Optimization of light conditions for growing well-improved potatoes in the laboratory

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Abstract. The paper examined the impact of different lighting sources on the growth and
development of the Antonina and Solnechnyi potato varieties, grown in vitro. The object of the
experiment was pathogen free parent micro clones of potatoes, derived from apical meristem by
cultivation in the standard Murashige and Skoog medium with modifications. The following
sources of light were used: OSRAM luminescent lamps, with the predominant red and blue,
power 36 W; OSRAM luminescent lamps, cold daylight, power 36 W; TELEFUNKEN linear
light-emitting-diode (LED) lamps - 4200 K, white light, power 18 W; linear LED lamps
TELEFUNKEN - 2700 K, warm white light, power 18 W; LED lamps red-blue, diodes ratio 3:1,
power 32 W and LED lamps full-spectrum, power 32 W. The effect of these lighting sources on
the length of plants, time of root emergence, number of leaves / internodes, mass of the plants
and leaves, shoots and roots, and the surface area of the leaf plate was studied. The operating
costs for the studied lighting sources was calculated. It was discovered that for the cultivation of
the test tube plants varieties Antonina and Solnechnyi with the aim to obtain the maximum
number of new test plants, LED lamps 2700 K are the most suitable ones. For the cultivation of
test plants of the chosen varieties to prepare them for further transplantation on aero-hydroponic
system for producing mini tubers, full-spectrum LED lamps are recommended.

1. Introduction

Potato is one of the main crops, the world production of which is constantly increasing. In recent years,
there has been a decline in the yield of this crop. First, this is due to the unfavorable phytopathological
features of the cultivation regions of this crop and, consequently, to the loss of a high-quality seed
material. One of the main factors guaranteeing the achievement of the maximum possible yield of
potatoes is to obtain a high-quality source of healthy viral and bacterial-free planting material. The basis
of modern elite potato seed production in advanced economies is development of a high-quality
pathogen free planting material with application of the method of apical meristem and clonal micro
breeding in vitro, which allows both new and old varieties to be free from viral infection.

In vitro culture potato test tube plants are cultivated in the conditions of artificial lighting. Light
of different spectrums regulates morphogenesis and growth processes in potato plants in vitro [9-13].
Nowadays, luminescent lighting sources are widely used for growing ornamental plants and flowers, in
which most of the spectrum consists of red and green light. The green part of the spectrum is usually
reflected from the leaf surface, which results in low efficiency of these lamps [14]. The use of
luminescent lamps gives the best value for money in crop production. LED sources are a promising
direction in plant growing as they vary the spectrum of the light flow and are characterized by high light output and long-term lifetime [15-18].

The aim of the work: to identify the optimal light conditions for growing pathogen free potatoes of the Antonina and Solnechnyi in vitro varieties in the laboratory.

Tasks:
1. To examine the effect of different lighting sources on the morphometric features of the potato plants varieties Antonina and Solnechnyi in vitro.
2. To determine the cost-effectiveness of the used lamps.

2. Materials and methods
The work was carried out in Siberian Research Institute of Agriculture and Peat – branch of the Siberian Federal Scientific Center of Agro-Bio Technologies of the Russian Academy of Sciences (Tomsk). The object of the experiment - pathogen free parent micro clones of potatoes solanum tuberosum L. varieties Antonina and Solnechnyi, derived from apical meristem by cultivation in the standard Murashige and Skoog medium with modifications. The preparation and cultivation of the plants was carried out under the recommendations of L. N. Trofimets [19].

Antonina is an early ripe variety, with a period from planting to the beginning of the formation of the commodity harvest 60-70 days. The skin of tubers is slightly rough, yellow, pulp - light yellow. The Antonina potato tubers are oval with medium-depth eyes. The tuber weight is 104-153 grams. The starch content is 15.9-19.4%. This variety is resistant to potato cancer, moderately susceptible to golden potato cyst nematode and late blight pathogen [20].

The Solnechnyi variety is medium-ripe, suitable for processing for potato products. Commodity yield -is 178-290 centners per hectare. The tuber is rounded with medium-depth eyes. The skin is smooth, yellow. The pulp is yellow. The mass of the commodity tuber 139-290 grams. The starch content is 14.4-16.0%. It has good taste. The marketability is 85-98%. The keeping capacity is 94%. Resistant to the pathogen of potato cancer, weakly affected by golden potato cyst-forming nematode. It is included in the State Register of the Russian Federation West Siberian (10) region.

For potato improvement, a search for basic clones in the field conditions was implemented.

Requirements for the plant, intended for selection: the plant has typical morphological structure for this variety, healthy in appearance, well developed, with a characteristic number of shoots for the variety. The tubers have typical morphological features for this variety without signs of spindle-likeness, perfectly healthy in visual estimation; characteristic for the variety is the number of standard-sized tubers and transition from large to small.

The yield of each selected clone was put in a separate package and deposited for storage. After the end of the rest period, thermotherapy was carried out - in a thermostat for 40 days at the temperature 37°C. The tubers, placed for thermotherapy, did not have the external signs of infection, late blight, sclerotiarism risoctonia and were carefully washed.

After the thermotherapy, the tubers were sprouted to produce etiolated sprouts with the size 2 to 3 cm. The tubers were placed on the racks in the dark place, at the temperature of 18 - 22°C and relative humidity around 75%.

Isolation of apical meristems was carried out in a microbiological box sterilized with bactericidal lamps. Sprouts were separated from the carefully washed tubers and sterilized in ethanol for 10 minutes, followed by three times rinsing sterile H₂O. The sterilized sprouts were placed in a sterile Petri dish and a few drops of autoclave water was added to prevent them from drying out. The meristem of 100 - 250 microns without leafy rudiments (primordium) was dissected with a conventional thin needle, clamped in the holder and transported to the surface of the nutrient medium in the test tube, then it covered with a cotton plugs over the flame of the burner and put in a test tube holder. After filling the test tube holder with the test tubes, it was covered with a cellophane cap to prevent the nutrient medium from drying out, signed and put in the light chamber with constant light and moisture-temperature modes.

After the emergence of a full-fledged test plant from the meristem, its microclonal reproduction and the beginning of the experiment was performed. Microclonal reproduction of potato test plants was
carried out by micropropagation in sterile laminar boxes. Before the beginning of the experiment all the micro plants were diagnosed by real-time PCR method in the laboratory of diagnosis and quality control of seed potatoes.

During the experiment, the nodal cuttings were cultivated at 20-22 degrees Celsius, with a photo period of 16/8 hours light/darkness, in the test tubes for 28 days using the following types of lighting:
1. OSRAM Fluora luminescent lamps, with the predominant red and blue, power 36 W.
2. OSRAM luminescent lamps, cold daylight, power 36 W.
3. TELEFUNKEN linear LED lamps – 4200 K, white light, power 18W.
4. TELEFUNKEN – linear LED lamps – 2700 K, warm white light, power 18 W
5. LED lamps red-blue, diode ratio 3:1, power 32 W.
6. Full-spectrum LED lamps, power 32 W.

35 plants of each variety were grown with each lighting source. Repetition was triplicate. During the experiment on 3d, 7th, 14th, 21st, 28th days were measured indicators that characterize the development of plants: length of the plant, presence of the root, number of leaves and presence of internodes per plant.

On 28th day the total mass of the plant, the mass of leaves and roots and the surface area of the leaf plate were measured. The emergence of the roots was determined visually at certain intervals. The height was measured with a dimensional ruler from the base of the plant to the top point. The number of leaves was determined by calculation on one test plant. The mass of plants with leaves, mass of leaves and roots were determined by weighing on the laboratory scales. To determine the surface area of the leaves, scanned images of the leaves were used, they were processed with the help of a special program “ImageJ”.

The results were statistically processed with the help of the software package for Windows Statistica 8.0. The Mann-Whitney U-test was used to compare the studied values.

## 3. Results and discussion

The results of studying the impact of different lighting sources on the height of plants at different stages of their development are presented in tables 1 and 2. In tables 1, 2 and beyond, the studied lighting sources are numbered as follows:

1 – linear luminescent lamps Fluora;
2 - linear luminescent lamps of cold daylight;
3 - linear LED lamps 4200 K;
4 - linear LED lamps 2700 K;
5 - LED lamps red and blue (diode ratio 3:1);
6 - LED lamps full-spectrum.

### Table 1. The impact of different lighting sources on the height of pathogen free test tube plants of the Antonina variety, cm.

| Lighting source | 3 days       | 7 days       | Cultivation time | 14 days       | 21 days       | 28 days       |
|-----------------|--------------|--------------|------------------|--------------|--------------|--------------|
| 1 (control)     | 0.25±0.02    | 1.03±0.05*   | 4.94±0.18***     | 8.65±0.24    | 10.19±0.23   |
| 2               | 0.27±0.02    | 1.22±0.06    | 6.12±0.22        | 8.58±0.27    | 9.81±0.28    |
| 3               | 0.28±0.02    | 1.23±0.07    | 6.28±0.23        | 8.34±0.24    | 9.12±0.23*   |
| 4               | 0.25±0.02    | 1.15±0.06    | 6.00±0.17        | 8.92±0.25    | 10.10±0.24   |
| 5               | 0.20±0.02*   | 0.79±0.04*** | 4.41±0.19***     | 8.13±0.23    | 9.14±0.21*   |
| 6               | 0.27±0.02    | 1.06±0.05*   | 5.12±0.22**      | 8.17±0.27    | 9.08±0.25*   |

Notes: *- differences are valid with p<0.05 compared to control; **- differences are valid with p<0.01 compared to control; ***- differences are valid with p<0.001 compared to control.

Using the red-blue LED lamps in the cultivation of Antonina variety potatoes resulted in the reduction of the plant height (9.14 cm compared to 9.81 cm in control) on 28th day of the cultivation, with a lag in growth observed from the beginning of the plant cultivation. LED lamps 4200 K also caused a decrease in the plant height (9.12 cm compared to 9.81 cm in control) on 28th day of the cultivation. Under the
full-spectrum LED lamps, the reduction in the plant height compared to the control version was observed throughout the entire growing period and on 28th day (9.08 cm compared to 9.81 cm in control).

Table 2. The impact of different lighting sources on the height of pathogen free test tube plants of the Solnechnyi variety, cm.

| Lighting source | 3 days       | 7 days       | 14 days      | 21 days      | 28 days      |
|-----------------|--------------|--------------|--------------|--------------|--------------|
| 1               | 0.10±0.01*** | 1.00±0.06*** | 4.67±0.16*** | 7.88±0.21*** | 10.25±0.26*  |
| 2 (control)     | 0.17±0.02    | 1.57±0.12    | 6.25±0.02    | 9.15±0.32    | 11.07±0.31   |
| 3               | 0.17±0.02    | 1.40±0.08    | 5.83±0.22    | 8.95±0.26    | 11.11±0.26   |
| 4               | 0.13±0.02**  | 1.68±0.11    | 7.60±0.30*** | 11.21±0.34***| 13.13±0.30***|
| 5               | 0.09±0.01*** | 0.89±0.06*** | 3.83±0.18*** | 6.94±0.25*** | 8.68±0.22*** |
| 6               | 0.19±0.02    | 1.36±0.09    | 5.39±0.26*   | 9.04±0.32    | 10.15±0.35   |

Notes: *- differences are valid with p<0.05 compared to control; **- differences are valid with p<0.01 compared to control; *** - differences are valid with p<0.001 compared to control.

In the cultivation of the Solnechnyi variety potato test plants with the red-blue LED lamps, there was a statistically significant reduction in the plant height which was observed during the entire growing period (8.68 cm compared to 11.07 cm in control on 28th day). The plants under LED lamps 2700 K, on the contrary, had a better height, starting on 14th day of the cultivation. The differences remained and on 28th day (13.13 cm compared to 11.07 cm in control). The plants grown under the linear luminescent lamps Fluora were less high throughout all growing period (10.25 cm compared to 11.07 cm in control on 28th day).

The results of measuring the number of leaves/internodes in the cultivation of test tube plants using different lighting sources are presented in tables 3 and 4.

Table 3. The impact of different lighting sources on the number of leaves/internodes of pathogen free test tube plants of the Antonina variety, pcs.

| Lighting source | 3 days      | 7 days      | 14 days     | 21 days     | 28 days     |
|-----------------|-------------|-------------|-------------|-------------|-------------|
| 1               | 0.26±0.06   | 2.15±0.10   | 4.47±0.08** | 6.06±0.08   | 7.15±0.11   |
| 2 (control)     | 0.19±0.04   | 2.16±0.09   | 4.71±0.10   | 5.88±0.11   | 7.05±0.16   |
| 3               | 0.22±0.06   | 2.30±0.09   | 5.15±0.08** | 6.17±0.07   | 7.08±0.12   |
| 4               | 0.15±0.04   | 2.27±0.09   | 4.83±0.08   | 6.33±0.08** | 7.31±0.10   |
| 5               | 0.15±0.05   | 2.05±0.11   | 4.57±0.10   | 5.91±0.08   | 6.77±0.08   |
| 6               | 0.35±0.07   | 1.96±0.10   | 4.78±0.09   | 6.06±0.10   | 6.60±0.11*  |

Notes: * - Notes: *- differences are valid with p<0.05 compared to control; **- differences are valid with p<0.01 compared to control; *** - differences are valid with p<0.001 compared to control.

Therefore, when growing test tube plants under full-spectrum LED lamps, there was observed a decrease in the number of leaves and internodes (6.60 pieces compared to 7.05 pcs. in control for the Antonina variety and 7.37 pieces compared to 8.01 pcs. in control for the Solnechnyi variety) on 28th day of the cultivation. In this case, other cultivation periods are not of important practical importance, because micropropagation is recommended to be performed exactly on the 28th day.
Table 4. The impact of different lighting sources on the number of leaves/internodes of pathogen free test tube plants of the Solnechnyi variety, pcs.

| Lighting source | 3 days | 7 days | 14 days | 21 days | 28 days |
|-----------------|--------|--------|---------|---------|---------|
| 1 (control)     | 0.01±0.01 | 1.73±0.12*** | 5.20±0.10 | 6.60±0.07 | 8.01±0.13 |
| 2 (control)     | 0.06±0.02 | 2.44±0.13 | 5.14±0.00 | 6.81±0.12 | 7.40±0.13 |
| 3               | 0.08±0.04 | 2.21±0.13 | 5.33±0.12 | 6.94±0.11 | 8.05±0.11 |
| 4               | 0.02±0.01 | 2.29±0.13 | 5.30±0.13 | 6.59±0.11 | 7.94±0.12 |
| 5               | 0.00±0.00 | 1.85±0.13** | 4.91±0.11** | 6.45±0.12 | 7.75±0.12 |
| 6               | 0.05±0.02 | 2.28±0.13 | 5.05±0.12* | 6.63±0.12 | 7.37±0.12*** |

Notes: *- differences are valid with p<0.05 compared to control; **- differences are valid with p<0.01 compared to control; *** - differences are valid with p<0.001 compared to control.

The effect of different lighting sources on the morphometric parameters of cultivated plants is shown in tables 5 and 6.

Table 5. The impact of different lighting sources on morphological parameters of the pathogen free test tube plants of the Antonina variety on the 28th day of the cultivation.

| Type of lighting | Mass of sprout, gr | Mass of leaves, gr | Mass of roots, gr | Surface area of leaf plates, mm² |
|------------------|--------------------|--------------------|-------------------|----------------------------------|
| 1                | 0.31±0.01          | 0.14±0.01          | 0.18±0.01         | 100.65 ± 3.53                   |
| 2 (control)      | 0.31±0.01          | 0.15±0.01          | 0.18±0.01         | 111.35 ± 3.56                   |
| 3                | 0.28±0.02          | 0.15±0.01          | 0.14±0.01*        | 105.07 ± 3.79                   |
| 4                | 0.32±0.01          | 0.14±0.01          | 0.12±0.01*        | 106.35 ± 3.48                   |
| 5                | 0.34±0.01*         | 0.18±0.01*         | 0.28±0.01***      | 128.77 ± 4.97                   |
| 6                | 0.33±0.01          | 0.15±0.03          | 0.25±0.01***      | 144.04 ± 4.58***                |

Notes: *- differences are valid with p<0.05 compared to control; **- differences are valid with p<0.01 compared to control; *** - differences are valid with p<0.001 compared to control.

Table 6. The impact of different lighting sources on morphological parameters of pathogen free test tube plants of the Solnechnyi variety on the 28th day of the cultivation.

| Type of lighting | Mass of sprouts, gr | Mass of leaves, gr | Mass of roots, gr | Surface area of leaf plates, mm² |
|------------------|---------------------|--------------------|-------------------|----------------------------------|
| 1                | 0.31±0.01           | 0.15±0.01          | 0.16±0.01         | 113.41 ± 3.59                   |
| 2 (control)      | 0.33±0.01           | 0.14±0.01          | 0.17±0.01         | 114.82 ± 3.46                   |
| 3                | 0.31±0.02           | 0.14±0.01          | 0.13±0.01***      | 107.63 ± 3.63                   |
| 4                | 0.35±0.01           | 0.15±0.01          | 0.12±0.01***      | 109.39 ± 3.86                   |
| 5                | 0.42±0.01***        | 0.24±0.01***       | 0.22±0.01***      | 150.23 ± 4.84***                |
| 6                | 0.38±0.01*          | 0.16±0.01          | 0.19±0.01         | 124.69 ± 3.79                   |

Notes: *- differences are valid with p<0.05 compared to control; **- differences are valid with p<0.01 compared to control; *** - differences are valid with p<0.001 compared to control.

Thus, the use of red-blue LED lamps in the cultivation of the Antonina variety potatoes resulted in the increase in the mass of plants with leaves (0.34 gr compared to 0.31 gr in control) due to the increase in the leaf mass (0.18 gr compared to 0.15 gr in control) and increase in the mass of the root system (0.28 gr compared to 0.18 gr in control) on 28th day of the cultivation.

The use of 4200 K linear LED lamps and 2700K linear LED lamps resulted in reduction of the root mass of the plants (0.14 gr and 0.12 gr, respectively, compared to 0.18 gr in control). When growing test tube plants under full-spectrum LED lamps, there was an increase in the root mass (0.25 gr compared to 0.18 gr in control) and of the area of the leaf plate (from 111.35 mm² in control to 144.04 mm² in the test plant).
When growing test tube plants of the Solnechnyi potato variety with the help of red-blue LED lamps, there was a statistically significant increase in the mass of the plants with leaves (0.42 gr compared to 0.33 gr in control) due to the increase in the leaf mass (0.24 gr compared to 0.14 gr in control), in the root mass (0.22 gr compared to 0.17 gr in control) and the leaf plate area (from 114.82 mm\(^2\) in control to 150.23 mm\(^2\) in the test plant). The use of 4200 K linear LED lamps and 2700K linear LED lamps resulted in reduction of the root mass (0.13 gr and 0.12 gr, respectively, compared to 0.17 gr in control). Growing plants under full-spectrum LED lamps led to the increase in the mass of the plants with leaves (0.38 gr compared to 0.33 gr in control) due to the mass increase of the shoot (0.22 gr compared to 0.19 gr in control).

The dynamics of rhisogenesis under different lighting sources is shown in tables 7 and 8.

**Table 7.** The impact of different lighting sources on the plant number of the Antonina potato variety with emerged roots on different days of the cultivation.

| Type of lighting | Cultivation time |
|------------------|------------------|
|                 | 3 days | 7 days | 14 days | 21 days | 28 days |
| 1                | 14     | 103    | 105     | 105     | 105     |
| 2 (control)      | 23     | 99     | 105     | 105     | 105     |
| 3                | 28     | 104    | 105     | 105     | 105     |
| 4                | 21     | 98     | 105     | 105     | 105     |
| 5                | 15     | 100    | 105     | 105     | 105     |
| 6                | 15     | 98     | 105     | 105     | 105     |

As can be seen from the tables, no differences in the time of root growth under different lighting sources was observed.

Summing up, it can be noted that various sources of lighting caused changes in the morphology of cultivated plants, and the variety specificity was observed.

Luminescent lamps Fluora did not meet the expectations and did not show any positive effects; in the test of the Solnechnyi variety even caused a statistically significant reduction in the plant height on the 28th day of the cultivation.

The use of 4200K linear LED lamps resulted in the mass reduction of the root system of potato plants. In addition, it was noted that the plants of the Antonina variety had a lower height on the 28th day of the development.

The mass of the root system of the plants grown under linear LED lamps 2700 K was less. In addition, the use of these lamps resulted in an increase in the plant height of the Solnechnyi variety.

LED red-blue lamps caused reduction in the height of the plants of both varieties, as well as increase in the mass of leaves and roots of the plants.

LED full-spectrum lamps caused a decrease in the number of internodes, increase in the surface area of the leaves of both varieties. In addition, the Antonina variety plants grown under these lamps were shorter than the plants in control and had a more developed root system. The plants of the Solnechnyi variety were characterized by a heavier shoot.
None of the studied sources of lighting caused any increase in the number of internodes, the most important indicator in the process of microclonal reproduction, and full-spectrum LED lamps even caused its decline.

When calculating the cost-effectiveness of the lamps, the following characteristics were considered: the cost of one lamp, the recommended lifetime, light flow and power consumption. In calculation the operating costs of one month, were considered different types of lamps, necessary to illuminate one section of the experimental rack in accordance with the requirements of the in vitro method for growing potato plants. The results of the calculation are shown in Table 9.

Table 9. Calculation of the operating costs for different types of lamps.

| The type of lamp | Cost, ruble. | Lifetime, hours | Light flow, lm | Power consumed, W | Costs for 1 month of operation, ruble. |
|-----------------|-------------|-----------------|----------------|-------------------|--------------------------------------|
| 1               | 555         | 20 000          | 1 400          | 36                | 348.5                                |
| 2               | 100         | 13 000          | 2 500          | 36                | 175.6                                |
| 3               | 950         | 50 000          | 1 800          | 18                | 141.9                                |
| 4               | 600         | 50 000          | 1 800          | 18                | 133.2                                |
| 5               | 6 000       | 10 000          | 4 000          | 32                | 412.6                                |
| 6               | 6 000       | 10 000          | 5 000          | 42                | 367.2                                |

There are two main areas in the process of growing pathogen free test tube potatoes, which are important: 1. Microclonal potato reproduction to increase the number of test tube plants 2. Development of test tube plants for further planting on aero hydroponic system in order to obtain pathogen free mini tubers. Plant requirements for these areas of potato cultivation are different. In the first case, the important factor is the breeding coefficient, achieved by increasing the number of internodes and reducing the time of growing the plant ready for further micropropagation. In the second case, the plants, later grown on aero hydroponic system should be distinguished by a well-developed root system and photosynthesis system. So, approaches to growing plants for these two purposes may be different.

Table 9 shows that the cheapest to operate are 2700 K LED lamps. The most common cold daylight luminescent lamps (the one accepted in the experiment for control) are 32 percent more expensive in operation than 2700 K LED lamps. A number of authors [21-24] also note, that the cost-effectiveness of LED lamps compared to luminescent ones is higher. Their higher cost-effectiveness is achieved by reducing electricity costs and much longer lifetime of light diodes. In addition, LED lamps do not contain dangerous substances and almost do not emit heat, which makes it easier to maintain the optimal temperature in the rooms for growing tube plants.

The use of 2700K LED lamps did not result neither in increase nor reduction of the number of internodes, but the operation of these lamps is the least expensive, so this type of lamps can be recommended for the cultivation of the Antonina and Solnechnyi varieties of tube plants, aimed at getting the greatest amount of plants.

To achieve the second goal, which is cultivation of tube plants with subsequent transplantation on aero hydroponic system, according to the results of the performed research, the most suitable are full-spectrum LED lamps because they help to increase the mass of the root system and the surface area of the leaf plate. In addition, they are cheaper to operate compared to the red-blue diode lamps that cause mentioned above effects.

4. Conclusion
After studying 6 different sources of lighting for growth and development of well-improved potato plants of the Antonina and Solnechnyi varieties in vitro in the laboratory, and after calculating the operating costs for the studied lighting sources, it was concluded that the most cost-effective for growing plants with the aim of speedy increase in their number are LED lamps 2700 K. At the same time, for plant cultivation with subsequent transplantation on aero hydroponic installation and development of mini-tubers the most suitable are full-spectrum LED lamps.
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