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Investigations

Efficiency of Microtubers Application in the Production of Original Potato Seeds

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Abstract: The development of the technology of mass microtubers cultivation *in vitro* based on innovative methods with respect to potato varieties allowed for use in Kazakhstan has an important theoretical and practical significance for the development of seed production in the Republic. The goal is to develop a technology for mass cultivation of potatoes microtubers *in vitro* based on biotechnology methods and to assess the possibility of their use as a starting material in the seed-growing process of potatoes. Research methods are generally accepted in plant biotechnology. For the first time, studies are carried out on the mass cultivation of microtubers *in vitro* and their testing in the field, which determines the relevance and scientific novelty. The practical significance of the project is due to the determination of the most optimal timing for *in vitro* production of microtubers for the original seed production of potatoes in Kazakhstan. It was revealed that the induction of potato microtubers *in vitro* allows to accumulate and preserve the healthy planting material of valuable varieties during the entire period without additional transplantations, creating a kind of bank, to simplify and reduce the cost of forwarding the planting material. By regulating the photoperiod, lowering the temperature and modifying the nutrient medium, the tuber formation process was induced. A conclusion is made about the possibility of using microtubers as a planting material in the seed production of potatoes.

Keywords: Potato, Original Seed Production, Microtubers, Regenerant, Culture Medium, Photoperiod, Cultivation

Introduction

In the original potato seed production, an important link is the production of the original healthy material. Improving the technology of accelerated reproduction at laboratory and in field conditions is one of the topical issues in the seed production of potatoes.At present, the introduction of improved potato varieties into production is proceeding slowly. In this regard, it is advisable to use various methods of accelerated reproduction, which make it possible to obtain high multiplication factors in the production of original potato seeds (Basiev, 2015; Deryabin, 1997). One of the ways to solve this problem is to use microtubers induced *in vitro* as a health improvement material.

Because of their small size and smaller mass, the use of microtubers as a planting material reduces the cost of planting, storage (less storage capacity required) and seed transportation (Makarov, 1990; Matevossian *et al*., 1990).

The production of microtubers also has a number of advantages over the existing method of microclonal reproduction of test plants.

When propagating by microtubers, the multiplication factor (up to 7-8 times) and labor productivity increase, year round viral and diseases free micro and minitubers are produced, at the same time there is a real possibility of accelerated reproduction and wide introduction of new varieties of Kazakhstan breeding (Tokbergenova, 2016).

The method of cultivation of microtubers *in vitro* is actively being developed in a number of foreign

countries (USA, Canada, Great Britain, France, Denmark, the Netherlands, South Korea, China) in order to use it as a planting material in potato seed production. In the UK, by the company "Gudron and Innes" and in France a technology for year-round production of "basic" microtubers with a diameter of 4-12 mmwas developed (Shukurova *et al*., 2007; Hannapel, 2007).

According to the forecasts of scientists CIP (International Center for Potatoes, Peru), tubers obtained *in vitro*, are widely used in various schemes for the production of seed tubers. A group of American scientists at the University of Wisconsin studied the growth and development of tubers of early and late maturing varieties in the field conditions (Wang and Hu, 1985; Wareh *et al*., 1989; Wareing *et al*., 1980).

According to many authors for tuber formation in test tubes, the duration of the light and dark periods of the day, as well as the temperature factor are very important. Conditions of a short day and low temperatures contribute to tuber formation, a long day and high temperatures slow this process down up to its full braking (Balashova, 2015b; TPPOERSP, 2011; Anisimov and Smolegovets, 2008). The mechanism of photoperiodic induction of tuber formation is based on a change in the ratio and level of phytohormones.

All the plant hormones known at the present time-auxins, gibberellins, abscisins, cytokinins participate in the regulation of tuber formation. The data of many researchers indicate that when phytohormones inductors are injected into the culture medium *in vitro*, effective stimulation of tuber formationwas obtained (Rogrigues-Falcon *et al*., 2006; Koksharova, 2004; Anisimov *et al*., 2014).

Achieving the optimal ratio of factors that stimulate the formation of tubers in potatoes, you can get more of them and in the earlier periods. Such microtubers, used as a virus-free planting material, are the "ideal end product of micropropagation of potatoes", convenient for storage and transportation (Owes *et al*., 2014).

Researchers at the Korean Research Institute of Bio Science and Biotechnology have indeed achieved the goal of developing an unexplored and innovative technology in which the production efficiency is at least 100 times higher than in traditional technology. Currently, this technology is industrialized in Korea and 30 million microtubers of different species are produced and distributed among numerous local and foreign farmers with excellent field test results (Owes *et al*., 2014; Pelacho *et al*., 1994).

With the purpose of improving the technology of inducing biotechnological microtubers *in vitro* - the sources of the virus-free planting stock of seed potatoes, since 2002 a series of experiments have been carried out in the laboratory of biotechnology of the Kazakh Scientific Research Institute. The main attention was paid to the photoperiodic factors of tuber formation, the influence of certain components of the nutrient medium on it, as well as the temperature regimes during storage of microtubers.

Kazakhstan scientists developed a laboratory regulation on the accelerated induction of potato microtubers *in vitro* with a complex of biotechnological parameters (Tokbergenova *et al*., 2010).

Based on the foregoing, undoubtedly, the development and introduction of the technology of mass production of microtubers for the subsequent production of virus-free seed can produce a green revolution in potato cultivation. The use of healthy microtubers in seed production will allow farmers and farms to obtain a high yield and hence profit.

Materials and Methods

Laboratory and field experiments and the processing of their results were carried out in accordance with existing recommendations and guidelines.

The object of the study was the new varieties of potato selection of the Kazakh Scientific Research Institute of Potato and Vegetable Growing: Aksor, Zhanaisan, Tamyr, Tokhtar and Nerli.

Improvement of plant material was carried out by the method of culture of the apical meristem in combination with thermotherapy using the methodical recommendations of (Rakhimbaev, 1985; Kataeva and Butenko, 1983).

Plants-regenerants, reached the height of the test tube, are grafted in aseptic conditions. Before passivation on the nutrient medium for tuber formation, the cuttings were tested for viruses by the method of enzyme immunoassay (ELISA).

The middle parts of the cuttings of the sanitized plants were placed in sterile jars with the Murashige-Skoog nutrient medium containing tuber inducers.

To accelerate the process of tuber formation *in vitro* and improve their quality at the laboratory, a set of techniques were used.

During the experiments, the following factors were studied: Photoperiod - 16 h and constant darkness; concentration of 6-Benzylaminopurine (6-BAP) – 0,5; 1,0; 2,0 и 3,0 mg/L; Concentration of Indoleacetic Acid (IAA) -125 mg/L, adenine - 40 mg/L and kinetin concentration - 1 mg/L.

During the work on selection of nutrient media, solid, agar media were tested. In a nutrient medium, an elevated concentration (80,000 mg/L) of sucrose was used as a control, as inducers of the formation of microtubers were used: Benzylaminopurine, kinetin and adenine, that are, the components that together regulate the process of tuber formation of micro-plants *in vitro*. The results were determined as the tubers appeared.

After transferring on the nutrient medium, cuttings in vessels (jars) were cultivated in a phytotron at a temperature of 22°C, with a lighting of 3-5 thousand lx,

with a 16-h photoperiod for 3, 7 and 15 days. Then they were transferred to a constant darkness.

The experience was laid to study the influence of the mass of microtubers on the productivity of the seed material under in vivo conditions.Experiment variants: 100-200; 200-400; 400-600 mm. The influence of the microtuber planting scheme on the productivity of seed in field conditions is determined. Trials were by variants: 70×5; 70×10; 70×15; 70×20; 70×25 and 70×30 cm.

The number of microtubers from each variety is 100 pieces. Repetition of the experiment is 4-fold.

Statistical Analysis

The obtained data of laboratory and field experiments were statistically processed by means of dispersion analysis according to the experimental procedure (Dospekhov, 1985). In this case, the smallest significant difference was calculated.

Results and Discussion

Possibility of using the growth regulator of 6-Benzylaminopurine (6-BAP) from the cytokinin group, varying the concentration from 0.5 to 2.0 mg/L has been studied to accelerate the process of *in vitro* tuber formation and improve their quality. As a control, the Murashige-Skoog base medium was used with a sucrose concentration of 80,000 mg/L. Accounting for the beginning of microtubers formation was carried out visually, upon their appearance on plants.

The results of the study showed that, when 6-BAP at a concentration of 2 mg/L was added to the nutrient medium, the regenerating plants of the varieties Aksor, Zhanaisan, Tokhtar and Nerli formed microtubers for 55-63 days, that at the level of the control variant, for which the given index was 56-64 days (Fig. 1).

According to Fig. 1, using 6-BAP was not acceptable to accelerate the microtubers formation *in vitro*.

Many authors confirm that auxins and cytokinins in combination promote accelerated tuber formation *in vitro*, since such a combination of phytohormones is possible in a culture medium designed to induce microtubers (Ogluzdin, 1981; Ostapenko, 1981).

In this regard, the experience on the modification of the nutrient medium with phytohormones (indolylacetic acid 0.125 mg/L, adenine - 40 mg/L and kinetin - 1 mg/L) for induction potato tuber formation *in vitro* has been laid.

The results showed, with a joint action, phytohormones contributed to the formation of microtubers after 20-27 days, and in the control variant-56-64 days, respectively. The time dependence of tuber formation *in vitro* on the varietal characteristics of potato was also established (Fig. 2).

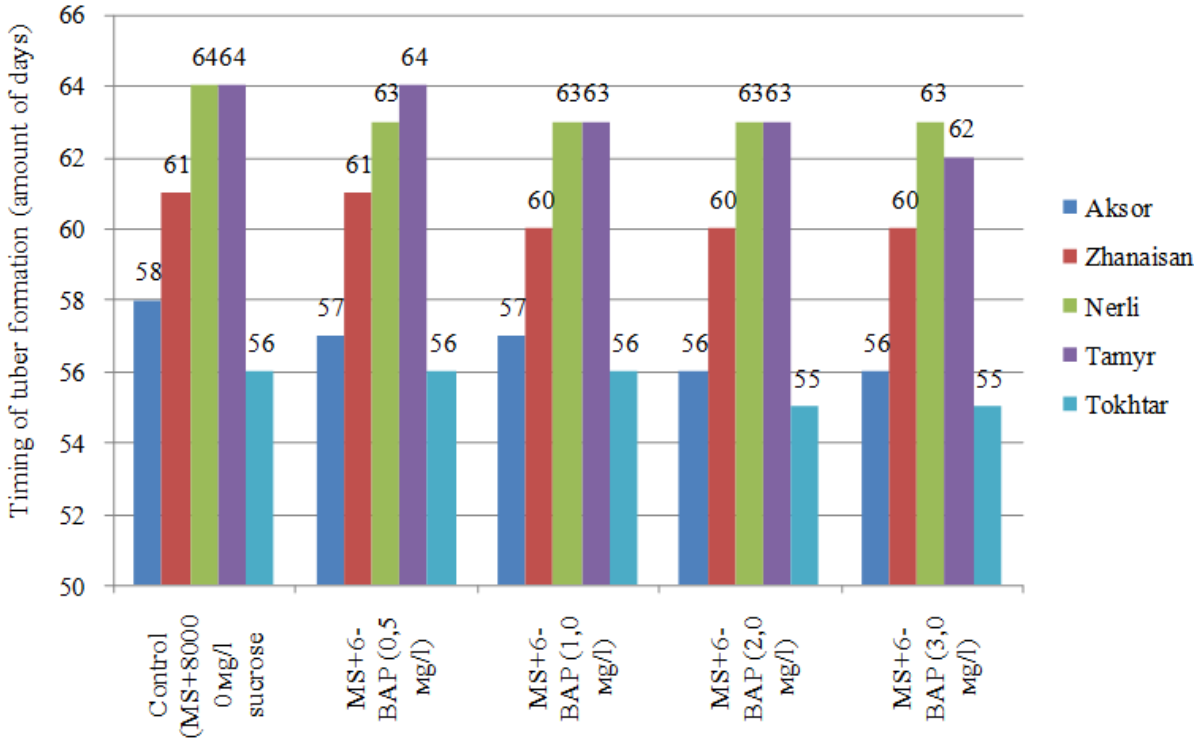

Fig. 1: Effect of 6-BAP on *in vitro* potato tuber formation

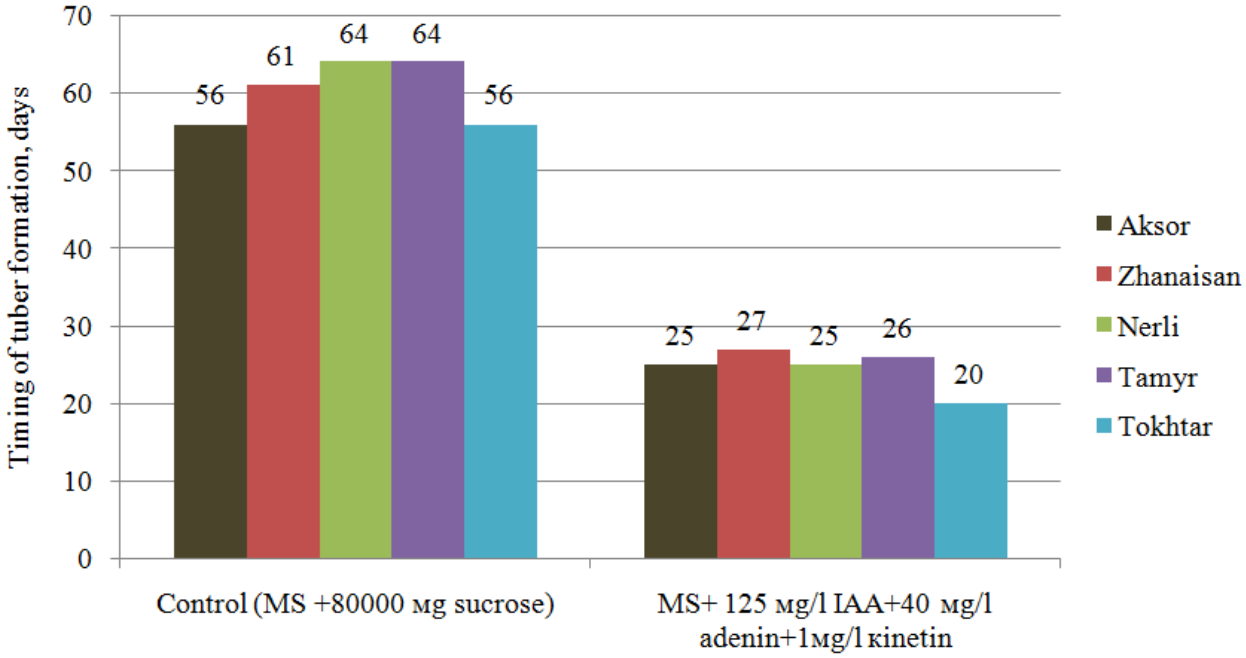

Fig. 2: The timing of *in vitro* potato tuber formation, depending on the composition of the nutrient medium

The earliest terms of tuber formation on the experimental variants were observed in the middle-ripening Tokhtar variety, in which the plants formed microtubers on the 20th day, the middle-ripening varieties of Aksor and Nerli formed microtubers on the 25th day. In the varieties Tamyr and Zhanaisan tuber formation was observed at a later date, on the 26-27th day.

The experiments showed that for all tested varieties the optimal medium for accelerated formation of microtubers turned out to be a medium containing IAA - 125 mg/L, adenine - 40 mg/L and kinetin - 1 mg/L.

It should also be noted that the abovementioned phytohormones positively influenced the increase in the number, size and accumulation of mass of microtubers induced *in vitro*.

By the number, size and mass of the potato microtubers, the accounting were carried out on the 20th day after the appearance of nodules (Table 1).

The number of microtubers per plant in the experimental variant was up to 2.1 pieces, depending on the genotype of the potato, while on the control variant it was 1.0 piece. In all tested varieties, the size of microtubers increased by 2.2-6.0 mm compared to the control variant, which was from 6.0 mm to 9.0 mm.A similar trend was observed in the mass of microtubers - from 490.0 mg to 692.0 mg, which is important in the system of potatoes seed production.

When studying inducers of tuber formation, the varietal feature of potato was clearly distinguished. It was established that the highest tuber capacity for the timing and quantity of microtubers was noted in plants of the middle ripening variety Tokhtar, which in the medium containing IAA, adenine and kinetin appeared microtubers on 20.0 days in an amount of 2.1 pieces per plant, that is, the maximum number of tubers among all tested varieties. By weight and size, it was inferior to the middle-ripening varieties of Nerli and Tamyr.

Hormonal regulation of tuber formation *in vitro* is associated with a number of factors, including the complex effect of the duration of the photoperiod with inducers of microtuber formation (Balashova, 2015a).

In this regard, we considered the options: A-plants-regenerants, cultivated with a 16-h photoperiod for 15 days and transferred to a constant darkness); B-regenerative plants cultivated with a 16-h photoperiod for 7 days and transferred to a constant darkness; C-regenerative plants cultivated for a 16-h photoperiod for 3 days and transferred to a constant darkness.

The photoperiod in combination with the nutrient medium had a significant effect on the intensity of tuber formation (Table 2).

The maximum values for the number and mass of microtubers were obtained by reacting a 16-h photoperiod for 3 days with transfer to a constant darkness and a concentration of phytohormones (indolylacetic acid-125 mg/L; adenine - 40 mg/L; kinetin- 1 mg/L). At the same time, the number of microtubers per plant of varieties Aksor and Tokhtar, was 1.9-2.1, the mass of these microtubers was 462.0-489.4 mg, respectively.

The next stage is the experience of study the effect of microtuber mass on *in vivo* seed productivity.

Table 1: Effect of phytohormones on the yield of *in vitro* potato micro tubers

Varieties	Treatments	Amount of microtubers per 1 plant, piece	Size of microtuber, mm	Mass of 1 microtuber, mg
Aksor	Control (MS+80000 mgsucrose)	1,0	4,0	295,0
	MS +125 mg/L IAA + +40 mg/L adenine +1mg/L kinetin	1,67	6,8	526,0
SD				0,81
Zhanaisan	Control (MS +80000 mgsucrose)	1,0	3,8	205,0
	MS +125 mg/L IAA + +40 mg/L adenine +1mg/L kinetin	2,0	6,0	490,0
Nerli	Control (MS +80000 mgsucrose)	1,0	3,9	330,0
	MS +125 mg/L IAA + +40 mg/L adenine +1 mg/L kinetin	1,9	7,8	692,0
SD				1,02
Tamyr	Control (MS +80000 mgsucrose)	0,92	3,0	332,0
	MS +125 mg/L IAA + +40 mg/L adenine +1 mg/L kinetin	1,8	9,0	647,9
Tokhtar	Control (MS +80000 mgsucrose)	1,0	3,2	200,0
	MS +125 mg/L IAA + +40 mg/L adenine +1 mg/L kinetin	2,1	7,6	561,3
SD				0,62

Table 2: *In vitro* tuber formation of potato depending on the photoperiod and nutrient medium, 2015-2016

Varieties	Cultivation conditions, photoperiod	Phytohor-mones nutrient medium concentration in	Amount of microtubers per 1 plant, piece	Mass of 1 microtuber, mg
Aksor	Cultivation of plants with a 16-h photoperiod for 15 days and transferring them to a constant darkness	MS +125 mg/L IAA + +40 mg/L adenine +1 mg/L kinetin	0,9	178,0
	Cultivation of plants with a 16-h photoperiod for 7 days and transferring them to a constant darkness		1,0	429,2
	Cultivation of plants with a 16-h photoperiod for 3 days and transferring them to a constant darkness		1,09	462,0
SD				1,1
Tokhtar	Cultivation of plants with a 16-h photoperiod for 15 days and transferring them to a constant darkness	MS +125 mg/L IAA + +40 mg/L adenine +1 mg/L kinetin	0,9	210,0
	Cultivation of plants with a 16-h photoperiod for 7 days and transferring them to a constant darkness		1,0	350,0
	Cultivation of plants with a 16-h photoperiod for 3 days and transferring them to a constant darkness		1,2	489,4
SD				1,23

On the basis of a comparative evaluation of the productivity and quantitative yield of the seed material using different microtubers in mass, the highest values were obtained when planting *in vitro* microtubers with a mass of 400-600 mg. In this variant, depending on the genotype, the productivity indicators were 1.0-1.2 times higher than in *in vitro* variants of microtubers weighing less than 400-600 mg. Depending on the varietal characteristics, the mass of tubers obtained from micro-tubers 6.0-9.0 mm in size was 395.4-415.4 g/bush, however, the quantitative indices of some varieties in this variant were less. For example, with the planting of microtubers 6,0-9,0 mm, the number of tubers per 1 bush in the varieties Aksor and Tokhtar was 10.0-13.1, which is by 0.3-1.9 pieces fewer than in the variant with microtubers of the size 4,0-6,0 mm (Table 3).

Table 3: Plant productivity and quantitative yield of minitubers depending on the size characteristics of *in vitro* microtubers, 2015-2016

Mass of *in vitro* microtubers, mg	Mass of tubers, g/bush				
	Aksor	Zhanaisan	Nerli	Tamyr	Tokhtar
100-200	355,2	366,1	398,3	385,9	392,6
200-400	395,4	407,5	412,5	400,1	415,4
400-600	410,2	451,0	462,3	501,0	500,0
SD	0,93	2,08	1,02	0,88	1,09
Amount of tubers, piece/bush					
100-200	10,3	15,3	11,9	10,7	15,0
200-400	10,0	16,4	12,7	11,5	13,1
400-600	10,0	16,7	12,9	12,0	13,1

Table 4: Influence of *in vitro* microtubers planting scheme on seed productivity in field conditions, g/bush, 2015-2016

Experienced options	Varieties					
	Alliance	Aksor	Zhanaisan	Nerli	Tokhtar	Tamyr
70×5 cm	334,5	202,8	298,6	176,3	157,3	153,8
70×10 cm	356,8	256,6	360,7	258,0	182,4	179,6
70×15 cm	381,4	324,7	361,2	325,7	208,3	246,8
70×20 cm	405,7	460,5	480,2	419,6	457,6	419,7
70×25 cm	558,2	497,5	519,6	500,8	536,8	518,5
70×30 cm	615,2	500,7	543,1	557,8	552,2	631,0
SD	2,49	1,9	1,62	1,46	1,02	1,29

In the experiment with the use of various planting schemes for *in vitro* microtubers, the most optimal performance indicators were the variants with a planting scheme of 70×30 cm. Productivity of tubers from 1 bush on this variant was up to 631,0 g/bush (Table 4).

Conclusion

The results of the study on phytohormone 6-BAP on the acceleration of *in vitro* microtubers induction showed that the growth regulator of the cytokinin group did not give a positive effect. In this case, the regenerating plants of the studied varieties formed microtubers within 55-63 days, which was at the level of the control variant, in which the given index was 56-64 days.

In all studied varieties, when 125 mg/L of IAA,40 mg/L of adenine and 1 mg/L of kinetin were added to the nutrient medium the dynamics of tuber formation was different from control (MS + sucrose 80,000 mg/L). The number of microtubers per plant in the experimental variant was up to 2.1 pieces, the size of microtubers increased by 2.2-6.0 mm compared to the control, the mass of microtubers increased to 692.0 mg, which is important in the system of seed potato production.

The photoperiod in combination with the nutrient medium had a significant effect on the intensity of tuber formation. The maximum values for the number and mass of microtubers were obtained by reacting a 16-h photoperiod for 3 days with transfer to a constant darkness and a concentration of phytohormones. At the same time, the number of microtubers per plant of varieties Aksor and Tokhtar, was 1.9-2.1, the mass of these microtubers was 462.0-489.4 mg, respectively.

The research results on the effect of the weight of microtubers on the productivity of seed in *in vivo* conditions have shown that with increasing mass, the productivity of the seed material increases.

The most optimal scheme for planting microtubers *in vivo* was 70×30 cm.

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Author's Contributions

All authors participated in all experiments, coordinated the data.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of

the other authors have read and approved the manuscript and there are no ethical issues involved.

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Abbreviations

KazSRIPVG– Kazakh scientific research institute of potato and vegetable growing, MS –Murashige-Skoog, 6-BAP-6-Benzylaminopurine, IAA-indolylacetic acid, t–ton, mg – milligram, ha– hectare, t/ha–ton per hectare.