Review on Agriculture and Rural Development 2022 vol. 11 (1-2) ISSN 2677-0792
DOI: 10.14232/rard.2022.1-2.98-104

THE GENETIC DIVERSITY ASSESSMENT OF NEW POTATO VARIETIES OF DIFFERENT MATURITY GROUPS BY SSR MARKERS

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ABSTRACT
The purpose of study was to assess the genetic diversity of potato varieties of different maturity groups by SSR markers. Twenty-four potato varieties of different maturity groups (very early, yearly, medium, late) were investigated by 8 SSR markers (STM0019, STM3009, STM2005, STM2028, STM3012, STM3023, STM5136, STM5148) for genetic diversity assessment. To assess the genetic diversity of studied varieties, the cluster analysis was performed and the genetic distances between varieties were determined.
As results of PCR analysis from 6 to 10 alleles were determined with an average of 7.88 alleles. The most polymorphic marker was STM2028 with PIC 0.89. The lowest value of PIC (0.78) was obtained for STM3012 marker. For other studied markers values of PIC were from 0.82 to 0.88. It was found, that the most similar varieties are varieties with genetic distance 3.74: Pravda and Riviera, Pravda and Vzirets. The most different varieties turned out to be Vzirets and Lilly, ESMEE and Opilla varieties with genetic distance 6.00. Thus, it was found that the major of studied varieties formed cluster grouts according their maturity groups.

Keywords: molecular genetic polymorphism, DNA analysis, genetic distances, cluster analysis

INTRODUCTION

Ukraine is one of the five world leaders in potato (*Solanum tuberosum* L.) production – 22 million tons of tubers on an area of 1.5 million hectares. Today, a variety has become a strong realization factor of achievements of scientific and technological progress in agronomy. The potato cultivation in almost all regions of Ukraine requires the creation and introduction varieties, which belong to different ripeness groups for providing high productivity (SEMENCHUK, 2020). Around 40 varieties are applied for DUS (distinctness, uniformity and stability) examination annually. In this case the increasing of number new varieties requires the involving the additional molecular methods for manage refence collection within DUS test. A rapid and robust method for variety identification to aid the management and maintenance of existing variety collections and for the screening of new candidate varieties would therefore be highly desirable. There are three main ways to characterize the genetic diversity of a crop plant: morphology, pedigree analysis, and molecular analysis. Molecular analysis has the advantage of eliminating confounding environmental and pleiotropic effects while providing more easily quantifiable data for large collections (MCGREGOR ET AL., 2000; BORNET ET AL., 2002). Microsatellite markers (simple sequence repeats - SSR) are very suitable for this purpose as they are highly discriminatory, the profiles obtained are reproducible in different laboratories, their analysis can be automated and the results can be stored and analyzed easily (GHISLAIN ET AL., 2004; REID ET AL., 2011; PRYSIAZHNIUK ET AL., 2019). Thus, SSR markers were used to genotype new potato varieties developed through the repeated selection of tubers derived from true botanical seeds, as well as their maternal lines (TILLAULT AND YEVTUSHENKO, 2019). A set of 24 microsatellite markers were used for investigating forty-

seven resistant and susceptible to late blight Indian potato cultivars to assess genetic diversity present among them (PATIL ET AL., 2020). WANG ET AL. (2019) developed and genotyped a diverse population containing 292 potato genotypes was using 30 SSR markers covering the entire potato genome. KISHINE ET AL. (2017) reported that set of 8 tetra-nucleotide SSRs was used for discrimination of 72 potato varieties obtained from Japan and the United States. Within international project, which aimed constructing an integrated database that would include microsatellite genotypes and morphological characteristics specific to potato varieties in the European Union Common Catalogue, approximately 1,000 varieties of potato were collected and genotyped using 9 SSR markers. The results of these studies show that nearly all varieties (99.5%) had a unique genotype except for some mutants (COTE ET AL., 2013). In our previous studies there were investigated potato varieties of Ukrainian breeding by 4 SSR markers (PRYSIAZHNIUK ET AL., 2018a). As well as a correlation between SSR markers and morphological features by genetic distances on the basis of polymorphism of twelve potato varieties developed in Institute of Potato Studies of National Academy of Sciences of Ukraine was estimated (PRYSIAZHNIUK ET AL., 2018b). Considering the proved efficiency of combining the morphological and molecular data for potato DUS test, it is actual to evaluate both new varieties and varieties which are already registered by morphological traits by SSR markers to obtain genetic profiles of studied varieties.

Thus, the purpose of study was to assess the genetic diversity of potato varieties of different maturity groups by SSR markers. The data generated will be used for variety identification and future breeding programs.

MATERIALS AND METHODS

Twenty-four potato varieties of different maturity group were assessed. All studied varieties were included into State register of plant varieties suitable for dissemination in Ukraine (*Table 1*).

Table 1. List of the potato varieties studied, including their maturity group, origin and year of registration

Maturity group	Varieties	Origin	Year of registration
Very early	Riviera	Foreign	2007
	Vzirets	Ukrainian	2017
	Duma	Ukrainian	2017
	SANIBEL	Foreign	2019
	Prada	Foreign	2020
Early	Svitanok Kyivs'kyi	Ukrainian	1987
	Bellarosa	Foreign	2003
	Paroli	Foreign	2019
	Zhytnytsia	Ukrainian	2020
	Opillia	Ukrainian	2020
Medium	Yavir	Ukrainian	2000
	ESMEE	Foreign	2017
	Constance	Foreign	2017
	Solokha	Ukrainian	2016
	Granada	Foreign	2018
	PREDSLAVA	Ukrainian	2017
	Lilly	Foreign	2020
	Rodynna	Ukrainian	2020

	Kniazha	Ukrainian	2020
Late	Picasso	Foreign	1998
	Sluch	Ukrainian	2014
	Toscana	Foreign	2015
	Belmonda	Foreign	2016
	Challenger	Foreign	2018

Molecular genetic analysis was carried out in Laboratory Molecular Genetic Analysis of Ukrainian Institute for Plant Variety Examination (Kyiv, Ukraine) in 2021. DNA was extracted from seedling of each variety in two replicates using a modified cetyltrimethyl ammonium bromide (CTAB) extraction method (PRYSIAZHNIUK ET AL., 2018A). PCR (polymerase chain reaction) was done using eight SSR markers: STM0019, STM2005, STM2028, STM3009, STM3012, STM3023, STM5136, STM5148 (REID ET AL., 2009).
Each PCR reaction was performed in a total volume of 10 µl containing 0.25 µM of each primer, 1.8 mM $MgCl_2$, 250 µM dNTPs, 1 U Taq DNA Polymerase and 1×PCR buffer. Amplification was carried out using the thermocycler Sure Cycler 8800 (Agilent, USA) in following thermocycling conditions: 1 cycle of 95 °C for 5 min, followed by 40 cycles of 95 °C for 45 s, 50 °C (60 °C for STM3012 and STM5136 marker) for 30 s, 72 °C for 1.5 min and a final extension step for 1 cycle of 7 min at 72 °C s. The PCR products were separated by capillary electrophoresis with Fragment Analyzer (Agilent Technologies, USA) and dsDNA 910 Reagent Kit, 35-1,500 bp. The data was proceeded using software PROSize 2.0.
To characterize the genetic structure of the studied varieties the allele frequencies and polymorphism information content (PIC) were calculated (REID ET AL., 2009; PRYSIAZHNIUK ET AL., 2019). The genetic distances between potato varieties were calculated based on Nei &Li's similarity coefficient using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with Euclidean distances. STATISTICA 12 software (trial version) was used for data proceeding (EVERITT *ET AL*., 2011).

RESULTS

Twenty-four potato varieties were analyzed in this study using 8 SSR markers. As results of PCR analysis from 6 to 10 alleles were determined by each marker in studied potato varieties with an average of 7.88 alleles (*Table 2*).

Table 2. Marker name, allelic variation and PIC of SSR markers used in analysis potato varieties

SSR marker	Number of alleles	Allelic variation (bp)	PIC
STM0019	10	155-262	0.88
STM3009	8	134-192	0.84
STM2005	7	142-194	0.85
STM2028	10	275-414	0.89
STM3012	6	163-233	0.78
STM3023	6	164-222	0.82
STM5136	8	205-274	0.86
STM5148	8	416-500	0.86

The ranges of the band sizes corresponded to the expected ranges (REID ET AL., 2009). The majority of 8 amplified markers were polymorphic. It was determined that one allele was detected in Picasso variety by STM5136 marker, and one allele was identified by

STM5148 marker Duma variety. Overall, from 2 to 4 alleles were revealed by each marker. All markers demonstrated high PIC values (0.78-0.89). The most polymorphic marker was STM2028 with PIC 0.89. The lowest value of PIC (0.78) was obtained for STM3012 marker. Thus, the obtained high PIC values, indicate that the identified alleles are evenly represented among potato varieties.

Based on SSR analysis, genetic distances between potato varieties were calculated with the Nei &Li's similarity coefficient using UPGMA method (*Figure 1*).

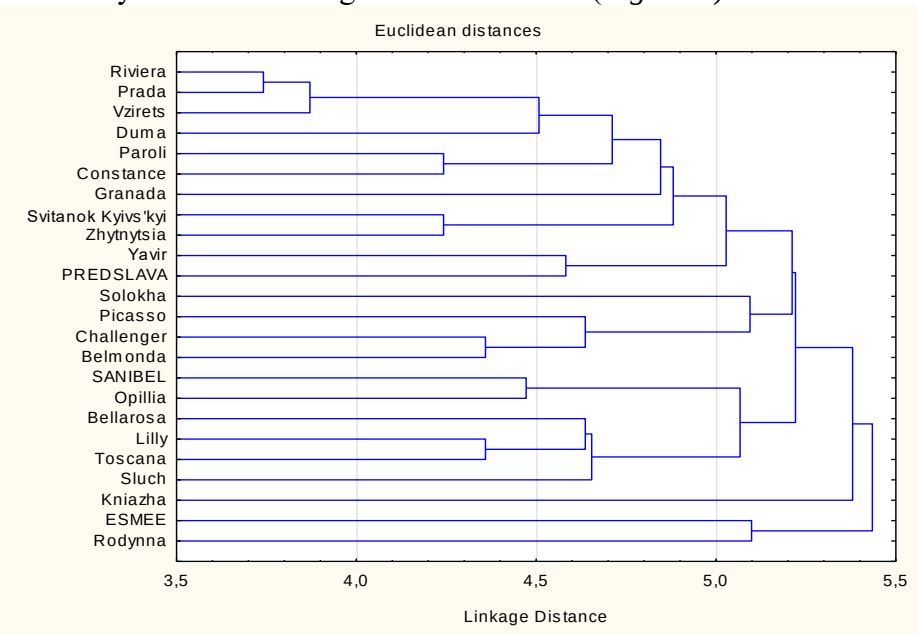

Figure 1. Dendrogram of potato varieties by SSR markers

As results of cluster analysis eight clusters were obtained. In most cases, potato varieties formed clusters according to their maturity groups. Thus, Riviera and Prada, Prada and Vzirets varieties were the most similar, genetic distance was 3.74. Vzirets and Duma varieties were adjacent to Riviera and Prada varieties cluster. All of these four varieties are varieties of very early maturity group. The separate clusters were formed with varieties of medium maturity group (Yavir, PREDSLAVA, Solokha and Kniazha, ESMEE and Rodynna varieties). The genetic distances between Yavir, PREDSLAVA and Solokha varieties were ranging from 4.58 to 5.57. It was determined that genetic distances which were observed between Kniazha, ESMEE and Rodynna varieties, were 5.10-5.66. In addition, the one separate group contained varieties of late maturity group (Picasso, Challenger, Belmonda). The genetic distances between these varieties appeared to be 4.36-4.69.

Svitanok Kyivs'kyi and Zhytnytsia varieties represented cluster of early maturity group with similarity value 4.24. According to obtained results, other varieties of early maturity group were distributed into clusters with varieties of medium and very early group (Paroli and Opillia respectively). Although, Bellarosa variety was adjacent to cluster group of medium and late maturity group varieties, it showed rather high similarity to Opillia - variety of early maturity group (genetic distance is 4.58). It was found that the most distinct varieties by SSR markers were Lilly and Vzirets, ESMEE and Opilla with genetic distances 6.00. Overall, Kniazhna variety appeared to be the most distinct variety in relation to other studied variety, genetic distances were ranging from 5.00 to 5.83. Moreover, the dendrogram showed that Kniazhna variety was clustered separately from other studied potato varieties. The obtained distribution indicates the high level of

differential ability of marker system and possible association SSR markers with genes which are connected with maturity traits (D'HOOP ET AL., 2008; DANAN ET AL., 2011).
The potato varieties did not form specific groups according to their originating. However, several varieties of different maturity groups of Ukrainian breeding were included into the one cluster group. Thus, Svitanok Kyivs'kyi, Zhytnytsia (early maturity group), Yavir, PREDSLAVA and Solokha (medium maturity group) which were clustered into one cluster group, originated by Ukrainian breeders. As well as Bellarosa (early maturity group), Lilly and Toscana (late maturity group) varieties are developed in foreign countries.

DISCUSSION

Our results demonstrated that, the values of allele numbers were ranged from 6 to 10 alleles per locus. FAVORETTO ET AL. (2011), evaluating 38 potato genotypes from two collections of commercial cultivars with 10 SSR loci, identified 46 alleles with an average of 4.6 alleles. BRAUN & WENZEL (2004), studying 69 varieties from Germany by 26 SSR markers, found 128 alleles and a mean number of alleles of 5.12. TIWARI ET AL. (2018) were analyzing allelic variations in Indian potato varieties. Total of 155 SSR alleles of 12 markers were scored in 48 varieties with alleles per locus varied from 4 to 35. Thus, the values of allele numbers found in this study are therefore consistent with the literature whereas a smaller number of genotypes was evaluated using smaller number of SSR markers.
In this study, the PIC obtained SSR were 0.78-0.89. The high PIC values were showed also in DUAN'S ET AL., (2019) study. Authors observed PIC values ranging from 0.64 to 0.93 in the evaluation of 217 potato varieties with 20 SSR loci. Similar values were obtained by TIWARI ET AL., (2018), PIC values ranged between 0.53 to 0.92. According to the data obtained by REID ET AL,. (2009) the PIC values calculated for the same markers range from 0.79 to 0.98 and are very similar to values presented in this study. Therefore, the PIC level depends on the set of primers used and the material evaluated. Thus, studied potato varieties which belong to different maturity groups and have different originating, have a wide genetic basis and showed high variability. COTE ET AL., (2013) were evaluated reference potato variety DNA collection at the Canadian Food Inspection Agency (CFIA) by SSR markers used in this study. The authors proved that method successfully differentiated 200 potato reference DNAs of the Canadian collection with the exception of 10 groups that were most likely clonal variants. In this study, 24 potato varieties were differentiated by 8 SSR markers including varieties which have similar origination. The obtained results showed highly discriminative power of the used microsatellite markers.
There are reported that used SSR markers demonstrated high capability to distinguish between different genotypes. As result of TIWARI'S ET AL. (2018) study, potato varieties were clustered into mainly three groups at the cutoff point (0.30) based on the Jaccard's similarity coefficient (0.22-0.63) using UPGMA method by SSR analysis. The authors managed to highlight the most similar varieties among 48 Indian potato varieties using 14 SSR markers. It was concluded that SSR market set would be a useful tool for varietal identification, testing of true-to-type genotype and DUS characterization of potato (TIWARI'S ET AL., 2018). According to results obtained by KARURI ET AL., (2010), who were investigating 89 sweet potato genotypes by SSR markers, studied genotypes were divided into two major groups. The genotypes did not form specific groups according to geographic regions and genotypes that shared a common name did not show genetic similarities. In this study potato varieties in the most cases were included into the clusters according to their maturity group. Besides, it can be observed groups of clusters

represented the varieties origination. The similar result was obtained by RAHMAN ET AL., (2022). They developed a DNA fingerprinting profile of 12 potato cultivars grown in Pakistan using 214 informative SSR markers. The authors showed that cluster analysis results fit well with pedigree/parentage information. Thus, in accordance with the reported results the set of eight SSR markers demonstrate high discriminating ability to assess the genetic diversity of potato varieties of different maturity groups.

Thus, the genetic diversity assessment would be of immense help for the breeders to improve potato variety characteristics through selection of efficient and diverse parents. Molecular profiling by means of microsatellites is very suitable for enlargement and management of potato reference collections

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