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**Physiological response of *Dieffenbachia maculata* “Compacta”and *Fittonia* *verschaffeltii* under three different sources of continuous artificial lighting**

**Eva María Almansa1, Antonio Espín2, Rosa María Chica3 and María Teresa Lao1\***

*1Agronomy Department of Higher Polytechnic School and Experimental Science College, University of Almeria, Agrifood Campus of International Excellence ceiA3. Ctra. Sacramento s/n, La Cañada de San Urbano, 04120, Almería, Spain;* \*e-mail: [mtlao@ual.es](mailto:mtlao@ual.es)

*2Civil Engineering Department. E.T.S Ingenieros de Caminos, Canales y Puertos.  
University of Granada. Campus de Fuentenueva. C/ Severo Ochoa s/n 18071 Granada. Spain.,* e-mail: [aespin@ugr.es](mailto:aespin@ugr.es)

*3 Engineering Department. Superior Polytechnic School and Faculty of Experimental Sciences. University of Almeria. CEIA3. Ctra. Sacramento s/n. La Cañada de San Urbano 04120 Almería. Spain.* e-mail: [rmchica@ual.es](mailto:rmchica@ual.es)

***Abstract.*** The use of fluorescent lamps in ornamental greenhouses, presents wide possibilities to improve ornamental crops. *D. maculata*  Schott*“*Compacta” and *F. Verschaffeltii* Coëmare ornamental small plants, attractive for their coloured leaves and ideal for shady places. The experimental design consisted of three continuous artificial lighting treatments (standards fluorescent lamps, standards fluorescent lamps mixed with Light Emitting Diode –LED- and high efficiency fluorescent lamps) with four replicates per treatment. Spectrum of light radiation sources, plant parameters: fresh and dry biomass partitioning, indole-3-acetic acid, proline, starch and reduced sugars were measured. Standard fluorescent lamps increase the biomass of both species related with high PAR (Photosynthetically Active Radiation). The fluorescent enriched with a mixture of red and blue LEDs provides to *D. maculata* greater capacity for synthesis of auxin. *F. Verschaffeltii* presents in this lighting mix lower indole-3-acetic acid concentration related with higher root dry weight than the other treatments. The different lighting treatments have not produced significant effects in the proline production. *D. maculata* and *F.* *verschaffeltii* have an inverse behaviour regarding the starch storage, indicating a different sensitivity to different spectral regions. These are the parameters B:R (Blue:Red) and R:FR (Red:Far-red) that determine the behaviour of these species to a light treatments with artificial light sources.

***Key words****:* Fluorescent lamp; LED; Auxin; Proline; Carbohydrates.

**INTRODUCTION**

The most useful lamps in nurseries are fluorescent lamps, specially, conventional and high efficiency fluorescents. *D. maculata*  Schott*“*Compacta” and *F. Verschaffeltii* Coëmbelong to the *Araceae* and *Acanthaceae* familys respectively; both are popular plants of indoor foliage due their beautiful pigments in leaves (Pataky, 2001; Buldewo and Jaufeerally-Fakim, 2002) and ideals for shady places (Ojasti, 2001).

Auxin or indole-3-acetic acid (IAA) is a phytohormone that takes part improving the rooting because it induces the cellular development and stimulates the growth of the roots (Hartmann et al., 2002; Buitrago and Ramírez, 2003). Auxin levels are related to quality (Kurepin et al., 2007a) and intensity (Kurepin et al., 2008) of radiation. The auxin can be synthesized in the leaves and travel for polar transport promoting the growth of the plants (Barbez et al., 2012). The light is an environmental factor that has a strong effect in the lateral transport of the IAA and therefore in the distribution of the auxin in the plant (Salisbury and Ross, 1994).

Proline has demonstrated to provide protection against the free radical ones induced by the solar damage (Alia et al., 1991; Alia et al., 1993). Alia et al. (1997) showed that proline is involved in reducing the 1O2 level, and thereby prevents damage to thylakoid membranes during exposure to bright light.

Pardha Saradhi et al. (1995) reported the accumulation of proline in plants exposed to ultraviolet (UV). The PAR interception by the leaves is the main process for biomass production (Plénet et al., 2000). Plants must achieve a balance between carbon assimilation, carbohydrates storage and plant growth. Smith and Stitt (2007) describe the existence of regulatory mechanisms that coordinate the supply and use of carbon, and the likely role of sugar signalling, through new approaches to control the level of starch synthesis and degradation in leaves.

By now there were only imported a lighting adapted to the human eye, not taking into account important to the canopy as the intensity of radiation received, quality of light, spectrometric distribution of this radiation and the duration of light aspects. Responding to a need for knowledge of the preferences of environmental users, Scott (1993) studied commonly preferred visual attributes of interiors. People prefer environments that are stimulating and involving, and include natural contents and balanced opportunities for “prospect” and “refuge”. The aim of this work is to know how the quality of light affects physiologically and morphologically two species of ornamental plants, *D. maculata* and *F.* *verschaffeltii*, cultivated under two artificial light sources, conventional fluorescent and high efficiency fluorescent, and the complement one of them with LEDs (Light Emitting Diodes).

**MATERIALS AND METHODS**

The experiment was carried out with two ornamental species: *D.* *compacta* and *F. verschaffeltii* in a controlled environment chamber (temperature of 25 ºC and 55% of relative humidity). Into the chamber there are an essay table with 3 light levels (one per treatment).

The experimental design consisted of three continuous artificial lighting treatments (Table 1) with 4 plants per replication and 4 replications per treatment.

The spectrum of light radiation sources in each treatment was measured at the canopy level with spectroradiometer Licor 1800 (LI-COR inc. PO Box 4425 Lincoln, Nebraska 68504 USA). Growth parameters were registered at the end of the cultivation, 60 days after the beginning of the trial. Each plant of each replication and treatment was sampled at the end of the trial. Roots, stems and leaves were weighted separately on a COBOS series CSC scale (precision 0.01 g) to determine fresh weight. Afterwards, the material were decontaminated with a 1% non ionic detergent solution and rinsed three times in distilled water (Wolf, 1982) and then blotted on filter paper and were dried in a NüveE FN500 oven (range 30 to 300 ºC) at 60ºC for 48 h to determine dry weight. Extraction for determination of proline, auxine, sugars and starch was made by grinding fresh roots, stems and leaves with 95% + 70% (v/v) ethanol. After filtering and centrifugation the at 5500 rpm for 10 min, proline was measured using the ninhydrin method and sugars with antrone reactive (Irigoyen et al., 1992); auxine was quantified by Salkowski method (Tien et al., 1979) by means of colorimetric methods using a spectrophotometer (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). The residue was dried up for 48 h at 40 ºC for the determination of the starch concentration, via the incubation with a-glucoamilase, measuring the resulting sugars (Irigoyen et al., 1992).

Tukey’s multiple range tests were used to test the difference between more than two means at the 0.05 significance level using the statistics software Statgraphics (Stat-Point, Herndon, VA) plus 4.0.

**RESULTS AND DISCUSSION**

***Spectral quality of light sources***

Agronomic characterization of light treatments according to Baille et al. (2003) is presented in table 2. There are important differences between power lamps specifications and experimental values, which could be related with type of luminary and physical distribution in space of these light sources.

The combination of standard fluorescent lamps with LED modules (TLD + LEDs) reduces UV (ultraviolet) received by the plants. This is considered an advantage for plants and operators. UV radiation perceived by phytochromes and blue radiation receptors (B) regulate the adaptive responses of the plant to high irradiance conditions (Wellman, 1983). These responses are related with differential accumulation of carbohydrates and/or products of secondary metabolism (Ballaré et al., 1995). In addition, workers can suffer eye damage by UV radiation if the lamp exceeds of 104 J·m-2, with a exposure limit time of 8 h, according to Directive 2006/25/EC of artificial optical radiation (UNE-EN 62471 photobiological safety of lamps; Considerations for General Lighting Products).

Phytochromes play host to the bands of R (Red) and FR (Far-red), and they are related to organogenesis, photomorphogenesis and adaptation to the length of the photoperiod and the adjustment to the changing irradiance conditions (Deng, 1994).

The red region (R) of fluorescent lamps plays an important role in promoting the development of plant, independently of photoperiodism and vernalisation processes (Kasajima et al., 2007). TL5 emits the highest radiation in this band. But the combination of fluorescents with blue and red LEDs (TLD+LEDs) appears to be beneficial for young plants, especially in continuous light as reported by Homma et al. (2009) in *Camellia sinensis* L., because blue light only or blue mixed with red light indicated that blue light-containing irradiation produced higher plant biomass (Matsuda et al., 2008). The lamps essayed present higher value of FR band, similar to levels reported by Sato et al. (2009) where the use of red light (> 0.7 µmol·m-2·s-1) and continuous light promoted a higher biomass of *Eustoma grandiflorum*. High FR light used as supplemental lighting, reduces symptoms of leaf tomato damage caused by white light (Globig et al., 1997). TL5 presents the highest values FR, R:FR and the lowest B:R of lamps essayed. The distribution of biomass between root and aerial parts, length of petioles and leaf morphology depend on the ratio R:FR and also on the ratio B:R (Kasperbauer and Hunt, 1990; Benavides, 1998). The lower R:FR causes a reduction in the proportion of phytochromes that are in the active (Pfr) form and this reduction, in turn, stimulates stem elongation (Ballaré et al., 2006). High B:R ratios are able to reduce the height of gardenia plants (Lykas et al., 2008). On the other hand, the respiratory rate and accumulation of non-structural carbohydrates depend on relative enrichment of Red light compared to Blue or UV, both of two decreased respiration and photosynthetic capacity (Kowallik, 1982; Britz and Adamse, 1994).

TLD increased the biomass of both species by high PAR (400-700 nm) that activated the rate of photosynthesis and the formation of storage organs, the greater biomass and greater root weight in agreement with Akira et al. (1988).

The near-infrared radiation (NIR) is related to stomatal conductance independently of leaf temperature, favouring the stimulation of photosynthesis and transpiration in leaves (Pieruschka et al., 2010). TL5 lamps presented the highest NIR. Consistent with Kurepin et al. (2007b), we have found chlorosis in plants due to the combination of low PAR and high R:FR ratio under standard fluorescent enriched with LEDs treatment.

***Biomass evaluation***

No significant differences of fresh weight were found between treatments in different organs, for the both studied species(see Fig. 1 a). Plants under TLD+LEDs present lower dry weight in both species (Fig. 1 b) due to the lowest PAR, in accordance with Akira et al. (1988).

The partitioning dry weight in root, stem and leaf of both species are presented in Fig. 2. Both species present a differential behaviour that could be related with the differential sensitivity of light spectral regions. In *D. compacta* (Fig. 2 a) does not present significant differences of dry weight partitioning under light treatments. *F.* *verschaffeltii* (Fig. 2 b) leaves dry weight do not present significant differences between treatments. Nevertheless, there is a significant (P<0.05) increasing of root dry weight related with a decrease of stem dry weight under TLD + LEDs lamps. It could be due to the spectral balance R:FR (17.65) and the relative content of blue light on the red (B:R=1) according with Kasperbauer and Hunt (1990) and Benavides (1998) in *Gossypium hirsutum* L., *Lactuca sativa* L. and *Spinacia oleracea* L. respectively.

***Endogenous IAA levels in leaves***

There are significant differences between treatments and species responses. The differences between species could be related with the plant sensitivity of different band of the spectrum. On the other hand, auxins are synthesised in shoot apex and young leaves (Ljung et al., 2005) but they are transports to roots. *D. compacta* presents greater capacity for synthesis of auxin under TLD+LEDs (Fig. 3), this results are agree with Behringer and Davies (1992), they found a positive relationship between high FR and elevated IAA in seedlings of *P. sativum* andKurepin et al. (2007b), considering PAR radiation delivered at low levels combined with a high ratio R:FR produced significantly lowered endogenous indole-3-acetic acid (IAA) levels.  *F.* *verschaffeltii* presents in TLD + LEDs lower IAA concentration but higher root dry weight that could be related with high auxin transport capacity. In this sense, Mapelli et al. (1978) consider that there are species sensitive to the proportion between red and blue light (B:R = 1) provided by the light source, along with UV and NIR. The effects of these spectral regions favour the development of plant by cell polar transport produced from the leaves (of synthesis) to the roots.

***Proline production as evidence of stress lighting***

The different lighting treatments have not produced significant effects (NS) in leaf proline concentration in both species studied (Fig. 4). However, both species are different in production. *F.* *verschaffeltii* presents higher concentration of proline than *D. compacta*. Singh and Pandey (2011) measured proline content in *Pistia stratiotes L.* leaves under lighting stress conditions. Proline concentration of 0.80 µg·g-1 FW was found for control treatment, similar to *F.* *verschaffeltii*. ***Total carbohydrates: reduction sugars and starch***

There were significant differences in sugars between treatments and species responses, but not significant differences (NS) were found in starch (see Fig.5). Carbohydrate concentrations of *D. Compacta* were higher than *F.* *verschaffeltii.*  Photosynthetic carbon assimilation only occurs under the action of light, but the growth and maintenance of plants require carbon throughout the day-night cycle (Nozue and Maloof, 2006). In *Arabidopsis* (Caspar et al., 1985; Gibon et al, 2004; Lu et al., 2005) and many other plant species (Geiger et al., 2000), products of photosynthetic carbon assimilation in the light is split between sucrose (immediately available for growth) and starch, which accumulates in the leaves during the day. At night, starch is degraded to produce sucrose (Smith and Stitt, 2007). Starch accumulation in leaves is higher than sugars in both species. This may be because there is not photoperiod, so it does not degrade the starch and it’s accumulated as needed for growth. However, the production of carbohydrates occurs in reverse between species, significant differences between light treatments (Fig. 5). Carbohydratesof *D. compacta* could be related with PAR. However, *F.* *verschaffeltii* presents the opposite behaviour. It can be related with protective UV-B mechanisms: epidermal pigments, mainly flavonoids, acting to absorb UV-B radiation, while minimizing absorption of photosynthetic wavelengths (Caldwell et al. 1983, 1989) and epicuticular waxes, and hairs of some species have been reported to contain UV-B-absorbing compounds (Skaltsa et al. 1994). High UV-B levels develop glaucous or pubescent leaves (Barnes et al. 1996). UV protection have could be developed in TLD and TL5 and the reflectance of potentially damaging UV radiation can substantially reduce the amount of PAR arriving at the leaf surface (Holmes and Keiller, 2002) related with a lower carbohydrate synthesis. Also the highest PAR:NIR ratio in TLD + LEDs, related with the stimuli of NIR on leave temperature and respiration (Umbach et al., 2008) could contribute to increase carbohydrates.

**CONCLUSIONS**

The sensibility of *D. compacta* and *F. verscafeltti* to spectral quality of light treatments was different considering *F. Verscafeltti* more sensible than *D. compacta.*

The use of TLD lamps supplemental with LEDs reduced the plant size due to low PAR value. Effect related to the experimental design placement of lamps, produces plants with greater root mass and more compact. They can relate to the spectral enrichment because the LEDs on the parameters R:B and R:FR and its effect on the concentration of auxin and their transport. Also, it was observed a significant increase of carbohydrates concentration related with PAR in *D. compacta*. The high ratio starch/sugars may be due to the use of continuous light. Species studied did not exhibit symptoms of stress, valued in function of proline concentration.

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Table 1. Lighting per treatments arranged by level. The treatments were maintained during the 24-hour photoperiod.

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Lighting** | **Power (W)** | |
| TLD | Fluorescent TLD 18W (4 light Philips TCS097 x 2 lamps) | | 144 |
| TLD + LEDs | Fluorescent TLD 18W (3 light Philips TCS097 x 2 lamps) + Pure Blue and Pure Red Mix-Light-Emitting Diodes (BR-LEDs) RGB (4 lines ALUM 40 x 25 LED SMD RGB x 9 W with console DN-RGB FIBER LIGHT) | | 144 |
| TL5 | High Efficiency Fluorescent TL5 35W (2 light MAXOS 4M691 x 2 lamps) | | 140 |

*TL5: Tubular Lamp with the diameter of the bulb in eighths of an inch 5/8”; TLD: Standard Tubular Lamp Don’s bulb; TCS: Transmission Control System; BR-LEDs: Blue Red Light Emitting Diodes; ALUM: Aluminum; LED: Light Emitting Diode; SMD: Surface Mount Device; RGB: Red, Green and Blue colors; DN: Denon electronics brand.*

**Table 2.** Agronomic characterization of the radiation received by the different lighting treatments on plant canopy with high efficiency fluorescents, standard fluorescents and standard fluorescents enriched with red (636 nm) and blue (470 nm) LEDs.

|  |  |  |  |
| --- | --- | --- | --- |
| Radiation (µmol·m-2·s-1) | | | |
| Wavelength (nanometers) | **TLD** | **TLD+LEDs** | **TL5** |
| UV (300-400) | 0.81 | **0.41** | 1.89 |
| B (400-500) | **30.52** | 26.22 | 20.16 |
| R (600-700) | 34.58 | 26.26 | **39.61** |
| FR (700-800) | 2.92 | 1.49 | **4.14** |
| PAR (400-700) | **111.96** | 77.32 | 98.43 |
| NIR (700-1100) | 4.21 | 2.12 | **7.32** |
| TOTAL (300-1100) | 116.0 | 79.31 | 106.84 |
| PAR:TOTAL | 0.97 | 0.97 | 0.92 |
| PAR:NIR | 26.58 | **36.49** | 13.45 |
| B:R | 0.88 | **1.00** | 0.51 |
| B:FR | 10.45 | 17.62 | **4.87** |
| R:FR | 11.84 | 17.65 | **9.58** |

*UV: Ultraviolet; B: Blue; R: Red; FR: Far Red; PAR: Photosynthetic Active Radiation; NIR: Near Infrared.*





**Fig. 1.** *D. compacta* and *F.* *verschaffeltii* total fresh (a) and dry (b) weights (g) of 59 days grown under light irradiances: low photosynthetically active radiation (PAR) provide by artificial lightings (standard fluorescents TLD, standard fluorescents TLD with LEDs and high efficiency fluorescents TL5). The error bars indicate one SE of the mean, and mean values with the same letter do not differ significantly at P < 0.05 based on Tukey’s multiple comparison test.



**Fig. 2.** *D. compacta* (a) and *F.* *verschaffeltii* (b) weights (%) of 59 days grown under light irradiances: low photosynthetically active radiation (PAR) provide by artificial lightings (standard fluorescents TLD, standard fluorescents TLD with LEDs and high efficiency fluorescents TL5).



**Fig. 3.** Indole-3-acetic acid (IAA) levels in leaves harvested from 59 days grown under light irradiances: low photosynthetically active radiation (PAR) provide by artificial lightings (standard fluorescents TLD, standard fluorescents TLD with LEDs and high efficiency fluorescents TL5). Mean values with the same letter do not differ significantly at P = 0.05 based on Tukey’s multiple comparison test. FW, fresh weight.



**Fig. 4.** Proline levels in leaves harvested from 59 days grown under light irradiances: low photosynthetically active radiation (PAR) provide by artificial lightings (standard fluorescents TLD, standard fluorescents TLD with LEDs and high efficiency fluorescents TL5). The error bars indicate one SE of the mean. FW, fresh weight.



**Fig. 5.** Leaf’s starch and sugars contents in *D. compacta* and *F.* *verschaffeltii* of 59 days under artificial lightings (standard fluorescents TLD, standard fluorescents TLD with LEDs and high efficiency fluorescents TL5). Plants were grown in a no cycle light-dark (24 h continuous lighting). Mean values in sugars with the same letter do not differ significantly at P < 0.05 based on Tukey’s multiple comparison test. NS were founded to starch accumulation. FW, fresh weight.