

# Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success



Filippos Bantis<sup>a</sup>, Theoharis Ouzounis<sup>b</sup>, Kalliopi Radoglou<sup>a,\*</sup>

<sup>a</sup> Department of Forestry and Management of the Environment and Natural Resources Democritus University of Thrace (DUTH), Pantazidou 193, 68200, Nea Orestiada, Greece

<sup>b</sup> Horticulture and Product Physiology Group, Department of Plant Sciences, Wageningen University, PO Box 630, 6700 AP Wageningen, The Netherlands

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## ABSTRACT

The morphological and phytochemical characteristics of two *Ocimum basilicum* cultivars (Lettuce Leaf, and Red Rubin–mountain Athos hybrid) under artificial lighting were investigated. Four LED light treatments [AP673L (high red and high red:far-red), G2 (high red and low red:far-red), AP67 (moderate blue and red and low red:far-red), and NS1 (high blue and green, high red:far-red and 1% ultraviolet)] with different colors mixing UV, blue, green, red and far-red, and fluorescent tubes (FL, high blue, green and red:far-red) as Control were used in the growth chambers for 28 days under PPFD of  $200 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$  for all treatments at plant height. G2, Control and AP67 treatments for Lettuce Leaf, and G2 for Red Rubin hybrid had higher growth rate. Roots of Lettuce Leaf were significantly longer under AP673L compared to NS1, while Red Rubin hybrid showed no significant differences. Total biomass was significantly greater under NS1, AP67 and G2 compared to the Control, for both cultivars. For both Lettuce Leaf and Red Rubin hybrid, root:shoot ratio (R/S) was favored under NS1, whereas the Control had the lowest impact. Leaf area of both cultivars was greater under the Control. Root growth capacity evaluation was also assessed. Seedlings of Lettuce Leaf cultivated under the effect of the Control and AP673L, and seedlings of Red Rubin hybrid grown under AP673L (mainly) quickly developed new root system. This could offer the advantage of fast exploitation of larger soil volume after transplanting. Total phenolic content of Lettuce Leaf was significantly higher under NS1 compared to the rest of the treatments, while in Red Rubin hybrid, NS1 had significantly higher total phenolic content compared to the Control and G2. Our study demonstrates that LEDs variably affected growth characteristics and increased total phenolic content compared to conventional fluorescent light for these two *O. basilicum* cultivars.

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## 1. Introduction

Until today fluorescent, metal-halide and incandescent lamps are used as supplementary lighting for crop production. Even though these sources induce an increase of daily photosynthetic flux levels, they are not as energetically efficient as desired. Spectral quality can affect plant growth and development (Schuerger et al., 1997) and the aforementioned light sources do not offer the

option of spectral manipulation. Therefore, a light source with high energy-conversion efficiency that offers the possibility of spectral setting would be of great value. Research has already been conducted using light-emitting diodes (LEDs) as a primary source of light irradiation for plant growth chambers in space programs (Barta et al., 1992). LED light technology presents a number of advantages including long life, small volume, low heat emission, adjustable light intensity, high energy-conversion efficiency and wavelength specificity (Massa et al., 2008; Morrow 2008; Schuerger et al., 1997). Therefore, several plant species such as sweet basil and lemon balm (Fraszczak et al., 2014), pepper (Schuerger et al., 1997), pea (Wu et al., 2007), cucumber and tomato transplants (Brazaityte et al., 2010, 2009), lettuce (Kim et al., 2004; Li and Kubota 2009; Lin et al., 2013; Ouzounis et al., 2015) and rapeseed (Li et al., 2013), as well as ornamental plants such as roses, campanulas, chrysanthemums and *Phalaenopsis* (Ouzounis et al., 2014a,b)

**Abbreviations:** DWL, dry weight of leaves; DWS, dry weight of shoots; DWR, dry weight of roots; LED, light-emitting diode; NDWR, dry weight of new roots; NRL, new root length; RGC, root growth capacity; R/S, root:shoot ratio; TPC, total phenolic content.

\* Corresponding author. Fax: +30 2310929806.

E-mail addresses: [radoglou@fri.gr](mailto:radoglou@fri.gr), [kradoglo@fmenr.duth.gr](mailto:kradoglo@fmenr.duth.gr) (K. Radoglou).

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**Table 1**  
Spectral distribution and red:far-red (R:FR) ratio for the five light treatments.

Light treatments	<400 nm	400–500 nm	500–600 nm	600–700 nm	700–800 nm	R:FR
Control (FL)	0%	35%	24%	37%	4%	5.74
AP673L	0%	12%	19%	61%	8%	5.56
G2	0%	8%	2%	65%	25%	2.51
AP67	0%	14%	16%	53%	17%	2.77
NS1	1%	20%	39%	35%	5%	8.16

have already been tested under LED light sources with promising results, although varying among the different species.

Basil (*Ocimum basilicum* L.) is an annual culinary and medicinal herb originating from India and Southeast Asia. Apart from being cultivated in Asia, Africa and South America it is also cultivated in the Mediterranean countries (Makri and Kintzios, 2008). It is also cultivated as an ornamental plant and its leaves are used as a seasonal food in dry and fresh form (Lee et al., 2005). Basil is rich in phenolic compounds such as rosmarinic, caffeic, chicoric and tartaric acids (Kwee and Niemeyer, 2011; Lee and Scagel, 2009; Zgora and Glowniak, 2001). One of the most important cultivars is “Lettuce Leaf” (*O. basilicum* var. *crispum*). Its large crinkled leaves have a sweet sense which makes it ideal for salad and cooking use. “Red Rubin” basil (*O. basilicum* var. *purpurascens*) is a purple-leaf cultivar with strong flavor. Its leaves are edible and are also used in cooking. Although basil is an important culinary and medicinal herb only a few studies have been conducted using LEDs as a lighting source.

Little information is available regarding the relationship between the light absorbed by basil and the mechanisms underlying the physiology and secondary metabolism under the influence of different light spectra. Secondary metabolites are formed in order for the plant to overcome potential stressful conditions. In plant tissues such as stems and leaves, the secondary metabolite synthesis can change due to environmental, physiological, biochemical and genetic factors (Wink, 2010; Zhao et al., 2005) with light being one of the most influential factors (Kopsell et al., 2004; Kopsell and Sams, 2013). One of the main groups of secondary metabolites is the phenolic group. This group is among the most ubiquitous groups of secondary metabolites in the plant kingdom and represents an example of metabolic plasticity enabling plants to adapt to biotic and abiotic environmental changes (Wink, 2010). Their concentration depends on season and varies at different stages of growth and development (Seigler, 1998; Wink, 2010). Phenolics are pigments exhibiting radical scavenging activity, as well as protective activity against fungi, bacteria, viruses and insects (Lattanzio et al., 2006; Seigler, 1998). Physiological changes are triggered by exposure to varying wavelengths (Samuolienė et al., 2013). Red light is the primary light source affecting biomass production and elongation through the phytochrome photoreceptor (Sager and McFarlane, 1997). Blue light also affects photomorphogenic responses (e.g., regulation of leaf flattening and compact appearance) through phototropins and cryptochromes acting in an

independent and/or synergistic manner with the phytochromes (de Carbonnel et al., 2010; Kozuka et al., 2013).

The research hypothesis was that pre-cultivating basil seedlings under the effect of lights with varying irradiation wavelengths would differently affect morphological and phytochemical properties. In addition, different LED spectra would trigger various morphological and phytochemical reactions among different basil cultivars. The objective of this research was (1) to investigate the effect of LED lighting on morphological and phytochemical characteristics of two basil cultivars during pre-cultivation in mini-plug containers, (2) to determine the best light quality treatment for pre-cultivation of two basil cultivars, and (3) to evaluate the transplant success.

## 2. Materials and methods

### 2.1. Plant material, growth conditions and light treatments

Seeds of “Lettuce Leaf” basil (LL) and “Red Rubin” basil cultivars were supplied from a nursery (Geniki Fytotechniki of Athens, Athens, Greece) in 2012. Additionally, basil seeds were collected from mountain Athos, Greece in 2012. The “Red Rubin” and “mountain Athos” cultivars were hybridized (RR). The experiment was held in November and December 2013. In total, 100 basil seeds per cultivar and treatment were hydrated for 24 h and two seeds were transferred into each cell of the mini-plug containers. After three days the germination rate of the “LL” cultivar was 85%, whereas the “RR” had a 55% germination rate. One week after the sowing, excessive seedlings were removed leaving one seedling per mini-plug cell (50 per cultivar and treatment). The mini-plug plastic container trays (QP D 104 VW QuickPot<sup>®</sup>, Herkplast-Kubern, Germany) with identical dimensions (310 × 530 mm, density: 630 seedlings/m<sup>2</sup>; 27 cc) were filled with a stabilizer peat (Preforma PP01, Jiffy<sup>®</sup> Products, Norway) containing a binding agent in order to facilitate the process of transplanting.

We examined the application of conventional fluorescent lighting (FL) or LED lighting for the first four weeks after sowing. In total, 50 seedlings per cultivar and treatment were used. After sowing, basil seedlings were transferred to environmentally controlled growth chambers for 28 days under five different light treatments as described in Table 1. The white fluorescent lamps (Osram, Fluora, Munich, Germany) were used as the Control treatment. Valoya (Valoya Oy, Helsinki, Finland) LED lights generate a wide

**Table 2**  
Morphological and developmental parameters of *Ocimum basilicum* “LL” and *Ocimum basilicum* “RR” grown under the five different light treatments with the same abbreviations as in Table 1. Average values ( $n = 10$ ,  $\pm$ SE) followed by different letters within a row differ significantly ( $\alpha = 0.05$ ).

Cultivars	Parameters	Light treatments				
		Control (FL)	AP673L	G2	AP67	NS1
<i>Ocimum basilicum</i> “LL”	Shoot height (cm)	3.22 ± 0.19 <sup>a</sup>	2.34 ± 0.26 <sup>b</sup>	3.52 ± 0.16 <sup>a</sup>	3.42 ± 0.33 <sup>a</sup>	2.43 ± 0.13 <sup>b</sup>
	Root length (cm)	4.03 ± 0.12 <sup>ab</sup>	5.69 ± 0.67 <sup>a</sup>	4.50 ± 0.26 <sup>ab</sup>	4.52 ± 0.36 <sup>ab</sup>	3.73 ± 0.34 <sup>b</sup>
	Root/Shoot ratio	0.09 ± 0.01 <sup>c</sup>	0.29 ± 0.02 <sup>b</sup>	0.26 ± 0.03 <sup>bc</sup>	0.35 ± 0.09 <sup>b</sup>	0.57 ± 0.04 <sup>a</sup>
	Leaf area (cm <sup>2</sup> )	10.02 ± 0.39 <sup>a</sup>	6.56 ± 0.54 <sup>b</sup>	6.56 ± 0.38 <sup>b</sup>	6.91 ± 0.64 <sup>b</sup>	5.70 ± 0.38 <sup>b</sup>
<i>Ocimum basilicum</i> “RR”	Shoot height (cm)	4.09 ± 0.25 <sup>bc</sup>	3.50 ± 0.28 <sup>c</sup>	5.46 ± 0.22 <sup>a</sup>	5.08 ± 0.28 <sup>ab</sup>	3.93 ± 0.30 <sup>bc</sup>
	Root length (cm)	4.59 ± 0.44 <sup>a</sup>	6.23 ± 0.42 <sup>a</sup>	4.42 ± 0.37 <sup>a</sup>	4.94 ± 0.36 <sup>a</sup>	6.53 ± 0.78 <sup>a</sup>
	Root/Shoot ratio	0.16 ± 0.03 <sup>c</sup>	0.49 ± 0.11 <sup>ab</sup>	0.34 ± 0.03 <sup>bc</sup>	0.46 ± 0.04 <sup>ab</sup>	0.61 ± 0.04 <sup>a</sup>
	Leaf area (cm <sup>2</sup> )	8.25 ± 0.80 <sup>a</sup>	5.59 ± 0.36 <sup>b</sup>	6.70 ± 0.44 <sup>ab</sup>	6.05 ± 0.29 <sup>b</sup>	7.05 ± 0.39 <sup>ab</sup>

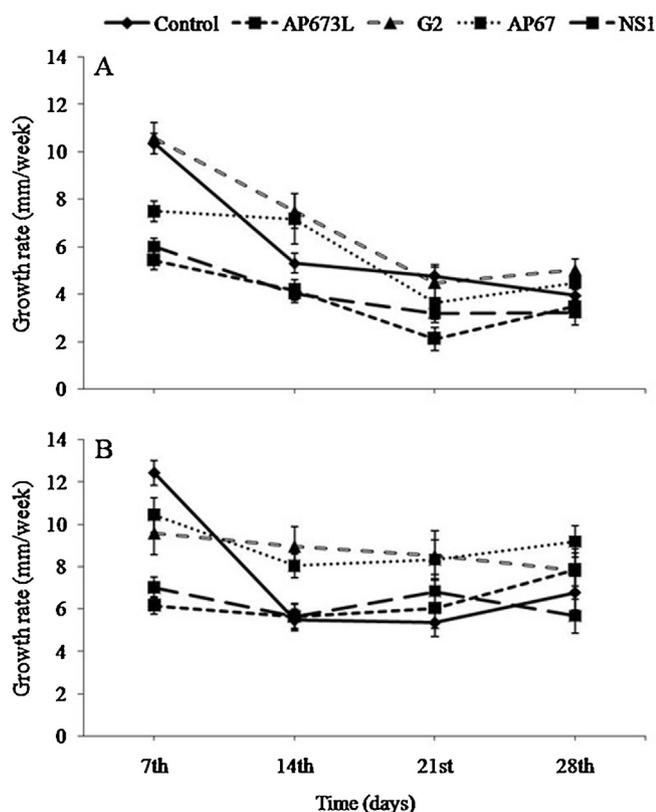


Fig. 1. Growth rate from the 7th, 14th, 21st and 28th day of *Ocimum basilicum* “LL” (A) and “RR” (B) seedlings grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values ( $n = 10$ )  $\pm$ SE. Error bars represent the SE.

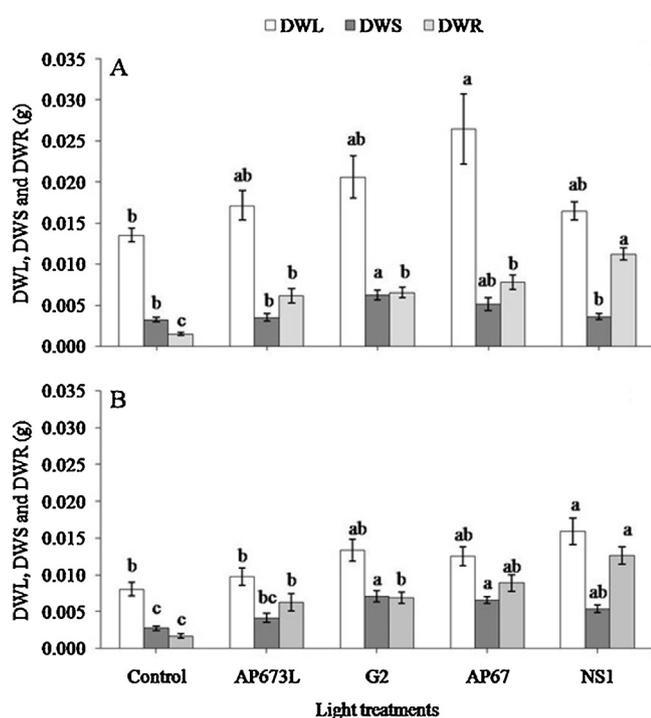


Fig. 2. Dry weight of leaves (DWL), shoots (DWS) and roots (DWR) (g) of *Ocimum basilicum* “LL” (A) and “RR” (B) seedlings grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values ( $n = 10$ )  $\pm$ SE. Error bars represent the SE. Bars followed by a different letter within a parameter differ significantly ( $\alpha = 0.05$ ).

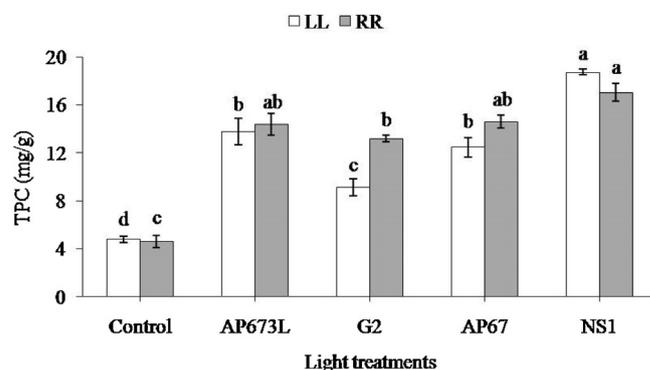


Fig. 3. Total phenolic content (TPC) of *Ocimum basilicum* “LL” and “RR” seedlings grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values ( $n = 5$ )  $\pm$ SE. Error bars represent the SE. Bars followed by a different letter within a cultivar differ significantly ( $\alpha = 0.05$ ).

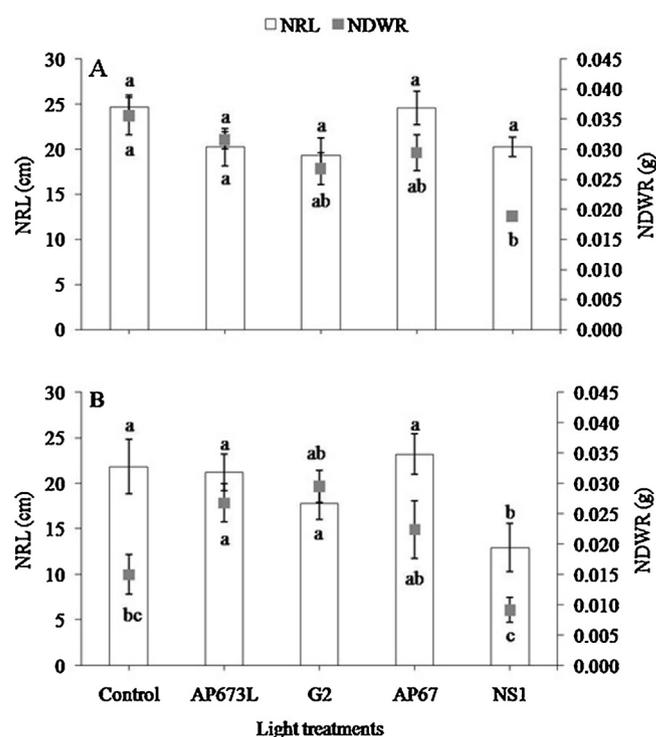


Fig. 4. New root length (NRL) and new dry weight of roots (NDWR) of *Ocimum basilicum* “LL” (A) and “RR” (B) seedlings after 31 days in the RGC water bath after grown under the five different light treatments with the same abbreviations as in Table 1. Data are mean values ( $n = 6$ )  $\pm$ SE. Error bars represent the SE. Bars and dots followed by a different letter within treatments differ significantly ( $\alpha = 0.05$ ).

continuous spectrum with a mixture of ultraviolet (UV, <400 nm), blue (B, 400–500 nm), green (G, 500–600 nm), red (R, 600–700 nm) and far-red (FR, 700–800 nm). It is worth mentioning that G2 emits high proportion of red and far-red light which makes it unpleasant to the user’s eyes when working inside the growth chambers. Environmental conditions in the chambers were set at a 20 °C/15 °C day/night temperature, air relative humidity (RH) of 80  $\pm$  10% and 14 h photoperiod. Photosynthetic photon flux density (PPFD) was maintained at around 200  $\pm$  20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for all treatments at plant height. Watering was applied every day with automated water sprinklers and the containers were rotated frequently in order to ensure equal growth conditions.

## 2.2. Morphological and developmental measurements

Height growth rate measurements were conducted during the four-week experimental period (28 days). Specifically, shoot height was measured on a weekly basis with the first measure taking place 7 days after sowing. After the four-week experimental period 10 randomly selected seedlings per cultivar and mini-plug container were sampled. Morphological characteristics such as leaf number, shoot height, root length, leaf area, leaf dry weight (DWL), shoot dry weight (DWS) and root dry weight (DWR) were measured. Additionally, root:shoot dry weight ratio (R/S) was estimated. The samples were dried in a drying oven for three days at 80 °C before weighing and the leaf area (cm<sup>2</sup>) of every plant was measured on fresh leaves by leaf area meter LI-3000C (LI-COR biosciences, Lincoln, USA).

After four weeks six randomly selected seedlings for each treatment were transferred in containers placed on top of stainless steel boxes (35 cm × 26 cm × 8 cm) having a 1:1 mixture of peat and sand and placed in a water tank in order to assess root growth capacity (RGC) (Mattsson, 1986). The water tank is used in order to maintain a stable temperature (20 ± 1 °C) for the root system. Environmental conditions in the RGC room were set at 20 °C temperature; air relative humidity (RH) of 60 ± 10% and 14 h photoperiod. Photosynthetic photon flux density (PPFD) was maintained at around 300 μmol m<sup>-2</sup> s<sup>-1</sup> for all treatments at plant height. Watering was applied every two days. Seedlings were harvested after 31 days. The characteristics measured were new root length (NRL) and dry weight of new roots (NDWR) after a three-day stay in a drying oven at 80 °C. The shoot height and root length, as well as the NRL were calculated with a Powerfix (Milomex, Pullox-hill, UK) digital caliper.

## 2.3. Quantification of total phenolic content (TPC)

Four weeks after initial germination, basil shoots and leaves were stored in polyethylene bags at -80 °C until processed. Specifically, five samples of shoots and leaves from four-five seedlings per cultivar and treatment were used (1 g fresh basil per treatment). TPC of the extracts was measured using the Folin–Ciocalteu colorimetric assay (Singleton and Rossi, 1965) with gallic acid as calibration standard, by a UV–vis spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA). Shoots and leaves weighing 1 g were ground using a mortar and pestle and then extracted into 10 mL of 80% aqueous methanol. This was followed by centrifugation at 16,000 × g for 15 min and at 4 °C. Aliquot of 2.5 mL of Folin–Ciocalteu's reagent was added and the mixture was vortexed for 20–30 s. Aliquot of 2 mL of 7.5% sodium carbonate solution was added after 1 min and the mixture was then vortexed for 20–30 s. Samples were incubated in a thermoblock for 5 min at 50 °C. The absorbance of the colored reaction product was measured at 760 nm versus a blank consisting of 500 μL of methanol, 2.5 mL of Folin–Ciocalteu's reagent and 2 mL of 7.5% aqueous sodium carbonate. The TPC in the extracts was calculated from a standard calibration curve obtained with different concentrations of gallic acid (correlation coefficient: R<sup>2</sup> = 0.998) and the results were expressed as mg of gallic acid equivalent per g (mg GAE/g) of fresh basil.

## 2.4. Statistical analysis

Statistical analysis was performed using SPSS (SPSS 15.0, SPSS Inc.). Growth rate data were analyzed using repeated measures. After the four-week experimental period data were analyzed by analysis of variance (ANOVA). Mean comparisons were conducted using a Bonferroni test at  $\alpha = 0.05$ .

## 3. Results

### 3.1. Growth rate

The development and morphogenesis of the two cultivars were variably affected by the different light conditions. On the 7th day after sowing, LL seedlings grown under the Control and G2 lights had significantly higher growth rate compared to the rest of the treatments. Furthermore, AP67 showed significantly greater values than AP673L treatment. On the 14th day, both NS1 and AP673L LEDs showed significantly lower growth rate than G2 and AP67 lights. On the 21st day, the Control and G2 lights exhibited significantly higher values compared to AP673L. No significant differences were found on the 28th day between the light treatments (Fig. 1A).

On the 7th day after sowing, RR seedlings grown under NS1 and AP673L lights had significantly lower growth rate compared to the Control and AP67 light treatments. On the 14th day, G2 exhibited higher growth rate than the Control, NS1 and AP673L light treatments. No significant differences were observed on the 21st day with the Control showing the lowest average values among all the light treatments. However, on the 28th day, significant differences were found only between NS1 that exhibited the lowest values, and AP67 light treatment (Fig. 1B).

### 3.2. Number of leaves, shoot height, root length, root:shoot ratio and leaf area

At day 28, leaf number of both cultivars was not affected by the different light treatments, with LL and RR forming around 4 and 5 leaves respectively (data not shown). Shoot height of LL was variably affected by the different light treatments. Seedlings grown under the Control, G2 and AP67 exhibited significantly higher shoots compared to AP673L and NS1 treatments. Regarding the root length, significant differences were found only between AP673L that exhibited the highest average value and NS1 treatment (Table 2). As for RR, significantly higher shoots were observed under G2 compared to AP673L, Control and NS1 lights, while AP67 promoted higher shoots than AP673L light. Pre-cultivation of basil seedlings showed no significant effect on the root length at any of the different light treatments (Table 2). For the LL seedlings the results revealed significant differences for R/S ratio between NS1 that showed the greatest values, and the rest of the light treatments. Furthermore, the Control showed significantly lower R/S ratio compared to AP67 and AP673L lights (Table 2). The R/S ratio of the RR seedlings was higher under NS1 light compared to the Control and G2. Additionally, AP673L and AP67 lights exhibited significantly greater values than the Control (Table 2). Regarding the leaf area, it is reduced by LED light independently from spectra and cultivar tested. LL had significantly greater leaf area under the Control compared to all other treatments (Table 2). As for the leaf area of RR seedlings, the Control fluorescent lamp promoted greater leaves' formation compared to AP673L and AP67 lights (Table 2).

### 3.3. Dry weights (DW)

For LL seedlings, there were significant differences regarding the DWL only between AP67 that showed the greatest values and the Control. G2 induced the highest values for the DWS compared to the Control, AP673L and NS1 light treatments. DWR of *O. basilicum* seedlings benefited under the LEDs, especially under NS1 light that induced the heavier roots compared to the rest of the treatments. Furthermore, the Control induced lighter roots compared to AP673L, G2 and AP67 lights (Fig. 2A). Finally, total biomass was significantly lower under the Control compared to AP67, G2 and NS1.

The results showed significant differences between the light treatments for the DWL of RR. NS1 light induced heavier leaves than the Control and AP673L lights. As for the DWS, plants grown under G2 and AP67 lights exhibited greater values than the Control and AP673L lights, while the Control also induced lighter shoots than NS1. NS1 also promoted greater DWR compared to the Control, AP673L and G2 lights. In addition, the Control also induced lighter roots than AP67, G2 and AP673L lights (Fig. 2B). Total biomass was significantly greater under NS1 compared to the Control and AP673L treatments. In addition, the Control induced the formation of significantly less total biomass compared to G2 and AP67 light treatments.

### 3.4. Total phenolic content (TPC)

Different light qualities after the cultivation of LL into the growth chambers resulted in significantly higher TPC for seedlings grown under NS1 compared to all other treatments. Further, the Control showed lower TPC values compared to AP673L, AP67 and G2 lights. Finally, G2 light induced significantly less total phenolics than AP673L and AP67 lights. The results of RR showed significantly lower TPC for the Control compared to the rest of the treatments. Additionally, NS1 light exhibited significantly higher TPC than G2 light (Fig. 3).

### 3.5. Root growth capacity (RGC)

After 31 days of cultivation LL seedlings exhibited no significant differences in the NRL formation at any of the different light treatments. The measurements also revealed significantly lower NDWR for seedlings grown under NS1 compared to those grown under the Control and AP673L (Fig. 4A). For the RR seedlings, after 31 days in the RGC water tank NS1 induced significantly lower NRL than AP67, Control and AP673L. Additionally, roots of seedlings pre-cultivated under NS1 were found significantly lighter compared to G2, AP673L and AP67. Finally, Control seedlings formed significantly lighter new roots compared to G2 and AP673L (Fig. 4B).

## 4. Discussion

Light spectrum strongly affects plant growth and development (Whitelam and Halliday, 2007). A great deal of different spectral distributions has been applied up to now in confined environments in order to characterize the effect of LEDs on numerous plants (Li and Kubota 2009; Johkan et al., 2010; Chen et al., 2014; Fraszczak et al., 2014). In general, the cultural conditions applied during this experiment for basil proved appropriate, as it was confirmed by the absence of morphological and developmental abnormalities during plant growth in the mini-plug containers. In both cultivars, AP673L and NS1 (the treatments with high R:FR ratio) induced the lowest growth rate resulting in the formation of the shortest shoots. The seedlings of the two cultivars grown under the same light treatments exhibited different responses, indicating that shoot height is species and/or cultivar dependent. Higher shoots were formed under G2 and AP67 (the treatments with low R:FR ratio) for both cultivars. It is no surprise that R and FR light have such effects as it has been reported that R light via R:FR ratio on phytochrome affects stem elongation, leaf area, germination and photomorphogenic responses of plants (Hogewoning et al., 2010; Sager and McFarlane, 1997). Earlier work has shown that high R:FR ratio reduced dry weight, plant height and leaf area compared to natural light in chrysanthemum, tomato, and lettuce (Mortensen and Strømme, 1987), as well as in begonia and campanula (Mortensen, 1990). Later work has shown that sweet basil grown for 28 days under white LED induced shorter plants compared to FL lamps (Fraszczak et al., 2014). In lettuce plants, R LED lighting induced longer stems

compared to the B, RB LEDs and the FL treatment (Chen et al., 2014). On the contrary, rapeseed plantlets showed no differences regarding the aforementioned parameter. However, in the same experiment rapeseed plantlets grown under RB LED (B:R = 3:1 and B:R = 1:1) exhibited greater root length values than plants cultivated under R LED and under FL light (Li et al., 2013).

Both for LL and RR, seedlings grown under the Control formed less total biomass compared to G2, AP67 and NS1 (treatments with relatively high R light portion) LEDs. Rapeseed plants grown under BR LED (B:R = 3:1 and B:R = 1:1) also exhibited greater total biomass, while FL promoted the lightest dry plants (Li et al., 2013). In roses and chrysanthemums increasing R light ratio resulted in greater biomass and taller plants (Ouzounis et al., 2014a). For both cultivars, G2 followed by the AP67 light recipes (treatments with high R portion and low R:FR ratio) promoted the greatest DWS. FL light after 45 days from sowing also induced the lowest DWS in red leaf lettuce, while the greatest values were found under the B LED and the BR (B:R = 1:1) LED lights (Johkan et al., 2010). Furthermore, DWR for both basil cultivars was favored under NS1 light treatment (high R:FR ratio and 1% UV). The results are consistent with red leaf lettuce which also formed greater DWR under the R and the BR (B:R = 1:1) LED treatments and lower under the FL light after 45 days from sowing (Johkan et al., 2010). On the contrary, FL light promoted the formation of more DWR in lettuce plants compared to the R, B and RB (R:B = 1:1) LED treatments (Chen et al., 2014), as well as compared to the RB LEDs in hydroponically grown lettuce (Lin et al., 2013). In chrysanthemums total biomass was increased with higher B light ratio, whereas in roses more R light induced the formation of greater total dry weight (Ouzounis et al., 2014a). All these results indicate that plant responses to LED lighting are species and/or cultivar dependent.

For both cultivars, leaf area of the seedlings was benefited under the Control. Studies with hydroponically grown lettuce also revealed greater leaf area for plants cultivated under FL compared to plants grown under RB LED (Lin et al., 2013), whereas cool white FL lamps induced the formation of smaller leaves compared to RGB treatment but larger compared to the RB LED (Kim et al., 2004). However, sweet basil after 28 days did not exhibit significant differences between the FL and white LED treatments (Fraszczak et al., 2014). In *Phalaenopsis* greater leaf formation was observed with increasing R light (Ouzounis et al., 2014b) which has been also reported for cucumber (Hogewoning et al., 2010). In roses greater leaf area was observed with increasing R light, while in chrysanthemums and campanulas greater values were observed under 20%B/80%R and white light respectively (Ouzounis et al., 2014a). As mentioned in the introduction, R and B lights acting in an independent and/or synergistic manner, affect photomorphogenesis. From the results it appears that leaf area is reduced under LEDs, whereas DWL is increased. This could be the result of higher carbohydrates and primary metabolites accumulation suggesting that LED light affects photosynthesis activity. Previous studies have shown that combinations of R and B light are favorable for plant growth and greater carbohydrates production in plants (Yorio et al., 2001; Ouzounis et al., 2014b).

Seedlings grown under low irradiation elongate quickly and form compact root system that does not absorb sufficient water and nutrients leading to plant growth decrease. Therefore, seedlings with low R/S ratio are not suitable for active growth (Johkan et al., 2010). Our results suggest that seedlings grown under the Control form inadequate roots compared to the plants grown under the rest of the treatments. In general, LEDs that emit more B light promoted greater R/S ratio indicating that B light affects this parameter. B light is reported to suppress stem elongation leading to more compact plants (Folta and Childers, 2008). Results of R/S ratio in the literature are contradicting. Lettuce plants treated with FL showed greater S/R dry weight ratio (lower R/S) (Johkan et al., 2010) com-

pared to the LED treatments, whereas in other studies with lettuce, RB (R:B = 1:1) LED treatment promoted greater S/R dry weight ratio (lower R/S) compared to the FL light (Chen et al., 2014; Lin et al., 2013).

It has been reported that both morphological characteristics and RGC parameters are species dependent (Kostopoulou et al., 2010). A careful selection of quality seedlings is important for an increase of the survival rate and the growth rate after transplanting (Radoglou, 2001). Further, selecting high quality seedlings can potentially mitigate the harmful effects of stressful environmental conditions resulting in successful establishment in the field, while using low quality seedlings can lead to unsuccessful transplanting even under favorable environmental conditions (Radoglou et al., 2009). The potential for new root growth is critical for a rapid absorbance of water and minerals after transplanting. RGC is a performance feature for quality assessment (Mattsson 1996, 1986) and can be defined as the ability of a seedling to increase the size of its root system through the formation of new roots and the elongation of already present roots (Mattsson, 1986). Even though the NS1 light regime (relatively high B portion with the highest R:FR ratio and 1% UV) promoted the formation of heavier roots compared to the other light qualities for both cultivars, this was not the case after the RGC transplant, with NS1 seedlings having less new root biomass for both cultivars. The quick root system development under the effect of the Control and AP673L for LL basil, and mainly under AP673L for RR basil may offer the seedlings an advantage regarding the fast exploitation of larger soil amount after transplanting.

The highest TPC was obtained under NS1 light quality (relatively high B portion with the highest R:FR ratio and 1% UV) which was 3.9 times higher and 3.7 times higher than the Control's for the LL and the RR respectively. In plant tissues, phenolic compounds are reported to act protectively against UV radiation (Lattanzio et al., 2006), which might explain the increased total phenolic formation under NS1 light regime. Previous studies have shown that secondary metabolites are increased with additional B light, even though the B LED has been applied only as supplemental light (Ouzounis et al., 2014a), while secondary metabolites were reportedly enhanced in carrots and grapes under UV-B radiation (Glässgen et al., 1998; Pezet et al., 2003). Our results indicate that LED applications with high B light portion create a physiological state that induces the accumulation of phenolic compounds. As far as concern the mechanism behind the increase of secondary metabolites under B light, it has been reported that the activity of phenylalanine ammonia-lyase (PAL, a key enzyme in the phenylpropanoid pathway) was stimulated (Heo et al., 2012). Moreover, PAL gene expression was activated by monochromatic B LED lighting in lettuce (Son et al., 2012). However, there are also contrasting examples in the literature, indicating that the amount of secondary metabolism under artificial lighting is light and species dependent. For example, no essential difference was found in pigment content in *Dieffenbachia amoena*, *Ficus elastica* and Boston lettuce (Heo et al., 2010; Martineau et al., 2012). Consequently, the production of phenolic compounds depends concurrently on the light environment, the physiological, and biochemical factors.

## 5. Conclusion

Two *O. basilicum* cultivars (LL and RR) were grown under artificial LED and FL lighting. For both cultivars, total biomass was increased under LED lighting, while root:shoot ratio was favored under NS1 (high blue, high red:far-red and 1% UV) in comparison with fluorescent lighting. Leaf area was favored by the Control in both LL and RR. The treatments with high red and high red:far-red (AP673L) and moderate blue and red with low red:far-red (AP67)

triggered greater new root length compared to NS1, but only in RR. Total phenolic content was also higher in treatments enriched with blue light. For both cultivars, root growth capacity was variably affected by the different light treatments. Our study demonstrates that artificial LED lighting enhanced (even though variably) the growth and increased total phenolic content of two *O. basilicum* L. cultivars compared to fluorescent light, but the effects were cultivar dependent.

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## References

- Barta, D.J., Tibbitts, T.W., Bula, R.J., Morrow, R.C., 1992. Evaluation of light emitting diode characteristics for a space-based plant irradiation source. *Adv. Space Res.* 12, 141–149.
- Brazaityte, A., Duchovskis, P., Urbonaviciute, A., Samuoliene, G., Jankauskiene, J., Kasiuleviciute, B., Bliznikas, Z., Novickovas, A., Breive, K., Zukauskas, A., 2009. The effect of light-emitting diodes lighting on cucumber transplants and after-effect on yield. *Zemdirb-Agric.* 96, 102–118.
- Brazaityte, A., Duchovskis, P., Urbonaviciute, A., Samuoliene, G., Jankauskiene, J., Sakalauskaite, J., Sabajeviene, G., Sirtautas, R., Novickovas, A., 2010. The effect of light-emitting diodes lighting on the growth of tomato transplants. *Zemdirb-Agric.* 97, 89–98.
- Chen, X., Guo, W., Xue, X., Wang, L., Qiao, X., 2014. Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromatic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). *Sci. Hortic.* 172, 168–175.
- de Carbonnel, M., Davis, P., Roelfsema, M.R.G., Inoue, S.-i., Schepens, I., Lariguet, P., Geisler, M., Shimazaki, K.-i., Hangarter, R., Fankhauser, C., 2010. The Arabidopsis PHYTOCHROME KINASE SUBSTRATE2 protein is a phototropin signaling element that regulates leaf flattening and leaf positioning. *Plant Physiol.* 152, 1391–1405.
- Folta, K.M., Childers, K.S., 2008. Light as a growth regulator: controlling plant biology with narrow-bandwidth solid-state lighting systems. *HortScience* 43, 1957–1964.
- Fraszczak, B., Golcz, A., Zawirska-Wojtasiak, R., Janowska, B., 2014. Growth rate of sweet basil and lemon balm plants grown under fluorescent lamps and LED modules. *Acta Sci. Pol.* 13, 3–13.
- Glässgen, W.E., Rose, A., Madlung, J., Koch, W., Gleitz, J., Seitz, H.U., 1998. Regulation of enzymes involved in anthocyanin biosynthesis in carrot cell cultures in response to treatment with ultraviolet light and fungal elicitors. *Planta* 204, 490–498.
- Heo, J.W., Kang, D.H., Bang, H.S., Hong, S.G., Chun, C., Kang, K.K., 2012. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. *Kor. J. Hortic. Sci. Technol.* 30, 6–12.
- Heo, J.W., Lee, Y.B., Kim, D.E., Chang, Y.S., Chun, C., 2010. Effects of supplementary LED lighting on growth and biochemical parameters in *Dieffenbachia amoena* 'Camella' and *Ficus elastica* 'Melany'. *Kor. J. Hortic. Sci. Technol.* 28, 51–58.
- Hogewoning, S.W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., Harbinson, J., 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *J. Exp. Bot.* 61, 3107–3117.
- Johkan, M., Shoji, K., Goto, F., Hashida, S., Yoshihara, T., 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience* 45, 1809–1814.
- Kim, H.-H., Goins, G.D., Wheeler, R.M., Sager, J.C., 2004. Stomatal conductance of lettuce grown under or exposed to different light qualities. *Ann. Bot.* 94, 691–697.
- Kopsell, D.A., Kopsell, D.E., Lefsrud, M.G., Curran-Celentano, J., Dukach, L.E., 2004. Variation in lutein,  $\beta$ -carotene, and chlorophyll concentrations among *Brassica oleracea* cultivars and seasons. *HortScience* 39, 361–364.
- Kopsell, D.A., Sams, C.E., 2013. Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *J. Am. Soc. Hortic. Sci.* 138, 31–37.
- Kostopoulou, P., Dini-Papanastasi, O., Radoglou, K., 2010. Density and substrate effects on morphological and physiological parameters of plant stock material of four forest species grown in mini-plugs. *Scand. J. For. Res.* 25, 10–17.
- Kozuka, T., Suetsugu, N., Wada, M., Nagatani, A., 2013. Antagonistic regulation of leaf flattening by phytochrome and phototropin in *Arabidopsis thaliana*. *Plant Cell Physiol.* 54, 69–79.
- Kwee, E.M., Niemeier, E.D., 2011. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. *Food Chem.* 128, 1044–1050.

- Lattanzio, V., Lattanzio, V.M.T., Cardinali, A., 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato, F. (Ed.), *Phytochemistry: Advances in Research*, Research Signpost, Trivandrum, India, pp. 23–67.
- Lee, J., Scagel, C.F., 2009. Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. *Food Chem.* 115, 650–656.
- Lee, S.-J., Umano, K., Shibamoto, T., Lee, K.-G., 2005. Identification of volatile components in basil and thyme leaves and their antioxidant properties. *Food Chem.* 91, 131–137.
- Li, H., Tang, C., Xu, Z., 2013. The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. *Sci. Hortic.* 150, 117–124.
- Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67, 59–64.
- Lin, K.-H., Huang, M.-Y., Huang, W.-D., Hsu, M.-H., Yang, Z.-W., Yang, C.-M., 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*). *Sci. Hortic.* 150, 86–91.
- Makri, O., Kintzios, S., 2008. *Ocimum* sp. (basil) botany, cultivation, pharmaceutical properties, and biotechnology. *J. Herbs Spices Med. Plants* 13, 123–150.
- Martineau, V., Lefsrud, M.G., Tahera-Naznin, M., Kopsell, D.A., 2012. Comparison of supplemental greenhouse lighting from light emitting diode and high pressure sodium light treatments for hydroponic growth of Boston lettuce. *HortScience* 47, 477–482.
- Massa, G.D., Kim, H.-H., Wheeler, R.M., Mitchell, C.A., 2008. Plant productivity in response to LED lighting. *HortScience* 43, 1951–1956.
- Mattsson, A., 1986. Seasonal variation in root growth capacity during cultivation of container grown *Pinus sylvestris* seedlings. *Scand. J. For. Res.* 1, 473–482.
- Mattsson, A., 1996. Predicting field performance using seedling quality assessment. *New For.* 13, 223–248.
- Morrow, R.C., 2008. LED lighting in horticulture. *HortScience* 43, 1947–1950.
- Mortensen, L., Strømme, E., 1987. Effects of light quality on some greenhouse crops. *Sci. Hortic.* 33, 27–36.
- Mortensen, L.M., 1990. Effects of temperature and light quality on growth and flowering of *Begonia x hiemalis* Fotsch. and *Campanula isophylla* Moretti. *Sci. Hortic.* 44, 309–314.
- Ouzounis, T., Fretté, X., Rosenqvist, E., Ottosen, C.-O., 2014a. Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanula. *J. Plant Physiol.* 171, 1491–1499.
- Ouzounis, T., Fretté, X., Ottosen, C.-O., Rosenqvist, E., 2014b. Spectral effects of LEDs on chlorophyll fluorescence and pigmentation in *Phalaenopsis* 'Vivien' and 'Purple Star'. *Physiol. Plant* 154, 314–327.
- Ouzounis, T., RaziParjikolaei, B., Fretté, X., Rosenqvist, E., Ottosen, C.-O., 2015. Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids, and pigments in *Lactuca sativa*. *Front. Plant Sci.* 6, 19, <http://dx.doi.org/10.3389/fpls.2015.00019>.
- Pezet, R., Perret, C., Jean-Denis, J.B., Tabacchi, R., Gindro, K., Viret, O., 2003. Viniferin, a resveratrol dehydromer: one of the major stilbenes synthesized by stressed grapevine leaves. *J. Agric. Food Chem.* 51, 5488–5492.
- Radoglou, K.M., 2001. Understanding planting establishment in constraint environment. In: In: Proceedings of Scientific meeting Hellenic Forestry Society, October 17–20, 2000, Kozani, Greece, pp. 206–212 (in Greek with English summary) Greece: Hellenic Forest Society.
- Radoglou, K., Dini-Papanastasi, O., Kostopoulou, P., Spyroglou, G., 2009. Forest regeneration material: state of the art and a new European approach for pre-cultivated planting stock production. In: Poljaković-Pajnik, L., Tobolka, A.T. (Eds.), *Forestry in Achieving Millennium Goals*. Novi Sad, Serbia: Old Commerce, pp. 17–24.
- Sager, J.C., McFarlane, J.C., 1997. Radiation. In: Langhans, R.W., Tibbits, T.W. (Eds.), *Plant Growth Chamber Handbook*. North Central Region Research Publication, Iowa State University Press, pp. 1–29.
- Samuolienė, G., Brazaitytė, A., Sirtautas, R., Viršilė, A., Sakalauskaitė, J., Sakalauskienė, S., 2013. LED illumination affects bioactive compounds in romaine baby leaf lettuce. *J. Sci. Food Agric.* 93, 3286–3291.
- Schuerger, A.C., Brown, C., Stryjewski, E.C., 1997. Anatomical features of pepper plants (*Capsicum annuum* L.) grown under light-emitting diodes supplemented with blue or far-red light. *Ann. Bot.* 79, 273–282.
- Seigler, D., 1998. *Plant Secondary Metabolism*. Kluwer Academic publishers, Dordrecht, p. 759.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158.
- Son, K.-H., Park, J.-H., Kim, D., Oh, M.-M., 2012. Leaf shape, growth, and phytochemicals in two leaf lettuce cultivars grown under monochromatic light-emitting diodes. *Kor. J. Hortic. Sci. Technol.* 30, 664–672.
- Whitelam, G., Halliday, K., 2007. *Light and Plant Development*. Blackwell Publishing, Oxford, p.313.
- Wink, M., 2010. *Functions and Biotechnology of Plant Secondary Metabolites*. Annual Plant Reviews, 3. Wiley-Blackwell, Oxford, p.410.
- Wu, M.-C., Hou, C.-Y., Jiang, C.-M., Wang, Y.-T., Wang, C.-Y., Chen, H.-H., Chang, H.-M., 2007. *Food Chem.* 101, 1753–1758.
- Yorio, N.C., Goins, G.D., Kagie, H.R., Wheeler, R.M., Sager, J.C., 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortScience* 36, 380–383.
- Zgorka, G., Glowniak, K., 2001. Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. *J. Pharm. Biomed. Anal.* 26, 79–87.
- Zhao, J., Davis, L.C., Verpoorte, R., 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.* 23, 283–333.