Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 24, issue 1, 2014, pp. 139-142

POTATO GERMPLASM VITROPRESERVATION, USING LIGHT EMITTING DIODES AS LIGHT SOURCE

Liviu Pop[1], Dorina Cachiţă[1], Adriana Petruş-Vancea[2], Cornelia Purcărea[2]
[1]Western University "Vasile Goldiş" – Arad
[2]University of Oradea

ABSTRACT. In this experiment we tried to reduce the consumption of electric energy used in biotechnological vitroconservation process, in order to reduce the costs of preservation in gene banks 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, if illuminated with low intensity LED light, the potato inocula have registered a high growth retard, whether they were kept at +24°C, or at +4°C, the differences being minimal. This technique could be used to reduce the energy expenses and it also could be extended to any other plant.

KEYWORDS: *Solanum tuberosum* L., "*in vitro*", CFL, LED, preservation, gene bank

INTRODUCTION

Last century, the extinction of some plant species was observed. First warnings came since 1936 (Vavilov, cited by Dodds, 1991) but, at that time, nobody paid attention to this issue. Today we know that maintaining the biodiversity at least at the present level is an absolute priority. As a response to this problematic reality, many organizations, including FAO (Food and Agriculture Organization), notify that vegetal resources must be preserved in the best and safety condition possible, to avoid genetic erosion and species extinction. It was encouraged living collection to be started, where the plants having high genetic value to be maintained and, when necessary, to be used for ecological reconstruction (Sasson, 1993). The new economic concepts - bioeconomics, launched by Georgescu-Roegen (Roegen, 1979; Mayumi, 2001; Bonaiuti, 2011) and Brown's eco-economics (Brown, 2001) - came to accomplish the ideas of a sustainable economy, in order to leave a healthy environment heritage to the next generations.

International Board for Plant Genetic Resources (***IBPGR, 1985) has evaluated the vitropreservation in aseptic conditions as being realistic and efficient. They recommend living collections establishment, so to have anytime available plant germplasm (Cachiţă and Halmagyi, 2005). Vitropreservation is an alternative method to preserve genetic materials. Today, livestock management represents a multifunctional activity (Bogdan et al., 2010) which implies multidisciplinary research teams, acting together against hunger, for a better world. *In vitro* cultures can be considered also simplified experimental systems, which permit a sequential study of different morphogenetic programs (Toma and Toma, 2003).

Potato (*Solanum tuberosum* L.), known also as the second bread of humanity, is an important plant to be preserved; it's always needed as food, as seedling or as primary matter. The most important varieties are already stored in living collections, in gene banks, but the preservation is costly and, by this experiment, we intended to reduce the electricity consumption, in order to reduce the preservation costs, without using growth retardants (Wescott, 1981).

MATERIALS AND METHODS

Light is an indispensable factor for photoautotrophic organisms, needed to any kind of process regarding plants. Usually, 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 the lowest level yet, and 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 or preservation costs.

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 have offered enough light to be used to illuminate different objects, parks or buildings. They were also used as a light source in vitrocultures, replacing the old CFL tubes. Jao and Feng (2003) have designed a growth box with red and blue LEDs and in 2004 they used flashing light to potato vitrocultures and have obtained a good growth at a pulse frequency of 720 Hz.

In this experiment we exposed *potato* vitrocultures at LED continuous light with diminished intensity, and have been 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 stalk fragments (Rennali, 1997) and were placed in presterilized recipients (vol.=50 ml, height=6.5 cm; Ø=2.5 cm) containing standard Murashige and Skoog (1962) media, having Heller macroellements (Gautheret, 1959) and glycine, without growth regulators. The pH of the media was adjusted to 5.5, before autoclaving at 121°C for 30 min (Cachiţă et al, 2004).

The resulted experimental variants were as following:

**Correspondence: Liviu Pop, "Vasile Goldiş" Western University of Arad, Arad, Romania*
liviu.pop@bioresearch.ro
Article published:March 2014

V_0 (control variant) – CFL white light [16.2 µMoles/m^2/s (1200 lux)] at +24°C (75.2°F);

V_1 – LED white light [1.62 µMoles/m^2/s (120 lux)] at +24°C (75.2°F);

V_2 – LED white light [1.62 µMoles/m^2/s (120 lux)] at +4°C. (39.2°F).

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 (1200 lux) at their base. The others were put in growth boxes, and there was one LED above each bottle, at 1 cm distance (Fig.1). The light intensity was adjusted to 1.62 µMoles/m^2/s (120 lux). The photoperiod was set to 16h light from 24h for all experimental variants.

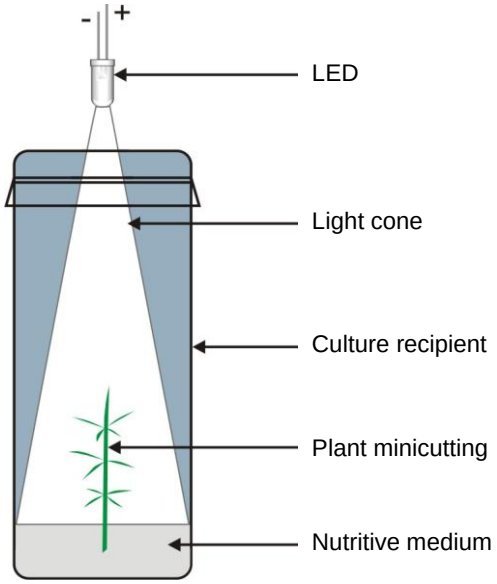

Fig.1 The schematic of LED illumination.

RESULTS AND DISCUSSION

The experiment lasted for *8 weeks* and the survival percentage is presented below (Fig.2). 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.

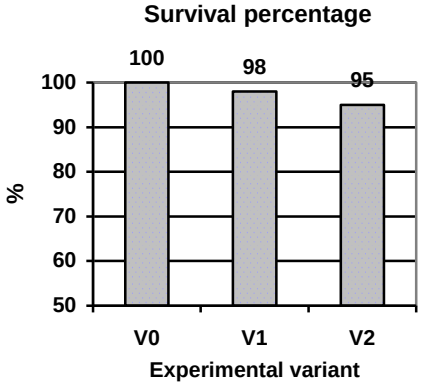

Fig.2 The survival percents of phytoinocula

The plantlets had different growth and development, according to the type of light used for illumination and the wavelength (Table 1). The statistical significance of differences was calculated by *t-test* for two tailed strings with unequal variances, using MS-Excel.

The stalk length touched the highest value at V_0 variant (CFL), fact that have been expected, considering the higher light intensity used at the level of inocula in this variant. Related to the control, all differences have very good statistical significance, the inocula that grew under low intensity light have presented a very tiny elongation.

Referring to number of leaflets, these were well formed under CFL light, but at low intensity light leaflets were found only at the V_1 variant, in early stage and very few (around 3 pieces). The inocula on V_2 did not form any leaflets in this time interval.

The sprouting was manifested at control variant (V_0), but new sprouts regenerative capacity was weak, with an average of 2.1at V_0 and 2.0 at V_1. Lower temperature (at V_2) has stopped this process.

Rooting is an important issue if we consider a further acclimatization, but also for a proper *in vitro* plant feeding. This process was observed to all experimental variants, but, if we consider control variant's value as 100%, then on V_1 we had 82.35% (statistically distinct significant) and on V_2 only 35.29% (statistically very significant).

Tubergenesis process was not observed as being present during this experiment.

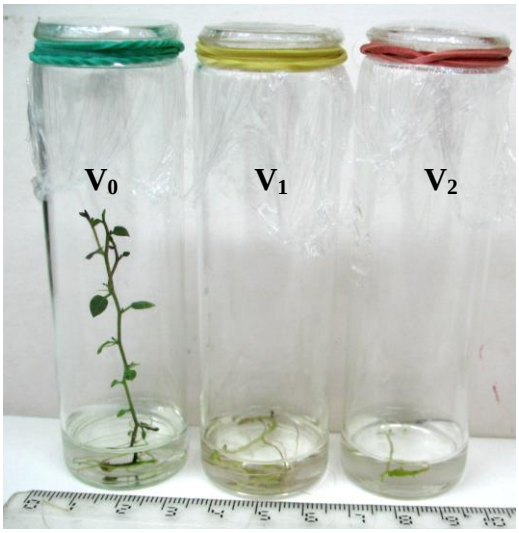

Fig. 3 The look of *Solanum tuberosum* L. vitrocultures at 8 weeks of experiment. V_0 (control variant) – CFL white light [16.2 µMoles/m^2/s (1200 lux)] at +24°C; V_1 – LED white light [1.62 µMoles/m^2/s (120 lux)] at +24°C; V_2 – LED white light [1.62 µMoles/m^2/s (120 lux)] at +4°C

Table 1

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

ID	Experimental variant Parameters (average values)	V_0	V_1	V_2
1	***Stalk length (cm)***	8.3±0.32	2±0.14	1.2±0.10
	Variance	0.1044	0.0200	0.0111
	Statistical significance	n/a	***	***
2	***Leaflet number***	19.2±1.22	3±0.66	0
	Variance	1.5111	0.4444	0
	Statistical significance	n/a	***	***
3	***Sprout number***	2.1±0.32	2±0.66	0
	Variance	0.1000	0.4444	0
	Statistical significance	n/a	***	***
4	***Root length (cm)***	3.4±0.18	2.8±0.24	1.2±0.17
	Variance	0.0356	0.0600	0.0289
	Statistical significance	n/a	**	***

At the end of experiment, the inocula from V_1 and V_2 variants were moved under CFL light, at the same conditions as V_0 were before. In a few days the regenerating processes were observed, and the growth has developed very well, in keeping with development that V_0 had during this experiment.

CONCLUSIONS

According to the observations made during this bioassay, we can conclude that LEDs are suitable devices for *Solanum tuberosum* L. vitrocultures illumination in vitroconservation purposes and keeping this species in living collections.

We also can claim that potato vitropreservation can be made even at ambient temperature (+24°C) because the differences regarding organogenesis, at low intensity light, have registered small differences between +4°C and +24°C, especially when we refer at stalk length, the most important parameter for enlarging the time between subcultivations.

The inocula preserved in these conditions can be easily reactivated and cultivated in a normal state.

Using LEDs for vitroconservation activities, we can save electricity and a lot of space, because there is a very small volume necessary to make this practice. It is explained by the fact that LEDs are placed at few millimeters distance above culture bottles, while CFLs must be very far from these, because their light intensity cannot be controlled.

ACKNOWLEDGEMENTS:

This work was co-financed from the European Social Fund through Sectorial Operational Program Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 "*Postdoctoral school for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by eco-sanogenesys*" with grant financing from European Union.

REFERENCES

Bogdan, AT, Miresan V, Sonea A, Chelmu S, Boboc, V, Surdu I, Burlacu R, Diaconescu D, Strateanu A, *Prospects for Development of Livestock Production between 2010–2100 Based on Integrated Rural Bioeconomics and Ecoeconomics*. Recent Researches in Business Administration, Finance and Product Management, 100-105, 2010.

Bonaiuti M, *From Bioeconomics to Degrowth: Georgescu-Roegen's 'New Economics' in Eight Essays*. Ed. Taylor and Francis, 2011.

Brown L, *Eco-Economy: Building an Economy for the Earth*, W. W. Norton & Co., NY, 81, 2001.

Cachiţă CD, Deliu C, Tican RL, Ardelean A, *Tratat de biotehnologie vegetală*, vol.I, Editura Dacia, Cluj-Napoca, 2004.

Cachită, CD, Halmagyi A, *Vitroconservarea resurselor vegetale*. Al XIV-lea Simpozion Naţional de Culturi de Ţesuturi şi Celule Vegetale.Editura Alma Mater-Sibiu, 1-17, 2005.

Dodds JH, *Conservation of plant genetic resources – the need for tissue culture*. In vitro Methods of Conservation of Plant Genetic Resources, E Dodds, J.H., hapman and Hall, 1991.

Gautheret RJ, *La culture des tissus végétaux : techniques et réalisations*. Masson Edit., 1959.

Jao RC, Fang W, *An adjustable light source for photo–phyto relate research and young plant production*. Applied Engineering in Agriculture, Vol. 19(5), 601–608, 2003.

Jao RC, Fang W, *Effect of frequency and duty ratio on the growth of potato plantlets in vitro using Light-emitting Diodes*. Hortscience 39(2), 375-379, 2004.

Mayumi K, *The origins of ecological economics: the bioeconomics of Georgescu-Roegen.* Routledge, 2001.

Murashige T, Skoog F, *A revised medium for rapid growth and bioassays with tobacco tissues cultures.* Physiologia Plantarum, 15, 155-159, 1962.

Ranalli P, *Innovative propagation methods in seed tuber multiplication programmes.* Potato Research , 40, 439-453, 1997.

Roegen GN, *The entropy law and the economic process*, Harvard University Press, 1971.

Sasson A, *Biotehnologii şi dezvoltare.* Ed. Tehnică, 2003.

Toma C, Toma I, *Citodiferenţiere şi morfologie vegetală*. Ed. Corson, 2003.

Wescott RJ, *Tissue culture storage of potato germplasm.2 Use growth retardants*. Potato Research. 24, pp. 343-352, 1981.

***IBPGR – IBPGR *Advisory Committee on in vitro Storage*, Report of the First Meeting, IBPGR, Rome, 1983.