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Species-specific differences in synthesis of flavonoids and phenolic acids under increasing periods of enhanced blue light

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ABSTRACT

Species-specific differences in synthesis of flavonoids and phenolic acids were studied under lengthening periods of enhanced blue light in a greenhouse experiment in Northern Finland in the autumn of 2012. The aim was to compare red- and blue- weighted light spectra in relation to biosynthesis of the compounds. The species studied were red leaf lettuce and basil. There were five treatments for these inter-lighting LED manipulations using traditional high pressure sodium lamps as background light sources. Two treatments were exposed for the entire experimental period with (1) red- and (2) blue-weighted light for 48 days. The other three treatments were initiated with red- weighted light, but after each subsequent 12 day sub-period, one red-weighted treatment was switched to blue, resulting in the following treatments: 48, 36, 24, 12 and 0 days under enhanced blue light. Flavonoid and phenolic acid biosynthesis in plants were found to be species dependent. The most abundant compound in red leaf lettuce was cichoric acid (a dicaffeoyltartaric acid) while rosmaric acid (an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid) dominated in basil. Other compounds detected also varied between the species. Red leaf lettuce was much more responsive to supplemental blue light. Based on these results, it is suggested that both blue and red light may be needed to regulate the accumulation of phenolics in basil. Some of the compounds detected accumulated continuously as a function of the spent time under supplemental blue light in red leaf lettuce, but not in basil.

1. Introduction

Optimal plant growth requires both blue and red ranges of the light spectrum, because the two absorption peaks of chlorophyll molecules occur at these wavelengths. Thus commercially available greenhouse lights, which make use of new LED techniques, build their spectrum accordingly. However, there are also other light-driven processes in plants. Photomorphogenesis modifies a plant's form and shape using specific parts of the light spectrum (Cashmore, 2006) and specific wavelengths may enhance production of certain phytochemicals in plants (e.g., Li and Kubota, 2009).

Precise allocation of limited resources to increase competitive ability and defense is critical for a plant's survival (e.g., Ballaré, 2014). Spectral light composition may provoke "trade-off" between optimal growth and defense systems. Enhanced blue light (400–500 nm), for example, may strongly increase the biosynthesis of phenolic compounds (Taulavuori et al., 2013) as well as epidermal flavonoids (Hoffmann et al., 2014). The essential oil content of basil plants grown under blue light has been found to be between 1 and 4 times higher than those grown without blue light (Amaki et al., 2011). Phenolic compounds are an essential part of a plant's defense system (e.g., Bennet and Wallsgrove, 1994). On the other hand, blue light is a factor limiting growth (Taulavuori et al., 2005; Sarala et al., 2007, 2011). Relatively high quanta of red light (around 660 nm), or a high red to far-red ratio, act principally in similar ways—suppressing growth and stimulating the defense system via jasmonic acid (JA) biosynthesis. Effects of blue and red light obviously share some mechanisms, since shady environments with low levels of these lights cause shade-avoidance syndrome through increasing activity of phytochrome-interacting factors (PIFs) (Ballaré, 2014, and references therein).

It is known that biosynthesis of certain phytochemicals is stimulated by exposure to a specific light spectrum, although the overall mechanism is not well understood. Most of the thousands of phytochemicals have been found to be genus- or species-specific (e.g., Julkunen-Tiitto, 1989; Crozier et al., 1997; Pichersky et al.,

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2006; Cheynier et al., 2013). The specific question here concerns temporal changes during the life span of a plant: how soon is the seedling responsive enough to produce phytochemicals under light manipulation? Basil, for example, is able to accumulate anthocyanins in its early stages (8 days) of development, and the concentrations are highest prior to flowering (Phippen and Simon, 1998). Juvenile leaves in high-light environments commonly appear red as a result of anthocyanin accumulation, produced due to their photo-protective role (Hughes et al., 2007). While many phytochemicals are accumulated after growth has finished, many of them are also accumulated in large amounts during the juvenile stages (e.g., Bar-Peled et al., 1993, and references therein; Julkunen-Tiitto et al., 1996; Laitinen et al., 2002).

Son and Oh (2013) proposed that a mixture of blue and red lights enhance quality and yield in lettuce. They tested several blue to red ratios, and found the best yield with a red-weighted spectrum, and highest production of total phenols and total flavonoids by a blue-shifted spectrum. The aim of our work was to experimentally compare the effects of blue- and red-weighted spectra on two species, and to examine responses in phytochemical production when extending the period for supplemental blue light. Hence, plants were exposed to a variable number of days of supplemental blue light. The species studied were edible herbs that exhibit different growth forms and are commonly used as food (i.e., red leaf lettuce and basil). One treatment received supplemental blue light throughout the experiment (48 days). In the other treatments, the plants received first red-weighted light for a differing number of days, because the red is thought to be optimal for growth under LED exposure (Son and Oh, 2013). After each 12 days period, one treatment was changed to receive supplemental blue light, i.e., after 12, 24 and 36 days. One treatment remained as a red-weighted control throughout the experiment. Thus the supplemental blue light periods were 0, 12, 24, 36 and 48 days, in order to investigate temporal changes in phytochemical accumulation. It was hypothesized that (1) the possible responses are species-specific, (2) and blue light rather than red is behind the phenolic acid and flavonoid biosynthesis. It was also hypothesized that (3) certain flavonoids accumulate as a function of time, (4) while the developmental stage of the plant may affect the response (juvenile vs. older plants).

2. Materials and methods

The experiment was performed in the greenhouse of the Botanical Gardens, University of Oulu, Northern Finland (65°N). The two species studied were red leaf lettuce (*Lactuca sativa var* Lollo Rossa) and basil (*Ocimum basilicum* Cv genovese gigante). Lettuce with a rosette-like growth form (*Lactuca* sp.) has a relative low-flavor, but is widely used as a basic component of garden salads. Basil (*Ocimum* sp.) in turn is a culinary herb with leafy, erect stems. The red leaf lettuce was chosen for the study instead of green leaf lettuce due to its natural capability to produce anthocyanins, which is indicative of significant flavonoid biosynthesis. Our preliminary comparison between red and green cultivars (Lollo Rossa vs. Iceberg, respectively) confirmed this (unpublished). There are also other studies indicating that red leaf lettuce is more productive in phenolic compounds (e.g., Neocleous et al., 2014). Basil was chosen for the studied species as it is also known to be rich in phenolic compounds (Lee, 2010, and references therein).

The seeds were sown in 9×9 cm pots (0.75 l), filled with commercially available fertilized peat substrate (Berner Greencare, NPK 12-6-14, conductivity 20 m Sm^{-2}), on the 18th October 2012. Germination was carried out at +20 °C/RH 80% with a 9 h daily dim light period. The pots (6 of each species) were inserted into 25×35 cm boxes. There were 5 treatments levels and each treatment consisted of 4 replicate boxes ($n = 4$).

The experiment began on 2nd November and continued for 48 days as in our previous experiment, during which the outdoor light conditions (photoperiod, light intensity) were minimal, and decreased markedly over the experimental time (Taulavuori et al., 2013). The growth conditions in the greenhouse were as follows: temperature +18 °C, RH 60% and light intensity 300 μmol m^{-2} s^{-1} (sodium Philips Master Son-T Pia Green Power 400 W, high pressure sodium lamps) with 16 h photoperiod. The plants were watered at 3 day intervals with tap water and at the same time each rectangle shaped plot below the lamp system was rotated clockwise to equalize any light scattering effects. Thus the full rotation of the plots continued for 12 days, which was chosen as the temporal treatment unit of the experiment. Since the experiment continued for the 48 days, there were 5 treatments—exposure of 0, 12, 24, 36 and 48 days to enhanced blue light.

The specific light spectra were given by 120 W LED unit interlighting systems placed between traditional high pressure sodium (HPS) lamps (400 W) and plants, serving thus as supplemental light sources modifying the light spectrum. The LED units were around 60 cm above the plants and the HPS lamps were approximately 150 cm above the plants. Commercially available LED unit systems were used (Led Finland) to generate red-weighted growth light for optimal growth conditions (e.g., Son and Oh, 2013). The LED composition included 720 nm (1.8%), 660 nm (50%), 630 nm (30%), 450 nm (11%), 430 nm (3.6%), 410 nm (0.9%) and 3500 K white (2.7%). The specific, enhanced blue light spectrum boosted the range at 400–500 nm, which is described in detail elsewhere (Taulavuori et al., 2013), and was given as supplemental light to the HPS, in the same way as the red-weighted light. The spectra of both systems are shown in Fig. 1. At the beginning of the experiment, four treatments consisting of four replicate boxes ($n = 4$) received the red-weighted light (RW), and the fifth treatment received the enhanced blue light (EB). After each 12 days temporal unit, there was a switch in one treatment plot from red to blue: the RW LED system was replaced with the EB. Therefore, the experiment consisted of the following five treatments after 48 experimental days: 48, 36, 24, 12 and 0 days under enhanced blue light (see Fig 2. for the experimental design).

The experiment was terminated on the 20th December. Chlorophyll fluorescence ratio (Fv/FM) was first measured with a portable chlorophyll fluorometer (Walz PAM 2000) from 4 randomly chosen dark-adapted individuals of both species, per replicate box (Taulavuori et al., 2011). The 4 measurements were averaged for the value of one replicate in each treatment ($n = 4$). The shoot length of each basil plant was also measured with a ruler

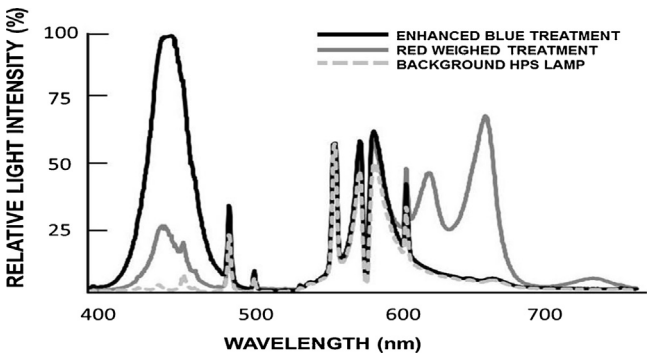

Fig. 1. Spectral compositions in EB (enhanced blue) and RW (red weighted) treatments, and background HPS (high pressure sodium) lamp (1 m above treatment lamps).