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TUBERIZATION AS A METHOD TO PRESERVE A VALUABLE GENE POOL OF *SOLANUM TUBEROSUM* L. FROM UKRAINIAN SELECTION BEING CULTIVATED *IN VITRO*

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Introduction

Microtubers of *Solanum tuberosum* L. gained being cultivated in vitro, are widespread for mass intensified propogation of stronger test-tube material within system of elite seed farming, storage and propagation of stronger material, breading the new valuable forms due to tissue culture method, propagation of unique regenerants, as a result of distant somatic hybridization in terms of experiments at transformation and cell selection [1, 3, 6, 8, 10]. Microtubers are also used for safe carry during introduction, transport and exchange by gene pool between selection organizations, distribution taking quarantine steps of trial an examination.

Safekeeping of seed gene pool that belongs to cultures with vegetative propagation, including potatoes, isn`t possible as pubescence changes genetic composition of cultivars, presented by highly heterozygous genotypes. Among modern technology of gene pool safekeeping under controlled conditions there are introduction of different genotypes *in vitro*, plant rehabilitation after diseases, micropropogation, monitoring of phytosanity status of plants, genetic typing, middle-term safekeeping *in vitro*, cryopreservation and long-term cryokeeping [2].

Safekeeping of potato gene pool takes more than field collection, doublet specimens *in vitro* are necessary as well. One of the principal approaches in this case is keeping collection by way of slow-growing test-tube plants [4, 7, 11, 13]. Safekeeping *in vitro* collection under optimal growth conditions (+20-23°C) demands frequent transport of microplants into new nutrient medium what upvalues its storage and enhances chance of plants infection, especially it concerns untested specimens for pathogen. Various methods are applied to increase interval between passages. These methods are based on growth impairment of tested-tube plants. Getting and safekeeping under lower temperature of storage organ plants, including microtubers is one of the methods to slowdown culture growth. Characteristics of formed microtubers during the last growth phase are mainly determined by genotype and as a result demand differentiated conditions of initiation and tubers formation *in vitro*.

Research objective is to investigate tuberization peculiarities of different genotypes in Ukrainian selection, optimize regimes of their medium-term storage.

Objects and methods of the research

Researches had been carried out in laboratory of plant biotechnology at National University of Bioresources and Nature management of Ukraine for 2010-2013. Potato tubers of the following cultivars were chosen as research objects: early ripe cultivars – Serpanok and Povin; middle-early – Oberig and Zeleny Gay; mid-season – Kalynovskaya and Bylina; middle-late – Chervona Ruta and Dherelo Polesya.

Intermediate internodes of germinated tubers of 1-2 sm with one couple of leaves that contain meristematic tissue were used to get stools. Received aseptic shoots were separated from primary explantats and cultivated in modified nutrient medium Murasige-Skuga (MS) [1, 5, 9, 12].

The way of microtubers formation were investigated in nutrient mediums with different contents of sucrose (4-9%), phytohormones (indoleacetic acid – 0,1-0,4mg/l), kinetin (0,5-1,5 mg/l), mesoinosit (110-120 mg/l). Effect of daylight length and temperature were studied allowing for duration of photoperiod (14-16, 8-10 hours), illumination (lack of illumination, 3-4 klx, 6-8 klx), temperature regimes of middle-term storage of microtubers (+2-4, 6-8, 8-10°C).

Modified medium by D.P. Ostapenko was used to determine peculiarities of plant tuberization [6]. Mature tubers were kept in a coolroom for 4-6 months under various controlled temperatures.

Tables presented below contain arithmetical mean value from received values and standard deflection (SD). Application software Statistika 5.1 and Microsoft Office XP® for Microsoft Windows® were used for statistic processing of study results.

Results and discussion

Plant tuberization of *Solanum tuberosum* L. is a highly coordinated process, that includes morphologic, physiologic and biochemical variations of plants on different stages of ontogenesis. Stages of tuberization are stolon induction and initiation, stolon growth and its branching, the end of stolon growth, induction and initiation of tubers, growth and ripening of tubers [3, 13]. Carbohydrate and hormone factors are among principal conditions of these processes. These factors effect on photoperiodic reactions, complex of biochemical processes. Tuberization is preceded by increasing of photosynthetic activity, accumulation of assimilate fund in stems and intensive transport of carbohydrates towards tubers [1, 3, 9].

In terms of our researches induction of stolon formation took place in 5-6 days after laterals occurrence in case of stem explants cultivation under conditions of diffuse light 0,5-1 klx in medium MS, added by kinetin of 0,5 mg/l and 2-4% of sucrose. Cytokinins effect became apparent due to intensive formation of laterals and development of stolons. Later for 3-5 weeks after thickening of stolon subapical zone formation of microtubers occurred. Hereafter intensity of tuberization reduced due to their formation period finished and their size enlarged. Microtubers, as a rule, were formed on stolons and from nodes on stems (fig.1).

Fig.1 Peculiarities of *Solanum tuberosum* microtubers formation being cultivated *in vitro*

Sometimes having removed apical dominance had the following sequences: slowdown of axial growth, slowdown of shoot growth and microtubers formation in atriums of stem explantats within non-hormonic medium MS with 2% content of sucrose during 16 hours of photoperiod (fig.2).

Fig.2 Microtuber formation having removed apical dominance within non-hormonic medium MS

Differences in induction of tuberization had cultivar correlation (fig.3). A number of plants, that produced microtubers, ranged from 68,7 – 98,2%. Culyivars Oberig, Zeleny Gay, Chervona Ruta and Kalinovskaya were characterized by the highest capacity to form microtubers with rate 1,7-2,1 microtubers per 1 plant; cultivars Bylina and Dzherelo Polesya – 1,2 and 1,4 relatively; cultivars Serpanok and Povin – 1,1 and 1,0. At the same time microtubers were ovel and oblong with various colours (from dark-green to dark-purple depending upon genotype) and size (3-1 mm). During further clonal micropropagation of somaclones with insignificant morphological differences capacity to form tubers hardly differed from parent forms. Microtuberization of dwarfish and bushy forms with small leaves and weakened apical dominance wasn`t much pronounced (insignificant thickening of stolons).

Fig. 3 Microtubers of Solanum tuberosum, cultivars of Ukrainian breeding: Early ripe – Serpanok (1) and Povin (2), middle-early – Oberig (3), mid-season – Kalinovskaya (5) and Bylina (6), middle-late – Chervona Ruta

Belonging to this or that group of ripening didn`t effect much on formation of microtubers *in vitro*. Each group included cultivars with high, middle and low capacity to form microtubers. All study cases presented induction of microtuberization during the first month. According to statistic data reliable regularities didn`t take place between plants of different ripening groups (table).

Table

Peculiarities of microtubers formaton depending upon genotype

Cultivars	Initiation of tuberization, day	Formation of microtubers, %	Average weight of microtubers, (mg)	Productivity of tuberization, units	Fraction classification of microtubers, %		
					large, 8-10	*middle, 5-7 mm*	*small* ,

					mm		*till 5 mm*
Early-ripe							
Serpanok	18,3	75,3	148±12	1,1±0,02	15,2	50,5	34,3
Povin	25,6	68,7	114±15	1,0±0,01	13,3	34,6	52,1
Middle-early							
Oberig	17,5	85,1	236±21	1,9±0,03	45,1	35,2	19,7
Zeleny Gay	19,9	92,8	363±20	1,7±0,01	25,4	43,7	30,9
Mid-season							
Kalinovskaya	21,3	81,6	270±12	2,1±0,02	38,7	39,2	22,1
Bylina	19,1	81,4	178±17	1,2±0,04	30,2	41,5	28,3
Middle-late							
Chervona Ruta	14,0	87,3	287±18	1,8±0,02	29,4	36,8	33,8
Dzherelo Polesya	16,7	77,4	218±14	1,4±0,03	20,0	36,1	43,9

Medium Ms, supplemented by kinetin – 0,5-0,8 mg/l indoleacetic acid – 0,1-0,2 mg/l, mesoinosit – 100-110 mg/l, sucrose – 4-9%, had stimulative effect on process of tuberization. For cultivars, inclined to tuberization *in vitro,* sucrose concentration made 4-6%, while for cultivars which have not so easy way of these processes sucrose content is 6-8% [2,4]. Maximum number of plants with tubers was registered in medium with kinetin 0,5-0,8 mg/l, reducing or increasing concentration of these hormones tended to decrease of microtubers.

Minimum number of microtubers formed under conditions of 16-hours of photoperiod, further cultivation during 1,5-2,0 months under controlled conditions (14-16 hours photoperiod, illumination 6000-8000 lx, temperature +20-22°C) induced germination of forming tubers. Abatement of illumination caused considerable growth of specimens with microtubers. 8-hours photoperiod and controlled temperature (+19-20°C) during first 8-10 days with further cultivation in the dark, favored formation of some small microtubers (fig.4). During dark period formation of microtubers practically didn`t occur.

High intensity of microtuber formation was actual during first 10-12 days under conditions of 8-hours photoperiod, but later - illumination 3-4 klx (diffusal light) and controlled temperature +19-21°C.

Fig.4 Peculiarities of *Solanum tuberosum* tuberization having different illumination regimes

The most favorable temperatute for keeping microtubers in coolroom during 4-6 months was +2-4°C. At the same time by the end of storage small shoots occurred, which didn`t reduce high vital capacity of tubers which were planted into sterile soil later (fig.4). Plants with mirotubers formed 1-2 stems with 5-10 internodes. Plant establishment ranged from 80-89%.

Fig.4 Sprouting of potato microtubers after long storage *ex vitro*

Taking into consideration natural physiological period of microtubers rest, which was artificially prolonged due to permanent specimen storage under low temperatures above zero (+2-4°), and then delayed sprouting of microtubers under these conditions, direct cycle of specimens storage is possible to extend much.

Conclusions

Effect of potato cultivar characteristics on process of microtuber formation was determined as a result of this research. In this way cultivars Oberig, Zeleny Gay, Chervona Ruta and Kalinovskaya presented the highest capacity to microtuber formation.

Optimal conditions of tuberization for Ukrainian breeding genotypes were determined as well allowing for cultivation in medium Ms, supplemented by kinetin 0,5-0,8 mg/l, indoleacetic acid – 0,1-0,2 mg/l, mesoinosit – 100-110 mg/l, sucrosw – 4-9 %. Initiation of tuberization for cultivars Oberig, Zeleny Gay, Chervona Ruta and Kalinovskaya were more intensive keeping plants under conditions of 8-hours photoperiod and controlled temperature +19-20°C during the first 8-10 days with further cultivation under condition of diffusal light (3-4 klx).

Collection *in vitro* is kept in gene bank with controlled temperature +2-4°C with a lack of illumination during 4-6 months.

Keeping in collection *in vitro* highly productive and adaptive to local climatic and soil conditions potato cultivars that have different vegetative period (early maturation) and purpose is an important stage in elite seed farming. Optimization of potato tuberization induction is practical and necessary part in researching the new valuable plant forms of this culture being cultivated *in vitro.*

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Klyachenko O.L., Boroday V.V. Tuberization as a method to preserve a valuable genotype of *Solanum Tuberosum* l. from Ukrainian selection being cultivated *in vitro* // Bull. of the State Nikit. Botan. Gard. – 2015. – № 116. – P. 60-65.

The most favorable conditions of tuberization for different *Solanum tuberosum* genotypes from Ukrainian selection were determined in terms of the research. Initiation of tuberization was more intensive if plants were kept under conditions of 8-hours photoperiod and controlled temperature +19-20°C during the first 8-10 days with further cultivation under conditions of diffused light (3-4klux). Within research it became possible to investigate tuberization peculiarities of gene pools with different ripening terms, get microtubers of 3-11mm and 114-287mg and optimize regimes of microtubers medium-term storage.

Key words: *Solanum tuberosum L; tuberization in vitro; microtubers; genotype*