



# Application of wide-spectrum light-emitting diodes in micropropagation of popular ornamental plant species: a study on plant quality and cost reduction

Natalia Miler<sup>1</sup> · Dariusz Kulus<sup>1</sup> · Anita Woźny<sup>1</sup> · Dominika Rymarz<sup>1</sup> · Magdalena Hajzer<sup>1</sup> · Krzysztof Wierzbowski<sup>1</sup> · Robert Nelke<sup>1</sup> · Liwia Szeffs<sup>1</sup>

Received: 20 February 2018 / Accepted: 18 September 2018 / Published online: 9 October 2018 / Editor: Wagner Campos Otoni  
© The Author(s) 2018

## Abstract

In the present study, the applicability of four wide-spectrum light-emitting diodes (LEDs) emitting warm light (AP67, AP673L, G2, and NS1) was determined for the micropropagation of five popular ornamental plant species: *Chrysanthemum* × *grandiflorum*, *Gerbera jamesonii*, *Heuchera* × *hybrida*, *Ficus benjamina*, and *Lamprocapnos spectabilis*. Plantlets were grown in a growth room with a 16-h photoperiod. The photosynthetic photon flux density was set at 62–65  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The composition of the media and subculture timing were adjusted to the needs of each species tested. The results were compared to the cool daylight-emitting fluorescent (FL) control (TLD 36W/54). In most of the species studied (except for *F. benjamina*), the highest propagation ratios, or ratios similar to the FL control, were observed under the red- and far-red-abundant G2 LEDs. NS1 spectrum (with the highest proportion of blue and green light) was also efficient for *G. jamesonii* and *L. spectabilis*, and it provided a similar propagation ratio as the FL control. Light quality affected shoot length, number of leaves, callus regeneration, and the biosynthesis of chlorophyll. This influence, however, was species-dependent. Lighting conditions did not affect the dry matter and rooting in most of the species tested, except for *G. jamesonii*. The substitution of FLs with G2 LEDs can result in a 50% reduction of annual electricity costs, while the application of NS1 lamps can generate savings of up to 75%. In conclusion, the G2 LED lighting system seemed to be the most suitable in terms of propagation efficiency, plantlet quality, and cost reduction.

**Keywords** *Chrysanthemum* × *grandiflorum* · *Gerbera jamesonii* · *Heuchera* × *hybrida* · *Ficus benjamina* · *Lamprocapnos spectabilis*

## Introduction

Micropropagation is an efficient technique for mass production of ornamental and medicinal plants, some woody species (such as *Musa sapientum* L., *Pseudotsuga menziesii* (Mirb.) Franco, *Hevea brasiliensis* Muli. Arg.), energetic and oil plants, and some vegetatively propagated vegetable crops (such as *Asparagus officinalis* L. and *Solanum tuberosum*

L.) (Dickson *et al.* 2011; Kumar and Reddy 2011; Zhang *et al.* 2012; Baghel and Bansal 2015). For this purpose, explants are cultured on synthetic media in growth rooms or phytotrons, and in controlled environmental conditions. Among numerous factors such as medium composition, gas exchange in the culture vessel, ambient temperature, and explant characteristics, light is one of the most important parameters for successful *in vitro* plant production.

Even though plants kept under *in vitro* conditions are usually mixotrophic, and their photosynthesis efficiency is limited (the carbon source is supplied in the form of sugar from the medium), light still has a key influence on genes and enzymes activity, and the growth of explants (Lin *et al.* 2011; Azmi *et al.* 2016; Manivannan *et al.* 2017). Some recent studies showed that lighting conditions have a significant shift on plant primary and secondary metabolism at the genetic level, such as the kinetics of chlorophyll accumulation and

✉ Dariusz Kulus  
dkulus@gmail.com

<sup>1</sup> Faculty of Agriculture and Biotechnology, Laboratory of Ornamental Plants and Vegetable Crops, UTP University of Science and Technology, 6 Bernardyńska St, 85-029 Bydgoszcz, Poland

synthesis, and production of phenolics, flavonoids, and pigments (Darko *et al.* 2014; Reis *et al.* 2015; Bantis *et al.* 2016). On the other hand, light that is too intense can lead to the release of harmful free oxygen radicals and damage cells (Solymosi and Schoefs 2010). Therefore, the proper selection and optimization of light parameters are crucial in every plant tissue culture system.

To date, several light sources have been utilized in plant tissue culture laboratories including high-pressure sodium, metal halide, and fluorescent lamps, with the latter light sources being most popular (Gupta and Jatothu 2013). Fluorescent lamps (FLs) are versatile, as they provide a wide light spectrum (350–750 nm), which is applicable to numerous plant species, although their disadvantages include high electricity consumption, unstable radiation parameters, and considerable heat emission (Bello-Bello *et al.* 2017). Light-emitting diodes (LEDs), on the other hand, have the flexibility to deliver specific wavelengths that may yield faster or more favorable results for plant researchers and growers.

The most important advantages of LEDs include low power consumption, long lifespan, and low radiant heat directed at the plant and less heat overall, which allows LEDs to be located much closer to the plant. This close proximity provides a higher concentration of photons that leads to better photosynthetic productivity and, at the same time, air-conditioning costs in the growth room are reduced. Another benefit is that LEDs emit consistent light regardless of temperature conditions, unlike FLs that are very sensitive to the ambient temperature and airflow (Gupta and Jatothu 2013). Moreover, their construction allows for precise spectrum control and the use of monochromatic light which is valuable for research and industrial purposes, but impossible to obtain with traditional FLs. Finally, disposal of LEDs is more environmentally friendly compared to mercury-containing FLs (Morrow 2008; Bantis *et al.* 2016; Woźny and Miler 2016). The bottleneck of using LEDs is the high lighting unit price. However, high upfront costs are paid back in the form of electricity savings (Gupta and Jatothu 2013). Moreover, the cost of LEDs continues to decrease.

There are many studies focused on LED light spectrum optimization with several ornamental and usable plant species (Azmi *et al.* 2016; Bantis *et al.* 2016; Bello-Bello *et al.* 2016). Most of the research focused on various narrow-spectrum red:blue light ratios, which coincide to two areas of the chlorophyll absorption curve, and thus, are essential for photosynthesis (Gupta and Jatothu 2013; Manivannan *et al.* 2017). However, in plants, there are carotenoids, xanthophylls, and phenolics, the so-called accessory pigments that also absorb radiation (Esteban *et al.* 2015). In terms of plant production, there are only a few applications in which a pure monochromatic light spectrum yields good growth results, such as to keep plants compact or to delay plant growth. Moreover, all wavelengths between 400 and 700 nm, and also the far-red area above

700 nm, contribute to photosynthesis, and carry information about the plant's surroundings (Zhen and van Iersel 2017).

Previous studies led to the conclusion that each plant species requires a specific wavelength range for optimal growth (Gupta and Jatothu 2013; Baghel and Bansal 2015). However, companies producing plants *in vitro* usually propagate many different species, and the profile of their production changes as the year goes by. Therefore, there is an urgent need to use efficient lighting systems, with a wide wavelength range, that can be utilized with various plant materials.

The aim of the present study was to compare the morphogenetic and biochemical responses of explants to FLs and LEDs, and to determine the most cost-efficient lighting system for the *in vitro* production of *Chrysanthemum* × *grandiflorum* /Ramat./Kitam., *Gerbera jamesonii* Bolus, *Heuchera* × *hybrida* L., *Ficus benjamina* L., and *Lamprocapnos spectabilis* Fukuhara. In contrast to many other studies, in the present research, the utility of four wide-spectrum LED lamps that emit warm light was investigated. It was assumed that the application of LEDs could increase the micropropagation ratio and, at the same time, decrease the production costs of the above-mentioned species. Despite the fact that *Lamprocapnos* and *Heuchera* species are popular in traditional cultivation *in vivo* and valuable from the medicinal and economical point of view (McCutcheon *et al.* 1994; McNulty *et al.* 2007), the information on their *in vitro* culture systems is scarce, which makes the present study very important.

It was found that the application of LEDs in micropropagation can reduce electricity costs from 50 to 75%. LED lamps are less successful with *F. benjamina* but are suitable for effective *in vitro* propagation of *C. grandiflorum*, *G. jamesonii*, *H. hybrida*, and *L. spectabilis*.

## Materials and methods

**Plant material** Five popular ornamental plant species were used in the experiment, which included *Chrysanthemum* × *grandiflorum* ‘Polka,’ *Ficus benjamina* ‘Natasha,’ *Gerbera jamesonii* ‘Suri,’ *Heuchera* × *hybrida* ‘Florists Choice,’ and *Lamprocapnos spectabilis* ‘Gold Heart.’ Axenic cultures of the analyzed plants (except chrysanthemum), 10- to 12-wk-old, were obtained from the commercial plant tissue culture laboratory (Vitroflora, Łochowo, Poland). Chrysanthemum axenic cultures of the same age were provided by the *in vitro* gene bank of the Laboratory of Ornamental Plants and Vegetable Crops, Faculty of Agriculture and Biotechnology, UTP University of Science and Technology, Bydgoszcz, Poland.

**Multiplication of plant material** To obtain the required number of explants, the plantlets were multiplied by cutting into different explant types, according to the specific growth habits of

the individual plant species, 0.5–1.0-cm-long single-nodes for *C. grandiflorum* and *L. spectabilis*, 1-cm single-rosettes for *G. jamesonii* and *H. hybrida*, and 3-cm whole single-shoots for *F. benjamina*. The duration of *in vitro* multiplication (one propagation cycle) was set up individually for each species, due to differences in growth dynamics, and was as follows: *C. grandiflorum* 10 wk, *F. benjamina* 12 wk, *G. jamesonii* 8 wk, *H. hybrida* 13 wk, and *L. spectabilis* 9 wk.

Plant tissue culture media, for all species tested, were based on components of MS culture medium (Murashige and Skoog 1962), supplemented with an increase by half concentration of calcium chloride and iron sulfate, with 3% (*w/v*) sucrose and solidified with 0.8% (*w/v*) agar (Biocorp, Warsaw, Poland), except for *F. benjamina* 0.6% agar was used. The pH was adjusted to 5.8 with 0.1 M HCl and 0.1 M NaOH (Chempur, Piekary Śląskie, Poland) after adding all media components (Chemia, Bydgoszcz, Poland), prior to autoclaving at 105 kPa and 121°C for 20 min. Plant growth regulator (PGRs; all from Sigma-Aldrich®, St. Louis, MO) supplementation was as follows: no PGRs for *C. grandiflorum*; 4.0 mg L<sup>-1</sup> 6-benzyladenine (BA) and 30 mg L<sup>-1</sup> adenine sulfate for *F. benjamina*; 3.0 mg L<sup>-1</sup> kinetin for *G. jamesonii*; 0.1 mg L<sup>-1</sup> BA and 0.1 mg L<sup>-1</sup> indole-3-acetic acid (IAA) for *H. hybrida*; 0.25 mg L<sup>-1</sup> BA and 0.25 mg L<sup>-1</sup> IAA for *L. spectabilis*. The media (40 mL) were distributed into 350-mL glass jars and sealed with plastic caps. Five explants were inoculated vertically into one jar.

The cultures were maintained in uniform lighting conditions under standard cool daylight TLD 54/36W fluorescent tubes with color temperature of 6200 K (Koninklijke Philips Electronics N.V., Eindhoven, the Netherlands), photosynthetic photon flux density (PPFD) of 62–65 μmol m<sup>-2</sup> s<sup>-1</sup>, and a 16-h photoperiod. The temperature was set at 23 ± 0.5°C, while the relative humidity was at 60%.

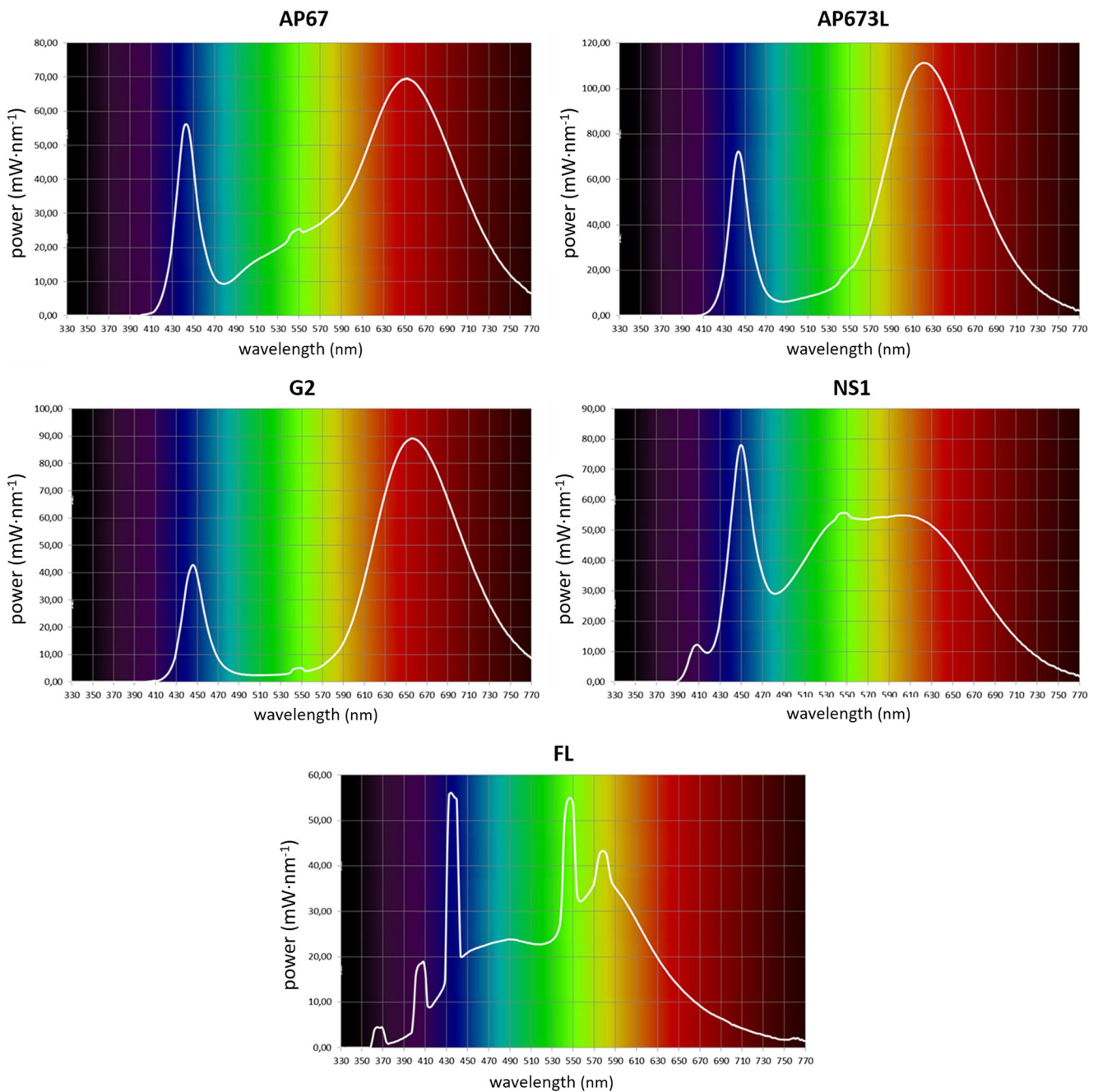
**Influence of light conditions** After multiplication, the explants (as above) were cultured in the same media, temperature, and humidity conditions for another propagation cycle. The cultures were maintained under LED tubes emitting four spectrum combinations: AP67, AP673L, G2, and NS1 (T8 tubes, L-series, Valoya, Helsinki, Finland, Table 1). Control plants were cultured under fluorescent tubes (as above). The light spectrum for each lamp was measured with a MK 350S spectrometer (UPRTEK, Zhunan, Taiwan) (Fig. 1). The PPFD was measured using a FR-10 photometer (Optel, Opole, Poland) at the central area of an empty shelf surface and was established for every tube type at 62–65 μmol m<sup>-2</sup> s<sup>-1</sup> and a 16-h photoperiod. The shelf size was 400 × 1200 mm (0.48 m<sup>2</sup>). To obtain an accurate PPFD value, various numbers of tubes (one or two) were used per one shelf (Table 2). The distance from the light source to the empty shelf surface was also adjusted (Table 2). To avoid light contamination, shelves were separated with white screens produced by a local manufacturer.

**Analyzed parameters** After a given time, plantlets of each species were carefully taken out of the culture vessels and measurements were taken. Micropropagation efficiency was defined as (1) the number of single-node offspring explants (similar to the primary ones described in the multiplication of plant material) that could be excised from a single produced shoot of *C. grandiflorum* and *L. spectabilis*, (2) the number of single-rosettes that could be excised from a multishoot of *H. hybrida* and *G. jamesonii*, and (3) the number of whole single-shoots that could be excised from a multishoot of *F. benjamina*. Shoot length (for *C. grandiflorum* and *L. spectabilis*) or multishoot length (for *H. hybrida*, *G. jamesonii*, and *F. benjamina*) was measured, and the number of leaves and roots were counted. The length of the longest root was evaluated. Total fresh weight of callus tissue (if

**Table 1.** Applied LEDs spectrum data

Color	Length (nm)	LED source			
		AP67	AP673L	G2	NS1
		LED light composition			
Ultraviolet (%)	< 400	0	0	0	1
Blue (%)	400–500	14	12	8	20
Green (%)	500–600	16	19	2	39
Red (%)	600–700	53	61	65	35
Far-red (%)	700–800	17	8	25	5
PAR (μM photons m <sup>-2</sup> s <sup>-1</sup> )	400–700	83	92	75	94
CCT (K)		2500	2000	n.a.	4800
B:G ratio		1.2	1.8	25.9	0.7
R:FR ratio		3.3	5.5	3.1	10.4

CCT correlated color temperature, B:G blue:green light ratio determines the effectiveness of the blue light response, R:FR red:far-red light ratio determines the ratio between active phytochromes (Pfr) and inactive phytochromes (Pr), n.a. not applicable



**Figure 1.** Spectral characteristics of the lamps used in the experiment, generated with a MK 350S spectrometer (UPRTEK, Zhunan, Taiwan).

present) was noted. Dry matter content in leaves (*C. grandiflorum*, *H. hybrida*, *G. jamesonii*, and *F. benjamina*), or in a whole shoot (*L. spectabilis*), was calculated as (dry matter ÷ fresh weight) × 100.

Chlorophyll content index (CCI) was measured in healthy and fully developed leaves of *C. grandiflorum*, *H. hybrida*, and *G. jamesonii* with a portable chlorophyll meter (CCM-Plus, Opti-Sciences Inc., Hudson NH). To calculate the CCI, the device measures optical absorbance in two different wavelengths (653 nm per chlorophyll and 931 nm per near-infrared). *Ficus benjamina* and *L. spectabilis* leaves were too small to take the

measurements properly; therefore, the CCI is not available for those species.

The electricity consumption costs were evaluated on the basis of an average energy price in the European Union in 2017 for the medium standard industrial consumption band, with an annual electricity consumption between 500 and 2000 MW h<sup>-1</sup>, 0.11 euro per 1 kW h<sup>-1</sup> ([http://ec.europa.eu/eurostat/statistics-explained/index.php/Electricity\\_price\\_statistics](http://ec.europa.eu/eurostat/statistics-explained/index.php/Electricity_price_statistics)).

**Statistical analysis** The study was conducted as five single-factor independent experiments in a completely randomized

**Table 2.** Characteristic of lighting used in the experiment in terms of installation, power, and energy consumption

Lamp	Number of tubes required to obtain 62–65 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Distance from the light source to shelf surface (cm)	Power consumption ( $\text{W h}^{-1}$ )		Energy consumption ( $\text{kW h}^{-1}$ )	
			Per tube	To obtain 62–65 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD	Per day	Per year
FL	2	41	36	72	1.15	419.75
AP67	2	41	18	36	0.58	211.70
AP673L	1	45	18	18	0.29	105.85
G2	2	38	18	36	0.58	211.70
NS1	1	30	18	18	0.29	105.85

design, with five or six replicates. Each replicate consisted of five to ten plantlets, depending on the species. A total of 150 plantlets of *C. grandiflorum*, 250 plantlets of *F. benjamina*, 150 plantlets of *G. jamesonii*, 250 plantlets of *H. hybrida*, and 150 plantlets of *L. spectabilis* were studied. Results were evaluated with a one-way analysis of variance (ANOVA). Means were compared using the Tukey's HSD test ( $P < 0.05$ ).

## Results and discussion

Light quality directly affects the success of a plant tissue culture system. Controlling the light quality and knowing which part (or combinations) of the spectrum areas are involved in different processes enables production of plants with desired characteristics (Bantis *et al.* 2016). The commonly applied FL lamps usually lack far red light, which is important for the plant development such as stem elongation and phytochrome activity, whereas they are abundant in green and yellow light, which are less efficient for plants (photosynthetically active radiation; PAR = 20–30%) (Shin *et al.* 2008; Fig. 1). Therefore, in the present study, wide-spectrum LEDs, which have a higher PAR share (75–94%; Table 1), with different combinations of various colors were applied. The recorded results were very often species-specific, and dependent on the parameter tested.

The most important aspect of a micropropagation protocol is its effectiveness, which is measured as the number of explants, such as microshoots and single-nodes, obtained from a single donor plant (the so-called propagation ratio). In previous studies, it was reported that the impact of light quality on this parameter is species-specific (Gupta and Jatothu 2013). Therefore, it is difficult to indicate a single light spectrum suitable for the propagation of every plant species. For example, Bello-Bello *et al.* (2016) reported that blue and red LED light alone decreased the proliferation of *Vanilla planifolia* Andrews, while white LED, the mixture of blue and red LED, and FL provided the highest propagation ratio. As for *Panax vietnamensis* Ha et Grushv., the propagation ratio

under red:blue LED light in 60:40 ratio was reported to be two times higher than under FL (11.21 and 5.8, respectively; Nhut *et al.* 2015). On the other hand, FLs, white LED, and blue LED light increased the *G. jamesonii* 'Rosalin' propagation ratio (Gök *et al.* 2016).

In the present study, the propagation ratios differed depending on the species and lamps used (Table 3). The highest propagation ratio value for *C. grandiflorum* was reported under FL and G2 LEDs. Similarly, the G2 combination provided the highest propagation ratio for *H. hybrida*. This might suggest that far-red light (not present in the FLs) is essential for the species multiplication. For *G. jamesonii*, the highest propagation efficiency was obtained under AP63L, as well as under G2 and NS1 lamps. *Lamprocapnos spectabilis* produced a similar number of secondary explants under FL and most of LEDs, except for AP673L. Surprisingly, the propagation ratio for *F. benjamina* was significantly higher under FLs compared to all LEDs used. This could result from the absence of UV or cool light in the LEDs (Fig. 1). These results show that it is possible to effectively replace FLs with LEDs in four out of the five tested plant species. The G2 spectrum was favorable in most of the species tested, probably due to the high blue:green (B:G) and low red:far-red light (R:FR) ratios (Table 1), which provide a higher portion of active phytochromes (Pham *et al.* 2018).

Light quality affects the growth of *in vitro* plants. For example, blue LED light enhanced shoot elongation in *V. planifolia* (Bello-Bello *et al.* 2016) but inhibited the growth of *Chrysanthemum* plantlets (Kurilčik *et al.* 2008). Likewise, in the present study, the spectrum composition affected shoot parameters in most of the plant species analyzed, except for *H. hybrida* (Fig. 2, Table 3). This influence was species-specific. The longest shoots of *C. grandiflorum*, with the highest number of leaves, were produced under FL and G2 spectra, probably due to the high share of red light, which was already reported to promote the elongation of internodes (Gupta and Jatothu 2013; Baghel and Bansal 2015). *Ficus benjamina* shoots grown under all LED lamps tested were considerably shorter than those grown under FL. However, shoots cultured

**Table 3.** Influence of light quality on the morphogenetic and biochemical response of *in vitro* explants under fluorescent lamps (FL) and wide-spectrum LEDs after a single propagation cycle

Lamp	<i>Chrysanthemum</i> × <i>grandiflorum</i> (10 wk)*	<i>Ficus benjamina</i> (12 wk)	<i>Gerbera jamesonii</i> (8 wk)	<i>Heuchera</i> × <i>hybrida</i> (13 wk)	<i>Lamprocapnos spectabilis</i> (9 wk)
Propagation ratio**					
FL	9.2 a	8.5 a	6.2 bc	5.3 b	2.7 a
AP67	5.5 c	4.6 b	6.1 c	5.4 b	2.2 ab
AP673L	5.0 c	4.3 b	9.2 a	4.9 b	1.3 b
G2	8.6 ab	4.5 b	7.8 abc	7.3 a	2.2 ab
NS1	6.5 bc	5.7 b	8.1 abc	4.4 b	1.6 ab
Shoot length (cm)					
FL	5.6 a	2.8 a	6.6 a	3.8 a	1.55 a
AP67	2.8 b	1.0 b	4.0 c	3.9 a	1.51 ab
AP673L	2.5 b	1.3 b	4.8 bc	3.6 a	0.83 bc
G2	5.7 a	1.3 b	4.3 c	3.6 a	1.19 abc
NS1	3.7 b	1.5 b	6.1 ab	3.4 a	0.70 c
Number of leaves in plantlets					
FL	13.8 ab	6.3 a	5.2 ab	6.8 a	7.1 a
AP67	11.8 bc	3.8 b	5.0 ab	6.0 a	5.1 ab
AP673L	10.8 c	4.9 ab	5.0 ab	7.1 a	3.6 b
G2	15.1 a	4.4 b	4.1 b	5.1 a	4.5 b
NS1	12.0 bc	5.0 ab	5.4 a	6.8 a	3.6 b
Chlorophyll content (CCI) in leaves					
FL	18.9 ab	n.a.	18.5 ab	6.4 ab	n.a.
AP67	21.0 a	n.a.	23.7 a	9.4 ab	n.a.
AP673L	20.1 ab	n.a.	14.7 b	5.2 b	n.a.
G2	16.4 b	n.a.	17.0 b	9.6 a	n.a.
NS1	17.8 ab	n.a.	14.5 b	5.8 ab	n.a.
Dry matter (%) in plantlets					
FL	7.35 a	18.49 a	14.46 a	2.66 a	15.35 a
AP67	7.60 a	19.29 a	14.32 a	1.54 a	17.69 a
AP673L	7.75 a	14.78 a	11.70 b	2.16 a	18.84 a
G2	7.73 a	22.13 a	14.46 a	2.20 a	19.33 a
NS1	7.22 a	21.52 a	14.31 a	1.28 a	16.93 a
Number of roots per plantlet					
FL	3.7 a	Absent	0.9 a	4.8 a	Absent
AP67	3.4 a	Absent	0.2 b	6.4 a	Absent
AP673L	2.9 a	Absent	0.4 b	6.4 a	Absent
G2	3.2 a	Absent	1.1 a	5.9 a	Absent
NS1	2.1 a	Absent	1.1 a	5.9 a	Absent
Root length (mm)					
FL	13.4 a	Absent	31.0 a	3.3 a	Absent
AP67	12.3 a	Absent	16.2 ab	4.0 a	Absent
AP673L	10.6 a	Absent	8.8 b	3.8 a	Absent
G2	12.6 a	Absent	16.0 ab	3.9 a	Absent
NS1	11.8 a	Absent	13.4 ab	3.6 a	Absent
Fresh weight (g) of callus regenerated at the base of shoots					
FL	Absent	0.68 b	0.73 a	Absent	0.03 b
AP67	Absent	1.14 a	0.49 b	Absent	0.06 ab
AP673L	Absent	1.25 a	0.70 a	Absent	0.08 a
G2	Absent	0.98 ab	0.64 ab	Absent	0.07 a

**Table 3.** (continued)

Lamp	<i>Chrysanthemum</i> × <i>grandiflorum</i> (10 wk)*	<i>Ficus benjamina</i> (12 wk)	<i>Gerbera jamesonii</i> (8 wk)	<i>Heuchera</i> × <i>hybrida</i> (13 wk)	<i>Lamprocapnos spectabilis</i> (9 wk)
NS1	Absent	1.05 a	0.80 a	Absent	0.06 ab

\*Duration of a single propagation. \*\*Number of offspring explants that could be excised from a single produced shoot (*C. grandiflorum*, *L. spectabilis*) or multishoot (*F. benjamina*, *G. jamesonii*, *H. hybrida*). n.a. data not available. Means in columns followed by different letters are significantly different at  $P < 0.05$  according to Tukey's test

under AP673L and NS1 produced the same number of leaves as the control, but the plantlets had shorter internodes (Table 3). The compact habit of the *F. benjamina* plantlets can be considered a positive change desired by both the consumers and producers. *Gerbera jamesonii* multishoots were of similar height under FL and NS1 lamps. As for leaves, only the far-red abundant G2 combination led to a significant decrease in their number (Table. 3). The height of *H. hybrida* multishoots and the number of leaves were not influenced by the light quality. As for *L. spectabilis*, shoots grown under FL, AP67, and G2 lamps were the longest, but the number of leaves was lower under most LED spectra compared to FLs, except for AP67.

Light directly affects the plant cell metabolic activity. The synthesis of numerous important primary and secondary metabolites takes place in plastids, such as chlorophyll and terpenoids, or requires their activity (phenylpropanoids). Therefore, by controlling lighting conditions, it is possible to stimulate or inhibit the production of those compounds (Ibrahim and Jaafar 2012). In the present study, the highest CCI was recorded in FL- and AP67-cultured plants of all tested species, and also in NS1 and AP673L LEDs for *C. grandiflorum*, as well as G2 and NS1 for *H. hybrida* (Table 3). It was previously reported that blue LED light alone increased chlorophyll content in *F. benjamina*, *Anthurium andraeanum* Linden ex André, and *Phalaenopsis* sp. (Werbrouck *et al.* 2012; Bello-Bello *et al.* 2017). However, in the present study, the NS1 lamp, with an increased blue light content, did not improve the results. This could be explained by the fact that in the earlier studies, monochromatic lights were used, while in the present research, a combination of different wavelengths was applied. In particular, the presence of the green light portion of a spectrum impairs the blue light response (Folta and Maruhnich 2007). As for *V. planifolia*, white LED light provided the highest chlorophyll content (Bello-Bello *et al.* 2016), which could also be considered for future research with the species used in the present study. Nevertheless, it can be assumed that light quality may affect the production of metabolites in the species tested, although more experiments in this field are necessary.

The share of dry matter was affected by light quality only in *G. jamesonii*, which showed the lowest dry matter percentage under AP673L LEDs (Table 3). The poor effectiveness of this spectral combination is surprising, as AP673 lamps had one of

the highest PAR share (92%). This can be explained by the fact that the tissues are cultured on a nutrient medium and photosynthesis probably is not even occurring at the tested light intensities. Another reason, of inherent internal growth limitations, was suggested by Kirschbaum (2011). In contrast, in other studies in which LED lamps with peaks in particular wavelengths were used, the influence of the light on the dry matter was evident. For example, in *Chrysanthemum* and *Gerbera*, red light decreased microshoot dry matter (Kurilčik *et al.* 2008; Gök *et al.* 2016). On the other hand, in *V. planifolia*, the application of blue or red LED light alone resulted in the lowest dry matter, while FL, white LED, and blue:red mixture (1:1) increased this parameter (Bello-Bello *et al.* 2016). The lack of significant differences in dry matter content in other plant species tested here could have resulted from non-sufficient gas exchange in the culture jars (Chen and Chen 2002), or from the presence of multichromatic wavelengths.

The root number and length were affected by light quality only in *G. jamesonii* (Tables 3). Therefore, it can be concluded that the species is the most vulnerable to light conditions. The greatest number of roots was produced under G2, NS1, and FL lamps, but the longest roots were obtained under the FL control. Roots regenerated under LEDs were somewhat shorter, which could be the impact of warm light. This result is positive from a production point of view, as it facilitates further acclimatization to *ex vitro* conditions. *Ficus benjamina* and *L. spectabilis* did not produce roots at all, irrespective of the lighting conditions.

Callus is undesirable in classical micropropagation techniques involving growth from lateral or apical buds, because it may be a source of unpredictable somaclonal variation (Miler and Zalewska 2014; Miler and Kulus 2018). On the other hand, novel transgenic or micropropagation methods, such as somatic embryogenesis, may require callus proliferation at a certain stage. Therefore, various factors (including light quality) that enhance callus regeneration were studied (Nhut *et al.* 2015). Werbrouck *et al.* (2012) observed that blue LED light stimulated callus formation in *F. benjamina* 'Exotic.' On the other hand, Nhut *et al.* (2015) reported that yellow LED light with the wavelength of 570–590 nm promoted callus development in *Panax vietnamensis*. However, red, blue, and mixtures of red:blue LED light inhibited callus proliferation. Similarly, in the present study, callus

**Figure 2.** Plantlets cultured *in vitro* under fluorescent tubes (FL) and LEDs with different wavelengths; *bar* = 3 cm.





**Table 4.** Comparison of lighting electricity costs with reference to different light sources used in plant micropropagation: fluorescent tubes (FL) and wide-spectrum LEDs

Lamp	Annual cost of lighting (euro)				Per plant in a single propagation cycle**
	Per shelf*	Per m <sup>2</sup>	Per plant	Per 1 × 10 <sup>6</sup> plants	
FL	47.85	99.67	0.60	600,000	0.12
AP67	24.13	50.26	0.30	300,000	0.06
AP673L	12.07	25.14	0.15	150,000	0.03
G2	24.13	50.26	0.30	300,000	0.06
NS1	12.07	25.14	0.15	150,000	0.03

\*Shelf size 400 × 1200 mm (0.48 m<sup>2</sup>). \*\*Average duration of a single propagation cycle was estimated as 10.4 wk., based on the mean growth dynamics of the species tested

regeneration was affected by light quality in most species tested (Table 3). With *F. benjamina* and *L. spectabilis*, callus weight was the lowest under the FL control, while for *G. jamesonii*, it was lowest under AP67 lamps. This could result from the relatively low B:G and R:FR ratios. No callus was formed by *C. grandiflorum* and *H. hybrida* plantlets. Therefore, LED lamps could be considered in organogenesis studies in *F. benjamina* and *L. spectabilis*, and indirect somatic embryogenesis research if the embryogenic potential of callus is confirmed.

High costs are the main limiting factors of commercial laboratory plant production (Kulus 2015). The expenditure on electricity is one of the highest among various expenses in micropropagation (Chen 2016). It can constitute from 20% to even 60% of the total costs, depending on the country and the local climate (Tomar *et al.* 2007). Lighting control consumes a gross share of this pool (up to 85%) (Kulus 2015). Fortunately, the development of LED technology can greatly reduce those costs, as demonstrated in the present research. The replacement of FLs with LEDs can result in a daily power consumption decrease to 0.29 kW h<sup>-1</sup> (Table 2). Consequently, replacing FLs with G2 LEDs can provide a 50% reduction of annual costs of electricity for lighting, while the application of NS1 lamps can result in 75% cost savings (Table 4). The production electricity costs of a single microshoot can decline to even 0.03 euro. This demonstrates the legitimacy of applying LEDs in plant tissue culture laboratories. In future studies, it would be beneficial to analyze how LEDs lower the heat emission and reduce the cooling costs in the growth room.

## Conclusions

Light quality affects the laboratory production of plants, and thus, also plant breeding or genetic modification programs. The application of wide-spectrum LED lamps in micropropagation allows producers and researchers to mix wavelengths that effectively promote consistent and healthy

plant growth. In most of the species tested (except for *F. benjamina*), the common denominator was G2, because it provided a propagation ratio as high as the FL control for *C. grandiflorum*, *G. jamesonii*, *L. spectabilis*, and even higher than FL with *H. hybrida*. This could be due to a high share of both red and far-red light (65 and 25%, respectively). Therefore, it can be concluded that the rate of photosynthesis was optimal when both red and far-red wavelengths were given together, rather than the sum of the rates when given apart. The second most-efficient lamp type was the blue- and green-light-abundant NS1, because it gave a similar propagation ratio as the FL control for *G. jamesonii*, *H. hybrida*, and *L. spectabilis*. This demonstrated the importance of those two lights in plant morphogenesis *in vitro*. The application of LED technology in plant production process also affects the economy, by bringing tremendous savings (at the level of up to 75%) in annual electricity expenses and consumption, which are significant in the total *in vitro* production costs.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## References

- Azmi NS, Ahmad R, Ibrahim R (2016) Fluorescent light (FL), red led and blue led spectrums effects on *in vitro* shoots multiplication. *J Teknologi* 78:93–97. <https://doi.org/10.11113/jt.v78.9032>
- Baghel S, Bansal YK (2015) *In vitro* regeneration of oil yielding plants - a review. *J Essent Oil Bear Plants* 18:1022–1050. <https://doi.org/10.1080/0972060X.2014.971068>
- Bantis F, Ouzounis T, Radoglou K (2016) Artificial LED lighting enhances growth characteristics and total phenolic content of

- Ocimum basilicum*, but variably affects transplant success. *Sci Hortic* 198:277–283. <https://doi.org/10.1016/j.scienta.2015.11.014>
- Bello-Bello JJ, Martinez-Estrada E, Caamal-Velazquez JH, Morales-Ramos V (2016) Effect of LED light quality on *in vitro* shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews). *Afr J Biotechnol* 15:272–277. <https://doi.org/10.5897/AJB2015.14662>
- Bello-Bello JJ, Perez-Sato JA, Cruz-Cruz CA, Martinez-Estrada E (2017) Light-emitting diodes: progress in plant micropropagation. *InTech* 6:93–103
- Chen C (2016) Cost analysis of plant micropropagation of *Phalaenopsis*. *Plant Cell Tissue Organ Cult* 126:167–175. <https://doi.org/10.1007/s11240-016-0987-4>
- Chen C, Chen J-J (2002) Measurement of gas exchange rates in plant tissue culture vessels. *Plant Cell Tissue Organ Cult* 71:103–109. <https://doi.org/10.1023/A:1019909520132>
- Darko E, Heydarizadeh P, Schoefs B, Sabzalian MR (2014) Photosynthesis under artificial light: the shift in primary and secondary metabolism. *Philos Trans R Soc Lond Ser B Biol Sci* 369:20130243. <https://doi.org/10.1098/rstb.2013.0243>
- Dickson I, Okere A, Jammadin E, Olayode M, Olatunde F, Abiodun S (2011) *In-vitro* culture of *Hevea brasiliensis* (rubber tree) embryo. *J Plant Breed Crop Sci* 3:185–189
- Esteban R, Barrutia O, Artetxe U, Fernández-Marín B, Hernández A, García-Plazaola JI (2015) Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. *New Phytol* 206:268–280. <https://doi.org/10.1111/nph.13186>
- Folta KM, Maruhnich SA (2007) Green light: a signal to slow down or stop. *J Exp Bot* 58:3099–3111. <https://doi.org/10.1093/jxb/ern130>
- Gök KM, Şan B, Bayhan AK (2016) Micropropagation of gerbera (*Gerbera jamesonii* Bolus) under different color of light-emitting diodes. *J Nat App Sci* 20:468–474. <https://doi.org/10.19113/sdufbed.13860>
- Gupta SD, Jatothu B (2013) Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnol Rep* 7:211–220. <https://doi.org/10.1007/s11816-013-0277-0>
- Ibrahim MH, Jaafar HZ (2012) Primary, secondary metabolites, H<sub>2</sub>O<sub>2</sub>, malondialdehyde and photosynthetic responses of *Orthosiphon stamineus* Benth. To different irradiance levels. *Molecules* 17:1159–1176. <https://doi.org/10.3390/molecules17021159>
- Kirschbaum MUF (2011) Does enhanced photosynthesis enhance growth? Lessons learned from CO<sub>2</sub> enrichment studies. *Plant Physiol* 155:117–124. <https://doi.org/10.1104/pp.110.166819>
- Kulus D (2015) Selected aspects of ornamental plants micropropagation in Poland and worldwide. *Life Sci* 4:10–25. <https://doi.org/10.13140/RG.2.1.5086.8082>
- Kumar N, Reddy MP (2011) *In vitro* plant propagation: a review. *J Forest* 27:61–72
- Kurilčik A, Canova MR, Dapkuniene S, Zilinskaite S, Kurilčik G, Tamulaitis G, Duchovskis P, Zukauskas A (2008) *In vitro* culture of *Chrysanthemum* plantlets using light emitting diodes. *Cent Eur J Biol* 3:161–167. <https://doi.org/10.2478/s11535-008-0006-9>
- Lin Y, Li J, Li B, He T, Chun Z (2011) Effects of light quality on growth and development of protocorm-like bodies of *Dendrobium officinale* *in vitro*. *Plant Cell Tissue Organ Cult* 105:329–335. <https://doi.org/10.1007/s11240-010-9871-9>
- Manivannan A, Soundararajan P, Park YG, Wei H, Kim SH, Jeong BR (2017) Blue and red light-emitting diodes improve the growth and physiology of *in vitro* grown carnations ‘Green Beauty’ and ‘Purple Beauty’. *Hortic Environ Biotechnol* 58:12–20. <https://doi.org/10.1007/s13580-017-0051-2>
- McCutcheon AR, Ellis SM, Hancock REW, Towers GHN (1994) Antifungal screening of medicinal plants of British Columbian native peoples. *J Ethnopharmacol* 44:157–169. [https://doi.org/10.1016/0378-8741\(94\)01183-4](https://doi.org/10.1016/0378-8741(94)01183-4)
- McNulty J, Poloczek J, Larichev V, Werstiuk NH, Griffin C, Pandey S (2007) Discovery of the apoptosis-inducing activity and high accumulation of the butenolides, menisdaurilide and aquilegiolide in *Dicentra spectabilis*. *Planta Med* 73:1543–1547. <https://doi.org/10.1055/s-2007-990264>
- Miler N, Kulus D (2018) Microwave treatment can induce chrysanthemum phenotypic and genetic changes. *Sci Hortic* 227:223–233. <https://doi.org/10.1016/j.scienta.2017.09.047>
- Miler N, Zalewska M (2014) Somaclonal variation of chrysanthemum propagated *in vitro* from different explant types. *Acta Sci Pol Hort Cult* 13:69–82
- Morrow RC (2008) LED lighting in horticulture. *HortSci* 43:1947–1950
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Nhut DT, Huy NP, Tai NT, Nam NB, Luan VQ, Hien VT, Tung HT, Vinh BT, Luan TC (2015) Light-emitting diodes and their potential in callus growth, plantlet development and saponin accumulation during somatic embryogenesis of *Panax vietnamensis* Ha et Grushv. *Biotechnol Biotechnol Equip* 29:299–308. <https://doi.org/10.1080/13102818.2014.1000210>
- Pham VN, Kathare PK, Huq E (2018) Phytochromes and phytochrome interacting factors. *Plant Physiol* 176:1025–1038. <https://doi.org/10.1104/pp.17.01384>
- Reis A, Kleinowski AM, Klein FRS, Telles RT, do Amarante L, Braga EJB (2015) Light quality on the *in vitro* growth and production of pigments in the genus *Alternanthera*. *J Crop Sci Biotechnol* 18:349–357. <https://doi.org/10.1007/s12892-015-0074-0>
- Shin SK, Murthy NH, Heo WJ, Hahn JE, Paek YK (2008) The effect of light quality on the growth and development of *in vitro* cultured *Doritaenopsis* plants. *Acta Physiol Plantae* 30:339–343. <https://doi.org/10.1007/s11738-007-0128-0>
- Solymosi K, Schoefs B (2010) Etioplast and etio-chloroplast formation under natural conditions: the dark side of chlorophyll biosynthesis in angiosperms. *Photosynth Res* 105:143–166. <https://doi.org/10.1007/s1120-010-9568-2>
- Tomar UK, Negi U, Sinha AK, Kumar P (2007) An overview on economic factors influencing micropropagation. *My Forest* 43:523–534
- Werbrouck S, Buyle H, Geelen D, Van Labeke MC (2012) Effect of red-, far-red- and blue-light emitting diodes on *in vitro* growth of *Ficus benjamina*. *Acta Hortic* (961):533–538. <https://doi.org/10.17660/ActaHortic.2012.961.70>
- Woźny A, Miler N (2016) LEDs application in *ex vitro* rooting and acclimatization of chrysanthemum (*Chrysanthemum × grandiflorum* /Ramat./ Kitam.). *Electron J Polish Agric Univ* 19:#02
- Zhang XQ, Sun Y, Hu KH, Chen B, Hong CT, Guo HP, Pan YH, Zheng BS (2012) Micropropagation and plant regeneration from embryogenic callus of *Miscanthus sinensis*. *In Vitro Cell Dev Biol - Plant* 48:50–57. <https://doi.org/10.1007/s11627-011-9387-y>
- Zhen S, van Iersel MW (2017) Far-red light is needed for efficient photochemistry and photosynthesis. *J Plant Physiol* 209:115–122. <https://doi.org/10.1016/j.jplph.2016.12.004>