UDC 631.53.01:633.491

METHODS OF INTENSIFYING THE TUBERIZATION PROCESS OF POTATO SEEDS *IN VITRO* CULTURE

H. S. Balashova, Yu. O. Lavrynenko, R. A. Vozhehova, B. S. Kotov

Institute of Irrigated Agriculture NAAS of Ukraine
Kherson region, village of Naddniprianske, Ukraine, 73483
E-mail: izz.ua@ukr.net

Received on May 26, 2017

Aim. To determine optimal factors of impacting the tuberization intensity of the plants of mid-early potato variety Nevska *in vitro*. **Methods.** Complex application of the laboratory, mathematical-statistical, calculative-comparative methods and systemic analysis. **Results.** The experimental data on the impact of photoperiod and acidity of the culture medium (pH) on the tuberization induction in culture *in vitro* were presented. The dependence of tuberization intensity on pH of the culture medium was demonstrated. **Conclusions.** Optimal indices of tuberization intensity and economic efficiency were obtained during *in vitro* cultivation of the plants of mid-early Nevska variety at the photoperiod of 16 hours and pH of the culture medium of 5.3: tuberization intensity was 83.0 %, the mass of the average microtuber was 612.5 mg, the mass of microtubers per one plant was 514.4 mg, the number of microtubers with the mass over 350 mg – 75.4 % with the production profitability of 100 % and microtuber cost price of UAH 8.08 (USD 0.3).

Keywords: microclonal reproduction, tuberization induction, acidity of the culture medium, photoperiod.

DOI: 10.15407/agrisp4.02.037

At present, the process of curing potatoes from the viral infection has been worked out sufficiently which allows curing any variety. However, the issue of searching for optimal ways of reproducing the cured plants and obtaining the sufficient amount of high quality seeder material is yet to be solved. The most qualitative results are received using the methods of accelerated reproduction of plants *in vitro*, due to which it is possible to obtain several thousand using one plant within a short period of time. A great advantage of this method is the possibility of obtaining enough seed material of newly-created or cured varieties quickly and thus ensuring their timely introduction into the plant growing system [1]. In practice, the microclonal reproduction is performed via plant cuttings cultivation on a nutrient medium in a controlled environment [2–4].

The microclonal reproduction of potato plants *in vitro* culture allows obtaining genetically homogeneous seed material with a high coefficient of reproduction (10^5–10^6), which is viruses – free due to the use of meristem culture. It is also reducing the duration of the multiplication process, accelerating the transfer from plantlets to reproductive phase of development.

The impact of different factors of morphogenesis *in vitro* in many potato varieties has been intensively studied [5–9]. The process of tuberization *in vitro* may be regulated by a number of endo- and exogenous factors (excess of assimilants, hormonal state of plants, temperature regime, photoperiod, light intensity, pH and composition of culture medium) which is the basis for obtaining high quality material in primary cultivation of potatoes.

It is known that a cell functions within a narrow framework of concentration fluctuations for hydrogen ions. The value of pH impacts the structure and activity of biological macromolecules, proteins-enzymes first and foremost, stability and assimilation of a number of culture medium components, such as heteroauxin, vitamins B_1 and B_6. The availability of different iron compounds to tissues also depends on pH value.

The second, no less relevant factor, impacting the obtaining of the high quality initial material *in vitro* is lighting. The formation of chlorophyll and morphogenesis are impossible without it. Potato plants are particular about light. When there is shortage of light, there is some yellowing of leaves, plant stem

is getting thinner and thus the mass of microtubers is reduced [10].

Thus, the study of the interaction of such factors, relevant for tuberization induction, as pH of the culture medium and lighting regime becomes urgent for the purpose of process optimization and reduction of the cost price of obtained microtubers.

The aim of the studies is to determine optimal factors of impacting the tuberization intensity of the plants of mid-early potato variety Nevska *in vitro*.

METHODS OF STUDIES

To study the most efficient regime of tuberization in culture *in vitro* for mid-early potato variety Nevska, an experiment was conducted in conditions of microclonal laboratory. The study was conducted in terms of the photoperiod (10 and 16 hours – control) and acidity of the culture medium (4.3 – control; 4.8; 5.3).

The method of thermo-chemotherapy in combination with the culture of apical meristem was used *in vitro* to obtain initial potato plants, improved via the biotechnological method. The experiments were conducted by common methods [11–13]. The economic efficiency of the production of improved initial material in culture *in vitro* was estimated based on actual cost price of microtubers according to technological cards.

RESULTS OF INVESTIGATIONS

On the 20th and 40th days of observing the development of plants, the dependence of the height of plants on the photoperiod *in vitro* was established. For instance, on the 20th day of the study the plants were 0.1 cm higher on average with 16 light hours compared to 10 hours (Table 1). On the 40th day, this index was 44.4 % higher with longer light duration compared to 10 hours, amounting to 2.6 cm.

The number of internodes on days 20 and 40 of the cultivation was higher for 16 h photoperiod, amounting to 4.1 and 5.8 items on average respectively, compared to 3.8 and 5.2 – in case of 10 light hours. Nevska variety potato belongs to mid-early varieties. Therefore, only 5.1 % microtubers were formed on day 20 of the cultivation with 16 light hours, with even a lower number for 10 hours – 3.6 %. On day 40 of the observations, the photoperiod had almost no impact on the tuberization: 12.1 % of plants formed microtubers with 16 hours and 11.1 % – with 10 light hours.

There was inconsiderable impact of pH of the culture medium on the height of plants and number of internodes on days 20 and 40 of the cultivation. On day 20, the height of plants was 4.0; 4.7 and 4.2 cm (pH 5.3; 4.3 and 4.8 respectively), and the number of internodes – 3.8; 4.1 and 3.9. On day 40 of the studies, the height growth of plants was 2.1; 2.2 and 2.4 cm with the number of internodes 5.3; 5.7 and 5.6 (pH 5.3; 4.3; 4.8), respectively.

The acidity of the culture medium impacts the intensity of tuberization. On day 20 of the cultivation it was higher at pH=5.3, amounting to 5.6 % compared to 4.4 and 3.2 % at pH=4.3 and 4.8 respectively. On day

Table 1. The impact of acidity (pH) of the culture medium and photoperiod on growth, development, and tuberization of Nevska variety potato plants *in vitro*

Photoperiod, hours	pH of the culture medium	Day of cultivation									
		20				40				60	80
		Height of plants, cm	Number internodes, it.	% of plants which formed		Height growth of plants, cm	Number of internodes, it.	% of plants which formed			
				Stolons	Microtubers			Stolons	Microtubers	Microtubers	Microtubers
10	4.8	4.3	3.9	94.5	4.5	1.8	5.3	82.6	17.4	33.5	69.4
	4.3	4.6	3.9	96.6	3.4	1.9	5.3	92.2	7.8	11.7	44.9
	5.3	3.8	3.6	97.0	3.0	1.7	5.0	91.8	8.2	23.7	64.7
16	4.8	4.0	3.9	98.2	1.8	2.9	5.9	92.3	7.7	20.5	60.5
	4.3	4.7	4.3	94.6	5.4	2.5	6.1	88.0	12.0	30.8	60.7
	5.3	4.1	4.0	91.8	8.2	2.4	5.5	83.5	16.5	34.7	83.4

40, 2.5–2.7 % fewer microtubers were formed with the decrease of pH level to 4.3.

When the photoperiod duration and acidity of the culture medium were combined, the highest plants (4.7 cm) were noted with 16 light hours and pH = = 4.3 and the lowest (3.8 cm) – with 10 light hours and pH = 5.3 on day 20 of the cultivation.

On day 40 of the study, the maximal height growth was observed for 16 hours and pH = 4.8, amounting to 2.9 cm, and the minimal – for 10 hours and pH = = 5.3–1.7 cm. The number of internodes on days 20 and 40 was the highest for 16 photoperiod hours and pH = 4.3–4.3 and 6.1, and the lowest – 3.6 and 5.0 – for 10 light hours and pH = 5.3 respectively.

The smallest amount of microtubers on days 20 and 40 of the cultivation was formed at 16 light hours and pH = 4.8–1.8 and 7.7 %, and the highest amount on day 20 – at 16 light hours and pH = 5.3–8.2 %, and day 40 – at 10 light hours and pH = 4.8–17.4 %.

On day 60 of the cultivation with photoperiod duration of 16 hours, regardless of the acidity of the culture medium, 28.7 % of microtubers were formed, and at 10 hours – 23.0 %. The best indices of tuberization were noted at the acidity of 5.3–29.2 % compared to 27.0 and 21.3 % at 4.8 and 4.3 respectively.

When photoperiod and acidity of the culture medium were combined, more microtubers were formed at 16 light hours and pH = 5.3–34.7 %. The lowest index was noted for 10 light hours and pH = 4.3–11.7 %.

On day 80 of the study the intensity of tuberization increased considerably. For instance, it was 68.2 % on average for 16 light hours compared to 59.7 % for 10 light hours (Table 2).

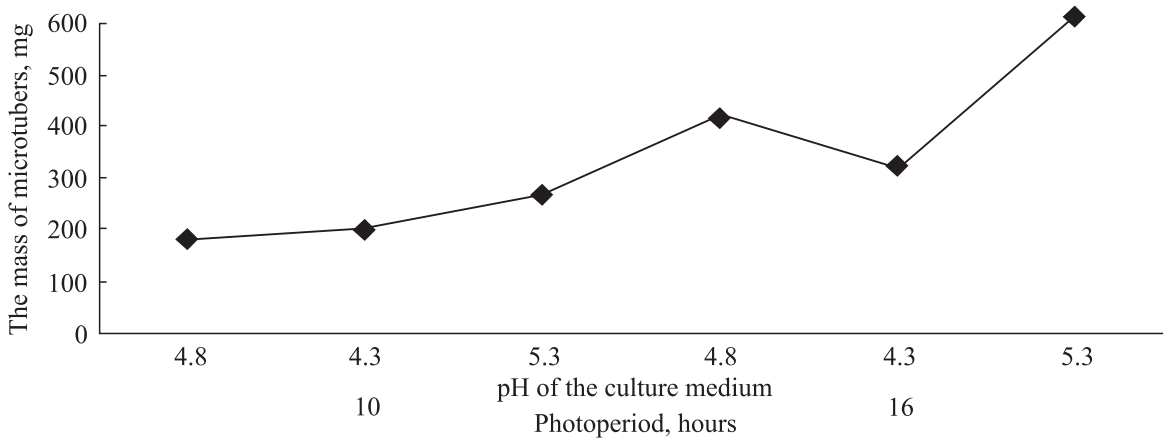
The duration of photoperiod impacted the formation of the mass of an average microtuber and the yield of microtubers with the mass over 350 mg considerably. The correlation coefficients (r) were 0.716 + 0.221 and 0.720 + 0.219, respectively. The mass of an average microtuber at 16 light hours was 234.5 mg higher compared to 10 light hours, amounting to 450.7 mg. 55.1 % of microtubers with the mass over 350 mg were obtained at the photoperiod duration of 16 hours and 18.9 % – at 10 hours.

The photoperiod duration had average impact on the mass of microtubers per plant, the correlation coefficient (r) was 0.549 ± 0.264. The mass of a microtuber per 1 plant at the photoperiod of 16 hours was 320.0 mg which is 175.5 mg higher compared to 10 hours.

There was an average correlation between the tuberization intensity, the mass of an average microtuber, the mass of microtubers per one plant and pH of the culture medium (r = 0.469 ± 0.279; 0.425 ± 0.286; 0.528 ± ± 0.269, respectively). The highest number of microtubers was formed at pH = 5.3–74.1 % compared to 52.8 and 65.0 at pH = 4.3 and 4.8 respectively. The best indices of the mass of an average microtuber was formed at pH = 5.3–439.2 mg compared to 262.7 and 298.5 mg at pH = 4.3 and 4.8 respectively. The mass of microtubers per one plant is maximal at

Table 2. The performance of Nevska variety potato plants in culture *in vitro* depending on the acidity (pH) of the culture medium and photoperiod duration

Photoperiod, hours	pH of the culture medium	Mass of average microtuber, mg	Mass of microtuber per 1 plant, mg	Yield of microtubers with the mass over 350 mg, %	Number of plants, which formed microtubers, %	Number of microtubers per one plant, it.
10	4.8	181.4	129.4	11.6	69.4	0.70
	4.3	201.4	105.8	17.9	44.9	0.45
	5.3	265.8	198.3	27.3	64.7	0.65
16	4.8	415.7	254.5	52.2	60.9	0.61
	4.3	324.0	191.0	37.9	60.7	0.61
	5.3	612.5	514.4	75.4	83.4	0.83
Multiple correlation index		0.890	0.895	0.866	0.926	
HIP_{05}, mg	A	49.6	58.8			
	B	30.5	23.3			



The impact of photoperiod and acidity of the culture medium on the formation of microtubers of Nevska potato variety in culture *in vitro*

pH = 5.3, amounting to 356.4 mg. With such acidity, the yield of microtubers with the mass over 350 mg was 51.3 % compared to 27.9 and 31.9 % at pH = 4.3 and 4.8, respectively.

The impact of the interaction of investigated factors on the productivity indices was strong, the multiple correlation index was 0.890; 0.895; 0.866 and 0.926. At the interaction of pH of the culture medium and photoperiod duration, the higher tuberization intensity was noted at pH = 5.3 and 16 light hours – 83.4 % and the lowest – 44.9 % at 10 light hours and pH = 4.3. The best indices of the mass of an average microtuber were obtained at 16 light hours and pH = = 5.3–612,5 mg and the lowest – at 10 hours and pH = 4.8–181.4 mg.

The mass of a microtuber per one plant was also the highest at 16 light hours and pH = 5.3 – 514.4 mg and the lowest – at 10 hours and pH = 4.3 – just 105.8 mg. The highest yield of microtubers with the mass over 350 mg was at pH = 5.3 and 16 photoperiod hours – 5.4 %, and the lowest – at pH = 4.8 and 10 light hours – 11.6 % (Figure).

While using 16 light hours, the cost price of one microtuber decreased by 13.8 % compared to 10 hours (Table 3). Using pH of the culture medium of 5.3 while cultivating the plants *in vitro*, the cost price of a microtuber was 8.7 and 29.7 % lower compared to pH of 4.8 and 4.6, respectively, regardless of photoperiod duration.

CONCLUSIONS

The maximal indices of Nevska variety potato plant productivity *in vitro* were obtained at 16 photoperiod hours and pH of the culture medium of 5.3: the intensity of tuberization – 83.0 %, the mass of an average microtuber – 612.5 mg, the mass of microtubers per one plant – 514.4 mg, the number of microtubers with the mass over 350 mg – 75.4 %, the cost price of a microtuber – UAH 8.08 at the profitability of production of 100 %.

Table 3. The economic efficiency of the cultivation of potato microtubers of the mid-early potato variety Nevska in the culture *in vitro* depending on photoperiod and acidity of the culture medium

Photoperiod, hours	pH of the culture medium	Number of microtubers per one plant, it.	Expenses per one plant, UAH	Cost price, UAH/microtuber	Operating profit or loss, UAH/microtuber	Profitability, %
10	4.8	0.70	6.18	9.18	6.82	79
	4.3	0.45	6.28	15.54	0.46	14
	5.3	0.65	6.38	10.64	5.36	62
16	4.8	0.61	6.48	11.32	4.68	50
	4.3	0.61	6.58	11.09	4.90	48
	5.3	0.83	6.68	8.08	7.90	100

Прийоми інтенсифікації процесу бульбоутворення насіннєвої картоплі в культурі *in vitro*

Балашова Г.С., Лавриненко Ю.О., Вожегова Р.А., Котов Б.С.
e-mail: izz.ua@ukr.net
інститут зрошуваного землеробства НААН
м. Херсон, сел. Наддніпрянське, Україна, 73483

Мета. Визначити оптимальні фактори впливу на інтенсивність бульбоутворення рослин *in vitro* середньораннього сорту картоплі Невська. **Методи.** Комплексне використання лабораторного, математично-статистичного та розрахунково-порівняльного методів і системного аналізу. **Результати.** Наведено експериментальні дані щодо впливу фотоперіоду та кислотності живильного середовища (pH) на індукцію бульбоутворення в культурі *in vitro*. В процесі дослідження встановлена залежність інтенсивності бульбоутворення від pH живильного середовища. **Висновки.** Оптимальні показники інтенсивності бульбоутворення та економічної ефективності отримано при культивуванні рослин *in vitro* середньораннього сорту Невська за фотоперіоду 16 годин та pH живильного середовища 5,3: інтенсивність бульбоутворення становила 83,0 %, маса середньої мікробульби 612,5 мг, маса мікробульб на одну рослину – 514,4 мг, кількість мікробульб масою понад 350 мг – 75,4 % за рентабельності виробництва 100 % та собівартості мікробульби 8,08 грн.

Ключові слова: мікроклональне розмноження, індукція бульбоутворення, кислотність живильного середовища, фотоперіод.

Приемы интенсификации процесса клубнеобразования семенного картофеля в культуре *in vitro*

Балашова Г.С., Лавриненко Ю.А., Вожегова Р.А., Котов Б.С.
e-mail: izz.ua@ukr.net
Институт орошаемого земледелия НААН
г. Херсон, пос. Надднепрянский, Украина, 73483

Цель. Определить оптимальные факторы влияния на интенсивность клубнеобразования растений *in vitro* среднераннего сорта картофеля Невский. **Методы.** Комплексное использование лабораторного, математико-статистического и расчетно-сравнительного методов и системного анализа. **Результаты.** Приведены экспериментальные данные относительно влияния фотопериода и кислотности питательной среды (pH) на индукцию клубнеобразования в культуре *in vitro*. В процессе исследования установлена зависимость интенсивности клубнеобразования от pH питательной среды. **Выводы.** Оптимальные показатели интенсивности клубнеобразования и экономической эффективности получено при культивировании растений *in vitro* среднераннего сорта Невский при фотопериоде 16 часов и pH питательной среды 5,3: интенсивность клубнеобразования составила 83,0%, масса среднего микроклубня – 612,5 мг, масса микроклубней на одно растение – 514,4 мг, количество микроклубней массой более 350 мг – 75,4% при рентабельности производства 100% и себестоимости микроклубня 8,08 грн.

Ключевые слова: микроклональное размножение, индукция клубнеобразования, кислотность питательной среды, фотопериод.

REFERENCES

1. *Riznyk VS.* Improvement of potatoes: problems and prospects. *Kartopliarstvo*. Kyiv*, 1997;27:182–190.*
2. *Vermenko YuYa, Andrushko OM, Oliynyk VP.* Reproduction of elite potato seeds on the basis of the initial seeder material, improved by the biotechnological method. *Bulletin of the Lviv State Agrarian University*. 2001;5:374–385.
3. *Kilchevsky A, Lemesh V, Sycheva E.* Plant genetics and biotechnology in Belarus. *J Biotechnology;* 2016;231:18p.
4. *Zaman MS, Quraishi A, Hassan G, Raziuddin, Ali S, Khabir A, Gul N*. Meristem Culture of Potato (*Solanum tuberosum* L.) for production of virus-free plantlets *J Biological Sci,* 2001;1(10):898–899.
5. *Xiaofeng M, Yongping W, Mengxi L, Jianmin X, Zhigang X.* Effects of green and red lights on the growth and morphogenesis of potato *(Solanum tuberosum L.)* plantlets *in vitro*. *Scientia Horticulturae*. 2015;190:104–109.
6. *Konovalova GI.* Using biotechnological methods and techniques in modern potato seed production. *Actual problems of science and technology: Questions of potato growing*. Moscow, 2006;332–336.
7. *Matskevich VV, Liashchenko SA.* Features of the regeneration of potato plants from stalks, depending on the lighting and substrate. *Potato growing, in-dept. scientific thematic collection.* Kyiv, 2008;37:98–110.
8. *Dobranszki J*, *Magyar-Tábori K, Hudak I. In vitro* tuberization in hormone-free systems on solidified medium and dormancy of potato microtubers magyarne. *Fruit, vegetable and cereal science and biotechnology 2 (special Issue 1),* 2008;82–94.
9. *Sarkar D.* The signal transduction pathways controlling in plant tuberization in potato : an emerging synthesis. *Plant Cell Reports*, 2008;**27**:1–8.
10. *Kitaya Y, Fukuda O, Kozai T, Kirdmanee C*. Effects of light intensity and lighting direction on the photoautotrophic growth and morphology of potato plantlets *in vitro*. *Scientia Horticulturae*, 1995;**62**(1–2):15–24.
11. *Bondarchuk AA*. Scientific basis of seed potatoes in Ukraine. Kyiv, Bila Tserkva Book Factory. 2010;400 p
12. *Kutsenko VS, Osypchuk AA, Podhaietskyi AA*. Methodological recommendations regarding the investigations with potatoes. Ed. V.V. Kononuchenko. Nemishayeve, Institute of Potato Production, 2002;183 p.
13. *Murashige T, Skoog F*. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol Plant*. 1962;**15**(3):473−97.