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Accelerated Reproduction of Potatoes under Conditions of the RNO - Alania

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Abstract.

Healthy seed grains can be obtained in a variety of ways, but the most assured quality is provided by in vitro reproduction using the apical meristem technique. Thus, in order to improve the quality of the potato seed tubers from viral diseases and their accelerated reproduction, the plant survival *in vitro* and the reproduction factor of different varieties were studied. The research was carried out in the laboratory of the Department of Plant Growing of the Gorsky State Agrarian University (GSAU) of the Republic of North Ossetia-Alania (RNO-Alania), the Russian Federation (RF) on a nutrient medium proposed by scientists from the All-Russian Research Institute of Potato Farming (VNIIKh) based on Murasige-Skuga for the cultivation of potato plants. The Volzhanin, Zhukovsky rannii, Udacha and Kolobok potato varieties were studied on the plant height, the number of internodes, and the length of roots and internodes. The beginning of growth from the day of the grafting and the beginning of the root system formation was noted, and the reproduction factor and the yield of plants from one initial sample were determined. The maximum reproduction factor (5.6) was established for the Zhukovsky rannii variety.

Keywords: potatoes, virus-free seed production, test-tube plants, grafting, reproduction factor.

INTRODUCTION

Potato (Solanum tuberosum L.) is one of the most important food crops, on which valuable theoretical material has been accumulated for the real development of cellular technologies necessary for producing highly reproductive seed tubers for agricultural production [1; 2].

This is achieved through the seed production, the task of which is to multiply the high reproduction seeds of regionalized varieties and hybrids. The conditional seed tubers of higher reproductions improve their sowing and varietal qualities and also increase the yield. At the same time, various methods are used to improve the potato seed tubers. One of the main indicators reducing the seed features of tubers is the infection of tubers with viral diseases. The fight against them lies, first of all, in the correct organization of seed production. In doing so, it is necessary to apply a range of activities aimed at obtaining healthy planting material and its preventive inspection, taking into account the biology of viruses, their spread and harmfulness [1; 3; 4; 5; 6].

Techniques and methods of healing regionalized varieties from viral diseases should be mandatory in the seed production system. The improvement of seed potatoes may be achieved by early and thorough selection.

The improvement of virus-infected varieties must begin with the removal from the planting material of clearly sick tubers that are affected by severe viral diseases, small, non-typical in form for the variety. Selection of healthy tubers markedly reduces the percentage of sick plants in the field. It has been established by many researchers that tubers from sick plants have weakened germination energy, which is particularly evident at lower temperatures ($8\text{-}10^0$) in the first 15-20 days of germination [7; 8; 9].

The main techniques for the cultivation of healthy potatoes are the clonal selection, the timely and thorough phytocleaning of all seed production plots. However, selections and cleaning according to external signs do not exclude the emergence of plants infected with viruses in a latent (latent) form [10; 11].

Currently, in many countries (the Netherlands, Poland, Germany, the Czech Republic, Slovenia, etc.) seed production is based on the selection of a virus-free (free of XVK, SBC, MBK, YVK, ABK, etc.) material, using serological, immunoenzyme (EIA) and PCR method, which allows detecting latent forms of the disease. The PCR-diagnostics is also being introduced in our country into

the practice of institutions and farms growing the potato super-elite and elite [6; 8; 12].

Domestic and foreign experience has shown that by applying EIA and PCR diagnostic methods during selection, it is possible to improve the planting material of many regionalized varieties [1; 2; 3; 12].

Methods of the cell and tissue culture contribute to an accelerated solution of fundamental and applied problems of the plant experimental biology. Potatoes are a convenient object for biotechnological research and, in particular, for studying nontraditional techniques for obtaining the maximum amount of virus-free minitubers for further production of highly reproductive seed tubers [8; 13; 11; 14; 15].

The study of plant survival *in vitro* and the reproduction factor of various potato varieties were carried out with the goal of improving the seed tubers of potato from viral diseases and accelerating their reproduction [10; 16].

Healthy seed grains can be obtained in a variety of ways, but the most assured quality is provided by in vitro reproduction using the apical meristem technique. The method of growing shoots culture combined with the subsequent cloning of microplants and the cultivation of minitubers ensures high quality of the elite, but it is quite costly.

Therefore, the actual problem facing plant selection breeders is the following: reducing the costs of the planting material reproduction by optimizing the composition of the nutrient medium, using the completion of growing of potato seedlings in greenhouse conditions after the last grafting of microplants (for better adaptation in in vivo conditions in the production of minitubers). In addition, it was envisaged to ensure the maximum output of the first field generation tubers (by way of regulation of the nutrient medium), the selection of soil mix and the determination of the optimum level of mineral nutrition.

In view of the foregoing, we tested the survivability of newly isolated explants and observed their growth and development in test tubes and in greenhouse conditions in the future.

MATERIALS AND METHODS.

The study of the increase in the reproduction factor of different potato varieties in laboratory conditions on the basis of the apical meristem had been carried out for more than 7 years.

The experiment was carried out in the laboratory on a single nutrient medium proposed by scientists of the All-Russian Research Institute of Potato Framing (VNIIKhH) based on Murasige-Skuga for cultivating potato plants, with the following composition of macrosalts (mg/l): NH_4NO_3 – 1650; KNO_3 – 1,900; $CaCe_2x2H_2O$ – 440; $MgSO_4{\times}7H_2O$ – 370; KH_2PO_4 – 170; Na_2 EDTA– 37,3; $FeSO_4{\times}7H_2O$ – 27.8. The composition of microsalts was: H_3BO_3 – 6.2; $MnSO_4{\times}4H_2O$ – 22.3; $ZnSO_4{\times}4H_2O$ – 8.6; KJ – 0.83; $CuSO_4{\times}5H_2O$ – 0.025; $Na_2MoO_4{\times}2H_2O$ – 0.25; $CoCl_2{\times}6H_2O$ – 0.025. Vitamins were presented as follows: Meso-inositol - 100; Nicotinic acid - 0.5; Pyridoxine - 0.5; Thiamine - 1.0; Sucrose - 30,000; Casein hydrolyzate – 1,000. Growth regulators were: GK - 1.0; Kinetin - 0.01; IUC - 2.0; Agar – 10,000, developed by the VNIIKh method (2011).

Different varieties of potatoes were studied - Volzhanin, Zhukovsky rannii, Udacha, and Kolobok. 20 plants of each variety were taken in four replicates. The height of plants, the number of internodes, the length of the roots, the length of the internodes were measured, the beginning of growth from the day of the grafting and the beginning of the root system formation were noted. Then, the reproduction factor and the yield of plants from one initial sample on the original nutrient medium were determined.

RESULTS

The genetic bank of healthy test-tube plants contained 10,890 plants of the Volzhanin-St, Zhukovsky rannii, Udacha, Kolobok, Golubizna, and Bars varieties.

After checking for the content of viral diseases, the immunoenzymatic analysis of potato plants allowed detecting that all plants grown in vitro were free from five viruses (XBK, SBK, MBK, ZBK, YBK) and groups (XBK+SBK+MBK) during all years of the study.

When studying different potato varieties, we found that they were developed in different ways. For example, from planting to the beginning of the growth of plants in test tubes, 2.5 days passed for Zhukovsky rannii variety and 2.9 days for Udacha variety. We also found that the root formation according to the varieties corresponded to the maturing period. Early varieties provided earlier plant development in test tubes than medium or medium late ones (Table 1).

The total growth and development of test-tube plants occurred within 20-25 days, during this period it was formed from 5.8 pcs. of internodes on the control variant for the Volzhanin variety up to 7.1 pcs. for the Kolobok variety. The maximum length of the stem of 8.7 cm was observed in the Udacha variety.

Table 1. Growth and development of test-tube plants depending on the variety

Varieties	Days from the beginning (after grafting)		Plants before grafting			
	growth	root formation	number of internodes, pcs.	stem length, cm	root length, cm	length of internodes, cm
Volozhanin. St.	3.2	3.8	5.8	6.8	2.7	1.0
Zhukovskiy rannii	2.5	2.9	6.8	8.6	2.9	1.3
Udacha	2.9	3.5	6.7	8.7	2.6	1.2
Kolobok	3.0	3.6	7.1	8.3	2.5	1.1

Table 2. The reproduction factor and the yield of test-tube potato plants of different varieties on the original nutrient medium of VNIIKh

Varieties	Initial number of test-tube plants, pcs.	Reproduction factor	Number of plants after grafting, pcs.	Difference to control, %
Volozhanin. St.	25	3.9	98	
Zhukovskiy rannii	25	5.6	140	42
Udacha	25	4.7	118	20
Kolobok	25	4.2	105	7

The length of the internode is considered to be one of the most important indicators in the *in vitro* culture. For the grafting of test-tube plants, the average length of the internode is more important than the short or the long one. Short internode makes the grafting of plants very difficult, therefore many grafts become unsuitable for planting. The reverse trend is observed in case of the internodes outgrowing. In this case, too, there is a lot of waste, and it is necessary to cut them several times, which turns the planted graft into the conditioning form. But all this requires a lot of time.

The results of the studies showed that the Zhukovsky rannii (6.8 cm) and Undacha (6.7 cm) varieties had formed the longest internodes, which exceeded that of the Volzhanin control variety by 1.0 and 0.9 cm, respectively.

According to our research, it was found that not all varieties reacted equally to the same nutrient medium. This can be explained by the adaptability feature of each genotype to the source of nutrition.

During normal development, a large number of healthy internodes are formed and, as a rule, more grafts emerge for further reproduction. One test tube allows obtaining 625 plants after 4 graftings with a reproduction factor of 5 (Table 2).

As the data in Table 2 show, the maximum reproduction rate (5.6) was noted in the Zhukovsky variety, which exceeded the control by 1.7, and one test tube plant allowed obtaining 22% more healthy plants after 4 graftings. The remaining varieties were inferior to it, although the studied indicator was higher than the control one.

CONCLUSION

1. Variety differences in the length of the internodes were established in the growth of the test-tube plants in vitro in the Murasige-Skuga nutrient medium.
2. The early Zhukovsky variety showed the best indicators of the length of the internodes (6.8 cm), the stem (8.6), and the reproduction factor (5.6).
3. By controlling the amount and quality of the nutrient medium, the yield of microtubers can be regulated.

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