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Analysis of diversity and genetic interactions of potato varieties (*Solanum tuberosum* L.) based on morphological characteristics and SSR markers

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Purpose. To estimate a correlation between SSR-markers and morphological features by genetic distances on the basis of polymorphism of potato varieties. **Methods.** PCR analysis, cluster and correlation-regression analysis. **Results.** The results of analysis of potatoes varieties polymorphism based on molecular genetic and morphological marker features are presented. Varieties polymorphism was assessed on four microsatellite markers: STM0019, STM3009, STM3012, STM5136 and 42 morphological markers. According to the results of PCR analysis, it was determined that the frequency of identified alleles ranged from 0.021 to 0.33, the index of polymorphism on the average for studied markers was 0.76. Using cluster analysis, the genetic distances between varieties based on SSR and morphological markers were determined, and similar varieties and varieties that differ among themselves were identified. According to the obtained distribution, the correlation between the genetic distances by Mantel test are estimated, namely, a regression, in which the variables are similarity matrices, summing the pairwise similar values between the sampling points was defined. As a result of the estimation of genetic distances between the investigated genotypes of potatoes, it was revealed that the varieties 'Skarbnytsia' and 'Yavir' were the most similar by markers STM0019, STM3009, STM3012, STM5136. The distance between these varieties was 2.45, while the greatest distance – 3.74 was found among the varieties 'Levada' and 'Dovira', 'Fantaziia' and 'Dovira'. According to marker morphological signs, the least value of genetic distances was 8.9 for varieties 'Slovianka' and 'Poliske Dzherelo', the most distant ones were 'Okolytsia' and 'Dovira', with values of 18.2. It was revealed that the potato variety 'Dovira' with the value of genetic distances of 18.2 and 3.74 for morphological and SSR markers respectively was the most distant from other investigated genotypes. The correlations were not found by Mantel test as a result of the analysis of genetic distances based on SSR-markers and morphological signs. **Conclusions.** The use of a complex of studies containing a description of morphological features and microsatellite markers is promising for identifying potato varieties, creating collections of well-known varieties and determining their differences.

Keywords: *potatoes; genetic polymorphism; cluster analysis; Mantel test; correlation links.*

Introduction

Potatoes (*Solanum tuberosum* L.) is the main source of nutrition in many regions of the world and characterized by high levels of carbohydrates, proteins and vitamin C [1–3]. Genetic differentiation between individual genotypes or populations is evaluated at the level of quantitatively inherited phenotypic features, monogenic characteristics that are subjected to selective pressure (for example, signs of disease resistance), molecular markers, etc. Information on correlations that exist between different levels is important for several reasons. In particular, from an evolutionary point of view, the study of links between genetic diversity and morphological differentiation will help to find the mechanisms responsible for this differentiation. While from the standpoint of conservation of genetic resources – it promotes the search for similar signs of different genotypes or populations and combinations of genes, and predicts their heterosis based on selection [4–5]. Annually in

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Ukraine state testing for distinctness, uniformity and stability (DUS) of about 40 varieties of potatoes is held. At the same time, the increase in the number of new varieties introduced into production causes the need to control the diversity of modern varieties through reliable and modern methods for their differentiation.

Over the past thirty years, the results of the study of interaction between morphological features and DNA markers have been highlighted in a number of publications [6–8]. European organizations for plant variety evaluation, such as GEVES (France) and Naktuinbouw (the Netherlands), use the definition of correlations for the evaluation of new varieties and the formation of their collections [6, 8–12].

The purpose of the research is to assess the correlation between SSR markers and morphological features by genetic distances based on the polymorphism of potato varieties.

Materials and methods

Twelve varieties of potato selected at the Institute of Potato Studies of the National Academy of Sciences of Ukraine were studied: ‘Poliske Dzherelo’, ‘Dorogyn’, ‘Oktolytsia’, ‘Dovira’, ‘Lileia’, ‘Levada’, ‘Yavir’, ‘Chervona ruta’, ‘Skarbnytsia’, ‘Obrii’, ‘Fantaziia’ and ‘Slovianka’.

The morphological features of potato varieties were described in the course of varieties testing for the purpose of their state registration. The DUS method numbers 42 marker signs of potato varieties, which cover the qualitative and quantitative characteristics of the sprouts grown by light, stems, leaves, buds, inflorescences and tubers. Varieties were evaluated using the method of visual assessment, measurements and calculations, depending on the type of signs detection using the DUS method [13]. According to the method, the degree of signs detection was denoted by numerical values from 1 to 9. As a result of digital coding, matrices were constructed on which genetic distances were calculated.

DNA was isolated from two-week sprouts of 500 mg potato varieties with CTAB (cetyltrimethylammonium bromide), purified with chloroform-isoamilic alcohol and precipitated with isopropyl alcohol [13–15]. Extracted DNA was stored at a temperature of +4 °C during the process.

The molecular genetic polymorphism of potato varieties with specific primers: STM0019, STM3009, STM3012, STM5136 selected on the basis of the analysis of the polymorphic index [12, 14–17] (Table 1), was investigated based on four microsatellite loci (MC loci).

The reaction mixture contained 100 ng DNA, the component concentration was: 1 Buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.01% Triton X-100), 2.5 mM $MgCl_2$; 200 µM deoxynucleotide triphosphates (dNTF), 0.5 µM of each of the primers and 1 unit. act. Taq polymerase. The total volume of the mixture was 20 µL.

PCR (Polymerase Chain Reaction) was performed using the BioRad IQ5 (USA) amplifier. Parameter for amplification for the examined potato markers: initial denaturation – 95 °C – 5 minutes, 40 cycles: denaturation – 45 s for 95 °C, hybridization – 30 s for 50–60 °C, elongation – 90 s for 72 °C, final elongation – 7 minutes for 72 °C. The visualization of the amplification reaction products was carried out by electrophoresis in a 4% agarose gel in 0.5 x TBE (tris borate buffer solution) according to the method [15, 20] for the intensity of the electric field of 5 V/cm. The size of ampilicons was determined using the TotalLab v.2.01 computer program.

On the basis of definite alleles, PIC (polymorphism information content) was calculated and binary data matrices were produced, with “1” for presence of the corresponding allele and “0” for its absence. The genetic distances between the studied varieties were determined using a computer program STATISCA 12 (trial version) based on cluster analysis. Varieties were grouped by SSR-markers with the use of unweighted pair-group method using arithmetic averages (UPGMA), by morphological features with the use of Single Linkage method with the calculation of Euclidean distances [21–24].

Table 1

Characterization of SSR loci primers

MC locus	Primer sequence 5′ ……. 3′	GC-content, %	Motif	Hybridization temperature, °C	Expected amplicon size, bp
STM0019	F – aataggtgtactgactctcatg	39,1	(AT)7, (AT)10, (AT)4, (GT)5, (GC)4, (GT)4	50	99–206
	R – ttgaagtaaaagtcctagtatgtg	33,3			
STM3009	F – tcagctgaacgaccactgttc	45,5	(TC)13	50	110–140
	R – gatttcaccaagcatggaagtc	52,4			
STM3012	F – caactcaaaccagaaggcaaa	42,9	(GT)4, (CT)8	60	180–225
	R – gagaaatgggcacaaaaaaca	38,1			
STM5136	F – gggaaaaggaaaagctcaa	42,1	(AGA)5	60	240–280
	R – caacactatcgccatctccttt	45,8			

The correlation between the investigated SSR markers and the morphological characteristics of potato varieties was determined based on genetic distances using the Mantel test with XLSTAT 2018 computer program [25–26].

Results

Alleles of the expected size were obtained by PCR on four SSR markers with specific primers (Fig. 1).

According to obtained amplicons with STM3012 marker, 12 alleles of sizes 175–224 bp were identified among the studied varieties. For the other investigated loci from five (markers STM3009, STM5136) to twenty alleles (marker STM0019) were identified. The allele frequencies were from 0.021–0.21 for the STM3012 marker, and 0.08–0.33 for the STM3009 and STM0019 markers (Fig. 2). It was found that the greatest number of alleles – 20 was identified by the marker STM0019, of sizes 98–258 bp. For the markers STM3009 and STM5136, five alleles with size range of 164–172 and 240–267 bp respectively were determined.

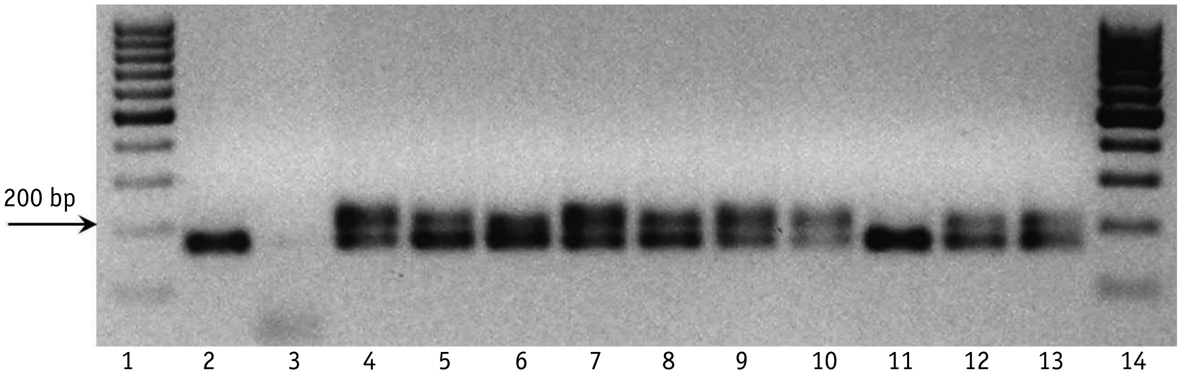


Fig. 1. Electropherogram of DNA amplification products of different potato varieties based on STM3012 marker: 1, 14 – molecular weight marker 100 bp DNA Ladder O'GeneRuler (Thermo Scientific); 2 – 'Poliske Dzherelo'; 3 – 'Dorohyn'; 4 – 'Okolytsia'; 5 – 'Dovira'; 6 – 'Lileia'; 7 – 'Levada'; 8 – 'Yavir'; 9 – 'Chervona Ruta'; 10 – 'Skarbnytsia'; 11 – 'Obrii'; 12 – 'Fantaziia'; 13 – 'Slovianka'

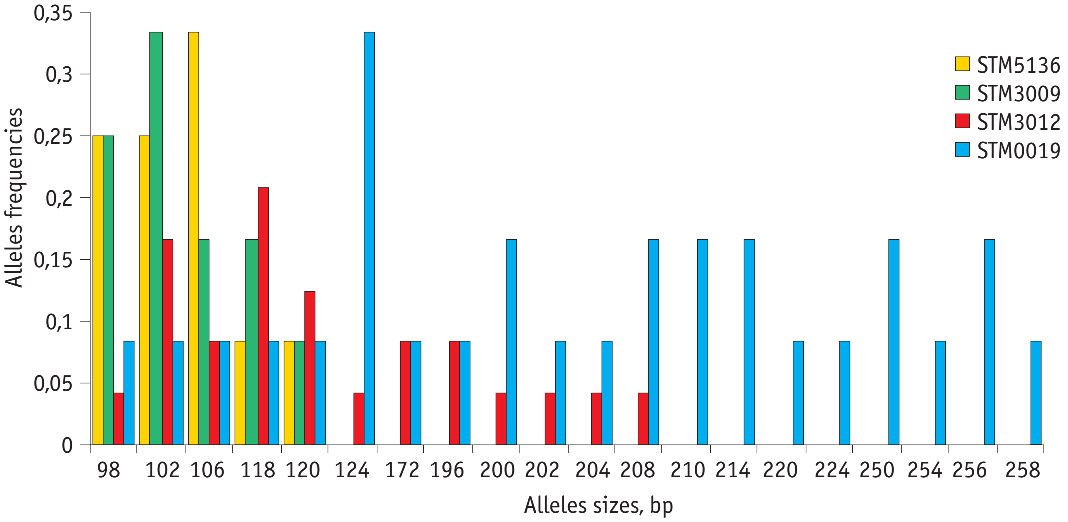
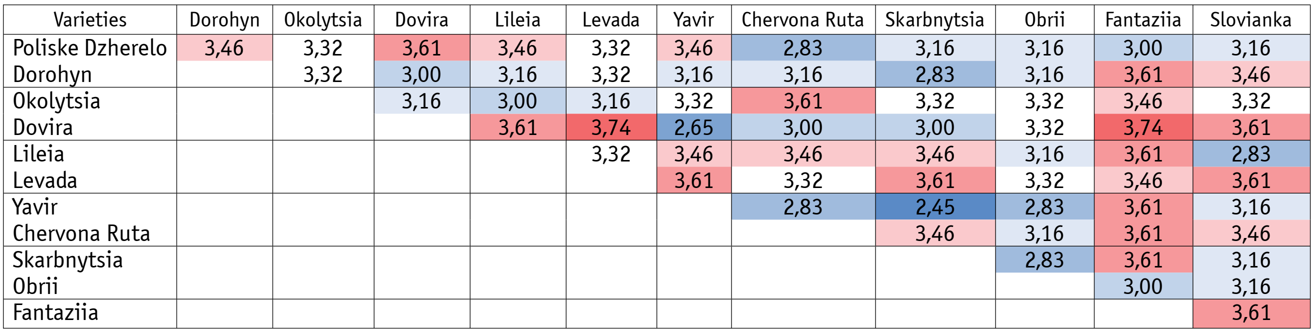


Fig. 2. Distribution of identified alleles by size and frequency for the investigated potato varieties based on STM0019, STM3009, STM3012, STM136 markers

For the studied loci, the average value of the polymorphism index of the locus (RIS) is 0.76, with the highest index obtained for the marker STM3012 – 0.88. The listed level of PIC indicates a uniform distribution of identified alleles and sufficient resolution for differentiation of investigated potato varieties.

To study the polymorphism of 12 varieties of potatoes based on SSR markers and morphological features, a cluster analysis was performed and genetic distances between varieties were calculated (Fig. 3).

As a result of cluster analysis and calculation of genetic distances between studied potato varieties, the greatest distance was noted between 'Levada' and 'Dovira', 'Fantaziia' and 'Dovira' varieties – 3.74. Considering that with the decrease in the digital value of genetic dis-



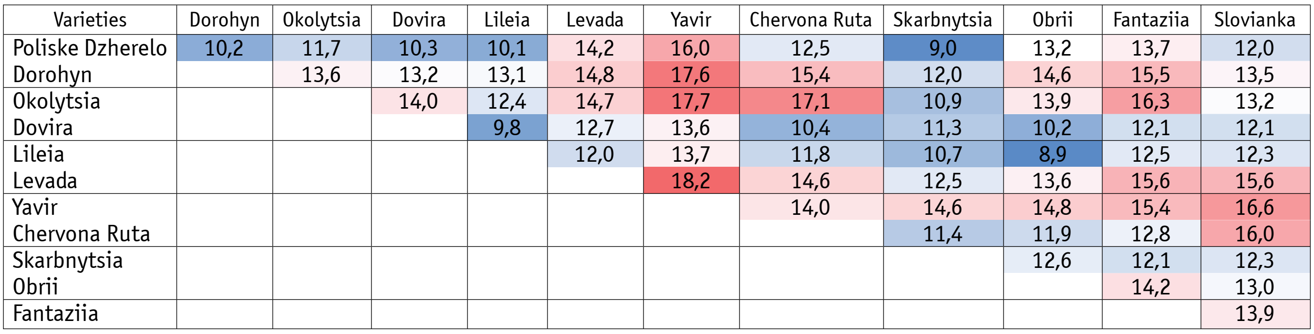
Varieties	Dorohyn	Okolytsia	Dovira	Lileia	Levada	Yavir	Chervona Ruta	Skarbnytsia	Obrii	Fantaziia	Slovianka
Poliske Dzherelo	3,46	3,32	3,61	3,46	3,32	3,46	2,83	3,16	3,16	3,00	3,16
Dorohyn		3,32	3,00	3,16	3,32	3,16	3,16	2,83	3,16	3,61	3,46
Okolytsia			3,16	3,00	3,16	3,32	3,61	3,32	3,32	3,46	3,32
Dovira				3,61	3,74	2,65	3,00	3,00	3,32	3,74	3,61
Lileia					3,32	3,46	3,46	3,46	3,16	3,61	2,83
Levada						3,61	3,32	3,61	3,32	3,46	3,61
Yavir							2,83	2,45	2,83	3,61	3,16
Chervona Ruta								3,46	3,16	3,61	3,46
Skarbnytsia									2,83	3,61	3,16
Obrii										3,00	3,16
Fantaziia											3,61

Fig. 3. Genetic distances between investigated potato varieties based on SSR markers
(the color reflects genetic distances in descending order where blue (red) denotes large (small) distances, respectively)

tances the affinity between varieties increases, the genotypes with the value of 2.45 – 'Skarbnytsia' and 'Yavir' were the most close ones. Among other varieties, the values of genetic distances ranged from 3.61 to 2.65.

It is known that objects of cluster analysis with zero values of genetic distances are considered to be identical. Taking into account the obtained data, the studied varieties are different according to the proposed marker system. Taking into account that the investigated varieties of potatoes are the result of selective work of one institution, the proposed marker system is effective for the differentiation of closely related objects.

According to the set of marker morphological characters of the studied potato varieties, a cluster analysis was performed and genetic distances were calculated (Fig. 4).



Varieties	Dorohyn	Okolytsia	Dovira	Lileia	Levada	Yavir	Chervona Ruta	Skarbnytsia	Obrii	Fantaziia	Slovianka
Poliske Dzherelo	10,2	11,7	10,3	10,1	14,2	16,0	12,5	9,0	13,2	13,7	12,0
Dorohyn		13,6	13,2	13,1	14,8	17,6	15,4	12,0	14,6	15,5	13,5
Okolytsia			14,0	12,4	14,7	17,7	17,1	10,9	13,9	16,3	13,2
Dovira				9,8	12,7	13,6	10,4	11,3	10,2	12,1	12,1
Lileia					12,0	13,7	11,8	10,7	8,9	12,5	12,3
Levada						18,2	14,6	12,5	13,6	15,6	15,6
Yavir							14,0	14,6	14,8	15,4	16,6
Chervona Ruta								11,4	11,9	12,8	16,0
Skarbnytsia									12,6	12,1	12,3
Obrii										14,2	13,0
Fantaziia											13,9

Fig. 4. Genetic distances between investigated varieties of potatoes based on morphological markers
(the color reflects genetic distances in descending order where blue (red) denotes large (small) distances, respectively)

The investigation revealed that the most distant by morphological features are 'Okolytsia' and 'Dovira' varieties, the numerical value of which was 18.2. 'Lileia' and 'Dorogyn' with distances of 17.6 and 17.7, respectively, were quite distant from 'Okolytsia'. The most related varieties were 'Slovianka' and 'Poliske Dzherelo' and 'Chervona Ruta' and 'Levada' with values of genetic distances of 8.9 and 9.0. 'Lileia', 'Skarbnytsia' and 'Poliske Dzherelo' were quite close to 'Levada' (10.1–10.3).

Cluster analysis according to selected indicators shows a different distribution of genotypes and their degree of affinity. To determine the objective approaches to the examination of varieties, a complex evaluation of different characteristics is required. The correlation between genetic distances obtained by SSR analysis and morphological features was evaluated using Mantel test (Pearson's correlation) [9]. As a result of the analysis, the p-value and r(AB) indices for the significance level $\alpha = 0.05$ were determined, which according to the interpretation of the test, allows to accept one of the analysis hypotheses of the presence (Ha) or absence of correlation (H0) (Fig. 5).

According to the analysis, the H0 hypothesis about the absence of correlation is assumed under the condition $p > \alpha$, that is the calculated value $p = 0.765$ significantly exceeds the significance level $\alpha = 0.05$.

Therefore, the alternative hypothesis Ha of the presence of correlation was rejected and the zero hypothesis H0 [6, 27–28] was adopted. The correlation coefficient [r(AB)] and the normality of the data distribution along the matrixes of genetic distances are presented in Figure 6. The diagram form indicates the normality of the data distribution and the reliability of the sample, the correlation coefficient by Mantel was $r = 0.035$. According to the hypothesis H0, a reliable correlation by the Mantel test between the genetic distances for SSR

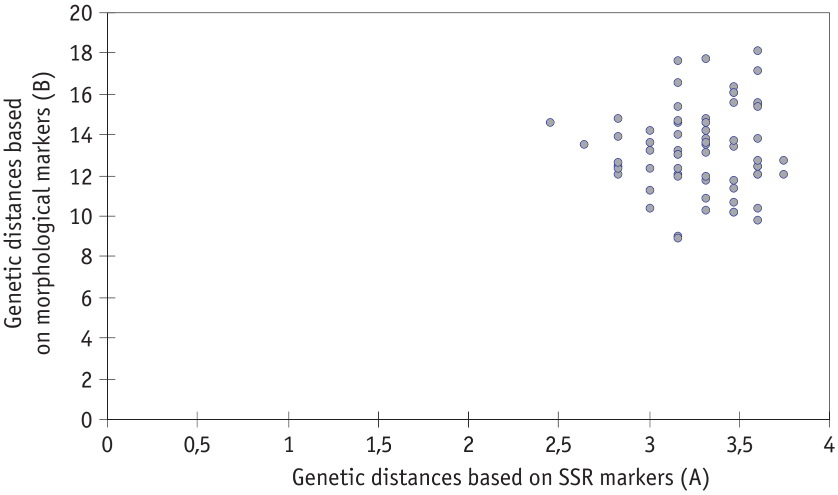


Fig. 5. Correlation between genetic distances based on SSR markers and morphological features of potato varieties

markers and morphological features in the investigated varieties of potatoes was not detected. The results of the absence and presence of correlations between genetic distances were obtained by Karuri H. W. et al. [29] during the study of the genetic diversity of sweet potato varieties, as well as Darvishzadeh R. [30] in the study on sunflower varieties.

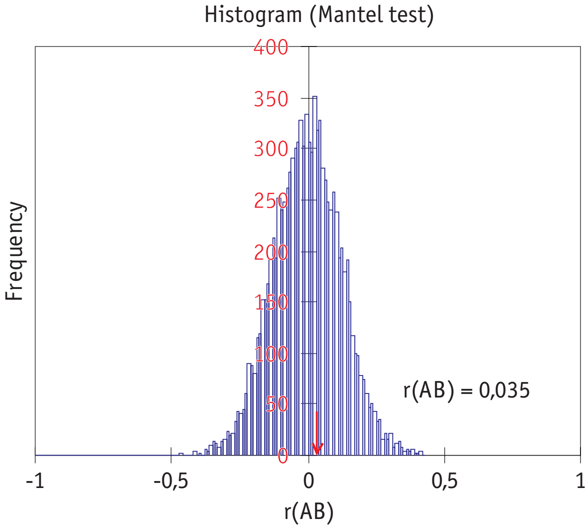


Fig. 6. Results of graphical analysis of data distribution by Mantel test results for potatoes varieties by genetic distances

However, the distribution obtained by genetic distances allowed detecting the difference between the studied potato varieties. Consequently, due to the analysis of genetic distances for the four SSR markers and morphological features, it is determined that the most distant from the two marker systems is 'Dovira variety'. For other studied varieties, differentiation by these criteria varied, which indicates the possibility of applying different approaches to the definition of varietal differences.

Conclusions

The molecular genetic polymorphism of 12 varieties of potatoes based on four microsatellite markers and 42 morphological features used for the description of the variety during the examination on DUS was investigated.

According to the results of the cluster analysis, it was revealed that the varieties 'Skarbnytsia' and 'Yavir' were the most similar by loci STM0019, STM3009, STM3012, STM5136; the genetic distance between them was 2.45. The obtained results were used to create a database of molecular genetic polymorphisms of the studied potato varieties for further identification.

Regarding the distribution of varieties according to morphological features, it was found that the lowest value of the genetic distance was 8.9 between 'Slovianka' and 'Poliske Dzherelo'. The most distant, both morphologically and by SSR markers, was 'Dovira' variety with a genetic distance of 18.2 and 3.74, respectively.

Using the Mantel test, it was found that there is no correlation between the genetic distances for SSR analysis and marker morphological features. The results of the assessment of the varieties distribution allow the use of studies containing a description of morphological features and microsatellite markers for the creation of collections of well-known varieties and determination of varietal differences in the process of DUS testing.

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Використана література

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Присяжнюк Л. М.[1*]**, Кляченко О. Л.**[2]**, Діхтяр І. О.**[1]**, Симоненко Н. В.**[1] Аналіз різноманіття та генетичних взаємодій сортів картоплі (*Solanum tuberosum* L.) на основі морфологічних ознак та SSR-маркерів. *Plant Varieties Studying and Protection*. 2018. Т. 14, № 3. С. 277–284. https://doi.org/10.21498/2518-1017.14.3.2018.145292

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Мета. Оцінити кореляційні зв’язки між SSR-маркерами та морфологічними ознаками за генетичними дистанціями на основі поліморфізму сортів картоплі. **Методи.** ПЛР-аналіз, кластерний та кореляційно-регресійний аналізи. **Результати.** Наведено результати аналізу поліморфізму сортів картоплі за молекулярно-генетичними та морфологічними маркерними ознаками. Поліморфізм сортів оцінювали за чотирма мікросателітними маркерами: STM0019, STM3009, STM3012, STM5136 та за 42 морфологічними маркерними ознаками. За результатами ПЛР-аналізу визначено, що частота ідентифікованих алелів становила від 0,021 до 0,33, індекс полімофності в середньому за досліджуваними маркерами – 0,76. За допомогою кластерного аналізу визначено генетичні дистанції між сортами за SSR- та морфологічними маркерами, виявлено подібні та сорти, які різнилися між собою. Відповідно до отриманого розподілу оцінено кореляційні зв’язки між генетичними дистанціями за Mantel test, а саме визначено регресію, в якій змінними є матриці подібності, що підсумовують попарні подібні значення між місцями вибірки. Унаслідок оцінювання генетичних дистанцій між дослідженими генотипами картоплі встановлено, що найподібнішими за маркерами STM0019, STM3009, STM3012, STM5136 виявилися сорти ‘Скарбниця’ та ‘Явір’, відстань між якими становила 2,45, тоді як найбільшу відстань – 3,74 виявлено між сортами ‘Левада’ та ‘Довіра’, ‘Фантазія’ та ‘Довіра’. За маркерними морфологічними ознаками найменше значення генетичних дистанцій – 8,9 спостерігалося для сортів ‘Слов’янка’ та ‘Поліське джерело’, найвіддаленішими виявилися ‘Околиця’ та ‘Довіра’, значення для яких становить 18,2. Встановлено, що сорт картоплі ‘Довіра’ зі значенням показників генетичних дистанцій 18,2 та 3,74 за морфологічними та SSR-маркерами відповідно виявився найвіддаленішим від інших досліджених генотипів. У результаті проведеного аналізу генетичних дистанцій за SSR-маркерами та морфологічними ознаками кореляційних зв’язків за Mantel test не виявлено. **Висновки.** Застосування комплексу досліджень, які містять опис морфологічних ознак і мікросателітних маркерів є перспективним для ідентифікації сортів картоплі, створення колекцій загальновідомих сортів та визначення їх відмінностей.

Ключові слова: *картопля; генетичний поліморфізм; кластерний аналіз; Mantel test; кореляційні зв’язки.*

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Присяжнюк Л. М.[1*]**, Кляченко О. Л.**[2]**, Дихтяр И. А.**[1]**, Симоненко Н. В.**[1] Анализ разнообразия и генетических взаимодействий сортов картофеля (*Solanum tuberosum* L.) на основе морфологических признаков и SSR-маркеров // Plant Varieties Studying and Protection. 2018. Т. 14, № 3. С. 277–284.
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Цель. Оценить корреляционные связи между SSR-маркерами и морфологическими признаками по генетическим дистанциям на основе полиморфизма сортов картофеля. **Методы.** ПЦР анализ, кластерный и корреляционно-регрессионный анализы. **Результаты**. Приведены результаты анализа полиморфизма сортов картофеля по молекулярно-генетическим и морфологическим маркерным признакам. Полиморфизм сортов картофеля оценивали по четырем микросателлитным маркерам: STM0019, STM3009, STM3012, STM5136 и 42 морфологическим маркерными признакам. По результатам ПЦР-анализа определено, что частота идентифицированных аллелей составляла от 0,021 до 0,33, индекс полимофности локуса в среднем по исследованным маркерам – 0,76. С помощью кластерного анализа определены генетические дистанции между сортами по SSR- и морфологическим маркерам, отмечены сходные и различные сорта. В соответствии с полученным распределением проведена оценка корреляционных связей между генетическими дистанциями по Mantel test, а именно определена регрессия, в которой переменными выступают матрицы сходства, которые суммируют попарные сходные значения между местами выборки. В результате оценки генетических дистанций между исследованными генотипами картофеля установлено, что наиболее сходными по маркерам STM0019, STM3009, STM3012, STM5136 оказались сорта ‘Скарбныця’ и ‘Явир’, расстояние между которыми составляло 2,45, тогда как наибольшее расстояние – 3,74 отмечено между сортами ‘Левада’ и ‘Довира’, ‘Фантазия’ и ‘Довира’. По маркерным морфологическим признакам наименьшее значение генетических дистанций – 8,9 наблюдалось для сортов ‘Славянка’ и ‘Полиське джерело’, отдаленными оказались ‘Околыця’ и ‘Довира’, значение для которых составляет 18,2. Установлено, что сорт картофеля ‘Довира’ со значением показателей генетических дистанций 18,2 и 3,74 по морфологическим и SSR-маркерами соответственно оказался самым удаленным от других исследованных генотипов. В результате проведенного анализа генетических дистанций по SSR-маркерам и морфологическим признакам корреляционных связей по Mantel test не обнаружено. **Выводы.** Применение комплекса исследований, включающих описание морфологических признаков и микросателлитных маркеров является перспективным для идентификации сортов картофеля, создание коллекций общеизвестных сортов и определения их различий.

Ключевые слова: *картофель; генетический полиморфизм; кластерный анализ; Mantel test; корреляционные связи.*

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