CONTRIBUTION TO *Solanum tuberosum* L. TUBERGENESIS, VITROCULTIVATED UNDER ULTRABRIGHT COLOR L.E.D.

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Abstract. This experiment had the main purpose to reduce the consumption of electric energy used in biotechnological vitroculture processes, in order to obtain cheaper seedling and keep the environment cleaner. To achieve this goal, we replaced CFLs with ultrabright LEDs, and, as biologic experimental model, we used *Solanum tuberosum* L. inocula. Within 8 weeks we found that, at the same light intensity, the vitroplantlets grown under white light LEDs are totally similar to those cultivated under CFL white light. We have also found that colored light, generated by LEDs, especially the red and blue ones, determine the tubergenesis at potato vitroplantlets in 6-8 weeks and at a normal sucrose concentration. This technique could be used to produce really fast and at low cost potato seedling and can be extended to any other plant, too.

Keywords: *Solanum tuberosum*, *in vitro*, CFL, LED, wave length, tubergenesis.

INTRODUCTION

"Converting our economy into an eco-economy is a monumental undertaking" [6]. The food production necessity is running up very fast and the statistics say that, within a few years, 80% of it must be fulfilled by increasing the techniques efficiency and only 20% by agriculture expansion [3], because the number of human beings is now about 7 billion and growing. This issue, addicted to the energy crisis and global warming, leads to a complex question: "How can we feed an enlarging number of people [13], with less energy consumption and without a negative impact to the environment?" A possible answer is a sustainable bioeconomy [5, 16, 24], based on environmental factors preservation and food biosecurity, in relationship to climate change [1], actually the new paradigm – eco-bioeconomics. Today, livestock management represents a multifunctional activity [4] which implies multidisciplinary research teams, acting together against hunger, for a better world.

One of the most important elements in life's algorithm is the *potato*, known as the second bread of humanity. Originated in Peru, it was worldwide spread, cultivated, exploited and researched. The need for seedling is increasing year after year, but, because of diseases present in cultivars, a seed renewal is required each second year. The best method to produce minitubers is biotechnology. By culturing *in vitro* we can produce low cost seedling, in a large amount, and also virus free plants [8]. Regarding *potato*, we are interested to obtain microtubers that will be used *ex vitro* to produce minitubers, which can be planted in field as a *healthy* seedling. A higher production must be obtained by applying others experimental results to this goal, as replacing the sucrose with honey [19, 20], using ultra sounds [12], adding salicylic acid at the culture media in order to stimulate chlorophylls biosynthesis [17, 22], using nanocomposyte magneto-fluids, which have a synergic effect with phytohormons [2, 7], using the temporary immersion system [25], or any other method that could lead us to a higher productive capacity.

The mentioned methods offer the opportunity to enrich the microtubers quantity and, some of them, to shorten the time interval needed to yield. In our experiment we were concerned to reduce the energy consumption and, implicitly, to reduce the negative impact to environment. In the last decade, the technology progress came forth with new possibilities in lighting techniques. Light Emitting Diodes (LED) became quickly an important element in this work area. Finally, the ultrabright LEDs offered enough light to be used to illuminate different objects, parks and buildings. They were also used as a light source in vitrocultures, replacing the old CFL tubes. A growth box with red and blue LEDs was designed [14] and in 2004, using flashing light to potato vitrocultures, a good growth was obtained at a pulse frequency of 720 Hz [15].

MATERIALS AND METHODS

Usually, cool fluorescent lamps (CFL) are used to illuminate the vitrocultures. It is a classic method, cheaper than using incandescent bulbs, but still not very convenient, because the consumed electricity is not at a low level yet, so the emitted infrared radiation increases the temperature in the growth rooms, fact that requires cooling devices which increases the electricity consumption and also the production costs. In 1941, an intermittent light system was proposed, to be used in vitrocultures [27], and many experiments that followed this were focused to establish the proper interchange between light and dark. Some research shown that highest yielding can be obtained with a 40 ms dark period [9] and another team, in 1985, observed that a flashing light, having 2 ms light and 198 ms dark, reduces the photosynthesis [26].

In this experiment we exposed *potato* vitrocultures at LED continuous light [21] having different wave length, and compared to the ones illuminated with CFL tubes. The biologic material was taken from a *Solanum tuberosum* var. GARED *in vitro* culture. The inocula consisted in single node fragments of stalk [23] and were placed in presterilized recipients (vol.=50 ml,

height=6.5 cm; Ø=2.5 cm) containing standard Murashige and Skoog (1962) media [18], having Heller macroellements [10] and glycine, without growth regulators. The pH of the media was adjusted to 5.5, before autoclaving at 121°C (250°F) for 30 min [8].

The resulted experimental variants were as following:

V_0 (control variant) – CFL white light
V_1 – LED white light (380 to 740 nm spectrum)
V_2 – LED red light (670 nm)
V_3 – LED yellow light (580 nm)
V_4 – LED green light (540 nm)
V_5 – LED blue light (470 nm)

After inoculation, the bottles corresponding to V_0 were placed on shelves under CFL white light, at a proper distance in order to get a 16.2 µMoles/m^2/s light intensity at their base. The others were put in growth boxes (Fig. 1), and there was one LED above each bottle, at 1 cm distance (Fig. 2). The light intensity was set to 16.2 µMoles/m^2/s.

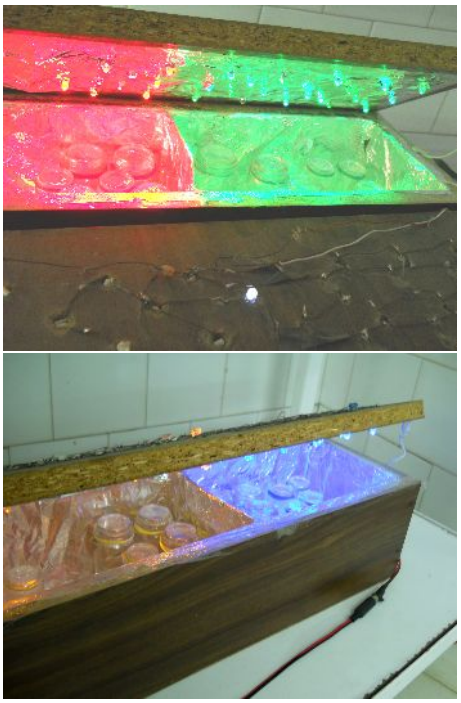

Figure 1. LED-based culture boxes.

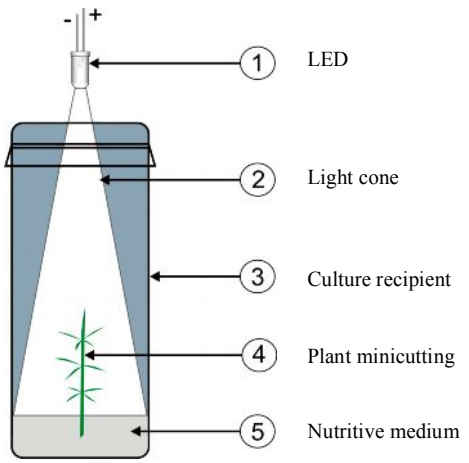

Figure 2. The schematic of ultrabright LED illumination.

RESULTS

The experiment lasted 8 weeks and the survival percentage is presented below (Fig. 3) The survival percent was good on the most of variants, being over 95% (Fig. 3), fact that show the LEDs are proper devices to illuminate the vitrocultures.

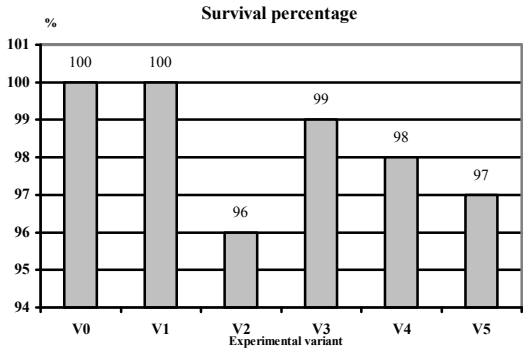

Figure 3. The survival percents of phytoinocula.

The plantlets had different growth and development, according to the type of light used for illumination and the wavelength (Table 1). The statistic significance of difference related to control variant (V_0) was calculated by T-test, for two tailed strings with unequal variances. The used software was MS-Excel. Three repetitions were made for each variant.

The stalk length touched the highest value at V_1 variant (white LED), but very close to the one measured at control variant (CFL). V_2, V_3, V_4 and V_5 manifested a slower growth, the smallest plantlets being found at V_2 (red LED). Related to the control, all differences had very good statistic significance.

Referring to number of leaflets, the red light have determined only a half of the average number, with about 10 pieces, all the inocula found at the rest of variants having around 20.

The sprouting was best manifested under red and yellow LED light, where the benefit, related to the CFL light, was 65% and 103%, but we cannot consider the sprout benefit at V_3 (yellow LED) as being relevant, because of the short stalks found at the level of this variant.

Rooting is an important issue if we consider a further acclimatization, but also for a proper *in vitro* plant feeding. This process was well represented to all experimental variants, the LEDs determining longer roots than CFL, but the values of the number of root filaments were very near-by, being about 5-6 at each variant. The longest roots were developed under yellow and green LED light, where the benefit, related to control variant, was 29.16%.

The tubergenesis takes place usually after a longer period of time and higher concentration sucrose (about 80-90 g/l) is used to achieve this goal. Under CFL light the tubergenesis was not observed, and under white LED it was only sporadically. This process took place allover the V_2, V_3, V_4 and V_5 variants, but the red LEDs manifested the best stimulation effect in tuber production.

Table 1. The monitored parameters of *Solanum tuberosum* L., at 8 weeks of vitroculture.

ID	Variant / Parameter	V_0 CFL white	V_1 LED white	V_2 LED red	V_3 LED yellow	V_4 LED green	V_5 LED blue
							
1	Stalk length average(cm)	6.3±0.20	6.8±0.24	3.0±0.27	4.3±0.16	4.4±0.11	4.5±0.18
	Statistic significance	-	***	***	***	***	***
2	Average leaves number	19.2±2.19	20.0±2.26	9.8±0.64	22.2±0.63	22.5±1.08	16.4±0.96
	Statistic significance	n/a	ns	***	***	***	***
3	Average sprout number	3.2±0.63	5.3±0.94	3.3±1.15	6.5±2.12	3.0±1.05	2.5±0.70
	Statistic significance	n/a	***	ns	***	ns	**
4	Roots length average (cm)	2.4±0.35	2.8±0.21	3±0.70	3.1±0.31	3.1±0.30	2.7±0.36
	Statistic significance	n/a	***	***	***	***	***
5	Average roots number	5.3±1.25	5.5±0.70	6.5±	6.2±	5.6±	5.4±
	Statistic significance	n/a	ns	**	*	ns	ns
6	Average tubers number	0	0.25±0.14	4.2±1.13	2.4±1.17	2.2±0.91	3.8±0.78
	Statistic significance	n/a	***	***	***	***	***

Legend: V_0 - white fluorescent light; V_1 - white LED; V_2 - red LED; V_3 - yellow LED; V_4- green LED; V_5 - blue LED; ns- non significant difference ($p \geq 0.1$); *- significant difference ($0.05 \leq p < 0.1$); **- distinctly significant difference ($0.01 \leq p < 0.05$); ***- very significant difference ($p < 0.01$); n/a - not applicable.

DISCUSSION

In some experiments, microtubers were obtained within 6 weeks, with 80 % sucrose concentration, using temporary immersion system and 6.75-8 µMoles/m^2/s light intensity, with benzylaminopurine (BAP) and coumarine added, but before it, the stalks were cultivated 5 weeks for elongation [25], so they needed 11 weeks for tubers yielding. In our case, we obtained a reduced number of tubers, but in only 8 weeks, and here the main advantage is diminished energy consumption. According to our observations, made during this experiment, we can conclude that LEDs are suitable for *Solanum tuberosum* vitrocultures illumination. White LEDs, at all monitored parameters, brought forth at least same good results as CFL tubes, but the energy low consumption recommend them to be used instead CFLs. In 2009, a typical 13 Watt LED ensemble emitted 450 to 650 lumens, which is equivalent to a standard 40 Watt incandescent bulb. In 2011, LEDs have become more efficient, so that a 6 Watt LED ensemble can easily achieve the same results [11].

Colored LEDs, especially the red ones (670 nm), and blue ones (470 nm) can stimulate tubergenesis at *potato* vitroplantlets, contributing this way to a fast seedling production at low cost.

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