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Effects of different irradiation levels of light quality on Chrysanthemum

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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 h 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 a lower light intensity were observed, we saw a decrease in leaf thickness for blue, red + blue and multispectral white light but not for the red wavelength. 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 an inhibition of 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.

1. Introduction

Bedding plants and also 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 photo-morphogenesis for a targeted plant response (Goto, 2012; Tennessen et al., 1994). Plants are able to use spectral wavelengths within the range of 400–700 nm for photosynthesis (Davis and Burns, 2016). Light perception of photosynthetic pigments (chlorophylls and carotenoids) peaks in the red and blue light region (Johnson, 2016). It is thus widely accepted to supply light to plants with blue and/or red wavelengths to sustain plant growth and development.

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 UVA/blue absorbing cryptochromes and phototropins (Ahmad, 1999). 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, 2017). This is well known in natural environments. Shade

Abbreviations: Chl, chlorophyll; F_v/F_m, potential quantum yield; NPQ, non-photochemical dissipation of absorbed energy; P_{fr}/P_{total}, proportion of phytochrome in its far-red light absorbing form; PPFD, photosynthetic photon flux density; Φ_{PSII}, quantum yield of photosystem II electron transport

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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 are influenced by light, which were documented in numerous species with respect to various light environments (Hogewoning et al., 2010; 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., 2015; Schuerger et al., 1997). Supplemental blue light promoted the stem and internode elongation in cut chrysanthemum without any inhibitory effect on flower bud formation (Jeong et al., 2014). Likewise, the stem length in *Tagetes*, an often-used bedding plant, was higher under monochromic blue light compared with fluorescence lamps. In *Salvia*, plants supplemented with far-red increased their stem length while it was significantly inhibited under red light (Heo et al., 2002).

However, limited 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. Low light intensities should saturate the reaction of phytochrome and cryptochrome 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. In Belgium daily light integrals range from 10 to 20 mol $m^{-2} day^{-1}$ for the production of rooted chrysanthemum cuttings (February to April). 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. Materials and methods

2.1. Plant material and experimental set-up

The experiment was performed in a climate chamber at Faculty of Bio-Science Engineering, Ghent University. Rooted chrysanthemum cuttings (Chrysanthemum *morifolium* 'Staviski'; Gediflora nv, Belgium) were planted in 0.3 L 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 °C and vapor pressure deficit ranged between 0.5–0.8 kPa. 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) were applied (Table 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) (Fig. 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 leaf.

2.2. Leaf anatomy

Leaf samples 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 microscopy (IX81, Olympus Inc., Tokyo, Japan). Leaf thickness, palisade, spongy parenchyma thickness and epidermis thickness were analyzed with ImageJ software (ImageJ 1.48 v, NIH, USA).

2.3. Stomatal characteristics and stomatal conductance

Stomatal characteristics were determined using a nail polish print method on the leaf abaxial side as described by Mott and Michaelson (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). The third fully developed leaf was chosen for measurements. Four positions on the abaxial side of each leaf were measured and the average result was used as the stomatal conductance of this leaf.

2.4. 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 0.6 s saturating light pulse (3450 μmol $m^{-2} s^{-1}$) was given to obtain the minimal and maximal fluorescence yield (F_0 and F_m). Then, leaf was light adapted 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 PSII (F_v/F_m) was calculated using $F_v/F_m = (F_m\text{-}F_0)/F_m$; PSIIoperating efficiency (Φ_{PSII}) was calculated as $\Phi_{PSII} = (F_{m'}\text{-}F_s)/F_{m'}$ 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).

2.5. Pigments content

Leaf chlorophyll content was determined according to Lichtenthaler and Buschmann (2001). 150 mg fresh leaf was grinded using liquid nitrogen and extracted in 80% acetone overnight at −20 °C.