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Problems of Managing Potato Plant Growth Under Micropropagation for Primary Seeds

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Abstract

This study shows the possibility of controlling the growth of potato microplants when adding the Murashige-Skoog (MS) nutrient medium chlorocholine chloride (CCC) to the composition at a concentration of 0.05-0.25 mg · L^{-1}. Varietal specificity of the reaction of potato plants to the preparation was revealed. At optimal concentrations of CCC, plants with a stem length of 7-10 cm in 250 ml flasks were grown from single-node microcuttings for 1.5 months. Microplants were removed from the flasks using 20 cm scissors and unsterilized tweezers. The roots were shortened to 2-3 mm and the basal part of the stems was treated with a chalk-based paste containing 0.04% indolylbutyric acid (IBA). Plants were planted on 36 mm diameter Jiffy 7 peat pellets directly in the laboratory. The trays with the material were transferred to the greenhouse at the end of April at a temperature of 20-25 °C and placed in the conditions of the upper fine irrigation. After 3-4 days, mass root formation began in the plants, and another week later the seedlings were ready for use. By then the height of the plants was 10-15 cm, and the roots were clearly visible on the surface of the peat pellets. Planting these seedlings in a technological greenhouse to obtain minitubers was convenient and inexpensive.

Keywords: potato, microcuttings, microtubers, minitubers, chlorocholine chloride, peat pellets

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1. Introduction

In the practice of crop production, the fact that the defeat of crops, affected by phytopathogenic viruses, significantly decreases the yield and quality of products of many crops is well known. In this regard, countries engaged in the cultivation of large volumes of marketable potatoes are compelled to carry out systematic work on monitoring this class of pathogens. Their reliable identification, improvement of approaches to obtaining and efficient propagation of virus-free material using methods of helthing and meristem culture allows us to successfully develop primary potato seed production.

In this system, two main technological schemes of clonal micropropagation of potatoes are prevalent [1, 2]. The most commonly used technique has become a classic of biotechnology. This is in vitro cutting, which has been used almost unchanged for several decades. This method allows obtaining test-tube plants with 5-8 nodes from single-nodecuttings in 3-6 weeks, which provides an increase in the volume of propagated material of 5-8 times per one passage [3, 4, 5]. To withdraw the material from the propagation and to transfer it directly to the primary seed system, it must be transferred to non-sterile conditions. Therefore, special preventive measures are required to protect plants from all possible pathogens, because of which the death of plants is possible. However, the percentage of losses that is not always significant at the same time can be reduced for objective organizational and technological reasons. The planting of sterile non-adapted microplants is associated with the need to move large quantities of tubes to the site of planting. It is also associated with significant amounts of manual labor during planting and caring for them in extremely uncomfortable protected soil conditions.

The second scheme is associated with obtaining microtubers in vitro and also has a number of serious limitations. During cultivation, the parameters of nutrient media and levels of abiotic factors of cultivation are very often important. In this case, it is necessary to monitor the physiological status of the microtubers before planting in the greenhouse, and the correct selection of the ratio of the components of the substrate is necessary to optimize plant growth [6, 7]. Compliance with these requirements is necessary to ensure the effectiveness of the subsequent tuberization process. Thus, the technological solution to obtain microtubers uniform in size and in predictable quantities, in predicted time intervals, is very problematic.

The approaches we have developed to control the growth of potato microplants are focused on improving the technology of growing minitubers. Through the use of seedlings on peat pellets obtained in comfortable laboratory conditions, planting in a greenhouse is greatly simplified.

2. Methods and Equipment

The laboratory part of the research was carried out in the biotechnological laboratory of the Peoples' Friendship University of Russia (RUDN) in 2017-2019. The objects of research were potato varieties of the European selection Riviera, Arizona, Evolution, Twinner, Alouette, kindly provided for study by Agriko-Eurasia LLC (Moscow). To propagate plant material in the amount necessary for the experiment, the method of

clonal micropropagationwas used. The Murashige-Skoog prescription was used in the preparation of the basic nutrient medium at the propagation stage [8]. Against the background of kinetin at a concentration of 0.02 mg · L^{-1}, chlorocholine chloride (CCC) in the concentration range from 0.01 to 0.25 mg · L^{-1} was included as an additional growth-regulating additive. For propagation, 250 ml flasks were used, which were covered with sterile cotton-gauze disks at each transplant. After this, the containers were sealed with a thin plastic film leaving a ventilation hole. 12one-node cuttings were planted in each flask. The passage duration was 1.5 months. The temperature was kept within + 15 ± 0.1 ℃ by the climate chamber KBW 240 BINDER (Germany). In light room No. 1 within + 18 ± 2 ℃ and in light room No. 2 within + 21 ± 2 ℃ the temperature was maintained by household air conditioners.Light room No. 2 was used in an experiment with CCC.Illumination of 5000 lux and daylight hours of 16 hours were the same when using fluorescent lampsin all cases. To plant microplants for adaptation, they were removed from the flasks, the roots were shortened to 0.2-0.3 cm and washed from the remnants of the agar medium. After that, they were planted with tweezers in pre-moistened Jiffy 7 peat pellets with a diameter of 36 mm through the prepared planting holes. To stimulate rhizogenesis, the landing holes were pre-filled with chalk-based paste containing indolylbutyric acid (IBA) at a concentration of 0.04%. Peat pellets with microplants were placed in growth cassettes of 0.5 x 0.3 m in size, which were transferred to a greenhouse with a temperature of + 24 ± 4 °C for a period of 2 weeks. After the plants reached a height of 10-15 cm, they were transported and planted in a technological greenhouse for growing minitubers. In each variant, the repetition was 5-fold (1 flask as a repetition). Observations were made of the dynamics of microcuttings growth with an interval of 2 weeks.

3. Results

Potatoes are widely grown in the temperate zone as one-year food crop, for which a number of effective cultivation technologies are currently developed [9, 10]. The biological feature of the culture is the good dynamics of plant growth and development, determined by the prevailing temperature conditions during the growing season. It is well known that the optimum temperature for the growth of stems are temperatures from + 16 ° C to + 22 ° C [11]. Maintaining the same temperature range, as a rule, is provided during the cultivation of potatoes in vitro. In this temperature range, potato microshoots grow very actively, which is an important element of the technology of micropropagation of the material by periodically dividing it into single-node microcuttings. However, with

clonal micropropagation, phenotypic and morphological differences between varieties in shoot growth rate are very often manifested, which greatly complicates the prediction of the propagation results [12].That is, for plants to achieve equal sizes and a state of development, different passage durations, different temperature conditions, as well as the most acceptable hormonal background are demanded [13, 14]. This is very inconvenient technologically, since the material needs to be planted simultaneously and in a short time. In primary seed production, this is associated with the operation of seasonal greenhouses, where relatively standard technologies for caring for such plants are implemented. In order to withstand planting dates and comply with the requirements for the quality of microplants, it is very important to properly organize the preliminary work, which is carried out in a biotechnological laboratory. The initial experience regarding the growth rate of sterile cultures and the potential multiplication rate of varieties is extremely relevant. Several approaches can be used to solve this problem. The first of them, which we studied, is the regulation of the growth rate by temperature.

Initially, it was noted that at temperatures above + 21 ° C the buds on microcuttings of potatoes germinate already for 3-4 days and the shoots that appear grow very quickly, forming thin stems with long internodes and small leaves. Only in the upper part of the shoot the leaves acquire a normal size and the material could be used for further propagation. The use of large cultivation vessels for planting microcuttings made it possible to create more comfortable growth conditions for plants than they are in test-tubes. This provided a convenient access to the internal volume and, accordingly, to the plants, which made it possible to extract shoots with the help of long scissors without injuring them. However, the secondary material after cutting turned out to be very heterogeneous qualitatively, which affected the dynamics of reproduction. The results of studying the growth dynamics as a function of temperature are shown in Figure 1.

As can be seen from Figure 1, a decrease in the cultivation temperature from + 21 ° C to + 15 ° C almost 2 times reduced the final stem length for each cultivar. But at the same time, quite obvious differences were noted between the studied varieties at all temperature conditions.

In the next experiment, the effect of chlorocholin chloride, known as a substance with a growth inhibitory activity was studied as an alternative to regulating the growth rate of microshoots [15]. The study was conducted in the concentration range of 0.01-0.25 mg $\cdot$ L^{-1} with five varieties. An obvious varietal specificity of the reaction of microcuttings to the presence of the drug in the nutrient medium was established. The Riviera variety (Figure 2b) turned out to be the most sensitive to the preparation,

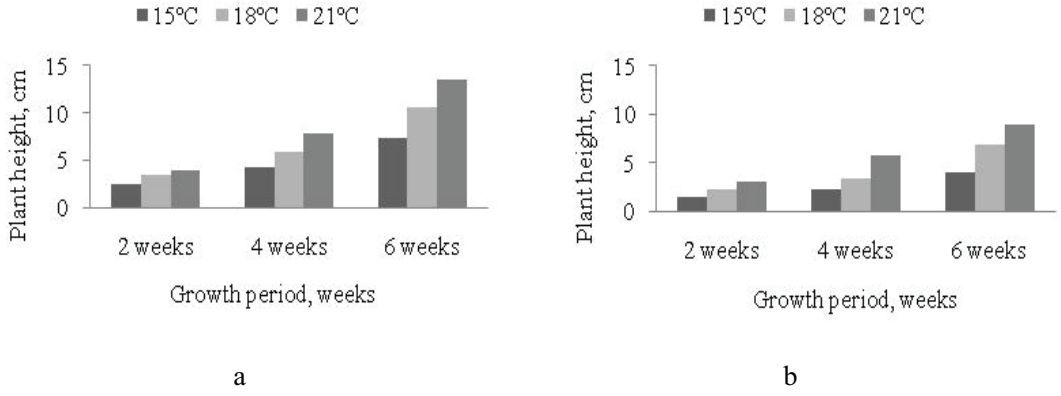

Figure 1: Growth dynamics of shoots of various potato varieties depending on temperature: cultivar Arizona (a), cultivar Riviera (b).

for which a concentration of chlorocholin chloride of 0.05 mg · L^{-1} already had an optimal growth-regulating effect. Over 6 weeks of cultivation, microplants reached a height of 7-10 cm, had a size of internodes and 6-8 well-developed leaves, convenient for cutting. We applied the technique that we used earlier, the microplants were cut of near base with long surgical scissors, were removing from the flasks, and then by scissors were dividing them into single-node microcuttings [16]. In contrast, the Arizona variety (Figure 2a) was the weakest of all other varieties to respond to the presence of the drug in the medium. The most effective concentration was 0.2 mg · L^{-1}, at which the microplants reached optimal sizes for subsequent work. Varieties Alouette and Evolution (Figure 2d) were similar in sensitivity to chlorocholin chloride and approached the Riviera variety. The concentration of 0.1 mg · L^{-1} was found to be optimal for them. In the Twinner variety (Figure 2c), good microplant conditions were achieved at a concentration of 0.15 mg · L^{-1}. A higher concentration already strongly inhibited growth, and a decrease in values led to the overgrowth of plants.

4. Discussion

The ultimate goal of the study was to clarify the possibility of preparing potato seedlings from micro plants grown using the described procedures. The plants extracted from the flasks had a rigid stem, a well-developed leaf apparatus and a powerful root system. Seedlings were prepared using Jiffy 7peat pellets. The root system was shortened to a length of 0.2-0.3 cm for planting convenience. It is known that potato culture is prone to rapid additional root formation and therefore additional stimulation with auxin-containing paste, which was used during planting, further accelerated the process of rhizogenesis.

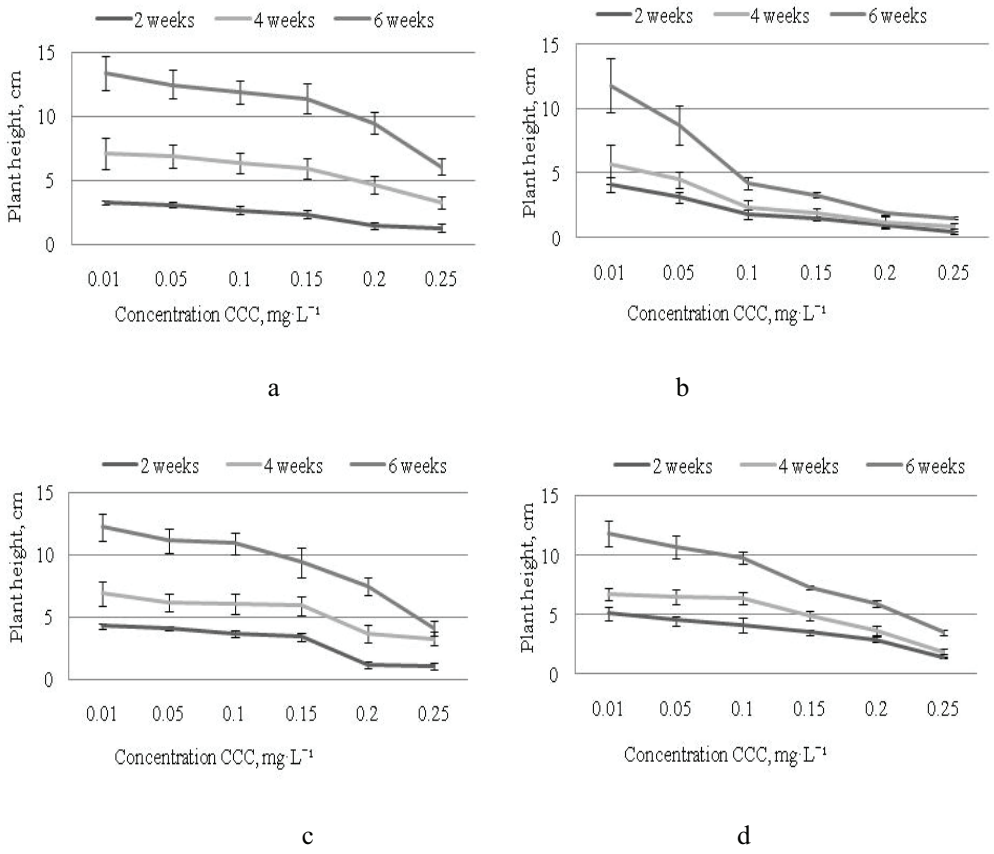

Figure 2: The effect of the concentration of chlorocholin chloride(CCC) in the composition of the nutrient medium on the potato shoot length depending on the cultivation duration: cultivar Arizona (a), cultivar Riviera (b), cultivar Twinner (c), cultivar Evolution (d)

Applying it to the basal part of microplants, the period of adaptation to non-sterile conditions ensured the activation of root growth after 3-4 days. Within 2 weeks, the plants grew actively and reached a height of 10-15 cm. In this state, they were successfully planted in a technological greenhouse for growing minitubers.

Clarification of the possibility of controlling the growth rate of microplants by adding chlorocholine chloride to the composition of the nutrient medium and selecting its optimal concentrations against the background of kinetin allows us to predict the final result of reproduction with a high degree of accuracy.

5. Conclusion

Laboratory preparation of potato seedlings on peat pellets allows you to significantly changethe technology of growing minitubers in primary seed production. The changes relate, first of all, to the most labor-consuming and high-cost stage associated with the planting of microplants in protected ground. The previously implemented scheme very

significantly limited the productivity of such workand their effectiveness consequently. When providing top fine automated watering, no additional planting operations are required. Plants placed in pots initially exist autonomously on a peat pellet, but within 3-4 days the root system begins to develop a new volume of substrate, the planting hole is gradually silted, which further enhances the root formation. Thesurvivalrateof-suchseedlingsis 100%.

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Conflict of Interest

The authors have no conflict of interest to declare.

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