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of the Institute of Plant Biology and Biotechnology

БИОЛОГИЯ ЖӘНЕ МЕДИЦИНА СЕРИЯСЫ

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СЕРИЯ БИОЛОГИЧЕСКАЯ И МЕДИЦИНСКАЯ

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APPROBATION OF ACCELERATED BIOTECHNOLOGICAL PRODUCTION OF ELITE SEED POTATOES FOR ENSURING SEED INDUSTRY IN THE REPUBLIC OF KAZAKHSTAN

Abstract. Production of healthy seed mini-tubers is the basis for obtaining high-quality potato seed material. The technology of pilot production of elite seed potatoes on a small scale has been developed and tested (approbated) in order to accelerate the production of domestic potato seed material. The prospects of this technology application are shown with use/availability of modern greenhouse complexes at the first stages of the technological process of "super-superelite" category of minituber material production. Virus free plants-regenerants of domestic high-yielding "Aksor" cultivar were obtained from a small number of tubers and propagated with the use of micrograftage method. Minitubers for the production of super-superelite seed material were obtained from the regenerant plants under conditions of closed (isolated) soil/ground in the first year. In the second year of the project superelite potato seeds were obtained from super-superelite minituber material under field conditions and were transferred to the elite seed-growing farms for further production of seeds of "elite" category in the third year of the project implementation.

Features of this technology consist in a continuous process of obtaining healthy test-tube plants *(in vitro*) and minitubers within 3 years. Conducting regular excavation of tuber material during the cultivation of plants in the greenhouse leads to an increase in the amount of minitubers harvest from one generation, as well as to cyclical production of super-superelite material 2 times a year. Results are the following: reduction of the period of elite seeds production to 3 years, a low demand for production areas, a slowdown in the norms of seed material consumption and a high coefficient of its reproduction.

Key words: potato, *in vitro* cultures, virus free plants-regenerants, microclonal propagation, potato original seeds, minitubers, potato elite seed material.

Potatoes are the most important food, technical and fodder crops. In addition, being the most important and significant vegetable source of food energy, vitamins, minerals and antioxidants among forbs (non-grain plants) in the world, potato considered as a unique product for healthy nutrition. Potato tubers contain on the average 76-78% of water, 17-19% of starch, 1-2% of proteins, and about 1% of minerals and vitamins [1]. In Kazakhstan, the priority direction of potato use is its production to ensure food security of the current and future generations of the country. Potato production in the country by profitability is one of the most promising branches of agriculture (from 50% to 300%) over the past few years [2].

Nowadays, the issue of producing high-quality seed material remains one of the most acute in this industry for a number of reasons. Firstly, not all regions of the republic own seed-growing farms for the production of original and elite seed potato material, and those that are - do not cover all the needs of potato growing farms with high-quality seeds of local cultivars. Secondly, most of the territory of our country is not suitable for potato cultivation due to difficult climatic conditions [3]. At present, more than 190 thousand hectares of land resources in the territory of Kazakhstan are occupied for potato production,

and the main regions in which the largest gross harvest of potatoes are recorded are Almaty, South Kazakhstan, Pavlodar, Zhambyl and North-Kazakhstan regions [4]. With the annual domestic production of potatoes, oriented only to the domestic market, consumers' needs are covered by only 50-60%, while the rest of the market is occupied by imported potatoes of very different quality from Russia, Kyrgyzstan, China and Pakistan [5,6]. The lack of a high-quality initial planting material of domestic potato varieties in the country makes it necessary to import potato seeds of foreign selection cultivars that are not adapted to local climatic conditions, quickly lose their qualitative characteristics, become susceptible to diseases, thereby reducing its yields and further reproduction becomes impractical [7] . Thus, in order to increase the efficiency of potato growing in the republic, it is necessary to organize a high-tech and cost-effective system of virus-free potato seed production.

The existing scheme for the production of "superelite" and "elite" categories seed potatoes by the traditional way requires from 4 to 6 years, and seed potatoes grown in the field are exposed to a high risk of infection with viral and bacterial diseases, which to a considerable extent reduces its quality and terms of use in further reproductions.

The risk of minituberous material contamination significantly reduces due to its cultivation in the greenhouse complex, and elite seed potato material production reduces as well by one year with use of the new scheme of accelerated production of healthy elite seed material on a biotechnological basis.

The accelerated scheme of healthy elite seed material obtainment was developed at the laboratory of Plant Bioengineering and concludes the following: virus free regenerant-plants and minitubers of domestic high-yielding potato cultivar will be obtained from a small number of tubers and propagated in the first year of the project, followed by production of minitubers, super-superelite and superelite seed material under closed and open soil conditions in the second year, and production of elite potato seeds from superelite tuberous material on fields on third year. Elite potato seeds then will be transferred to the elite seed-growing farms for further potato production.

Pilot biotechnological production of virus-free elite seed potato products of domestic varieties on the basis of cultivation technology developed in the laboratory allowed to evaluate experimentally the profitability of this technology, to reveal the weak and strong aspects of this scheme of seed production, and also to evaluate the prospects of such an approach for solving the problem of providing potato growing and peasant farms with elite seed material of potatoes of domestic cultivars.

Clone selection of the initial potato tuber material, isolation of meristem material and obtaining of healthy test-tube plants under *in vitro* conditions was carried out in each repeated cycle during each year.

Potato of domestic cultivar “Aksor” obtained from originator-variety at Kazakh National scientific research institute of potato and vegetable growing (KazNIIKO) was used as an initial potato tuber material. Cultivar characteristics: relatively heat resistant, drought-resistant, medium-ripening and medium-yielding. The productive potential of yield is in the range of 55 t / ha. Relatively resistant to diseases, of universal use [8].

Primary test-tube regenerant plants were obtained using method of apical meristems isolation out of healthy sterilized potato tubers in combination with thermotherapy [9]. Further apical meristems were transferred to the universal Murasige Skoog (MS) mediums and were cultivated in a room with regulated light and temperature conditions [10, 11] (Figure 1).

Microclonal reproduction of potato primary test-tube plants were performed by standard method of micro-graftage [12].

The test-tube potato regenerant plants were tested for infection with PVY, PVM, PRVL viruses using potato virus detecting diagnostic kits. Evaluation of the ELISA results was performed on a photometer at a wavelength of 450 nm. As a result of testing, the absence of a viral infection was detected in 19 of 21 batches (15 samples each) of test-tube potato plants. The batches of infected with virus M potato plants were eliminated.

The virus-free plants were replicated for their further transfer to *ex vitro* conditions and the production of potato minitubers in a closed ground/soil. 350 potato primary test-tube plants of “Aksor” cultivar were obtained from 19 lines in the first year. In the second and third year there were produced 252 primary test-tube plants from 21 lines and 800 test-tube plants from 7 lines of “Aksor” potato.

Figure 1 – Test-tube plants production from potato apical meristems

At the first stage of rooting and adaptation to the natural light and temperature conditions, potato regenerant plants were transplanted into individual plastic cups with an autoclaved soil mixture (peat - soil - sand in a ratio of 1:1:0,1). Plants were washed with a MS cultural medium, transplanted into aseptic soil and placed in a light-climatic room with an 18-hour light day, humidity 70%, lighting 3000-5000 lux, and temperature: day +25°C/night +22°C. Watering was carried out as the soil dries up with a Knopp modified nutrient solution for normal growth and development of plants.

The second adaptation stage of cultivation was conducted 3 weeks after planting test-tube plants into the soil/ground. Humidity in the climatic room was reduced to 56%, the temperature regime remained the same, watering of the plants was performed twice a week. The percentage of survived potato plants at this stage composed 92% from the number of plants that passed the first stage of adaptation. Adapted potato test-tube plants were planted in the greenhouse complexes of the elite seed-growing farm "Orken" to produce the original seeds.

Potato minitubers (original seeds) production

5400 pcs or 270 kg of harvest of “Aksor” potato minituberous material were obtained from test-tube plants during their cultivation in closed soil in the first year at the site of elite seed-growing farm “Orken”.

The harvest of potato minitubers material of “Aksor” cultivar were collected in the amount of 2000 pcs in winter period at “Orken” farm’s greenhouse complex from 2500 healthy test-tube plants in the second year.

For a continuous cycle of minitubers production, all the adapted test-tube potato plants were planted in the open ground in spring of the following year. Due to force majeure financial and climatic conditions in the summer of 2016 and 2017, during the second and third year of the project implementation, 90 kg and 52 kg of potato minituber material were obtained from 5000 healthy test-tube plants under the conditions of the greenhouse and field, respectively (Figure 2).

Figure 2 – Harvest from “Aksor” cultivar minitubers

Super-superelite seed material production from original seeds in greenhouse complexes.

In the first year of the project potato minituberous material were obtained out of 2,500 healthy test-tube plants on the fields of the "Orken" peasant farm which was stored from October to December. 3500 pcs of potato minitubers out of 5400 saved and maintained their viability due to natural loss during the storage period.

In January of the second year a minituberous potato seed material was placed on germination at a temperature of +18°C + 20°C for 14 days and planted in a greenhouse complex of the “Orken” elite seed-growing farm in order to produce super-superelite potato seed material. All necessary agrotechnical steps were carried out during the cultivation of plants in the greenhouse in winter period and a crop of super-super-elite potato was harvested in an amount of 150 kg and placed for short-term storage for further planting in the spring.

Production of "super-superelite" seed material from original seeds under field conditions on the second and third years

2000 pcs of potato minitubers of “Aksor” cultivar obtained in greenhouses in the winter period were planted into the open ground on the second year of the project. 81 kg of potato minitubers were planted under field conditions in the third year. All required agrotechnical measures were performed during cultivation of plants on the fields. The harvest of seed potatoes of the "super-superelite" category was collected in the second decade of October of the second and third years (Figure 3). 480 kg of super-superelite potato material were obtained in autumn of second year of project realization which composes 80% of initially expected harvest. Seed potato of super-superelite category were then stored for their further planting in spring period.

Figure 3 – “Super-superelite category seed potato of “Aksor” cultivar

1000 kg of “super-superelite” category potato were collected in autumn of the third year which composed 100% of initially expected harvest. Seed potato material of “super-superelite” category were transferred to the “Orken” farm for the storage and assessment of the quality of obtained seed potato.

“Superelite” category seed material production in the field conditions and their transfer to the elite seed-growing farms of Almaty region for the production of elite seeds and evaluation of its quality

150 kg of seed material of the “super-superelite” category of “Aksor” cultivar were planted to the open ground in the second year considering unfavorable spring weather conditions. 432 kg of “super-superelite” seed material of “Aksor” variety from the previous year planted in the third year.

Harvesting of potatoes was carried out in the second decade of October of the second year and in 3-4 decades in October of the third year (Figure 4).

In the second year the harvest of potato of superelite category of “Aksor” cultivar composed 315 kg. For the third year the harvest of potatoes of this category was about 4000 kg. Obtained superelite seed material was transferred to the seed-growing peasant farm “Orken” for further production of elite seeds and assessment of its quality.

Figure 4 – Collection of harvest from superelite category potato

Elite seed material production. The seed material of the "superelite" category in the amount of 283 kg was planted in the field at the end of May 2017considering the negative spring weather conditions. All required agrotechnical measures were performed during cultivation of plants on the fields. The harvest collection of potato seed material of “elite” category in the amount of 5000 kg was performed in first decade in October. Elite seed material was transferred to the “Orken” elite seed-growing farm for the evaluation of seed quality for further use in the production of elite seeds.

Thus, on the basis of experimentally obtained results from pilot technology, it was shown that the new scheme for accelerated biotechnological production of the healthy elite seed material proved the possibility of reducing the production period of the "elite" seed material to three years on the basis of obtaining minituber and super-superelite potato seed material in winter periods under greenhouse conditions.

The key factors for the successful implementation of the testing production scheme are the availability of an efficiently operating greenhouse complex for the cultivation of first-generation test-tubes plants and adult plants.

The results of the first year of approbation showed the success of the applied biotechnological methods for obtaining and replicating on an industrial scale of the healthy test-tube regenerant-plants. The results of the second year of studies confirmed the prospects of cultivation of plants in a greenhouse complex for the production of minituber seed material and super-superelite. The effectiveness of technology for obtaining seed material, taking into account the adjustment of quantitative indicators for the third year, was as follows (in percent from expected): 100% (5 tons) of “elite” category seed material, 80% (4 tons) of “superelite” -100% (1 ton) of “super-superelite and 2% (1800 pieces/52 kg) of original seed material (minitubers).

Summarizing, the obtained results indicated the prospects of using this accelerated technology for the production of elite seed potatoes taking into account the identified weak points (lack of financing and the influence of climatic factors), which allows us to further assume the possibility of effective implementation of this technology in production/industry.

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ҚАЗАҚСТАН РЕСПУБЛИКАСЫНЫҢ ТҰҚЫМ ӨНДІРІСІН ҚАМТАМАСЫЗ ЕТУ МАҚСАТЫНДА ЭЛИТТІ ТҰҚЫМДЫҚ КАРТОПТЫҢ БИОТЕХНОЛОГИЯЛЫҚ ӨНДІРІСІНІҢ ЖЫЛДАМДАТЫЛҒАН АПРОБАЦИЯСЫ

Аннотация. Сауықтырылған тұқымдық мини-түйнектерді өндіру картоптың жоғары сапалы тұқымдық материалын алудың негізі болып табылады. Картоптың тұқымдық отандық материалын жылдамдата алу үшін азғана өндірістік масштабта элиталық тұқымдық картоптың пилоттық өндіріс технологиясы жасап шығарылды және қабылданды. Бұл технологияны жаңа заманға сай жылыжай құрылғылары болған жағдайда «суперсуперэлита» категориясына жататын минитүйнектік материалды алудың технологиялық процесінің бірінші сатысында пайдаланудың болашағы бар екендігі көрсетілді. Түйнектің азғана мөлшерінен микроқалемшелеу әдісімен картоптың отандық өнімділігі жоғары «Ақсор» сортының сауықтырылған регенерант-өсімдіктері алынды және көбейтілді. Бірінші жылы регенерант-өсімдіктерден жабық топырақ жағдайында супер-суперэлиталық тұқымдық материал алу үшін минитүйнектер алынды. Екінші жылы егістік жағдайында супер-суперэлитаның тұқымдық материалдарынан картоптың суперэлиталық тұқымы алынды және элиталық тұқым шаруашылығы жобасының үшінші жылдық жобасына өндіруге «элита» категориясына жататын тұқым берілді.

Бұл технологияның ерекшелігі сауықтырылған пробиркалық өсімдік (*in vitro)* пен минитүйнектерді 3 жыл бойы үздіксіз алуға негізделген. Жылыжайда өсімдікті өсіру кезінде түйнектік материалды жүйелі түрде қазып алу. Бір ұрпақтан минитүйнектердің өнімінің санының артуы. Супер-суперэлиталық материалды жылына екі рет алу мүмкіндігі. Элиталық тұқым өндіру уақытының 3 жылға дейін қысқаруы, өндіріс аумағының қажеттілігінің азаюуы, тұқымдық материалдың шығынының төмендеуі және оны көбейту коэффициентінің жоғары болуы осы технологияның нәтижелері болып табылады.

Түйін сөздер: картоп, *in vitro* өскен клеткалар, сауықтырылған регенерант-өсімдік, микроклондау арқылы көбейту, картоптың бастапқы тұқымы, мини түйнек, картоптың элиталық тұқымдық материалы.

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АПРОБАЦИЯ УСКОРЕННОГО БИОТЕХНОЛОГИЧЕСКОГО ПРОИЗВОДСТВА ЭЛИТНОГО СЕМЕННОГО КАРТОФЕЛЯ ДЛЯ ОБЕСПЕЧЕНИЯ СЕМЕНОВОДСТВА В РЕСПУБЛИКЕ КАЗАХСТАН

Аннотация. Производство оздоровленных семенных мини-клубней является основой для получения высококачественного семенного материала картофеля. Для ускоренного получения отечественного семенного материала картофеля разработана и апробирована технология пилотного производства элитного семенного картофеля в малых промышленных масштабах. Показана перспективность ее применения при наличии современных тепличных комплексов на первых этапах технологического процесса получения миниклубневого материала категории «суперсуперэлита». Из небольшого числа клубней получены и размножены методом микрочеренкования безвирусные растения-регенеранты отечественного высокоурожайного сорта картофеля «Аксор». Из растений-регенерантов в условиях закрытого грунта в первый год получены миниклубни для производства супер-суперэлитного семенного материала. Из клубневого материала супер-суперэлиты на второй год в полевых условиях получены суперэлитные семена картофеля и переданы в элитсемхоз для производства семян категория «элита» уже на третий год выполнения проекта.

Особенности данной технологии заключаются в непрерывном процессе получения оздоровленных пробирочных растений (*in vitro)* и миниклубней в течение 3-х лет. Проведение регулярной выемки клубневого материала во время культивирования растений в теплице приводит к увеличению количества урожая миниклубней с одного поколения, а также цикличному получению супер-суперэлитного материала 2 раза в год. Результатами являются сокращение сроков производства элитных семян до 3 лет, низкая потребность в производственных площадях, снижение норм расхода семенного материала и высокий коэффициент его размножения.

Ключевые слова: картофель, культуры *in vitro*, безвирусные растения – регенеранты, микроклональное размножение, оригинальные семена картофеля, миниклубни, элитный семенной материал картофеля.

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