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IN VITRO POTATO VARIABILITY AND ITS EFFECT ON TUBER NUTRITIONAL PROPERTIES

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Abstract

A comparative analysis of nine somaclonal Dutch selection “Alladin” pota-to variants test results was carried out using a modified Murashige-Skoog medi-um with different concentrations of hormones, vitamins and growth stimulants. As a result of the work carried out, 100% callus formation providing from leaf explants and more than 90% shoot formation without the use of additional steps associated with their rooting optimal protocols for *in vitro* cultivation of pota-toes were defined. According to morphological characteristics 9 initial lines were selected among the Dutch selection of “Alladin” potato variety regenerated plants using these protocols. The tubers of the second reproduction obtained from

these lines were used for the analysis of biochemical parameters (starch, soluble sugars, proteins). As a result, lines with the maximum starch content were identified, 2 lines out of 9 exceeded the control sample by 3.3% and 10.7%. The protein con-tent in the tubers of 7 somaclonal variants from 1% to 10.6% exceeded this indi-cator in the control. In comparison to the control, the sugar content was signifi-cantly lower (from 37.9 to 54%) in 3 studied samples. The results obtained are of great practical interest for further breeding studies to consolidate them and create new dietary and table varieties of potatoes. Based on the obtained somaclonal variants, new promising potato varieties adapted to the soil and climatic condi-tions of Northern Kazakhstan can be obtained.

Key words: potatoes; *Solanum tuberosum*; *in vitro* culture; somaclonal var-iability; starch; total protein; free sugars.

Basic position and Introduction

Potato is one of the main agricultural crops of universal use, both for food purposes and for technical processing. The average consumption of potatoes per capita in the world is 120-130 kg per year. World potato production in 2018, ac-cording to the FAO, amounted to 368.247 million tons [1].

According to the World Potato Congress dated February 20, 2020, Ka-zakhstan is included in the list of 25 world leaders in terms of potato cultivation and is in 20th place (3.807 million tons), the main leaders are China (90.321 mil-lion tons), India (48.529 million tons), Ukraine (22.504 million tons), Russia (22.395 million tons), USA (20.607 million tons) [2].

According to the Bureau of National Statistics, in 2020, 4 million tons of potatoes were produced, 359.6 thousand tons were exported, and 45.8 thousand tons were imported. Security due to domestic production amounted to 108.5%. There are 95 kg of potatoes per capita per year [3].

Currently, on the territory of the Republic of Kazakhstan, varieties of Dutch and German selection are widely used in industrial potato growing, which have a high yield (up to 780 c/ha), resistance to a wide range of fungal and bacterial diseases. However, these varieties of potatoes are not intended for long-term storage, they are demanding on moisture and fertilizers, in case of insufficiency of which their yield drops sharply. In the advanced farms of the Republic, the yield of Dutch varieties is approximately 350 c/ha, which is 50-70 c/ha higher than the varieties of local selection [4].

In this regard, it seems promising for the agro-industrial complex of the Republic to involve Dutch potato varieties as high-yielding ones in the breeding process. Obtaining on the basis of Dutch varieties of new potato lines that are re-sistant to growing in the conditions of the Northern region of Kazakhstan. Ob-taining new varieties of potatoes with a reduced sugar content, as well as a high content of starch and other nutrients, will ensure a combination of high yields of this important agricultural crop with a longer storage period of tubers and an in-crease in their nutritional value [5].

According to published scientific data, spontaneous mutagenesis can be the most effective breeding method for obtaining new lines of potato (*Solanum tu-berosum L.*) [6]. The appearance of "somatic" mutations can be caused by the activation of transposons that cause a change in the expression of the plant's own genes, the appearance of point mutations. The use of such cells and tissues in in vitro culture enables researchers to obtain new variants of plants characterized by new economically valuable traits [7, 8, 9].

In this regard, the purpose of this study is to obtain somaclonal potato var-iants based on the Dutch selection "Aladdin" using an in vitro culture and evalu-ate them for such economically valuable traits as the content of starch, soluble sugars and protein.

Materials and Methods

The research was carried out on the basis of the Scientific Research Plat-form for Agricultural Biotechnology of NJSC "KATU named after S. Seifullin". Potato tubers of 9 somaclonal lines of the second reproduction of the Aladdin variety (A-R3-1) were used as the starting material. Leaf explants were used to in-duce primary potato regenerants. Cultivation was carried out on a nutrient medi-um of Murashige and Skoog [10] with modifications, mg/l: biotin, folic acid (0.01-0.05), NAA (0.1-0.5), 2,4-D (1 .0-3.0), kinetin (0.01-0.05). The medium for regeneration contained ferrulic acid (0.01-0.05), ascorbic acid (0.5-3.0), IAA (0.1-0.6), 6-BAP (0.5-2.5), IMC (0.1 -0.5).

For adaptation, regenerated plants were

transferred into pots with perlite sand for 10-15 days, then into pots with a mixture of peat and sand in a ratio of 3:1, and after rooting, into natural field conditions. First reproduction tubers were tested for phenotypic traits (flesh color, skin color) and selected samples were used to obtain second reproduction tubers, which were used to evaluate the content of free sugars, starch and total protein.

The content of starch in potato tubers was determined by acid hydrolysis [11]. Inverted sugar was determined using the DNS method [12].

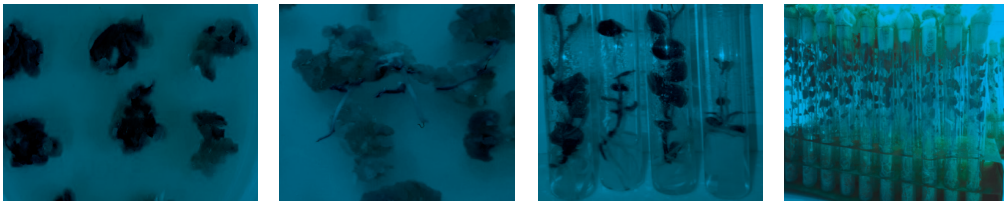
To determine the content of free (inverted) sugar, which is represented in potato tubers by a mixture of glucose (up to 65%) and fructose, the glucose oxi-dase method was used using the Vital commercial clinical kit [13].

Protein content was determined by alkaline lysis [11]. The amount of pro-tein was determined by the Lowry method [14].

For each variant of biochemical analysis, one potato tuber was used; meas-urements were carried out in triplicate. The calculation of the amount of protein and starch was carried out relative to 100 g of fresh weight of tubers and ex-pressed in grams and percent, respectively. For statistical data processing, Excel Microsoft 2007 was used.

Results

Based on a comparative analysis of the results of testing 9 nutrient media with the addition of various components for the induction of callus and organo-genesis, the most optimal options were selected from the leaf explants of the Alladin potato variety (results not shown). The maximum number of explants in which callus induction was observed was noted on MS-4 and MS-6 media. It was on this basis that MS-6 medium was chosen for further experiments, under which conditions callus formation was observed in all cultured explants (100%) on days 7–8 (Figure 1, A). MS-5 medium was chosen as the optimal medium for organo-genesis from the obtained potato calli, on which the highest percentage (92%) of the yield of regenerated plants was noted (Figure 1 B and C). It should be noted that organogenesis in this environment did not require additional manipulations to induce rhizogenesis.



A B C D

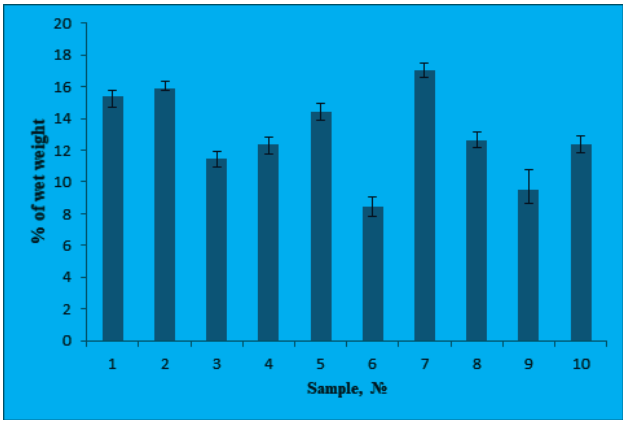
(A), regeneration in potato culture (B) and regenerative plants induced from them (C), obtained microclones of regenerative plants for rooting in soil (D)

Figure 1 – Callusogenesis in in vitro culture from leaf explants of Aladdin potato variety

As a result of the studies, 815 regenerated plants were rooted (Figure 1, D), from which, after their transfer to the field, 3548 tubers of the first reproduction were obtained. Based on the results of the morphological analysis, samples were selected that differed from each other and from the control in the color of the pulp (white in the control, pale yellow and yellow in the samples) and the color of the peel (light purple uniform in the control, light purple with dark purple eyes and deep purple in samples).

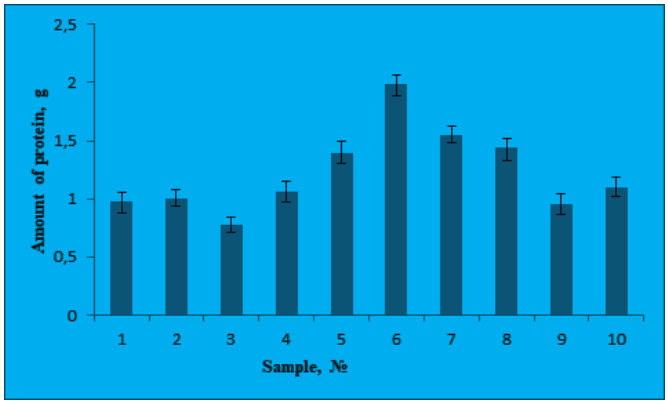
So, as a result of the studies, the optimal composition of the media for the induction of callusogenesis and organogenesis was selected when cultivating leaf explants of potato variety "Aladdin" in vitro, the tubers of the first reproduction were tested according to morphological characteristics, 9 initial lines were select-ed that differ in these characteristics from the control (variety "Aladdin"). The tu-bers of the second reproduction were used to analyze some biochemical parame-ters - the amount of starch, protein and sugar content.

Figure 2 shows the results of testing 9 somaclonal potato lines for starch content.



K-control (variety "Aladdin"); 1-9 - somaclonal lines; ns - notsignificant; * p < 0.05; **p<0.01.
Figure 2 – Total starch content in somaclone potato tubers obtained on the basis of the Aladdin variety, (%)

As can be seen in the presented diagram (Figure 2), among the samples of potato tubers of somaclonal lines, variability in starch content was observed. The range of variability for this trait ranged from 8.4% to 17%. Two lines (No. 1 and No. 6) exceeded the control value for the Aladdin variety (15%) in this indicator. It should be noted that line No. 6 was characterized by an atypical color of the tuber pulp, and line No. 1 was found to have an excess height of the aerial parts of plants compared to the control (data not shown). The obtained data are con-sistent with the literature data, for example, Slavin J.L. showed that fresh pota-toes contain about 20% dry matter, of which 60-80% is starch, and 70-80% of this starch is amylopectin. The dependence of the starch content in potatoes on the genotype and growing conditions was shown [16].



K-control (variety "Aladdin"); 1-9 - somaclonal lines; ns - notsignificant; * p< 0,05
Figure 3 – Total protein content in somaclone potato tubers obtained on the basis of the Aladdin variety, (g/100g fresh weight)

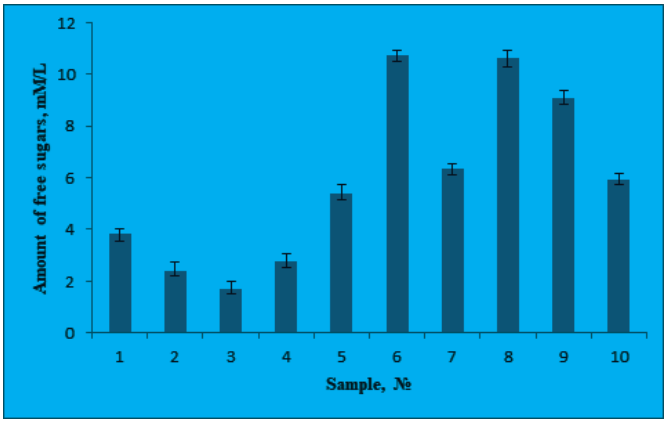
According to such an indicator as the protein content in tubers, the studied samples of somaclonal potato lines varied from 0.78 g to 1.96 g. As can be seen in the presented diagram (Figure 3), most of the studied samples in this indicator exceeded the control values, which amounted to 0.97 g. The exception was sam-ples No. 2 (0.78 g) and No. 8 (0.97 - at the control level). According to modern data, potato protein is considered as the main contender for hypoallergenic and is equated with dietary foods [17]. According to Gorissen et al. potato protein con-tains on average 39.5% higher essential amino acids compared to wheat, soy, corn and eggs [18].

One of the important characteristics that researchers focus on when creat-ing new varieties of potatoes is the content of free sugars in the pulp of tubers. It is from this indicator that the keeping quality of potato tubers directly depends and, accordingly, the increase in the duration of their

shelf life. Free sugars in po-tatoes are represented by a mixture of easily water-soluble sugars (glucose, fruc-tose, sucrose, maltose), as well as their phosphate esters.

Based on the fact that, according to international standards, units of mM/l are accepted to characterize the content of free sugars in potato tubers and the maximum range for this indicator is determined from 83-110 mM/l, the excess of which is not desirable for breeders, we used these units to characterize somaclonal variants to exclude potato lines whose free sugar content exceeds the recommended values.

Figure 4 below shows data on the content of the level of free sugars in the obtained somaclonal variants, compared with the control.



K-control (variety "Aladdin"); 1-9 - somaclonal lines; ns - notsignificant;
* p<0,05; **p<0,01; *** p<0,001.

Figure 4 – The total content of free sugar in somaclone potato tubers obtained on the basis of the Aladdin variety, (mM/l)

The results of assessing the content of free sugars in potato tubers of the studied somaclonal lines revealed only three samples (No. 1-No. 3), in which the values of this indicator were lower than the control (3.84) and ranged from 1.72 to 2.77 mM/l. The remaining samples of tubers in terms of the content of free sugars exceeded the control values. In samples of such lines as No. 5, No. 7 and No. 8, the excess in these indicators was 2.5-3.0 times higher compared to the control. Based on foreign data, the amount of sugar is affected by the stage of de-velopment of potatoes, for example, *Chen, J. Y. et al.* showed the dependence of sugar content in tubers at different stages of tuber maturation, so the highest con-tent of sucrose, glucose and fructose (7.65; 2.22 and 0.37 mM/l, respectively) was noted 21 days after the onset of tuberization, the number of which decreases by the rate of tuber development. It was found that the average tuber weight nega-tively correlates with the content of sucrose, reducing sugars (glucose and fruc-tose), and total sugars [19].

Discussion

Based on the data of domestic and foreign researchers, it is known that starch has a direct effect on the storage and keeping quality of tuber material, as well as the quality indicators of potatoes [20, 21, 22]. The starch content depends on the precocity of varieties, so late-ripening varieties are characterized by maxi-mum values for this indicator. During storage, the amount of starch in the tubers decreases as a result of its hydrolytic decomposition to sugars.

The level of sugar content in potato tubers directly affects the quality of the finished product and its stability during storage. During storage, the interaction of reducing sugars and amino acids produces melanoidins. This group of sub-stances causes a darkening of the product, a deterioration in taste, a decrease in the content of vitamins. A noticeable deterioration in the quality of the finished product occurs when the sugar content is 5–6%, which in units according to in-ternational standards corresponds to 83-110 mM/l [23].

The protein content in potatoes is on average 2 g. 2/3 of the protein sub-stances of potatoes are tuberin, which contains almost all essential amino acids, which makes it an important dietary product.

The starch content in potato tubers varies depending on the variety and line - a varietal trait. Similar studies were carried out by Burlov

S.P. et al. among the mid-ripening varieties and hybrids studied by them, the maximum value was not-ed in the variety of the Russian selection Granat - 17.9%, Krinitsa - 19.6%, Zeku-ra - 16.7% Ladozhsky - 17.2%, as well as in the hybrid 22009 - 16.6% [14].

In terms of protein content, potatoes are superior to many other agricultur-al crops. Depending on the variety, the protein content ranges from 1.0 to 2.9%. Potato protein contains 19 amino acids necessary for the human body, including lysine, methionine, threonine, and tryptophan [24]. In the studied variety Alad-din, the protein content was less than 1%, while in the somaclonal variant No. 5 this figure reached 1.97%. In the works of Russian authors, the protein content in the tubers of the varieties Krinitsa, Ladozhsky, Sarma is 1.94%. According to re-cent studies, potato protein is attracting increasing attention as a source of pro-tein for human consumption, especially as a source of hypoallergenic protein and a selective component against cancer cells. There is growing evidence that potato protein can be used in many future nutraceuticals and food products [9].

Based on the data obtained, line No. 2 of the Aladdin variety can be used for further study on the consolidation of the obtained traits, as well as for the creation of new dietary and table varieties of potatoes adapted for growing in the conditions of Northern Kazakhstan.

Conclusion

Based on the studies, it becomes obvious that among the 9 studied somaclonal potato lines obtained by in vitro cultivation of leaf explants of the Aladdin variety, four lines (No. 4-No. 7) are of interest in terms of increased pro-tein content in tubers, and, therefore, and nutritional value per 100 g of product. According to the content of starch, as the main indicator of potato quality, lines No. 1 and No. 6 are of interest for further research. Whereas, in terms of such an indicator as the content of free sugars, three lines (No. 1-No. 3) are of interest, in which these values are minimal compared to the control and allow us to hope for the possibility of increasing the storage time of tubers with further selection re-finement of these lines.

The studied samples of lines No. 1 and No. 6 are of interest for their further use as table and dietary varieties, since these samples differ significantly from the control variant in the content of starch and proteins. In addition, these options also contain a relatively low amount of sugars, which will positively affect the keeping quality of tubers. Sample No. 2 with the lowest sugar content, a slightly lower amount of protein and starch compared to the control, can also be used to create a table potato variety.

As a result of the research, 9 somaclonal variants of the Aladdin potato va-riety were obtained and analyzed in terms of nutritional characteristics. The re-sulting somaclones differed in biochemical composition. In terms of starch content, the values ranged from 8.4% to 17%. Two lines (No. 1 and No. 6) exceeded the control value for the Aladdin variety (15%) in this indicator.

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КАРТОПТЫҢ IN VITRO ӨЗГЕРГІШТІГІ ЖӘНЕ ОНЫҢ ТҮЙНЕКТЕРІНІҢ ТАҒАМДЫҚ ҚАСИЕТТЕРІНЕ ӘСЕРІ

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Түйін

«Аладдин» голланд селекциясының тоғыз сомаклоналды картоп нұсқасын сынау нәтижелеріне гормондардың, витаминдердің және өсу стимуляторларының әртүрлі концентрациясы бар модификацияланған Мурашиге-Скуга ортасын қолдану арқылы салыстырмалы талдау жұмыстары жүргізілді. Жүргізілген жұмыстардың нәтижесінде картопты *in vitro* өсірудің оңтайлы хаттамалары таңдалып алынды және онда жапырақ эксплантарынан 100% каллус түзілуін және тамырланумен байланысты қосымша қадамдарды қолданбай 90% астам өркеннің түзілуін қамтамасыз ететіні анықталды. Осы хаттамаларды пайдалана отырып, голландиялық «Аладдин» селекциясының картоп сортының негізінде бірінші репродукцияның регенерант өсімдіктері алынды және олардың ішінде морфологиялық сипаттамаларына сәйкес 9 бастапқы түрі таңдалды. Осы бастапқы түрден алынған екінші репродукцияның түйнектері биохимиялық көрсеткіштері (крахмал, еритін қанттар, белоктар) талданды. Нәтижесінде крахмал мөлшері ең жоғары түрлер анықталды, 9 түрдің ішінде 2 зертетелген картоптың түрлерінде крахмал мөлшері бойынша

бақылау үлгісінен 3,3% және 10,7% артық екені көрсетілді. Зерттелетін сомаклондық 7 нұсқаның түйнектеріндегі ақуыз мөлшері 1%-дан 10,6%-ға дейінгі көрсеткіш көрсетіп, бақылаудағы көрсеткіштерден жоғары болды.Зерттелген 3 үлгіде қант мөлшері бақылаумен салыстырғанда айтарлықтай төмен болды (37,9-дан 54%-ға дейін). Зерттеу барысында алынған нәтижелер картоптың жаңа диеталық және асханалық сорттарын шығарусаласында және селекциялық зерттеулер үшін үлкен тәжірибелеік қызығушылық туғызып отыр. Алынған сомаклондық нұсқалар негізінде Солтүстік Қазақстанның топырақ-климаттық жағдайына бейімделген жаңа перспективалы картоп сорттарын алуға болады.

Кілтті сөздер: картоп, *Solanum tuberosum, in vitro,* каллусогенез, органогенез, сомаклоналды өзгергештік, крахмал,жалпы ақуыз, байланыспаған қанттар.

ИЗМЕНЧИВОСТЬ КАРТОФЕЛЯ IN VITRO И ЕЕ ВЛИЯНИЕ НА ПИЩЕВЫЕ СВОЙСТВА КЛУБНЕЙ

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Аннотация

Проведен сравнительный анализ результатов тестирования девяти сомаклональных вариантов картофеля сорта голландской селекции «Алладин» с использованием модифицированной среды Мурасиге-Скуга с различной концентрацией гормонов, витаминов и стимуляторов роста. В результате проведенной работы были подобраны оптимальные протоколы для культивирования картофеля *in vitro,* обеспечивающие 100% образование каллуса из листовых эксплантов и более 90% образования побегов без применения дополнительных этапов, связанных с их укоренением. С использованием данных протоколов на основе сорта картофеля голландской селекции «Алладин» получены растения-регенеранты первой репродукции, среди которых по морфологическим признакам отобрано 9 исходных линий. Клубни второй репродукции, полученные от этих линий, были использованы для проведения анализа по биохимическим показателям (крахмал, растворимые сахара, белки). В результате были выделены линии с максимальными значениями содержания крахмала, из 9 линий 2 линии превышали по содержанию крахмала контрольный образец на 3,3% и на 10,7%. Содержание белка в клубнях 7 сомаклональных вариантов от 1% до 10,6% превышало данный показатель в контроле. В 3-х исследованных образцах содержание сахаров было значительно ниже (от 37,9 до 54%) по сравнению с контролем. Полученные результаты представляют большой практический интерес для дальнейших селекционных исследований по их закреплению и созданию новых диетических и столовых сортов картофеля. На основе полученных сомаклональных вариантов могут быть получены адаптированные для почвенно-климатических условий Северного Казахстана новые перспективные сорта картофеля.

Ключевые слова: картофель; *Solanum tuberosum;* культура in vitro; сомаклональная изменчивость; крахмал; общий белок; свободные сахара.