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’, ‘Fantazia’ and ‘Dovira’. According to marker morphological signs, the least value of genetic distances was 8.9 for varieties ‘Slovianka’ and ‘Poliske Dzerelo’, the most distant ones were ‘Okolysia’ 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
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**


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-isoamyl 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 MgCl2; 200 µM deoxynucleotide triphosphates (dNTP), 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**

<table>
<thead>
<tr>
<th>MC locus</th>
<th>Primer sequence 5’…… 3’</th>
<th>GC-content, %</th>
<th>Motif</th>
<th>Hybridization temperature, °C</th>
<th>Expected amplicon size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>STM0019</td>
<td>F – aatagtgtagctgcactctag</td>
<td>39,1</td>
<td>(AT)7, (AT)10, (AT)4, (GT)5, (GC)4, (GT)4</td>
<td>50</td>
<td>99–206</td>
</tr>
<tr>
<td></td>
<td>R – ttgaatgtaagctgcactctag</td>
<td>33,3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STM3009</td>
<td>F – tcagctgaacgaccactgtt</td>
<td>45,5</td>
<td>(TC)13</td>
<td>50</td>
<td>110–140</td>
</tr>
<tr>
<td></td>
<td>R – gatttcaccaagcatggaagtc</td>
<td>52,4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STM3012</td>
<td>F – caactcaaaccagaaggcaaa</td>
<td>42,9</td>
<td>(GT)4, (CT)8</td>
<td>60</td>
<td>180–225</td>
</tr>
<tr>
<td></td>
<td>R – gagaaatggggcagaaaaaaca</td>
<td>38,1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>STM5136</td>
<td>F – gqaaaagggggcaggctg</td>
<td>42,1</td>
<td>(AGA)5</td>
<td>60</td>
<td>240–280</td>
</tr>
<tr>
<td></td>
<td>R – caacactacgcgccatctcttt</td>
<td>45,8</td>
<td></td>
<td></td>
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</tr>
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</table>
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.

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-
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).

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Dorohyn</th>
<th>Okolytsia</th>
<th>Dovira</th>
<th>Lileia</th>
<th>Levada</th>
<th>Yavir</th>
<th>Chervona Ruta</th>
<th>Skarbnytsia</th>
<th>Obrii</th>
<th>Fantaziia</th>
<th>Slovianka</th>
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<td>3.46</td>
<td>3.32</td>
<td>3.61</td>
<td>3.46</td>
<td>3.32</td>
<td>3.46</td>
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<td>3.16</td>
<td>3.16</td>
<td>3.00</td>
<td>3.16</td>
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<td>3.00</td>
<td>3.16</td>
<td>3.32</td>
<td>3.32</td>
<td>3.16</td>
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<td>3.16</td>
<td>3.16</td>
<td>3.61</td>
<td>3.46</td>
</tr>
<tr>
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<td>3.16</td>
<td>3.32</td>
<td>3.61</td>
<td>3.32</td>
<td>2.83</td>
<td>3.00</td>
<td>3.00</td>
<td>3.32</td>
<td>3.74</td>
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<td>3.74</td>
<td>2.85</td>
<td>3.00</td>
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<td>3.00</td>
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<tr>
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<td>2.83</td>
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<td>Yavir</td>
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<tr>
<td>Obrii</td>
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<td>3.61</td>
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<td>3.61</td>
<td>3.16</td>
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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 ‘Polskie Dzherelo’ and ‘Chervona Ruta’ and ‘Levada’ with values of genetic distances of 8.9 and 9.0. ‘Lileia’, ‘Skarbnytsia’ and ‘Polskie 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 (H$a$) or absence of correlation (H$0$) (Fig. 5).

According to the analysis, the H$0$ 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 H$a$ of the presence of correlation was rejected and the zero hypothesis H$0$ [6, 27–28] was adopted. The correlation coefficient $r(AB)$ and the normality of the data distribution along the matrices 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 H$0$, a reliable correlation by the Mantel test between the genetic distances for SSR
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. 5. Correlation between genetic distances based on SSR markers and morphological features of potato varieties](image)

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

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 ‘Skarpnytsia’ 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 Dzhe-
relo'. The most distinct, 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.

References


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8. Vinnytsia Lviivs’koho natsionalnoho universytetu. [in Ukrainian]


**Використана література**


2. Opipari T. M., Slobozjan C. O., Grzič P. R. Zasutrisanje mikro-...
Мета. Оцінити кореляційні зв'язки між ССР-маркерами та морфологічними ознаками за генетичними дистанціями на основі поліморфізму сортів картоплі. Методи. ПЦР-аналіз, кластерний та кореляційно-регресійний аналіз. Результати. Наведено результати аналізу поліморфізму сортів картоплі за молекулярно-генетичними та морфологічними маркерами. Поліморфізм сортів оцінювали за чотирма мікростатейними маркерами: STM0019, STM3009, STM3012, STM5136 та за 42 морфологічними маркерами ознаками. За результатами ПЦР-аналізу визначено, що частота ідентифікованих алелей становила від 0,021 до 0,33, індекс поліморфності в середньому до досліджуваних маркерів становив 0,76. За допомогою кластерного аналізу визначено генетичні дистанції між сортами за ССР- та морфологічними маркерами, виявлені подібні та сорти, які різились між собою. Відповідно до отриманого розподілу оцінено кореляційні зв’язки між генетичними дистанціями за Mantel test, а саме визначено регресію, в якій змінились 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 ‘Явир’, відстань між якими становила 2,45, тоді як найбільшу відстань – 3,74 виявили між сортами ‘Лева’ та ‘Довіра’, ‘Фантазія’ та ‘Довіра’. За маркерними морфологічними ознаками найменше значення генетичних дистанцій – 8,9 спостерігались для сортів ‘Слов’янка’ та ‘Поліське джерело’, найвіддаленишими виявилися ‘Околиця’ та ‘Довіра’, значення для яких становить 18,2.

Висновки. Застосування комплексу досліджень, які містять опис морфологічних ознак і мікростатейних маркерів є перспективним для ідентифікації сортів картоплі, створення колекції загальновідомих сортів та визначення їх відмінностей.

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