

CRESCIMENTO DE TUBERCULOS DE SEMENTE DE BATATA SEM VÍRUS EM PLANTIO AEROPÔNICO**GROWING OF VIRUS-FREE POTATO SEED TUBERS IN THE AEROPONIC PLANT****ВЫРАЩИВАНИЕ ПОСАДОЧНОГО МАТЕРИАЛА КАРТОФЕЛЯ НА БЕЗВИРУСНОЙ ОСНОВЕ С ИСПОЛЬЗОВАНИЕМ АЭРОПОННОЙ УСТАНОВКИ**

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RESUMO

As batatas como cultura alimentar são de grande importância no mundo. As infecções virais estão entre as principais razões por trás das más propriedades das sementes do material de plantio, baixa qualidade e baixo rendimento da colheita da batata. O uso de sementes sem vírus é uma das formas com alto potencial para aumentar os índices de rendimento e a eficiência do cultivo de batata. A aeropônia é uma área promissora para o cultivo de batatas sem vírus. O estudo teve como objetivo avaliar o potencial e melhorar a tecnologia do cultivo de tubérculos de batata sem doenças usando plantação aeropônica. A tecnologia aeropônica da produção de semente de batata é uma maneira segura e econômica de produzir mini tubérculos. As práticas aeropônicas exigem menos água e energia por unidade de volume de material de sementes em comparação com as práticas convencionais de cultivo. A tecnologia elimina doenças transmitidas pelo solo e danos causados por pragas. Condições artificiais, como iluminação adicional, podem ser facilmente fornecidas em estufa para o cultivo de variedades cultivadas em diferentes regiões. Cálculos econômicos mostraram que a tecnologia aeropônica pode ser praticável na produção de sementes de batata em larga escala.

Palavras-chave: *planta aeropônica; mini tubérculos; batatas; material de sementes; produção de sementes.*

ABSTRACT

Throughout the world, potatoes, as a food crop, are very important. One of the main reasons for the poor quality of planting material, yield and potatoes themselves are viral infections. The use of virus-free seed material is one of the high-potential ways to increase the yield and efficiency of potato production. Aeroponics is a promising direction in obtaining a virus-protected crop. This study aimed to assess the potential and improve the technology for growing healthy mini-tubers of potatoes using the aeroponic method, which is a safe and economical method. Compared to the usual method of growing crops, aeroponics assumes lower water and energy costs per unit of production, as well as excludes soil diseases of the plant and does not allow damage to the tuber caused by pests. For growing different varieties of crops in different regions, artificial conditions such as additional lighting in greenhouses can be easily provided. In this study, economic calculations have shown that, from a practical point of view, Aeroponics technology may be appropriate for large-scale production of seed potatoes.

Keywords: *aeroponic plant; mini-tubers; potatoes; seed material; seed production.*

АННОТАЦИЯ

Во всем мире картофель, как продовольственная культура, имеет очень большое значение. Одной из основных причин низкого качества посадочного материала, урожайности и самого картофеля являются

вирусные инфекции. Использование безвирусного семенного материала является одним из высокопотенциальных путей для повышения урожая и эффективности картофелеводства. Аэропоника – это перспективное направление в получении защищенного от вирусов урожая. Данное исследование имело за цель оценить потенциал и усовершенствовать технологию выращивания здоровых мини-клубней картофеля с использованием аэропонного метода, являющегося безопасным и экономичным способом. По сравнению с обычной методикой выращивания сельскохозяйственных культур, аэропоника предполагает более низкие затраты воды и энергии на единицу продукции, а также исключает почвенные заболевания растения и не допускает повреждений клубня, вызванных насекомыми-вредителями. Для выращивания разных сортов сельскохозяйственных культур в различных регионах, такие искусственные условия, как дополнительное освещение в теплицах, могут быть легко обеспечены. В рамках данного исследования, экономические расчеты показали, что, с практической точки зрения, технология аэропоники может быть целесообразной при крупномасштабном производстве семенного картофеля.

Ключевые слова: аэропонная установка; миниклубни; картофель; семенной материал; семеноводство.

1. INTRODUCTION:

Potato (*Solanum tuberosum*) ranks third in the world among the most important food crops after rice and wheat; 374 million tons of potato tubers are produced in the world. However, potato yields are significantly below the yield potential in most developing countries, mainly due to poor potato seed properties (Tessema and Dagne, 2018). Due to biological characteristics, potatoes are more susceptible to viral and viroid diseases than other crops (Anisimov *et al.*, 2014). Viral diseases are among the major causes of poor potato seed properties and low productivity (Priegnitz *et al.*, 2019). As a result of multi-year reproduction, potatoes tend to accumulate diseases, mainly viral ones. The viral infections, in turn, lead to degeneration in potatoes (Li *et al.*, 2018; Dupuis *et al.*, 2019). The potato aphids and Colorado beetles transmit viruses from diseased to healthy plants, then viruses spread from one generation to another through vegetative reproduction of potatoes (Tessema and Dagne, 2018). Potato yields and storage capacity tend to decrease with each subsequent planting. Global potato losses from viral infections are 90 million tons, and the yield is reduced by 40-50%; tuber losses during storage can reach 15-20% (Adikini *et al.*, 2016; Khelifa, 2019). Such factors as the pathogen type and strain, stability rate of the variety, growing and weather conditions determine potato yield losses. Mild viral diseases reduce the yield by an average of 10-20%; potato yields drop by 70-85% in severe viral diseases. Starch values fall by 0.8-4.6% in diseased tubers compared to healthy tubers. The diseased potato tubers have lower values of crude protein, vitamin C, B₁, B₂ (Ateş *et al.*, 2019; Inglis *et al.*, 2019; Ogero *et al.*, 2019).

Therefore, the use of the virus-free seed material is one of the high-potential ways for

increasing yield indices and efficiency of potato growing (Anisimov *et al.*, 2014; Mbiri *et al.*, 2015; Wang *et al.*, 2017; Tessema and Dagne, 2018). There are many methods for improving potato seed material (thermotherapy, chemotherapy). Thermotherapy is the treatment of plants, shoots, and tubers with high temperatures (+37 - +42 °C) for pathogen inactivation. Chemotherapy relies on the introduction of Virazole at 20-50 mg/l concentration into the nutrient medium, where apical meristems are grown. The main objective of reproducing the virus-free source material was to achieve the maximum net reproduction with the lowest risk of repeated viral infection and to produce material suitable for planting in the open ground (Anisimov *et al.*, 2009; 2014). Currently, to solve the problem, researchers use new techniques such as clonal propagation of meristem cultures and year-round reproduction of the source material in the closed environment (Lakhiar *et al.*, 2018; Singh *et al.*, 2019). These are the main methods of seed material reproduction used in potato seed tuber production: improving seed material through tissue cultures and selecting the most virus-free lines; clonal propagation of micro-plants in the laboratory environment; growing disease-free mini-tubers on the protected ground or in hydroponic modules; selection of healthy source plants and clones in the field based on visual assessment and laboratory testing methods for viral, viroid and bacterial infection (Martirosyan, 2014; Oves *et al.*, 2014; Khutinaev *et al.*, 2016).

Aeroponics is a promising area for growing virus-free potatoes (Mateus-Rodríguez *et al.*, 2012; Sumarni *et al.*, 2013; Rykaczewska, 2016; Abdul *et al.*, 2018; Hajiaghaei Kamrani *et al.*, 2019;). Aeroponics is a type of hydroponics: plants are grown suspended with their roots periodically sprayed with a nutrient-rich solution. The basic principle of the aeroponic plant growing

is atomizing the plant with a mineral-rich nutrient water solution in the closed or semi-closed environment (Martirosyan, 2014; Rykaczewska, 2016b; Wang *et al.*, 2017).

Aeroponics provides opportunities for the selection of seed tubers without interrupting the vegetative growth of potatoes. The sufficient root zone provided full visual monitoring and easy access to the plant root system, careful management of the roots during multiple harvests of mini-tubers. Therefore, the number of seed tubers harvested from one potato plant was several times larger, which increased the net reproduction of high-value potato seed tubers (Mateus-Rodriguez *et al.*, 2013; Basiev *et al.*, 2019; Rykaczewska, 2016b). On average, 805 - 900 mini-tubers could be picked from the area of 1 m² (Abdullateef *et al.*, 2012; Rykaczewska, 2016b). Aeroponics allows researchers to develop fully automatic plant growing systems much more straightforward than substrate cultivation systems (Gabitov *et al.*, 2018; 2018b).

So far, this technique is not widespread and has not been adequately studied, especially in Russia. Several countries have recommended aeroponic cultivation as the most effective and convenient practice for growing plants than soil and other soilless techniques. Aeroponics based cultivation of potato seed tubers requires less water and energy (Tessema and Dagne, 2018). Aeroponics is recommended as a modern plant cultivation technique for potato seed production by the International Potato Center (CIP) (Mateus-Rodriguez *et al.*, 2013).

Although the approaches to the issue are generally the same, there is no standard practice on applying aeroponics for growing potato mini-tubers (Oraby *et al.*, 2015; Khaksar *et al.*, 2018; Zhuravleva *et al.*, 2018; Kaur *et al.*, 2019;). In this regard, the study aimed to assess the potential and improve the technology of growing disease-free potato seed tubers using an aeroponic plant.

2. MATERIALS AND METHODS:

The study was conducted in the laboratory of potato breeding and seed production at Bashkir State Agrarian University.

The source tubers were taken from visually healthy potato plants without frank disease symptoms. Potato tubers were placed in the thermal chamber, where the temperature was raised daily by 2°C from 25°C to 37°C during the first week. The potato tubers were kept in the thermal chamber for 14 weeks at a relative

humidity of 90%. For reducing the saprophytic microorganism population, the explants were washed with soap solution, rinsed with tap water then with reagent water type IV, and then treated with 70% ethanol solution. 0.1% diacid solution was used as a sterilizing solution. After sterilization, the explants were washed with distilled water.

The explants were appropriately tested for viral, viroid, bacterial diseases. So the material could be used for future grafting during the year, thereby reducing the costs of buying new disease-free material. The study used the Polymerase chain reaction (PCR) to diagnose viral diseases. This method offers high sensitivity as it has the advantage of producing results within 24 hours for some infections.

The cultivated plants were then grown in a nutrient medium (Figure 1).

The modified MS nutrient medium contained mineral salts and growth stimulants. PVP (polyvinylpyrrolidone) (5000-10000 mg/l) was used as an anti-oxidant. The nutrient medium was sterilized in an autoclave at 120 °C and 1 atm for 20 min. Micro-plants were grown at 3 thousand lx (16-hour photoperiod) at 25 °C and 70% of relative humidity. Then the potato micro-plants were placed in the aeroponic plant.

The study used the FTA 60 aeroponic phytotron for growing potato mini-tubers of the Alekseevsky variety (Khamaletdinov *et al.*, 2018). The FTA-60 aeroponic phytotron comprises a tray equipped with an aeroponic system (3); a rack (3); a pump station (1); a hydraulic storage unit (1); a solenoid valve (1); a container for spray solution (1); a digital timer (1); a submerged type pump (1). The phytotron can accommodate 60 plants. The plants were placed on a lightproof cover of the container with a built-in siphon tube, which was periodically one-quarter filled with the nutrient solution. The stem and leaves were above the cover and received lighting while the roots were under the cover. 1/5 of the roots were submerged in the solution, and the rest were suspended in the air between the solution and the cover. The hanging roots were recurrently sprayed with the nutrient solution through the atomizers fixed to the cover.

The study used the LED 101 W lamp under the 4:3 red and blue ratio for vegetative growth and 109 W lamp under the 3:1 ratio for tuber formation.

CFX96 Real-Time PCR Detection System was employed in the Polymerase chain reaction (PCR) method to diagnose potato virus infection.

3. RESULTS AND DISCUSSION:

The production of disease-free seed potato tubers consisted of three major stages: microcloning and growing of test-tube plants, cultivation of mini-tubers in the aeroponic phytotron, and planting potato tubers in the greenhouse.

The explant was isolated and introduced into the culture at the first stage. Under sterile conditions, the apical meristem, a culture of actively dividing cells, was isolated from the tip growth region and put in a nutrient medium. Apical meristems of 200-400 microns were isolated by applying micro-cuts to the plants in LAMSYSTEMS laminar flow cabinet under aseptic conditions using a 20x binocular magnifier.

The test tube plants were small (the length was 10 cm, the weight was 3-5 g), so the first three days of cultivation were crucial (Figure 2).

The study showed that the plants needed special care (watering and lighting) in that period. The root zone was kept moistened by periodic spraying of the nutrient solution. For spraying, the study used a high-pressure pump and atomizers. The plant was fixed with a soft clip or in lattice pots at the top of the container. Plants grown in the aeroponics system have a more robust root system than plants grown in the soil medium. The aeration of the roots resulted in quick absorption of nutrients. Thorough spraying of the nutrient solution is crucial in the process. It is necessary to cover all the roots with the solution droplets. The optimal droplet size for a large number of plants was 20-100 microns. This range allows small droplets to saturate the air, maintaining the required humidity level, and large droplets to fall on the plant roots and be absorbed. The solution was sprayed in the following order: at first, the nutrient solution was sprayed for 60 seconds every 15 minutes during the two weeks after planting, and then for 30 seconds every 15 minutes. This scheme eliminated drying out of the root system as the plant absorbed nutrients continuously, and reduced operating costs by saving electricity during the pump idle time.

Lighting and temperature regulation, as well as the composition of the nutrient solution, are essential in the aeroponic cultivation practice. At the initial stage (days 1-10), the lighting period was 24 hours. From day 11, the lighting period was reduced to 18 hours per day. By day 15, the plants gained vegetative weight and had a developed root system of light color. The length of

lighting was gradually reduced by 1 hour per day from day 16 to day 24. By the end of the period, the length of lighting was 10 hours per day. When the lower leaves dried (days 77-80), the light phase was reduced to 6-8 hours per day to stimulate tuber growth; this increased necrosis of the top and stimulated outflow of plastic substances from the top into the tubers.

The optimal temperature for the first three potato growth stages was 22-24/16-18°C day/night (Adikini *et al.*, 2016). The second half of the growing season (stages 4,5) requires decreasing temperature to 18-20°C during the day and to 14-16 °C at night (Adikini *et al.*, 2016). The temperature requirements were met in the study.

'Basaplant' soluble fertilizers served as a basis for the nutrient solution. 'Basaplant 15-10-15 +ME' and 'Basaplant 8-12-24 +ME' soluble fertilizers were used in 0.7:0.3 ratio during the first period of the plant growing. The solution-specific conductance was 0.8-0.9 millisiemens (mSm), the acidity (pH) was 7.7-6.9 from day 1 to day 16. The acidity level gradually decreased to 6.3 by day 14 and 5.7 by day 16. The optimal acidity level (pH 6,9-6,7) was maintained by adding the tap water with a high level of calcium salts and sodium bicarbonate. However, the yellowing of the lower leaves was observed in some plants on day 13; the number of plants with yellowed leaves increased on day 15. Therefore, the concentration of the nutrient solution was increased from day 17 to day 20, and the specific conductance was 1.1 mSm.

Ferrous sulfate was added to the nutrient solution to eliminate iron-induced chlorosis. However, the resultant solution appeared ineffective. Therefore, on day 20, the solution was enriched with NPP compounds such as ammonium nitrate, potassium sulfate, and double superphosphate as well as micro-fertilizers such as hydrogen borate and potassium permanganate. The specific conductance raised to 1.8 mSm, and the level was maintained to day 31.

'Basaplant 8-12-24 +ME' soluble fertilizer was applied from day 32 to day 51. The fertilizer contains little nitrogen (8%), phosphorus (12%), and much potassium (24%). The specific conductance reached 1.8-1.9 mSm, so the solution concentration was decreased by adding water until the specific conductance fell to 1.3-1.4 mSm.

'Basaplant 15-5-30' fertilizer was applied at the next stage (from day 54 to harvesting). The specific conductance was maintained at 1.3-1.4

mSm from day 54 to day 62. Then, the concentration of the nutrient solution was gradually decreased from day 63 to day 74, so the specific conductance fell to 1.1 mSm, from day 75 to day 79 the index fell to 1.0-0.9 mSm. The solution-specific conductance was kept constant (0.7-0.9 mSm) till the end of potato harvesting.

The study demonstrated that concentration indices should be monitored daily and be kept at the optimum level via adding fertilizers or water; the solution acidity should be managed by changing the nutrient solution or adding calcium carbonate or phosphoric acid. The optimum acidity for potato growing should be maintained at 6.8-7.4. The nutrient solution should be replaced at least twice a month.

Harvesting of potato mini-tubers started on day 96. The harvesting was over on day 108.

Thirty-four mini-tubers were picked from one potato plant (Figure 3). The maximum weight of a tuber was 78 g. The yield of the optimum sized tubers (20-30 mm in diameter) was 72.1 percent. Large tubers (more than 30 mm) were 9.3 %, small tubers (15-20 mm) were 11.4%, and smaller tubers (< 15 mm) were 7.2 %.

The yield of the optimum sized mini-tubers was 969 pcs from 1 m² of the FTA-60 aeroponic plant. The study analysed the costs for aeroponic production of seed potato mini-tubers, and the analysis showed that the cost of one seed potato minituber was 10 roubles.

Production of seed potato mini-tubers allows it to grow potato mini-tubers free from viral infections. The aeroponics technology optimizes access to air and carbon dioxide for potato growth as opposed to practices involving a substrate medium. The yield of the optimum sized mini-tubers was 969 pcs from 1 m² of the FTA-60 aeroponic plant. This figure is slightly higher than indices obtained by some growers *in regular medium* (805-900 mini-tubers per 1 m²) (Abdullateef *et al.*, 2012; Mateus-Rodriguez *et al.*, 2013).

Aeroponic production of potato mini-tubers requires systematic monitoring for temperature, humidity, light intensity, nutrients, pH, and mSm indices of the nutrient solution, spraying length, and time. The study used controls to adjust the parameters. Wireless sensors introduced into the aeroponic technology can ensure early detection of the parameter fluctuations. So, the farmer could monitor several parameters without using laboratory instruments (Lakhari *et al.*, 2018).

The optimal temperature for the first three

potato growth stages was 22-24/16-18°C day/night. The second half of the growing season (stages 4,5) requires decreasing temperature to 18-20°C during the day and to 14-16°C at night (Adikini *et al.*, 2016). The temperature parameters are consistent with the findings obtained by other researchers (Oraby *et al.*, 2015). The density of planting in the aeroponic unit is crucial for successful plant growth. In the study, tubers were planted at a density of 40 plants per 1m². The plant density of 60 plants per m² showed the most significant results (Abdullateef *et al.*, 2012).

Several researchers have proved that aeroponics-based technology is effective in potato seed production (Mateus-Rodriguez *et al.*, 2013; Martirosyan, 2014; Rykaczewska, 2016b; Tessema and Dagne, 2018). At the same time, some growers point out (Chang *et al.*, 2012; Tican *et al.*, 2017) that the hydroponics technology is also a successful strategy for the production of seed potatoes. The hydroponic system employs a circulating nutrient solution; it has a positive effect on the average weight of mini-tubers per plant. Moreover, aeroponics technology requires a constant power supply and trained maintenance personnel throughout the crop growing season. Unreliable power supply and expensive boiler-based sterilization methods for growing media hinder the successful application of aeroponics technology. Mini-tubers produced in the sand culture hydroponic system were larger than grown based on the conventional aeroponics technology. The initial cost of the hydroponic unit and the cost of the sand hydroponics system per season is lower than that of the conventional aeroponics system. Sand hydroponics can replace the traditional system of quality seed potato production (Mbiri *et al.*, 2015) and general potato growing techniques in crop growing (Rodríguez-Delfín, 2011).

4. CONCLUSIONS:

The aeroponic practice of seed potato production is a safe and economical way to produce mini-tubers. Less water and energy per volume unit of seed material are needed for aeroponic systems compared to the hydroponic growing practice. The aeroponic technology optimizes access to air and carbon dioxide for potato growth as opposed to practices involving a substrate medium. Aeroponics can prevent disease transmission as the diseased plant can be quickly removed. The technology eliminates soil-borne diseases and pest damage. Artificial conditions such as additional lighting can be easily

provided in the greenhouse for cultivating crop varieties grown in different regions.

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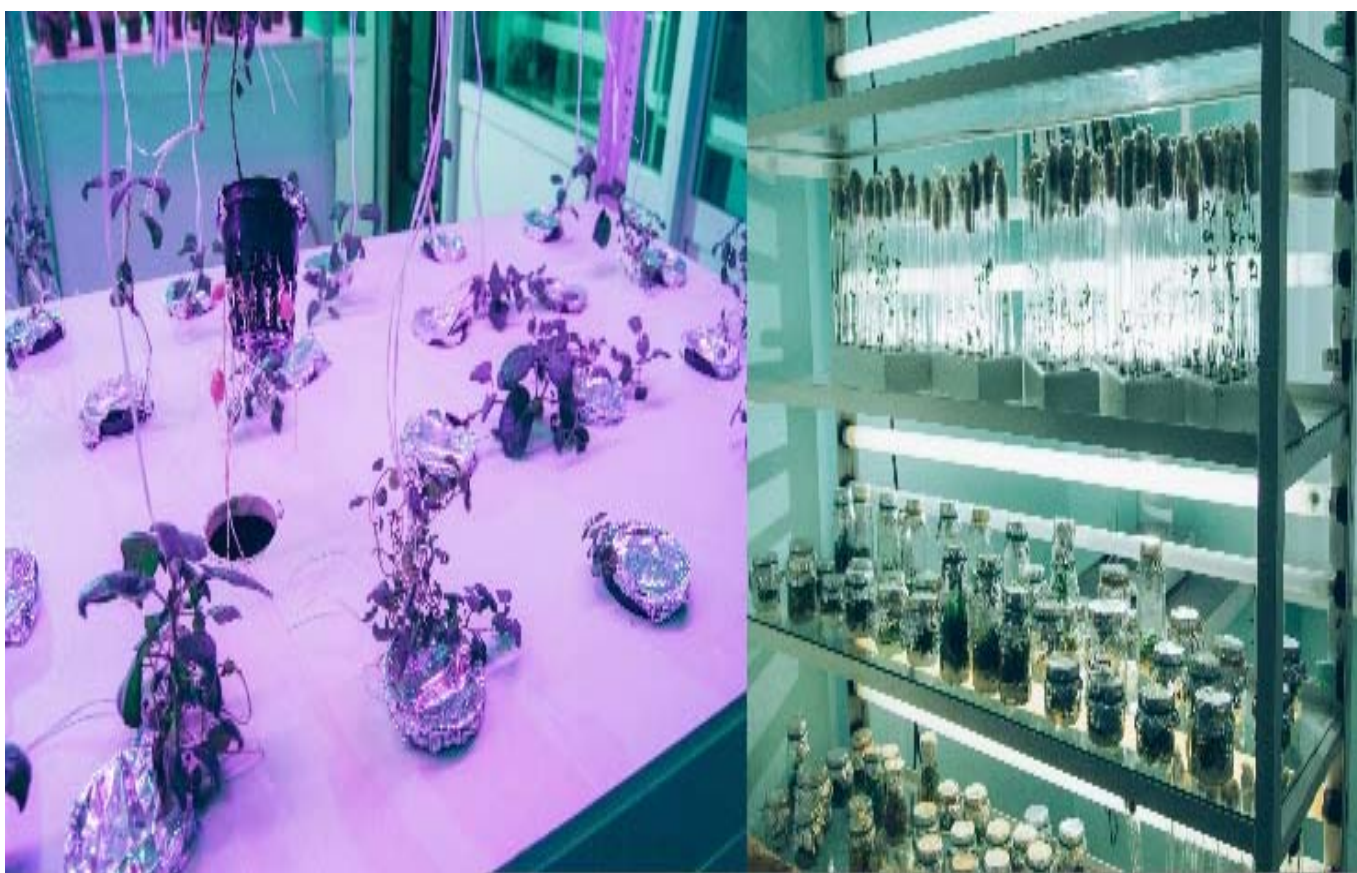


Figure 1. Potato growing place



Figure 2. Potato samples in test tubes



Figure 3. Mini potato tubers grown in the aeroponic plant.