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Research article

Analysis of gas exchange, stomatal behaviour and micronutrients uncovers dynamic response and adaptation of tomato plants to monochromatic light treatments

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ABSTRACT

Light spectrum affects the yield and quality of greenhouse tomato, especially over a prolonged period of monochromatic light treatments. Physiological and chemical analysis was employed to investigate the influence of light spectral (blue, green and red) changes on growth, photosynthesis, stomatal behaviour, leaf pigment, and micronutrient levels. We found that plants are less affected under blue light treatment, which was evident by the maintenance of higher *A*, g_s, T_r, and stomatal parameters and significantly lower *VPD* and T_{leaf} as compared to those plants grown in green and red light treatments. Green and red light treatments led to significantly larger increase in the accumulation of Fe, B, Zn, and Cu than blue light. Moreover, guard cell length, width, and volume all showed highly significant positive correlations to g_s, T_r and negative links to *VPD*. There was negative impact of monochromatic lights-induced accumulation of Mn, Cu, and Zn on photosynthesis, leaf pigments and plant growth. Furthermore, most of the light-induced significant changes of the physiological traits were partially recovered at the end of experiment. A high degree of morphological and physiological plasticity to blue, green and red light treatments suggested that tomato plants may have developed mechanisms to adapt to the light treatments. Thus, understanding the optimization of light spectrum for photosynthesis and growth is one of the key components for greenhouse tomato production.

1. Introduction

Stomatal behaviour can be influenced by light quantity and quality, CO_2 concentration, temperature, humidity, soil water availability, mineral nutrition, and atmospheric pollutants (Assmann, 1999; Assmann and Shimazaki, 1999; Chen and Blatt, 2010; Hills et al., 2012; Mott, 2009; Shimazaki et al., 2007). Light is used as the energy source for photosynthesis and also perceived as a signal (Lin and Cheng, 1997). Alterations in light quality affect plant morphogenesis and photosynthesis with variable effects among species (Hogewoning et al., 2010; Terfa et al., 2013), and many spectral responses of plants are regulated via photoreceptors, such as phytochromes, cryptochromes, and phototropins, which alter the expression of a large number of genes (Barnes et al., 1997; Whitelam and Halliday, 2007).

Blue light (450–495 nm) regulates the formation of chlorophyll, stomatal opening, photo-morphogenesis, growth of internodes and cell expansion, and phototropism (Briggs and Christie, 2002; Christie, 2007; Shimazaki et al., 2007; Takemiya et al., 2005; Zeiger and Hepler, 1977). Several blue light photoreceptors have been identified (Whitelam and Halliday, 2007) in stomatal guard cells. For instance, UV-A and blue light responses are mediated via cryptochromes and phototropins (Ballaré, 1999; Christie, 2007). Blue light stimulated photoreceptors are phosphorylated and bind to 14-3-3 proteins, leading to proton extrusion and membrane hyperpolarisation, and K^+ and anion uptake into stomatal guard cells (Assmann, 1999; Assmann and Shimazaki, 1999; Mott, 2009; Shimazaki et al., 2007; Takemiya et al., 2005). As a result, guard cells swell and bend, thereby pushing the stomatal pore open and enabling gas exchange between the leaf interior and the

Abbreviations: A, Net CO_2 assimilation; g_s, stomatal conductance; T_r, transpiration rate; *VPD*, vapour pressure deficit; T_{leaf}, leaf temperature.

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atmosphere (Roelfsema and Hedrich, 2005). Green light (510–585 nm) shows distinct effects on seedling germination, stem elongation, leaf expansion, leaf hyponasty, apical dominance, photosynthesis, and biomass accumulation (Ballaré, 1999; Schmitt and Wulff, 1993; Wang and Folta, 2013; Whitelam and Halliday, 2007). Green light reverses the blue light-induced stomatal opening and deactivates guard cell solute uptake (Talbott, 2006; Talbott et al., 2002). Recent studies suggested that, among the known photoreceptors, cryptochromes may absorb green light (Lin et al., 1995; Liu et al., 2008) and the green light response relies on both photosynthesis-dependent and -independent mechanisms; however, green light photoreceptors are not fully understood (Wang and Folta, 2013). Responses to red light (600–740 nm), mediated by phytochromes, are important for the development of the photosynthetic apparatus and are associated with the morphological adaption of plants to the environment (Ballaré, 1999; Christie, 2007). Red enriched environments impose a distinct inhibition on internode elongation, promote lateral branching, delay floral initiation, and alter chlorophyll and carotenoid contents (Demmig-Adams and Adams, 1992; Schmitt and Wulff, 1993; Wang and Folta, 2013; Whitelam and Halliday, 2007). Red light-induced stomatal closure is achieved via a net result of loss of K^+ and solutes from guard cells (Chen et al., 2004; Zeiger, 1990). In addition, stomatal opening in isolated epidermal strips and activation of plasma membrane H^+-ATPase in guard cells are also reported to be stimulated by red light (Olsen et al., 2002; Serrano et al., 1988). Therefore, a prolonged period of monochromatic light treatments is likely to affect the yield and quality of greenhouse tomato. However, detailed studies on the contributions of photosynthetic and stomatal responses to different light regimes are still lacking.

Micronutrients, such as manganese (Mn), copper (Cu), zinc (Zn), iron (Fe), boron (B) and nickel (Ni), influence numerous physiological functions in plants including stomatal regulation, photosynthesis, disease resistance, cell wall formation, osmoregulation, translocation of other minerals as well as participating in various enzymatic reactions (Clarkson and Hanson, 1980; Marschner, 1995; Welch and Shuman, 1995). Acute deficiency or toxicity of micronutrients in plants leads to perturbations in the normal physiological functions of plants (Li et al., 2010). However, few studies have focused on the tomato plant's ability to absorb and interact with crucial micronutrient concentrations under different light spectra.

Although mechanisms of stomatal response to light have been studied intensively (Assmann, 1999; Assmann and Shimazaki, 1999; Ballaré, 1999; Barnes et al., 1997; Briggs and Christie, 2002; Chen et al., 2004; Christie, 2007; Demmig-Adams and Adams, 1992; Schmitt and Wulff, 1993; Shimazaki et al., 2007; Zeiger, 1990), there are still many unexplored areas such the long-term response and adaptation of stomata and micronutrients to different light spectra. The overarching hypothesis of this study is that different light spectra will lead to the reduction of stomatal opening, photosynthesis, micronutrient accumulation and growth in tomato plants. Therefore, the aims of this study were to: 1) elucidate the dynamic regulation of blue, green and red light on stomatal behaviour and gas exchange; 2.) investigate the effects of blue, green and red light on leaf pigment and micronutrient accumulation; and 3) decipher the links among stomatal behaviour, gas exchange, pigment, and micronutrients in tomato.

2. Materials and methods

2.1. Growth conditions and light spectral treatments

Seeds (*Solanum lycopersicum* L. *cv*. Black Krim) were sown and germinated in a compost potting mix (Debco Pty Ltd, VIC, Australia). The germinated seeds were grown for two weeks and uniform and healthy seedlings were then transplanted into 5-L pots. A full strength Hoagland's solution was applied to the plants in Week 3 followed by a slow release fertiliser Osmocote (Scotts Australia, NSW, Australia) in Week 4 before commencing light treatments. The glasshouse growth conditions were 26 ± 2 °C and 60% relative humidity (RH) during the day, 22 ± 2 °C and 70% RH at night with a 16 h/8 h light/dark cycle. The plants were well watered to avoid drought stress during the entire experiment.

Five-week-old plants were used for light spectral treatments with light boxes made from 3 mm thick blue, green and red Perspex (All Plastics Pty Ltd, NSW, Australia). Control (white light) was provided by a 600 W broad spectrum growth lamp (LUCAGROW, GE Lighting, Hungary). The light boxes were attached beneath lamps and to black PVC plastic sheets, which can block non-target light sources on plants from the sides while the Perspex light boxes and lamps provide the desired light spectra. Light transmission of the Perspex was assessed using a UV–visible spectrophotometer (Cary, Melbourne, Australia). Irradiance on top of leaves was monitored with an LI-250A light meter (Li-Cor Inc. Lincoln, NE, USA), and the height of the lamps were adjusted to ensure that plants received 300 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) in the control and 100 μmol m^{-2} s^{-1} of PAR in the blue, green and red light treatments. Plant height and leaf numbers were measured weekly. Harvest was undertaken from Weeks 5–9 by sampling four plants from each treatment for dry weight and micronutrient analysis.

2.2. Gas exchange measurement

Gas exchange measurement was made according to Chen et al. (2005) and O'Carrigan et al. (2014). Net CO_2 assimilation (A), stomatal conductance (g_s), transpiration rate (T_r), vapour pressure deficit (*VPD*), and leaf temperature (T_{leaf}) of the third fully-expanded leaves were determined over five weeks with an LI-6400XT infrared gas analyser (Li-Cor Inc. Lincoln, NE, USA). The conditions in the measuring chamber were controlled at a flow rate of 500 mol s^{-1}, a saturating PAR of 1500 μmol m^{-2} s^{-1}, 400 mmol mol^{-1} CO_2, and a relative humidity of 60–70%.

2.3. Stomatal assay

Stomatal assays were carried out essentially as described in Chen et al. (2012), Eisenach et al. (2012), and O'Carrigan et al. (2014). Abaxial epidermal strips of the third fully expanded tomato leaves were peeled, immediately immersed in a buffer and mounted on glass slides for micro-imaging. Four images of each epidermal strip were taken using a Leica microscope (Leica Microsystems AG, Solms, Germany) attached with a Nikon NIS-F1 CCD camera and a Nikon DS-U3 controller (Nikon, Tokyo, Japan). The experiments were repeated five to eight times weekly. Images were analysed with the Nikon NIS Element software (Nikon, Tokyo, Japan). Stomatal pore area was calculated by assuming an oval pore shape, and guard cell volume was estimated and calculated according to Chen et al. (2010), Eisenach et al. (2012) and Meckel et al. (2007). Stomatal density was calculated as the number of stomata per mm^2, and stomatal index was calculated as ([number of stomata]/[number of epidermal cells + number of stomata]) × 100 (Kubínová, 1994).

2.4. Measurements of leaf pigment

Chlorophyll *a*, *b* and carotenoid contents were measured according to Arnon (1949) and Mak et al. (2014) using the same third, fully-expanded leaves were collected weekly following gas