FORESTRY IDEAS, 2016, vol. 22, No 1 (51): 49–64

ENHANCED GROWTH CHARACTERISTICS OF *CASTANEA SATIVA* MILL. SEEDLINGS UNDER LED LIGHTING WITH CONTINUOUS SPECTRUM DURING INDOOR CULTIVATION

Sonia Smirnakou and Kalliopi Radoglou*

Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace (DUTH), Pandazidou 193, 68200 Orestiada, Greece. E-mails: sosmirnakou@gmail.com, kradoglo@fmenr.duth.gr*

Received: 04 January 2016 Accepted: 05 May 2016

Abstract

Previous studies have demonstrated that monochromatic LEDs could be used as an alternative to conventional lighting sources for cultivation of several species in artificial environments, which have led to satisfactory results. However, the application of LED lights of continuous spectrum as an artificial light source in plant growth chambers has not been tested yet. This study examines the influence of five different LED light qualities (L20AP67, AP673L, G2, AP67 & NS1) that emitted a mixture of continuous spectrum based on various percentages of ultraviolet, blue, green, red, far-red and infra-red radiation or Fluorescent light (FL as a control) on growth of sweet chestnut seedlings into mini-plug containers during one month indoor cultivation. Leaf characteristics of chestnut seedlings were better promoted under LEDs by means of faster leaf formation of greater leaf area that showed significantly higher stomata and epidermal cell number compared to the FL light. Therefore among LEDs G2, NS1 and AP67 showed the greatest effect by inducing significantly higher stomatal density (SD), stomatal index (SI%) and cell density (CD) compared to the FL light and L20AP67 LED. Similar shoot development was found irrespective of light spectrum; however, significantly longer roots were formed by the L20AP67 than the FL light that showed the shortest. Further, root system architecture analysis revealed that NS1 and AP673L LEDs produced seedlings with significantly higher root fibrosity index compared to the FL. Dry weight accumulation especially of the shoots and roots of the seedlings treated with the AP673L was the highest by far. In contrast, FL light demonstrated the lowest dry weight mass thus exhibited the lowest R/S ratio compared to LEDs. These results provide new strategies for using LEDs of continuous spectrum for adequate cultivation protocols into growth chambers for *Castanea sativa* Mill. and possibly other forest tree species.

Key words: chestnut, growth chamber, LEDs, light quality, photomorphogenesis.

Introduction

Plant growth and development is the result of the interaction between genotype and environment. This interaction is perceived by specialized photosensory receptor proteins that adapted to signals of the incident light spectrum due to best utilization. In the artificial plant production, light quality, quantity, and duration constitute the information that will ultimately contribute to plant productivity and quality (Singh et al. 2011, Barrero et al. 2012).

Until today, a wide variety of artificial lighting has been used in horticulture including incandescent, fluorescent and high intensity discharge lights (Sager et al. 1982). Incandescent lighting is typically high in the red and infrared wavelengths. Fluorescent lights produce more white light but the fixtures must be located very close to the plants. High intensity discharge (HID) lights, such as high-pressure sodium (HPS) and metal halide, have been used in growth chambers to supplement natural sunlight and increase photosynthetic rates (Seelye and Mullan 2010). Due to the large amount of electrical energy required, adding lights to increase photosynthesis is for most reforestation services and native plant nurseries, economically impractical (Downs 1977, Warrington et al. 1976).

Light emitting diodes (LEDs) are the newest light source being used in controlled environments and greenhouse plant culture, which are solid-state, durable, lightweight, long-lasting, and come in selectable narrow-waveband emissions such as red and blue that can be matched to the absorption spectra of plant pigments by eliminating other wavelengths found within normal white light, thus reducing the amount of energy required for power (Goins et al. 1997, Kim et al. 2005, Landis et al. 2013).

Plant growth under the combination of blue and red light has been studied, for instance, in lettuce, spinach, komatsuna (Japanese mustard spinach) and radish (Yorio et al. 2001, Hanyu and Shoji 2002, Ohasi-Kaneko et al. 2007). The combination of red and blue light was an effective lighting source to produce plant biomass, but also the combination of green, blue and red light was effective (Kim et al. 2004, Pardo et al. 2013). Additionally it has been shown in cucumber plants, which were grown under different combinations of red and blue light supplied by light-emitting diodes (LEDs), that light quality by itself can induce photosynthetic and morphological properties in leaves that normally occur at high light intensities, although the plants were grown under low light intensity (Hogewoning et al. 2010). On the other hand, the number of stomata, rate of photosynthesis and transpiration, and stomatal conductance increased progressively with increasing photosynthetic photon flux density (PPFD) in plantlets of *Withania somnifera* L. (Lee et al. 2007). Also raising the PPFD from 25 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased the average number of stomata and stomatal length and reduced stomatal frequency in barley (Kubinova 1991). Most of the studies with LED lighting were performed in controlled environment growth chambers, where the main environmental parameters, such as temperature, humidity, CO_2 concentration and photosynthetic daily integral can be controlled independently of external influences. Unfortunately, successful lighting strategies in phytotrons not necessarily produce the same results in greenhouse conditions (Pinho et al. 2007), especially when variable daylight effect is involved. Therefore individual experiments should evaluate the background growth conditions in conjunction with the natural effect lighting.

Sweet chestnut (*Castanea sativa* Mill.), the only native species of *Castanea* genus in Europe, is distributed in majority of the Mediterranean countries, extending from Caucasus to Italy, France, Spain, Portugal and south England. Thus, this wide-range distribution throughout southern Europe highlights the ability of the species to adapt to varying environmental conditions (Lauteri et al. 1997, Martin et al. 2010). Chestnut is one of the multipurpose species of major economic importance in the Mediterra-

nean basin, valued not only for fruit and timber but also for its contribution to the landscape and environment. Because of the multipurpose characteristics of the species, chestnut populations have been affected by clonal propagation and silvicultural practices. This along with the changes in land use and the accelerating dynamics of global and climatic changes have resulted in a fragmentation of habitats, a reduction of population size and probably a loss in biodiversity (Conedera et al. 2004).

The objective of this study was to examine the effects of LEDs with continuous spectrum on seedling growth and quality characteristics of sweet chestnut seedlings and to compare their responses with those grown under fluorescent conventional light.

Materials and Methods

Plant material and culture conditions

Castanea fruits were collected from natural stands located in Petrokerasa, Zagliveri, Thessaloniki, Greece (40°32'40.86" N, 23°15'26.31" E). Prior to 24 h hydration of the nuts the spiny involucres were removed. In order to achieve higher germination, we stratified the nuts in the refrigerator at 2–5 °C in plastic bags filled with moist vermiculite for five months. Only pre-germinated nuts were used, in order to maximize the uniformity of seedlings in trays.

Pre-germinated nuts were sown into each cavity of the mini-plug plastic trays (QP60/7R QuickPot®, Herkuplast-Kubern GmbH, Ering, Germany) of size 310×530 mm; cell size 46×75 mm; volume 95 cc; 363 plants/m^2 and filled with enriched peat soil substrate (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany) mixed with perlite on the surface. In total 60 seedlings were randomly selected, specifically 10 seedlings of each of two mini-plug container trays per light treatment were used.

Mini-plug trays were transferred for a cultivation period of 21 days to environmentally controlled growth chambers. There were six different light treatments: fluorescent (FL) treatment with white fluorescent lamps, Osram Fluora Philips-TLD (36W/54 daylight) as the reference light, and five light treatments provided by Valoya LED lights spectra (Anonymous 2016): L20AP67, AP673L, G2, AP67 and NS1. The selected light spectrum percentages are shown in Table 1. Both chambers reach 2 m height and consist of three shelves. Each shelf has 1.20 m length, 0.60 m height, and 0.55 m depth. The distance from the lights to the top of the seedlings in the mini-plugs is 0.40 m. In chamber 1, at the first shelve four L20AP67 tubes were placed. In the middle one (of chamber 1 only) four fluorescent lamps were placed as the reference lighting type, with 30 cm space between them. On the bottom shelf AP673L light was used. In Chamber 2 the top shelf had the G2 light, the second – the AP67 light and third shelf – the NS1 light.

The environmental conditions inside the chambers were 17 h photoperiod, 150±10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD), 80±10 % relative air humidity (RH), and 20 °C/15 °C day/night temperature. Irradiance and PPFD of light sources were quantified at plant height with a LI-1800 portable spectroradiometer (LI-COR, Lincoln, Nebraska, USA) with the sensor at ≈25 cm from the light tubes in 10 different spots through the growth chamber shelves. Watering was applied twice a day by automatic sprinklers followed by full rotation of the

Table 1. Spectral distribution and red:far-red ratio of the five light treatments.

Light treatments	Continuous Spectrum data including different percentages out of 400–800 nm, %					R:FR ratio
	380–400 nm	400–500 nm	500–600 nm	600–700 nm	700–800 nm	
FL	0	34.8	24.1	36.7	4.4	5.7
L20AP67	0	10.5	26.2	48.9	14.4	2.9
AP673L	0	11.9	19.3	60.5	8.3	5.6
G2	0	7.7	2.4	64.4	25.5	2.5
AP67	0	13.8	15.1	53	18.1	2.7
NS1	1.8	20.2	38.9	35.7	5.2	8.2

trays in order to ensure uniform growth conditions.

Measurements during the cultivation period

During the 21 days cultivation period in growth chambers, 10 seedlings per light treatment were randomly selected, marked and measured for their growth rate and leaf number. Growth rate based on the height increment gained of each seedling every seven days.

Measurements at the end of the cultivation period

Leaf level

The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis 1990). The abaxial epidermis of the leaf was cleaned first by using a degreased cotton ball, and then carefully smeared with nail varnish in the mid-area between the central vein and the leaf edge, for approximately 20 min. The thin film (approximately 15 mm × 10 mm) was peeled off from the leaf surface, mounted on a glass slide, immediately covered with a cover slip, and then lightly pressured with finepoint tweezers. All the impressions were taken from at least five leaves for each light treatment and examined under a light microscope with camera attachment (40×/0.75 magnification) using the Axio Vision program (47.1). Four fields per slide were randomly selected and photographed. Stomata and epidermal cells were counted on the photographs and the stomatal density (SD), stomatal index (SI%) and cell density (CD) were calculated. Stomatal index was estimated using the formula $[s/(e + s)]\cdot 100$ where s is number of stomata and e is number of epidermal cells (Salisbury 1927). The guard cells were not included in the number of epidermal cells. Cell density was calculated as the total number of cells ($e + s$) per unit area of leaf. Further leaf area was measured by the device LI-3000C Portable Area Meter (LI-COR Biosciences); Chlorophyll content index (CCI), defined as the ratio between leaf transmis-

sion percentages at 931 nm and 653 nm (Anonymous 2015), was measured by the portable CCM-200 (Opti-Sciences, Inc., NH 03051 USA). The CCM sensor area is 0.71 cm^2 and was placed randomly on the leaf mesophyll, avoiding the mid-vein. Five measurements were taken (per leaf) that were averaged to provide a single chlorophyll content index (CCI) per leaf. The saturation pulse method associated with the pulse-amplitude-modulation technique was applied for chlorophyll fluorescence measurements using a MINI-PAM fluorometer (Heinz Walz, Effeltrich, Germany). The tip of the fiber optics was located 1.0 cm from and 60 ° to the leaf surface. The effective quantum yield was calculated as $\Delta F/F_m' = (F_m' - F)/F_m'$, where F and F_m' are the fluorescence yield before and after the saturation pulse is applied on the leaf, respectively.

Plant growth parameters

Shoot height (SH), root length (RL), leaf dry weight (LDW), shoot dry weight (SDW) and root dry weight (RDW) were measured. SH and RL were defined as the distance from the top of the root plug to the upper and lower end of a seedling, respectively. LDW, SDW and RDW were assessed after oven drying at 70 °C for 48 h. The root-to-shoot ratio (R/S) was calculated on a dry weight basis.

Root architecture analysis

After being washed away gently, the remained soil particles from the roots of five selected *Castanea* seedlings for each of the light treatments were used. They were scanned and loaded in GiA Roots (Galkovskyi et al. 2012), which is a software tool to automate and facilitate the large-scale analysis of root networks. GiA Roots was used to quantify the structure of the plant root system by measuring the number of lateral roots in order to estimate root density. After counting the lateral roots, we proceeded computing their density with the following formula: Root density = Length of primary root / No of lateral roots (Bors-Oprişa et al. 2011). Following root architecture of seedlings was also defined by the number of FOLRs (First Order Lateral Roots) greater than 1 mm in diameter originating along the length of the taproot and at the base of the taproot. Further, selected seedlings were individually evaluated for their root fibrosity, classified on a1 to 5 scale by using an index designed by Hatchell and Muse (1990) and modified by Wilson et al. (2007) (Table 3).

Statistical analysis

Statistical analysis was conducted with IBM SPSS Statistics for Windows, Version 20.0. Collected data every seven days such as mean growth rate and leaf number under the different light treatments were analyzed using general linear model (GLM) repeated measurements. Each subject in the design was, hence, measured three times. At the end of the cultivation period collected data was analyzed using general linear model (GLM) multivariate analysis. Significant differences were established by multiply comparisons test with Bonferroni correction at $p < 0.05$.

Results and Discussion

Cultivation of the tested broad-leaved species showed significant morphological and physiological adjustments after 21 days of growing under different light qualities, reaffirming the reliance and the sensitivity of

Table 2. Growth rate of *Castanea sativa* seedlings grown under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments, every seven days (for a total of 21 days experimental period).

Light treatments	Growth rate			Leaf number		
	7th day	14th day	21st day	7th day	14th day	21st day
FL	1.58±0.54b	0.33±0.16a	0.15±0.08a	6.29±1.25a	7.71±0.95a	8±3.36b
L20AP67	2.81±0.60a	0.23±0.13a	0.29±0.24a	7.43±1.61a	8.57±2.14a	11.14±2.73a
AP673L	1.74±0.70b	0.28±0.20a	0.22±0.12a	6.43±2.14a	9.57±1.13a	10±1.52ab
G2	1.84±0.41ab	0.46±0.23a	0.07±0.05a	6.43±1.98a	8.71±1.38a	11.14±3.57a
AP67	1.78±0.23ab	0.21±0.14a	0.24±0.18a	6.43±2.63a	9.57±7.09a	10.43±2.77ab
NS1	1.39±0.27b	0.38±0.21a	0.21±0.21a	6.2±1.34a	8.57±1.90a	9.57±2.57ab

Note: Different small letters within the column indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 10$) ± SE.

Table 3. Rating system for root fibrosity of Castanea sativa under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Light treatments	Rating	Fibrosity class	Description of root system appearance
FL	3	Moderate	3–5 2nd order long roots; moderate density of higher order long and short roots
L20AP67	4	High	>5 2nd order long roots; moderate density of higher order long and short roots
AP673L	>5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots
G2	5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots
AP67	>5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots
NS1	>5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots

Note: The rating is based on visual assessment of the approximate number and type of high order lateral roots per 10 cm segment of primary first order lateral roots (those with a diameter > 1 mm, branching from the taproot). Rating: 1 – Very low (no second order long roots; zero or few short roots present); 2 – Low (one–three second order long roots; low density of higher order long and short roots); 3 – Moderate (three–five 2nd order long roots; moderate density of higher order long and short roots); 4 – High (> five 2nd order long roots; moderate density of higher order long and short roots); 5 – Very high (> five 2nd order long roots; high density of higher order long and short roots). Long roots are > 5 mm and are likely to contain branches of the next highest order. Short roots are < 5 mm; they do not support roots of higher order (Hatchell and Muse 1990).

the seedlings to the inherent need of light especially in early developmental stages.

Castanea seedlings showed significant differences in height growth rate only the first week (7th day) of the experimental period (Table 2). L20AP67 showed significantly higher height increment of 67.61 %, 56.03 % and 47 % compared to NS1, FL and AP673L lights, respectively, while no significant differences were found for the rest of the lights (Table 2). Considering only the light quality effects, height increment was more enhanced by L20AP67 and G2 throughout the indoor cultivation, than NS1 and FL. A possible explanation for the higher values of the growth rate induced by the L20AP67 and the G2 could be the low R:FR ratios (2.9 and 2.5). Low R:FR ratio is an indication of shading by neighbouring plants and according to studies on the mechanisms that control the shade avoidance responses, the phytochrome system controls the distribution of auxin in different cell layers, thus shaded plants reduce cell expansion in their leaves and enhance cell elongation in the stems (Morelli and Ruberti 2002). Nevertheless, both L20AP67 and G2 showed also significantly faster leaf expansion of 11 leaves than the FL that showed the lowest of eight leaves (Table 2). According to Nhut et al. (2003), strawberry plantlets showed that the number of leaves was higher under blue plus red LEDs than of those treated with fluorescent or red LEDs alone.

It is well known that light causes also alternations in the stomata movements of plants. Unfortunately, there are few studies about the effects of light quality on this subject, especially for the parameters examined in this study. Stomatal opening and closure is affected by light; for instance Talbott et al. (2006) observed that blue and red light stimulated stomata opening, whereas green light inhibited it. It has been also reported that an increase in light intensity, decreases the number of epidermal cells and increases stomata number, index and size (Fernandez and Mujica 1973). Schoch et al. (1984) reported that blue and far-red light reduced the stomatal index, while red light increased it *in Vigna sinensis*. Kim et al. (2004) showed that blue and red light increased the stomata size and decreased the stomata number of chrysanthemum plantlets, while blue and far-red had the opposite effect. Furthermore, Lee et al. (2007) found that white light increased the stomata number and size, while blue light reduced the mentioned parameters of *Withania somnifera* plantlets. Treatments with UV-B can also affect stomatal conductance, altering the rate of water loss by transpiration and uptake rate of CO_2 for photosynthesis, while stomatal closure occurs rapidly even at low UV-B levels in some UV-B sensitive seedlings such as in Mono Maple (*Acer Mono Maxim*) (Tevini and Teramura 1989, Yao and Liu 2006). Nevertheless, G2 had the highest red (600–700 nm) and far-red (700–800 nm) percentages of 64.4 % and 25 %, respectively, while at the same time had the lowest blue (400–500 nm) percentage of 7.7 % that induced the highest impact on the hypostomatous character of *Castanea* leaves for the following parameters. Likewise, NS1 has the highest percentage of 38.9 % in blue-green region (500–600 nm) and it is the only that covered the UV band of 1 % (<400 nm) than the rest of lights. Consequently G2 showed significantly higher number of stomata (data not shown) and thus significantly higher SD of 251 stomata/mm^2, compared to the FL and L20AP67 (Fig. 1) that showed the lowest among all the lights of 137 stomata/mm^2 and 139 sto mata/mm^2, respectively (Fig. 1). The rest of the light treatments showed also higher

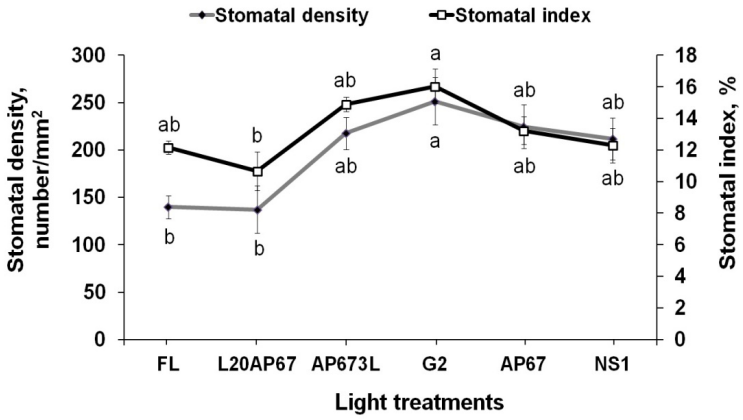

Fig. 1. Stomatal density (SD) and Stomatal index (SI) (%) on the abaxial leaf surface of Castanea sativa seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

SD than the latter ones such as 225 stomata/mm^2, 217.7 stomata/mm^2 and 211.7 stomata/mm^2, for the AP67, AP673L and NS1 (Fig. 1). Furthermore, G2 also induced significantly higher SI% of 16.03 % compared to the L20AP67 that showed of 10.6 %. No significant differences were found for the SI% among the rest of the lights; however, they still showed higher values, such as 15 %, 13.2 % and 12.15 % for the AP673L, AP67 and FL lights, respectively, than the L20AP67 (Fig. 1). Additionally NS1 and AP67 had significantly created more epidermal cells compared to the FL conventional light (data not shown), thus significantly higher CD was also found (1714 and 1688.7) than 1140 induced by the FL (Fig. 2). Higher number of epidermal cells and CD were found for the rest of the light treatments than the FL of 1573.5, 1452, and 1276 induced by the G2, AP673L and L20AP67 (Fig. 2).

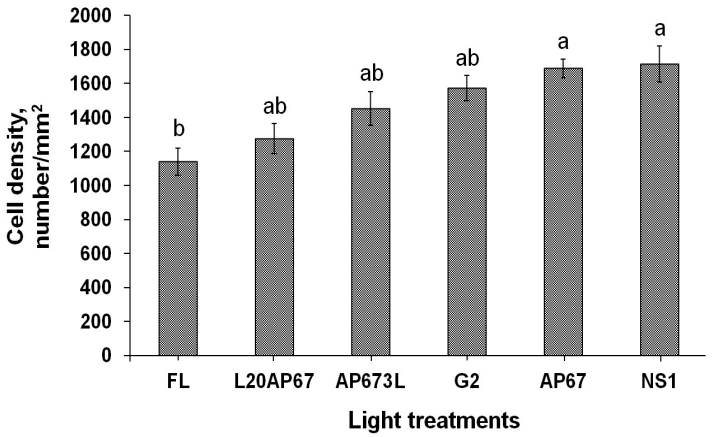

Fig. 2. Cell density on the abaxial leaf surface of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 5$) ± SE.

Leaf area of chestnut seedlings under different light treatments was similar; however, it was benefited more under LEDs than the FL light. Specifically, higher leaf area was formed under the

AP673L with 269.42 cm^2, followed by the NS1, L20AP67, AP67, G2, and FL with 216.75 cm^2, 215.8 cm^2, 203.37 cm^2, 188.55 cm^2 and 178,42 cm^2, respectively (Fig. 3). Johkan et al. (2010) found that red lettuce plants irradiated with red light increased their leaf area by 33 % than the fluorescent light, but those treated with blue LED lights decreased by more than 9 %. Also in another study on lettuce growth promotion under supplemental far red lighting was associated with the increase in leaf area (Kubota et al. 2012). Following L20AP67 had significantly higher CCI of 13.72 compared to the NS1 that had CCI of 6 (Fig. 4); while the rest of the lights had CCI values of 10.7, 9.42, 8.47 and 8.2 for the FL, G2, AP673L and AP67, respectively (Fig. 4). According to Randall and Lopez (2014), relative chlorophyll content for Pelargonium and Salvia seedlings was 21 % and 15 % higher when those grown under 70:30 red:blue LEDs than under high pressure sodium lamps (HPS). *Castanea* seedlings showed no significant differences for the effective quantum yield between the light treatments; however higher values of F_v/F_m close to 0.81 were found for t

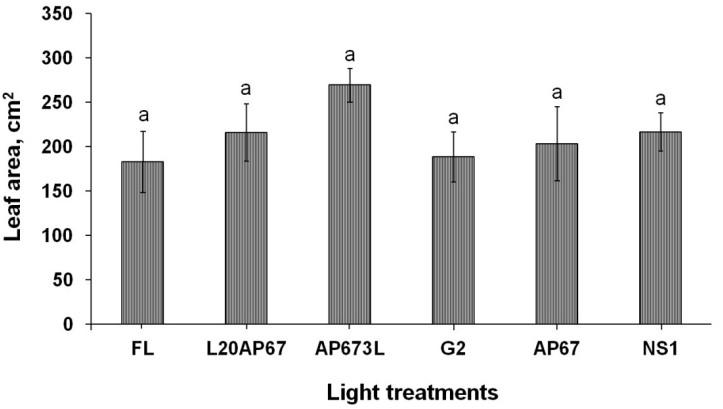

Fig. 3. Leaf area of Castanea sativa seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: No significant differences were found. Data are mean values (n = 10) ± SE.

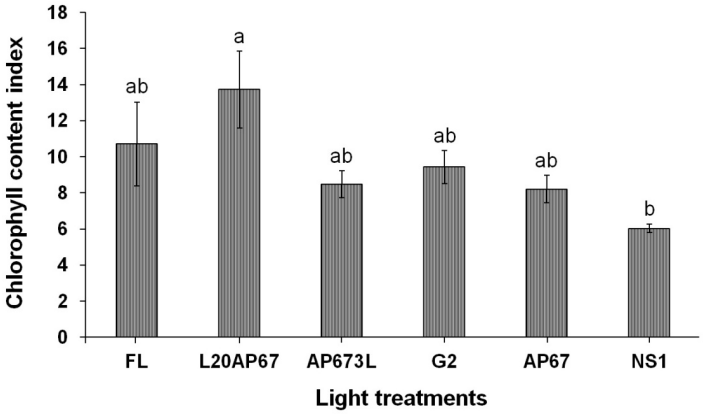

Fig. 4. Chlorophyll content index (CCI) of Castanea sativa seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 10$) ± SE.

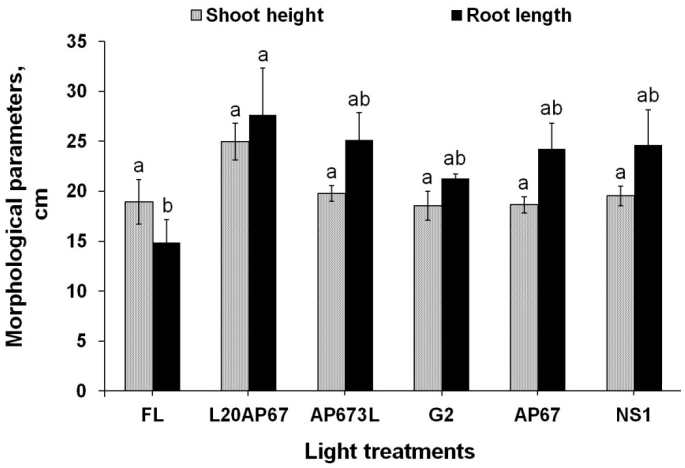

Fig. 5. Shoot height (SH) and root length (RL) of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 10$) ± SE.

he L20AP67 and the FL lights (data not shown). Despite the fact that the values found under the rest of the lights were less than the optimal value of 0.832 for F_v/F_{max}, typical of well-functioning photosynthetic apparatus (Björkman and Demmig 1987), however, this should not be a surprise as the parameter varies with species and environmental conditions (Björkman and Demmig 1987, Cha-um et al. 2010).

Shoot height of *Castanea* seedlings showed no significant differences between the different light environments; however, L20AP67 exhibited higher SH of 25 cm (Fig. 5). The rest of lights such as the AP673L, NS1, FL, AP67 and G2 showed similar shoot development of 19.78 cm, 19.53 cm, 18.94 cm, 18.64 cm and 18.53 cm, respectively (Fig. 5). Our findings were in accordance with the results of another study on the development of zygotic embryos and seedlings of *Castanea crenata* S. et Z., which showed that the hypocotyl development was not significantly affected by light quality as well (Park and Kim 2010). Shorter shoot height by 18 % was found for *Rosa* × *hybrida*, cv. Toril plants under LED lighting with 20 % blue and 80 % red compared to HPS lamps (Terfaa et al. 2013), which is in accordance with findings on several species on the inhibition of internodes growth caused by blue radiation (Folta et al. 2003, Dougher and Bugbee 2004). Maas and Bakx (1995) also showed that a decreased proportion of blue light increased the shoot length of 'Mercedes' roses. That was not the case in our findings, since the influence of light may vary from species to species, therefore the proportion of blue radiation for each of the different light treatments used showed no significant effect. However, lettuce seedlings treated with blue LEDs showed four-fold longer roots than those treated with broad-spectrum white LED or red LED alone (Kook et al. 2013). Our results revealed that L20AP67 showed significant effect on the root formation since induced the longest roots for sweet chestnut seedlings of 27.64 cm (60 % longer) compared to the FL light that induced the shortest of 14.87 cm (Fig. 5), while the rest of LEDs showed similar root length of 25.13 cm, 24.62 cm, 24.21 and 21.28 cm for the AP673L, NS1, AP67 and G2,

respectively (Fig. 5).

Root architectural analysis reveals a formal description of root systems that has important ecological applications since it reflects root plasticity responses to environmental heterogeneity and edaphic constraints to plant productivity and determines the function of roots in mechanical support of the shoots (Fitter et al. 1991, Lynch 1995, McPhee 2005). Plants can actively sense light via their roots as well, by means of the analysis both of the spectrum and the intensity by using several photoreceptors such as phytochromes, which had maximum absorption wavelength in the red and far-red spectrum that ultimately affects the root elongation and lateral root formation in *Arabidopsis* seedlings (Takano et al. 2001; Raya-Gonzalez et al. 2014). Our findings revealed that *Castanea sativa* seedlings showed denser root system under the effect of LEDs, especially benefited by the G2 LED maybe due to its higher percentage in the red region (600–700 nm) and far-red region (700-800 nm), with a ratio of 1.52, than the FL light that showed the lowest ratio of 0.85 (Fig. 6); the rest of LEDs showed root density ratios of 1.45, 1.41, 1.30 and 1.13 induced by the NS1, AP67, AP673L and L20AP67, respectively. According to Nhut et al. (2003), the highest root number of strawberry plants was found under the effect of 70 % red plus 30 % blue LEDs. High root system fibrosity is considered a parameter that improves field survival and early growth of seedlings (Schultz and Thompson 1996, Wilson et al. 2007). Also the number of FOLR remained significantly higher (more than 30) under all LED treatments inducing threefold increase compared to the conventional FL light (Fig. 7). Further AP673L and NS1 LEDs obtained significantly higher number of FOLR with diameter >1 mm and categorized to the highest fibrosity class compared to the seedlings grown under the FL light that characterized with the least fibrous root system (Fig. 7 and Table 3).

The use of monochromatic LED lights supplemented to conventional light sources such as fluorescent or HPS that stimulated t he dry weight mass of several species was

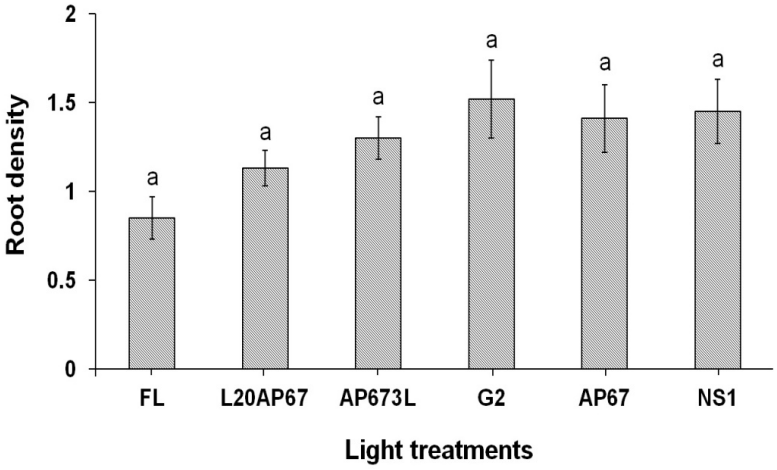

Fig. 6. Root density of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.
Note: No significant differences were found. Data are mean values (n = 5) ± SE.

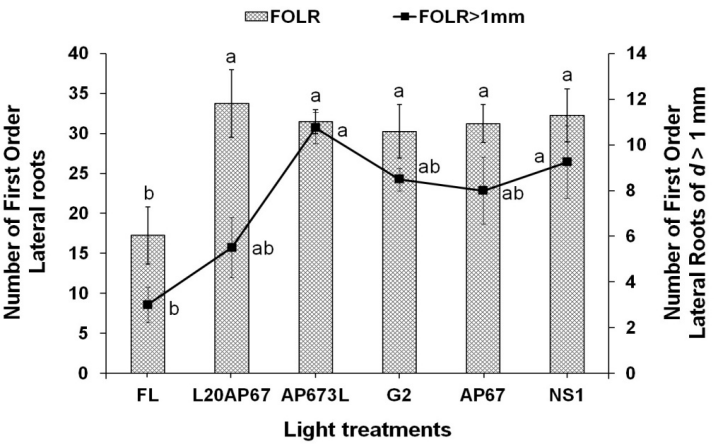

Fig. 7. Number of First Order Lateral Roots (FOLR) & number of First Order Lateral Roots of *d* > 1 mm of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.
Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 5$) ± SE.

investigated by several researchers (Hoenecke et al. 1992, Brown et al. 1995, Johkan et al. 2010, Li et al. 2012, Muneer et al. 2014). In this study the LED lights with continuous spectrum showed a predominately dry weight mass accumulation than the fluorescent con ventional light. Specifically, AP673L LED light induced significant increase of 85 % and 90.15 % in the SDW and RDW compared to the FL light (Table 4). The rest of LEDs also showed higher SDW and RDW than the FL (Table 4). As for the LDW, LEDs showed higher dry weight accumulation than the FL; however, no significant differences were found (Table 4). The relative allocation of dry weight matter to roots or shoots has been considered a key factor in plant strategies regarding water use and is very important for seedling performance and survival in the field (South 2000). Consequently

Table 4. Leaf, shoot, root dry weight (LDW,SDW,RDW) and root-to-shoot ratio (R/S) of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Light treatments	LDW	SDW	RDW	R/S ratio
FL	0.78±0.29a	0.53A±0.21b	0.99±0.28b	0.8±0.20b
L20AP67	0.99±0.34a	1.03±0.36ab	1.79±0.74ab	0.86±0.19ab
AP673L	1.41±0.24a	1.31±0.16a	2.61±0.44a	0.97±0.22ab
G2	0.98±0.34a	0.81±0.25ab	1.75±0.67ab	0.97±0.31ab
AP67	1.19±0.22a	1.00±0.22ab	2.21±0.41ab	1.01±0.18a
NS1	1.03±0.14a	0.95±0.37ab	1.80±0.88ab	0.88±0.27ab

higher allocation to the roots was obtained under LEDs than the FL, especially for the AP67 that showed significantly higher R/S ratio of 1 that characterized a more balanced allocation of dry weight mass both for the above and below parts of the plants (Table 4).

Conclusion

In the present study, the optimum light quality among LEDs of continuous spectrum and conventional fluorescent light for growing robust and healthier seedlings in controlled environment were investigated. Indeed sweet chestnut seedlings showed positive changes in growth, especially triggered by the LED light qualities compared to fluorescent light. Particularly, L20AP67 light stimulated the height growth rate, leaf, shoot and root development; G2 induced high cell division by means of higher stomatal density and index; NS1 and AP673L induced higher root fibrosity; AP673L increased significantly the shoot and root dry weight accumulation and LEDs in general induced more balance seedlings especially those cultivated under the AP67 by means of greater R/S ratio. However, further investigation needs to be done on the cultivation of forest tree species growing into growth chamber illuminated by LED lights and find out whether the possible differences between the treatments will be maintained after transplantation at the nursery or outplanting to a field site.

Acknowledgments

The authors wish to thank the European Commission for having provided funds to conduct this research, supported by the ZEPHYR, contract no: FP7308313

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