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Rooting and Adaptation of *Solanum tuberosum* L. under Ex Vitro Conditions after Exposure to Different Sucrose Concentrations in the In Vitro Nutrient Medium †

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Abstract: The composition of nutrient media intended for the formation of morphogenic callus in tissue culture is a key element of the intermediate link in the production of plants in vitro. The six potato varieties of the Ukrainian breeding of different ripeness groups: early-ripening, medium-early, medium-ripening were used as the research object. Fragments of the apical meristem of plant organs were selected for the introduction of in vitro in the culture.Parts of stems with axillary buds were used to produce callus tissue. The Murashige and Skoog media (Murashige and Skoog, 1962) with sucrose content modifications of 30 and 60% were used to induce callus genesis and growth of the resulting callus. The control version of the medium contained 10% sucrose. The resulting material was planted in the open ground on the experimental plots of Sumy National Agrarian University. The results of the studies indicate a generally positive, but uneven effect on the rooting nature of test tube plants of the varieties used. Individual variants of potato cultivars of different ripeness groups grown on a medium of both 10 and 30% and 60% sucrose content are considered to be promising for further reproduction and use in the breeding process.

Keywords: nutrient medium; in vitro; potato cultivars (varieties), sucrose content; meristem; plant engraftment

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1. Introduction

Recently, the main condition for effective potato growing is the creation of a scientifically based seed production system, since without high-quality planting material, it is practically impossible to obtain stable yields of commercial products.

Significant difficulties arise out of the implementation of breeding programs for the creation of varieties. Firstly, there is a need to use improved source material (without pathogenic infections). Secondly, it is expedient to preserve a healthy state of plants in the process of subsequent reproduction, and thirdly, to ensure a maximum reduction in the reproduction period of the source material.

Despite a significant amount of research in this area, the results are scattered and often of a contradictory nature. A seed production system, which is based on the use of

active methods of recovery, namely the apical meristem method (tissue culture method), has been developed, and is applied nowadays [1–4].

It is the potato plants obtained from fragments of the apical meristem that not only increase productivity and quality indicators [5], but also underlie the fight against viral diseases [6,7], since viruses do not have the ability to penetrate the apical meristem. In the mid-50 s of the last century, the French G. M. Morel and C. Martin [8] obtained potato plants from the apical meristem with a size of 100–200 MK, free from viruses X and A. In the 70 s, dozens of varieties were improved and introduced into the seed production process in different countries [9,10], including in Ukraine [11] using this method.

Currently, the issue on finding optimal ways to propagate improved plants and obtain a sufficient amount of high-quality seed material remains in abeyance. This task is most efficiently solved by methods of accelerated plant reproduction in vitro, thanks to which several thousand can be obtained from one plant in a short period of time. A great advantage of this method is the ability to quickly develop the required amount of seed material of newly created, adapted or improved varieties and thereby ensure their timely introduction into the seed production system. In the practical work microclonal reproduction is carried out through propagation by cutting of plants in artificial conditions in a nutrient medium [8–10,12].

The influence of various factors on in vitro morphogenesis in many potato cultivars has been studied by a number of authors [13–17]. Scientific works are mainly devoted to solving and studying certain methodological issues. However, for almost each cultivar, it is necessary to select individual conditions for in vitro morphogenesis.

The composition of nutrient media used for the formation of morphogenic callus in tissue culture, as an intermediate link in the production of plants in vitro, does not lose its relevance. Therefore, the problem of the influence of the composition of nutrient media, in particular sugar, on the rooting and engraftment of planting material of test-tube potato plants ex vitro is extremely important. The solution of this problem has been the basis for conducting our research.

2. Materials and Methods

The research was carried out in 2020-2021 in the educational and scientific laboratory of in vitro biotechnological research of Sumy National Agrarian University, taking into account all the requirements of the research methodology (Dospekhov; "Methodological Recommendations for Conducting Research with Potatoes" [18,19]. The experiment was performed with the use of the six potato cultivars of different ripeness: early-ripening—Riviera (Netherlands), Kiranda (China), medium-early—Pliushka (Ukraine), Studentska (Ukraine), medium-ripening—Hrenada (Ukraine), Kniahunia (Ukraine), and fragments of the apical meristem of plant organs were selected for the introduction of in vitro in the culture.

To obtain the initial seed material improved by the biotechnological method, the method of thermo-chemotherapy was used in combination with the culture of apical meristems according to the "Methodological Recommendations for Conducting Research with Potatoes" [19], the methodological recommendations "Optimization of the Processes of Improvement, Reproduction and Protection of Seed Potatoes from Viral Infection" [20] and "Use of Biotechnological Methods and Techniques in Modern Potato Seed Production" [21]; the propagation of the initial seed material obtained using the biotechnological method was carried out in the Murashige and Skoog (MS) nutrient medium (Murashige and Skoog, 1962) with a sugar content of 10%. Cuttings were transplanted to a nutrient medium with a sugar content of 10%, 30% and 60%. The grown test-tube plants were planted out in a soil mixture in a greenhouse. The first accounting was carried out on the 10th day to determine the percentage of rooted plants of the crop. The second accounting was carried out to determine the adaptation of *Solanum tuberosum* L. plants on the 40th day (or on the 10th after planting out in the field).

3. Results

The results of the research indicate a different nature of callus genesis and soil adaptation of test-tube plants of potato cultivars of different ripeness groups, depending on the concentration of sucrose in the nutrient medium (Figure 1).

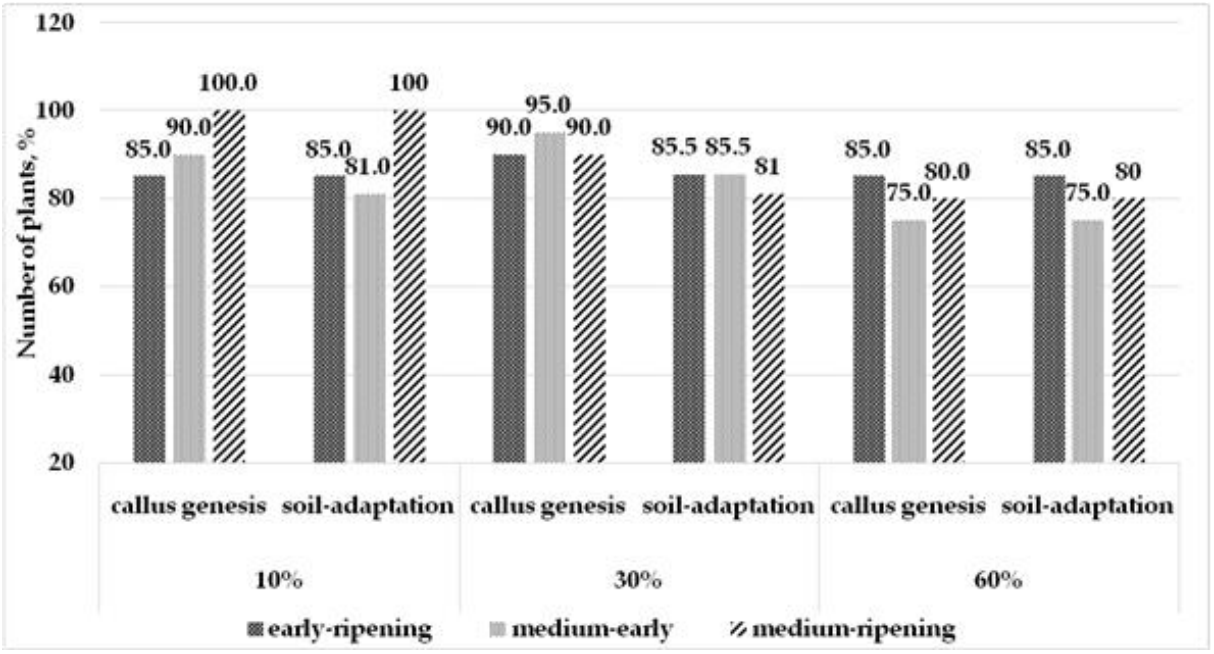

Figure 1. Callus genesis and soil-adaptation of test-tube plants of potato cultivars of different ripeness groups.

4. Discussion

The highest percentage of rooted plants is observed in a medium with 10% and 30% sucrose content; higher sugar concentrations have negatively affected the rooting of test-tube plants. But the reaction of the varieties themselves (depending on the ripeness group) to the increase in sucrose content is not the same. Thus, early-ripening varieties (Riviera and Kiranda) take root best at a sugar concentration of 30%, and the nature of rooting at high and low concentrations is slightly lower, but almost the same. In the group of medium-early varieties, which includes Pliushka and Studentska, the largest number of plants also takes root at a sucrose concentration in the nutrient medium of 30%, and an increase in the concentration has negatively affected rooting. The rooting of plants of the middle ripeness group (Hrenada and Kniahunia) occurs best at a sucrose concentration of 10%, and an inversely proportional relationship is found (Figure 1).

The adaptation of test-tube plants after being replanted in the ground is also quite peculiar. For example, in varieties of the early ripeness group, it is the same, and the effect of sucrose concentration is not noticed. In varieties of the medium-early ripeness group, adaptation occurs best after growing them in the medium with the 30% sucrose content, and the worst—in the medium with high sugar content. The adaptation of potato varieties of the medium-ripening group is best after growing them in the medium with minimum sucrose content, while increasing its concentration proportionally reduces the number of adapted plants

5. Conclusions

We have found that the effect of sucrose concentration in the nutrient medium on the rooting and adaptation of potato varieties is quite uneven, while there are differences in ripeness groups. Early and medium-early varieties take root better in the medium with the sugar content of 30%, and medium-ripening varieties react negatively to an increase

in sucrose content. Thus, in the breeding work, during microclonal reproduction of potatoes, it is worth paying attention not only to the composition of the nutrient medium, but also to the ripeness group of the variety. Thus, it is most advisable to use nutrient media with the sucrose content of 10 and 30 percent in microclonal potato propagation, which is also confirmed by the nature of plant adaptation in the open ground.

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References

1. Konovalova, H.I.; Bobryk, A.A.; Semenova, Z.A. Production of Source Seed Material. Potato Growing: Science. Scientific Papers of the All-Russian Research Institute of Potato Farming. **2000**, *10*, 215–221.
2. Oliinyk, V.P. Productivity of potato seed material reproduced from healthy mini–tubers of various origins. *Potato Growing* **1999**, *29*, 174–177.
3. Cassells, A.C. In Vitro Induction of Virus-Free Potatoes by Chemotherapy. *Biotechnol. Agric. For.* **1987**, *3*, 40–50.
4. Mellor, F.C.; Stace–Smith, R. Virus free potatoes through meristem culture Biotechnology in Agriculture and Forestry. *Potato* **1987**, *3*, 30–39.
5. Truskinov, Y.V.; Frolova, D.V. Meristemic potatoes: features and problems of production and use. In *Papers of the International Scientific and Practical Conference, Part I*; Potato Institute Republican Unitary Enterprise of the National Academy of Sciences of Belarus: Minsk, Belarus, 2003; pp. 322–329.
6. White, P.R. *Handbook of Plant Tissue Culture*; Jajues Cattell Press: 1943; Volume 4, pp. 791–794.
7. Gautheret, R.J. *Manuel Technigue de Culture des Tissues Vegetaux Paris, Masson et Cie*; 1942; pp. 231–243.
8. Morel, G.M.; Martin, C. Guerison des plantesatteintes de maladies a virus; par culture de meristems apicaus. *Rep. Int. Hort. Congr. Nertherland*s **1955**, 303–310.
9. Kawakami, K.; Tomida, G.; Tamaka, S. Ageing rate in term of relative produktivity of potato seed tuber. In Proceedings of the 7th Trienniol Conference of the European Assotiation for Potato Research, 1978; pp. 52–54.
10. Zaman, M.S.; Quraishi, A.; Ghulam, H. Culture of potato (*Solanum tuberosum* L.) for production of virus-free plantlets. *J. Biol. Sci.* **2001**, *1*, 898–899.
11. Ostapenko, D.P.; Kuchko, A.A. Meristem culture and increasing its effectiveness in improving the health of potatoes from viral infection. Protection of potatoes from viral diseases in seed production: Scientific papers of the All–Russian Research Institute of Potato Farming. **1977**, *30*, 58–63.
12. Trofimets, L.N. *Biotechnology in Potato Growing*; Moscow, 1989; 45p.
13. Ostapenko, D.P.; Moroz, I.Kh.; Kononuchenko, V.V.; Reznik, V.S. *Obtaining Potato Microtubules in vitro and Forming the Elite on Their Basis: Methodological Recommendations*; Kiev, 1990; 27p.
14. Khromova, L.M. Callus and morphogenesis in potato tissue culture. In *Research on the Cellular Selection of Potatoes*; Moscow, 1984**;** pp. 81–88.
15. Dobránszki, J.; Tábori, K.; Hudák, I. In Vitro Tuberization in Hormone—Free Systems on Solidified Medium and Dormancy of Potato Microtubers Magyarné. In *Potato I. Fruit, Vegetable and Cereal Science and Biotechnology 2 (Special Issue 1)*; Benkeblia, N., Tennant, P., Eds.; 2008**;** pp. 82–94.
16. Ewing, E.E.; Struik, P.C. Tuber formation in potato: induction, initiation, and growth. *Hortic. Rev.* **1992**, *14*, 89–198.
17. Sarkar, D. The signal transduction pathways controlling in plantatuberization in potato: an emerging synthesis. *Plant Cell Rep.* **2008**, *27*, 1–8.
18. Dospekhov, B.A. Methodology of field experience. Moscow. *Agropromizdat* **1985**, 351p.
19. Kutsenko, V.S.; Osipchuk, A.A.; Podhaetskii, A.A. *Methodological Recommendations for Conducting Research with Potatoes*; Nemeshaievo, Ukraine, 2002; 183p.

20. *Optimization of Methods of Improvement, Reproduction and Protection of Seed Potatoes from Viral Infection: Methodological Instructions*; Belorus Research Institute of Plant Protection: Minsk, Belarus, 1996; 16p.
21. Konovalova, G.I. Use of biotechnological methods and techniques in modern potato seed production. In *Actual Problems of Science and Technology: Issues of Potato Production: Scientific Papers*; Moscow, 2006; pp. 332–336.