

## Efficiency of use of light-emitting diodes for clonal microreproduction of sweet potato and peppermint in crop in vitro

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**The purpose.** To determine efficiency of application of state-of-the-art LED-systems for clonal microreproduction in crop *in vitro* of individually selected, with high biochemical parameters of clones of sweet potato and peppermint. **Methods.** Methods of cultures of organs and tissues — culture of somatic tissues and cells, clonal microreproduction, measuring-weight, mathematical-statistical. **Results.** At use of light-emitting diode lamps of red and dark blue colors in tubed plants they observed increase of average length of shoot: for peppermint — on 31,2%, for sweet potato — on 10,6 % in comparison with control alternative (cultivation under luminescent lamps). The maximum multiplication ratio of peppermint has provided genotype M.r-17/4 — 7,5±0,6 pieces (control) and 7,9±0,6 pieces — cultivation under LED-lamps. At plants of sweet potato cultivated under light-emitting diode lamps owing to augmentation of length of internodes from 5,2 mm (control) to 6,7 mm the multiplication ratio has increased from 5,5 (control) to 5,8 pieces. Application of light-emitting diode lamps of red and dark blue colors for tubed clones of peppermint and sweet potato has provided tall multiplication ratio of perspective for selection and seeds growing of genotypes of well crops. Spectral make-up of light-emitting diodes according to biological features of crop and stage of morphogenesis requires the further optimization. **Conclusions.** Use of light-emitting diode lamps of red and dark blue colors placed on light-emitting diode tape in combination 2 1, provides necessary illuminance for growth of plants of tubed clones of peppermint and sweet potato at the stage of their mass reproduction and reduces expenses for the electric power for 62,5 %.

**Key words:** *initial stock, illumination, rep-rodution, efficiency, morphogenesis, power saving.*

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Growing and processing niche crops is a promising direction for diversifying production for both small farms and large companies. Allows you to develop agricultural products for sale on the domestic and foreign markets. In Ukraine, they have formed a traditionally traditional set of crops, but now there is a demand for new species with high content of biologically valuable ingredients.

Sweet potatoes (*Ipomoea batatas* L.), a plant of the *Convolvulaceae* family, originating in the tropical regions of Central and South America, are among the most promising for mass cultivation in Ukraine. It is characterized by a high nutritional value of root tubers, which, besides starch (which contains amylose, predominates amylopectin), contain vitamins C and B, glucose, calcium, magnesium, carotene, folic acid [1]. Sweet potatoes we have so far little known to vegetable producers. Due to the high potential of productivity in the soil-climatic conditions of our country (from 40 to 100 t/ha), there are valuable medicinal and dietary properties of root tubers and high export potential (for 10 years the export of culture in Europe has increased by 6 times). The question of introducing sweet potatoes on the territory of Ukraine is relevant and timely.

A promising culture is also peppermint (*Mentha piperita* L.). Due to its unique biochemical composition, the plant is a source of essential oils. It has unsurpassed antioxidant, ant allergic, antiviral, antibacterial, antimicrobial and anticancer properties. Therefore, peppermint is widely used in the

perfume, pharmaceutical and food industries [2]. Due to the constantly growing demand in the world for natural essential oils, a large-scale production of its raw materials is promising [3].

Seeds easily propagate most of the known varieties of mint. However, the production of modern commercial genotypes, with high content and quality of the beneficial oils multiplied exclusively in a vegetative way. Because they are polyploid sterile forms. Because of this, commercial production of gardening material in the country's culture has not yet reached the proper level.

Economic efficiency and stability of vegetable growing is largely determined by the quality of planting material. Depending on the biometric indices of the seedlings, the genetic potential of the variety, the clone, their productivity and quality. For vegetative multiplication of crops - important is the survival of the seedlings, the balance of the bushes on the strength of growth and development, the beginning of fruiting, yield and merchantability.

According to traditional technology, mainly vegetative organs carry out multiplication of sweet potato and peppermint. However, this method does not meet modern requirements because of its low coefficient and constant accumulation of planting material of dangerous pathogens. Application of the same clonal micropropagation in culture in vitro allows in a short time to reproduce unique samples of new high-yield genotypes, including sterile and polyploid forms and obtain a genetically homogeneous source seedlings. Involvement in the breeding work of introductory samples, which have undergone the culture in vitro, have prevented the spread of dangerous quarantine pests, disease and weed killers in the regions. [4].

They are a reliable source for obtaining experimental material for plant breeding [5]. The method has ensured multiplication of planting material throughout the year and does not depend on environmental conditions. In addition, the use of cellular technologies in vitro is now a more technical solution for the propagation of planting material. Because it ensured the development of niche through reinvestment of profits in technology that provided value added with a quick payback period.

The essence of the method is the activation of lateral and apical meristems on the developed, in accordance with the biological and morphological features of culture, nutrient media. The general technology of reproduction of genotypes in culture in vitro includes the following steps. Selection and sterilization of primary explants; introduction into culture in vitro; proliferation and induction of development of shoots; rooting of test tubers on nutrient media and substrates; their adaptation from in vitro conditions to in vivo conditions; grooming of seedlings to the standard [6].

The effectiveness of the biotechnological stage of plant propagation in a culture in vitro depends, first, on the development of effective standardized protocols of this process. It requires a thorough analysis of economic efficiency at all its stages [7]. It was determined that in the structure of the cost of reproduction of regeneration plants in the culture of isolated cells and tissues in vitro a significant part fell on the wage tariff fund, the depreciation of laboratory equipment, the costs of preparing nutrients and electricity.

The problem of finding and using efficient energy sources together with the development and implementation of energy-saving technologies in Ukraine today is extremely relevant both for research institutions and for commercial in vitro laboratories. Taking into account the significant growth of electricity tariffs over the last ten years, it will be necessary to search for new energy-saving ones. At the same time, more efficient lighting devices will be needed at the stage of mass reproduction of test tubes.

One of the important indicators of energy-saving characteristics of light devices is light transmission. In the most widespread fluorescent lamps, this figure is 60 L/W, in LEDs - 100 L/W. The service life of the fluorescent lamps DRL does not exceed 6000 hours, while the modern LED-light up to 1 000 000 hours. Modern LED (LED) technologies allow you to create light of any color and intensity. Using LEDs of different colors and in different proportions, you can provide the lighting necessary for plants in certain phases of their development and biological characteristics. Red light-emitting diodes (720-600 nm) are considered as the main source for photosynthesis, which influences the rate of development of plants. Blue rays (490-380 nm) also stimulate the formation of proteins and regulate the rate of development of plants [8, 9]. However, to optimize the mode of illumination of the test material (intensity and combination

of spectra) it is necessary taking into account the biological features of culture and the stage of morphogenesis.

**Materials and methods of research.** Work on this theme was performed at the Laboratory of genetics, genetic resources and biotechnology of The IVM of NAAS in 2006-2008. In laboratory and field conditions in accordance with ISO 17025 [10]. In the course of research in culture in vitro used standard methods of sterilization of nutrients, tools and laboratory equipment [11].

The source material is 3 varieties-sweet potato intruducts and 3 genotypes of peppermint. For obtaining sterile potato sprouts, donor organs - shoots sprouted on sand bubbles and young peppermint shoots were sterilized in 30% sodium hypochlorite solution with a treatment exposure of 25 minutes. Washed them with sterile distilled water. Multiplied viable planting material by activation of lateral and apical meristems on agarized nutrient media MS [12], supplemented with 3% sucrose, vitamins and growth regulators [13]. Derived from lateral and apical meristem shoots were divided into cuttings, which included part of the stem with a leaf and lateral kidney. Cuttings were planted in a test tube with a solid nutrient medium MS.

The plants were regenerated in a specially equipped room, at a temperature of 22-24 °C and a 16-hour photoperiod. Two types of lamps illuminated the test material: fluorescent (control) and LED red and blue located on the Philips LED ribbon in combination of 2x1. After 45 days, they carried out the account of the development of test tubes and determined the biometric indices. The height of the shoots (mm), the number of leaves (pieces), the length of the interstitial (mm), and the length of the root (mm), the weight of the plant (g). Experiments were performed in 3-fold repetition, the number of explants of one repetition - 20 pcs.

The economic efficiency of the cultivation of test plants was determined according to the methodological recommendations "Determination of the economic efficiency of research results in vegetable growing" [14]. The statistical processing of the results obtained was carried out using the program Statistica 6.0, Excel 2010.

**Results.** Types of lighting significantly influenced the morphological characteristics of the sample clones of genotypes-introducers of mint and sweet potatoes.

In all genotypes of mint under the LED-lamps of red and blue, the average length of the shoot increased from 47.1 (control variant) to 61.8 mm, which is 31.2% higher than the control index (Table 1). A similar trend was observed in all genotypes. The maximum lengths for both types of illumination provided sample M.p-17/4 (67.9 and 45.9 mm), the minimum values were M.p-16/3 - 55.4 ± 6.1 and 45.9 mm, respectively. By the way, previous studies on potatoes have determined [15] that during the cultivation of test tubes under red and blue LED-lamps in plants, the content of chlorophyll "a" and chlorophyll "b" increased. It is probable that due to the increase of photosynthetic activity, an increase in biometric indices of regenerated plants.

The number of leaves after four weeks of cultivation of the plants-regenerants of mint under blue-red light exceeded the control variant and was an average of 6.7 pcs/plant. Regenerants under fluorescent lamps - 6.4 pcs/plant. This indicator is closely related to the regeneration rate of multiplication factor. During further in vitro culture, the obtained shoots were divided into cuttings, which had a part of the stem with a leaf and a lateral kidney. Therefore, according to our data, the maximum average reproduction rate provided the genotype M.p-16/3 - 7.5 ± 0.6 pcs (control) and 7.9 ± 0.6 pcs - for cultivation under LED-lamps.

In the natural environment, reducing the level of lighting of plants is usually accompanied by a decrease in temperature and an increase in relative humidity of air. As a result, the intensity of respiration and the evaporation of water by leaves decreases, as a result - the energy consumption is reduced. Therefore, reducing the intensity of photosynthesis does not harm the plant. However, during the cultivation of plants under artificial conditions, including in an in vitro culture, where there is a different level of their illumination, other parameters of the medium (temperature, humidity) are unchanged.



Table 1. Influence of types of illumination on morphological indices of plants-regenerants of genotypes-introducers peppermint and sweet potato in the culture in vitro

№ for biotechnology catalog	Shoots length mm		Number of sheets pcs		Length of internode, mm		Length of root mm		Weight of plant g	
	white (control)	blue-red	white (control)	blue-red	white (control)	blue-red	white (control)	blue-red	white (control)	blue-red
peppermint										
<i>M.p.-16/1</i>	43,8±4,0	62,2±6,8	6,7±0,5	7,4±0,7	6,5±0,6	8,4±0,9	84,8±7,9	74,5±7,2	1,4±0,1	1,6±0,1
<i>M.p.-16/3</i>	45,9±4,3	55,4±6,1	7,5±0,6	7,9±0,6	6,1±0,5	8,0±0,8	80,5±7,6	86,3±9,2	1,8±0,2	1,7±0,1
<i>M.p.-17/4</i>	51,6±5,5	67,9±7,1	5,1±0,4	5,9±0,6	10,0±0,9	11,5±1,2	66,7±7,1	80,9±8,1	1,5±0,1	1,3±0,1
Average	47,1	61,8	6,4	6,7	7,5	9,3	77,3	80,6	1,6	1,5
sweet potato										
<i>I.b.-Or/17</i>	70,1±6,5	71,6±7,0	5,8±0,3	6,1±0,8	5,4±0,5	7,7±0,7	77,7±7,6	74,2±7,6	1,0±0,1	0,89±0,05
<i>I.b.-Bo/17</i>	60,8±6,3	67,3±6,9	5,4±0,5	6,0±0,5	5,3±0,5	6,9±0,7	49,6±5,6	59,7±5,6	1,2±0,1	1,14±0,1
<i>I.b.-Mu/17</i>	65,5±6,8	69,3±7,0	5,1±0,35	5,4±0,4	4,9±0,4	5,4±0,6	70,3±6,3	71,4±6,9	1,3±0,1	1,05±0,2
Average	65,4	69,4	5,4	5,8	5,2	6,7	65,9	68,4	1,2	1,0

Because of these regeneration plants, to get lighter are elongate in internodes and petioles. There was an increase in the average length of internodes of plants-regenerants of mint at 24.0% (from 7.5 mm in control, up to 9.3 mm under blue-red LEDs).

The level of illumination of plants also affected the development of the root system of mint regenerants. In the experiment, it developed even more intensively than above ground organs. The average length of the roots of genotypes after cultivation under colored LED-lamps was 80.6 mm, control - 77.3 mm. The maximum values belonged to samples M.p-16/3 and M.p-17/4 -  $86.3 \pm 9.2$  mm and  $80.9 \pm 8.1$  mm, respectively. Significantly lower than control, the data of sample M.p-16/1 -  $74.5 \pm 7.2$  mm (control -  $84.8 \pm 7.9$  mm).

An important integral indicator that more fully characterizes the provision of plants with all the necessary components during cultivation in an isolated culture is their average mass at the end of the passage. This feature in the experiment varied depending on the genotype. Under the blue-red light in samples M.p-16/3 and M.p-17/4, the weight of the plant was  $1.7 \pm 0.1$  and  $1.3 \pm 0.1$  g, respectively, and was lower than control. In the sample M.p-16/1, a similar index was  $1.6 \pm 0.1$  g and was higher than control ( $1.4 \pm 0.1$  g). Thus, the application for illumination of test tubes of mint pepper LEDs in red and blue, on an LED ribbon in a combination of 2x1, ensured their growth and development in culture in vitro. For genotypes in which plants-regenerators lag behind the mass, it is necessary to correct the spectral composition of the LEDs. During the cultivation of sweet potato plants under the red and blue LEDs, the main biometric indices in the microparticles of the introductive genotypes were also higher. Thus, the average length of the shoots of culture under the LED-lamps exceeded the indices of plants, under white luminescent light (69.4 and 65.4 mm respectively). The average indices of the length of the root, the number of leaves and the length of the nodules of the introductive genotypes of the new niche culture after cultivation under colored LED-lamps were respectively 68.4 mm, 5.8 pcs, 6.7 mm, and were higher than control. Depending on the genotype, the number of leaves has changed from  $5.4 \pm 0.4$  pcs in the sample I.b.-Mu/17 to  $6.1 \pm 0.5$  pcs in I.b.-Or/17. LED-lamps provided a larger length of interchanges - from 5.2 mm (control) to 6.7 mm higher reproduction rate - from 5.5 pcs (control) to 5.8 pcs from one plant regenerator. The average mass of test tubes at the end of the passage was quite variable, depending on the genotype: all specimens under blue-red light were weighed 1.0 g, which was 0.2 g less than control (1.2 g) due to elongation

The cost structure for various methods of lighting in vitro collections of peppermint and sweet potato mint test plants showed that during the cultivation of 10 thousand probiotic plants for standard illumination by fluorescent lamps for 4 weeks, the costs amounted to 15,963.54 UAH. The use of modern LED lighting fixtures for the same number of regenerating plants was UAH 5988.0 per one passage and provided a reduction in the cost of 62,5 %. Additional advantages of using LED-systems in biotechnological laboratories are their high light output and long service life. LED modules, in addition, emit less heat, and creates a uniform distribution of light over the test material, which reduces the cost of air conditioning premises in the summer. They do not contain harmful substances (such as mercury), so there are no problems with their disposal.

Thus, the application for illumination of test tubes of mint pepper and bitter LEDs of red and blue, on a LED ribbon in a combination of 2 x 1, provided a high reproduction rate for niche cultures promising for breeding and seeding. The spectral composition of the LEDs in accordance with the biological peculiarities of the culture and the stage of morphogenesis needs further optimization.

## References

1. Doliński R. (2013). Micropropagation of sweet potato (*Ipomoea batatas* L.) from node explants. *Acta Sci. Pol., Hortorum Cultus*. 12 (4). P. 117-127.
2. Fialová S., Tekel'ová D., Švajdlenka E., Potůček P.I. (2014). The variability of secondary metabolites in *Mentha × piperita* cv. 'Perpeta' during the development of inflorescence *Acta Fac. Pharm. Univ. Comen. LXI*. (2). P. 21–25.

3. Brun N, Voirin B. (1991). Chemical and morphological studies of the effects of aging on monoterpenes composition in *Mentha × piperita* leaves. *Can J Bot.* 69. pp. 2271-78.
4. Riabchun V.K. (2002). Problemy ta perspektyvy zberezhennia henofondu roslyn v Ukraini. [Problems and perspectives of preservation of the gene pool of plants in Ukraine]. Kharkiv. 38 p. [in Ukraine].
5. Hrodzynskiy A.Y. (1971). Allelopatyia y yntroduktsyia rasteniy. [Allelopathy and plant introduction]. *Bul. GBS AN SSSR.* 81. pp. 45–50. [in Russian].
6. Kalinin F.L. (1980). Metody kultury tkanei v fiziologii i biokhunii rastenii. [Methods of tissue culture in plant physiology and biochemistry]. Kyiv: Naukova dumka. 488 p. [in Russian].
7. Ivchenko T.V. (2013). Vykorystannia kultury apikalnykh merystem dlia pryskorenoho rozmnozhenia mistsevykh form chasnyku. [Use of apical meristem culture for accelerated reproduction of local forms of garlic]. *Bulletin of Kharkiv Agrarian University.* Kh. 5. P. 179–183. [in Ukraine].
8. Chervinskyi L.S., Loienko S.V. (2017). Perspektyvy vykorystannia svitylnykyv na osnovi svitlo diodiv dlia roslyn v sporudakh zakrytoho hruntu. [Prospects for using light fixtures based on light diodes for plants in closed-ground buildings]. *Scientific Journal TDTU.* 2. P. 1-6. [in Ukraine].
9. James M. Stephens. (2014). Grow your own vegetables without soil. *UF/IFAS Extension.* 2. P. 2-4.
10. ISO/IEC 17025:2006 Natsionalnyi standart Ukrainy. Zahalni vymohy do kompetentnosti vyprobuvalnykh ta kalibruvalnykh laboratorii. [National standard of Ukraine. General requirements for the competence of testing and calibration laboratories]. [in Ukraine].
11. Ivchenko T. V., Vitsenia T.I., Bashtan N.O. (2013). Metodychni rekomendatsii po zastosuvanniu kultury izolovanykh tkanyn v selektsii ovochevykh roslyn. [Methodical recommendations on the application of the culture of isolated tissues in the selection of vegetable plants]. Merefa: IOB NAAN. 48 p. [in Ukraine].
12. Murashige T.A. (1962). Revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol.plant.* 15. P. 473–497.
13. Ivchenko T.V., Mozghovska H.V., Vitsenia T.I., Bashtan N.O. (2018). Metodychni pidkhody do selektsiinoho protsesu ta nasinnytstva batatu (*Ipomoea batatas* L.). [Methodical approaches to the selection process and seedlings of sweet potatoes]. Kharkiv: Pleiada. 34 p. [in Ukraine].
14. Vyznachennia ekonomichnoi efektyvnosti rezultativ naukovo-doslidnykh robit v ovochivnytstvi: metodychni rekomendatsii. [Determination of economic efficiency of research results in vegetable growing: methodical recommendations]. 2001. Kh.: KhNAU im. V. V. Dokuchaieva. 27 p. [in Ukraine].
15. L.M. Reshotko, S.V. Derevianko, O.O. Dmytruk, I.V. Volkova. (2016). Vplyv riznykh spektriv vyprominiuvannia na rist ta rozvytok ozdorovlenykh roslyn kartopli v kulturi in vitro. [Influence of various spectra of radiation on the growth and development of healthy potato plants in culture in vitro]. *Agricultural Microbiology.* 24. P. 73–78. [in Ukraine].