Original Article

NEW LIGHT SOURCES FOR *in-vitro* POTATO MICROPROPAGATION

NOVAS FONTES DE LUZ PARA MICROPROPAGAÇÃO in vitro *DE BATATA*

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ABSTRACT: This research work objective was to optimize the micropropagation of potato cultivars through the use of new light sources in the growth rooms. Treatments consisted of three potato cultivars (Asterix, Catucha and Macaca), and five light sources (blue, green and red LEDs; Growlux and white fluorescent lamps). The explants consisted of nodal segments containing one bud, isolated from plantlets grown *in vitro*. The experimental design was completely randomized arranged in a 3x5 factorial, with eight replications. Each experimental unity consisted of a flask with five explants. Three 28-day consecutive subcultures were carried out in MS semi-solid medium, in growth-room under controlled conditions (temperature = 25±2 ºC; photoperiod = 16 hours; light intensity = 20 μmol m^{-2} s^{-1}). At the end of each subculture, the bud number per plantlet, plantlet length and internode length were evaluated. After the third subculture, the concentrations of carotenoids and *a*- and *b*-chlorophylls were also determined. Different micropropagation efficiencies were found among potato cultivars grown *in vitro* conditions: 'Macaca' was the most and 'Catucha' the least responsive cultivar. The growth room light sources differently affected the potato plantlet development: red and green LEDs were the most and least recommended for plantlet development, based on the results of bud number per plantlet, plantlet length, and leaflet concentrations of *a*- and *b*-chlorophylls and carotenoids.

KEYWORDS: *Solanum tuberosum* L. Tissue culture. Light emission diodes. LEDs. Explant.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is worldwide the most consumed vegetable and important source of carbohydrates, fibers and potassium for human feeding (ABBA, 2014). In Brazil, potato is an economically and socially relevant crop with yearly average production of 3.5 million t over a harvest area of 140 thousand hectares (AGRIANUAL, 2013). The main available cultivars used are: 'Asterix' characterized by rough pink tuber epidermis, light yellow pulp, nematode resistance and common scab (*Streptomyces* spp.) tolerance; 'Catucha' characterized by smooth yellow epidermis, yellow pulp, high yield potential and late blight (*Phytophthora infestans*) resistance; and 'Macaca' characterized by rough dark purple epidermis, white pulp, short dormancy cycle and green tuber low susceptibility (ABBA, 2014).

Potato is vegetatively propagated using virus free seed potatoes that are fundamental to reach high yields. Virus free seed potatoes are obtained by means of tissue culture techniques, which represent 17 to 21% of the total crop costs (AGRIANUAL, 2013). Several studies have been made to optimize the micropropagation process, mainly related to culture medium composition (ABDULLATEEF et al., 2009). However, tissue culture environments might be improved by using new available light sources that might represent lower production costs, especially for seed potato production (SEABROOK, 2005; LI et al., 2010).

Light is the radiant energy source for photosynthesis process, which regulates plant development. Chlorophyll is the most important pigment in photosynthesis, since it is responsible for light energy capture and transformation in chemical energy (WU et al., 2007). Plants use the photosynthetically active radiation that is the light wavelength between 390 and 760 nm (visible light) (STREIT et al., 2005). Therefore, photosynthesis efficiency is influenced by light quality, or else, light wavelength, light duration and intensity (NHUT et al., 2002).

Although natural sunlight can be used as source of energy for plants grown in micropropagation laboratories, the white fluorescent lamps are worldwide the most used. Recently developed light emission diodes (LEDs) have been pointed out by authors as potential sources of light for *in-vitro* environments cultivation. LEDs are characterized by specific wavelengths, small mass and volume, long useful life, low heating and highly efficient light generation process (60%), and do not contain mercury or other hazard element to the environment (YEH; CHUNG, 2009). For this reason, interesting research works have been carried out with several plant species grown under LEDs, such as cherry (MULEO; THOMAS, 1997), banana

Received: 20/05/14
Accepted: 20/05/15

(NHUT et al., 2002), strawberry (ROCHA et al., 2010), cotton (LI et al., 2010), among others.

This work aimed to optimize the *in-vitro* propagation of three potato cultivars, using new light sources in the culture environment.

MATERIAL AND METHODS

The experiment was carried out in the Tissue Culture Laboratory of Embrapa-Temperate Climate, at Pelotas, Rio Grande do Sul, Brazil, using explants from meristems. Nodal segments with one bud and 10 mm long were used, originated from three successive subcultures in MS medium (MURASHIGE; SKOOG, 1962) supplemented with 100 mg L^{-1} of myo-inositol, 30 g L^{-1} of sucrose and 7 g L^{-1} of agar, pH 5.8, cultivated in growth room under white fluorescent lamps.

Explants were transferred to 250 mL glass flasks, 6.5cm diameter x 13cm high, containing 40 mL of culture medium described by Pereira et al. (2005). Under such conditions, three successive 28 day long subcultures were carried out at constant temperature (25 ± 2ºC) and light intensity (20 µmol m^{-2} s^{-1}) with photoperiod of 16 hours. After each subculture period, explants with similar characteristics of the ones used in the first subculture were transferred to fresh culture medium of equal treatment. This procedure (three successive subcultures) was done to minimize the effect of white fluorescent lamps in the initial explant production.

Treatments consisted of three potato cultivars (Asterix, Catucha and Macaca cv) and five light sources, as follows: blue LEDs-EDEB 3LA1 470 nm, green LEDs-EDET 3LA1 530 nm, red LEDs-EDER 3LA3 630 nm, Growlux fluorescent lamps and white fluorescent lamps (control). Treatments followed a completely randomized design arranged in a 3 x 5 factorial (cultivars x light sources) with eight replications. Each experimental unit consisted of a flask with five explants.

In the end of each subculture, the number of buds per plantlet, average plantlet length (mm) and average internode length (mm) were evaluated. The three subculture average values were considered data entries for statistical analysis. In the end of the third subculture, plantlet leaf samples (100 mg of fresh leaf tissue) were collected from the different treatments for analysis (concentration determination) of carotenoids and (*a* and *b*) chlorophylls in 80% acetone extracts. The pigment quantification was made by spectrophotometry (*a*-chlorophyll at 663 nm; *b*-chlorophyll at 645 nm; and carotenoids at 470 nm), according to Lichtenthaler (1987).

The results were submitted to analysis of variance and means were compared by Duncan test ($p < 0.05$). The bud number per explant data was transformed in the square root of (x+0.05), that is, $(x + 0.5)^{½}$. The other variables were not transformed.

RESULTS AND DISCUSSION

All three potato cultivars (Asterix, Catucha and Macaca cv) showed good *in-vitro* growth under the five studied light sources (blue, green, red LEDs, Growlux and white fluorescent lamps), without problems of contamination, oxidation or callus formation. According to Pereira et al. (2005) and Abdullatellf et al. (2009), such results might be attributed to the easy *in-vitro* propagation of *Solanum tuberosum* species. Also, in all treatments, no plantlet tuber formation was observed, which usually impairs explant multiplication, since tuber formation occurs at the expense of energy reserves (SEABROOK, 2005). Similarly, during *in vitro* propagation, no abnormal plantlet morphological characteristics were observed. Changes in plant morphology indicate physiological impairments or somaclonal variation.

In the present research, the analysis of variance showed interactions between potato cultivars and light sources for the variables: average bud number per plantlet, average plantlet length and average internode length (Table 1).

Higher bud number per plantlet was obtained with Macaca cv under most light sources studied, except for the blue LEDs, under which no differences were found among cultivars. Also, 'Macaca' did not differ from 'Asterix' under white fluorescent and Growlux lamps (p<0.05). On the other hand, 'Catucha' was the least responsive cultivar *in vitro,* once it showed the least bud number per plantlet under most light sources, except for the blue LEDs (Table 1). Wilson et al. (1993) also demonstrated genetic differences among four potato cultivars (Kennebec, Norland, Denali and Superior) as concerned to their responses (bud number per plantlet) under different sources of light.

In general, 'Macaca' showed the longest plantlets, but it did not differ from 'Catucha' under blue and green LEDs and Growlux lamps (p<0.05). And 'Asterix" showed the shortest plantlets under all sources of light (Table 1).

Table 1. *In vitro* explant development of three potato (*Solanum tuberosum* L.) cultivars under different sources of light.

Sources of light	Cultivar		
	Asterix	**Catucha**	**Macaca**
	Average bud number per plantlet		
Blue LEDs -EDEB 3LA1	6,39[1] a A	6,20 a A	6,63 a B
Green LEDs-EDET 3LA1	4,44 b B	3,87 c C	5,18 a C
Red LEDs-EDER 3LA3	6,33 b A	5,21 c B	7,57 a A
Growlux lamps	6,79 a A	6,22 b A	7,21 a A
Fluorescent lamps	6,44 a A	6,37 b A	7,33 a A
Average	6,08	5,57	6,78
CV (%)	3,18	3,18	3,18
	average plantlet length **(mm)**		
Blue LEDs-EDEB 3LA1	35,94 b C	43,78 a D	48,61 a D
Green LEDs-EDET 3LA1	53,70 b B	78,21 a B	76,44 a C
Red LEDs -EDER 3LA3	66,92 c A	101,20 b A	127,98 a A
Growlux lamps	53,70 b B	80,30 a B	84,25 a B
Fluorescent lamps	38,20 c C	59,52 b C	73,72 a C
CV (%)	10,41	10,41	10,41
	average internode length **(mm)**		
Blue LEDs-EDEB 3LA1	5,65 b D	7,09 a D	7,26 a E
Green LEDs-EDET 3LA1	11,86 c A	19,51 a A	14,85 b B
Red LEDs -EDER 3LA3	10,41 c B	19,29 a A	16,45 b A
Growlux lamps	7,51 c C	12,93 a B	11,53 b C
Fluorescent lamps	5,96 b D	9,29 a C	10,06 a D
CV (%)	11,06	11,06	11,06

'Catucha' showed the longest internodes under all studied light sources, but it did not differ from 'Macaca" under blue LEDs and white fluorescent lamps (p<0.05). And 'Asterix' showed the *in-vitro* worst performance, with the shortest internodes (Table 1).

As concerned to the light source effect on the *in-vitro* plantlet development, it was observed that red LEDs, white fluorescent and Growlux lamps induced higher bud number per plantlet in Macaca cv; blue LEDs, white fluorescent and Growlux lamps induced higher bud number per plantlet in 'Catucha'; and blue and red LEDs plus white fluorescent and Growlux lamps induced higher bud number per plantlet in 'Asterix'. Green LEDs negatively affected the bud number per plantlet in all three cultivars (Table 1).

Such results demonstrated the positive effect of white fluorescent and Growlux lamps and red and blue LEDs on the *in-vitro* bud number per plantlet of the studied potato cultivars. According to Folta and Maruhnich (2007), red and blue lights induce faster plantlet growth, even faster than white light does, meanwhile green light that is absorbed by phytochromes and cryptochromes, act influencing events that reduce vegetative development. Furthermore, Wu et al. (2007) reported that red light spectrum emission is near the point of maximum absorption by chlorophylls and phytochromes and it is important for photosynthetic apparatus development and for starch accumulation; and that blue light is relevant for chloroplast development, chlorophylls formation and stomata opening.

Red LEDs induced longer plantlets in all three studied potato cultivars; plantlet length plus bud number per plantlet are the most important variables to be evaluated for the *in-vitro* propagation process. Average plantlet length showed intermediate values under Growlux fluorescent lamps and green LEDs, and the least values under white fluorescent lamps and blue LEDs (Table 1). Kim et al. (2004) and Rocha et al. (2010) had already observed longer plantlets of chrysanthemum and strawberry when grown under red LEDs than under other sources of light. With other potato cultivars, Wilson et al. (1993) obtained longer plantlets under red LEDs, compared to plantlets grown under white fluorescent lamps or even under blue LEDs. Petiole and plantlet length has been associated to red light, which was observed to stimulate and enhance plant species cell lengthening (WILSON et al., 1993). In general, longer plantlets (since not resultant from etiolating) are considered

the best in the micropropagation process, because they are easily separated and acclimated, and besides, they contain higher bud number. Villavicencio et al. (2007) observed that potato plantlets of 51 to 70 mm long presented 91% survival rate after acclimatization, meanwhile plantlets with less than 30 mm long showed only 77% survival rate.

White fluorescent lamps and blue LEDs induced shorter plantlet internodes in all three potato cultivars studied. Longer internodes were found under red and green LEDs for 'Catucha'; under red LEDs for 'Macaca'; and under green LEDs for 'Asterix.

Muleo e Thomas (1997) also observed longer internodes in cherry plantlets grown under red LEDs than under white fluorescent lamps and blue LEDs. In the same way, Kim et al. (2004) found longer internodes in chrysanthemum plantlets grown under red LEDs.

LEDs provide radiant energy for better potato explant and plantlet development in laboratory rooms, and also, they show the advantage of longer useful life that may reach 100,000 hours, meanwhile fluorescent lamps present an average useful life of 8,000 hours, and incandescent lamps of 1,000 hours (ROCHA et al., 2010). According to these authors, another advantage is the energy saving, since LEDs present high energetic efficiency (50%) compared to fluorescent lamps (20%) and incandescent lamps (5%). This fact directly reduces the plantlet production costs, once the growth room illumination is responsible for 65% of energy power costs of a tissue culture laboratory (YEH and CHUNG, 2009).

Interaction between potato cultivars and light sources for the (*a* and *b*) chlorophyll concentrations was found ($p<0.05$). Red LEDs induced higher (*a* and *b*) chlorophyll concentrations in all three studied cultivars. However, no chlorophyll differences were found for 'Catucha' and 'Macaca' under red LEDs and white fluorescent lamps; and for 'Catucha' under blue, red LEDs and Growlux lamps. On the other hand, green LEDs provided the least chlorophyll concentrations in all cultivars (Table 2). Furthermore, although distinct *a*- and *b*-chlorophyll concentrations among potato cultivars were found, induced by different light sources, there always was the same response for each chlorophyll type in relation to the light source used (Table 2). Besides, almost all treatments presented a:b chlorophyll ratio higher than 2 (a:b > 2) that is close to the ratio found in plants grown under natural sunlight (3:1) (STREIT et al., 2005). According to these authors, *a*-chlorophylls are important for the photosynthesis first stage (photochemistry), meanwhile *b*-chlorophylls act in the radiant energy capture process and transference to the reaction centers.

In the present work, significant *a*- and *b*-chlorophyll differences ($p<0.05$) were observed among cultivars, under each source of light studied, with 'Macaca' always ranked on top, except for the white fluorescent lamps (Table 2).

Table 2. Leaf (*a* and *b*) chlorophyll concentrations (mg g^{-1}) in plantlets of three potato (*Solanum tuberosum* L.) cultivars, grown *in vitro* under different sources of light.

Sources of light	**Cultivar**		
	Asterix	**Catucha**	**Macaca**
	Chlorophyll *a* (mg g^{-1} of fresh tissue)		
Blue LEDs-EDEB 3LA1	0,97 a B	1,05 a A	1,04 a B
Green LEDs-EDET 3LA1	0,49 a C	0,34 a B	0,45 a C
Red LEDs -EDER 3LA3	1,65 a A	1,16 b A	1,63 a A
Growlux lamps	1,13 a B	1,25 a A	1,19 a B
Fluorescent lamps	0,95 c B	1,36 b A	1,76 a A
Average	1,04	1,03	1,21
CV (%)	6,56	6,56	6,56
	Chlorophyll *b* (mg g^{-1} of fresh tissue)		
Blue LEDs-EDEB 3LA1	0,44 a B	0,46 a A	0,45 a B
Green LEDs-EDET 3LA1	0,21 a C	0,14 a B	0,20 a C
Red LEDs -EDER 3LA3	0,72 a A	0,53 b A	0,79 a A
Growlux lamps	0,55 a B	0,51 a A	0,52 a B
Fluorescent lamps	0,54 b B	0,59 b A	0,86 a A
CV (%)	4,35	4,35	4,33

Carotenoids concentrations did not significantly differed among Asterix, Catucha and Macaca cultivars, but significantly differed among light sources ($p<0.05$) (Table 3). Highest carotenoids concentrations (0.64 and 0.57 mg g^{-1} of fresh tissue) were found in plantlets grown under white fluorescent lamps and red LEDs, respectively. However, red LEDs did not significantly differed from blue LEDs and Growlux lamps, for this variable. Wu et al. (2007) had already observed higher carotenoids accumulation in peas grown under red light. In the present work, the least carotenoids concentrations were obtained under green LEDs, corroborating the results of Rocha et al. (2010), when studying *in vitro* culture of strawberry. In general, green light is considered a less relevant type of energy for photosynthesis mainly due to its low absorption coefficient (KIM et al., 2004). It is highlighted that chlorophylls and carotenoids synthesized by plants are essential pigments: in the photosynthesis process (light absorption); in preventing plants from photo-oxidation; in the coloration of plants; and besides, as precursors of vitamins and antioxidants (WU et al., 2007).

Table 3. Carotenoid concentrations (mg g^{-1}) in plantlets of three potato (*Solanum tuberosum* L.) cultivars, grown *in vitro* under different sources of light.

Sources of light	**Carotenoid (mg g^{-1} of fresh tissue)**
Blue LEDs-EDEB 3LA1	0,56 b
Green LEDs-EDET 3LA1	0,14 c
Red LEDs -EDER 3LA3	0,57 ab
Growlux lamps	0,53 b
Fluorescent lamps	0,64 a
CV (%)	4,33

CONCLUSIONS

A genetic effect of potato cultivar in the process of *in-vitro* propagation was evident, since 'Macaca' showed higher bud number per plantlet, followed by 'Asterix' and at last 'Catucha'.

LEDs (light emission diodes) can be used as source of light in substitution to the white fluorescent lamps in tissue-culture growth rooms for *in-vitro* potato propagation.

The light source of growth rooms influenced the potato explant development *in vitro*: red LEDs were the most and green LEDs the least recommended for plantlet vegetative development and pigment synthesis (*a*- and *b*-chlorophylls and carotenoids).

ACKNOWLEDGEMENTS

The authors are thankful to CNPq (Brazilian National Council for Scientific and Technological Development) for the research grants and project financial aid.

RESUMO: Este trabalho objetivou otimizar a micropropagação de cultivares de batata por meio do uso de novas fontes de luz no ambiente de cultivo. As cultivares avaliadas foram Asterix, Catucha e Macaca e as fontes de luz foram LEDs azuis, LEDs verdes, LEDs vermelhos, lâmpadas Growlux e lâmpadas fluorescentes brancas. Como explantes foram utilizados segmentos nodais contendo uma gema, isolados de brotações estabelecidas *in vitro*. O delineamento experimental foi inteiramente ao acaso em um fatorial 3 x 5 (cultivar x fonte de luz), com oito repetições por tratamento. Cada unidade experimental foi composta por um frasco contendo cinco explantes. O experimento compreendeu três subcultivos consecutivos de 28 dias em meio semi-sólido MS a 25±2 ºC, 16 horas de fotoperíodo e intensidade luminosa de 20 μmol m^{-2} s^{-1}. Ao final de cada subcultivo foram avaliados o número de gemas produzidas por explante, o comprimento das brotações e o comprimento dos entrenós. Ao final do terceiro subcultivo, determinaram-se, ainda, as concentrações de carotenoides e de clorofilas *a* e *b*. Verificou-se efeito da cultivar na eficiência do processo de propagação de batata, sendo a 'Macaca' a mais e a 'Catucha' a menos responsiva *in vitro*. A fonte de luz do ambiente de cultivo afetou o desenvolvimento dos explantes de batata. Os LEDs vermelhos e os verdes foram, respectivamente, os mais e os menos indicados tanto para o desenvolvimento vegetativo quanto para a formação de clorofilas *a* e *b* e de carotenoides.

PALAVRAS CHAVE: *Solanum tuberosum.* Cultura de tecidos. Diodos emissores de luz. LEDs. Explante.

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