic blue light seemed able to compensate for the lack of red light, as higher concentrations of chlorophyll and carotenoids under monochromatic blue resulted in unaffected F_v/F_m and Φ_{PSII} compared to either white light or a combination of red and blue. Blue light has previously been shown to promote biosynthesis of chlorophyll pigments (Hoffmann et al., 2015a; Olle and Viršile, 2013; Sæbø et al., 1995).

Both photochemical quenching (q_P) and the quantum yield (Φ_{PSII}) demonstrate the effective operating efficiency of PSII. Under a constant light fluence we observed a steady value for the beginning and middle part of the photoperiod, when malic acid decarboxylation provided saturating concentrations of intercellular CO_2 for photosynthesis activity (Winter and Lesch, 1992). Towards the end of the photoperiod, the internal pool of malic acid has been consumed, leading to a decrease of Φ_{PSII} and q_P (Adams et al., 1989). The steep decrease of qP and Φ_{PSII} at the transition of day to night corresponds to the termination of photosynthetic electron

transport simultaneous with Calvin cycle inactivation (Pollet et al., 2009). During the light period, q_P, as a measure of the proportion of open PSII reaction centers, is always lower under R while no differences between the other light treatments were observed. It is, however, difficult to interpret heat dissipation (NPQ) for R as F_v/F_m values were lower compared to the other light treatments. Although the values for non-photochemical quenching are low, RB resulted in average in the highest NPQ values during the day, W in the lowest and for B a steady increase during the light period was observed.

After 8 weeks under the respective monochromatic light treatments (100 µmol m^{-2} s^{-1}) the different phases in Osmond's framework could all be distinguished. Upon the onset of the day short Phase II was observed in the four light treatments. Ceusters et al. (2014) reported only a phase II under W and low-fluence B in the obligate CAM *Aechmea*, while this phase was absent under low-fluence R. In C3 plants it is generally accepted that blue light triggers stomatal opening through the blue light photoreceptors, phototropin and cryptochrome (Boccalandro et al., 2012; Liscum et al., 2003; Shimazaki et al., 2007). This signaling pathway is supposed to be weaker or absent in CAM species (Lee and Assmann, 1992). In addition, recent transcriptomic analyses of the consecutive CAM plant *Agave americana* did not reveal a prominent role regarding stomatal regulation (Abraham et al., 2016). However, in *Aechmea* low fluence B clearly induced early morning stomatal opening and phase II occurred while this was not the case for low fluence R (Ceusters et al., 2014). The different response to R in phase II between the results of Ceusters et al. (2014) in *Aechmea* and our data might also be due to the applied fluence levels which were 10-fold higher in our treatments (10 and 100 µmol m^{-2} s^{-1} respectively). It is without doubt that the role of blue light to induce stomatal opening at the transition of the dark to the light phase needs further investigation, but different mechanisms might exist in CAM species. There are several hypotheses about stomatal opening mechanisms of CAM, such as the internal CO_2 concentration, leaf-air vapor pressure deficit and photoperiodic circadian rhythm (Lee, 2010; Males and Griffiths, 2017).

The rate of decarboxylation under R during the photoperiod was consistently lower compared to the other light treatments and malic acid breakdown persisted until 4 hours after dusk. Together with higher basal levels of malic acid, red illumination might cause a lowered ME activity in the leaf mesophyll cells or bring about an

impaired malate efflux out of the vacuole. B and RB on the other hand brought about an acceleration of malic acid consumption in comparison to control plants under white light. Starch was the main carbohydrate storage to fuel nocturnal carboxylation in *Phalaenopsis* and was synthesized during the photoperiod under all light treatments but to a higher extent under white light.

Nocturnal CO_2 uptake during Phase I showed an overall reduction of about 25 % under R compared to the other treatments. As a consequence the nocturnal malic acid turnover was also negatively affected under R. Since our measurements indicate that intrinsic activity of PEPC was not statistically different among the light treatments, the restricted availability of storage carbohydrate (i.e. starch) under R is likely to cause this important penalty. The availability of carbohydrate storage is generally considered to be a major limiting factor for malate synthesis and consequently for the magnitude of dark CO_2 uptake in CAM plants under different environmental conditions (Borland and Dodd, 2002; Ceusters et al., 2010, 2011).

Phase IV CO_2 assimilation was also observed in all treatments (Table 5.2). We recorded an earlier stomatal opening in phase IV under blue light and RB leading to a longer duration of phase IV (Table 5.2, Figure 5.4). This earlier start of phase IV may be explained by the low intercellular CO_2 concentration induced by the end of malate breakdown which was earlier in B and RB, thus resulting in the reopening of stomata to initiate Phase IV (Males and Griffiths, 2017).

The significant decrease in photosynthetic efficiency, diel CO_2 uptake and turnover of starch under monochromatic red light was also reflected in biomass accumulation, but to a lesser extent. R grown *Phalaenopsis* produced about 15% less biomass in comparison to white illuminated plants (Table 5.4). Ouzounis et al. (2015) even found a ± 25% decrease of leaf fresh weight for *Phalaenopsis* under R in a daylight background. Leaf area and specific leaf area were not affected by light quality in our treatments. For a similar period of 8 weeks *Phalaenopsis* ‘Vivien’ increased its leaf area with an increasing blue light fraction while ‘Purple Star’ was not affected (Ouzounis et al., 2015a) indicating the species dependency for effects of light quality on leaf development.

5.5 Conclusion

In conclusion, long-term monochromatic red light induced disorders in the development of Photosystem II in comparison with treatments including blue light. This was reflected in reduced maximum quantum yield, quantum efficiency, chlorophyll and carotenoid content, starch and malate formation and gas exchange and biomass. The present study stressed the importance of blue light quality in regulating an efficient Photosystem II. Blue light influenced the diel CAM rhythm and enhanced malate metabolism. Further investigations on the stomatal behavior explaining the effects of light quality on the regulation of CAM phases are needed.

Chapter 6

Acclimation of *Chrysanthemum* and *Spathiphyllum* to summer greenhouse conditions after LED light pre-production phase

This chapter is based on:

Zheng L., Steppe K. and Van Labeke M.C. Acclimation of *Chrysanthemum* and *Spathiphyllum* to summer greenhouse conditions after LED light pre-production phase. Submitted to Frontiers in Plant Science. Under review.

Author contribution:

LZ and MCVL conceived and designed the experiments. LZ performed the experiments, analyzed the data and drafted the manuscript, MCVL and KS critically revised the manuscript.

Abstract

Light is one of the most important environmental factors affecting plant development and behavior. Acclimation of young plants that were grown for a period solely under LED lights to greenhouse conditions might depend on their initial light quality treatment. In this study, we chose two plant species: *Chrysanthemum* (sun species) and *Spathiphyllum* (shade species), and pre-conditioned them in the growth chamber for four weeks under four light qualities: blue (B, peak at 460 nm), red (R, peak at 660 nm), red with blue (RB, 60% R with a peak at 660 nm and 40% B with a peak at 460 nm) and white (W, 300-800 nm) at 100 µmol m^{-2} s^{-1}. The applied light quality influenced both leaf characteristics and leaf photosynthetic performance. Monochromatic light (R and B) limited leaf development of both *Chrysanthemum* and *Spathiphyllum*, which resulted in lower leaf mass per area when compared to multispectral light (RB for *Chrysanthemum*, RB and W for *Spathiphyllum*). Leaves that developed under R had a lower photosynthetic efficiency in both species. On the first day of transfer to high natural light levels in the greenhouse, R and B pre-conditioned leaves of both species resulted in inhibition of photosynthesis. After 1 week of acclimation, *Chrysanthemum* leaves that had developed under B acclimated to sunlight at a similar level of RB though this was not the case for R pre-conditioned leaves. Even after 1 month of development in the greenhouse, R pre-conditioned *Chrysanthemum* plants resulted in a lower dry mass accumulation when compared to the other light quality treatments. *Spathiphyllum* leaves (shade species) showed a decrease in ETR_{max} after one week of acclimation and this was most pronounced for the R pretreatment. In contrast to *Chrysanthemum*, no effects on dry weight of *Spathiphyllum* after one month in the greenhouse with respect to the light quality pretreatments were observed.

6.1 Introduction

Light is an indispensable energy source for plant growth though it may also be a stress-causing factor. Plants exhibit a remarkable adaptability and plasticity to changing light conditions by varying the organization of their photosynthetic apparatus and adapting anatomical structures in newly formed leaves. For example, sun leaves are thicker and have a well-developed palisade layer with a high proportion of columnar cells to arrange all chloroplasts along the cell surface (Bukhov et al., 1995). Plant leaves that developed under a specific irradiance are adapted to this light environment and anatomical changes are limited after maturation (Milthorpe, 1959; Oguchi et al., 2003). High plasticity in photosynthetic acclimation of mature leaves would be advantageous when irradiance suddenly increased such as under sun-flecks in natural ecosystems (Oguchi et al., 2005) or from relative low light intensities in climate controlled vertical farming systems to the more dynamic greenhouse environment as might be the case in ornamental production.

Shade-adapted leaves have more chlorophyll-containing light-harvesting proteins relative to light-using enzymes involved in electron transport and metabolism, meaning that photosynthesis saturates at lower irradiances. When low-light acclimated leaves were exposed to a higher irradiance, increases in maximum photosynthetic rate have been observed (Naidu and DeLucia, 1997; Oguchi et al., 2003), though these leaves may not achieve the assimilation level of leaves that developed under high irradiance (Frak et al., 2001). Moreover, when shade leaves are exposed to high light intensities, this results in light stress as the absorbed excessive light energy cannot be used for CO_2 fixation. Light acclimation processes in plants act to dissipate this excess excitation energy and optimize photosynthesis under variable light conditions. This energy excess is directly dissipated as light emission by fluorescence (Φ_{NO}) or as heat by non-photochemical quenching (Φ_{NPQ}). The light energy absorbed by Photosystem II (PSII) is thus divided into three fractions: $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{NO} = 1$ (Kramer et al., 2004). Failure to dissipate and quench the energy excess can be highly damaging to plants, and is often visible as chlorosis, bleaching or bronzing of leaves (Karpiński et al., 2013).

Not only light intensity but also light quality can influence leaf anatomy (Arena et al., 2016). Monochromatic red light generally results in a decrease of leaf thickness

(Shengxin et al., 2016; Zheng and Van Labeke, 2017a). In addition, modifications in leaf structure, such as enlargement of palisade cells or development of multiple layers of palisade cells, which were observed under enrichment of blue light (Shengxin et al., 2016; Zheng and Van Labeke, 2017b), can favor photosynthetic acclimation (Abidi et al., 2013; Calzavara et al., 2015; Sanches et al., 2016). Increasing the blue photon fraction increases the Chl *a/b* ratio (sun-leaf characteristic) (Abidi et al., 2013), which is consistent with decreases in the size of the PSII light-harvesting antenna complex (Bailey et al., 2001). Different light spectral qualities induce differences in the ratio of Photosystem II to Photosystem I (Walters and Horton, 1994). When leaves that were formed under different spectral qualities are transferred to natural full spectrum light, they may acclimate in a different way or at a different rate. Cucumber leaves with the 'red light syndrome', which indicates physiological disorder induced by monochromatic red light, recovered from photodamage after transfer to red+blue light within 4 days (Trouwborst et al., 2016). Also the anti-oxidative status is influenced by light quality (Ouzounis et al., 2014). For *Chrysanthemum*, Zheng and Van Labeke (2017b) found a higher proline level when leaves developed under monochromatic blue, and for certain cultivars higher H_2O_2 level.

Differences in leaf characteristics that developed under a specific light spectrum could potentially lead to differences in acclimation when plants are subjected to high irradiances. Compared to controlled conditions, plants in the greenhouse not only face changing light conditions, but also air temperature will fluctuate with respect to the ambient sunlight thus also influencing photosynthetic acclimation (Berry and Bjorkman, 1980; Yamori et al., 2014). We investigated the acclimation capacity of leaves of two ornamental species to summer greenhouse conditions that were pre-treated with relative low light intensities of blue, red, red+blue and multispectral white light. We selected two plant species with contrasting light saturation levels under natural conditions, being *Chrysanthemum* with light saturation levels between 500-600 µmol m^{-2} s^{-1} at leaf level (Weerakkody and Suriyagoda, 2015) and *Spathiphyllum* with light saturation levels between 200-300 µmol m^{-2} s^{-1} (Neretti, 2009). We hypothesized that the blue light fraction would be beneficial for the acclimation phase as blue light has a positive effect on the leaf anatomical development and has no negative effects on the photosynthetic performance. Our approach was by studying

both photosynthesis and chlorophyll fluorescence parameters, including the rapid light response curve, during the first week of acclimation. Additionally, we measured dry mass accumulation after 1 month in greenhouse conditions to rate the long term effect of light quality treatments.

6.2 Materials and Methods

6.2.1 Plant material

Rooted cuttings of *Chrysanthemum morifolium* 'Bolero' and young plants (= in vitro acclimated plants) of *Spathiphyllum wallisii* 'Alfetta' were selected as experimental plants. The experiment with *Spathiphyllum* started on 15 April 2016, while the experiment with *Chrysanthemum* started on 13 May 2016. At the start of the experiment the plants were transplanted in 0.3 L pots with commercial peat-based potting substrate (Van Israel nv, Belgium).

6.2.2 Light treatments during the first four weeks

Plants were subjected to four different light qualities for four weeks in a growth chamber at Ghent University, Belgium. Light was supplied with either LED lamps (Philips, Eindhoven, The Netherlands) and CID-800 programmable LED lighting system (CID Bio-Science, USA) for the polychromic RB light or with light emitting plasma lamps (Gavita Inc., the Netherlands) as a multispectral white light control treatment. Treatments were separated with non-reflective curtains. The light treatments were blue (B, peak at 460 nm), red (R, peak at 660 nm), red with blue (RB, 60%/40%, peak at 460 nm and 660 nm) and white (W, 300-800 nm), respectively. Light intensity at the top of the canopy level was set at 100 µmol m^{-2} s^{-1} by adjusting the distance of the lamps. The light uniformity was verified by five point measurements. The wavelength spectrum was recorded with a JAZ spectrometer (Ocean optics, FL, USA) (Figure 5.1). Plants received a photoperiod of 16 h. Air temperature in the growth chamber was maintained at ± 22 ℃, relative humidity at 60-70 %, and plants were irrigated and fertilized with water soluble fertilizer (N: P: K=4:1:2, EC=1.5 dS m^{-1}) twice a week.

6.2.3 Greenhouse conditions

After four weeks, plants were transferred to the greenhouse before sunrise (Melle, Belgium. 50°99'N, 03°78'E); this respectively on 17 May 2016 for *Spathiphyllum* and 16 June 2016 for *Chrysanthemum*. Air temperature was set at 22°C/18°C for day and night. Shading screens closed when irradiation was higher than 300 W m^{-2} (± 635 µmol PAR m^{-2} s^{-1}). Plants were daily irrigated and received once a week a fertigation (EC=1.5 dS m^{-1}). Temperature and irradiation were monitored (Table 6.1) and the temperature and light intensity change for the first (t_1) and eight day (t_8) of greenhouse transfer is shown in Figure 6.1.

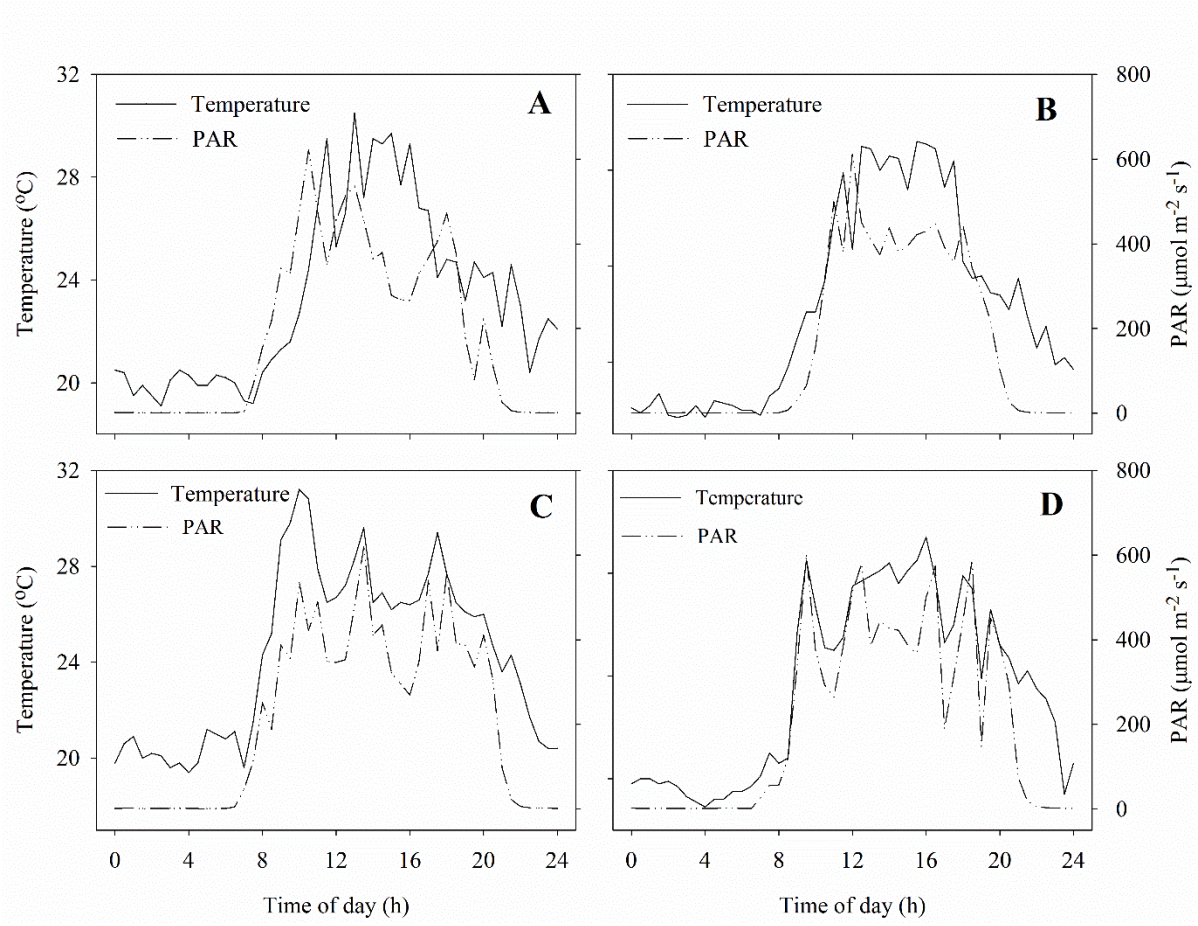


Figure 6.1 The climate condition of the greenhouse on the first day of greenhouse acclimation (t_1): A, *Spathiphyllum* (17/05/2016) and C, *Chrysanthemum* (16/06/2016) and after 8 days of greenhouse acclimation (t_8): B, *Spathiphyllum* (24/05/2016) and D, *Chrysanthemum* (23/06/2016). Light intensity was measured outside the greenhouse with a solarimeter, recalculated to PAR light and reduced by 10% to account for the transmission losses of the glass cover, for the time points that the shading screen was closed, light intensity was reduced by 45% (reduction of the screen). Temperature was measured inside the greenhouse compartment.

Table 6.1 The daily light integral (DLI) (mol m^{-2}) and mean day/night temperature (°C) conditions in the greenhouse during the acclimation phase. t1, t8 and t30: respectively the first, eighth and thirtieth day of acclimation.

Time point	*Chrysanthemum*		*Spathiphyllum*	
	DLI (mol m^{-2})	T (°C)	DLI (mol m^{-2})	T (°C)
t_1	18.70	26.5/20.3	16.42	23.5/20.0
t_8	17.99	26.8/22.1	15.56	25.0/20.0
t_{30}	17.26	28.2/19.9	17.02	25.2/20.8
Averaged (t_1-t_{30})	17.44	26.5/20.6	16.83	26.2/20.2

6.2.4 Photosynthesis and chlorophyll fluorescence measurements

Photosynthesis and chlorophyll fluorescence were measured the last day in the growth chamber (t_0) and in greenhouse conditions on successive days (day (=t) 1, 2, 5, 8 for *Chrysanthemum* and day 1, 2, 3, 4, 8 for *Spathiphyllum*) to study effects of leaf acclimation. For these measurements a fully developed leaf (four plants each treatment) under the light quality treatments of the growth chamber was labeled. This was the third fully expanded leaf from the apex for *Chrysanthemum* and the second leaf from the apex for *Spathiphyllum.* On the first day of transfer to the greenhouse (t_1), daily chlorophyll fluorescence pattern was also recorded (every 2 h from 10 am to 18 pm). After 1 month (t_{30}), photosynthesis and chlorophyll fluorescence were measured again but then on the youngest fully developed leaf with four replicates under greenhouse conditions in order to study if there were remaining effects on new developed leaves.

Leaf gas exchange was measured using the Li-6400 portable gas exchange system (LiCor Inc., Lincoln, NE, USA.). The CO_2 concentration entering the leaf chamber was adjusted to 400 µmol mol^{-1} supplied by a CO_2 gas container, leaf temperature was maintained at 22°C, PPFD at 600 µmol m^{-2} s^{-1}.

Chlorophyll a fluorescence was measured using a portable amplitude modulation fluorometer (PAM-2500, Walz, Effeltrich, Germany). The leaf was dark adapted for 30 min, after that, a 0.6 s saturating light (3450 µmol m^{-2} s^{-1}) was given to obtain the maximal and minimal fluorescence yield (F_m and F_0). Then, the leaf was light adapted

for 5 min with continuous actinic light at 600 µmol m^{-2} s^{-1} and saturating pulses every 25 s, the maximum (F_m') and the steady state fluorescence (F_s) signal were recorded. The actinic light was turned off and a far-red pulse was applied to obtain the minimal fluorescence after PSI excitation (F_0'). The calculation of F_v/F_m Φ_{PSII} and NPQ are given in 2.2.4. The sum of all yields for dissipative processes for the energy absorbed by PSII is unity: $\Phi_{PSII}+\Phi_{NPQ}+\Phi_{NO}=1$ (Kramer et al., 2004), Φ_{PSII} indicates the quantum yield of Photosystem II electron transport, Φ_{NPQ} is the quantum yield of non-photochemical quenching, while Φ_{NO} is the quantum yield of non-regulated energy dissipation.

The rapid light response curve (RLC) was determined with the portable amplitude modulation fluorometer (PAM-2500, Walz, Effeltrich, Germany) according to Ralph and Gademann (2005). Rapid light curve (RLC) measures the effective quantum yield as a function of irradiance in comparison with traditional light curves that use photosynthesis (P-I curves). RLC provides a reliable assessment of photosynthetic activity, by integrating the ability of leaves to tolerate light fluctuation (Ralph and Gademann, 2005). It was conducted on t_0 and t_8. F_0 and F_m were obtained as above from dark-adapted leaves. The leaves were exposed to a gradual increase of irradiance in eight steps with 10 s intervals ranging from 0 to 2000 µmol photons m^{-2} s^{-1}, each irradiance step was separated by a 0.8 s saturating flash. The fluorescence signal was recorded and the rapid light curve was fitted. ETR was calculated as $\mathrm{ETR} = \Phi_{\mathrm{PSII}} \times \mathrm{PAR} \times 0.5 \times 0.84$ with PAR as the actinic irradiance, 0.5 accounts for the fraction of excitation energy distributed to PSII and 0.84 was the assumed fraction of incident quanta absorbed by the leaf (Baker, 2008; White and Critchley, 1999). The light response of the plant was characterized by fitting the model of Platt et al. (1980) to ETR versus I (PPFD) curves and by estimating the parameters α (initial slope of the light curve), ETR_{max} (maximum ETR) and the light-saturation I_k (irradiance at the onset of light saturation) was calculated by $I_k=ETR_{max}/\alpha$.

6.2.5 Leaf chlorophyll content

Leaf chlorophyll content was analyzed on a leaf that fully developed under a light quality treatment (t_0) and the same position leaves after acclimation in the greenhouse (t_8) were sampled. Pigments were determined using the method described by Lichtenthaler and Buschmann (2001). For details see 2.2.5.

6.2.6 Growth analysis

Dry weight of the aerial part was determined after four weeks under the LED treatments (t_0) and after four weeks (t_{30}) in the greenhouse. Fresh weight was determined and then oven-dried at 85 °C for 3 days until a constant mass was reached to obtain the dry weight. The absolute growth rate (AGR, g day^{-1}), which defines the rate of increase of total dry weight per plant per day, was calculated between the two time points. A normalized factor for biomass increase was calculated as $(DW_{t30}-DW_{t0})/DW_{t0}$. Measurements were done in four replicates.

At the same time points, the third fully expanded leaf from the apex was taken on four lateral branches for each *Chrysanthemum* plant and the second leaf from the apex in *Spathiphyllum*, this in four replicates. Digital photos of each individual leaf on millimeter paper as reference were taken to analyze the leaf area with ImageJ (NIH, USA). After that, the leaves were oven-dried for 72 h to obtain the dry weight. Leaf mass per area (LMA) was calculated as leaf dry weight/leaf area. Plant height was measured with a ruler (accuracy at 1 mm) for *Chrysanthemum*. Because *Spathiphyllum* is a monocot and only rosette leaves were present/formed, height was not measured for this species.

6.2.7 Data analysis

Data are reported as means ± SE. Results were analyzed using SPSS statistical software Version 24 (SPSS Inc., Chicago, USA), figures were made using SigmaPlot 13.0 (Systat Software, Inc, USA). Homogeneity of variance was verified with Levene's test, analyses were carried out using one-way ANOVA and significant differences were separated with Tukey's HSD test (p=0.05).

6.3 Results

6.3.1 Characterization of the photosynthetic efficiency after four weeks under LED light (t_0)

Leaf photosynthetic capacity was assessed by the rapid light curve approach. The electron transport rate curves (ETR) follow the classical shape of the photosynthesis light response curves with a linear rise followed by a plateau for both species (Figure

6.3). However, depending on the light quality treatment, the light response was different for *Chrysanthemum* and *Spathiphyllum* (Figure 6.3, Table 6.2).

For *Chrysanthemum* the R and B light treatments reached a plateau at 500 µmol m^{-2} s^{-1} while RB and W saturated at higher light levels (800 µmol m^{-2} s^{-1}). The slope of the light limiting response (α) showed no significant influence and ranged between 0.391 and 0.413. ETR_{max} was greatest for leaves developed under W followed by RB and significantly lower for B and R. I_k was greatest for W followed by RB and R and significantly lower under B (Table 6.2). qP decreased with increasing light intensity. However, leaves that developed under W maintained a higher qP for each light level, followed by RB while no differences between R and B were observed. NPQ increased with increasing light intensity, heat dissipation was highest for W followed by RB and R while heat dissipation was lowest for R (Figure 6.3).

Analysis of CF quenching parameters was assessed for light intensities from 0 to 2000 µmol m^{-2} s^{-1}. The fluorescence yields of the photochemical processes (Φ_{PSII}) (Supplementary Figure 1; A, C, E and G), showed slightly steeper lines in the monochromatic R and B treatments for *Chrysanthemum* leading to a lower light intersection with $\Phi_{NPQ.}$ The other non-photochemical losses (Φ_{NO}) remained stable when the light irradiation was higher than 500 µmol m^{-2} s^{-1} but were lowest for the W adapted leaves. Decreases in Φ_{PSII} with increasing light intensity induced an increase in both thermal (Φ_{NPQ}) and other non-photochemical dissipation (Φ_{NO}) (Figure 6.2). Thermal dissipation was highest for W followed by RB and lowest for B and R for values of Φ_{PSII} lower than 0.6. Dissipation of energy to Φ_{NO} was lowest for W, followed by RB while it reached values around 0.5 for B and R when Φ_{PSII} ranged between 0.1-0.4.

For *Spathiphyllum* all light treatments reached a plateau at 800 µmol m^{-2} s^{-1} (Figure 6.3). The slope (α) of the light curve showed a similar trend, it was highest for W and significantly lower for R while RB and B had intermediate values. ETR_{max} was the highest under RB followed by W and B and lowest under R, I_k was unaffected by light quality (Table 6.2). The decrease in qP was not affected by light quality for light intensities up to 500 µmol m^{-2} s^{-1}; for higher light intensities qualities B tended to result in higher qP values. NPQ increased to a higher level for RB and W while heat dissipation was lower for leaves that developed under R and B (Figure 6.2).

Analysis of CF quenching parameters showed that Φ_{PSII} decreased steeper for W resulting in a lower light intersection with Φ_{NPQ} than the other light treatments (Supplementary Figure 2; A, C, E and G). Decreases in Φ_{PSII} with increasing light intensity induced an increase in both thermal (Φ_{NPQ}) and other-non photochemical dissipation (Φ_{NO}) (Figure 6.2). Thermal dissipation was lowest for R while no differences between the other light treatments were present. Dissipation of energy to Φ_{NO} was highest for R and reached values between 0.45-0.50 when Φ_{PSII} ranged between 0.1-0.5.

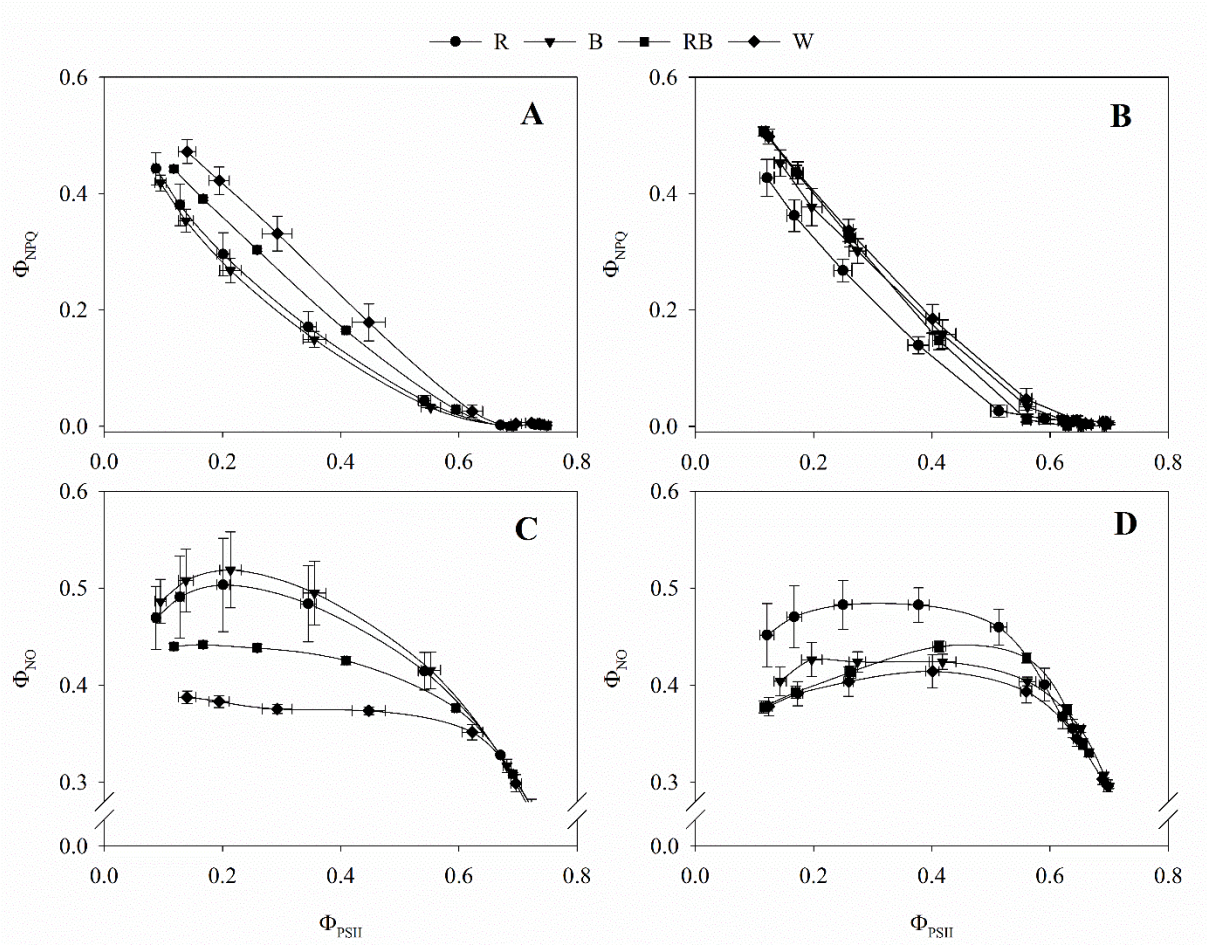


Figure 6.2 The non-regulated non-photochemical energy dissipation (Φ_{NO}) and the non-photochemical quenching (Φ_{NPQ}) versus the PSII operation efficiency (Φ_{PSII}) of fully expanded leaves that developed under different light qualities at t_0 for *Chrysanthemum* (left panel) and *Spathiphyllum* (right panel). R, red light; B, blue light; RB, red/blue (60%/40%); W, white light. Values are the means of four replicates with standard errors.

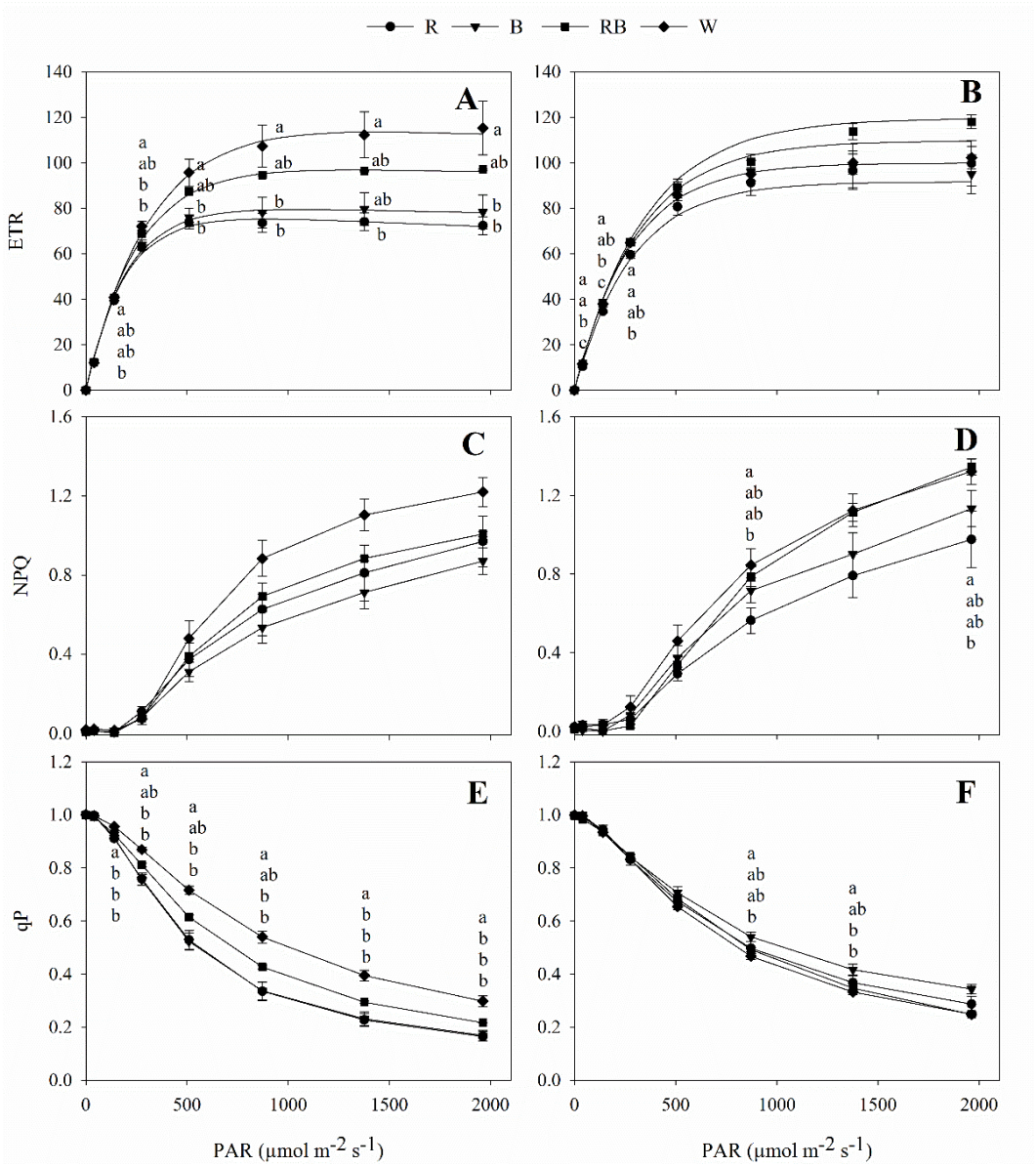


Figure 6.3 Rapid light curve of ETR, photochemical quenching (qP) and non-photochemical quenching (NPQ) in *Chrysanthemum* (left panel: A, C and E) and *Spathiphyllum* (right panel: B, D and F) at the first day of greenhouse acclimation (t_0) after a light quality pretreatment. R, red light; B, blue light; RB, red/blue (60%/40%); W, white light. Values are the means of four replicates with standard errors shown by vertical bars. Different letters indicate significant differences between the light qualities for a given PAR level, Tukey's HSD Test (P = 0.05). No significant differences between treatments when no letter is given.

Table 6.2 Rapid light curve (RLC) parameters for *Chrysanthemum* and *Spathiphyllum* after a four week light quality treatment (t_0) and after 1 week acclimation in the greenhouse (t_8)

Species	Light quality	t_0			t_8		
		ETR_{max}	α	E_k (µmol m^{-2} s^{-1})	ETR_{max}	α	E_k (µmol m^{-2} s^{-1})
Chrysanthemum	R	81.80 ± 3.05 b	0.391 ± 0.020 a	502.98 ± 42.01 ab	104.93 ± 14.32 a	0.366 ± 0.003 c	641.65 ± 70.36 b
	B	84.17 ± 6.05 b	0.413 ± 0.007 a	487.13 ± 43.37 b	127.27 ± 6.84 a	0.371 ± 0.001 bc	795.67 ± 39.15 ab
	RB	100.88 ± 1.40 ab	0.400 ± 0.001 a	601.99 ± 9.22 ab	160.16 ± 15.28 a	0.381 ± 0.002 ab	1028.29 ± 100.09 a
	W	114.05 ± 10.15 a	0.395 ± 0.004 a	688.94 ± 64.93 a	155.91 ± 16.37 a	0.390 ± 0.004 a	1012.89 ± 98.71 a
Spathiphyllum	R	91.70 ± 7.02 b	0.329 ± 0.017 b	671.90 ± 87.76 a	78.18 ± 1.81 b	0.320 ± 0.002 c	581.84 ± 15.85 ab
	B	109.83 ± 9.83 ab	0.353 ± 0.007 ab	746.14 ± 81.97 a	105.48 ± 10.54 a	0.350 ± 0.003 b	718.35 ± 71.92 a
	RB	119.78 ± 3.81 a	0.350 ± 0.001 ab	814.22 ± 25.61 a	95.17 ± 4.39 ab	0.355 ± 0.005 ab	638.66 ± 37.52 ab
	W	99.80 ± 3.47 ab	0.363 ± 0.006 a	655.09 ± 24.05 a	80.08 ± 2.98 ab	0.364 ± 0.002 a	523.20 ± 17.63 b

Data are means ± standard error of three replicates. Means followed by the same letter in each column means no significantly differ by Tukey test at $P<0.05$.

6.3.2 Short term responses to high light intensities in the greenhouse

The first day of acclimation to the greenhouse environment of *Chrysanthemum* was characterized by light intensities up to 600 µmol m^{-2} s^{-1} and temperatures above 30°C before 12:00 followed by a drop around noon followed by a short time increase again at 2:00 and 17:00 (Figure 6.1C). F_v/F_m was lowest for leaves that developed under R compared to other light qualities though this was not always significant (not at time point 12:00 and 16:00). Likewise Φ_{PSII} was mainly lower under R though this was not consistent for all time points. Overall NPQ did not significantly differ between the light qualities (data not shown) and values were low (< 0.15).

The first day of acclimation to the greenhouse environment of *Spathiphyllum* was characterized by light intensities lower than 600 µmol m^{-2} s^{-1} and temperatures above 28°C around12:00 followed by a drop to 25°C and fluctuations between 28-30°C until 16:00 (Figure 6.1A). The diurnal changes of F_v/F_m and Φ_{PSII} during the daytime are given in Figure 5. F_v/F_m was lowest under R for all light treatments; only at 12:00 RB had also a lower F_v/F_m value. For all measuring points, the highest value was found for leaves that developed under B. The first measuring point (10:00) is characterized by a sharp increase in natural PPFD. When measuring Φ_{PSII} at this time-point the lowest values were found for R and W followed by RB and highest values were observed for leaves that developed under for B. Two hours later (12:00) both B and R had the lowest values. During the afternoon (14:00 and 16:00) lowest values continued to be found under R compared to the other light quality treatments. Late afternoon, when both light intensity and temperature started to decrease no significant effects between the treatments were found. NPQ values were small (< 0.20) and did not significantly differ between the light qualities (data not shown).

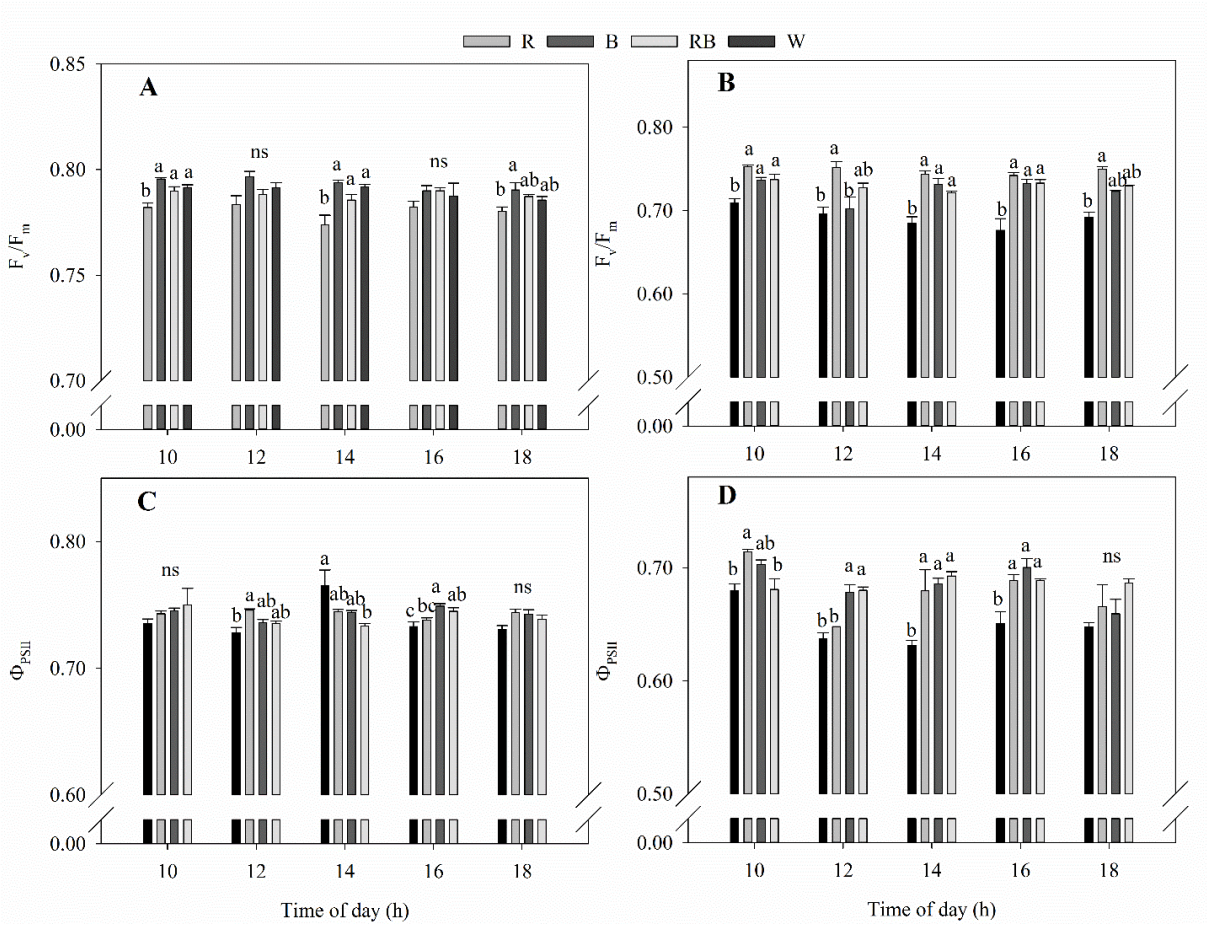


Figure 6.4 Diurnal change of chlorophyll fluorescence on the first day (t_1) of greenhouse acclimation for *Chrysanthemum* (left panel) and *Spathiphyllum* (right panel). R, red light; B, blue light; RB, red/blue (60%/40%); W, white light. Values are the means of four replicates with standard errors shown by vertical bars. Different letters indicate significant differences (at each time point) using Tukey's HSD Test (P = 0.05), ns: not significant.

6.3.3 Evolution of the photosynthetic acclimation during the first week of transfer to the greenhouse

Acclimation dynamics of photosynthesis (P_n), F_v/F_m and Φ_{PSII} are shown in Figure 6.5. Leaves that developed under different light qualities adapted to the fluctuating light and temperature conditions present in the greenhouse environment.

On the first day of the greenhouse transfer of *Chrysanthemum*, the photosynthetic rate was significantly lower under R (6.73 µmol m^{-2} s^{-1}) followed by B (8.56 µmol m^{-2} s^{-1}) while higher rates were measured for W and RB (10.64 and 10.71 µmol m^{-2} s^{-1}, respectively). In the following days *Chrysanthemum* leaves that had developed under

R and B acclimated to the greenhouse environment and P_n increased to respectively 9.03 and 11.82 µmol m^{-2} s^{-1} after one week. Despite this trend leaves under R yielded the lowest P_n values for all dates. For RB and W, P_n did not show a clear increasing trend. Likewise F_v/F_m of R grown plants was significantly lower compared to B on t_8, while Φ_{PSII} was lower for R compared with other light qualities at t_2 and t_5, no significant differences were present for Φ_{PSII} at t_8.

Photosynthetic rates were lower for *Spathiphyllum* compared to *Chrysanthemum* (Figure 6.5). At the first day of the transfer to the greenhouse the lowest P_n was observed under RB. If one looks, however, at the trend during the following days leaves developed under R yielded the lowest P_n and on average leaves that developed under B yielded the highest P_n. For all treatments an increase in P_n over time was noted indicating acclimation of the leaves to the greenhouse environment. After 8 days of acclimation no difference between B, RB and W was longer present.

F_v/F_m varied between 0.70 and 0.75 for *Spathiphyllum* (Figure 6.5). Light quality affected F_v/F_m: it was the greatest for B during the first four days after the greenhouse transfer followed by W; RB increased during the first four days and reached the same value as B after 8 days. After 1 week, F_v/F_m was still the lowest for R compared with other light qualities. Φ_{PSII} was significantly greater for RB and W compared with R and B the first day of transfer but was also characterized by fluctuations despite we measured between 9:00 and 10:00 when light intensities and temperature were still relative low. Eight days after the transfer to the greenhouse, lowest Φ_{PSII} values were still found under R and surprisingly also under W while B and RB had the highest values.

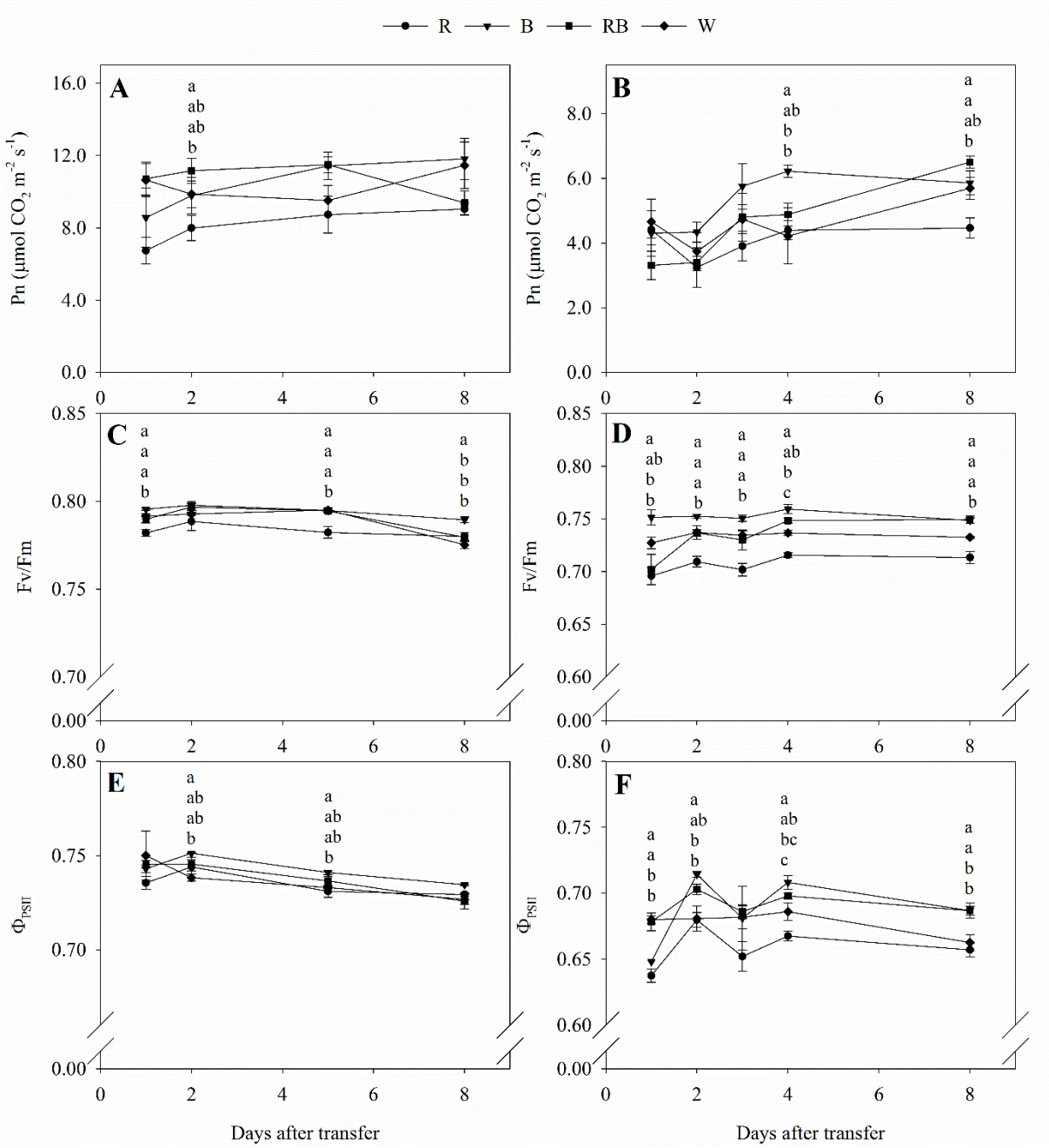


Figure 6.5 Photosynthetic rate and chlorophyll fluorescence parameters during acclimation in the greenhouse for *Chrysanthemum* (A, C and E) and *Spathiphyllum* (B, D and F). R, red light; B, blue light; RB, red/blue (60%/40%); W, white light. Values are the means of four replicates with standard errors shown by vertical bars. Different letters indicate significant differences between the light qualities per day using the Tukey's HSD Test (P = 0.05). No significant differences between treatments when no letter is given.

6.3.4 Rapid light curve (RLC) after 1 week of acclimation in the greenhouse (t_8)

Figure 6.6 shows the rapid light curve of ETR after 1 week acclimation. For *Chrysanthemum*, the ETR_{max} was highest for leaves acclimated under W and RB

followed by B and lowest under R (P=0.053). The initial slope of the light curve (α) was greatest under W followed by RB and decreased significantly for B and R. I_k was significantly lower under R compared with RB and W. For *Spathiphyllum*, ETR_{max} was the highest under B followed by RB and W and significantly lower for R; α was highest under W and RB and decreased under R, I_k was greatest for B declined under R and RB and was significantly lower for leaves acclimated under W.

For *Chrysanthemum*, qP decreased with increasing light intensity, though the lowest values were found for leaves that developed under R this from 500 µmol m^{-2} s^{-1} on followed by B while it was higher for RB and W. NPQ was significantly greater for leaves that developed under R compared to other light qualities at 140 µmol m^{-2} s^{-1}, while no significant difference was found at other light intensity points. In *Spathiphyllum*, qP was the lowest for R and W while it was the greatest for RB while B was intermediate below 276 µmol m^{-2} s^{-1}. R leaves generated more NPQ when light intensity was less than 1500 µmol m^{-2} s^{-1} though the differences were not significant except at 500 µmol m^{-2} s^{-1}. For the highest light intensities both R and B resulted in a higher heat dissipation though there was no statistical difference.

Analysis of CF quenching parameters was assessed for light intensities from 0 to 2000 µmol m^{-2} s^{-1}. After 1 week acclimation (t8), the decrease of Φ_{PSII} with increasing light intensity was still slightly steeper for monochromatic R and B treatments for *Chrysanthemum* leading to a lower light intersection with Φ_{NPQ} and Φ_{NO} (Supplementary Figure 1; B, D, F and H). Decreases in Φ_{PSII} with increasing light intensity induced an increase in both thermal (Φ_{NPQ}) and other non-photochemical dissipation (Φ_{NO}) (Figure 6.7). Thermal dissipation (Φ_{NPQ}) was not much different within light treatments when compared with t_1. The other non-photochemical losses (Φ_{NO}) were clearly lowest for R compared with other light qualities for a given value of Φ_{PSII}. At t8, the increase of Φ_{NO} was relatively low with increasing light intensity in comparison with t_1 (Φ_{NO} lower than 0.4).

Analysis of CF quenching parameters of *Spathiphyllum* showed that Φ_{PSII} decreased steeper for R and W resulting in a lower light intersection with Φ_{NPQ} than the other light treatments (Supplementary Figure 2; B, D, F and H). Decreases in Φ_{PSII} with increasing light intensity induced an increase in both thermal (Φ_{NPQ}) and other-non photochemical dissipation (Φ_{NO}) (Figure 6.7). Thermal dissipation (Φ_{NPQ}) was not

much difference within light treatment when compare with t_1. Dissipation of energy to Φ_{NO} was in a lower range of 0.35-0.40 in compare with it in t_1 (0.45-0.50), highest for W followed by RB ad lower in B and R when Φ_{PSII} ranged between 0.1-0.5.

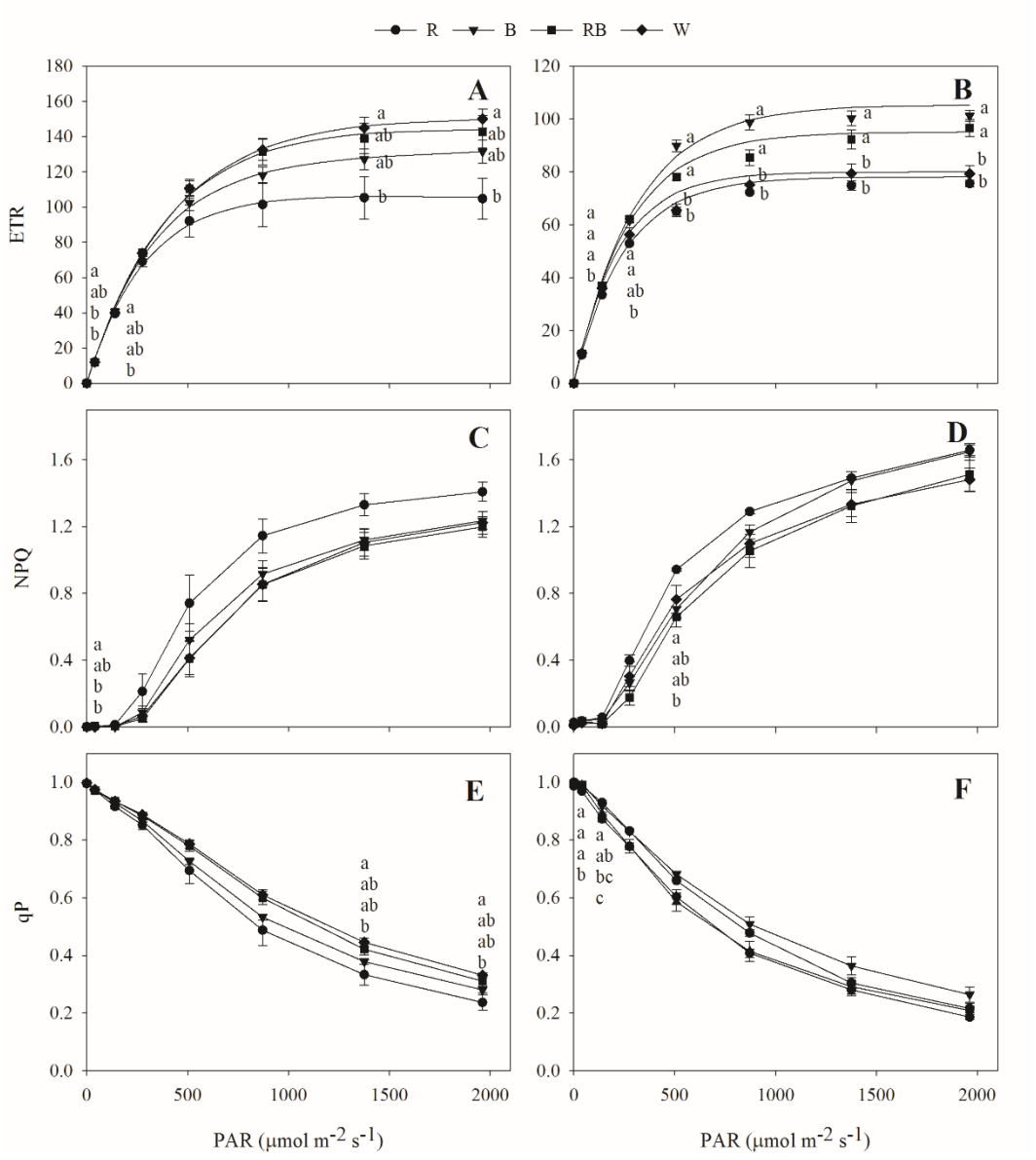


Figure 6.6 Rapid light curve of ETR, photochemical quenching (qP) and non-photochemical quenching (NPQ) in *Chrysanthemum* (A, C and E) and *Spathiphyllum* (B, D and F) after 8 days under greenhouse conditions (t_8). R, red light; B, blue light; RB, red/blue (60%/40%) light; W, white light. Values are the means of four replicates with standard errors shown by vertical bars. Different letters indicate significant differences between the light qualities for a given PAR level, Tukey's HSD Test (P = 0.05). No significant differences between treatments when no letter is given.

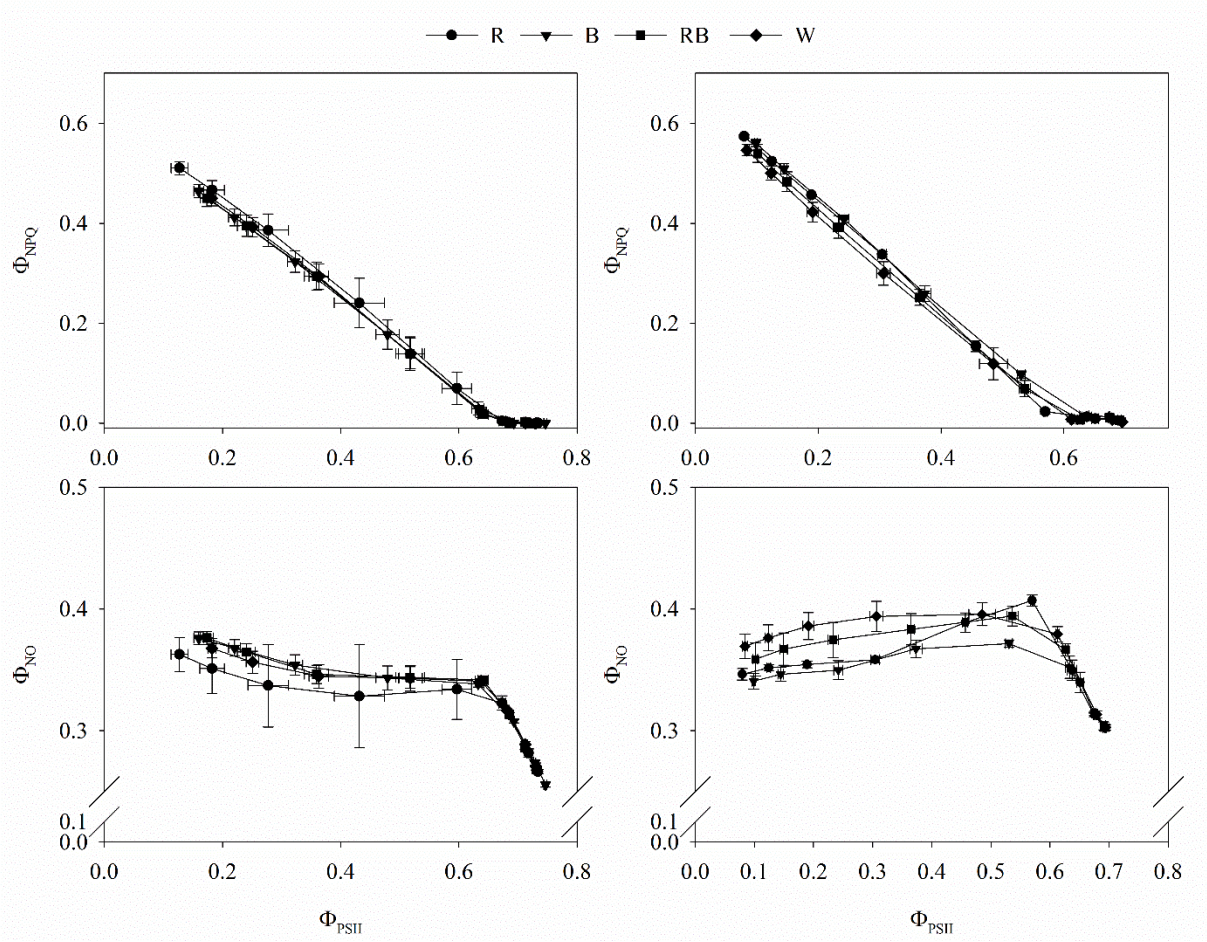


Figure 6.7 The non-regulated non-photochemical energy dissipation (Φ_{NO}) and the non-photochemical quenching (Φ_{NPQ}) versus the PSII operation efficiency (Φ_{PSII}) in fully expanded leaves that developed under different light quality after 8 days of greenhouse acclimation (t_8) of *Chrysanthemum* (left panel) and *Spathiphyllum* (right panel). R, red light; B, blue light; RB, red/blue (60%/40%); W, white light. Values are the means of four replicates with standard errors.

6.3.5 Chlorophyll content

Chlorophyll pigments were measured just before the transfer to the greenhouse and after 8 days of acclimation in greenhouse conditions (Table 6.3). At day 0 the light quality had affected the total chlorophyll content but not the Chl *a/b* ratio. The highest concentration was found under RB followed by W while lowest concentration was for both monochromatic B and R. After 8 days in the greenhouse differences of the light treatment had disappeared. For *Spathiphyllum* much lower total chlorophyll concentration and higher Chl *a/b* ratio in comparison with *Chrysanthemum* were observed. Light quality did not affect total content or the Chl *a/b* ratio. However, after 1 week of acclimation in the greenhouse, a significant decrease for leaves developed

under R were observed, while no significant changes in the other light treatments took place.

Table 6.3 Photosynthetic rate (P_n), F_v/F_m and Φ_{PSII} for *Chrysanthemum* and *Spathiphyllum* after one month of acclimation in the greenhouse (t_{30}).

Species	Light quality	P_n (µmol CO_2 m^{-2} s^{-1})	F_v/Fm	Φ_{PSII}
Chrysanthemum	R	11.04 ±1.20 n.s.	0.781 ± 0.009 n.s.	0.741 ± 0.004 n.s.
	B	11.94 ± 0.79	0.802 ± 0.002	0.748 ± 0.001
	RB	11.97 ± 0.50	0.794 ± 0.004	0.742 ± 0.002
	W	10.63 ± 0.56	0.802 ± 0.002	0.741 ± 0.001
Spathiphyllum	R	7.02 ± 0.43 n.s.	0.740 ± 0.005 n.s.	0.686 ± 0.005 n.s.
	B	7.80 ± 0.17	0.757 ± 0.005	0.703 ± 0.006
	RB	6.91 ± 0.17	0.753 ± 0.005	0.695 ± 0.003
	W	8.08 ± 0.34	0.739 ± 0.001	0.684 ± 0.004

Data are means ± error (n=4). n.s: no significant differences according to Tukey's HSD test.

6.3.6 Long term effects after 30 days in the greenhouse

After one month, new leaves had developed under the greenhouse conditions. As expected leaves did not differ with respect to their photosynthetic rate, F_v/F_m or Φ_{PSII} (Table 6.4). Differences between species were however evident with higher P_n, F_v/F_m and Φ_{PSII} in *Chrysanthemum* than *Spathiphyllum*.

Plant growth parameters prior to the greenhouse transfer and after a growth period of 30 days in the greenhouse were also recorded (Table 6.5). For *Chrysanthemum* no effect of the light quality treatments at t_0 was observed, though 30 days (t_{30}) later dry weight was significant lower for R, intermediate for B and RB and highest for the W pretreatment. This was also reflected in the absolute growth rates which were lowest after R and highest for W. Light quality also affected the shoot length: at t_0 the tallest plants were under B and W, while shoot length was reduced by both R and RB. At t_{30} the stimulating effect of B was still visible as found by total shoot length and height

increase. Leaf characteristics were also affected by light quality (Table 6.6), the lowest leaf expansion was under RB while B and W resulted in the highest leaf area. The highest LMA was under RB, followed by W and significantly lower LMA was observed under B and R. Newly formed leaves after 30 days as well as the LMA did not differ.

In *Spathiphyllum*, light quality did not affect the dry weight at t_0 nor at t_{30} though it tended to result in lower dry weight under R; as a result AGR was also relative similar (Table 6.5). Red light tended to result in the highest leaf area (t_0) though effects were not significant; no effects were observed for the newly formed leaves in greenhouse conditions (t_{30}) (Table 6.6). The greatest LMA was under W followed by RB and significantly lower under R and B at t_0. After 30 days no effects were visible. LMA was clearly higher in *Spathiphyllum* compared to *Chrysanthemum*.

6.4 Discussion

Plant leaves that develop under a specific light quality and intensity are adapted to this environment which include changes in structure, function and efficiency of the photosynthetic machinery (Wagner et al., 2008). These variations affect directly the efficiency of the photosynthetic light reactions and therefore acclimation to changes in the light environment. In natural environments such as a forest understory changes in the light environment may range from the transitory changes caused by sunflecks to the more sustained changes that occur when gaps are formed or when canopies develop. Acclimation changes to full sunlight involves several responses including minimizing photo-inhibition and an increase in photosynthetic capacity of leaves that previously developed in shade and/or maximize the production of newly formed leaves in a sunny environment (Lavinsky et al., 2014; Sanches et al., 2016).

Table 6.4 Total chlorophyll content and Chl*a/b* ratio for *Chrysanthemum* and *Spathiphyllum* after a four week light quality treatment (t_0) and after 1 week acclimation in the greenhouse (t_8).

Species	Light quality	t_0		t_8		P- value	
		Total chlorophyll (mg m^{-2})	Chl *a/b* ratio	Total chlorophyll (mg m^{-2})	Chl *a/b* ratio	Total chlorophyll (mg m^{-2})	Chl a/b ratio
Chrysanthemum	R	650.58 ± 19.18 b	1.28 ± 0.44 n.s.	885.29 ± 155.76 n.s.	1.37 ± 0.11 n.s.	0.003	0.321
	B	577.22 ± 63.36 b	1.29 ± 0.04	855.97 ± 90.99	1.36 ± 0.21	0.524	0.005
	RB	1104.95 ± 136.18 a	1.12 ± 0.12	1051.20 ± 105.96	1.25 ± 0.07	0.391	0.357
	W	811.49 ± 147.95 ab	1.28 ± 0.13	894.58 ± 84.60	1.11 ± 0.11	0.423	0.497
Spathiphyllum	R	328.33 ± 27.01 n.s.	2.48 ± 0.06 n.s.	250.42 ± 9.64 b	2.45 ± 0.01 n.s.	0.002	0.000
	B	352.66 ± 61.90	2.53 ± 0.07	345.39 ± 32.79 a	2.42 ± 0.02	0.550	0.137
	RB	318.79 ± 40.67	2.57 ± 0.05	330.05 ± 20.63 ab	2.50 ± 0.03	0.288	0.168
	W	349.29 ± 38.02	2.34 ± 0.04	369.76 ± 10.60 a	2.44 ± 0.08	0.034	0.961

Data are means ± standard error of four replicates. Means followed by the same letter in each column means no significantly differ by Tukey test at $P<0.05$. n.s.: not significant. P-value indicates the difference between t_0 and t_8 by a t-test.

Table 6.5 Biomass, a normalized factor for biomass increase, absolute growth rate (AGR), plant height and height increase of *Chrysanthemum* and biomass of *Spathiphyllum* after a four week light quality treatment (t_0) and after 1 month acclimation in the greenhouse (t_{30})

Species	Light quality	DW_{t0} (g)	DW_{t30} (g)	Normalized factor (g/g)	AGR (g day^{-1})	$Height_{t0}$ (cm)	$Height_{t30}$ (cm)	Height increase (cm)
Chrysanthemum	R	1.27 ± 0.03 a	5.67 ± 0.14 c	4.46	0.147	10.95 ± 0.33 b	15.83 ± 0.62 c	4.88
	B	1.37 ± 0.09 a	8.06 ± 0.40 b	5.88	0.223	16.50 ± 0.35 a	23.55 ± 0.35 a	7.05
	RB	1.70 ± 0.03 a	7.96 ± 0.20 b	4.68	0.209	11.45 ± 0.43 b	16.28 ± 0.56 c	4.83
	W	1.65 ± 0.18 a	9.21 ± 0.24 a	5.58	0.252	15.85 ± 0.21 a	20.25 ± 0.14 b	4.40
Spathiphyllum	R	1.98 ± 0.21 a	4.94 ± 0.20 a	2.49	0.099	-	-	
	B	1.98 ± 0.14 a	5.17 ± 0.17 a	2.61	0.106	-	-	
	RB	1.70 ± 0.14 a	5.41 ± 0.15 a	3.18	0.125	-	-	
	W	1.97 ± 0.13 a	5.53 ± 0.23 a	2.80	0.119	-	-	

Data are means ± standard error of four replicates. Means followed by the same letter in each column means no significantly differ by Tukey test at $P<0.05$.

Table 6.6 Leaf area (LA) and leaf mass area (LMA) of *Chrysanthemum* and *Spathiphyllum* after a four week light quality treatment (t_0) and after 1 month acclimation in the greenhouse (t_{30})

Species	Light treatment	Leaf area$_{t0}$ (cm^2)	Leaf area$_{t30}$ (cm^2)	LMA$_{t0}$ (g m^{-2})	LMA$_{t30}$ (g m^{-2})
Chrysanthemum	R	23.62 ± 0.62 ab	38.41 ± 1.30 a	25.71 ± 0.90 b	25.75 ± 0.59 a
	B	28.69 ± 0.83 a	37.88 ± 3.38 a	25.95 ± 0.91 b	26.91 ± 0.96 a
	RB	18.49 ± 1.69 b	34.90 ± 1.70 a	32.17 ± 1.54 a	25.54 ± 1.38 a
	W	27.55 ± 2.54 a	36.96 ± 1.92 a	25.98 ± 0.38 b	27.51 ± 3.00 a
Spathiphyllum	R	87.91 ± 7.34a	103.01 ± 3.03 a	43.52 ± 0.77 b	54.65 ± 1.52 a
	B	79.03 ± 2.96a	112.66 ± 5.71 a	43.43 ± 0.74 b	57.58 ± 1.90 a
	RB	69.48 ± 5.30a	101.82 ± 2.73 a	45.66 ± 2.00 ab	53.99 ± 3.09 a
	W	67.01 ± 5.51a	103.03 ± 5.00 a	51.93 ± 3.28 a	57.63 ± 6.77 a

Data are means ± standard error of four replicates. Means followed by the same letter in each column means no significantly differ by Tukey test at $P<0.05$.

In our study, we monitored the acclimation of leaves that developed under narrow band spectral light sources (R, B, RB) as well as a multispectral control (W) to the fluctuating light and temperature conditions of the greenhouse environment. Rapid light response curves (RLCs) provide information about the light saturation characteristics (White and Critchley, 1999). The effects of light quality on ETR_{max} differed between the species: for *Chrysanthemum* W and RB grown leaves resulted in higher values while for *Spathiphyllum* B and RB yielded the highest $ETR_{max.}$ These different responses are also reported in literature. A first group of plants reacts better to dichromatic or multispectral light for the development of the Photosystems as observed for *Sambucus nigra* (Cooney et al., 2015), lettuce (Fu et al., 2012; Wang et al., 2016) and cucumber leaves (Savvides et al., 2012). In this group W and RB grown leaves might be better adapted to the change to full-bright sunlight and therefore acclimate better, which is consistent with the photosynthetic rate (P_n) during the first day in the greenhouse for *Chrysanthemum* (Figure 6.3). A second group of plants responds positively to monochromatic blue or high percentages blue in the light spectrum as found for *Spathiphyllum*. Terfa et al. (2013) reported that higher blue ratios in the spectrum were beneficial in the development of the photosynthetic apparatus in *Rosa* × *hybrida* and Shengxin et al. (2016) suggested that rapeseed leaves grown under pure blue or a high blue photon ratio showed higher ability to utilize high photon fluxes. Leaves of pepper plants that developed under a higher blue light ratio better recovered after an UV stress treatment due to their higher amount of epidermal flavonols that work as an UV screen (Hoffmann et al., 2015b). The negative effects of R on photosynthetic performance of both *Chrysanthemum* and *Spathiphyllum* resulted in the lowest ETR_{max}. As already suggested by Tennessen et al. (1994), monochromatic R irradiation induces an imbalance of light energy distribution available for Photosystem I and II, which results in the inhibition of the photosynthetic performance and subsequent decline in photosynthetic efficiency.

On the first day of transfer to the greenhouse environment, leaves that developed under different light qualities might respond differently to this abrupt change in light environment. Zheng and Van Labeke (2017b) showed that under monochromatic B higher proline levels were present, and this compound is known for its protective function under abiotic stress (Koca et al., 2007). Photoinhibition induced by the much higher light intensities in the greenhouse (600-800 µmol m^{-2} s^{-1}) compared with the

pre-conditioning (100 µmol m^{-2} s^{-1}) might occur and in this experiment changes in F_v/F_m as a proxy of photoinhibition were monitored from 10:00 till 18:00. The response of both species was similar: the diurnal pattern distinguished acclimation stress for leaves developed under R but for the other light quality pretreatments no difference in their response was found. Φ_{PSII} decreased under R compared to the other light qualities in *Spathiphyllum*, although negative effects of R were less clear in *Chrysanthemum* (Figure 6.5). As leaves were under sunlight, the negative effects of R cannot be attributed to imbalances in light energy distribution between the two Photosystems PSII and PSI but are probably the result of the different leaf anatomy and thylakoid development under R. NPQ can improve the dissipation of excessive absorbed light energy as heat and therefore protect against photoinhibition. Effects of light quality on NPQ were mainly not significant (data not shown) in both *Chrysanthemum* and *Spathiphyllum*, which might indicate that both plant species used other ways to dissipate energy by other non-photochemical losses (Φ_{NO}).

Leaf anatomy can hardly change after full development (Oguchi et al., 2003). During the acclimation period of low to high light intensities chloroplasts enlarge to fill the space along with an increasing photosynthetic capacity, but without an increase in leaf thickness (Oguchi et al., 2005). Leaf mass per unit area of leaf (LMA) is regularly used in growth analyses and is affected by both anatomy (the number of cell layers and cell size) and cell content (Poorter et al., 2009). However, when we compared LMA of leaves developed under a specific light quality treatment (t_0) and in full sunlight (t_{30}) we found no striking differences ($P=0.56$) for *Chrysanthemum* though an increase ($P<0.01$) in LMA was observed for *Spathiphyllum* under high intensity greenhouse conditions.

The general trend we observed during the first week in the greenhouse was an increase in photosynthetic rate. One week acclimation was insufficient to restore the photosynthetic capacity of leaves developed under R of both *Chrysanthemum* and *Spathiphyllum* to the same levels as the other treatments. Based on the time course of F_v/F_m and Φ_{PSII} it seems that *Spathiphyllum* has more difficulties to acclimate to the new full spectrum environment than *Chrysanthemum*.

Comparing the parameters of the rapid light response curve (RLC) between day 0 and day 8, *Chrysanthemum* leaves acclimated differentially to the greenhouse

conditions. ETR_{max} and I_k increased for all light qualities though the light quality effects remained visible. RB and W reacted similar and reached the highest ETR_{max} values, yet the proportional increase was 60 % for RB, 36% for W, 47 % for B and 28% for R. The increase for R pre-treated leaves remained the lowest indicating that the recovery capacity for these leaves was hampered by their leaf structure as suggested by Oguchi et al. (2003). In contrast *Spathiphyllum* leaves had problems to acclimate to the higher light intensities of the greenhouse, only B pretreated leaves maintained the same level as t_0. The RLC parameters (ETR_{max}, α, I_k) tended even to decrease slightly for the other light qualities. We hypothesize that this effect is linked to the fact that *Spathiphyllum* is a facultative shade species (Gamboa et al., 2009) and a sudden change of low to high light intensities caused photo-oxidative stress.

Another approach to understand the acclimation differences is given by the quenching analysis prior to the greenhouse transfer (t_0). The increase in non-heat loss (Φ_{NO}) of ± 50% under R and B in *Chrysanthemum* and ± 45% under R in *Spathiphyllum* indicates their higher susceptibility for photodamage when leaves are exposed to higher irradiances. Lower Φ_{NPQ} ratios will result in reduced photoprotective effects as the rate of photodamage and excitation dissipation by basal dissipation mechanisms (Φ_{NO}) are correlated (Kato et al., 2003). Trouwborst et al. (2016) also reported a more pronounced Φ_{NO} under monochromic R than RB in cucumber leaves.

Eight days later the negative effects of R and the higher risk of photoinhibition are still visible in both species though to a lesser extent. In *Chrysanthemum* the pretreatment with monochromatic B had recovered, however, this was still not the case in *Spathiphyllum*. The thermal dissipation power, as assessed by Φ_{NPQ} was however restored in both species.

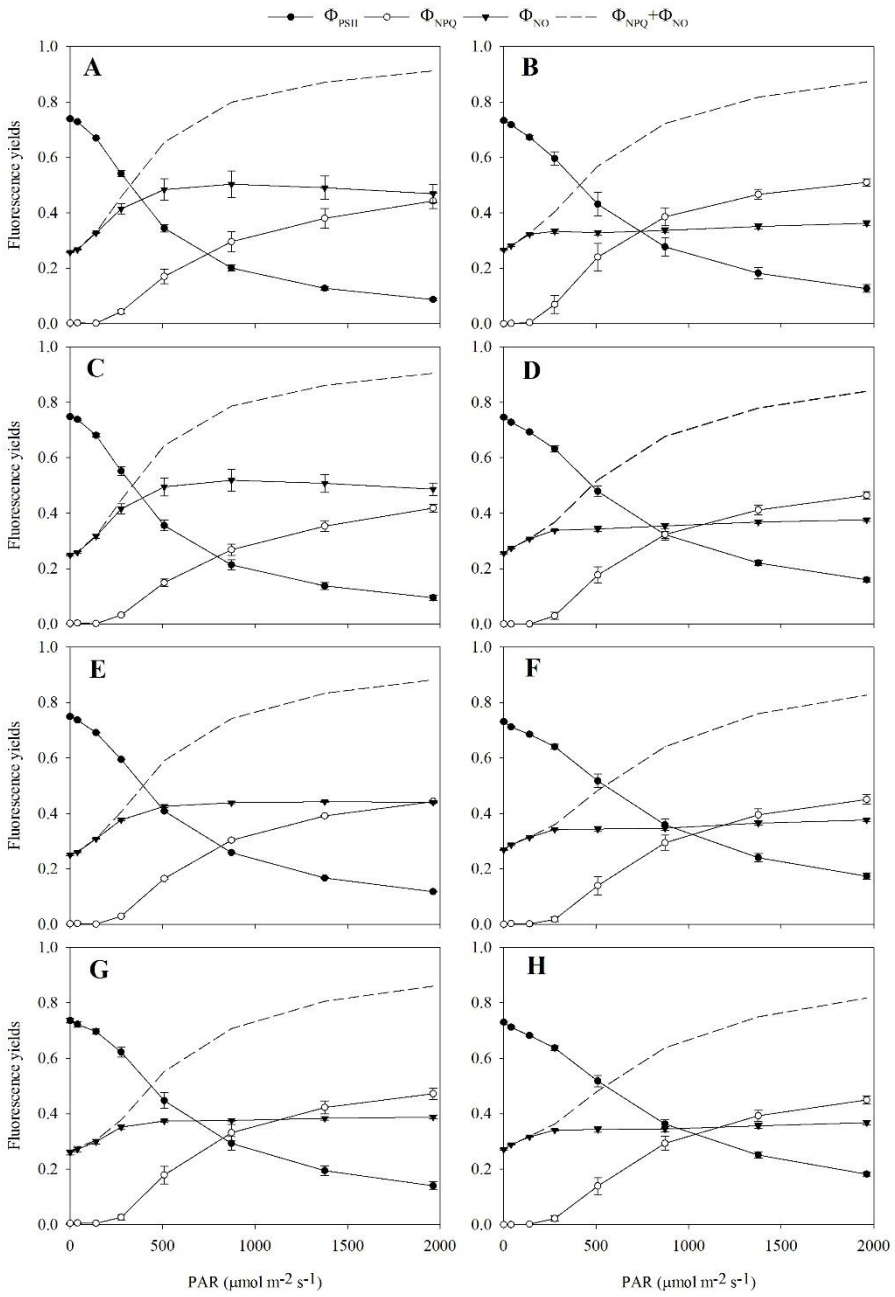
For *Chrysanthemum* a four-week light quality treatment did not affect the dry weight. However, after one month development in the greenhouse the cumulative effects of the acclimation period resulted in the highest biomass under W and lowest under R. B plants could acclimate to the greenhouse environment in a similar way as RB treated plants. Monochromic R as well as the RB pretreated *Chrysanthemum* plants continued their difference in plant architecture as we still recorded a reduced plant height after 1 month in greenhouse conditions. The cultivation during the young

phase under a spectral light quality thus opens opportunities to modify plant architecture for this species (Dierck et al., 2017). Smaller differences for *Spathiphyllum* were recorded, increase in biomass tended to be smaller for the monochromatic R and B.

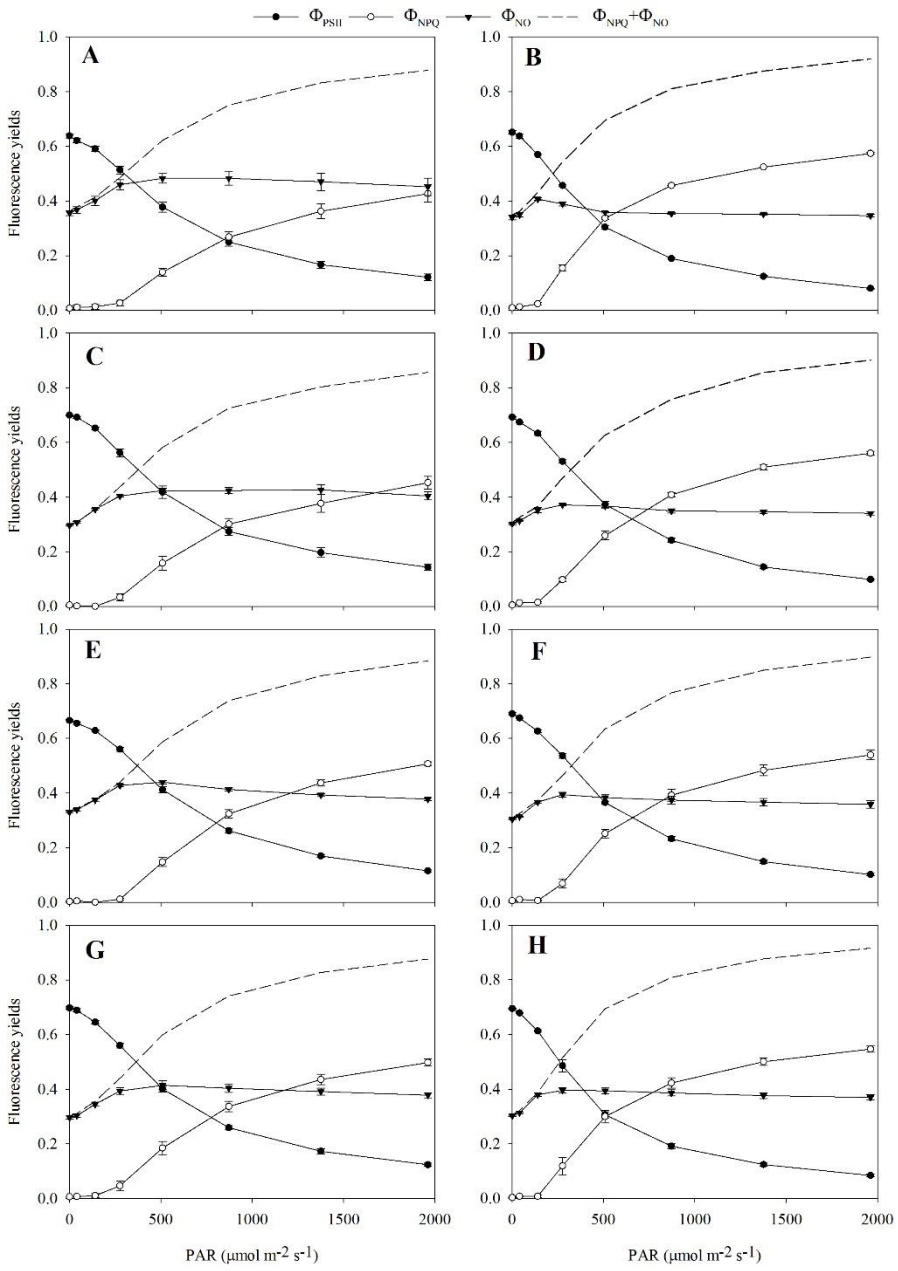
6.5 Conclusion

We show here for the first time acclimation properties of plants grown under narrow band R, B and RB to greenhouse conditions. We observed the responses of a sun species *Chrysanthemum* and a shade species *Spathiphyllum*. It is clear that light quality changed the leaf and thylakoid characteristics and this will influence the acclimation ability to a full spectrum greenhouse environment.

Leaves that developed under monochromatic red light in *Chrysanthemum* and *Spathiphyllum* were irreversibly inhibited in their photosynthetic functioning compared to the white control. We hypothesized that blue light would be beneficial in the adaptation phase but this was only partly observed. Blue light grown *Chrysanthemum* leaves acclimated at the same level (or higher) in comparison to RB and W and as assessed by P_n, F_v/F_m and Φ_{PSII}. However, looking at DW, it seems that pretreatment with monochromatic B does not yield the same biomass as W. Also in *Spathiphyllum* this tendency is observed. Light quality effects on photomorphogenesis are still visible after one month of transfer in full sun environment, which would be of interest for the future horticultural applications.



Supplementary Figure 1 The quantum yield fractions of three processes: PSII photochemistry (Φ_{PSII}), regulated non-photochemical heat dissipation (Φ_{NPQ}), and other non-photochemical losses (Φ_{NO}) of *Chrysanthemum* at t_0 (A, C, E and G) and t_8 (B, D, F and H) for the four different LED treatments: R, B, RB and W (upper to lower).



Supplementary Figure 2 The quantum yield fractions of three processes: PSII photochemistry (Φ_{PSII}), regulated non-photochemical heat dissipation (Φ_{NPQ}), and other non-photochemical losses (Φ_{NO}) of *Spathiphyllum* at t_0 (A, C, E and G) and t_8 (B, D, F and H) for the four different LED treatments: R, B, RB and W (upper to lower).

Chapter 7

General discussion and perspectives

7.1 General conclusion

Greenhouses in northern latitudes rely on supplemental lighting during winter months to achieve high-quality ornamentals. Improving light usage efficiency is an important objective in ornamental production. Application of LEDs in comparison to high-pressure sodium lamps (HPS) is potentially energy conserving. Furthermore, the wavelength specific light addition has potential to steer the morphology of the ornamentals such as compactness. Sole-source LED lighting could therefore be used to grow ornamental young plants (seedlings, rooted cuttings, acclimation of in vitro propagated plantlets) indoors in multilayer production or vertical farming systems. The last decade the effect of LED light on the general morphology and plant architecture was investigated in many ornamental species and cultivars though hardly any information on effects on physiological traits was considered.

The main purpose of this PhD was to study the effects of specific light qualities on the development and physiology of ornamental plants. In our experimental approach we applied monochromatic red (R) and blue (B) light, dichromatic red+blue light (RB) and compared selected plant anatomical and physiological traits with multispectral light (W). In most chapters, we applied these light qualities at intensities of 100 µmol m^{-2} s^{-1}, which is already a high level of supplemental light if applied in the ornamental production. In this final chapter, the main outcomes of the previous chapters will be discussed and linked together with photosynthesis.

Effects of light quality on photosynthesis and plant development

The most important role of light is to drive photosynthesis, yet the photosynthetic efficiency is unavoidable affected by the applied light quality. Chlorophyll fluorescence was used to estimate the photosynthetic activity by measuring different parameters of chlorophyll fluorescence kinetics throughout all the chapters. Photosynthetic activity is critically influenced by the spectral light quality, although there were species (all chapters) and cultivar (Chapter 4) depending influences. We evaluated the photosynthetic efficiency of nine *Chrysanthemum* cultivars in this study (Chapter 2, Chapter 4 and Chapter 6). F_v/F_m, which is widely used as proxy of photoinhibition, was not affected by light quality in some cultivars while in other cultivars F_v/F_m was greatest under either B or RB. An overall analysis taking all these

cultivars into account showed that B and RB resulted in a higher F_v/F_m in comparison with W and R. Surprisingly, there were wide genotype dependent differences. An overall decrease of Φ_{PSII} under W was found in chapter 4 for the eight cultivars, whereas it was only significantly decreased under R for 'Staviski' studied in Chapter 2, this indicates the genotyping dependent responses which is also found in the intraspecific response Φ_{PSII} (Figure 4.2), 'Lana' and 'Sunny' resulted in no significant difference. Species depended differences in their reaction to the light quality were also found. However, under blue light F_v/F_m and Φ_{PSII} were generally higher than for plants that developed leaves under monochromatic red. This red light response was not only present in C3 plants, but also in the study of CAM plant *Phalaenopsis* (Chapter 5). In *Phalaenopsis* we observed the acclimation of leaves to a given spectral quality. After about 5-10 days under monochromatic red a stable lower response of F_v/F_m and Φ_{PSII} compared to the other light qualities was observed (Figure 5.2).

These observations seem contradictory to the general consensus that monochromatic red light is utilized most efficiently for photosynthesis (McCree, 1971). However, these positive effects relate to short-term treatments with monochromatic red light and do not apply for leaves that developed under red light nor to a longer transient phase to solely red light treatments. These negative effects of monochromatic red light were also described as the 'red light syndrome' (Trouwborst et al., 2016) (Chapter 3) and reported in many species including cucumber (Hogewoning et al., 2010b; Savvides et al., 2012), tomato (O'Carrigan et al., 2014a) and rapeseed (Shengxin et al., 2016). We observed these negative effects of R not only at 100 µmol m^{-2} s^{-1}, but it also occurred for a light intensity of 40 µmol m^{-2} s^{-1} (Chapter 2). The 'red light syndrome' (Trouwborst et al. 2016) is characterized by suboptimal morphology, disordered photosynthetic machinery development and functioning, and aberrant gene expression and biochemistry. It suppresses and impairs photosynthesis resulting a low F_v/F_m, unresponsive stomatal conductance and a low maximum photosynthetic rate (P_{max}) (Hogewoning et al., 2010b; Savvides et al., 2012).

In contrast to the monochromatic red treatment, the studied ornamental species did not exhibit adverse effects for B. B exposed plant could develop compact leaves and resulted in even the greatest photosynthetic performance in *Ficus benjamina* and

Phalaenopsis compared to the other light treatments. Also in other studies plants grown under blue light exhibited photosynthetic rates similar to those of plants acclimating to high irradiance (Matsuda et al., 2008). It is well accepted that supplementary blue with red light increases the net photosynthesis (Goins et al., 1997), 7% of blue (W used in chapter 2, 3 and 4) could already eliminate the disorder induced by monochromatic red. Increasing the fraction of blue light in the spectrum is efficient in enhancing the photosynthetic performance in cucumber (Hogewoning et al., 2010b), lettuce (Hernández and Kubota, 2016) and rose (Terfa et al., 2013). One important function of blue light is regulating chloroplast movement, which is controlled by the blue-light receptors, phot1 and phot2. Both are responsible for the accumulation response of chloroplasts, while phot2 alone mediates the avoidance response (Christie, 2007). It is through chloroplast movement that plants maximize light capture (accumulation response) in weak light allowing efficient photosynthesis and avoid photoinhibition (avoidance response) under light stress.

Growth is the result of photosynthetic production and biomass accumulation. Light intensity and quality can change growth, fresh weight and ornamental value of horticultural crops, and thus greatly affect their market value. Light quality, through its effect on leaf anatomy and photosynthetic efficiency, influenced the plant biomass. The negative effects of monochromatic red light on the photosynthesis efficiency in all our studied species resulted in reduced biomass production (Table 7.1). This was shown for *Chrysanthemum* ‘Staviski’ (Chapter 2), *Chrysanthemum* ‘Bolero’ (Chapter 6), *Cordyline australis*, *Ficus benjamina* and *Sinningia speciosa* (Chapter 3) and also for the CAM plant Phalaenopsis (Chapter 5).

Leaf morphology and photosynthesis

In response to the ambient light environment, leaf anatomy changes to maximize photosynthesis**.** We studied the leaf anatomy of *Chrysanthemum* (Chapter 2 and Chapter 4) and three ornamental pot plants *Cordyline australis* (monocot), *Ficus benjamina* (dicot, evergreen leaves) and *Sinningia speciosa* (dicot, deciduous leaves) (chapter 3). It was a general trend that the leaf thickness of both dicot and monocot species was the smallest under R with exception for *Sinningia speciosa* (table 2.2 and 3.3) that showed no plasticity to the applied light quality. Histological characterization of leaves of these ornamentals (Figure 2.2 and Figure 3.3) showed

differences in the leaf anatomical development of the species. In *Chrysanthemum* an unclear boundary of palisade and spongy parenchyma for leaves developing under R was observed and this was also reported for the tropical plant *Alternanthera brasiliana* (Macedo et al., 2011). Monochromatic red light decreased either the palisade or the spongy parenchyma in *Ficus benjamina* and *Chrysanthemum*. It is generally observed that blue light stimulates the length of mesophyll cells. This confirmed the importance of blue light in the development of compact sun-type leaves. Under B, dichromatic RB and multi-spectrum W leaves developed with well-organized mesophyll tissues. Comparing 40 and 100 µmol m^{-2} s^{-1} for the leaf development of *Chrysanthemum* (Chapter 2), leaf thickness decreased even more under 40 µmol m^{-2} s^{-1} to allow more light absorption. R again was the exception, although also under low fluence R mesophyll did not well develop.

Modification of leaf anatomy by different light qualities resulted in differences in light absorption and thus differences in photosynthesis. The relation between leaf anatomy and photosynthesis was discussed in Chapter 3. Leaf anatomy influences the light penetration into the leaves, light distribution within leaves, thus the light absorption and use efficiency, potentially the gas and water conductance and leaf photosynthesis performance. It is tricky to define if thicker or thinner leaves are beneficial for photosynthesis. Under low light intensities, as in the understorey vegetation (shade), thinner leaves are a shade adaptation to improve light capture (Brodersen and Vogelmann, 2010). However, thicker leaves with thicker mesophyll tissue that contain more light harvesting pigments are reported to have a positive effect on the net photosynthesis (Agusti et al., 1994; Boardman, 1977). Further on, photosynthesis is influenced by other factors besides light capture. An important parameter in this respect is the stomatal conductance which is influenced by stomatal characteristics.

We also observed a species depended response of the stomatal development to light quality, two groups could be separated. We found no stomatal density differences in the *Chrysanthemum* (chapter 2) and *Cordyline australis* (chapter 3), while blue light positively affected the epidermal cell enlargement, which resulted in a stomatal index decrease when compared with R. In the other group, including *Ficus benjamina* and *Sinningia speciosa*, blue light increased the stomatal density. It is no surprise that both blue and red light mediate stomatal development through the additive function of

CRY1, CRY2, PHYB, and PHYA (Pillitteri and Torii, 2012). Light is an important factor that affects opening of stomata. A well-characterized blue light response is localized in the stomatal guard cells and is rapid and reversible (Shimazaki et al., 2007; Tallman and Zeiger, 1988). Blue light stimulated stomatal opening is mediated by the blue light receptors, the phototropins and cryptochromes (Talbott, 2002). Also for the three ornamental pot plants (Chapter 3) an increasing blue light ratio goes together with an increase of stomatal conductance. Stomatal conductance is one of the most important limitation of photosynthesis, though in Chapter 2 and Chapter 3 where we studied the stomatal conductance, no significant correlation between g_s and Φ_{PSII} was found. This is explained by the relative low light intensity (100 or 40 µmol m^{-2} s^{-1}), which is still below the threshold for gs to limit photosynthesis activity, it is far below the saturation point for plants (for instance, the saturation light intensity of chrysanthemum is 500-600 µmol m^{-2} s^{-1}, Weerakkody and Suriyagoda, 2015).

Photosynthetic pigments and secondary metabolism

The main pigments used in light harvesting are chlorophyll a, chlorophyll b and carotenoids. The capacity of light harvesting is crucial, especially in light-limited conditions, when the plants need to harvest every available photon. Therefore, chlorophyll content is to a certain level associated with the photosynthetic efficiency of the plant. Biosynthesis of chlorophyll is influenced by light quality and blue light has a positive effect on its biosynthesis (as explained in Chapter 4). Effects of light quality can best be studied under the same light intensity as also light intensity affects chlorophyll content. In our experiments, we found species-specific responses. B and RB treated *Phalaenopsis* increased the chlorophyll content compared to W and R (Chapter 5), B increased the chlorophyll content in *Sinningia speciosa* (Chapter 3). Different light qualities did not result in different chlorophyll contents in *Chrysanthemum* 'Staviski' (Chapter 2), *Ficus benjamina* (Chapter 3) and *Spathiphyllum* (Chapter 6). Within *Chrysanthemum* we found cultivar dependent variation for the genotypes (Chapter 4), a global cultivar analysis resulted in a decreased pigment content under R, B and RB compared to W. It is not surprise that in purple cabbage it was even reported that red light was beneficial for pigment and secondary metabolite accumulation (Yang et al., 2016). R at our applied intensities (100 µmol m^{-2} s^{-1}) did not impair chlorophyll biosynthesis either (Tripathy and Brown, 1995). Pigments are directly related to the photosynthesis, though it is not only the

chlorophyll content that influences photosynthesis. Less chlorophyll is not always linearly correlated with the photosynthesis efficiency, it is reported that less chlorophyll might be more efficient (Abadía et al., 1999; Sæbø et al., 1995).

Primary metabolism is directly involved in plant growth, while secondary metabolism comprises compounds produced in other metabolic pathways. In Chapter 4 we studied effects of light quality on this group of secondary metabolites and checked especially the intraspecies responses. We found that blue light increased the H_2O_2 accumulation, which is an important ROS species, while also for proline a high increase in leaves under monochromatic B was observed. The increase of free proline could contribute to the scavenging capacity of free radicals. Flavonoids and allied phenolic and polyphenolic compounds are an important group of secondary metabolites. They are considered as major antioxidant compounds in plants. Blue light has the potential to enhance the biosynthesis of flavonoids and phenolic compounds through the up-regulation of chalcone synthase (CHS) expression, which is the initial enzyme of flavonoid synthesis through the involvement of cryptochrome. However, also red light was reported to be active in the regulation of CHS expression though phytochrome A. These two potential pathways in the up-regulation of flavonoid and phenolic compounds explain the complex response of the *Chrysanthemum* cultivars to the light quality treatments. Depending on the studied cultivar, red or blue light enhance the biosynthesis of these metabolites. Our data thus clearly support that multiple photosensory pathways contribute to the up-regulation of flavonoids and phenolic compounds.

Photosynthetic acclimation beyond the young phase

If only a certain production phase is conducted under LED light such as the young phase of ornamental plants, the plants are inevitably under greenhouse conditions during the production phase. Based on the obtained knowledge in Chapters 2-5 it is clear that the light quality treatment changes the leaf and thylakoid characteristics, which will have its effects on the acclimation ability of the plants when subjected to the full spectrum and high-intensity sunlight. During the first week of the greenhouse transfer, the preliminary treatment with monochromatic R and B resulted in a lower photosynthetic capacity compared to RB or W in *Chrysanthemum*. However, B

leaves acclimated quickly to similar level as W while the leaves formed under R showed for a longer period a dysfunctioning of the photosynthetic apparatus.

Evaluation of the applied light spectra

Blue and red light are widely accepted in the application for both research and production, because they meet the absorption peaks of chlorophyll. According to the equation $E = hc/\lambda$, where E is the energy content of the photon (J), h is the Planck's constant, c is the speed of light, and λ is the wavelength (nm), blue photons contain more energy than red photons. Thus red LED light produces more efficient light photons than blue LEDs with the same energy input.

For short time application red light is more photon efficient because in the blue spectrum absorption of accessory pigments (such as carotenoids and anthocyanin) takes place next to chlorophyll absorption (Figure 1.2). Red LED light thus seems the optimal spectrum considering both energetic and photosynthetic efficiency. However, according to our findings, it is unwise to apply monochromatic red as sole light source for a longer time, as it causes dysfunction in photosynthesis and it limits leaf and stomatal development in certain species. Adding small amounts of blue light (7% in our case)could (partially) eliminate the disorder induced by monochromatic red. Therefore, from an energetic point of view, LED lights with a major fraction of red can be used if blue is added. The optimal blue light percentage needs to be further investigated, and will probably result in groups of plants needing lower or higher blue light fractions in the spectrum.

Table 7.1 Effects of light qualities applied on the ornamental species in this study. All given differences were obtained from the the comparison with the W treatment applied in each experiment.

Light	Species	Leaf anatomy	Growth	Stomata	Pigments	Chlorophyll Fluorescence	
R	*Chrysanthemum* "Staviski"	Leaf thickness and spongy parenchyma decreased	FW decreased	Stomatal density and index unaffected	No effect	Φ_{PSII}, qP, ETR and F_v/F_m decreased	Chapter 2
	Chrysanthemum 8 cultivars	Leaf thickness increased, leaf area decreased	n/a	n/a	Chl and carotenoids decreased	Φ_{PSII} increased	Chapter 4
	C. australis	Leaf thickness decreased	FW and DW decreased	Stomatal index decreased	Chl a and b decreased	Φ_{PSII}, ETR and F_v/F_m decreased	Chapter 3
	F. benjamina	Leaf thickness and palisade decreased. Leaf area decreased	FW and DW decreased	Stomatal index decrease. g_s decreased,	No effect	F_v/F_m decreased	Chapter 3
	S. speciosa	No effect	FW and DW decreased	Stomatal index and density decreased, g_s decreased	Increased Chl a and carotenoid	Φ_{PSII}, ETR and F_v/F_m decreased	Chapter 3
	Phalaenopsis	No effect	DW decreased	n/a	Chl and carotenoids decreased	Φ_{PSII}, ETR and F_v/F_m decreased	Chapter 5
	Spathiphyllum	No effect	No effect	n/a	No effect	F_v/F_m decreased	Chapter 6
B	*Chrysanthemum* "Staviski"	Palisade thickness increased	FW increased	No effect	No effect	ETR and F_v/F_m increased	Chapter 2
	Chrysanthemum 8 cultivars	Leaf thickness increased	n/a	n/a	Chll and carotenoids decreased	Φ_{PSII} and F_v/F_m increased	Chapter 4
	C. australis	Leaf thickness decreased,	n/a	Stomatal index decreased	No effect	Φ_{PSII} and ETR decreased, F_v/F_m and NPQ increased	Chapter 3
	F. benjamina	Leaf thickness, spongy and palisade increased. Leaf area increased	FW and DW increased	Stomatal index and density decreased	No effect	Φ_{PSII} and ETR increased	Chapter 3

Table 7.1 (continued)

Light	Species	Leaf anatomy	Growth	Stomata	Pigments	Chlorophyll Fluorescence	
B	*S. speciosa*	Palisade thickness increased. Leaf area unaffected	FW decreased	No effect	Chl and carotenoids increased	No effect	Chapter 3
	Phalaenopsis	Leaf area unaffected	DW decreased	n/a	Chl and Carotenoids increased	F_v/F_m increased	Chapter 5
	Spathiphyllum	Leaf area unaffected	Dry weight unaffected	n/a	Chl unaffected	F_v/F_m increased	Chapter 6
RB	*Chrysanthemum* "Staviski"	Palisade thickness increased	FW increased	No effect	No effect on pigment,	F_v/F_m increased	Chapter 2
	Chrysanthemum 8 cultivars	Leaf thickness increased, leaf area unaffected	n/a	n/a	Chl and carotenoids decreased	F_v/F_m and Φ_{PSII} increased, NPQ decreased	Chapter 4
	C. australis	Leaf thickness decreased. Leaf area unaffected	FW and DW decreased	No effect	Chl a and Chl b increased,	F_v/F_m and NPQ increased	Chapter 3
	F. benjamina	Palisade thickness decreased. Leaf area unaffected	No effect	Stomatal index decreased. g_s decreased	No effect	F_v/F_m increased	Chapter 3
	S. speciosa	Palisade thickness increased	DW increased	Stomatal index and density decreased g_s decreased	Chl and carotenoids increased	NPQ decreased	Chapter 3
	Phalaenopsis	Leaf area unaffected	DW unaffected	n/a	Chl increased	No effect	Chapter 5
	Spathiphyllum	Leaf area unaffected	DW unaffected	n/a	No effect	No effect	Chapter 6

n/a: not available. The *Chrysanthemum* 'Staviski' of Chapter 2 present in the table is the results at 100µmol m^{-2} s^{-1}

7.2 Future perspectives

This study focused primarily on the effects of light quality on different parameters related to functioning of the photosynthesis in selected ornamentals. We have focused on effects of monochromatic R, B while only one dichromatic treatment was included in this study. Based on the knowledge we gained, different ornamental species or different genotypes may react different with respect to the applied light quality. This suggests that optimization of the RB ratio will not result in one single strategy but that for the ornamental industry different light quality combinations will be needed. Optimization is not only necessary for horticultural traits as compactness and other photomorphogenic responses, but for traits linked with the primary (photosynthesis, biomass) and secondary (pigmentation) metabolites.

Perspectives for applied horticultural research could focus on production phases beyond the young vegetative phase. If plants remain their whole production cycle under artificial lighting, it would be interesting to study the effect of light quality on the flowering time, bud emerging, flower morphology, and how the flower color might be affected for the respective ornamental species.

The mechanisms underlying the physiology and secondary metabolism under the influence of different light spectra are still poorly studied with respect to solely LED light combinations. Perspectives for more fundamental research in plants in general might be an in-depth study of light quality ratios on the integrity of the chloroplast proteins, this in order to better understand the ‘red light’ syndrome we observed in both C3 and CAM metabolism. In addition, a study of the key enzymatic activities involved in CAM metabolism (malic enzyme, PEP carboxykinase, carbonic anhydrase etc) might be interesting in view of our observed effects in *Phalaenopsis*. Stomatal behavior of CAM plants is different from the well-known C3 model, further investigation in stomatal movement of CAM plants in response to the light spectrum would be interesting.

LED research is still at the start of its potential, now focusing mainly on R and B combinations. However, as technology advances, other efficient monochromatic LED lights (for instance green) within the visible light might become available for the ornamental sector. Plants grown under an enriched green environment show a shade

response as also found under far red. The interaction of blue and green light, which are percepted by the cryptochromes, might result in favorable traits for specific ornamentals. Furthermore, UV-A and UV-B are also an integral component of the sunlight and received increasing research interest in recent years. UV light, as a component of sun radiation, exerts a wide range of physiological responses in plants. Further research in applications of UV-A/UV-B light in horticultural production may be interesting.

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