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Led spectral composition effects on mycorrhizal symbiosis formation with tomato plants

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ABSTRACT

The present study discusses physiological and biochemical nature of relationships between below- and aboveground processes affected by three different lights spectra. Our objective was to characterize the effect of light quality on *Solanum lycopersicum* L. by determination of the mycorrhizal function, soil enzyme activities and plant performance (growth, photosynthesis, secondary metabolites and the enzymes linking nitrogen and carbon metabolism). A growth chamber experiment was conducted with young tomato plants and the light was provided by fluorescent tubes (white light, WL) and computer controlled light system that combines four lamps with 4 cm^2 arrays of red, green, blue, amber LEDs. The combinations were RB (Red 66%, Blue 33%) and RG (Red 66%, Green 33%). Under WL mycorrhizal fungi promoted soil fertility increasing enzyme activities and this was linked to the higher plant biomass accumulation. Also, we found the same upward trend between the values of root and soil alkaline phosphatase together with acid root phosphatase activity and accumulation of flavonoids in leaves. RB light stimulated shoot biomass production and gas-exchange parameters in inoculated tomatoes. The enhancement of soil urease activity, mycorrhizal development and nitrogen content in the leaves in parallel with nitrate reductase activity were as a consequence of RG lightening. Plant responses to the light quality and quantity precise manipulation and inoculation with beneficial soil microorganisms could be perspective in extending the production economic efficiency and nutrition potential of vegetables grown in controlled environments.

1. Introduction

Light is both a source of energy and information for green plants (Aphalo Pedro, 2006). Light quality and quantity affect plant growth and development in a complex way initiating signaling cascade of specific photoreceptors, which alter the expression of a large number of genes (Hogewoning et al., 2010). Light-emitting diodes (LEDs), which are characterized by relatively narrow-band spectra, are employed analyzing specific plant responses to the light quality (Olle and Viršilė, 2013). LEDs have exceptional potential as a single source or supplemental lighting systems for crop production. Their small size, durability, long operating lifetime, wavelength specificity, relatively cool emitting surfaces, and linear photon output with electrical input current make them ideal for use in plant lighting designs (Massa et al., 2008). Different studies covered partially the question of red, blue and green LED effects on the growth and photosynthetic activity of various plants. Red and blue light are essential in the lighting spectra for green vegetables and tomato, cucumber, and pepper transplants (Olle and Viršilė, 2013). The higher percentage absorption (90%) of blue or red light on leaves strongly influenced plant development and physiology (Terashima et al., 2009). The use of red LED light to stimulate photosynthesis has been widely accepted. The McCree curves (Sager and McFarlane, 1997) indicate that red wavelengths (600 to 700 nm) are efficiently absorbed by plant pigments, close to an absorption peak of chlorophyll (Massa et al., 2008). However, theoretically unprofitable spectral parts as green also have significant physiological effects on plants as a rapid increase in the growth rate of *Arabidopsis* seedlings (Olle and Viršilė, 2013). Plant biomass, internode length, branch number and leaf size are affected by light quality and quantity (Da Silva et al., 2016). It has been demonstrated that light regulated nitrate metabolism in plants and the exposure of plants to red and blue light at the same time stimulates the process of nitrogen assimilation in comparison to the exposure to only red or blue light (Wojciechowska et al., 2016).

Physiologically and ecologically, a significant amount of light penetrates the soil approximately 4–5 mm from the surface, eliciting some responses such as spore germination, root growth, and formation of mycorrhiza (Tester and Morris, 1987). This information has led some

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experts to hypothesize the function of LEDs on the mycorrhizal formation (Cruz, 2016). The arbuscular mycorrhizas represent the most pervasive symbiosis throughout the plant domain, which is significant for plant nutrition and ecosystem functioning. Variations in abiotic conditions such as light would have an essential effect on the symbiosis (Ballhorn et al., 2016) though plants have fine control mechanisms over mycorrhizal colonization of their roots (Sikes, 2010). Nagahashi and Douds (2004) demonstrated that blue light stimulates hyphal branching. The red-blue and only red illuminations stimulated the formation of arbuscular mycorrhizal fungi (AMF) spores in glass beads, whereas the blue treatment suppressed it (Cruz, 2016). AMF hyphae and spores secreted a specific protein glomalin which is quantified from the soil as glomalin-related soil protein (GRSP). GRSP plays important roles in AMF fungal effects on soil aggregation and properties (Rillig, 2004). AMF improve the soil enzyme activity since they influence soil carbon, nitrogen, and phosphorous cycling (Zhao et al., 2010). Respectively, the organic carbon transformation reflects by the soil invertase activity and is used as an indicator for nutrient conversion and energy metabolism (Nannipieri et al., 1990). Urease catalyzes the hydrolysis of urea into ammonia or ammonium ion depending on soil pH and carbon dioxide. Soil phosphatases are enzymes with a relatively broad specificity, capable of hydrolyzing various organic phosphate esters, and are involved in the P cycle (Yang et al., 2010). Tomato (*Solanum lycopersicum* L.) is a crop widely distributed in the world and is cultivated throughout the year. The young tomato plants are produced on a large-scale under monitored conditions to meet the increasing production seeking. The light is the major requirement affecting the growth of young tomato plants in a controlled environment to gain year-round high production and good quality (Fan et al., 2013).

Advantages make LEDs perfect for supporting plant growth in a controlled environment such as growth chamber. Depending on its own features, each plant species react differently to the light source and light wavelength and a detailed understanding of costs and benefits arising from the mycorrhizal symbiosis under varied light conditions is lacking. Whereas specific responses of plants to a spectrum may sometimes be predictable based on published research, the overall plant answer is generally difficult to hypothesize due to the complex interactions at different metabolic levels. Therefore, the objective of the present study was to reveal the effect of light treatments: white fluorescent light (WL), red-blue (RB) and red-green (RG) on *S. lycopersicum* L. growth and mycorrhizal symbiosis formation. Tomato plants were analyzed for photosynthetic performance, mycorrhizal colonization; glomalin – related soil proteins; enzyme activities in soil (urease, invertase and phosphatase activity); leaf chlorophyll content; flavonoids; nitrogen balance index; carbon-nitrogen metabolic status (nitrate reductase, glutamine synthetase, glutamate synthase and NADP-malic enzyme).

2. Material and methods

2.1. Experimental design

The experiment had a completely randomized block design with four replications that had the following treatments of non-inoculated and inoculated with mycorrhizal strain *Claroideoglomus claroideum* EEZ 54 tomato plants:

(1.) C-WL control, non-inoculated plants grown under white fluorescent lamp
(2.) AM-WL plants, inoculated with Claroideoglomus claroideum EEZ 54 grown under white fluorescent lamp
(3.) C- RB – non-inoculated plants grown under blue and red LEDs light (Red 66%, Blue 33%)
(4.) AM- RB – plants, inoculated with Claroideoglomus claroideum EEZ 54 grown under blue and red LEDs light (Red 66%, Blue 33%)
(5.) C- RG – non-inoculated plants grown under green and red LEDs light (Red 66%, Green 33%)
(6.) AM- RG – plants, inoculated with Claroideoglomus claroideum EEZ 54 grown under green and red LEDs light (Red 66%, Green 33%)

	white fluorescent lamp	Red 66% + Blue33% light	Red 66% + Green 33% light
non-inoculated plants	1. C-WL	3. C- RB	5. C – RG
inoculated plants with *Claroideoglomus claroideum* EEZ 54	2. AM-WL	4. AM- RB	6. AM- RG

Tomato plants (*Solanum lycopersicum* L. var. Karlik Shtambovy (*dw*)) were grown from seeds in 5 kg plastic pots filled with unsterilized soil/perlite substrate (3:1, v/v). All pots were adjusted daily to 60% water holding capacity. The soil (leached cinnamonic forest soil (*Chromic Luvisols*, FAO), 30–40 cm depth) had the following agrochemical characteristics: pH (H_2O) = 6.2; 8 mg kg^{-1} soil total mobile nitrogen (4.20 mg kg^{-1} N-NO_3^- and 3.80 mg kg^{-1} N-NH_4^+); 30 mg kg^{-1} soil P_2O_5; 120 mg kg^{-1} soil K_2O. The mycorrhizal strain (*Claroideoglomus claroideum*, ref. EEZ 54) was kindly provided by the AMF collection of Estación Experimental del Zaidín (CSIC Granada, Spain). Mycorrhizal inoculation was done by placing the seeds over a thin layer of the AMF inoculum (2 g kg^{-1} soil substrate) following the layering method (Jackson et al., 1972). The inoculum consisted of colonized roots and soil from 4-month-old oat pot cultures. Plants were cultivated in a growth chamber from April to July (till flowering stage) under two light regimes: RB (Red 66%, Blue 33%) and RG (66%, Green 33%). Light was provided by a computer controlled light system that combines four lamps with 4 cm^2 arrays of red (R), green (G), blue (B), amber (A) light-emitting diodes (LEDs) (Octa Light LTD, Bulgaria), capable of generating varied monochromatic light within the visible spectrum. White light (WL) used as a control, was supplied by fluorescent tubes (Lumilux L360W/640 and L360W/830, Osram, Germany). The total photosynthetic photon flux density was the same in each light treatment about 320 μmol m^{-2} s^{-1}. All plants were kept at 21–25 °C (night-day), a 15 h photoperiod and relative humidity 60–70%. The light treatments were separated by solid walls. At harvest, the root system was separated from the shoot and the biometrical data (plant height, a fresh and dry weight of shoots and roots) was determined.

2.2. Determination of root colonization and glomalin-related soil proteins

The extent of mycorrhizal root colonization was estimated using the gridline intersect method (Giovannetti and Mosse, 1980). To visualize the AMF colonization under a dissecting microscope, roots were cleared in 10% KOH and stained with 0.05% Trypan blue in lactic acid (v/v), according to Phillips and Hayman (1970). The extraction procedure for the soil proteins followed the method reported by Wright and Upadhyaya (1996). The soil (2 g) was mixed with 8 mL of 20 mM sodium citrate at pH 7.0. The samples were autoclaved for 30 min (121 °C) and immediately centrifuged at 5,000 x *g* for 15 min. The supernatant represented the easily extractable glomalin-related soil proteins (EE-GRSP). The procedure for extracting total (TE-GRSP) consisted of autoclaving 2 g soil in 8 mL of 50 mM sodium citrate at pH 8.0 for 60 min. Immediately, after autoclaving, centrifugation at 5,000 x *g* for 15 min was done; the supernatant was stored at 4 °C until needed for analysis. Glomalin quantification (EE-GRSP and TE-GRSP) was done by the method of Bradford (1976) using protein dye reagent and bovine serum albumin (BSA, Sigma) as a standard.

2.2 **Soil enzyme extractions and assays**

Urease (EC 3.5.1.5) activity was determined colorimetrically following incubation of soil with urea (aqueous solution) and citrate buffer according to modified method of Hoffmann and Teicher (1961).