Innovative technologies for accelerated reproduction of initial seed material from in vitro micro plants on the biotechnological module AGM-60

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Abstract. A biotechnological aerohydroponic module has been created for growing plants without using soil by creating a water-air nutrient medium in the root space, and a method has been developed for accelerated propagation and mass replication of improved potato minitubers, which allows increasing the planting density to 60 plants per 1 m^2, increasing the propagation coefficient to 50 or more tubers per plant, increasing the yield of tubers per unit area to 3 thousand or more pieces per 1 m^2. The device allows for continuous monitoring of root system development, differentiated plant nutrition by development phases, control of plant growth and development during vegetation, initiation and control of tuber formation, stage-by-stage collection of ripening tubers during their development, and an increase in the propagation coefficient per plant and per unit of conventional area.

1 Introduction

1.1 Relevance

The main task of modern seed-growing enterprises is to provide them with the initial seed material in the required volumes. At the same time, the initial material must have the highest quality characteristics and the most important stage of seed production is the improvement of the initial potato planting material using biotechnological methods. The technology of improvement and obtaining basic seed material in the form of potato minitubers in original seed production is mainly based on reliable and time-tested traditional cultivation in closed ground. However, growing minitubers in closed ground requires very significant costs and does not allow growing large volumes due to the excessively high labor intensity and high cost of the technology. Moreover, the use of soil does not exclude the risk of secondary re-infection of the material, and if an infection is detected, all the material will have to be rejected. In this regard, there is a problem of supplying seed farms with a sufficient amount of initial improved material of high quality.

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In turn. An increase in production will significantly reduce the cost of the final product and provide producers with cheap planting material.

Along with greenhouse technology, original seed production widely uses technologies of clonal propagation in vitro of micro plants in laboratory conditions with subsequent production of minitubers in controlled environment conditions. The method of mass production in vitro of microtubers in a test tube culture in laboratory conditions allows to significantly minimize costs, regulate the nutrient medium and change external conditions, but the tubers obtained in vitro are mainly very small 3-8 mm in diameter and weigh 0.2-0.7 g. In addition, in such conditions, one tuber is mainly formed from one plant. However, if special methods of in vitro tuber formation are used in clonal propagation of microtubers, it is possible to increase the number of tubers from one plant. A new method of clonal micropropagation of meristematic material has significantly increased the efficiency of obtaining test tube microtubers and made it possible to successfully use them for cultivation, but this method also does not solve the issue of simplifying and reducing the cost of the technological process and is also associated with significant costs of electricity and labor resources.

Thanks to new scientific research and technological innovations, new alternative technologies based on various types of hydroponics have come to the aid of producers. Hydroponics is a soilless method of cultivation, in which plants are grown in an aquatic environment.

Modern producers of original potato seeds are showing great interest in using new highly efficient production methods based on the use of hydroponic technologies. The technology of growing in a water-air nutrient medium reveals enormous potential in improving production and can significantly reduce all major production costs. The technology allows for an increase in the duration of tuber formation and the successful cultivation of microtubers measuring 10 g and above.

The most effective and promising direction of hydroponics is aeroponics, where plants are grown in the air space with the roots treated with a moisture-air mixture of nutrients. This method has long established itself as the most cost-effective and, in addition, problems associated with pests and adverse weather conditions are eliminated. With this method, with minimal energy costs, it is possible to obtain over 57 mini-tubers of potatoes larger than 10 mm. With a scientific approach, in aeroponics conditions, the nutrition regime is dynamically improved and the process of nutrient absorption itself is optimized, which makes it possible to control nutrition throughout the entire growing season. The use of high-tech aeroponic systems allows to maximize the seed multiplication coefficient without deteriorating quality, as well as significantly reduce the costs of cultivation and obtaining healthy seeds. Aeroponics has made it possible to obtain environmentally friendly crops all year round.

1.2 The purpose of the research

Creation of a highly efficient cultivation system for accelerated reproduction and mass replication of improved seed material in original seed production, increasing the efficiency of minitubers production with an increase in quantitative and qualitative characteristics, minimizing the costs of water and land resources, mineral substances, agrochemicals, reducing material, financial and labor resources, increasing the environmental friendliness and technological effectiveness of production.

2 Materials and methods

To study the aerohydroponic method of growing without the use of solid substrates in the conditions of a water-air nutrient medium, the aerohydroponic unit AGM-60 with an active-passive power supply system was created, allowing potato plants to be grown in the air space with control over the development conditions and direct control over the growth and tuberization processes during the growing season.

2.1 Purpose of the device

The created aerohydroponic units are intended as an alternative or primary method of producing the initial planting material in the original seed production of potatoes, as well as for growing products of various plant origins, such as seeds, various types of tubers, greens, fruits and vegetables.

2.2 Design features of the module

Figure 1 shows a model of an aerohydroponic device developed at the A.G. Lorkh Potato Research Center with a multi-loop active-passive nutrition system, designed to grow up to 60 tall or up to 400 low-growing herbaceous plants (Figure 1).

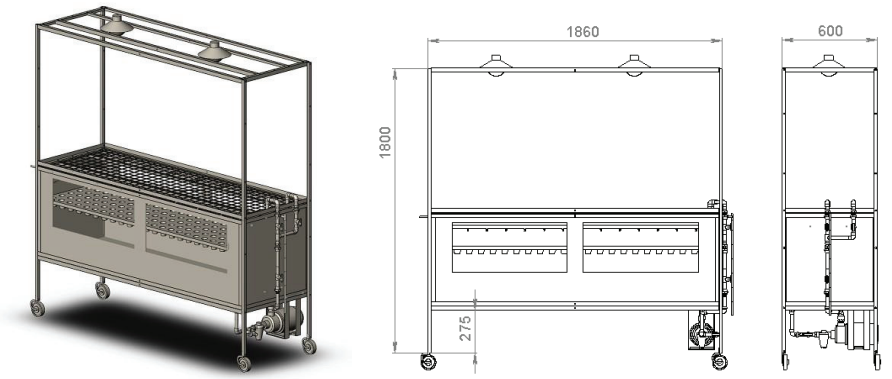


Fig. 1. Universal aerohydroponic module AGM with a multi-circuit active-passive power supply system.

The frame of the aerohydroponic box has dimensions of 600 mm in width, 1800 (+100) mm in height, 1860 mm in length. The dimensions are selected taking into account the use in closed rooms of buildings and greenhouse structures, with the possibility of carrying it through standard doors.

In the middle part of the frame there is a plastic aerohydroponic box with technological windows, through which visual monitoring and scheduled maintenance of the root system with tubers developing in the air are carried out during vegetation.

The power supply system in the aerohydromodule is combined, active-passive, consists of two power circuits - aeroponic and hydroponic. The aeroponic power supply system is active and periodically aerosolizes the root space with a water-air nutrient mixture due to a water pump that requires electricity (Figure 2).

The aeroponic system consists of a set of pipes and nozzles through which the nutrient solution is sprayed. The pump is located outside under the box. The water distribution

network itself is located immediately under the planting panel inside the aeroponic box. The hydroponic nutrition system is passive, energy-independent and provides nutrition to the roots on a permanent basis until the end of the growing season. The hydroponic nutrition system is an auxiliary and emergency nutrition system and in the event of an accident in the network or a pump breakdown, the supply of nutrition to the plants will not stop, and they will not experience a lack of moisture. The hydroponic system does not have any parts or units. It is represented by the hydroponic box itself with a nutrient solution inside. Plant nutrition occurs by flooding the roots. Both nutrition circuits are located directly in the root space inside the box. The box is covered from above with a rigid card made of aluminum or stainless steel mesh with a cell size of 100x100 mm (Figure 3).

Fig. 2. Aeroponic system AGM.

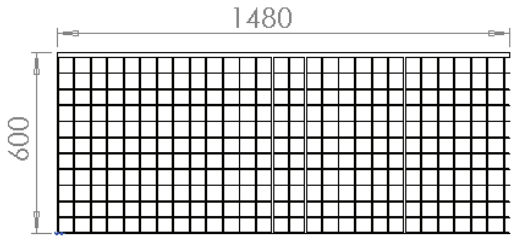


Fig. 3. Mesh card made of aluminum or stainless wire with 100x100 mm cells.

The net is covered with a black thick film, and a soft material made of foil-clad foamed polypropylene (penofol) 10 mm thick is laid on top of the film. Penofol is laid with the foil facing up in order to increase the reflective properties of the foil to improve the illumination of the lower tiers of plants. Penofol is a soft elastic material and is excellent as a reliable fixation for delicate stems of planted micro plants. The soft properties of penofol do not interfere with the growth and development of stems, and it reliably holds the plants in an upright state. The design of the planting panel allows you to plant plants in any pattern, starting from 3x3 cm and above. When growing potato minitubers from test-tube microplants on a planting panel with an area of 1.08 m sq., you can place up to 60 microplants in four rows of 15 plants each.

The active power supply system of the aeroponic circuit is controlled by a controller that turns on the pump at a time specified by the program. The control panel consists of a set of electrical equipment consisting of a two-channel timer, switches and electromagnetic

relays. The second channel of the timer and the second electromagnetic relay are activated when growing occurs under artificial lighting conditions, and then the second channel controls the lighting mode. The electrical control circuit of the active (aeroponic) power supply system is shown in Figure 4.

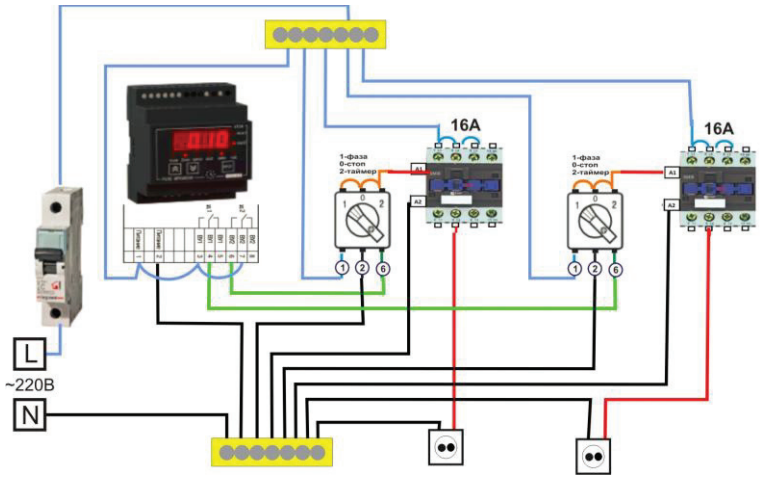


Fig. 4. Electrical control circuit of the active (aero) power supply system.

Technical characteristics of the aerohydroponic module:

Overall dimensions of the module (without rollers)	1950x600x1900 mm
Useful working area	1.06 sq. m
Module area with technological passages	2.12 sq. m
Module frame (anodized)	Aluminum
Aerohydrobox (wall thickness 4-6 mm)	HDPE plastic
Water displacement	up to 150 l
Number of tall plants to be planted	up to 60 pcs.
Number of herbaceous plants to be planted	up to 400 pcs.
Number of power circuits	2
Number of nozzles	30 pcs.
Water pump	1 pc.
Water pump power from 220V network (or from 24V network)	350 to 600 W, (or 100 W)
Water pump power	100 W,
Lighting fixtures (for indoor use)	(optional)
Power and lighting control	2-channel time relay

Experimental scheme. To determine the efficiency of the developed method for growing minitubers, 60 micro plants of the Red Scarlett potato variety were planted on the created aerohydroponic module AGM-60 with an area of 1.06 m^2. Testing was carried out on an open experimental site under natural lighting conditions, under the shelter of the installation with white covering material "Agrospan".

The installation was equipped with a high-pressure water pump with a power of 350 W and operating from a 220 V network. The supply of the nutrient mixture to the root space was carried out by two methods. Aeroponic nutrition was carried out by aerosolizing the root space with a water-air nutrient mixture through high-pressure fogging nozzles. Hydroponic nutrition was carried out directly, by immersing part of the roots in a nutrient solution.

Test-tube microplants were obtained from the clonal micropropagation laboratory of the "A.G. Lorkh Federal Research Center of Potatoes". The cycle of operations on the aerohydroponic installation began with the planting of test-tube plants directly onto the

module. Before planting, the plants were thoroughly washed from the remains of the agar medium to prevent the remains of agar-agar from getting into the nutrition system. Plant nutrition was carried out according to a differentiated scheme.

The following compositions of macro- and microsalts were used to feed plants at the stages of development

Table 1. Compositions of macro- and microsalts.

Elements	N	P	K	Ca	Mg	*EC*	pH
First 60 days	170	50	250	150	35	0.961	5.8-6.0
Stimulation	50	50	120	35	35	0.44	5.8-6.0
Fruiting	200	50	300	110	35	1.00	5.8-6.0

The content of microsalts in the solution according to Murasita-Skoog - Fe-EDTA (8), B (0.5), Mn (0.5), Zn (0.1), Cu (0.05), I (0.63), Co (0.006), Mo (0.1). The concentration of mineral substances was maintained within 0.7-1.3 g / l. The acidity of the solution was maintained at a pH of 5.5-6.5. pH was adjusted every 2-3 days. The solution was changed once a month. ELISA analysis, collection and storage of tubers according to the regulations developed in previous years.

At the initial stage of development, the plants received standard nutrition. When the plants entered the budding phase, the qualitative composition of the nutrient solution was changed in order to initiate and accelerate the tuberization process. After the plants entered the full tuberization phase, the qualitative composition of the nutrient solution was changed to the next solution. During the growing season, continuous monitoring of tuber development and staged harvesting were carried out in real time. Staged harvesting was carried out every 7-10 days when the tubers reached standard sizes of 20-25 mm. The harvested tubers were washed, dried and stored at a temperature of +3 +4°C.

The planting date for micro-plants was May 15. The last harvest was on October 15. The growing process lasted 153 days. The resulting mini-tubers were tested for seed quality in the first field generation nursery in comparison with tubers obtained using traditional technology.

3 Results and Discussion

The reason for the low yield was the abnormally cold summer of 2017. The entire European part of Russia was characterized by cold weather. June was marked by snowfall and night frosts and was the coldest month of the summer (www.kp.ru/daily/26690/3714864/). At the beginning of the month, solid precipitation in the form of snow pellets was observed. On June 3, the absolute minimum of the season was recorded (+3.2).

The installations were placed outdoors and covered with temporary awnings to protect from precipitation. The plants and the root part were exposed to sharp temperature changes during cold snaps and during the transitions between day and night temperatures, but due to extremely unfavorable conditions, the plants developed very slowly and began to gain weight normally only from the end of July. They began the process of tuber formation only in September.

Despite the extremely unfavourable weather conditions, the plants still showed high reproductive efficiency (Figures 5-6).

In 153 days of cultivating the Red Scarlett variety, 2968 minitubers were obtained from 60 micro plants, or an average of 34.7 pieces per bush, which is approximately 5-6 times more than with traditional cultivation in closed ground, where an average of 4-8 tubers are obtained from one plant (Table 2).

Fig. 5. Aerohydroponic installation AGM for growing under natural light. Variety Red Scarlett.

Fig. 6. Mini potato tuber harvest obtained in aerohydroponic systems under artificial lighting, Red Scarlett variety.

Table 2. Potato mini-tubers harvested in aerohydroponics conditions. Red Scarlett variety.

Number of plants in sample, pcs.	Harvest		Harvest structure						Average tuber weight, g	Reproduction coefficient. pcs./plant
	grams	pcs.	up to 10 mm	weight, g	10-20 mm	weight, g	20-30 mm	weight, g		
60	12247	2084	376	301	1496	8978	212	2968	5.9	34.7

When calculating mini-tubers, only mini-tubers corresponding to the standard sizes determined by GOST 33996-2016 "Seed potatoes. Specifications and methods for determining quality" were taken into account. The bulk of the crop was made up of tubers 10-20 mm in size. Tubers that did not have time to reach a size of 10 mm, the number of which averaged up to 25 pcs./bush, were not included in the calculation.

In the 1PP (field generation) nursery, a comparative analysis of the seed qualities of aerohydroponic tubers was carried out in comparison with greenhouse ones. Planting date is May 15, 2018. The year was characterized by favorable conditions, which affected the rapid growth and development of plants. Uniform, friendly shoots and development were noted for all variants on all experimental plantings.

Phenological observations in the experiment when comparing hydroponic and greenhouse tubers showed approximately the same results. The first shoots from aerohydroponic mini-tubers appeared by May 25, 12 days earlier than those from greenhouses (Table 3).

Table 3. Phenological observations and biometrics. Landing on May 15, 2018.

Technology	Number of days from planting to			Plant height, cm.	Avg. stems
	shoots	buds	flowering		
Aerohydroponics	10	35	55	31.4	4
Traditional	22	35	55	32.2	4

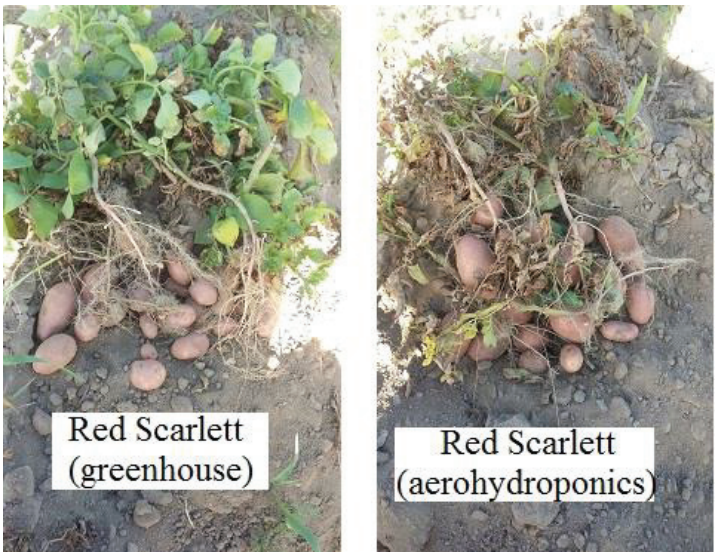
The emergence of shoots 12 days earlier is explained by the fact that by the time of planting, the aerohydroponic minitubers had sprouted strongly and practically already had real small green shoots. The greenhouse minitubers were also sprouted, but the length of the shoots did not exceed 3-5 mm. By mid-June, the greenhouse plants caught up with the aerohydroponic ones in growth and development. The subsequent picture of planting was almost the same everywhere. All plants formed on average the same number of stems, 4 pieces per bush, and the average plant height for all options was 31-32 cm.

When comparing the yield under the bush, the greenhouse minitubers gave a greater number of tubers under the bush - 11.7 versus 9.1 pcs./bush. At the same time, the average weight of a tuber under the aerohydroponic plants was 40 g versus 39.7 g from the greenhouse ones (Table 4).

Table 4. Comparison of 1PP yield from aerohydroponic and greenhouse. Red Scarlett variety.

Method of obtaining	No. of plants in sample, pcs.	Yield weight in sample, kg	Yield under bush, kg	Average tuber weight, g	Total tubers in sample, pcs.	Crop structure			Coefficient of size pcs/bush
						<30 mm	30-40 mm	>40 mm	
Greenhouse	25	16.6	0.66	39.7	292.7	39.3	184.7	68.7	11.7
Aerohydro	25	14.0	0.56	40.0	227.3	35.7	155.3	36.3	9.1

Mini-tubers obtained in aerohydroponics conditions have almost equal germination and yield, however, it should be taken into account that the main advantage of the aeroponics growing method is the ability to obtain several times more initial planting material of comparable quality, but with significantly lower costs. This makes it possible to increase the volume of valuable seed material in original seed production by almost 3-4 times and easily switch to a four- or even three-year seed production scheme (Figure 7).



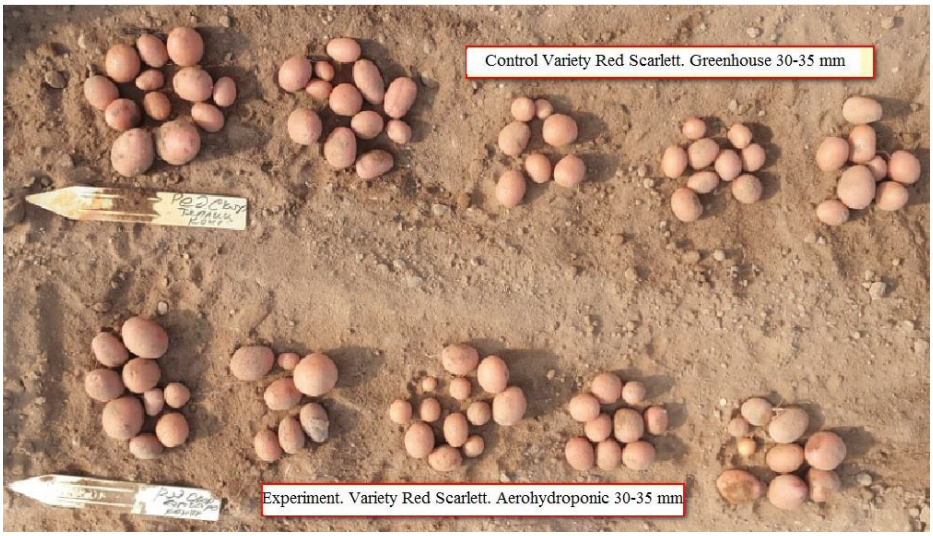


Fig. 7. Comparison of the yield in the first field generation obtained from greenhouse and aerohydroponic minitubers. Variety Red Scarlett.

4 Conclusion

The main advantages of the aerohydroponic module AGM-60 and the method of growing in water-air culture are determined:

- Growing plants on the universal module AGM occurs with or without the use of solid substrates in water-air culture, allowing you to actively influence the processes of plant growth and development in real time. The technology is resource-saving and environmentally friendly.
- The device can be used in any conditions of artificial and natural lighting. The device operates autonomously, with all the necessary equipment for safe plant cultivation. In case of emergency situations, the device is able to fully function for a long time without the risk of plant death.
- The device allows short-term crop rotations (up to 3-6 vegetation periods per year, depending on the crop being grown). The device can be used in research and development to study growing conditions, nutrition regimens, development of high-quality compositions of nutrient media, the effect of different light spectra on growth and development, searching for methods to accelerate growth and tuber formation processes, etc.
- The device allows for short-term crop rotations (up to 3-6 vegetation periods per year depending on the crop being grown).
- The device can be used in any conditions of artificial and natural lighting.
- The device operates autonomously and is equipped with all the necessary equipment for safe plant cultivation. In case of emergency situations, the device is capable of fully functioning for a long time without the risk of plant death.

Unlike substrate culture, cultivation in a water-air nutrient medium occurs without contact of the root system with a foreign solid substrate medium, where dangerous pathogenic bacteria can potentially develop. Aerosolization of the root space effectively supplies the roots with oxygen, moisture and nutrients, giving wide scope for scientific

research on the study of the efficiency of nutrient absorption and the initiation of growth and development processes.

Due to the exclusion of the use of solid substrates, expensive work and costs for the purchase of land, pots, boxes, inventory, etc. are excluded. There are no labor-intensive works associated with the maintenance of the substrate, related equipment and inventory. The absence of a substrate makes it possible to actively influence the growth-stimulating characteristics of the grown plants and control metabolic processes. During the growing season, it is possible to use a differentiated nutrition regime with control of plant development by development phases.

The AGM module can be effectively used in seed production programs for accelerated reproduction of the original seed material and in breeding programs for testing hybrids, where breeders will have the opportunity to reproduce up to two or three generations in one year while simultaneously selecting and evaluating the most promising breeding material.

The AGM module can be used in a wide segment of production when cultivating various economically valuable plants and medicinal crops. The growing process can take place in any conditions with natural or artificial lighting in isolated protective structures or year-round in closed rooms with an artificial microclimate.

The device can be used in research and development work to study growing conditions, nutrition regimes, development of high-quality compositions of nutrient media, the effect of different light spectra on growth and development, search for methods to accelerate growth and tuber formation processes, etc. The created aerohydroponic devices and the developed method of growing in water-air culture make it possible to increase the efficiency of production of the initial seed material and increase the quantitative output per plant and per unit of useful area.

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