IN VITRO DEVELOPMENT OF YELLOW LAPACHO (BIGNONIACEAE) USING HIGH-POWER LIGHT EMITTING DIODE [1]

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ABSTRACT – *Handroanthus ochraceus* (yellow lapacho) is a medicinal, ornamental and timber tree which can be propagated by *in vitro* culture. Conventional methods use fluorescent lighting (FL), whereas light emitting diode (LED) has been used for this purpose only recently. The aim of this work was to evaluate the effects of FL and high-power LED (HP-LED) on the *in vitro* multiplication and rooting of yellow lapacho at different irradiances (15 to 60 μmol $m^{-2}s^{-1}$). Epicotyls obtained from half-siblings was multiplicated in WPM (Woody Plant Medium) supplemented with 20 μM benzilaminopurine and 1 mM IBA (indolebutiric acid). For rooting, shoots were cultured for 3 days in ½WPM supplemented with 50 μM IBA and for 42 days in auxin-free ½WPM under HP-LED or FL lighting. Under HP-LED, the multiplication rate of shoots increased significantly (61%) from 20 to 40 μmol $m^{-2}s^{-1}$ respect to FL. Differences in abaxial stomatal density and size were observed between light sources at 20 μmol $m^{-2}s^{-1}$. High HP-LED irradiance produced the highest rooting percentage. In the rooting stage, the marginal means of treatments without factors interaction showed that HP-LED irradiances significantly increased shoot length by 20%, shoot fresh weight by 77% and shoot dry weight by 30% in comparison to the values under FL. The maximum values calculated from the regression curves were around 50 μmol m^{-2} s^{-1} for HP-LED for all parameters except root lenght whereas were around 20 μmol m^{-2} s^{-1} for FL for all parameters except fresh and dry weigth of shoot. Here we show that HP-LED lighting improve *in vitro* culture of *H. ochraceus*, reduced 81% energy consumption respect to FL and uses only a multispectral LED instead of different single color LEDs. Therefore, HP-LED could be useful for the micropropagation of tree species contributing to sustainable agriculture and ecological restoration of degraded areas.

Keywords: *Handroanthus ochraceus;* Irradiance; Micropropagation.

DESENVOLVIMENTO IN VITRO DE IPÊ-DO-CERRADO (BIGNONIACEAE) USANDO DIODOS EMISSORES DE LUZ DE ALTA POTÊNCIA

*RESUMO – **Handroanthus ochraceus** (ipê-do-cerrado) é uma árvore medicinal, ornamentais e um recurso de madeira que pode ser propagada por cultura **in vitro**. Os métodos convencionais de propagaçao usam iluminação fluorescente (FL) e recentemente os diodos emissores de luz (LEDs). Este trabalho avalia os efeitos do FL e do LED de alta potência (HP-LED) na propagação **in vitro** de ipê-do-cerrado em diferente irradiâncias de luz (15 a 60 μmol $m^{-2}s^{-1}$). Epicótilos obtidos de família de meios-irmãos foram multiplicados em WPM (Woody Plant Medium) suplementado com 20 μM de 6-benzilaminopurina e 1 μM AIB (ácido indol-butírico). Para o enraizamento, os brotos foram cultivados por 3 dias em ½WPM suplementado com 50 μM IBA e por 42 dias em ½WPM sem auxina sob iluminação HP-LED ou FL. Sob HP-LED, a taxa de multiplicação dos brotos aumentou significativamente (61%) de 20 para 40 μmol $m^{-2}s^{-1}$ em relação a FL. Foram observadas diferenças nas densidades e tamanhos estomáticas entre fontes de luz. A irradiância HP-LED elevada produziu o maior porcentagem de enraizamento. Na fase de enraizamento, as médias marginais dos tratamentos sem*

interação entre os fatores mostraram que as irradiâncias HP-LED aumentaram significativamente o comprimento da parte aérea em 20%, a parte aérea fresca em 77% e a massa seca da parte aérea em 30% em comparação com os valores da FL. Os valores máximos calculados a partir das curvas de regressão foram em torno de 50 μmol $m^{-2}s^{-1}$ para HP-LED para todos os parâmetros, exceto comprimento da raiz, enquanto em torno de 20 μmol $m^{-2}s^{-1}$ para FL, exceto fresco e seco peso de tiro. A iluminação HP-LED melhora a cultura ***in vitro*** *de* ***H. ochraceus****, reduz 81% o consumo de energia em relação à FL e usa um LED multiespectral em vez de diferentes LEDs monocromáticos. Portanto, HP-LED pode ser útil para a microprogação de árvores contribuindo a uma agricultura sustentável e para a restauração ecológica de áreas degradadas.*

Palavras-Chave: ***Handroanthus ochraceus****; Irradiância; Micropropagaçao.*

1. INTRODUCTION

Handroanthus ochraceus (Cham.) Mattos (=*Tabebuia ochracea* (Cham.) Standl.) "yellow lapacho" is a tropical forest tree native to South America belongs to the Bignoniaceae family with plenty beautiful yellow flowers. This species is used as a timber resource for woodworking and naval manufacturing as well as a medicinal and ornamental plant. Conventional propagation of this species is by seeds but their germinative capacity decreased to 47% in eight months after harvest. In adition, it suffers from irregular reproduction linked to unseasonal frosts and its seeds are depredated by insects. which may remove up to 95% of total production (Justiniano et al., 2000; Apóstolo et al., 2016).The agricultural frontier expansion and climatic changes have led to a reduction of the natural populations and motivated researchers to use biotechnological methods, such as micropropagation and mycorrhizal inoculation, to increase *Handroanthus* and *Tabebuia* species mass propagation (Silva, 2004; Huante et al., 2012; Larraburu and Llorente, 2015; Llorente et al., 2016).

Light affects plant morphogenesis as main factor. It may not only induce plant development but also induce photo-inhibition when leaves are exposed to more light than they can utilize (George et al., 2008). Usually, fluorescent light is used for plant micropropagation in growth chambers with irradiances between 25 and 150 mmol m^{-2} s^{-1} for a 16 h photoperiod. However, this illumination source has some disadvantages such as its short lifespan (10000 h) and large volume, and the fact that it produces heat, which leads to the need of an extensive cooling system and high maintenance costs (Jao and Fang, 2003; George et al., 2008).

Light emitting diodes (LEDs) have been used as an alternative light source for controlled-environment agriculture (Nhut et al., 2003; Astolfi et al., 2012; Gupta and Jatothu, 2013; Apostol et al., 2015; Riikonen et al., 2016). The high efficiency in energy conversion of LEDs reduces the heat emissions and thereby saves energy. LEDs show improved longevity (five to ten times longer lifespan than fluorescent light (FL) tubes), have a small mass and volume, are environmentally safer than FL tubes (they do not have mercury), and are made with recyclable material (Astolfi et al., 2012).

There are several LED types that emit light at different wavelengths. The range of emission wavelength affects the morphogenetic response of plant. For example, the emissions of blue and red LEDs match closely with the absorption peaks of chlorophyll *a* and *b*, and these wavelengths generate maximum photosynthetic efficiency, which in turn enhances bud development (Nhut et al., 2003). The effect of red and blue LEDs on the growth and development of plants of the genera *Zantedeschia* (Chang et al., 2003), *Phalaenopsis* (Jao and Fang, 2003), *Fragaria* (Nhut et al., 2003), *Lactuca* (Kim et al., 2004), *Mentha, Ocimum*, *Lens* (Sabzalian et al., 2014), and others has been studied by several researchers;. In addition, white and green LEDs have shown positive effects on the growth of some species such as *Lactuca sativa*, and *Solanum lycopersicum* (Kim et al., 2004; Johkan et al., 2012; Lu et al., 2012). In this sense, white HP-LED lamps can generate a multispectral light unlike that of the narrow light bands produced by single color LEDs.

Although LEDs are used as an artificial flexible lighting source for the growth of seedlings of forest species in the greenhouse (Apostol et al., 2015; Riikonen et al., 2016), their use on the growth of forest trees by plant tissue culture are limited (Astolfi et al., 2012; Gupta and Jatothu, 2013). Studies on the micropropagation

of woody species, such as *H. ochraceus*, using energy efficient LEDs could lead to reduced costs and more environmentally friendly growing practices. Thus, the aim of this work was to study the effect of HP-LED lighting at different irradiances on growth responses such as shoot and root development and stomatal characteristics of *H. ochraceus* and compare it with the response under fluorescent lighting, usually used in micropropagation.

2. MATERIALS AND METHODS

2.1. Reagents

IBA, BA, tyamine, glycine, nicotinic acid, pyridoxine, myoinositol, and agar were purchased from Sigma Chemical Co (St. Louis, MO). All other chemicals were obtained from Argentinean commercial sources and were of the highest purity available.

2.2. Plant material

H. ochraceus seeds were obtained from populations of adult trees from the northwest of Argentina (Orán, Salta, 23°08´10´´S 64°19´20´´W). Seeds were washed and disinfected with sodium hypochlorite solution according to Llorente et al. (2016). Axenic seeds were grown in Woody Plant Medium (Lloyd and McCown 1980) supplemented with 100 mg L^{-1} myoinositol, 20 g L^{-1} sucrose, and 7 g L^{-1} agar (WPM) and 5 g L^{-1} activated charcoal.

2.3. Multiplication

Epicotyls from successful *in vitro* germinations of half-siblings was selected for the multiplication stages. Epicotyls were cut (10 to 15 mm in length) and cultured vertically with the basipetal surface in contact with 65 mL of WPM supplemented with 20 μM BA and 1 μM IBA (multiplication medium) in 250-mL glass flasks. Subcultured shoots in the same medium were grown under FL or HP-LED at different irradiances. The multiplication rate (number of shoots per initial explant), node number, shoot length and visual observations through a transparent culture vessel of general aspect (presence/absence of hyperhydricity, necrosis, chlorosis or basal callus) were evaluated after 30, 45 and 60 days in all treatments.

2.4. Rooting

To reduce variability in the initial physiological status, ramdomly selected shoots from FL and from HP-LED multiplication experiments were respectively used for FL and HP-LED rooting treatments. Shoots of 4-week-old multiplication stage (20 mm in length) were cultured for 3 days in modified WPM [½WPM] (mineral salts at half-strength of standard concentration and 6 g L^{-1} agar) and supplemented with 50 μM IBA. Then, one shoot per flat bottom glass tube (100 × 25 mm) containing 15 mL auxin-free ½WPM was cultured for 42 days under the HP-LED or FL lighting treatments. At the end of the experiments, the following parameters were recorded for each light treatment: percentage of rooted shoots, fresh and dry weights and length of shoots and roots, and numbers of leaves and roots. The presence/absence of basal callus, hyperhydricity, and health of plants were also recorded.

2.5. Stomata analysis

After 60 days of culture, the second pair of leaves of three randomly selected shoots from *in vitro* multiplication assay at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were treated with an ascending ethanol gradient, dried using the critical point method, mounted and metalized with gold-palladium. Leaf epidermis were observed and photographed by scanning electron microscopy (SEM) Philips XL-30 TMP (SEM Service, MACN, Buenos Aires, Argentina). Pictures were processed using TSview software (Tucsen Imaging Technology Co., China). The stomatal density (numbers/mm^2) and stomata size in abaxial epidermis were measured using at least 50 stomata sampled randomly at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.6. Culture conditions

Shoots were incubated in two sections of a growth chamber with 55–60% relative humidity, 16-h photoperiod at 25 ± 2 ºC. One section had fluorescent cool daylight tubes (FL), whereas the other one had High Power warm white LED [HP-LED] as light source. The FL source was built on a metal rack (45 cm x 90 cm) using three Philips T8 tubes (220 V) and the HP-LED source was built on two aluminum plates (3 cm x 60 cm) with 12 HP-LEDs 1W (12 V) (Demasled-CE Rohs, Argentina). Each HP-LED module was splitted into four equal strips (3 HP-LEDs), each group wired in parallel with the remaining three. Shoots under FL and under HP-LED at different irradiances (15, 20, 30, 40, 50, and 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were evaluated. The different irradiances were obtained varying the height of the light source from 23 to 30 cm for HP-LED and from 32 to 38 cm for FL, respect to the base of the culture container. Irradiance was measured weekly over the course of the experiments with a lux-meter

digital (Schwyz SC105-1, Argentina) and expresed as $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF. The spectrum distribution and features of the light sources were provided by both manufacturers and showed emission peaks at 400, 440, 490, 550, 615 and 710 for FL and 475 and 550 nm for HP-LED. The spectral composition of HP-LED showed a lower red (600-700 nm) / blue (400-500 nm) light ratio (0.5) than FL (0.7). Also, HP-LED showed a higher green (500-600 nm) / red ratio (3.8) than FL (1.5) and a higher green / blue ratio (1.8) than FL (1.0). The energy consumption for FL and HP-LED source was measured by the multimeter Fluke 434 II (Everett, Washington, USA). Experimental plants were randomly assigned to each light treatment.

2.7. Experimental design and statistical analysis

Experiments were performed on the basis of a completely randomized factorial design considering the light source (FL and HP-LED) and irradiance (15, 20, 30, 40, 50, and 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as factors. The multiplication experiments consisted of five flask with five shoots for multiplication (n=25), whereas the rooting experiments consisted of 15 flat bottom glass tube with one shoots for rooting (n=15). All experiments were conducted three times. Analysis of variance with a factorial layout was carried out for all experiments. Means were compared using Tukey's test at 5% significance level when more than two means were analized. Normality of data was performed by Kolmogorov-Smirnov test and variace homegeinity by Levene test. Regression analysis for rooting parameter were performed. All data were evaluated using SPSS v.21.0 (IBM SPSS, Armonk NY, USA). To analyze global effects for factors and their interactions, a Growth Index (GI) adapted from Larraburu and Llorente (2015) was constructed using all the growth parameters as follows:

EQ-1

$$GI(k,t) = \sum_{i=1}^{8} (X(k,t,i) - GM(i))/SG(i)$$

GI(k,t): growth index in treatment t for case k;
X(k,i,t): value of parameter i for case k in treatment t;
GM(i): grand mean for growth parameter i;
SG(i): overall standard deviation for parameter i

3. RESULTS

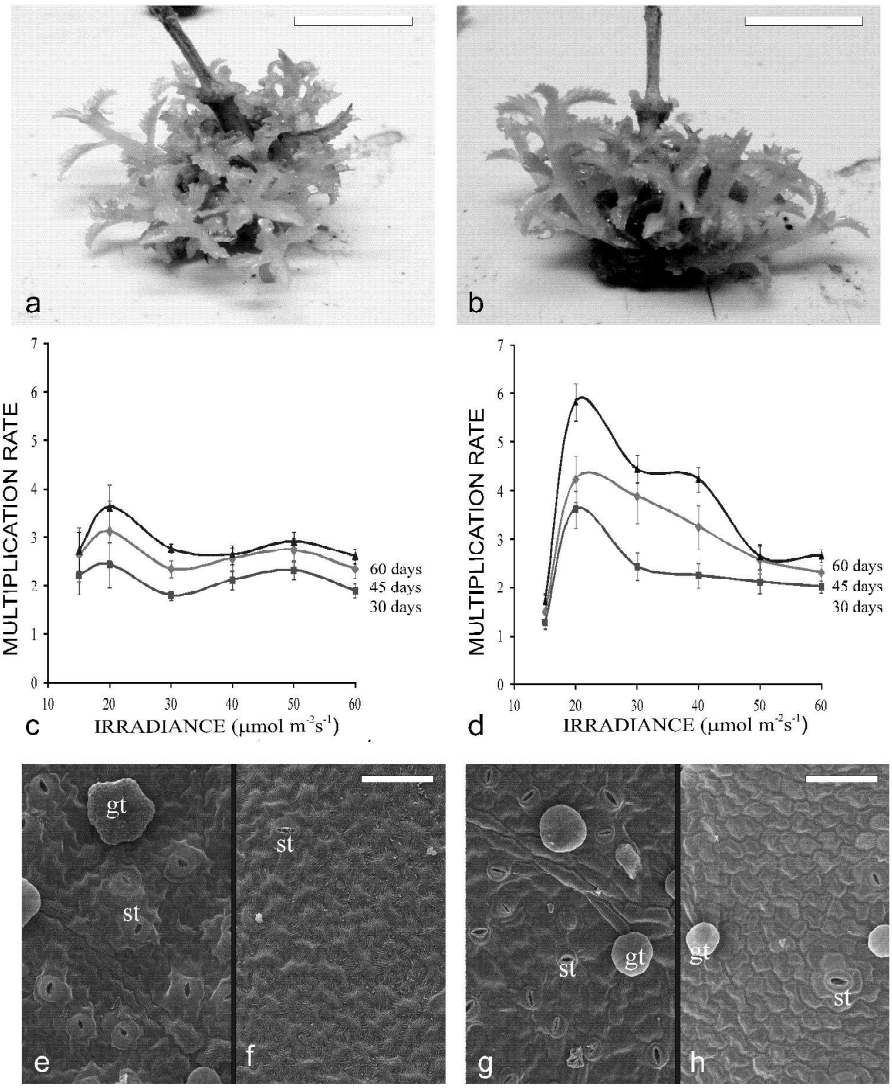
HP-LED and FL kept the same irradiance throughout the experiment. The energy consumption was 21.0 Wh for HP-LED and 112.2 Wh for FL. Both FL and HP-LED lighting resulted in multiple stem and leaf development of *H. ochraceus* without malformations, hyperhydricity, necrosis, chlorosis or basal callus during *in vitro* multiplication (Fig. 1 a-b).

All data shown normality and variace homogeninity. The factorial analysis showed that the shoot length was affected by light source and irradiance interaction only at 60 days of culture ($f= 2.8$, $p<0.01$) whereas the node number was affected at three times evaluated ($f= 4.1$ to 6.5, $p<0.05$). Light source mean of shoot length obtained under FL was significant higher ($p<0.05$) than HP-LED at all time evaluated. Also, under FL the shoot length showed a more uniform response than HP-LED at different irradiances. Light intensity mean (LIM) showed a reduction of shoot length and node number by 14 to 44 % under high irradiance (40-60 $\mu mol\ m^{-2} s^{-1}$) in comparison to 15 $\mu mol\ m^{-2} s^{-1}$ at 30, 45 and 60 days, although no significant differences ($p \leq 0.05$) were observed at 45 days of culture (Table 1).

Figure 1 a-d showed that HP-LED induced more proliferation of adventitious shoots than FL with significantly increased of the shoot multiplication rate by 61% from 20 to 40 $mmol\ m^{-2} s^{-1}$ after 60 days of culture as compared with FL. The highest multiplication rate occurred at 20 $mmol\ m^{-2} s^{-1}$, under both light sources. At this irradiance, the multiplication rate values were 3.6, 4.2, and 5.8 under HP-LED, and 2.4, 3.1, and 3.6 under FL, after 30, 45, and 60 days of culture, respectively (Fig. 1 c-d).

Leaves of *in vitro* multiplication shoots of *H. ochraceus* showed anomocytic stomata at both sides of the epidermis under both ligting treatments. The different light source influenced the stomata characteristics. Plants grown under HP-LED increase stomatal density (85% in abaxial and 152% in adaxial epidermis) and decrease between 9-23 % stomata size in both epidermis, respect to FL at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 1 e-f). All the differences were significantly at $p \leq 0.05$

All rooted shoots showed normal stem, leaf, and root development, without basal callus formation, and no hyperhydricity signs (Fig. 2 a). The ANOVA showed that leaf number, root length, fresh and dry root weights, and rooting percentage were significantly affected by the light source × irradiance interaction ($f= 2.8$ to 14.1, $p < 0.05$) whereas the other parameters evaluated were not affected (Fig. 2 c). Irradiances between 15 and 40 $\mu mol\ m^{-2} s^{-1}$ significantly reduced (76-89%) the rooting percentages ($p \leq 0.05$) in shoots grown under HP-LED compared with those grown under FL, which showed no significant differences at different irradiances (Fig. 2 b).



	Abaxial epidermis stomata			Adaxial epidermis stomata		
	Length	Width	Density	Length	Width	Density
LED	16.79±2.29 b	15.29±1.88 b	547.38±108.77 a	16.19±1.77 b	16.02±1.96 b	77.19±10.92 a
FL	18.44±1.58 a	18.88±2.04 a	295.09±49.64 b	18.23±1.98 a	18.72±2.15 a	30.51±5.56 b

Figure 1 – *In vitro* multiplication of *Handroanthus ochraceus* under two types of light source. (a) shoots under fluorescent light (FL); (b) shoots under high power light emitting diodes (HP-LED); bars: 1cm. Multiplication rate (number of shoots per inicial explant) as a function of irradiance after 30. 45. and 60 days under (c) FL and (d) HP-LED; bars represent standard error. e-i: abaxial (e-h) and adaxial (g,i) epidermis of leaves under 20 µmol m^{-2} s^{-1} irradiance FL (e-f) or HP-LED (g-h); gt: glandular trichomes; st: stomata; bars: 50µm. Table stomata features: different letters in the same column indicate significant differences between treatments ($p \leq 0.05$).

Figura 1 – *Multipliçao **in vitro** de **Handroanthus ochraceus** sob dois tipos de fontes de luz. (a) brotos sob luz fluorescente (FL); (b) brotos sob diodos emissores de luz de alta potência (HP-LED). Barras: 1cm. Taxa de multiplicação (número de brotos por broto inicial) em função da irradiância de luz após 30. 45 e 60 dias em (c) FL e (d) HP-LED; barras representam um erro padrão. e-h: epiderme abaxial (e-f) e adaxial (g-h) das folhas sob irradiância de 20 µmol m^{-2} s^{-1} FL (e) ou HP-LED(f); gt: tricomas glandulares; st: estômatos. Barras: 50µm. Características dos estômatos da tabela: diferentes letras na mesma coluna indicam diferenças significativas entre os tratamentos ($p \leq 0.05$).*

Table 1 – Mean comparison of growth parameters of *Handroanthus ochraceus* shoots during *in vitro* multiplication under different light sources and irradiances after 30, 45, and 60 days of culture.
Tabla 1 – *Comparação de meias de parâmetros de crescimento de brotos de* ***Handroanthus ochraceus*** *durante a multiplicação* ***in vitro*** *sob diferentes tipos de luz e irradiações após 30, 45 e 60 dias de cultura.*

Shoot length (mm)									
LI ($\mu mol.m^{-2}.s^{-1}$)	days	30			45			60	
	HP-LED	FL	LIM	HP-LED	FL	LIM	HP-LED	FL	LIM
15	11.4	10.9	11.1a	11.4	10.9	11.1a	14.7Aa	13.4Aabc	13.9a
20	9.1	9.8	9.2b	9.3	9.8	9.3a	10.0Bb	15.0Aa	10.4c
30	9.4	10.6	9.9ab	10.4	10.7	10.6a	12.2Bab	14.2Aab	13.1b
40	8.0	9.1	8.7b	9.8	10.2	10.1a	10.4Bb	12.1Abc	11.4bc
50	8.7	9.2	9.0b	9.1	10.2	9.8a	9.1Bb	11.3Ac	10.4c
60	8.4	9.4	9.0b	10.6	10.6	10.6a	10.7Bb	11.5Ac	11.2c
Light source mean	8.8B	9.5A		9.9B	10.4A		10.6B	12.2A	
CV	0.19			0.16			0.15		
Node number									
LI ($\mu mol.m^{-2}.s^{-1}$)	days	30			45			60	
	HP-LED	FL	LIM	HP-LED	FL	LIM	HP-LED	FL	LIM
15	1.8Aab	2.2Aa	2.1ab	1.8Aab	2.2Aa	2.1ab	2.7Aa	2.7Aab	2.7a
20	2.3Aa	1.9Aa	2.3a	2.4Aa	1.9Aa	2.3a	2.5Aab	3.0Aa	2.5ab
30	2.1Aab	2.0Aa	2.1ab	2.2Aa	2.1Aa	2.1ab	2.4Aabc	2.8Aab	2.5ab
40	1.2Bc	1.8Aa	1.6c	1.6Bb	2.1Aa	1.9b	1.6Bd	2.4Ab	2.1c
50	1.7Abc	1.7Aa	1.7bc	1.7Bab	2.1Aa	2.0ab	1.8Bcd	2.4Ab	2.2bc
60	1.6Ab	1.8Aa	1.7bc	1.9Aab	2.1Aa	2.0ab	2.0Bbcd	2.3Ab	2.2bc
Light source mean	1.8A	1.8A		2.0B	2.1A		2.1B	2.5A	
CV	0.71			0.56			0.45		

Means followed by different upper case letters in the lines differ from each other for the light source at 5% of significance level. Different lower case letters in the same column indicate significant differences between light intensity treatments by Tukey's test ($p \leq 0.05$). n= 75. Means that are not followed by letters show no significant interaction among the treatments. HP-LED: shoots cultured under High Power Light Emitting Diode. FL: shoots cultured under Fluorescent Light. CV: coefficient of variation. LI: light intensity. LIM: light intensity mean.

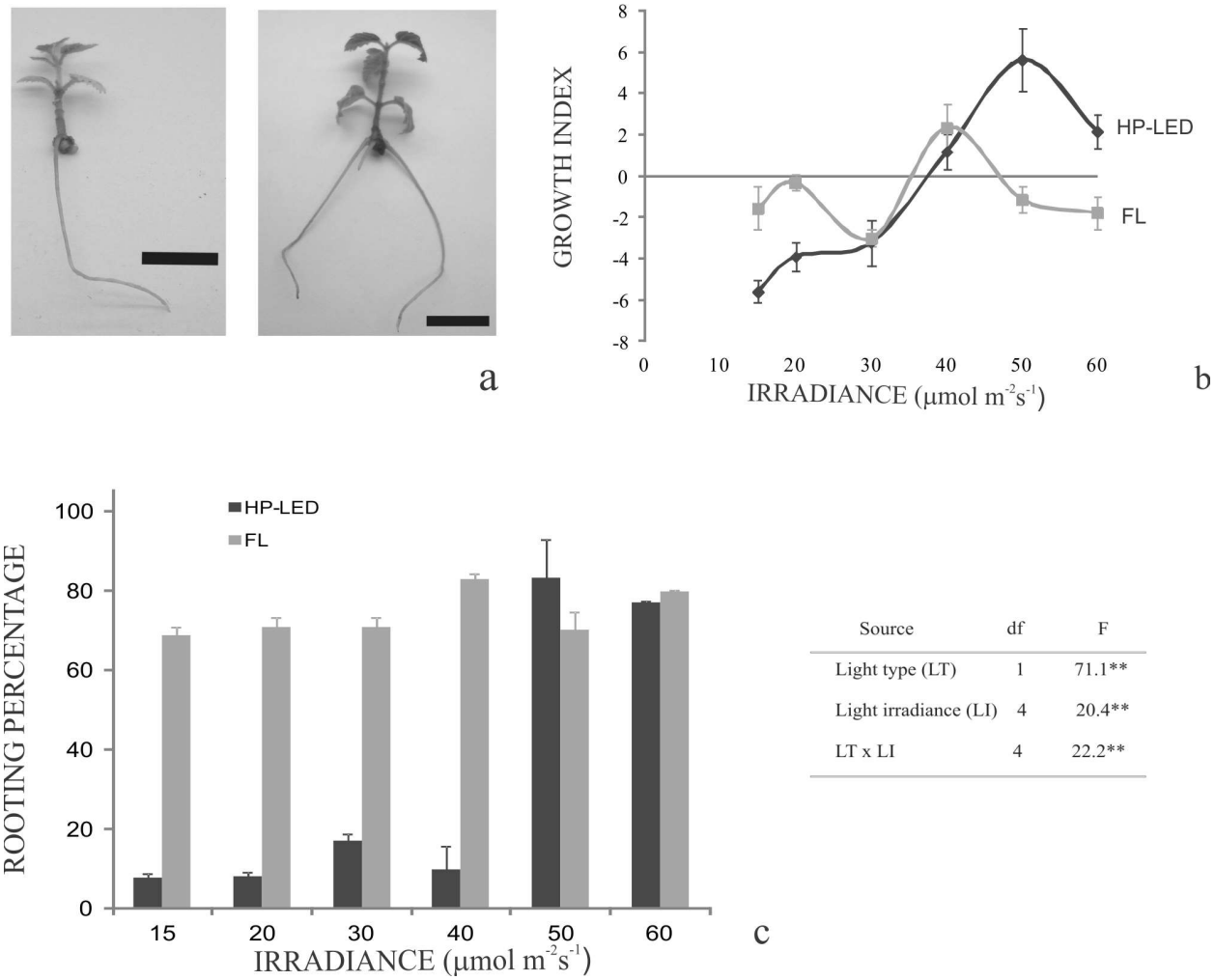
The means of light source treatments without factors interaction showed that HP-LED irradiances significantly increased shoot length by 20%, shoot fresh weight by 77% and shoot dry weight by 30% in comparison to the values under FL (Table 2). In addition, leaf number did not show significant differences among light intensities under HP-LED whereas it showed the highest values in low intensities under FL. Marginal mean of light intensity (LIM) for shoot lengh, fresh and dry weight of shoot and root were higher at upper intensity. Light source means of root parameters showed significant increases ($p \leq 0.05$) in number and length under FL whereas showed significant decreases in fresh and dry weigth.

The polynomial regression analysis for the light intensity for each light source indicated that the regression had a cubic adjustment in most parameters. The maximum values calculated from the regression curves were around 50 $\mu mol\ m^{-2}\ s^{-1}$ for HP-LED for all parameters except root lenght whereas were around 20 $\mu mol\ m^{-2}\ s^{-1}$ for FL for all parameters except fresh and dry weigth of shoot (Fig. 3).

The results described above were summarized in a growth index (GI) that shows a marked increase at 50 mmol $m^{-2}\ s^{-1}$ HP-LED respect to those obtained with the other HP-LED intensities and all FL intensities (Fig. 2 b).

4. DISCUSSION

HP-LED and FL were evaluated to select the best one for *H. ochraceus in vitro* culture. The multiplication rate obtained under HP-LED (20-40 $\mu mol\ m^{-2}\ s^{-1}$) was higher than that obtained under FL and was mainly due to the proliferation of adventitious shoots. Growth stimulation by LED has also been observed in *in vitro* cultures of other species. For example, beech (*Fagus*



Source	df	F
Light type (LT)	1	71.1**
Light irradiance (LI)	4	20.4**
LT x LI	4	22.2**

Figure 2 – *In vitro* rooting of *Handroanthus ochraceus* after 45 days of culture. (a) left: shoots under fluorescent light (FL); right: shoots under high power light emitting diodes (HP-LED); bar: 1cm. (b) Growth parameters index (GI) consisting of the sum of the standardized mean of all parameters of *H. ochraceus* plants analyzed as a function of irradiance of FL and HP-LED sources. (c) Rooting percentage as function of irradiance of FL and HP-LED sources and factorial analisis.

Figura 2 – *Enraizamento in vitro de* ***Handroanthus ochraceus*** *após 45 dias de cultura. (a) esquerda: brotos sob luz fluorescente (FL); direita: brotos sob diodos emissores de luz de alta potência (HP-LED); barra: 1cm. (b) Índice de parâmetros de crescimento (GI) consistindo na soma da média padronizada de todos os parâmetros das plantas de* ***H. ochraceus*** *analisadas como função da irradiância de luz das fontes FL e HP-LED (c) Percentagem de enraizamento em função da irradiância de luz de fontes FL e HP-LED e análise fatorial.*

sylvatica L.), holm oak (*Quercus ilex* L.), and wild cherry (*Prunus avium*) seedlings grown under a wide continuous spectrum LED showed significantly longer shoots than plants grown under FL and also beech had 40% and 110% greater shoot fresh and dry matter, respectively (Astolfi et al., 2012). Also, *Zantedeschia albomaculata* (calla lily) and *Vaccinium corymbosum* (highbush blueberry) shoot elongation as well as fresh and dry weights were significantly increased when cultures were kept under red LED in comparison to FL (Chang et al., 2003; Hung et al., 2016). Light quality influences plant development and physiology because it affects

Table 2 - Mean comparisons and factorial analysis of shoot and root growth parameters of *Handroanthus ochraceus* during *in vitro* rooting stage on different type and intensity of light after 45 days of culture.
***Tabela 2** - Comparações médias e análise fatorial dos parâmetros de crescimento aéreo e raiz de **Handroanthus ochraceus** durante o estágio de enraizamento **in vitro** em diferentes tipos e intensidade de luz após 45 dias de cultura.*

Light intensity	Leaf number			Shoot length (mm)			Shoot Fresh weight (mg)			Shoot Dry weight (mg)		
(µmol $m^{-2} s^{-1}$)	HP-LED	LF	LIM	HP-LED	LF	LIM	HP-LED	LF	LIM	HP-LED	LF	LIM
15	2.7 Ba	5.6 Aab	4.0 a	12.9	15.1	13.9 b	44.4	21.9	26.4 c	4.9	4.5	4.6 b
20	2.8 Ba	5.8 Aa	4.1 a	15.0	13.1	14.0 ab	49.0	24.8	30.6 bc	5.9	6.0	6.0 ab
30	3.1 Aa	2.3 Bd	2.7 b	15.7	13.8	14.7 ab	36.6	27.6	29.4 bc	5.4	5.1	5.1 b
40	3.9 Aa	4.6 Aabc	4.8 a	23.0	14.4	17.3 ab	58.4	38.3	41.2 ab	9.5	6.5	6.9 ab
50	4.4 Aa	3.3 Bcd	3.8 a	20.9	15.4	18.0 a	65.2	37.9	47.1 a	12.0	7.1	8.7 a
60	3.6 Aa	3.5 Abcd	3.5 ab	19.3	13.5	16.2 ab	53.1	23.7	37.1 ab	8.7	5.0	6.8 ab
Light source mean	3.5 B	4.0 A		17.1 A	14.2 B		50.1 A	28.3 B		7.3 A	5.6 B	
CV	0.46			0.12			0.15			0.33		

Light intensity	Root number			Root length (mm)			Root fresh weight (mg)			Root dry weight (mg)		
(µmol $m^{-2} s^{-1}$)	HP-LED	LF	LIM	HP-LED	LF	LIM	HP-LED	LF	LIM	HP-LED	LF	LIM
15	1.4	2.8	2.3 b	1.9 Bc	14.6Aab	8.4c	25.4 Ab	31.4 Ab	29.7b	3.5 Ac	3.7 Aa	3.6b
20	2.0	5.3	3.9 a	3.5 Bbc	20.9 Aa	12.0abc	32.9 Ab	33.4 Ab	33.2ab	3.9 Abc	4.8 Aa	4.5ab
30	1.7	2.5	2.3 b	6.0 Bab	18.4 Aa	14.3ab	37.1 Aab	33.1 Ab	34.0ab	4.3 Abc	4.1 Aa	4.1ab
40	2.8	3.6	3.1 ab	6.8 Bab	16.8 Aa	16.0a	26.7 Bb	47.6 Aa	43.0a	4.7 Abc	6.1 Aa	5.9a
50	2.9	3.3 4	3.1 ac	10.4 Aa	10.9 Ab	10.7bc	65.9 Aa	27.3 Bb	38.8ab	9.1 Aa	4.7 Ba	6.1a
60	2.1	3.1	2.5 b	14.3 Ba	17.4 Aa	15.6ab	42.9 Aab	33.0 Bb	38.2ab	7.2 Aab	4.7 Ba	6.0a
Light source mean	1.9 B	3.3 A		6.5 B	16.2 A		36.4 A	33.8 B		5.1 A	4.6 B	
CV	0.59			0.27			0.13			0.34		

Means followed by different upper case letters in the lines differ from each other for the light source at 5% of significance level. Different lower case letters in the same column indicate significant differences between light intensity treatments by Tukey's test ($p \leq 0.05$). n= 45. Means that are not followed by letters show no significant interaction among the treatments. HP-LED: shoots cultured under High Power Light Emitting Diode. FL: shoots cultured under Fluorescent Light. CV: coefficient of variation. LI: light intensity. LIM: light intensity mean.

the signaling cascade of specific photoreceptors which change the expression of some genes (Singh et al., 2015). Although the effect of light quality depends on the plant species, in general, blue light suppresses elongation and induces biomass production, green light affects leaf growth and shoot elongation, and red light stimulates photosynthesis, flowering and budding (Johkan et al., 2012; Singh et al., 2015). Previous studies have shown that the combination of red and blue LEDs enhances *Mentha* and *Fragaria* growth compared to other monochromatic LEDs (Nhut et al., 2003; Gupta and Jatothu, 2013; Sabzalian et al., 2014). Green light is the least absorbed by plants and is not sufficient to support their growth but, when used in combination with red and blue light, it shows some important physiological effects (Singh et al., 2015). In this sense, supplementation of green light with red and blue LED has been shown to enhance lettuce plant growth (Kim et al., 2004). Since blue light suppresses shoot elongation and red and green lights stimulate growth, the increase in the multiplication rate of *H. ochraceus* in HP-LED in comparison to FL could be related to the wider green light given that red light has less contribution in HP-LED. Moreover, FL induces oxidative processes (Astolfi et al., 2012), which could explain the decrease in the multiplication rate in comparison to that obtained under HP-LED.

The stomata status influence physiological activities such as photosynthesis and transpiration (Xiao-Jing et al., 2011). In general, an increase in stomatal density could allow plants to increase conductance for gas exchange and, thus photosynthetic performance with higher productivity (Schlüter et al., 2003). In this sense,

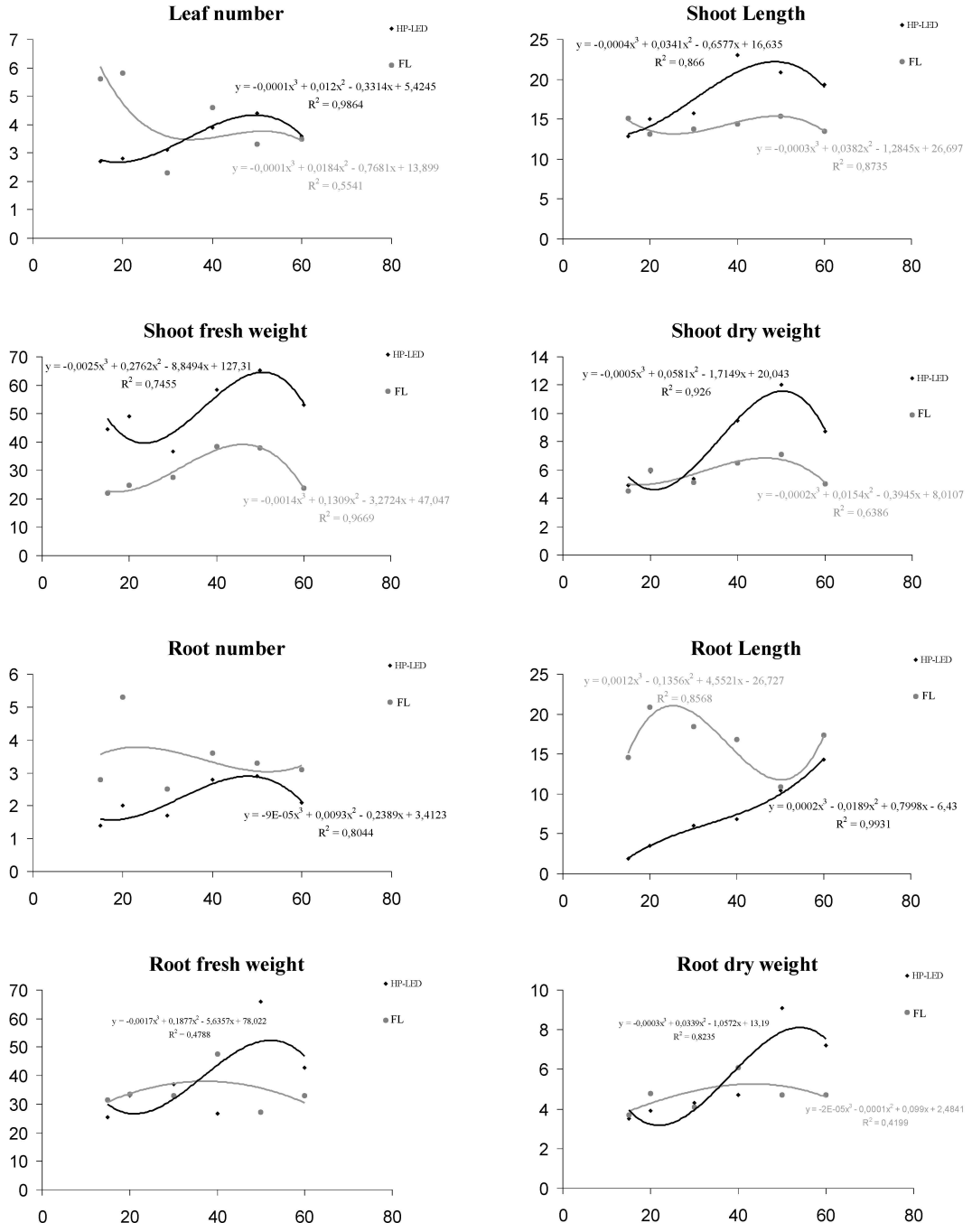


Figure 3 – *In vitro* rooting of *Handroanthus ochraceus* under two types of light source. Regression analysis of root parameter respect to irradiance for each light source. Significant regression curves are in the graphic (R^2>0.36). FL: shoots under fluorescent light HP-LED: shoots under high power light emitting diodes

Figura 3 – *Enraizamento **in vitro** de **Handroanthus ochraceus** sob dois tipos de fonte luminosa. Análise de regressão do parâmetro raiz quanto à irradiância para cada fonte de luz. Curvas de regressão significativas estão no gráfico (R^2> 0,36). FL: dispara sob luz fluorescente HP-LED: dispara sob diodos emissores de luz de alta potência*

the light source affected stomata density and size in both epidermis of *H. ochraceus*. The increase in stomata density under HP-LED was correlated to a higher multiplication rate, respect FL.

Plant growth and development is affected not only by the light source, but also by the irradiance because both affect photomorphogenesis. For example, Alvarenga et al. (2015) found that the growth of *Achillea millefolium* differed significantly under different FL irradiances, with high growth parameters at 27 μmol m^{-2} s^{-1}. Similar results have been observed in *Alocasia amazonica*, with a better growth response under 15 or 30 μmol m^{-2} s^{-1} FL than under higher irradiance (Jo et al., 2008). This matches that obtained in *H. ochraceus* grown under FL and HP-LED light sources, which showed a higher multiplication rate under 20 μmol m^{-2} s^{-1} and the highest shoot length and node number under 15-30 μmol m^{-2} s^{-1}. In contrast, previous studies have shown that the *in vitro* growth of strawberry (*Fragaria x ananassa*) under LED light (90% red + 10% blue) (Nhut et al., 2003) and of two orchids (*Phaius* spp and *Vanda* spp) under FL was better under 60-74 μmol m^{-2} s^{-1} (Soontornchainaksaeng et al., 2001). Although shoot morphogenesis is stimulated by light, the most favorable irradiance varies with the physiological and hormonal status of each species (George et al., 2008).

Rooting was also affected by light quality. HP-LED induced higher rooting percentage and growth index at 50 μmol m^{-2} s^{-1} than those obtained with the other HP-LED intensities and all FL intensities. In this sense, HP-LED 50 μmol m^{-2} s^{-1} produced the highest root weights, whereas lower irradiances significantly reduced them according to that observed in strawberry (Nhut et al., 2003). Also, *Z. albomaculata* root fresh and dry weigth increased, whereas its root number and root length decreased under blue LED 50 μmol m^{-2} s^{-1} in comparison to FL at the same intensity (Chang et al., 2003). On the other hand, the highest values of root number and root length of *H. ochraceus* were obtained under low FL irradiances (20 μmol m^{-2} s^{-1}) according to that observed in *Achilea millefolium* under 27 μmol m^{-2} s^{-1} FL (Alvarenga et al., 2015) and *Alocasia amazonica* under 30 μmol m^{-2} s^{-1} FL (Jo et al., 2008). High FL irradiance has been considered detrimental for rooting of some woody species because it may increase leaf temperature, transpiration, phenolic compound biosynthesis, peroxidase activity, and photo-oxidation (Fogaça and Fett-Neto, 2005; Tombesi et al., 2015), which would reduce the number and length of roots.

In addition, light intensity and quality determine asymmetric distribution of auxin which produces differential growth of plant organs (Kurepin and Pharis, 2014). Therefore, the positive effect of HP-LED on rooting percentage and root weight in *H. ochraceus* under high irradiances could be linked to increases of endogenous auxin levels, as it has been suggested for *Eucalyptus saligna* and *E. globulus* cuttings (Fogaça and Fett-Neto, 2005).

The differences between FL and HP-LED observed in *H. ochraceus* rooting may be related to the light spectral composition in according to the changes induced by the different blue/red ratios of LED and FL in strawberry (Nhut et al., 2003). These authors observe higher root development by decreasing LED red/blue ratio. In coincidence, HP-LED used in *H. ochraceus* rooting provided lower red/blue ratio than FL (0.5 and 0.7, respectively).

The low energy consumed by HP-LEDs results in savings of 81% compared with lighting costs of FL. Also, LEDs generate very little heat, thereby minimizing the need for an extensive cooling system in the plant growth chamber, and saving additional energy (Nhut et al., 2003). In this way, the use of HP-LED may allow achieving an ecological growth chamber with the same performance as a conventional chamber, without the environmentally hazardous components currently used in lighting with FL (Bourget, 2008).

5. CONCLUSIONS

The HP-LED irradiation system used in the present study showed several advantages over FL in the micropropagation of yellow lapacho (*H. ochraceus*), such as a significant increase in the shoot multiplication rate from 20 to 40 μmol m^{-2} s^{-1}, and of growth parameters and growth index from 50 to 60 μmol m^{-2} s^{-1} at the rooting stage. Then, HP-LED can be used as FL substitute on *in vitro* culture of *H. ochraceus*.

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