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Light quantity and quality supplies sharply affect growth, morphological, physiological and quality traits of basil

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ABSTRACT

Plants sharply adapt their growth and physiology to light availability. This study aimed at evaluating the effect of light quantity and quality manipulation on growth, morphological traits, pigment and secondary metabolites content in basil as well as comprehending the mechanisms which regulate such responses.

Two experiments were carried out under greenhouse in 2014 (spring transplanting, Spr_Tr) and 2015 (summer transplanting, Sum_Tr). On a complete randomized block design, plants of basil were exposed to three modifications of the transmitted solar radiation with colored plastic films: yellow (YF), green (GF) and blue films (BF), plus a control (Control).

Leaf pairs, axillary shoots, total fresh and dry biomass, specific leaf area, soil-plant analysis development, reflectance indices (Normalized Different Vegetation Index$_{670}$, NDVI$_{670}$, and Optimized Soil-Adjusted Vegetation Index, OSAVI), total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, single and total polyphenol content and radical scavenging activity were recorded and examined.

Shading induced stem elongation, a greater leaf area expansion and a lower leaf thickness; moreover, shaded plants increased chlorophyll accumulation (on average +29.4% and +21.6% during Spr_Tr and Sum_Tr, respectively). YF treatment allowed always the highest biomass accumulation (averaged over crop cycle: 2.1 and 3.4 g plant^{-1} during Spr_Tr and Sum_Tr, respectively).

OSAVI and NDVI$_{670}$ seem the more suitable indicators for chlorophyll accumulation. Light manipulation influenced specific phenolic compounds concentration. The application of colored films lowered rosmarinic and caftaric acids (by 29.8% and 33.2%, respectively, averaged over treatments and crop cycle). Antiradical activity was linearly correlated only with caffeic acid.

Light manipulation represents a promising tool for the manipulation of basil morphological, physiological and quality traits.

1. Introduction

Plants constantly modify their physiology and morphology to adapt to different environmental conditions. Among the environmental factors, which strongly influence plant growth and development, both quantity and quality of light transmitted to canopy play a major role (Shahak et al., 2004). Indeed, light represents both the primary source of energy and the most important regulatory factor in plant's cycle: i.e. seed germination, seedling establishment, transition to flowering and morphogenesis (i.e. stem elongation) (Folta and Carvalho, 2015; Galvão and Fankhauser, 2015).

Plants respond to light modified environments with physiological (photosynthetic rate, nutrient uptake) and biochemical (pigment and carbohydrate content) adaptations (Chang et al., 2008), which in turn

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); ANOVA, analysis of variance; BF, blue film; Car, carotenoids; Chl, chlorophyll; Chla, chlorophyll a; Chlb, chlorophyll b; CI$_{red\text{-}edge}$, chlorophyll index red edge; CRI$_{700}$, carotenoid concentration index$_{700}$; DAT, days after transplanting; DMF, N,Ndimethylformamide; DW, total dry weight; FW, total fresh weight; GAE, gallic acid equivalents; GDD, growing degree days; GF, green film; GNDVI, green normalized difference vegetation index; HCl, hydrochloric acid; HPLC, high performance liquid chromatography; I%, percentage of inhibition; LA, leaf area; LED, light-emitting diode; LSD, least significant difference; MCARI, modified chlorophyll absorption ratio index; NDVI$_{670}$, normalized difference vegetation Index$_{670}$; NIR, near-infrared; NumAS, axillary shoots; NumLP, leaf pairs on the main stem; OSAVI, optimized soil-adjusted vegetation index; PAR, photosynthetically active radiation; SED, standard error of the difference; SLA, specific leaf area; SPAD, soil-plant analysis development; Spr_Tr, spring transplanting; Sum_Tr, summer transplanting; TEAC, trolox equivalent antioxidant capacity; TPC, total polyphenol content; UV, ultraviolet; UV/vis, ultraviolet/visible; YF, yellow film

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are reflected into modification of plant growth, as well as into alterations of morphological and anatomical traits (Peralta et al., 2002). Low irradiance leads to more biomass allocated in leaves at the expense of roots optimizing leaf area per unit leaf biomass as well as maximizing light interception (Valladares and Niinemets, 2008). Shaded plants, indeed, have the thickness of mesophyll layer reduced (Terashima et al., 2005) and pigment density per unit leaf area increased (Xu et al., 2009).

In the last decades, studies on light manipulation have become more interesting due to the relationship between light and physiology of secondary metabolism under different light spectra and/or light intensities (Bantis et al., 2016). As already known in many horticultural and herbal crops, light quantity and quality plays an important role in the synthesis of many antioxidants such as phenolic acids, carotenoids, flavonoids, anthocyanins, and α-tocopherol (Stagnari et al., 2014; Bantis et al., 2016), mainly due to phytomorphogenic responses of phytochromes (Henschel et al., 2017).

Light manipulation is commonly applied using narrow-bandwidth light, such as that produced by light-emitting diode (LED)-based light sources (Samuolienė et al., 2016), coloured shade nets (Ilić and Fallik, 2017) and different coloured plastic covers or photoselective plastic films (Stagnari et al., 2014; Henschel et al., 2017). In particular, the use of photoselective cover materials (i.e. films or nets) could be a suitable agro-technological alternative to improve the quality of the edible products, of both horticultural and herbal crops. Beside plant protection from high rainfall, hail and frost (mainly films) as well as reduced physiological disorders and flower abortion (mainly nets), other important biochemical modifications could be obtained with the application of cover materials. Black and yellow nets increased the total antioxidant activity at harvest in oregano, while pearl nets favoured flavonoid (quercetin) accumulation in oregano, marjoram and coriander (Buthelezi et al., 2016). Blue cover film enhanced the levels of anthocyanins and ascorbic acid in fruits of strawberry, probably due to the antioxidant capacity of these compounds which act as an ultraviolet (UV) light filter (Henschel et al., 2017); conversely, UV black plastic films significantly lowered the total phenols and flavonoid glycosides contents in red leaf lettuce (García-Macías et al., 2007). On the other hand, Liu et al. (2015) observed higher biomass and camptothecin yield in Camptotheca acuminate, grown under red plastics.

Sweet basil (*Ocimum basilicum* L.) is an aromatic herb belonging to Lamiaceae family, which is extensively used fresh or dried to add a distinctive aroma and flavor to food, due to the strong content of essential oils; it contains high concentrations of phenolic compounds that contribute to its strong antioxidant capacity (Kwee and Niemeyer, 2011; Bufalo et al., 2015). Among these, rosmarinic acid represents the most prevalent basil's phenolic compound and it is associated with its functional properties; other important phenolic compounds are represented by caffeic acid derivatives, such as chicoric acid (Kwee and Niemeyer, 2011).

To date, in basil only few investigations on the effects of light manipulation on morphological and physiological traits, as well as on some biochemical compounds, have been carried out. Growth and yield performances of basil plants were investigated under both artificial LED or/and fluorescent lamps (Frąszczak et al., 2014; Bantis et al., 2016) and photoselective nets (Shahak et al., 2008). Regarding quality traits, the content of total volatile oils was strongly reduced under heavy shaded conditions (Chang et al., 2008), while the setting of specific spectra has been demonstrated to significantly influence phenolic content as well as antioxidant activity. According to Bantis et al. (2016), LED applications with high blue light portion induce phenolic compounds accumulation as well as red light has a preeminent role in the regulation of phenolic acids biosynthesis (Taulavuori et al., 2017). Irradiation (fluorescent lamps) with red and white rather than blue light, seems to favor higher rosmarinic acid accumulation (Shiga et al., 2009).

On these basis, the main objective of this study was to test the hypotheses that light quantity and quality manipulation – with colored cover films application (i.e. yellow, green and blue cover films) – on basil plants, should significantly affect: (i) growth and morphological traits (i.e. leaf biomass accumulation and expansion), (ii) pigment content, (iii) secondary metabolites content and activity (i.e. total polyphenols, phenolic compounds as well as antiradical activity). The main assumption of this research activity was that light sharply affects plant responses, consequently some agro-technologies can be conveyed to meet the consumers demand for edible herbs with high nutritional values.

2. Materials and methods

2.1. Plant material and growing conditions

Experiments were carried out at the greenhouse of the Agronomy and Crop Sciences Research and Education Center, University of Teramo (altitude 15 m above sea level; 42° 42′ N,13° 54' 10′ E) during two different crop growing seasons, from 4 April to 29 May 2014 (early spring transplanting, Spr_Tr) and from 28 May to 8 July 2015 (later spring transplanting, named as summer transplanting, Sum_Tr). The greenhouse was covered with a single layer of ethylene-vinyl acetate film (PATILUX) provided by P.A.T.I. S.p.A. (San Zenone degli Ezzelini, TV, Italy) and characterized by a natural ventilation system.

Seeds of sweet basil (*Ocimum basilicum* L. cv. Emily, Enza Zaden Italia S.r.l., Tarquinia, VT, Italy) were sown in a nursery substrate and maintained in a growth chamber until transplanting, which occurred 20 and 16 days after sowing in Spr_Tr and Sum_Tr, respectively. Uniformly sized seedlings (two-leaves stage) were transplanted into 9 cm side plastic pots, at a density of 1 plant per pot. Pots were filled with a mixture of peat-based compost (Terraplant® 2, COMPO Italia S.r.l., Cesano Maderno, MB, Italy), potting soil (HOCHMOOR HORTUS ESTIVO, Terflor S.r.l., Capriolo, BS, Italy), vermiculite and perlite at the ratio of 1.5:2:1:1 (v/v). The growth substrate was saturated with tap water before transplanting of seedlings and each pot was supplemented with 50 mL of fertilizer solution (Cifoumic 10-10-10, Cifo S.r.l., Bologna, BO, Italy) five days after transplanting (DAT). No insecticide or fungicide treatments were performed.

Starting from transplanting, the greenhouse's environmental conditions were constantly monitored with sensors of temperature and humidity connected to a data logger system (EM50 Data Collection System, Decagon Devices Inc., Pullman, WA, USA) (Fig. 1).

2.2. Treatments and experimental design

The experiments were arranged as a completely randomized block design with two replicates. Experimental treatments consisted on three modifications of the transmitted solar radiation achieved with the use of three different colored plastic films: a yellow film (named as YF), a green film (GF) and a blue film (BF); an uncovered treatment was included as control (Control). The colored films used in this study are actually made to be used for other purposes than the agricultural sector, but have been adapted to cover the basil plants through the support of a hand-made rigid and removable structure (1.5 m × 1.5 m) placed above the vegetation. The structures were covered from the top and sides to ~1 cm above the bottom of the pots to allow air circulation and secure proper temperature and humidity; light was not filtered from below. In particular, the colored films were purchased from a local store and provided by RiPlast S.r.l. (Pogliano Milanese, MI, Italy); yellow and blue films were 90 μM thick scratched polypropylene films, while green was a 80 μM thick polyvinyl chloride frosted film; thickness was measured by a thickness gauge (Vogel S.r.l., Leno, BS, Italy). The Fig. 2 shows a picture detail of the three films. The colored films were applied starting from transplanting and each treatment consisted of 98 pots, with 49 pots representing one experimental unit; pots were south-north (S-N) oriented.