Cultivation method for revitalized potato material in the system of original seed production

S A Buldakov, N A Shakleina, L P Plekhanova
Sakhalin Research Institute of Agriculture, 22 Gorky st., Yuzhno-Sakhalinsk, 693022, Russia
E-mail: sakhnii_sakhalin@mail.ru

Abstract. To arrange a system of virus-free potato seed production in the Sakhalin region, a series of studies undertaken to come up with a method towards potato material cultivation in film-gauze greenhouses. At the initial stage, the key agrotechnical methods were developed for growing test-tube microplants, including optimal planting plans – 70×15 cm (the highest yield derived from a standard mini-tuber fraction pcs/m²), 70×30 cm (for clone selection); spraying with insecticides (Aktellik – 3 l/ha, Rogor – 2 l/ha) and fungicides (Ridomil – 1 kg/ha, Tsineb – 2.4 kg/ha) to combat agents transmitting viral and fungal infections; chlorocholine chloride treatment (1.1%) to reduce the growth of the vegetative mass; serological analysis of plants for latent infection with viruses X, Y, S, M, F; burning tops with Reglon (2 l/ha) 2 weeks before harvesting. A set of agrotechnical methods used for producing healthy seed tubers on the Timiryazevskoye seed farm made it possible to reduce the elite cultivation cycle from 5 to 4 years, increase the yield by 57.2%, and reduce the production cost by 43.5%. Subsequently, the methods worked out for producing potato seeds were improved based on modern breeding varieties and phytoregulators (Azolen, Elena, Extrasol, Epin-Extra, Zircon) in spray mixtures with the fungicide Shirlan, contributing to an increased yield up to 34% and efficiency of a standard seed fraction up to 33%. Under production conditions, this method provided a conditionally net income of 450-700 thousand rubles/ha.

1. Introduction
A biotechnological method in potato growing, based on meristem-tip culture in vitro, is one of the key ways to produce original seeds [1, 2]. In a short time, it provides a large amount of homogeneous high-quality healthy material, thereby cutting back on the process of growing the elite and reducing labor costs [3].

Plant material cultivation in isolation is mostly aimed at increasing the yield of standard mini-tubers for subsequent establishment of temporary nurseries as per a seed production plan. To solve this task at the present stage, along with the development of agrotechnical methods, it is effective to use phytoregulators that not only stimulate productivity, but also have an adaptogenic effect on plants, thus enhancing resistance to diseases and stress factors [4, 5].

On Sakhalin Island, the development of a technological process for the production of the healthy elite began in the early 1980s. In this regard, at the first stage, it became necessary to study agrotechnical methods of growing a potato culture in vitro under cover.
The paper aims to develop and improve a scientifically grounded technology for growing plant material in the original seed production system.

2. Research Methods
A study was carried out from 1984 to 1987 at the Timiryazevskoye experimental production farm of the Sakhalin branch of Far Eastern Research Institute of Agriculture; from 2011 to 2013 – at Sakhalin Research Institute of Agriculture. Agrotechnical measures for the cultivation of potatoes in vitro were developed in film-gauze greenhouses. The study relied on test tube plants of zoned potato varieties Aurora, Kameraz, Nevsky, Priekulsky early, Ryabinushka that were obtained in biocenters: Leningrad Research Institute of Agriculture “Belogorka” and Russian Potato Research Center. Culture was propagated by microclonal cuttings on the Murashige-Skuga nutrient medium from January to May and was grown under phytotron conditions at a temperature of +22 °C, illumination – 5 thousand lux, photoperiod – 16-17 hours. The area of one greenhouse was 500 m². The soil consisted of three parts of peat compost and one part of sod land. The agrochemical properties of the soil were characterized by a low humus content – 2.8% and microelements: P₂O₅ – 16.8, K₂O – 13.4, N-NH₄ – 0.6, N-NO₃ – 3.5 mg per 100 g of dry soil; the soil reaction was slightly acidic – 5.1. Prior to planting, mineral fertilizers were applied (diammofoska 60-80 g/m²), the soil was milled, and furrows were cut. Microplants (2.5 thousand pcs.) were planted in the first ten days of June directly in greenhouse furrows previously watered and covered with soil up to one or two leaves. For better rooting, the plants were irrigated with a Knop solution of half concentration: Ca(NO₃)₂ – 0.5, KH₂PO₄ – 0.12, MgSO₄ – 0.12, KCI – 0.06, FeCl₃ – 0.006 g per 1 l of water. Subsequent care consisted in timely watering, loosening and hilling the soil.

Scientific research was based on the methods proposed by the Russian Potato Research Center (methods of research on potato culture, 1967) [6]. Potato blight lesion rates were determined using a 9-point scale of the CMEA International Classifier [7]. Latent infection of plants with viruses was evaluated through a serological method [8] and enzyme-linked immunosorbent assay [9]. The experimental data were mathematically processed by the dispersion method [10]. Economic [11] and energy efficiency were determined according to the variants of the experiment [12].

3. Results and discussion
The initial stage of research on the development of technological methods for growing potatoes in culture in vivo began in 1984.

A feeding area is pivotal for increasing the productivity of mini-tubers per unit area when test tube plants are grown in a greenhouse [13]. The authors experimentally studied a close-planting plan – 70×15 cm, and two spaced planting plans – 70×30 and 70×45 cm, for clone selection [14]. The findings showed that close-planting provided the highest yield derived from a standard seed fraction for both varieties (57.4-61.8%). With spaced plantings, this indicator slightly decreased to 57.0-49.2%, and the total yield increased to 797-1006 g/plant. The most efficient plan for individual plant selection was 70×30 cm planting plan that, as compared to 70×45 cm plan, provided the highest yield from the Nevsky variety – 59.9%, and the Kameraz variety – 54.0% (Table 1).

| Planting plan, cm | Nevsky variety (middle-early) | Kameraz variety (mid-season) |
|-------------------|-------------------------------|-------------------------------|
|                   | Productivity, g/plant         | Standard fraction, %          | Productivity, g/plant | Standard fraction, % |
| 70×15             | 787                           | 61.8                          | 651                   | 57.4                  |
| 70×30             | 918                           | 59.9                          | 722                   | 54.0                  |
| 70×45             | 1006                          | 57.0                          | 797                   | 49.2                  |
| LSD₀.₀5           | 88                            | -                             | 74                    | -                     |
Indoor favorable conditions promote excessive growth of the vegetative mass of potatoes, which leads to a longer growing season. In order to reduce intensive growth and increase the reproduction rate, experiments were carried out using growth-regulating drugs – chlorocholine chloride (CCC) at concentrations of 0.2, 1.1, 2.2%, Ivin (2,6-dimethylpyidine-1-oxide and biogenic trace elements) – 0.03, 0.6%, Hydroquinone (para-dihydroxybenzene) – 0.50, 0.25%. The plants were sprayed upon reaching 20 cm high and at the beginning of budding. Influenced by growth stimulators, the yield of potatoes increased by 19.7-25.5%, the number of tubers per plant increased by 1-2 pcs. The retardant chlorocholine chloride at 1.1% concentration hindered the growth of vines by 30 cm with a growing yield of tubers by 19.7%, changed potato mien, reduced stem growth by shortening internodes, and the plants became more compact in shape.

When flowers appear, all plants in the greenhouse were inspected for latent infection with viruses: X, Y, S, M, F. Sick plants were culled.

Along with new methods, a test tube culture of potatoes was grown by following standard measures used in the production of seed potatoes: threefold spraying with insecticides (Aktellik – 3 l/ha, Rogor – 2 l/ha) to protect from soil pests and vectors of viral infection; treatment with fungicides against late blight (Ridomil – 1 kg/ha, Tsineb – 2.4 kg/ha); chemical burning of tops by Region (2 l/ha) 2 weeks before harvesting [15].

The method developed for producing seed potatoes on the Timiryazevskoye experimental production farm made it possible to reduce the cultivation cycle of elite seeds from 5 to 4 years due to individual indoor clone selection, increase the yield of potatoes by 57.2%, reduce the cost of production by 43.5%, provide a net income of 8.9 thousand rubles/ha. In 1987, the first lot (262 tons) of the Priekulsky early variety superelite was sold [14].

Due to economic reforms in the 90s, the production of healthy potatoes in the region stopped and resumed since 1998, when a number of regional programs were adopted to restore the production of original seeds [16].

At the second stage of research (2011-2013), the agrotechnical methods for growing mini-tubers in greenhouses were improved based on varieties and phytoregulators of a new generation.

In modern conditions, the production of agricultural products using a new generation of phytoregulators is one of the priority areas. They are able to optimize nutrition, stimulate plant growth and development, increase crop resistance to stress factors and diseases, which increases the yield and quality of tubers without compromising agroecology [5]. Once applied in spray mixtures with other pesticides, they increase the efficiency of treatments and reduce production costs. To protect potatoes from fungal diseases, the experimental scheme included variants with a mixed use of phytoregulators and the fungicide Shirlan that, according to a number of authors, effectively protects potato plants and tubers from late blight [17].

The phytoregulators in Shirlan spray mixtures were examined on new zoned varieties – mid-early Ryabinushka, mid-season Aurora. Biological products were tested – Azolen (600 g/ha), Elena (600 g/ha), Extrasol (3 l/ha); growth regulators – Epin-Extra (80 ml/ha), Zircon (10 ml/ha) in spray mixtures with Shirlan fungicide (in a half dose – 0.25 l/ha). The plants were sprayed with preparations at a height of 15-20 cm, during budding and flowering.

The findings showed that all the target preparations in Shirlan spray mixtures had a positive effect on the biometric indicators of varieties. They improved plant height by 10-24%, number of stems – 20-44% and leaves – 22-46%, assimilation surface – 25-40%, which contributed to an increase in the productivity of both varieties by 12.9-34.2%, standard fraction yield – 10.0-32.9%. The highest indicators were found to be present after treatments with a spray Azolen/Shirlan mixture (Figure 1).

Under cover, potato plants during the growing season did not have any signs of fungal diseases on the vines. The enzyme immunoassay confirmed the absence of latent viral infection.

The tubers inspected a month after harvesting showed that spray mixtures of phytoregulators with a fungicide contributed to a decrease in rhizoctonia infection of mini-tubers: Ryabinushka variety – by 2.0-4.4%, Aurora – by 1.7-4.7%. The maximum protective effect was achieved when plants were
treated with a spray Elena/Shirlan mixture. In this variant, the smallest number of infected tubers was observed in both varieties – 2.3-3.0%.

![Figure 1. Productivity of potato microplants when influenced by phytoregulators in greenhouses, g/plant.](image)

The resultant healthy seed tubers were used for the next year to establish a nursery for the first field generation with subsequent reproduction according to the elite production plan.

A production indoor test of the Azolen/Shirlan spray mixture at the Korsakovsky State Farm (2013) confirmed its effectiveness. Net income from the use of the new technological method amounted to 0.74 thousand rubles/m², profitability – 84%. What is more, the energy income reached 61.5 GJ/ha, and the energy cost decreased to 1.77 GJ/t (versus 2.2 GJ/t through the no-treatment option).

4. Conclusion

Based on the results of multi-year scientific research (1984-1987), a method was developed for indoor growing healthy potato material, which made it possible to shorten the cultivation cycle of elite seeds, increase the yield by 57.2%, and reduce the cost of production by 43.5%. A new technological method (2011-2013) for the use of modern varieties and phytoregulators in spray mixtures with a fungicide, contributed to an increase in the total productivity, subject to a variety, by 12.9-34.2% and the yield derived from standard seed fraction – by 10.0-32.9%, reducing exposure to rhizoctonia-induced damage in mini-tubers. Additional income from the use of spray mixtures based on preparations with a fungicide amounted to 45-70 rubles/m².

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