Evaluation parameters of in vitro morphogenesis in the process of replication of potato starting material

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Abstract. In the process of replicating and growing the necessary volumes of in vitro material for original seed production, the period of formation of morphological structures and the achievement of microplants of standard characteristics is important. The existing method of regeneration assessment by the quantity of days from the moment of placement of micro stalks into a new nutrient medium does not allow a planned approach to the compilation and implementation of the clonal micropropagation program. The article proposes new methodological approaches to assessing in vitro potato material by the main phases of plant development in tissue culture: intensive growth (formation of 2-3 internodes), slow growth (4-6 internodes), and natural dying-off. The analysis of regenerative ability allows us to note that the effectiveness of the formation of morphological structures did not depend on the ripeness group of the studied variety samples. Presented results indicate the differentiated approach of varieties to the formation of morphological structures in the process of replication.

1. Introduction

In the biotechnological practice of potato culture could find a wide range of ways of assessing the formation of morphological structures in an in vitro culture. In studies of I.F. Golovatskaya et al. there are represented the results of the regeneration of potato mericlones starting from the second to the 27 day [1]. V.G. Darkhanova et al. and N.N. Chernysheva assessed in vitro morpho- and rhizogenesis on 15 and 30 days [2, 3]. While studying the process of accelerating the growth and development of potato regenerants in vitro, L.Yu. Guseva et al. evaluated micro plants on 25 and 30 days [4]. While studying the influence of growth regulator and temperature regime on the induction of the microtubules formation, G.S. Balashova conducted an assessment of the material in vitro on the 20 and 40 days [5]. In the practice of the Velikoluksky Agricultural Academy for many years has been applied a three-level assessment method on the 7, 14 and 21 [6]. A number of authors in their studies represent the effectiveness of the explants behavior in tissue culture only on the 21 day [7, 8]. In vitro morphogenesis depends on the varietal characteristics and according to G.A. Khadiga et al., 2015, not all varieties are able to form adult regenerants within 21 days [9]. In the process of studying the growth and development of micro stalks into plants, Anoop B. and Chauhan J.S. applied evaluation parameters of regenerants as and when internodes get form [10]. The project indicated that the maximum number of internodes was formed on the 35-40 day. By the same principle, L. Koleva et al. conducted observations in tissue culture [11]. The authors note that in order to form 4-5 internodes, it is necessary to keep microplants in
phytotron conditions for at least one calendar month. Similar results are presented in the projects of E.P. Venkatasalam, S. Mansoor, P. Rocha, I. Megrelishvili et al., J. Dobranszki, S. Ashrafzadeh and A. Shahid et al. [7, 8, 12, 13, 14, 15, 16]. The authors evaluated the growth and development of the biomaterial in tissue culture after 4 weeks from the moment of cutting grafting.

The tendency to use the temporal and morphometric factors to assess the microplants regeneration can be also found in projects of other authors. In process of determining the impact effectiveness of artificial light source on the process of plant regeneration, which was held in Belarusian State Academy, T.V. Nikonovich et al. evaluated the formation of morphological structures solely by biometric indicators [17]. In order to evaluate the effectiveness of growth regulators on plant morphogenesis in vitro, I.N. Anikina et al. in Toraigyrov University of Kazakhstan, relied on the height of microplants and the number of internodes formed [18]. R.A. Karnachuk et al. and A.V. Milekhina et al. [19, 20], in order to study the effect of different light spectra on in vitro regeneration, assessed the material by the number of formed internodes. In studies of S.V. Kushnarenko et al. in the process of optimizing the regimes of sterilization of plant material and nutrient media composition of 28 potato samples, the authors used only regenerants with 6-8 internodes formed [21]. The analysis of the literature source shows that the methodical plan uses a diverse approach to assessing the growth and development of microplants.

Research objective – to determine the morphogenesis characteristics of in vitro potato varieties of different maturity groups on the main phases of development of plants in tissue culture: rapid growth, slow growth, and the natural withering away.

2. Materials and methods

The object of research was 15 varieties of potato of different groups of ripeness. Early varieties- Eearly Zhukovsky, Red Scarlett, Udacha, Impala. Early to medium early varieties – Nevsky, Gala, Romano. Medium early varieties – Nakra, Golubizna, Skarb. Medium late to late varieties – Nikulinsky, Fioletovy, Golubizna, Asterix.

The experiment was carried out in 4 replications of 20 microplants. Micro stalks were placed into aseptic conditions on an agarized Murashige and Skoog medium with a sucrose content of 2%, after which the biomaterial was transferred to a phytotron with a photoperiod of 16 h and illumination of 4-5 thousand lux. In our studies, the growth and development of the biomaterial was evaluated by three main phases: intensive growth, slow growth, and natural dying-off. Development phases were determined visually, by the intensity of the color of leaf blades and roots, by the number of internodes, and by the increase in plant height. The most important part of the formative process is the phase of intensive growth. It includes two main stages: germination and formation of 2-3 internodes.

The phase of stunted growth occurs when the regenerated plants reach standard parameters. By this moment they form 4-6 internodes. Biomaterial with curved stems (overgrown microplants) is not recommended for planting on the substrate, but it is quite suitable for subsequent grafting. The duration of stunted growth phase mainly depends on the varietal characteristics and growth environment in a phytotron.

Physiological aging of in vitro material begins with the phase of the natural dying-off of microplants. Its occurrence is usually observed from the moment of total flow of the nutrient medium.

3. Results

The growth and development of an explant in tissue culture is determined by its regenerative ability. The study of this indicator in 15 potato varieties of different groups of ripeness showed that the formation of morphological structures was directly dependent on the varietal characteristics. The most morphogenic are the varieties that formed the standard regenerants within 20–25 days from the moment of planting micro stalks on a new nutrient medium. According to the observations results, varieties of different ripeness degree fall into this group. Thus, the varieties Zhukovsky Ranniy, Impala, Skarb, Nikulinsky, Asterix and Lorch were characterized as the ones with accelerated morphogenesis. The average period of morphological structures formation was noted in the Red Scarlett, Gala, Nakra
varieties, whose organogenesis was completed on the 30-35 day of passage. Late morphogenesis was characterized at samples in which the process of forming took 40 days, this group included the varieties Udacha, Golubizna, Fioletovy and Gigant (Table 1). The analysis of the regenerative capacity allows us to note that the effectiveness of morphological structures formation did not depend on the ripeness group of the studied variety samples.

### Table 1. Ontogenesis of biomaterial in in vitro culture, days.

| Variety      | Ripeness group | Intensive growth germination | 2-3 internodes | Stunted growth | Physiological aging |
|--------------|----------------|------------------------------|----------------|----------------|---------------------|
| Zhukovsky    | 3\(^a\)        | 3-4                          | 12-14          | 20-25          | 40-60               |
| Ranniy       |                |                              |                |                |                     |
| Udacha       | 3              | 5-6                          | 20-21          | 40-50          | 60-90               |
| Red Scarlett | 3              | 4-5                          | 15-20          | 30-50          | 70-100              |
| Impala       | 3              | 3-4                          | 12-14          | 20-25          | 40-60               |
| Nevskiy      | 4\(^b\)        | 3-4                          | 14-15          | 30-40          | 50-80               |
| Gala         | 4              | 3-4                          | 14-15          | 30-40          | 50-90               |
| Romano       | 4              | 4-5                          | 20-21          | 35-45          | 60-80               |
| Nakra        | 5\(^c\)        | 4-5                          | 20-21          | 35-45          | 60-90               |
| Golubizna    | 5              | 4-5                          | 20-21          | 40-50          | 70-100              |
| Skarb        | 5              | 3-4                          | 14-15          | 25-35          | 60-90               |
| Nikulinsky   | 6\(^d\)        | 3-4                          | 14-15          | 25-35          | 45-80               |
| Fioletovy    | 6              | 4-5                          | 20-21          | 40-50          | 70-100              |
| Gigant       | 6              | 4-5                          | 20-21          | 40-50          | 70-100              |
| Asterix      | 6              | 3-4                          | 14-15          | 25-35          | 50-80               |
| Lorch        | 7\(^e\)        | 4-5                          | 14-15          | 25-30          | 40-60               |

\(^a\) early.  
\(^b\) middle-early.  
\(^c\) mid-season.  
\(^d\) middle-late.  
\(^e\) late.

One of the main criteria for evaluating microplants when using them as a starting material and obtaining mini tubers is the ability to preserve standard characteristics. The longer time plants are able to maintain their standard characteristics, the more practical and effective work being done on replicating the biomaterial in an in vitro culture. The stunted growth phase of potato depended on the varietal characteristics of studied samples. According to the research results, the amplitude of its variation for the studied varieties ranged from 5 to 20 days. A short period of compliance with regulatory requirements is noted for the Zhukovsky Ranniy, Impala and Lorch varietes, the best indicators are noted for the Fioletovy and Red Scarlett varieties. These studies confirm the findings of L. Koleva (2012), S. Mansoor, (2017), Rocha P. (2015).

In the methodical plan uses a diverse approach to assessing the growth and development of the starting material in the form of microplants. The use of a time factor, which indicate the number of days from the moment of planting micro stalks into a new nutrient medium does not allow an objective assessment and planning of the periods of growth and development of various potato varieties in the laboratory. The development of explants depends on the varietal characteristics, and complete regeneration can vary from 20 to 45 days. Accordingly, the approach to the compilation of the clonal micropropagation program should also be differentiated.

In the process of replicating and growing original seed material, the period of compliance of microplants with the requirements of the standard is of great importance. Compliance of microplants with regulatory requirements is determined by the number of formed internodes, regardless of the time of
placement of the biomaterial on a new nutrient medium. Thus, for practical use in the process of clonal micropropagation, it is recommended to assess the in vitro morphogenesis by growth phases.

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