Ion-exchange substrates as the basis for growing seedlings of potato in tube culture

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Abstract. Studies had been carried out to study the possibility of using ionite substrates for growing potato seedlings in tube culture, to analyze the variability of hybrid potato populations by morphobiological and economically valuable characteristics depending on the substrate, to analyze the effectiveness of the selection of high-yielding forms at the early stage of the breeding process. The experiment included 7 different modifications of the nutrient medium composition. Seed germination, seedling survival rate, tuber formation rate, percentage of tuber-forming seedlings, number of tubers per seedling, size, and shape of tubers were taken into account. Quantitative assessment of factors in phenotypic manifestation of signs was carried out by the method of dispersion analysis. Sterile microtubers were stored at 2-4 °C in the dark before planting. As a result of the research, a different tuber-forming ability was found in seedlings of populations of different variants. The tube culture of seedlings ensures full survival of seedlings. For the growth and development of seedlings in a tube culture, the best of those studied was the nutrient medium based on the ion-exchange substrate IS-2V, containing 1% sucrose and 500 mg /1 CCC. Two-stage cultivation of seedlings (the initial increase in the above-ground mass, then the induction of tuberization) had a positive effect on the pace and quality of tuberization. 30–40 days after induction, tuberization was observed in 90–100% of seedlings, regardless of their origin, and the size of microtubers was on average 6.7–8.7 mm.

1. Introduction

In the implementation of the food program, great importance is attached to the application of scientific achievements [1]. In this regard, the role of research in the direction of accelerating and improving the efficiency of the breeding process to create highly productive forms of plants resistant to extreme environmental conditions, pests, pathogens, as well as suitable for mechanized cultivation [2,3].

For the development of seedlings, the most favorable is the environment containing the minimum amount of sucrose in the composition of which there are no growth-stimulating substances [4]. This indicates that sprouts and micro-seedlings do not need stimulants, since the seed itself is a nutrient base [5].

Some studies indicate that the main factors of tuber formation in the cultivation of plants in vitro culture are photoperiod, temperature, sucrose concentration in the medium [6]. The experiments of scientists indicate that the shortened photoperiod under in vitro conditions had a decisive effect on the process of tuber formation – shortening of the photoperiod caused an increase in the number of tubers [7].

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It is believed that tubers can be obtained in an environment designed for plant growth [8]. However, most researchers argue that for mass tuber formation it is necessary to use specially developed for this purpose nutrient media [9].

A number of researchers note that in the process of tuberous material reproduction obtained from test tubes microtubers, retains the advantages of the main features necessary for the successful conduct of the breeding process [10].

2. Purpose of research
The main purpose of research on this topic was to study the possibility of using ionite substrates for growing seedlings in a test tube culture.

3. Materials and methods of research
The studies used hybrid seeds obtained in the department of breeding and genetics VNIIKH named after A. G. Lorch. The experiment included the following combinations:

1. 374-55 x Aksenovsky (middle x early)
2. Nevsky x 733-65 (medium early x early)
3. Aksenovsky x Sotka (early x middle late)
4. 889-99 x Smena (medium late x medium)
5. 1169-2 x Zarevo (medium late x medium late)

Seedlings were grown in glass jars of 50 ml in the period from October to April. Research on the cultivation of seedlings in a test tube culture was carried out according to the guidelines for Trofimzu L. Sterilization was carried out by immersing the seeds for 1-2 s in alcohol and 7 minutes in a 0.1% diacide solution. The experiment included the following options:

1. Options AS-1 and AS-2. Seedlings were cultivated on an agar medium with a mineral base according to Murasige and Skoog in the modification of the Scientific Research Institute of Chemical Achievements named after A.G. Lorch.
2. Option P-IAS. Seedlings cultivated an agarized agar medium based on the ionic substrate IS-2V;
3. Options OP-IS and 1P-IS. Seedlings were cultivated on the medium based on the ionite substrate IS-2V;
4. Options 2P-IS and 3P-IS. Two-stage cultivation of seedlings on the medium, based on the ion-exchange substratum IS-2V;

The components of nutrient media are as follows:

1. AS-1 – vitamins by White, kinetin – 0.4 mg/l, adenine – 4 mg/l, sucrose – 80 g/l, agar – 7 g/l.
2. AS-2 – vitamins by Staba, casein hydrolysate – 40 mg/l, kinetin – 0.5 mg/l, alenin – 5 mg/l, sucrose – 80 g/l, agar – 7 g/l.
3. OP-IS – vitamins by White, kinetin – 0.4 mg/l, adenine – 4 mg/l, sucrose – 80 g/l.
4. P-IAS – vitamins by Staba, casein hydrolysate – 40 mg/l, kinetin – 0.5 mg/l, adenine – 5 mg/l, sucrose – 80 g/l, agar – 7 g/l.
5. 1P-IS – vitamins by Staba, casein hydrolysate – 40 mg/l, kinetin – 0.5 mg/l, adenine – 5 mg/l, sucrose – 80 g/l.
6. 2P-IS – vitamins by Staba, casein hydrolysate – 40 mg/l, gibberellinic acid – 0, 5 mg/l, heteroauxin – 1.5 mg/l, kinetin – 0.5 mg/l, adenine – 5 mg/l, sucrose – 20 g/l.
7. 3P-IS – chloroindole – 500 mg/l, sucrose 10 g/l.

In variants 2P-IS after 50 – 100 days and in 3P-IS after 50 days of cultivation of seedlings for inducing tuber formation, the concentration of sucrose in the medium was brought to 8%, and in addition, in 3P-is the concentration of kinetin – up to 0.5 mg/l, the concentration of adenine – up to 4 mg/l.

Variants AS-1 and OP-IS were grown in room lighting, the rest – at a photoperiod of 16 hours with illumination of 6000-8000 Lux at room temperature. After inducing tuber formation options 2P-IS and 3P-IS in 2017 for 3 days were grown in the dark, then – at room conditions.
Seed germination, seedling survival rate, tuber formation rate, percentage of tuber-forming seedlings, number of tubers per seedling, size, and shape of tubers were taken into account. Quantitative assessment of factors in phenotypic manifestation of signs was carried out by the method of dispersion analysis. Microtubers were stored at 2-4 °C sterile in the dark before planting.

To study the differences between the seedlings of forming and non-forming tubers in the test tube culture in yield, 10 seedlings of these two groups were cloned by microferencing in 2017, receiving 2 plants from each seedling.

Test-tube plants of seedlings and microtubers were planted in a film-gauze house in torfogrunt with a feeding area of 30x30 cm. In 2017, microtubers were planted in the open ground with a power area of 70x30 cm.

Tuberous reproductions of pot culture seedlings (first and second), as well as 2 tuberous reproductions of test tube culture were grown in the field. Nutrition area for 1 tuberous reproduction is 70x60 cm, for 2 reproductions – 70x30 cm. Field-planting was carried out in the first half of May. Hybrids of 1 tuberous reproduction were represented by one tuber, hybrids of 2 reproductions – by three tubers (selected by economic and valuable characteristics – by ten tubers). Among the studied hybrids, there were plots with parental forms and standard varieties (10 plants each). The following varieties were used as standards: Nevsky - mid-early, Zavorovsky - mid-season, Ramensky - mid-late.

4. Research results.

The germination of seeds according to the variants was taken into account 4 weeks after sowing. This was due to the fact that the germination seeds invariants containing sucrose in a concentration of 8% was somewhat delayed. In 2 weeks after sowing seeds in variants 2P-IS and 3P-IS, the nutrient media of which contain 2% and 1% sucrose, respectively, there was friendly germination, while in other variants – in 3-4 weeks (table 1).

| Table 1. Germination of seeds of different origin in tube culture on different media. |
|-----------------------------------|-------------|-------------|---------|
| Origin                           | Option      | Seeds sown, PCs. | Germination, % |
|----------------------------------|-------------|-------------|---------|
| 374-55 x Aksenovsky              | AS-1        | 150         | 82.3±3.1 |
|                                  | OP-IS       | 150         | 82.3±3.1 |
| Aksenovsky x Sotka               | AS-1        | 150         | 90.0±2.4 |
|                                  | OP-IS       | 150         | 91.6±2.3 |
| 1169-2 x Zarevo                  | AS-1        | 150         | 96.7±1.5 |
|                                  | OP-IS       | 150         | 98.7±0.9 |
|                                  | AS-2        | 25          | 88.0±6.5 |
| 374-55 x Aksenovsky              | 1P-IS       | 30          | 86.7±6.2 |
|                                  | 2P-IS       | 22          | 81.8±8.2 |
|                                  | P-IAS       | 20          | 90.0±6.7 |
|                                  | AS-2        | 25          | 84.0±7.3 |
| Aksenovsky x Sotka               | 1P-IS       | 40          | 95.0±3.4 |
|                                  | 2P-IS       | 27          | 96.3±3.6 |
|                                  | P-IAS       | 17          | 100.0    |
|                                  | AS-2        | 25          | 76.0±3.5 |
|                                  | 1P-IS       | 38          | 97.4±2.6 |
| 1169-2 x Zarevo                  | 2P-IS       | 40          | 100.0    |
|                                  | P-IAS       | 25          | 100.0    |
|                                  | AS-2        | 25          | 76.0±3.5 |
|                                  | 1P-IS       | 38          | 97.4±2.6 |
|                                  | 2P-IS       | 40          | 100.0    |
|                                  | P-IAS       | 25          | 100.0    |
| Nevska x 733-65                  | 2P-IS       | 20          | 95.0±4.9 |
| 889-99 x Smena                   | 2P-IS       | 20          | 100.0    |
| HCP05                            | 3P-IS       | 100         | 99.0±1.0 |
|                                  |             |             | 3.4 – 21.1 |
When comparing the variants of the test tube culture, it was found that the agar medium based on M-S (variants AC-1 and AC-2) did not have the same effect on the germination of seeds depending on their origin. So, in the combinations Aksenovskaya x Sotka and 1169-2 x Zarevo in these embodiments, the seed germination was below 0.7-24.0 %, compared to the options based on is-2 and 2 374-55 x Aksenovskaya was on their level.

With artificial lighting of 6000-8000 lux, full survival of seedlings in all variants as noted. In low-light indoor conditions, the percentage of seedling deaths in the AS-1 and OP-IS variants varied from 6.9 to 13.3% and from 51.9 to 53.3%, respectively, depending on the combination.

The test-tube culture based on the ion-exchange substrate IS-2V made it possible to increase the germination of seeds, as compared to the agar medium based on M-S, and to achieve the maximum survival rate of seedlings. There were two main ways to use test tube seedlings:

1. Transplanting them at the age of 30 days in pots with peat or ionite substrate for further cultivation.
2. The induction of tuber formation in tube culture.

More promising was to obtain microtubers in a test tube culture.

In this work, we evaluated 5 hybrid combinations of seedlings by tuber-forming ability in a test-tube culture on the ion-exchange substrate IS-2V.

As a result of the research, a different tuber-forming ability was found in seedlings of populations of different variants. This was primarily due to the light mode of growing seedlings and the composition of nutrient media.

**Table 2.** Characteristics of seedling populations by the ability of tuber formation in the test tube culture, depending on the substrate of cultivation.

| Origin             | Option | Total number of seedlings, pcs. | The number of seedlings with tubes, pcs. | % of tuberization |
|--------------------|--------|---------------------------------|------------------------------------------|------------------|
| 374-55 x Aksenovskaya | AS-1   | 112                             | 0                                        | 0                |
|                    | OP-IS  | 58                              | 12                                       | 20.7±5.3         |
| 374-55 x Aksenovskaya | AS-1   | 117                             | 0                                        | 0                |
| 374-55 x Aksenovskaya | AS-1   | 63                              | 9                                        | 14.3±4.4         |
| 1169-2 x Zarevo    | AS-1   | 135                             | 32                                       | 23.6±3.7         |
|                    | OP-IS  | 72                              | 12                                       | 16.7±4.4         |
| 374-55 x Aksenovskaya | 1P-IS  | 26                              | 2                                        | 7.7±5.2          |
|                    | 2P-IS  | 18                              | 17                                       | 94.4±5.4         |
| 1169-2 x Zarevo    | 1P-IS  | 18                              | 8                                        | 44.4±11.7        |
|                    | 2P-IS  | 21                              | 13                                       | 61.9±10.6        |
| 374-55 x Aksenovskaya | 1P-IS  | 38                              | 6                                        | 15.8±5.9         |
|                    | 2P-IS  | 26                              | 26                                       | 100.0±0          |
| 1169-2 x Zarevo    | 1P-IS  | 17                              | 10                                       | 58.8±11.9        |
|                    | 2P-IS  | 19                              | 9                                        | 47.4±11.5        |
| 374-55 x Aksenovskaya | 1P-IS  | 37                              | 10                                       | 27.0±7.3         |
|                    | 2P-IS  | 40                              | 36                                       | 90.0±4.7         |
| Nevsky x 733-65    | 2P-IS  | 25                              | 17                                       | 60.0±9.3         |
| 889-99 x Smena     | 2P-IS  | 19                              | 19                                       | 100±0            |
| HCP05              | 3P-IS  | 99                              | 99                                       | 100±0            |

19.5-30.9
When grown in 2015 in low light conditions, the seedlings were strongly drawn out and had an underdeveloped leaf apparatus. At the same time, on an agar medium (variant AS-1) in populations 374-55 x Aksenovsky and Aksenovsky x Sotka, tuberization was not observed in seedlings. Only in the population of 1169-2 x Zarevo in 32 seedlings out of 135, the formation of micro-tubers was observed, which amounted to 23.6% (Table 2).

Under the same conditions on a nutrient medium based on an ionic substrate (OP-IS variant), tuber formation was observed in all populations. Its percentage depending on the origin of seedlings varied from 14.3 to 20.7%.

Insufficient lighting, thus, lead to tuber formation only in a small number of seedlings in the environment of OP-IS in all, and on AS-1 only in individual populations, that is, only single genotypes were capable of tuber formation in a test tube culture when growing seedlings under room lighting. Therefore, artificial lighting was used for the cultivation of seedlings in the future.

Cultivation with a 16-hour photoperiod with illumination of 6000-8000 lux allows obtaining seedlings with a well-developed leaf apparatus. In all variants in 2017, regardless of origin, the formation of tubers was noted. At the same time, the nutrient medium of 2P-IS was more favorable. Already on the 90th day after sowing, the seeds in the population of 374-65 x Aksenovsky 5.6% and Aksenovsky x Sotka 15.6% of seedlings had tubers. After inducing tuber formation (after 100 days of cultivation) 94.4% of seedlings in the population of 374-55 x Aksenovsky, 90.0% of seedlings 1169-2 x Zarevo and all seedlings Aksenovsky x Sotka formed tubers. In other variants, the percentage of tuber formation is much lower.

**Table 3.** Characteristics of the populations of seedlings on the number, shape and the size of the tubers in the test tube culture.

| Origin                  | Option | A number of tubers per 1 seedling, pcs. | Tuber shape, score | The size of the tubers, mm |
|-------------------------|--------|----------------------------------------|-------------------|--------------------------|
| 374-55 x Aksenovsky     | OP-IS  | 1.0±0                                   | 3.0±0.3           | 5.6±0.7                  |
| Aksenovsky x Sotka      | OP-IS  | 1.0±0                                   | 4.8±0.3           | 4.5±0.4                  |
| 1169-2 x Zarevo         | AS-1   | 2.0±0.2                                 | 4.2±0.2           | 5.4±0.3                  |
|                         | OP-IS  | 1.2±0.1                                 | 4.0±0.3           | 4.0±0.4                  |
|                         | AS-2   | 2.1±0.4                                 | 3.4±0.4           | 5.8±0.8                  |
| 374-55 x Aksenovsky     | 1P-IS  | 1.5±0.7                                 | 3.5±0.3           | 3.5±0.7                  |
|                         | 2P-IS  | 1.2±0.1                                 | 3.2±0.2           | 8.7±0.4                  |
|                         | P-IAS  | 1.6±0.2                                 | 2.8±0.5           | 6.5±0.8                  |
|                         | AS-2   | 1.5±0.3                                 | 3.9±0.3           | 5.0±0.6                  |
| Aksenovsky x Sotka      | 1P-IS  | 1.6±0.2                                 | 4.5±0.3           | 4.3±0.5                  |
|                         | 2P-IS  | 1.5±0.1                                 | 3.9±0.1           | 7.9±0.3                  |
|                         | P-IAS  | 1.5±0.3                                 | 4.2±0.3           | 4.7±0.5                  |
|                         | AS-2   | 1.4±0.3                                 | 3.8±0.4           | 5.4±0.7                  |
| 1169-2 x Zarevo         | 1P-IS  | 1.2±0.1                                 | 4.6±0.2           | 4.1±0.4                  |
|                         | 2P-IS  | 1.5±0.1                                 | 3.9±0.1           | 7.6±0.2                  |
|                         | P-IAS  | 1.7±0.2                                 | 3.9±0.3           | 5.7±0.6                  |
| Nevsky x 733-65         | 2P-IS  | 1.2±0.1                                 | 3.7±0.2           | 7.3±0.6                  |
| 889-99 x Smena          | 2P-IS  | 1.4±0.2                                 | 3.9±0.3           | 6.7±0.5                  |
|                         | 3P-IS  | 1.3±0.1                                 | 4.0±0.1           | 8.1±0.3                  |
| HCPò8                   |        | 2.3                                     | 3.5               | 2.3                      |
The agar medium, both ordinary and based on IS-2V (variants AS-1, AS-2, and P-IAS), compared with the others, delays the rate of tuberization by an average of 3 weeks. Therefore, when growing seedlings in in tube culture in order to obtain microtubers, it is advisable to use non-agarized media, which make it possible to obtain microtubers in earlier periods.

In addition to the ability of the seedling population to form tubers in different variants of the test tube culture, the number, size, and shape of the tubers were analyzed (table 3).

By the number of tubers per plant between the variants of all populations, no significant differences were identified. In all variants, on average, 1 seedling accounts for 1-2,1 tuber. Also, the options do not differ in the form of tubers. Tubers of more rounded shape have seedlings of populations Aksenovsky x Sotka and 1169-2 x Zarevo.

Seedlings of variant 2P-IS of all three populations as a whole were characterized by larger tubers than seedlings of other variants (the differences are significant).

Analysis of the correlation between the size and shape of tubers shows that in the agar medium AS-2 in all populations this relationship is highly negative (the correlation coefficient ranged from -0.63 to -0.83). In seedlings on 2P-IS, there was a weakening of this connection. All this suggests that in tube culture when growing seedlings on the ion-exchange substrate IS-2V, it was possible to more accurately estimate populations by the shape of the tubers, since the shape of the tubers, in this case, was less dependent on their size.

When studying the degree of influence of the studied factors on the manifestation of signs, it was found that the variability of variations in the number of tubers by 41.2% due to the interaction of the cultivation substrate and the origin of seedlings. The size of tubers by 35.6% depended on the cultivation substrate and by 29.3% on the origin of the seedlings. The proportion of uncontrollable factors in the phenotypic manifestation of these characters was also large (32.6 - 40.6%).

In 2018, when growing seedlings of Nevsky x 733-65 and 889-99 x populations, tuber formation was induced after 50 days of seedling cultivation. The formation of tubers was observed already 70 days after sowing the seeds, and on the 90th day, all the seedlings of both populations had tubers, the average size of which was 6.7-7.3 mm. In this case, there was also a weak negative relationship between the size and shape of the tubers (from -0.24 to -0.31).

Analysis of the data obtained showed that for the growth and development of seedlings in a test-tube culture, the best medium among those studied in 3P-IS. This medium did not contain vitamins and growth regulators.

An insignificant dependence of tuber-forming ability on the group of maturity of parental forms was revealed. It's nature depends heavily on the variant of growing seedlings. So, on the usual agarized medium AS-2, the tuber-forming ability decreased in the following order: 374-55 x Aksenovsky (medium early x early) - 63.6%, Aksenovsky x Sotka (early x middle late) - 61.9%, 1169-2 x Zarevo (medium late x medium late) - 47.4%. In options 1P-IS and P-IAS the reverse picture was observed.

In variant 2P-IS, all seedlings of the Aksenovsky x Sotka populations, Nevsky x 733-65 and 889-99 x Aksenovsky tuberization percentage was 94.4%, and in 1169-2 x Zarevo - 90.0%. Consequently, the tuber-forming ability in variants of the ionite substrate largely depends not on the group of maturity of the parental forms, but on the composition of the nutrient medium and the conditions for growing seedlings in a test-tube culture.

Germination of microtubers obtained in test tube culture was of great importance for the preservation of all genotypes of seedling populations. As the data obtained show, the germination of tubers in 1 tuber reproduction, both when grown in closed and open ground, was quite high - from 73.7 to 100% (Table 4). 100% germination in closed ground was marked in option 2P-IS. Also, all the tubers rose in variants 1P-IS and P-IAS of the Aksenovsky x Sotka population and in the 1P-IS variant of the 374-55 x Aksenovsky population. The AS-1 and AS-2 variants in terms of germination of tubers were inferior not only to the 2P-IS, but also to the other variants of the ion-exchange substrate.

When growing 1 tuber reproduction in open ground, the germination of tubers was slightly lower compared to the closed ground. So, for the population of Nevsky x 733-65, it was 73.3%, for 889-99 x Smena - 88.1%. Apparently, this was due to the fact that under conditions of moisture deficiency, which
differed during the growing season during the years of research, a more reliable supply of water to plants was ensured in greenhouses.

**Table 4. Germination of tubers of test tube culture in 1 tuberous reproduction.**

| Option                  | Total planted, pcs. | Came up, pcs. | Germination of tubers, % |
|-------------------------|----------------------|---------------|--------------------------|
| **2017**                |                      |               |                          |
| 374-55 x Aksenovsky     |                      |               |                          |
| OP-IS                   | 12                   | 10            | 83.3                     |
| 1P-IS                   | 2                    | 2             | 100.0                    |
| 2P-IS                   | 17                   | 17            | 100.0                    |
| P-IAS                   | 8                    | 7             | 87.5                     |
| AS-2                    | 14                   | 12            | 85.7                     |
| Aksenovsky x Sotka      |                      |               |                          |
| OP-IS                   | 9                    | 7             | 77.8                     |
| 1P-IS                   | 6                    | 6             | 100.0                    |
| 2P-IS                   | 26                   | 26            | 100.0                    |
| P-IAS                   | 10                   | 10            | 100.0                    |
| AS-2                    | 13                   | 11            | 84.6                     |
| 1169-2 x Zarevo         |                      |               |                          |
| AS-1                    | 32                   | 28            | 87.5                     |
| OP-IS                   | 12                   | 11            | 91.7                     |
| 1P-IS                   | 10                   | 9             | 90.0                     |
| 2P-IS                   | 36                   | 36            | 100.0                    |
| P-IAS                   | 17                   | 16            | 94.1                     |
| AS-2                    | 9                    | 8             | 88.9                     |
| **2018**                |                      |               |                          |
| Nevsky x 733-65         |                      |               |                          |
| 2P-IS                   | 19                   | 14            | 73.7                     |
| 889-99 x Smena          |                      |               |                          |
| P-IS\(^a\)              | 143                  | 126           | 88.1                     |

\(^a\) – tubers of all variants are combined

Thus, the cultivation of seedlings on an ionite substrate in a test tube culture in comparison with the agar medium based on M-S allows increasing the germination of tubers in 1 tuber reproduction by 11.9 – 15.4%. In order to preserve all genotypes of the populations of test tube culture seedlings, 1 tuberous reproduction should be grown in closed soil conditions, or in the open ground, provided that the optimal soil moisture was reliably ensured.

5. **Conclusion**

The tube culture of seedlings ensures full survival of seedlings. For the growth and development of seedlings in a tube culture, the best of those studied was the nutrient medium based on the ion-exchange substrate IS-2V, containing 1% sucrose and 500 mg / l CCC. Two-stage cultivation of seedlings (the initial increase in the above-ground mass, then the induction of tuberization) has a positive effect on the pace and quality of tuberization. 30–40 days after induction, tuberization was observed in 90–100% of seedlings, regardless of their origin, and the size of microtubers was on average 6.7–8.7 mm.

Growing microtubers from a test-tube culture under greenhouse conditions allows preserving all genotypes in a population in 1 tuber reproduction.

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