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BORODAY V., Candidate of Agricultural Sciences, assistant professor,
KLYACHENKO O., Candidate of Agricultural Sciences, assistant professor
e-mail: veraboro@gmail.com

THE FEATURES OF INDUCED MORPHOGENESIS AND REGENERATION OF GENOTYPES *SOLANUM TUBEROSUM L.* OF UKRAINIAN SELECTION

The features of morphogenetic reactions of Solanum tuberosum L. in vitro for a number of modern potato cultivars of Ukrainian breeding, the effectiveness of which depends on the seasonal selection, type of explant, nutrient medium composition and genotype were studied. The composition of culture media and conditions for obtaining callus cultures, plants - regenerates, microtubers and induction processes of organogenesis in vitro were optimized. Study and optimization of conditions for inducing morphogenesis of cultured cells is an important part of work on the study of new forms of Solanum tuberosum L. in vitro.
Keywords: *morphogenesis, callus tissue, microtubers, regeneration, Solanum tuberosum L.*

Introduction. Potato (*Solanum tuberosum* L.) is one of the most important crops, which has considerable global significance. Genetic potential productivity of potatoes is far from exhausted, its increase can be achieved by studying of the morphological and physiological traits that contribute to increase the yield [2]. Among other crops, potatoes distinguished by the presence of rich genetic resources and the ease of transmission of inherited traits class. Almost all of the related wild species of *Solanaceae* can hybridize with *S. tuberosum* L. However, the spread of diseases caused by peculiarities of culture change and biological properties of pathogens, prevents obtaining high and stable yields of quality tubers. The rational combination of classical breeding and biotechnology techniques helps to speed up and improve the selection process by expanding the genetic spectrum obtained source material evaluation and selection of useful combinations that will result in new varieties with high biological and agronomic performance.

Influence of different factors on in vitro morphogenesis in many potato varieties studied by a number of authors [1, 2, 4-8, 10]. Scientific papers mainly devoted to the study and solution of some methodological issues. However, virtually every class should be selected for individual conditions morphogenesis *in vitro*. Therefore, the study and optimization of conditions for induction of morphogenesis potato cultured cells is relevant and important part of the work on the culture *in vitro* of new forms of plants of this crop.

The study of morphogenetic processes of *Solanum tuberosum* L. genotypes of Ukrainian selection *in vitro* was the aim of this work.

Materials and methods. The study was conducted in the laboratory of Plant Biotechnology, National University of Life and Environmental Sciences of Ukraine. The leaf and stem explants, microtubers and tubers of such cultivars: early grades Serpanok and Povin, middle early – Oberig and Zelenuy Gay, middle late - Kalynivka and Buluna, late-season – Chervona Ruta and Dgerelo Poliske were the objects of investigation. The intermediate microshoots of germinated tubers 1-2 cm long with one pair of leaves containing axillary meristematic tissue were used from mother plants. The traditional sterilizing agents in different concentrations and duration of exposure studied to optimize the conditions for obtaining aseptic cultures of *Solanum tuberosum.*

These aseptic shoots were separated from the primary explants and cultivated in the culture medium of Murashige - Skoog (MS) supplemented of kinetin at a concentration of 0.5mg/l [9].

The blade of the sterile leaf (0.5-0.8 cm^2), parts of stems and disks of microtubers (0.7-1.0 cm^2) were used for studying of callus formation. The scalpel incisions made in explants to increase surface proliferation. Callus tissue obtained by cultivating primary explants on modified medium MS supplemented by meso-inisit at a concentration of 180-200 mg/l; folic acid - 0.4-0.5 ml/l kinetin - 0.2-0.3 mg /l, 2.4l - 3.4 mg/l, casein hydrolyzate - 1g/l, adenine - 1 mg/l, glycine - 1 mg/l. Callus tissue cultured in absolute darkness with adjustable temperature +25 ºC, relative

humidity of 70-80% within 3-4 weeks. Increase in raw biomass (%) callus tissue was determined by L.A. Kucherenko [3]. Defining features of microtubers formation was carried out by a modified method D.P. Ostapenko (1990) [5].

Plants regenerates, which had well-formed shoots and the roots planted in pre- cooked in the oven substrate: black soil: peat: sand (2:1:1) and covered their glass case for increasing humidity and a better survival rate.

The table shows the arithmetic mean of the obtained values and their standard deviations (SD). Statistical analysis of the results was performed on a PC using software application Statistika 5.1 and Microsoft Office XP ® for Microsoft Windows ®.

Results and discussion. Manage selection process potatoes using tissue culture techniques involves the study of clonal micropropagation features, terms induction and maintenance processes of differentiation and dedifferentiation of plant tissues.

The sterile explants are the main condition for the successful cultivation of potatoes isolated tissues. We used different concentrations and duration of exposure traditional sterilizing agents - 70 % C_2H_5OH, 17% H_2O_2 and $Ca(ClO)_2$. The best option holding of primary explants according to our scheme was to turn out in 70 % C_2H_5OH for 1 min., 8 min. - 17% H_2O_2 and subsequent 3-fold washing in sterile H_2O for 10 minutes. As a result of optimizing the sterilization process was obtained to 92.4 % of viable cultures. Explants had minimal level of contamination by microorganisms (5.6%), kept green and accelerated ability to morphogenesis (1a, 1b). A more prolonged exposure in H_2O_2 solution destroyed plant tissue (1v). Cuttings of plants in vitro culture is one of the best methods for the rapid propagation of potatoes.

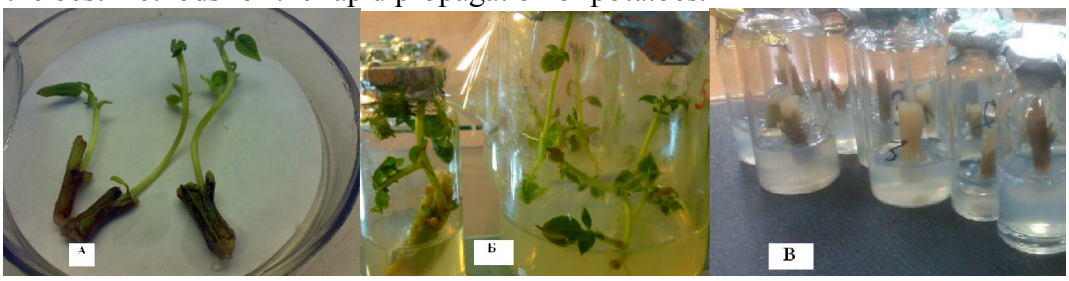

Fig. 1. Features introduction to the culture of *Solanum tuberosum* in vitro

Study morphogenic potential of different genotypes of potato showed that middle- early Green Guy characterized high multiplication factor - 144 intensive shoot growth (up to 15 cm in height) with evenly placed leaves, lots of internodes (6-11) to 1.5 cm long and well-developed root system. According to it, these characteristics were approaching middle- early and middle-late Kalynivka, Chervona Ruta and Dgerelo Poliske (2b). Povin had rapidly formed lateral shoots, plants were similar to bunch, had short internodes, shorter immature root (Fig. 2). In the middle early Serpanok and Povin observed weak shoot formation, very long and slow plant root growth.

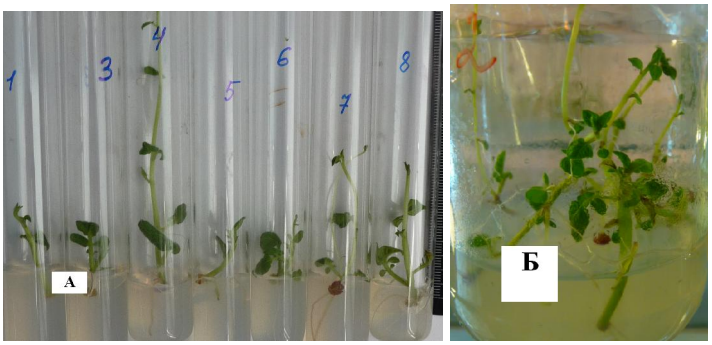

Fig. 2. Study of morphogenic potential of different genotypes *Solanum tuberosum*

Study of callus formation and morphogenesis of cultured tissues reveal the relationship with the biological characteristics of the initial variety and the rhythm of their growth and development.

Seasonal nature of callus formation and morphogenesis of plants was closely related to the features of ontogeny: morphogenetic activity marked by callus tissue at the beginning of February. As a result of the experiments revealed varietal of callus formation withdrawal in the process , which significantly affect the origin of explants (maximum effect obtained using leaf segments) and the composition of the culture medium. Thus the appearance of the callus is more dependent on their tissue of origin. The first signs of callus formation observed on 12-19-th day of cultivation, depending on the variety. Callus formation was observed in young leaves explants in the basal part of the field of tissue damage (Fig. 3), and stem explants in culture - the cut surface.

In studying the impact of such primary explants on callus formation it was found that the most intensive process that occurs when using leaf segments, and the least intense - microtubers drive. Callus formed on the leaf segments had a loose texture, light brown color unlike obtained in the interstices and observed negligible difference in intensity of callus formation (according 32,7-76,9% and 25,8-70,3%).

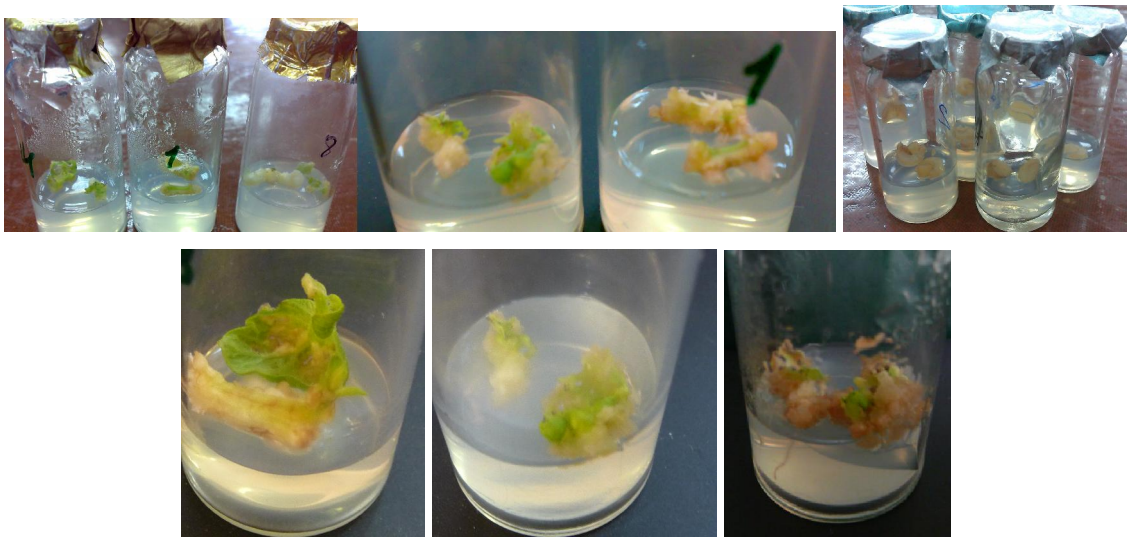

Fig. 3. Induction of callus formation of different explants types and *Solanum tuberosum* callus tissue

Study of initiation and morphogenesis of callus formation enabled detection of potato varieties with active callus formation and high morphogenetic potential. The most intensive growth of callus tissue was observed in Green Gay (75.2%), Kalinowskay (70.3 %) and Oberig (65.4 %) compared with varieties Serpanok, Povin and Buluna - 25.4 - 60.2% that can be successfully used in the cell selection of potatoes for resistance. The callus frequency formed in leaf explants, remained relatively high throughout the year and was 75,4-84,2 %, depending on the variety (Table 1). However, for all varieties was characterized by dependence process of callus formation from physiological age of leaves. The maximum frequency of callus formation (92,3-100 %) was observed when administered in culture isolated 1-3 pairs of leaves from shoot tips that were physiologically younger, while increasing age of leaves decreased in frequency of callus formation 10-24,2,0%. Using of optimized environments made possible the intensive growth of callus tissue. Puff segments studied varieties are the best explants for obtaining primary callus. Middle- grade Green Guy was characterized by the highest proliferative capacity of cells and less intense - the sort of flooding.

Study characteristics *in vitro* cultivation of different varieties of potatoes and lines is a prerequisite of high frequency plant regeneration and success of cell selection.

The undifferentiated callus tissue were formed *in vitro* by indirect morphogenesis and transfer to the medium for induce organogenesis, that gave rise to plants regenerated (Figure 4.).

Table 1

Frequency induction of callus formation potato genotypes depending on the type of primary explants

Cultivar	Frequency of callus formation, %		
	The blade of the sterile leaf	Parts of stems	Disks of microtubers
Serpanok	45,3±0,7	27,5±1,1	11,1±1,3
Povin	40,4±1,5	24,8±1,8	10,2±0,7
Oberig	82,1±1,3	53,4±0,9	18,4±1,1
Zelenuy Gay	95, 2±0,9	65,3±1,1	25,1±0,5
Kalinjvskaya	54,6±1,3	32,5±1,2	15,5±0,8
Buluna	57,1±1,5	30,1±1,3	14,2±0,9
Chervona ruta	83,2±1,2	51,7±0,8	19,8±0,6
Dgerelo Poliske	76,2±2,0	47,5±0,9	18,2±1,2

The basic process of reproductive development of potato plants is tuberization. The mass of bubbles formed in the final phase of their growth is largely determined by genotype and therefore require differentiated conditions of initiation and growth of bubbles *in vitro*.

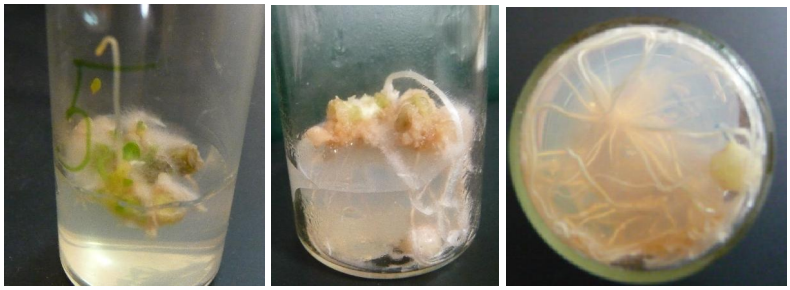

Fig. 4. Features of indirect plant morphogenesis *Solanum tuberosum* in vitro

During of tuberization one of the main conditions is carbohydrate and hormonal factors that influence the photoperiodic response of tuberization and growth responses as well as the complex biochemical processes. Initiation of tuberization preceding increase of photosynthetic activity, the accumulation of photosynthates fund in stems and intensive transport of carbohydrates in the direction of groundwater bodies. Slowing the growth of shoots was accompanied by the formation of active microtubers which were formed in the leaf axils or stem explants in culture medium directly on the shoots or stolon. The relationship between the intensity of shoot growth and intensity of tuberization was established. The induction of stolon formation took place at stem explants cultured under conditions of scattered light 0.5-1 KLK on MS medium supplemented kinetin at a concentration of 0,5 mg/l with 2% sucrose. This formation etiolated shoots with several internodes observed for 6-8 days. The morphological features of these shoots meet as the definition of stolons, but had diatrophic growth orientation [1, 8] (Fig. 5).

Fig. 5. Feature of *Solanum tuberosum* tuberization *in vitro*

The culture medium MS with the addition of kinetin - 0.5-0.8 mg/l, IAA - 0.1-0.2 mg/L, mezoinozit - 100-110 mg/l, sucrose - 8.9 % wasthe best for cultivation and tuberization. Green Gay, Oberig, Chervona Ruta and Dgerelo Poliske characterized by a high ability to form microtubers, which had an oval or elongated shape, different colors (from dark - green to dark - purple depending on the genotype) and sizes (from 4.8 mm). Statistically significant relationships between different groups of plants, maturation was observed (Table 3). Research of influence of duration of photoperiod on processes of tuberization shown that the initiation of tuberization took place more quickly under 8-hour photoperiod at a regulated temperature +19-20 ° C, only the first 8-10 days, and then - in the culture dark room (Fig. 6).

Table 3

The peculiarities of microtubers depending on gentotypes

Cultivars	Tuberi-zation, %	Initiation of tuberi-zation, day	The mean number of tubers, units	The mean weight of tubers, (mg)	The size of tubers, mm^2	The yield of tuberi-zation / shoot formation
Early						
Serpanok	75	35	1,5	150±21	<5	1,3
Povin	69	42	1,3	110±14	<5	1,0
Middle-early						
Oberig	83	25	2,0	237±23	5-7	2,3
Zelenuy Gay	90	20	3,5	370±28	7-10	3,3
Miidle-season						
Kalinivskaya	83	28	2,9	284±19	5-7	2,8
Buluna	81	29	1,8	185±22	5-7	1,7
Late-season						
Chervona Ruta	89	26	3,4	310±29	7-10	3,1
Dgerelo Poliske	79	27	2,2	221±19	5-7	2,0

Fig. 6. Feature of tuberization of *Solanum tuberosum* without lighting

Microtubers acquired pale green, later becoming light brown, depending on the genotype.

To study the adaptation of selected shoots developed from petioles of leaf plates and dark green in color, they were removed from the tubes, the roots carefully washed from the agar with distilled water balances, rinsed root 1% solution of potassium permanganate, planted in sterile soil

and covered with a glass cylinder. Thus the survival rate of potato plants for the studied genotypes was 87-95%.

Fig. 7. Adaptation of *Solanum tuberosum* to the conditions *in vivo*

Thus, the study of features of morphogenesis and plant regeneration *in vitro* is important not only for understanding the patterns of ontogeny of higher plants, but also for the successful development of methods of cell engineering of plants aimed at creating new forms of plants that combine the features of resistance to abiotic stress and pathogens with high performance and the quality of the product.

Conclusions.

1. The dependence of morphogenesis and plant regeneration in culture *in vitro* from the time of selection of material (seasonal). Better ability to morphogenesis and regeneration was observed in all the varieties with the introduction of explants in culture *in vitro* from February to March.

2. The most intensive process callus formation occurred on modified medium Murasihe - Skoog supplemented mezoinozit at a concentration of 180-200 mg/l folic acid - 0.4-0.5 ml/l kinetin - 0.2-0.3 mg/l, 2,4-D - 3.4 mg/l casein hydrolyzate - 1g/l adenine - 1 mg/l, glycine - 1 mg/l, using leaf segments, and the least intense – disks of microtubers.

3. The active of shoot and root formation observed by cultivation of plants in medium Murasihe - Skoog supplemented kinetin, BAR, IAA and 2,4 -D. The induction of stolons formation took place at using of stem explants cultured under conditions of diffuse light - 0.5-1 KLK on MS medium supplemented kinetin at a concentration of 0.5 mg/l with 2% sucrose.

4. The MS medium supplemented kinetin - 0.5-0.8 mg/l, IAA - 0.1-0.2 mg/l, mezoinozit - 100-110 mg/l sucrose - 8.9 % had stimulating effect on the tuberization processes.

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Анотація

Бородай В.В., Кляченко О.Л.

Особливості індукованого морфогенезу та регенерації генотипів Solanum tuberosum L. української селекції

Досліджено особливості морфогенетичних реакцій Solanum tuberosum L. у культурі in vitro для низки сучасних сортів картоплі вітчизняної селекції, ефективність яких залежить від сезонності відбору, типу експлантату, складу живильного середовища та генотипу. Оптимізовано склад живильних середовищ та умови одержання калюсних культур, рослин-регенерантів, мікробульб та індукції процесів органогенезу в культурі in vitro. Вивчення й оптимізація умов індукції морфогенезу з культивованих клітин є важливою складовою частиною роботи з вивчення в культурі in vitro нових цінних форм S. tuberosum L.

Ключові слова: *морфогенез, калюсні тканини, мікробульби, регенерація, Solanum tuberosum L.*

Аннотация

Бородай В.В., Кляченко О.Л.

Особенности индуцированного морфогенеза и регенерации генотипів Solanum tuberosum L. украинской селекции

Исследованы особенности морфогенетических реакций Solanum tuberosum L. в культуре in vitro для ряда современных сортов картофеля украинской селекции, эффективность которых зависит от сезонности отбора, типа эксплантата, состава питательной среды и генотипа. Оптимизирован состав питательных сред и условия получения каллусных культур, растений - регенерантов, микроклубней и индукции процессов органогенеза в культуре in vitro. Изучение и оптимизация условий индукции морфогенеза из культивируемых клеток является важной составной частью работы по изучению в культуре in vitro новых ценных форм Solanum tuberosum L.

Ключевые слова: *морфогенез, каллусные ткани, микроклубни, регенерация, Solanum tuberosum L.*