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GENOME VARIABILITY OF RUSSIAN POTATO CULTIVARS: AFLP-ANALYSIS DATA

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Abstract

Plant breeding success largely depends on knowledge of the genetic diversity and pedigree of cultivars, which is important for determining parental pairs for crossbreeding, donor genotypes of valuable traits and intraspecific homogeneity. AFLP is one of the popular methods for detecting genomic polymorphism and genotyping plant accessions, cultivars and lines. In addition to solving taxonomic and phylogenetic problems, the AFLP method is widely used to determine the variability, homogeneity, and the introgression and hybridity degree of *S. tuberosum* cultivars, reconstruct their pedigrees, and also to search for markers linked to various traits. Despite the importance of cultivar certification and inter-cultivar genomic variability assessment, in the Russian Federation, there are few studies on molecular marking of the potato domestic and foreign cultivars farmed in Russia. In the present work, the nuclear genome variability of 60 potato cultivars and five perspective clones was evaluated using the multilocus AFLP analysis. With primer combinations E35/M40 and E41/M35, 218 AFLP fragments were detected, 189 (86.7%) of which were polymorphic and 19 were unique for individual cultivars. Each of the 65 analyzed accessions was characterized by a specific AFLP spectrum. The genetic distances between the analyzed accessions varied widely from 0.37 to 0.77 with an average value of GD = 0.61. The species *Solanum stoloniferum*, used as an outgroup genotype, was most similar to the cv. Fioletovyi (GD = 0.59), and the greatest difference was to the cv. Aurora (GD = 0.80). Statistical analysis of the obtained AFLP data resulted in statistically insignificant clustering. On dendrograms constructed using the PAST and Structure v. 2.3.4 software, there was a tendency toward clustering (with low bootstrap support) of cultivars from the Lorch Potato Research Institute, and accessions with resistance to late blight, cyst nematode or PVY, as well as with yellow-coloured tuber peel. The high polymorphism level of the analyzed cultivars, the lack of their clear clustering and their "unstable" position at the dendrograms may be due to the current intensive exchange of breeding material, as well as to the increasing popularity of using wild potatoes in the potato breeding programs.

Keywords: *Solanum tuberosum*, potato, Russian cultivars, foreign cultivars, genomic polymorphism, tuber skin color, tuber flesh color, resistance, potato blight, cyst nematode, PVY, AFLP-clustering

A success of any breeding program largely depends on knowledge of the genetic diversity and pedigrees of plant cultivars, which is important for identifying parental pairs for crossing, donors of valuable traits and intravarietal homogeneity. Modern methods of molecular analysis make it possible to characterize the genotype, as well as to determine the degree of diversity within a cultivar and between cultivars of different geographical and breeding origin [1].

Currently, DNA genotyping of plants by assessing the polymorphism of both the entire genome and its functional regions (gene families, individual loci and genes) is becoming more and more relevant. In plants, DNA markers based on polymorphic DNA sequences obtained by molecular analysis methods are used to identify valuable genotypes, specific genes and chromosomal loci, as well as for the certification of cultivars and lines. DNA markers are not influenced by the environment and can be detected at any stage of development [2, 3]; therefore, their use makes it possible to overcome the disadvantages of protein markers in a number of breeding issues, including cultivars certification.

AFLP (amplified fragment length polymorphism) is a popular technique to detect genomic polymorphism and to genotype plant accessions, cultivars, and lines [4]. AFLP analysis makes it possible to assess the genome variability without determining the specific loci sequence, as well as to study an extensive, predominantly selectively neutral, part of the genome represented by unique and moderately repetitive DNA sequences [4]. High efficiency of AFLP markers has been shown in determining genetic distances and phylogenetic relationships at various taxonomic levels [5]. The frequency of its use evidences about the effectiveness of the method. AFLP is actively and successfully used to assess intervarietal variability in many agricultural crops, including wheat [6[, barley [7], peas [8, 9], and pepper [10, 11].

In potato research, AFLP technique was used to assess genetic diversity in existing world collections, for example, in wild species *Solanum microdontum* (GenBank USDA, USA) [12], *S. acaule* and *S. demissum* (CGN GenBank, the Netherlands) [13]. AFLP was used to revise 619 accessions of 13 wild potato species from the CPC (Great Britain) and NRSP6 GenBanks — The US Potato GenBank (USA) [14]. The method allowed assessing the levels of polymorphism of representative accessions of wild and cultivated potato species differing in geographic origin, ploidy and breeding system [14]. A number of taxonomic issues in the genus *Solanum* were solved using the AFLP method, e.g. the grouping of potato species in a series, previously proposed by Hawkes [15], has been revised; the effectiveness of AFLP for studying phylogeny of the genus *Solanum* and potato cultivars was shown [16], and the differences between taxa *S. americanum* and *S. nodiflorum* were confirmed [17].

In addition to addressing taxonomic and phylogenetic problems, the AFLP method is widely used to determine variability, homogeneity, degree of introgression and hybridity of *S. tuberosum* cultivars, to reconstruct their pedigrees, and also to search for markers associated with various traits. Thus, AFLP genotyping of 20 local Chilean cultivars was performed [18]. An analysis of 32 potato varieties cultivated in Scandinavian countries (NGB — Nordic Gene Bank, Sweden) showed that the collection consists of genetically and morphologically different clones, without any grouping by geographic origin [19]. AFLP analysis of 54 potato cultivars from the SASA GenBank (Great Britain) identified a group of 7 cultivars that were recommended for use in breeding programs in southern Italy [20].

Despite the significance of certification and assessment of intervarietal genomic variability, for potato cultivars of domestic and foreign origin cultivated in Russia, little is known about analysis and development of systems for molecular genotyping [21-24] or the determination of gene allelic variants for pathogen resistance [25-28].

The research aimed to assess the genomic variability of 60 potato cultivars and five perspective breeding clones of domestic and foreign breeding by the

AFLP method, as well as the effectiveness of AFLP analysis in genotyping potato varieties cultivated in Russia.

Materials and methods. Sixty domestic and foreign cultivars and five promising breeding clones of potato *S. tuberosum* (provided by the Lorkh All-Russian Research Institute of Potato Farming — VNIIKH, Moscow Province, Russia) were analyzed (a related species *S. stoloniferum* was an outgroup accession). Of the 60 varieties, 59 (or 90.77%) are included in the State Register of Breeding Achievements Allowed for Use (RF, 2020; http://reestr.gossortrf.ru/reestr/culture/159.html). The tubers were germinated in standard greenhouse conditions (23 °C/25 °C and 16 h/8 h day/night).

Genomic DNA was extracted from freshly harvested seedlings by the CTAB method [21, 29].

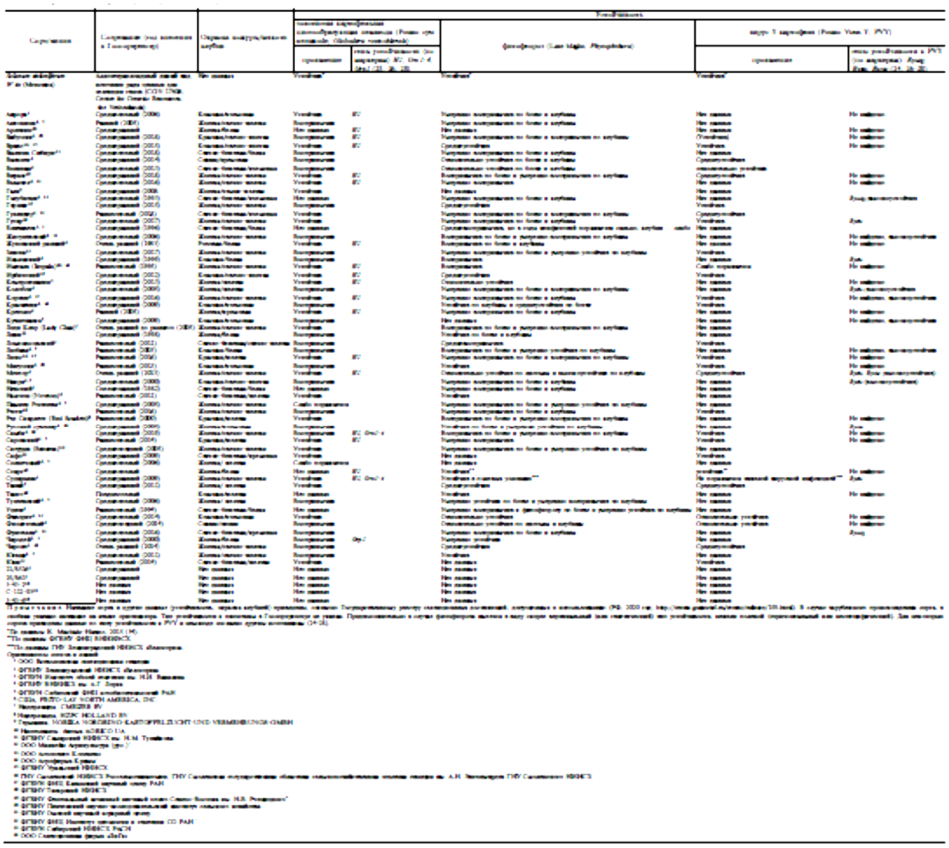
AFLP analysis was carried out according to a standard technique with hydrolysis of 350 ng of each accession genomic DNA with EcoRI and MseI restriction enzymes followed by ligation with EcoRI and MseI adapters [4] Selective amplification was carried out in two stages. The first step was a pre-amplification (denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, elongation at 72 °C for 1 min; 24 cycles) using adapter primers EcoRI+1 and MseI+1 [4] with a 3′-end selective nucleotide A. The second step was amplification using primers EcoRI+3 and MseI+3 with three selective nucleotides at the 3′-end. The results were visualized in a denaturing 6% polyacrylamide (a LI-COR 4300 gel analyzer, LI-COR operator manual, LI-COR, USA).

The obtained AFLP fragments were entered in MS Excel for calculation as binary matrices. Based on the constructed spectra and matrices, the variety-specific DNA markers were identified, the coefficients of pairwise genetic similarity between the accessions (GS) and the genetic distances (GD = 1 – GS) were calculated; cluster analysis was performed (by Neighbor Joining method and principal coordinates analysis), and groups of genetically similar accessions were identified using PAST software [30]. The genomic structure of the studied accessions was analyzed with Structure v. 2.3.4 (https://web.stanford.edu/group/pritchardlab/home.html) which allows identification of common genetic blocks and their ratio in each accession [31, 32].

Results. The description of the cultivars used in the study (originators, ripening dates, year of entry into the State Register, color of the tuber skin and flesh, resistance to golden potato cyst nematode, late blight, potato virus Y (PVY), resistance genes) are given in the Table 1 (see at http://www.agrobiology.ru).

The primer/enzyme combinations testing for multilocus AFLP genomic analysis of *S. tuberosum* cultivars. The restriction endonucleases EcoRI and MseI were used to digest DNA of the potato accessions, since it was previously shown that these enzymes provide the highest efficiency of AFLP analysis [10, 13, 18]. At the second step of amplification, seven combinations of EcoRI+3/MseI+3 primers differing in the composition of selective nucleotides at the 3′-end were tested using five cultivars (from different breeding centers), namely E35/M40 (E-ACA/M-AGC), E41/M35 (E-AGG/M-ACA), E41/M48 (E-AGG/M-CAC), E41/M45 (E-AGG/M-ATG), E12/M50 (E-AC/M-CAT), E32/T55 (E-AAC/M-CGA), and E32/T61 (E-AAC/M-CTA). Only two combinations, E35/M40 and E41/M35, could generate the polymorphic, clearly differentiated profiles with an optimal number of fragments and were subsequently used for AFLP labeling of 60 cultivars and five breeding clones of *S. tuberosum*.

1. Potato varieties and lines subjected to AFLP analysis (see at http://www.agrobiology.ru)



AFLP analysis of potato cultivars and breeding clones. AFLP analysis of 60 cultivars and five breeding clones of *S. tuberosum* and accession of wild species *S. stoloniferum* (an outgroup) detected 218 fragments (80-450 bp), 189 (86.7%) of which were polymorphic (Table 2). The E41/M35 primer combination was the most effective with 122 out of 139 obtained fragments variable (see Table 2). For some cultivars, unique fragments were found (19 in total).

2. AFLP analysis results for 65 potato cultivars and breeding clones

Primer combination	Number of fragments			
	total	polymorphic		unique
		total	%	
E35/M40	79	67	84,8	7
E41/M35	139	122	87,8	12
Total	218	189	86,7	19

The combinations E35/M40 and E41/M35 revealed the polymorphism of cultivars with greater efficiency than in a number of other studies. For example, AFLP analysis of 32 potato cultivars from the NGB GenBank with five EcoRI+3/MseI+3 combinations revealed 21-26 fragments of which only 4-18 were polymorphic [19]. Labeling of 22 potato cultivars (Chile) with five EcoRI+3/MseI+3 combinations showed only 26-71 polymorphic fragments out of 34-77 described [18]. AFLP genotyping of 25 potato cultivars from Iran with 16 primer combinations PstI+3/MseI+3 identified only 16-52 polymorphic fragments out of 19-53 [33]. It was previously reported that up to 80% of a standard AFLP pattern can serve as markers for detecting genetic polymorphisms at

restriction sites or within an excised fragment and, as a consequence, for determining the population structure and reconstructing the species phylogeny [4]. At the same time, careful selection of primer combinations can significantly increase the number of detected polymorphic bands. Thus, in some studies [18, 33], the percentage of polymorphic fragments in AFLP analysis of potato accessions is almost as high as in this study (75-100%) (see Table 2) while in other reports it varies from 17.4 to 78.3% [19].

Thus, it is obvious that the polymorphism revealed by the E35 M40 and E41/M35 primer combinations is so high that even one of the combinations would be sufficient for genotyping the analyzed potato accessions. As a result of AFLP analysis using E35/M40 and E41/M35 primers, each of 60 analyzed cultivars and five breeding potato clones was characterized by a specific AFLP pattern.

AFLP data statistical analysis. The analysis of the obtained data showed that genetic distances of the analyzed cultivars vary within wide limits, from 0.37 (between cv. Tanay and Yugan) to 0.77 (between cv. Aurora and Nakra) with an average of 0.61. *S. stoloniferum*, used as an outgroup, shows the greatest similarity with the cv. Fioletovii (GD = 0.59), and the greatest difference with the cv. Aurora (GD = 0.80).

Based on the AFLP analysis data, a dendrogram was obtained using the PAST program, where group 1, separated with low bootstrap support, comprised 14 cultivars of which half were of VNIIKH breeding origin, four (cv. Lady Claire, Red Scarlett, Impala and Saturn) were of Dutch breeding and three (cv. Aurora, Elizaveta and Charodei) were originated by other breeding centers (Fig. 1, see Table 1). Group 2 consisted of three cultivars of foreign (Gala) and domestic (Zhigulevskii and Safo) breeding (see Fig. 1). The cultivars of VNIIKKH (Meteor, Golubizna, Pamyami Rogacheva, Nakra and Velikan), Ural Research Institute of Agriculture (Gornyak), Tatar Research Institute of Agriculture (Reggi) and those bred in the USA (Newton) grouped in the third implicit cluster (group 3). It was the sister group to the outgroup accession, which, together with *S. stoloniferum*, included the cv. Fioletovii (see Fig. 1). All other analyzed cultivars formed a highly polymorphic cluster without reliable segregation into subclusters (see Fig. 1). Interestingly, the cv. Fioletovii had a high similarity (GD = 0.54-0.59) with nine cultivars, Fritella, Krasavchik, Lyuks, Irbitskii, Lina, Kortni, Virazh, Tanai, and Sarovskii. However, cv. Fioletovii grouped with the wild species *S. stoloniferum*.

When trying to group the cultivars by agronomic traits, country of origin or originator (see Table 1), we did not identify statistically significant groups.

However, it should be noted that not seeing the clustering by country of origin in the analyzed accession set may be because mainly the domestic cultivars were studied.

The outer group (cv. Fioletovii and *S. stoloniferum*) turned out to be resistant to late blight and PVY. Cv. Velikan, Gornyak, and Newton from group 3, which are closest to the outer group, are also resistant to late blight, and the subgroup that unites cv. Velikan and Gornyak is resistant to PVY. Group 2 cultivars are completely susceptible to late blight. In a large cluster, the subgroup of cv. Tanai, Yugana and Lina is resistant to late blight, and three other subgroups (subgroup 1 — cv. Favorit and Bravo; subgroup 2 — cv. Kolobok, Irbitskii, Start and Kortni, and subgroup 3 — cv. Lomonosovskii and Charoit) are resistant to PVY. The cultivars resistant to nematode *Globodera rostochiensis* show a slight trend to form a cluster. For the remaining subgroups of the large cluster and group 1, we did not find common traits.

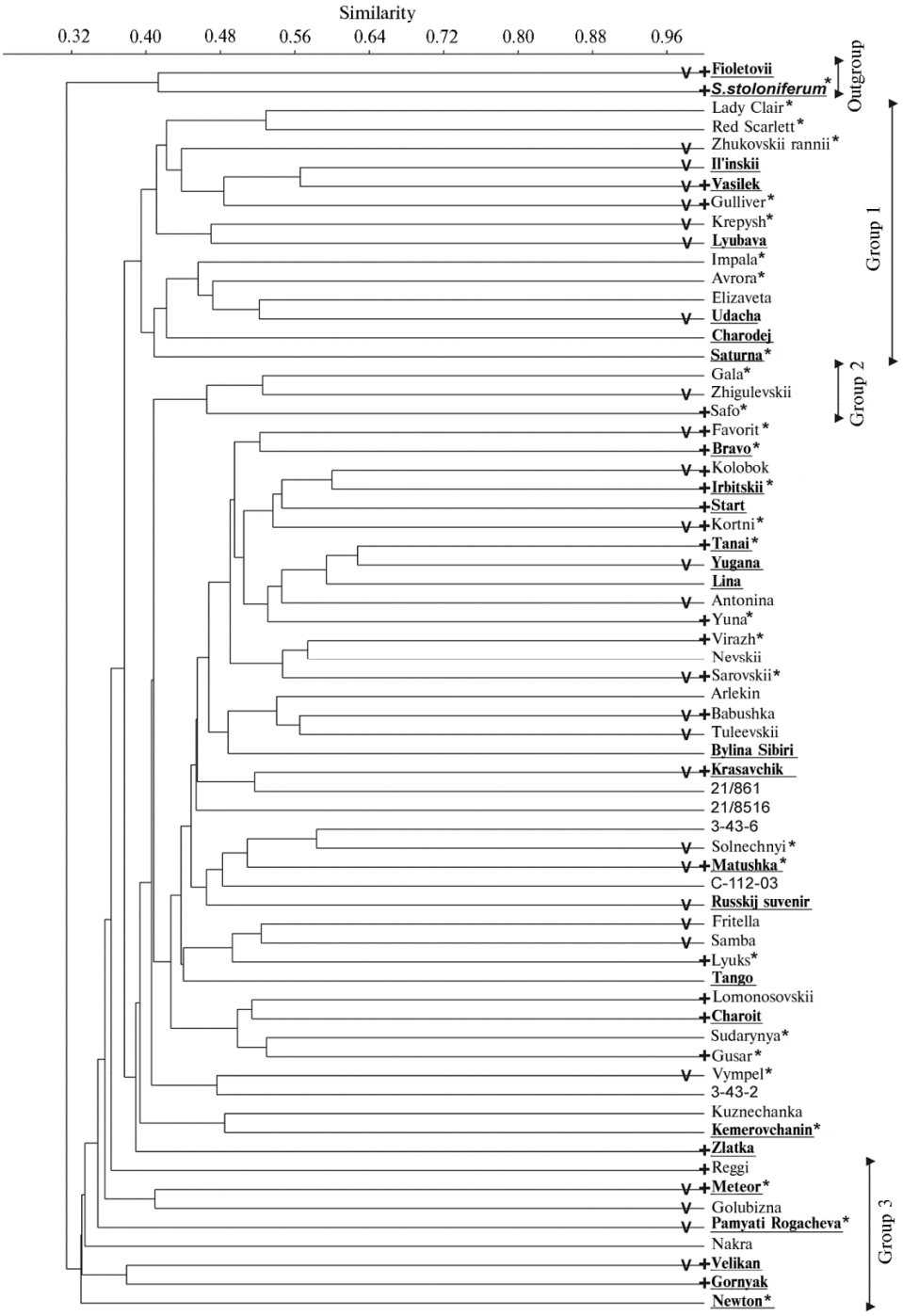


Fig. 1. Genetic diversity among 60 cultivars and five breeding clones of potatoes (AFLP analysis, Neighbor Joining method, PAST software). The cultivars resistant to late blight are highlighted in bold and underlined, the cultivars resistant to potato virus Y are marked with (+), to golden cyst nematode with (∗). The originator of the cultivars marked with (v) is Lorkh All-Russian Research Institute of Potato Farming.

Such a high polymorphism between the analyzed cultivars and the absence of statistically significant clustering may be due to the growing popularity of the wild potato species as genetic donors in recent decades. A total of 57 accessions, i.e. most of those we used in the study, are promising breeding clones and cultivars

(the entries in the Russian State Register of Varieties since 2000). The majority of these clones and cultivars are complex interspecific hybrids, in which wild potato species are often donors of economically valuable traits, e.g. resistance to pathogens, abiotic factors, etc. [34]. This is also evidenced by recent studies of potato cultivars originated from Russia and neighboring countries, which showed a relationship between a constant increase in the number of cultivars with rare and unique SSR loci alleles, on the one hand, and the use of interspecific hybridization, on the other hand [22].

The dendrogram obtained shows a tendency to clustering cultivars from Lorkh All-Russian Research Institute of Potato Farming (see Fig. 1). Earlier, SSR analysis of 41 domestic and foreign potato cultivars and 26 breeding accessions revealed clustering of the cultivars of related origin [23]. On the other hand, according to the AFLP analysis results, the cultivars Udacha and Lyubava, which have a common origin [22], belong to different clusters (see Fig. 1). In addition to cultivars, five lines from three originators (see Table 1) used in the study clustered with the cultivars of other originators (see Fig. 1). This may be the result of an intensive exchange of breeding material between breeding centers.

SSR analysis carried out by Kolobova et al. [23] demonstrated the possibility of potato cultivars clustering according to the tuber color. However, in the present work, such clustering (with low bootstrap values) was observed only for the cultivars with yellow tuber skin or flesh (see Fig. 1, Table 1). The cultivars Fioletovii and Vasilek with blue-violet tuber skin (the flesh of cv. Fioletovii tubers is also blue-violet) are distant from each other on the dendrogram (see Fig. 1).

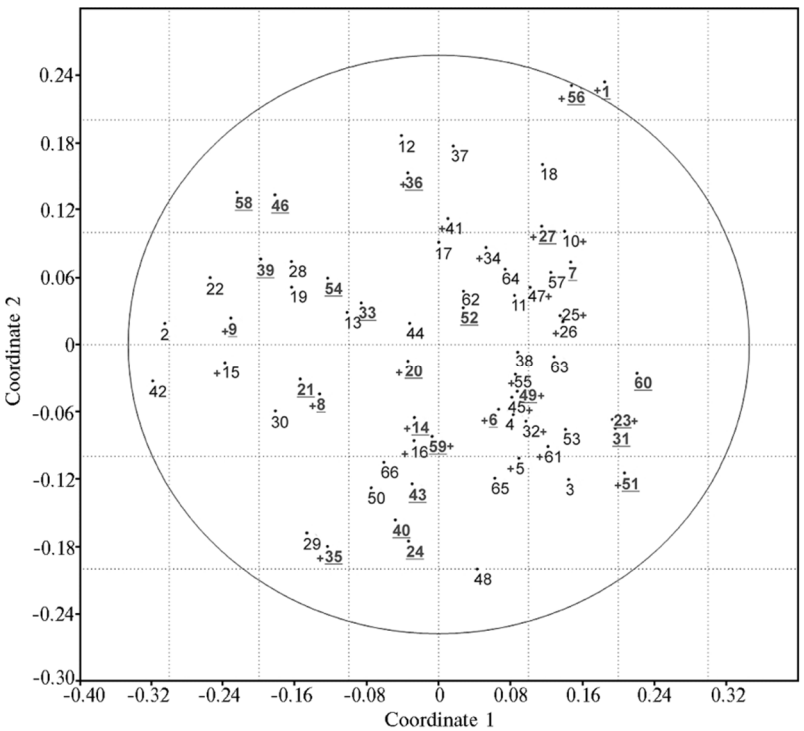


Fig. 2. PCA graph of AFLP analysis data for 60 cultivars and five breeding clones of potatoes. The numbering is as in Table 1. The outgroup comprises *Solanum stoloniferum* (1) and cv. Fioletovii (20). The cultivars resistant to late blight are highlighted in bold and underlined, those resistant to potato virus Y are marked with (+).

On the PCA graph, the analyzed cultivars form a single diffuse pool of genotypes, in which the same external group is distinguished as on the dendrogram,

however, the clustering of cultivars occurs in a slightly different way, although there is a noticeable convergence of the accessions resistant to late blight or to PVY (Fig. 2). It is interesting to note that a cultivar resistance, as it is described by the originator in the State Register does not always coincide with the research data. An example is the study of Klimenko et al. [26]. This is very probably due to the difficulty of visual determination of infection symptoms [26]. *S. stoloniferum* (the outgroup) is located on the PCA plot quite close to the rest of the *S. tuberosum* cultivars and accessions. The explanation may be that *S. stoloniferum* members have been quite often used in breeding programs as donors of resistance to various stresses [34].

The rather high general polymorphism of the analyzed cultivars, the absence of clear clustering and the "unstable" position of the accessions most likely result from the intensive exchange of breeding material, which is currently going on. When selecting parental pairs, breeders include accessions from various world breeding centers, which is confirmed by many studies. For example, SSR analysis of 113 domestic potato cultivars (80 accessions cultivated in Russia and 33 accessions from neighboring countries), including 12 cultivars that were studied in our work, showed no country-based clustering [22]. Even a morphologically little polymorphic collection of 32 potato cultivars grown in Scandinavian countries was not grouped according to the countries of origin as per the AFLP analysis results [19].

We also determined the genomic structure of potato accessions with Structure v. 2.3.4 software. Genomic structure analysis makes it possible to identify common genetic blocks and the ratio of such blocks in each accession to distribute the accessions into subgroups. In comparing the number of subgroups (k) from 2 to 15, the best result (LnLike = –23219.2) was obtained for k = 3.

The resulting graph shows the genomic structure of the studied 65 cultivars and breeding clones as different ratios of three blocks (Fig. 3). Any clear correlation between the ratios of the blocks and any of the considered features (see Table 1) was not revealed. There is some weakly expressed tendency of grouping cultivars resistant to nematodes (groups 1, 2, 3) and PVY (group 3), and cultivars having the same tuber color, namely those with yellow tuber skin, which is probably due to the prevalence of yellow tuber cultivars in the accession set) (see Fig. 3, Table 1).

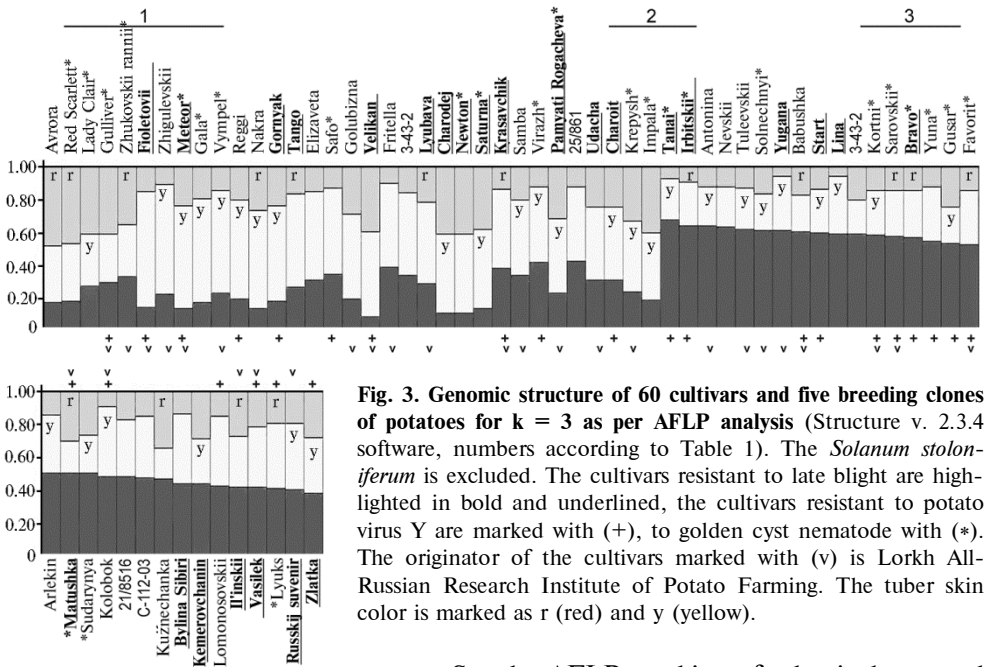


Fig. 3. Genomic structure of 60 cultivars and five breeding clones of potatoes for k = 3 as per AFLP analysis (Structure v. 2.3.4 software, numbers according to Table 1). The *Solanum stoloniferum* is excluded. The cultivars resistant to late blight are highlighted in bold and underlined, the cultivars resistant to potato virus Y are marked with (+), to golden cyst nematode with (∗). The originator of the cultivars marked with (v) is Lorkh All-Russian Research Institute of Potato Farming. The tuber skin color is marked as r (red) and y (yellow).

So, the AFLP marking of selectively neutral

regions in the genomes of 65 cultivars and promising breeding clones of potatoes, including modern domestic cultivars, revealed a high level of genomic polymorphism. No clear clustering was detected according to cultivar origin (a certain breeding center) or morphological traits. It has been shown that the AFLP analysis with the primer combinations we have chosen is promising for genotyping potato cultivars during the initial screening in collections and the primary selection for target traits for subsequent in-depth analysis.

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