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REGULAR ARTICLE

IN VITRO CULTIVATION PECULIARITIES OF DIFFERENT POTATO GENOTYPES OF UKRAINIAN SELECTION

*Oksana Leonidivna Klyachenko, Vira Vitaliyevna Boroday**

Adress: National University of Life and Environmental Sciences of Ukraine, Heroev Oborony st., 15, 03041, Kiev, Ukraine.

*Corresponding author: veraboro@gmail.com

ABSTRACT

In result of researches was found out that the highest morphogenetic potential had a middle-early Zeleniy Gay sort, which plants had high sprouts (to 15 cm) in height with a large number of internodes (6-11) with length to 1.5 cm, uniform allocated leaves, well developed root system, a high rate of reproduction - 145, intensive callusogenesis. According to these characteristics mid-season Kalinovskaya Ruta and Dzherelo Polesya sorts were approaching to it. These varieties can be recommended for further work in the direction of cell selection in vitro for resistance to fungal and bacterial diseases. The lowest morphogenetic potential had an early ripening Povin sort.

Keywords: potato culture *in vitro*, shoot formation *in vitro*, rooting *in vitro*, morphogenetic potential.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crop in Ukraine. An important direction in modern plant selection is creating of a fundamentally new and improved crop genotypes, including potatoes, with a single, group or complex resistance to biotic and abiotic stress factors, while maintaining and enhancing their productivity and

quality **(Бондарчук, 2007; Глеба, 1988; Захарчук, 2001; Кононученко, 2000; Кучко, 1998)**. Potato diseases with fungus and bacterial etiology have lead not only to a large yield loss (25 - 85%), but also accelerated sort's degeneration **(Адамова, 2002; Захарчук, 2001; Коновалова, 2006; Кононученко, 2000).** Potato is one of the most important crops, has a number of biological characteristics that significantly affect on approaches and methods of selection. It is one of the few crops with a high plasticity in the in vitro conditions. The rational combination of classical selection techniques with biotechnology techniques can solve tasks in a shorter period **(Банадысев, 2002; Леонова, 2009; Oleinik, 1998).**

Methodological approaches for obtaining of aseptic cultures, preparation of culture media with determined composition, compliance with photoperiodic and gas regimes of cultivation, sequence of cultivation methods in cell selection was developed in general regularities. They are changeable according to different potato varieties and types of pathogens. Furthermore, the main difficulty of selection factors, which have been chosen in biotechnological studies in this direction, is determining sublethal pathogens concentrations for a variety of explants on various stages of cultivation.

In this context, the method of genotypes and explants selection, which would be effective in the formation of morphogenetic structures in vitro culture, as well as methodology for callus and cell suspension cultures for potato selection are tasks of current interest.

The purpose was to examine peculiarities of different potato genotypes cultivation of Ukrainian selection, to determine the material with a high morphogenetic potential for further in vitro work in the direction of cell selection for resistance to fungal and bacterial diseases.

MATERIAL AND METHODS

Investigations were carried out in the Laboratory of Plant Biotechnology, National University of Life and Environmental Sciences of Ukraine.

The objects of the study were potato varieties from Institute of Potato NAAS of Ukraine: early ripening - Serpanok and Povin, middle-early - Oberig and Zeleniy Gay, mid-season - Kalinovskaya and Bylyna, middle-late – Chervona Ruta and Dzherelo Polesya.

The studies were carried out in several directions: 1) obtaining of aseptic plant varieties according to different ripening timeframes; 2) morphogenesis peculiarities: the intensity of root and callus formation of different potato varieties in vitro conditions using classical techniques **(Банадысев, 2002; Захарчук, 2001; Кучко, 1998; Мелик-Саркисов, 1989**; Методическое пособие. Семеноводство картофеля, контроль качества, сертификация,

2002; Методичні рекомендації щодо проведення досліджень з картоплею, 2002; **Різник, 1992; Oleinik, 1998**). For the stock plants obtaining were used intermediate micro cuttings of sprouted tubers' shoots with the length 1-2 cm, with one pair of leaves, containing the axillary meristematic tissue (Figure 1).

Figure 1 Germination of potato tubers for explants obtaining

Figure 2 Shoots formation from initial explants (Kalinovskaya variety)

Stem explants were sterilized in a solution of hydrogen peroxide with concentration of 17.5% for 8-10 min, and then washed with three portions of sterile water, placed in a Petri dish with blotting paper for drying and cultured on non-hormonal MS medium (Figure 2). Shoots were separated from initial explant and subcultivated on fresh MS medium with kinetin. Murashige & Skoog medium was used for plant growth in various modifications (Table 1).

Medium for shoot formation contained the cytokinin substance - kinetin (1:2) at a concentration 5 $mg.ml^{-4}$, which inducted development of axillary buds and stimulated growth of dormant organs. Prepared medium was bottled into tubes with length 10-15 cm and diameter 3 cm, autoclaved at a pressure 0.7-0.8 atm (115 °C) for 15 minutes.

Subsequently, potato plants were cultivated in a plant growth chamber at a temperature 24-26°C with a light intensity 2- 5 kilolux, 70% humidity and 16-hour photoperiod. Surveillance over plants on the abovementioned media was carried out in 14 days with plant examination every 7 days.

After 1.5-2 weeks of cultivation plants were grafted (micro-cuttings with length 1-2 cm in with one pair of shorten leaves, containing the axillary meristematic tissue), planted in a test tube on the growth medium in such a way is to place the part of the stem below the leaf

into the agar, and locate axillary buds on its surface. From one plant 5-8 cuttings were obtained. Cultivation of cuttings was made at a temperature 20-23°C, humidity 70-80%, 16-hour photoperiod and light intensity 3-4 kilolux.

From shoots, that were formed, an unlimited number of planting material can be obtained through the separating of shoots on cuttings and placing them on the nutrient medium.

Table 1 Different modifications of Murashige & Skoog medium

Components	Content in the medium, mg.ml^{-4}			
	Non-hormonal MS	MS for the shoots formation	MS for callusogenesis	Ms for the minitubers formation
Macro MS	1000	1000	1000	1000
Micro MS	100	100	100	100
Fe-chelate	500	500	500	500
Vitamins MS	100	100	100	-
Mesoinositol	-	-	20 k	10 k
Folic acid	-	-	50	-
White's vitamins	-	-	-	100
Kinetin	-	500	20	20
Casein hydrolyzate	-	-	10 k	-
Adenine	-	-	100	-
Glycine	-	-	100	-
2,4-D	-	-	300	-
IAA	-	-	-	10
NAA	-	-	-	5
Saccharose	-	-	2000-3000 k	2000-3000 k
Glucose	2000-3000 k	2000-3000 k	2000-3000 k	-
Agar	70-80 k	70-80 k	70-80 k	70-80 k
pH 5,6-5,8				

Callus tissue was obtained by cultivating stem explants on modified Murashige & Skoog medium laced with growth regulators: kinetin at concentration 20 mg.ml^{-4}, 2,4-D – 300 mg.ml^{-4}. Test tubes with planted explants were cultivated at a temperature 25ºC, humidity 70-80%.

Callus formation was studied through systematic observations of tissues growth and differentiation.

RESULTS AND DISCUSSION

As a result of sterilization process optimization, selection of hydrogen peroxide concentration and time of sterilization 70% of viable cultures were obtained. Cuttings of plants in vitro culture is the best method for the rapid potatoes propagation. This technique allows entirely eliminate reinfestation of plants in the process of reproduction, and also obtain a large amount of material for 2 - 3 months.

After 3-4 weeks of cultivation, the best plant growth was observed in middle-early variety Zeleniy Gay, plants developed with high shoots (up to 15 cm in height), uniform placed leaves, with a large number of internodes (6-11) up to 1.5 cm and well developed root system (Table 2 and 3).

Table 2 Averages of potato shoots length

Varieties	The height of sprouts in the day of planting, mm	The average height of shoots on the 14th day of observation, mm	The average height of shoots on the 21th day of observation, mm	The average height of shoots on 28th day of observation, mm
Serpanok	15	31	48	62
Povin	15	27	39	59
Oberig	15	42	61	77
Zeleniy Gfy	15	59	92	122
Kalinovskaya	15	48	75	93
Bylyna	15	32	51	69
Chervona Ruta	15	42	68	91
Jerelo Polesya	15	39	59	87

Table 3 Coefficient of reproduction in terms of potato variety

Varieties	Number of internodes, pcs	The length of internodes, cm	Plant height, mm	Rate of reproduction
Serpanok	4-6	1,12	59-62	85
Povin	3-5	1,02	45-59	72
Oberig	6-8	1,31	77-98	110
Zeleniy Gay	7-12	1,52	122- 148	145
Kalinovskaya	8-10	1,48	92-121	130
Bylyna	5-7	1,29	69-84	93
Chervona Ruta	6-9	1,41	91-119	122
Jerelo Polesya	4-7	1,26	75-84	102

Middle-early varieties Oberig and mid-season Bylina intensively formed side shoots, plants grew like a bouquet, had short internodes, formed root system incorrectly, had shorter undeveloped root.

In early ripening varieties Povin and Serpanok weak sprout formation, small length of the plant and the lack of roots was observed.

In the middle-late variety Chervona Ruta rooting passed with thickening and intense violet-brown coloration of the lower part of the shoot (Figure 3).

Zeleniy Gay and middle-late variety Jerelo Polesya had the most extensive root formation (Figure 4).

The reaction of potato plants on the varietal composition of medium is one of cultivation drawbacks. This often led to a significant slowdown in growth and development; meanwhile dieback of tops and crotching of plants.

The most difficult was to bring under the cultivation early ripening variety Povin. For further work in the direction of cell selection in vitro for resistance to fungal and bacterial diseases high morphogenetic potential had middle-early variety Zeleniy Gay.

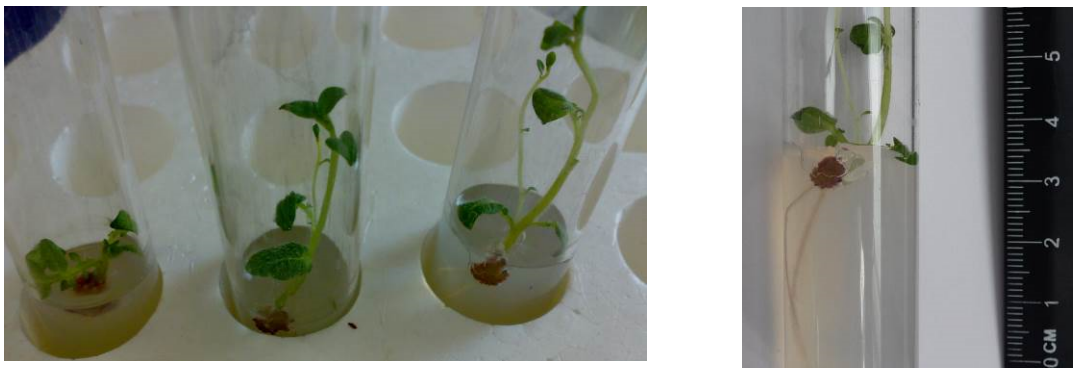

Figure 3 Roots formation in vitro (Chervona Ruta)

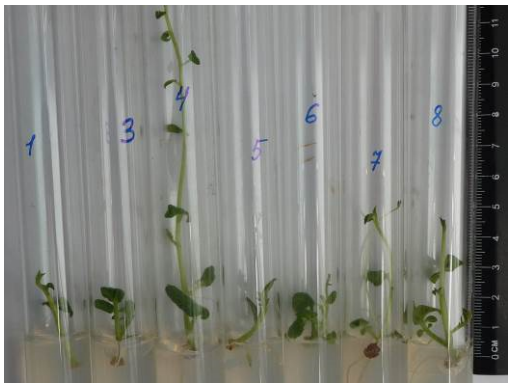

Figure 4 Shoot formation in vitro in different potato varieties according to ripening timeframe; (1- Early ripening – Serpanok; 3,4 – Middle-early varieties - Oberig and Zeleniy Gay; 5,6 - Mid-season Kalinovskaya and Bilina; 7,8 – Middle-late - Chervona Ruta and Jerelo Polesya)

An important indicator in assessing of morphogenesis peculiarities of different potatoes varieties was the rate of reproduction (Table 3). The highest rate of reproduction was observed in variety Zeleniy Gay - 144. In varieties Kalinovskaya and Chervona Ruta - 130 and 122, respectively, the smallest – in Povin variety - 72. The most intensive in Zeleniy Gay variety the callus formation was observed.

CONCLUSION

The highest growth rate of shoots was observed in middle-early varieties Zeleniy Gay, shoots grew less intense in mid-season and middle-late varieties Kalinovskaya, Chervona Ruta and Jerelo Polesya, the lowest growth rate observed in the early ripening Serpanok and Povin varieties. In general, early ripening Povin variety had low morphogenetic potential.

Middle-early Zeleniy Gay variety was characterized by a high rate of reproduction - 144, intensive callus formation. According to these characteristics, mid-season and middle-late varieties Kalinovskaya, Chervona Ruta and Jerelo Polesie were approaching to it. These varieties had high morphogenetic potential and can be recommended for further work in the direction of cell selection in vitro for resistance to fungal and bacterial diseases.

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