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THE GROWTH, PHOTOSYNTHETIC PARAMETERS AND NITROGEN STATUS OF BASIL, CORIANDER AND OREGANO GROWN UNDER DIFFERENT LED LIGHT SPECTRA

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ABSTRACT

Growth, morphological parameters, photosynthetic performance and nitrogen status were investigated in leafy herbs grown in light-limited time in a greenhouse under different light spectra emitted by LEDs. Fluorescence-based sensors that detect crop N status and maximum photochemical efficiency of photosystem II were used in this study. Four light treatments with the ratio of Red, Blue and White LEDs including 1) R40 + B50 + W10, 2) R70 + B20 + W10, 3) R70 + B20 + W10 + Far-Red and 4) White LEDs as control were used in this study. Dominant red light and/or white LED lights at 200 µmol m⁻² s⁻¹ at plant level and a 12 h photoperiod provided the most favourable conditions for plant growth and development compared to a high proportion of blue light (R40 + B50 + W10). However, plants grown under a high proportion of blue light had a higher chlorophyll index and nitrogen balance index (NBI) than under dominant red light treatments. Our study indicates the significant potential of fluorescence-based sensors in photobiology research as well as in the production of leafy herbs under LED lights.

Key words: chlorophyll fluorescence, herbs, greenhouse production, light, optical sensors

INTRODUCTION

Light is an essential source of energy for photosynthesis and an important environmental stimulus regulating plant growth and development. Growing systems of plants during the light-limited winter months require the application of additional light sources to improve photosynthesis and ensure plant growth and yield. Light-emitting diode (LED) lamps has attracted increasing interest as a primary source of irradiation or as supplemental lighting for plants due to high photosynthetically active radiation (PAR) efficiency, low energy consumption and long life. LED lamps can support the growth, development and quality of many crops cultivated in greenhouses [Morrow 2008, Olle and Viršile 2013, Stutte 2015, Bantis et al. 2018]. An exceptional property of LED lamps is possibility

Chlorophyll molecules absorb energy most strongly in the blue and red range, causing a molecular chain reaction known as the light-dependent reaction of photosynthesis [Wang et al. 2016, Yang et al. 2017]. Therefore, different blue and red LED light treatments

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of setting a desirable spectral composition for the targeted manipulation of metabolic responses in order to optimise photosynthesis performance and increase the amount secondary metabolites such as flavonoids to enhance food quality [Hasan et al. 2017]. Spectral composition management of LED lamps requires that photosynthetic efficiency and biomass accumulation are balanced with the mineral supply, especially nitrogen (N), since the leaf chlorophyll content, which is vital for photosynthesis, is strongly affected by leaf N.

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have been most often used to analyse photosynthetic performance of different plant species. Previous studies indicate that red LEDs generally stimulate biomass accumulation, stem elongation and increase leaf area [Johkan et al. 2010], while blue LEDs are more involved in chlorophyll production, the opening of stomata and photosynthesis [Muneer et al. 2014, Wang et al. 2015, Miao et al. 2016]. It is also known that far-red (>700 nm) used together with red light (680 nm) drives both photosystem I (PS I) and photosystem II (PS II), the so-called Emerson effect, and enhances the rate of photosynthesis [Lysenko et al. 2014] and biomass accumulation [Li and Kubota 2009]. Previous research also indicates that plants grown under multi-wavelength irradiation or white light show higher photosynthetic activity than those grown under monochromatic light [Kim et al. 2004, Yang et al. 2017].

Optical-based sensors are promising tools for the non-destructive monitoring of plant responses to different spectral compositions of LED lamps. Chlorophyll a fluorescence is a non-invasive measurement of PS II activity and photosynthetic performance and is a commonly used technique in plant physiology. Research conducted by Ahlman et al. [2017] indicates the suitability this method to optimise the LED light spectrum for plants. The recently developed Force-A Dualex Scientific + optical sensor provides indices of chlorophyll and flavonol contents and also calculate the nitrogen balance index (NBI) from these measurements. This device generates fluorescence in plant tissue for the estimation chlorophyll and flavonols and has been used for monitoring the N status of some crops. Studies by Tremblay et al. [2012], Padilla et al. [2015], Diago et al. [2016] and Agati et al. [2016] confirmed that the fluorescence indices of chlorophyll, flavonols and NBI are reliable indicators of crop N status in leaves of tomato, grapevine, white head cabbage and young apple trees.

Basil, coriander and oregano are culinary and medicinal herbs rich in antioxidants and nutrients. There is an increasing demand for fresh herbs, including during the winter, due to increasing interest in a healthy lifestyle and the consumption of healthier food. However, studies on the effect of LED lighting on photosynthesis and mineral nutrition in these plants in greenhouses are very limited. Supplementary lighting is used to increase plant growth and reduce production time during period with low levels of solar radiation. Recommended daily light integral (DLI) for commercial vegetables and herbs production range between 12 and 17 mol m⁻² d⁻¹ [Kozai et al. 2015]. There are no data that clearly indicate the optimal LED light spectrum for photosynthesis, biomass accumulation and, at the same time, crop nitrogen N status in these plants.

The aim of this study was to investigate the growth parameters, photosynthetic performance and nitrogen status of leafy herbs grown in a greenhouse under different light spectra emitted by LEDs. Fluorescence-based sensors that detect maximum photochemical efficiency of PS II and crop N status were used in this study. The results provide a practical basis for regulating the LED light spectrum for high-quality leafy herb production in greenhouses under low-level natural light conditions.

MATERIAL AND METHODS

Plant material and light treatments. Basil (Ocimum basilicum L.) 'Sweet Genovese', coriander (Coriandrum sativum L.) and oregano (Origanum vulgare L.) were used in the study. Seeds of the herbs were sown on 18 October 2018 in plastic 0.4 L single pots filed with a mixture of peat and perlite (3: 1, v/v, v)pH 6.0) and were set on ebb-and-flow benches. Ten days after sowing, excessive seedlings were removed, leaving four seedlings per pot. Plants were watered as needed to maintain adequate soil moisture. Fertigation was carried out twice a week using a nutrient solution containing minerals in the following concentrations (in mg L⁻¹): N-NO₃ 220, P 45, K 265, Ca 132, Mg 49, and microelements. The temperature during the day/night was 20/18°C and the relative humidity was 60–65%. The average daily light integral (DLI) inside the greenhouse during the experimental period averaged 4.6 mol m⁻² d⁻¹. The experiment was carried out during the period from mid-October 2018 to January 2019 and was terminated when the commercial value of the plants was reached. Coriander, basil and oregano were evaluated 9, 11 and 12 weeks after seed sowing, respectively.

The study was carried out in a greenhouse chamber $(5 \times 4 \text{ m})$ equipped with purpose-build LED arrays containing OSRAM diodes (Germany). Three

LED light treatments with different colour mixing was achieved using blue (450 nm), red (660 nm), far-red (730 nm) and white LEDs (cool-white 5000 K), such as 1) red + blue + white (40% + 50% + 10%), percentage share of R : B : W LEDs) as a high proportion of blue light treatment (treatment code R40 : B50 : W10), 2) red + blue + white (70% + 20% + 10%) as dominant red light treatment (treatment code R70 : B20 : W10) and 3) red + blue + white (70% + 20% +10%) + far-red (number of far-red diodes such as red ones) as dominant red light with FR treatment (treatment code R70 : B20 : W10R + FR). A lighting unit equipped only with white LEDs was used as the control treatment (W100). The relative spectral emission from white LEDs was not a homogenous mix of wavelengths; rather large peaks occurred in the blue region (peak at 440 nm, the direct contribution of the LED), with a further hump in the yellow/red region produced by the phosphor (peak at 565 nm). The combination of these wavelengths was a good approximation of white. Plants were grown under different spectra of LED lighting and the same photosynthetic photon flux density PPFD (200 µmol m⁻² s⁻¹) at plant level for 12 h per day, so that DLI inside the greenhouse was increased by 8.6 mol m⁻² d⁻¹ and in total with natural light amounted to 13.2 mol m⁻² d⁻¹. The light intensity was measured using a LI-COR quantum photometer Model LI-189 (USA).

Growth parameters. At harvest time, 20 plants were randomly selected for growth and morphological assessment within each treatment. Shoot fresh weight, plant height, length of internodes and total leaf area per plant were evaluated. Leaf area was measured using portable AM350 leaf area meter (ADC BioScientific).

Chlorophyll, flavonol and nitrogen balance indexes. An optical sensor was used in this study for the assessment of chlorophyll and flavonol compounds based on the measurement of UV absorbance of the leaf epidermis by the double excitation of chlorophyll fluorescence (The Force-A Dualex Scientific + instrument). The nitrogen balance index (NBI) was automatically calculated as the ratio of the chlorophyll index (CCI) to flavonol index (FLAV), i.e. NBI = CCI/FLAV. The device used in this study allows for non-destructive measurements of chlorophyll, flavonol contents and nitrogen balance in leaves, which makes it particularly suitable for photophysiological research [Cerovic et al. 2012]. For each light treatment, 20 young, fully expanded leaves were used for the assessment of the flavonol and chlorophyll indices.

Chlorophyll fluorescence parameters. Chlorophyll fluorescence, as an indicator of photosynthetic reactions, was analysed using a Mini PAM (Walz, Germany). For each light treatment, chlorophyll a fluorescence emission from the upper leaf surface of 20 intact, dark-adapted leaves (30 min) was measured. After dark adaptation, the fluorescence variables F_0 , F_{M} and F_{V}/F_{M} were determined. The minimal (F₀) fluorescence was recorded with modulated low intensity light below 0.1 μ mol m⁻² s⁻¹ without affecting the variable fluorescence. The maximal (F_M) fluorescence was estimated by a 0.8 s long saturation light pulse (2600 μ mol m⁻² s⁻¹) with 20,000 Hz frequency. The variable fluorescence (Fv) was calculated by the equation: $Fv = F_M - F_0$. The F_V / F_M ratio was obtained from the F_v and F_M and represent potential maximal photochemical efficiency of PS II. A 10 min exposure to actinic light (665 nm, 200 μ mol m⁻² s⁻¹) was used to measure steady-state fluorescence.

Statistical analysis. Statistical analysis was performed using STATISTICA software, version 13.1 (StatSoft Inc., USA). Data were analysed using oneway analysis of variance (ANOVA) and the treatment means were compared using Tukey's HSD test at $\alpha = 0.05$.

RESULTS

Plant growth and morphology. Shoot fresh weight, plant height, mean internode length and total leaf area of basil, coriander and oregano were strongly dependent on the spectrum of light emitted by LEDs; however, individual plant species responded differently to various LED light treatments (Fig. 1). The shoot fresh weight of basil was the greatest under white LEDs (W100) – control treatment (2.2 g), and was 11% lower under both dominant red light treatments (R70 : B20 : W10 and R70 : B20 : W10 + FR) and 23% lower under a high proportion of blue light treatment (R40 : B50 : W10) compared to white LEDs. For coriander, the greatest shoot fresh weight was recorded when red light was prevailing (2.2 g average for R70 : B20 : W10 and R70 : B20 : W10 + R). Under white LEDs





Fig. 1. Plant fresh weight (A), plant height (B), mean internode length (C) and total leaf area per plant (D) of basil, coriander and oregano grown under the four different light spectra treatments. Significant differences are marked by different letters at $\alpha = 0.05$ (Tukey's HSD test)

and a high proportion of blue light, the fresh weights of coriander were lower by 16% and 40% respectively, compared to both red light treatments. The shoot fresh weight of oregano was less dependent on the spectral distribution of LED lights; however, the fresh weight of plants exposed to a high proportion of blue light was 10% lower compared to other light treatments. Under both dominant red light regimes, coriander and oregano plants were the tallest (24 and 25 cm, respectively), followed by these under white LEDs and a high proportion of blue light treatments. Coriander and oregano grown under white LEDs were 20% and 26% smaller, and under a high proportion of blue light 44% and 47% smaller, respectively, as compared with both dominant red lights. For basil, there were no significant differences in plant height between white LEDs and the both red light treatments, but plants grown under a high proportion of blue light were 10% smaller than under the other light treatments. The increase in stem length and plants height of basil, coriander and oregano were generally dependent on internode elongation. Basil under white LEDs possessed the greatest total leaf area (46 cm), followed by these under both dominant red and a high proportion of blue light with a significant reduction 12% and 29%, respectively, compared with the values under white LEDs. Coriander and oregano grown under a high proportion of blue light had the lowest total leaf area, i.e. 19% and 17% lower, respectively, compared to the other treatments. There were no significant differences in the leaf area



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Fig. 2. Chlorophyll index (A), nitrogen balance index NBI (B), flavonol index (C), and the maximum quantum yield of PS II photochemistry FV/FM (D), minimal fluorescence F0 (E) and maximal fluorescence FM (F) of basil, coriander and oregano grown under the four different light spectra treatments. Significant differences are marked by different letters at $\alpha = 0.05$ (Tukey's HSD test)

of coriander and oregano between white LEDs and both dominant red light treatments.

Chlorophyll, flavonol and nitrogen balance indexes. Leaf chlorophyll index (CCI) and nitrogen balance index (NBI) in plants were closely related (Fig. 2 A, B, C). Average, white LEDs and a high proportion of blue band in light spectrum (R40 : B50 : W10) enhanced CCI and NBI compared to both dominant red lights (R70: B20: W10 and R70: B20: W10R + FR). CCI in basil grown under white LEDs and under a high proportion of blue light treatment was higher by 22% and NBI was higher by 17% than under both red light spectra. CCI in coriander exposed to white LEDs and a high proportion of blue band in light spectrum was higher as much as by 45% than under both red light spectra, and NBI under white LEDs and R40 : B50 : W10 was higher by 44% than under R70 : B20 : W10R + FR treatment. For oregano, the highest CCI as well as NBI were recorded under R40 : B50 : W10 treatment and the lowest under R70 : B20 : W10R + FR treatment. Differences in CCI and NBI between these treatments were 20% and 24%, respectively. Basil and coriander grown under a high proportion of blue band in light spectrum (R40 : B50 : W10) showed the maximum flavonol indices (1.8 and 0.9, respectively), which were 9% higher for basil and 20% higher for coriander compared to dominant red light treatment. There was no significant difference in flavonol index for oregano plants grown under different light spectra.

Chlorophyll fluorescence parameters. The maximum photochemical efficiency of PS II (F_v/F_m) evaluated at harvest time was significantly affected by the light spectra (Fig. 2 D, E, F). Under a high proportion of blue band in light spectrum, basil plants showed the greatest F_v/F_M (0.77), significantly lower under dominant red light with far-red (0.74) and dominant red light (0.70), which were reduced by 4% and 10% respectively, as compared with a high proportion of blue light. There were no significant differences in F_v/F_M between the white LEDs and a high proportion of blue light treatments in basil. Coriander grown under white LEDs and a high proportion of blue light showed the highest F_v/F_M (0.77) and the lowest under both red spectra (0.72), which was 7% lower compared to the white LEDs and a high proportion of blue light treatments. Oregano exposed to dominant red light showed

the highest $F_V/F_M(0.77)$ and the lowest under dominant red light with far-red (0.73), which was 5% lower than under dominant red light. Plants grown under white LEDs and a high proportion of blue light showed intermediate F_v/F_w values between dominant red and dominant red with far-red. A high proportion of blue light for basil and a high proportion of blue light as well as dominant red for oregano significantly contributed to the reduction of minimal fluorescence (F_{0}) compared to white LEDs treatment. For coriander, the opposite response was noted, where plants exposed to both red light spectra showed significantly higher F_o than under white LEDs as well as a high proportion of blue light. None of the spectral compositions increased the maximum fluorescence intensity (F_M) compared to LED White. Moreover, F_{M} values for basil exposed to a high proportion of blue light and both red light treatments were significantly lower than under white LEDs. For oregano grown under dominant red light with far-red, F_M values were significantly lower than under white LEDs.

DISCUSSION

Dominant red and/or white LED lights at 200 μ mol m⁻² s⁻¹ PPFD at plant level and 12 h photoperiod created more favourable conditions for plant growth and development than the high proportion of blue LED light; however slight differences were observed between plant species. Basil grown under white LED light had greater shoot fresh weight and total leaf area than under dominant red LED lights (irrespective of the share of FR), while coriander plants exposed to both dominant red LED lights had higher biomass and plants were taller than those grown under white LED light. For oregano, biomass production was similar under white LED light and dominant red LED lights, plants were significantly taller than under white light.

Many studies have indicated that the right proportion of red and blue light with predominant red light increased the yield of lamb's lettuce, lettuce, spinach and radish compared to red light alone [Yorio et al. 2001, Wojciechowska et al. 2015, Wang et al. 2016]. Red LED light at 200 μ mol m⁻² s⁻¹ for a 16 h photoperiod enhanced the yield of lamb's lettuce much more than solely blue light; however, the most effective treatment for biomass production was a mixture red and blue light, with the share of blue light not exceeding 30% [Wojciechowska et al. 2015]. The weight of coriander Coriandrum sativum increased when the share of blue light increased from 5% to 9% at 120 µmol m⁻² s⁻¹ and a 16 h photoperiod, and decreased when the share of blue light exceeded 17%, and was also the lowest under solely red light [Naznin et al. 2016]. For sweet basil Ocimum basilicum, a red to blue ratio of 0.7 was the most suitable for growth and plant development [Piovene et al. 2015]. For 'Caesar' sweet basil grown in controlled environment conditions, red and blue light (1:1) generated the same weight as red and blue, and the addition of another colour such as green or yellow increased plant weight compared to red/blue (1 : 1) treatment [Carvalho et al. 2016].

The light spectrum with a high proportion of blue band (R40 : B50 : W10) was the least effective for plant growth and biomass production and, under these conditions, the fresh weight of aboveground parts of basil, coriander and oregano were respectively 23%, 28% and 18% lower compared to white LED lighting. However, plants grown under a high proportion of blue light had higher chlorophyll and nitrogen balance index values than under dominant red lights. Chlorophyll content is an important indicator of plant nitrogen status and can be altered in response to the light environment. Long-term exposure of plants to blue light enhances 5-aminolevulinic acid synthesising activity [Kamiya et al. 1984], which is a key precursor in the chlorophyll biosynthesis pathway. The chlorophyll contents in plants increased under blue light [Ouzounis et al. 2015] and decreased under red light [Wang et al. 2016]; however, the opposite trends have also been found [Yang et al. 2017]. In the study, the lower NBI values for basil, coriander and oregano under dominant red LED light compared to a high proportion of blue LED light indicated a nitrogen deficient state in which the synthesis of organic compounds containing nitrogen, such as protein or chlorophyll, was reduced, and the synthesis of secondary flavonoid-type carbon metabolites increased [Cartelat et al. 2005, Cerovic et al. 2012, Tremblay et al. 2012, Padilla et al. 2015, Dong et al. 2020].

Flavonoids, including flavonols, are biologically strong natural antioxidants with high potential health benefits. Light exposure leads to broad changes in the

flavonoid biosynthesis pathway. Blue light promotes the expression of flavonoid pathway genes and the accumulation of flavonoids in plants. In our study, basil and coriander grown under a high proportion of blue LED light had significantly higher flavonol indices than under dominant red light, but this effect was not found in oregano plants. Our study reveals that the LED light spectrum used for lighting plants produced in a greenhouse in light-limited time can result in flavonol biosynthesis [Bantis et al. 2016, Dou et al. 2017] and blue light is more effective than red light; however, this effect is species-dependent [Matysiak and Kowalski 2019]. In recent years, attempts have been made to use the chlorophyll fluorescence technique for assessing plant photosynthetic performance under different light qualities [Ouzounis et al. 2015, Wang et al. 2016]. The F_V/F_M ratio estimates the maximum photochemical efficiency of PS II and is used as a stress indicator when plants are exposed to unfavourable conditions. The $\mathrm{Fv}/\mathrm{F}_{\mathrm{M}}$ of ~0.8 is typical for well-functioning apparatus, however this value depends on the plant species and environmental conditions [Hogewoning et al. 2012]. In our study, the maximum photochemical efficiency of PS II was in the range of 0.70-0.77 for basil 'Sweet Genovese', coriander and oregano. Walters and Currey [2019] showed that F_v/F_M for *O. basilicum* 'Nufar' and O. citriodorum 'Lime' increased from 0.5 to 0.84 as air temperature increased from 10.9 to 23.1°C whereas F_v/F_M for O. basilicum 'Sweet Dani' and O. tenuiflorum increased from 0.61 to 0.81, as temperature increased from 10.9 to 16.8°C. The research allowed to quantified nonstressed F_v/F_m values for basil near 0.8 when temperature were between 17-23 to 35°C depending on the species. Comparing these results with our achievements, it can be supposed that the basil 'Sweet Genovese' is a cold-sensitive plant, and the temperature in a greenhouse during winter time (20°C during the day) was slightly too low resulted in F_v/F_M values below 0.8. For Origanum vulgare, seasonal and altitudinal variations PS II photochemical efficiency was in the range 0.65-0.80 [Kofidis 2003]. In contrast, Murillo-Amador et al. [2015] showed that F_v/F_M was not affected by the environment treatments and this variable showed an average of 0.75 with the shade-enclosure and 0.76 with openfield, respectively.

In our study, the F_V/F_M ratio was overall higher in plants with a higher chlorophyll index and NBI, which indicates a permanent downregulation of PS II in plants with low chlorophyll contents and nitrogen deficiency status [Tremblay et al. 2012, Dong et al. 2020]. The highest values F_v/F_M for basil and coriander were observed under white (0.75 and 0.77) and a high proportion of blue light (both 0.77), which corresponded with the higher chlorophyll index and NBI values. For oregano, smaller differences were noted in F_V/F_M between light spectra; however, the lowest value of 0.73 was noted for plants grown under dominant red and far-red light; plants grown with these treatments had the lowest chlorophyll index and NBI. Muneer et al. [2014] showed that a lower rate of photosynthesis in red LEDs could be attributed to a low nitrogen content in lettuce leaves, due to the low chlorophyll content. Our results indicate that the reduction in F_v/F_m was partly associated with increased minimal fluorescence (F_0) , particularly noticeable in coriander grown under dominant red light. These plants had significantly lower chlorophyll indices compared to plants grown under white and a high proportion of blue lights. The minimal fluorescence F₀ comes from excited chlorophylls of the light harvesting antenna in PS II before excitation reaches the reaction centres. An increase in F_0 can be attributed to the physical separation of PS II from the associated pigment antenna, resulting in blocked electron transfer on the reductant side of PS II. On the other hand, a very low F_0 and F_M values were recorded under a high proportion of blue light in the three species indicate a low fluorescence signal, which usually means lower chlorophyll content. However, chlorophyll indexes indicate the highest chlorophyll content in this light conditions. This can be explained by the alteration of photosystem stoichiometry (PS I/ PS II ratio) upon changes in light quality. Zheng and Van Labeke [2017] and Landi et al. [2020] showed that the changes in light spectra may significantly alter the distribution of the light energy between two photosystems, modifying contribution of PS I fluorescence.

CONCLUSIONS

Based on our study, we can conclude that a spectral composition containing at least 70% red light and/or white LED light is the most favourable for biomass

production in basil, coriander and oregano grown in a greenhouse in light-limited winter time. However, a high share of blue light (40%) stimulates chlorophyll biosynthesis, improves NBI and enhances the maximum photochemical efficiency of PS II. Our study also indicated the significant potential of fluorescence-based sensors in photobiology research as well as in the production of leafy herbs under LED lights. Combined assessments of NBI and the maximum photochemical efficiency of PS II may provide valuable insights into the quality of leafy herbs and form a practical basis for regulating the LED light spectrum for high-quality leafy herb production in greenhouses.

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