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ASSESSMENT OF GENETIC DIVERSITY AMONG HEADED CABBAGE (*Brassica oleracea* L.) ACCESSIONS BY USING SSR MARKERS

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Abstract

In the Russian Federation, headed cabbage *B. oleracea* L. convar. *capitata* (L.) (both traditional cultivars and hybrids) is the most common cole crop. Application of DNA marker technologies allow rapid identification of valuable genotypes and their genetic relationships in order to produce genetically diverse breeding forms. Microsatellite (SSR) markers are widely involved in genetic identification and genotyping of crops. Particularly, these markers effectively reveal the polymorphism among and within cultivars of *B. oleracea* L. In the present work we have estimated for the first time the genetic relationship among local accessions of headed cabbage on the basis of SSR-loci polymorphism. The goal of the work was to reveal genetic relationship between breeding accessions of *B. oleracea* L. convar. *capitata* (L.) Alef. var. *capitata* L. f. *alba*, var. *capitata* L. f. *rubra*, and var. *sabauda* L. based on DNA typing and genetic classification using SSR markers, and to compare DNA data of studied genotypes with defined cabbage varietal and maturity groups. Twenty-four breeding accessions of headed cabbage including red and Savoy varieties from collection of the Federal Scientific Vegetable Center (FSVC) and also developed at FSVC were involved. Genomic DNA was extracted from young plant leaves at 2-3 leaf stage with DNA extraction kit Sorb-GMO (Syntol, Russia). Final DNA purity and concentration were identified with the SmartSpec Plus spectrophotometer (Bio-Rad, USA). Twenty-one microsatellite loci with known primer sequences were chosen to perform SSR analysis. The amplification was run in C1000 Touch thermocycler (Bio-Rad, USA). PCR products were separated in a 6 % polyacrylamide sequencing gel with the use of Sequi-Gen GT electrophoresis system (Bio-Rad, USA). The fragments sizes were detected in comparison with molecular weight markers GeneRuler100 bp plus DNA ladder (Thermo Fisher Scientific, USA). The digital images of electrophoregrams were analyzed with Image Lab 3.0 software (Bio-Rad, USA). STRUCTURE 2.3.4 (https://web.stanford.edu/group/pritchardlab/home.html) software was used to study population structure. The genetic distances were calculated using GenAlEx 6.5 software for Microsoft Excel by Nei's method. To construct the UPGMA dendrogram the algorithm of MEGA 5.2 program was used. As a result of analysis 103 alleles were obtained with an average 4.9 alleles per locus. PCR product sizes were between 130 and 410 bp. The PIC value varied from 0.3 to 0.9. Population analysis revealed six clusters to distribute all breeding accessions. Calculated Nei's genetic distances varied from 0.060 to 0.186. The UPGMA deprogram constructed on distances matrix reflected the origin of cabbage accessions taken. Thus, cultivars Belorusskaya 455, Podarok 2500, Amager 611 and Zimovka 1474 originated from Northwestern Europe were joined into one cluster, there was also hybrid Severiynka F_1 developed with the use of these cultivars. Early-maturing varietal group Ditmarskaya Raniya represented by cultivars Ijunskaya 3200, Stakhanovka 1513, Nomer Perviy Gribovskiy 147 formed a separate cluster which also included an early-maturing hybrid Avrora F_1 being of a partial origin from Ijunskaya 3200. Two breeding lines obtained from Avrora F_1 were genetically distant and disposed in another subcluster. Cultivars Slava 1305 and Slava 231 belonging to the separate varietal group Slava formed a branch of the dendrogram. Cultivar Parus and hybrids Zarnitsa F_1, Mechta F_1 developed relatively recently were disposed distantly from other accessions. Moscovskaya pozdnyaya 15 a local cultivar formed its own branch of the dendrogram. Three cultivars of Savoy cabbage were grouped together with sufficient genetic distance between each other, where a new early-maturing cultivar Moskovskaya kruzhevnitsa was more distant from others. The group of red cabbage accessions situated distantly from other clusters with great difference inside the group. The obtained results based on SSR

marker variation were in accordance with data on the origin of headed cabbage accessions confirming that they belong to defined varietal and maturity groups. This provides information for nearest breeding program for new cabbage breeding forms.

Keywords: *Brassica oleracea* L., headed cabbage, SSR markers, genetic identification, cultivar genotype polymorphism, varietal group

China, India and the Russian Federation are the world's largest cabbage producers, and Russia retains the leadership in cabbage consumption [1]. Cabbage is rich in antioxidants, e.g. polyphenols, anthocyanins, gallic, vanillic and coumaric acids, and has anti-inflammatory properties due to glutamine and flavonoids. Cabbage is also a rich source of vitamin C and glucosinolates [2].

Of the species *Brassica oleracea* L. (CC, $2n = 18$), the white cabbage *B. oleracea* L. convar. *capitata* L. Alef. var. *capitata* (L.) f. *alba* DC. is the most common in Russia, while the red-headed cabbage *B. oleracea* L. convar. *capitata* (L.) Alef. var. *capitata* (L.) f. *rubra* (L.) Thell., Savoy cabbage *B. oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L., cauliflower *B. oleracea* L. convar. *botrytis* (L.) Alef. var. *botrytis* L., broccoli *B. oleracea* L. convar. *botrytis* (L.) Alef. var. *italica* Plenck, Brussels sprouts *B. oleracea* L. convar. *gemmifera* (DC.) Gladis var. *gemmifera* DC., kohlrabi *B. oleracea* L. convar. *acephala* (DC.) Alef. var. *gongylodes* L., and leafy kale *B. oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L. are less popular. Numerous cultivars and high-yielding hybrids of *B. oleracea* have been produced due to breeding activities performed worldwide. The forms with the longest period of vernalization and frost resistance are a distinguishing feature of the convar. *capitata*. The initial biodiversity of cabbage originated in the Western Mediterranean Sea, Western and Northern Europe, and then spread to Eastern Europe, America, Asia (China, Japan), Australia and Africa. High yield and transportability were the properties of cabbage that contributed to widespread distributed of the crop. Cabbage varieties significantly differ in early maturity properties. The crop is not demanding for heat, positively responds to long daylight hours and cultivation technologies making the growing period longer [3].

Genetic characterization enables identification of parental forms with a high level of heterosis and a stable expression of economically valuable traits, which should be involved in crossing, thus allowing for a better use of plant resources. In addition, with the advent of a new breeding material, it is necessary to distinguish generated genotypes and identify the same or closely related ones. The use of SSR (simple sequence repeats) DNA markers facilitates the precise genetic typing of breeding samples. The reliability and detection of high genetic polymorphism are the advantages of microsatellite markers, which make them the most popular and universal in genetic studies of agricultural crops [4-6].

The database, which accumulates information on the variability of microsatellite loci in the genus *Brassica* L., including *B. oleracea*, contains 398 microsatellite markers [7]. Based on microsatellite loci, genetic relationships between species of the genus *Brassica* were established and confirmed [8-10]. Genetic analysis within the B. oleracea species revealed the relationships both between cabbage varieties, and varieties and breeding lines [11-14]. Phylogenetic studies of the *B. oleracea* were also based on the variability of microsatellites [15]. In a set of 91 commercial white cabbage genotypes, a total of 359 alleles grouped into six clusters were detected by 69 microsatellite markers. The first two clusters grouped the white cabbage genotypes, with broccoli, cauliflower, kohlrabi and kale varieties grouped in the remaining clusters [16]. In breeding samples of white cabbage, a high genetic variability was determined with accurate typing of each plant based on microsatellite polymorphism [17].

Thus, summarizing previous works, there is a scarcity of published literature on the genetic diversity of headed cabbage traditional Russian varieties and hybrids. Currently, the varieties used in breeding have been bred and zoned by E.M. Popova back in the 1940s at the Gribovskaya Experimental Station.

This work is the first to establish the genetic relationships between breeding samples of domestic head cabbage based on polymorphism of microsatellite loci. When comparing the three varieties, a close genetic relationship was found between the genotypes of Savoy cabbage and white cabbage.

The aim of our work was to identify genetic relationships between breeding samples of *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *capitata* L. f. *alba*, var. *capitata* L. f. *rubra*, and var. *sabauda* L. using SSR markers, and to compare the obtained data with assignment of the examined genotypes to cultivar types and maturity groups.

Materials and methods. Genomic DNA of 24 breeding samples of head cabbage (Genetic collection of the Federal Scientific Vegetable Center (FSVC) was extracted from leaves in the 2-3rd leaf phase by the CTAB buffer-based method using Sorb-GMO-B reagent kit (Syntol LLC, Russia) as per the manufacturer's protocol. Young leaves, from five plants per sample, were homogenized in an extraction buffer (a ball mill TissueLyser II, Qiagen, Germany; 26 Hz, 1560 vibrations per min, 1.7 min). The final DNA purity and concentration were determined spectrophotometrically (a SmartSpec Plus, Bio-Rad, USA). DNA solutions with $OD_{260/280}$ = 1.6-1.8 were used in PCR.

Twenty-one microsatellite loci with known primer sequences [11, 12] and polymorphic information content (PIC value) of 0.5 or above were involved in SSR analysis. For 25 µl PCR, 2.5 µl of 10× PCR buffer, 2.5 mM $MgCl_2$, 0.25 mM individual dNTPs, 0.3 µM of each primer, 1.5 U Taq DNA polymerase (Syntol LLC, Russia), and 3 µl of individual DNA template were mixed. The basal PCR protocol was as follows: 45 s at 92-95 °C (denaturation), 30 s at 52 to 58 °C, depending on primer pairs (annealing), and 30 s to 1 min at 72 °C (elongation). A 35-cycle amplification was performed (a C1000 Touch™ thermal cycler, Bio-Rad, USA).

Amplification products were separated by vertical electrophoresis (a Sequi-Gen GT system, Bio-Rad, USA) in a 6% polyacrylamide sequencing gel at 1600 V for 1.5-2 h. After electrophoresis, gels were stained (SYBR™ Safe DNA Gel Stain, Invitrogen, USA). The ChemiDoc XRS + system (Bio-Rad, USA) was used for gel documentation. The size of the amplified fragments was determined using GeneRuler100 bp plus DNA ladder (Thermo Fisher Scientific, USA). The digital photographs of electrophoregrams were analyzed using the Image Lab 3.0 software (Bio-Rad, USA).

For each SSR locus, the presence and absence of amplification product were designated as 1 and 0, respectively, to construct the final binary matrix. For each primer, the PIC value was calculated [18]. Population structures were analyzed using STRUCTURE 2.3.4 software (http://web.stanford.edu/group/pritchardlab/home.html) [19]; to determine the ΔK value, the STRUCTURE HARVESTER v0.6.1 program was applied (http://taylor0.biology.ucla.edu/structureHarvester/) [20]. The data analysis in STRUCTURE did not provide the number of clusters, so their probable number was set from 1 to 12, followed by a comparison of the K value for each option. Genetic distances were calculated in GenAlEx 6.5 (https://biology-assets.anu.edu.au/GenAlEx/Download.html) [21] by the Nei's method [22, 23]. The MEGA5.2 algorithm was used to construct the UPGMA dendrogram (https://mega.software.informer.com/5.2/) [24].

Results. Table 1 shows the samples of *B. oleracea* involved in the study.

1. A set of headed cabbage (*Brassica oleracea* L.) breeding samples involved in SSR analysis of genetic diversity (Genetic collection of the Federal Research Center for Vegetable Growing, FRCVG)

Name	Cultivar	Designation
Samples of *Brassica oleracea* convar. *capitata* (L.) Alef. var. *capitata* L. f. *alba* DC		
Amager 611	Amager	Ama
Avrora F_1	Ditmarskaya rannyaya	Avr
Avrora F_1 line 1	Ditmarskaya rannyaya	AvL1
Avrora F_1 line 2	Ditmarskaya rannyaya	AvL2
Belorusskaya 455	Belorusskaya	Bel
Zarnitsa F_1	Ditmarskaya rannyaya	Zar
Zimovka 1474	Langendeiskaya zimyaya	Zim
Iyunskaya 3200	Ditmarskaya rannyaya	Iju
Moskovskaya pozdnyaya 15/reproduction	Moskovskata posdnyaya	MoPo/MoPo1
Mechta F_1	Langendeiskaya zimyaya	Mech
Nomer pervyi gribovskii 147	Ditmarskaya rannyaya	N1
Parus/reproduction	Dutch group	Par/Par4
Podarok 2500	Belorusskaya	Pod
Severyanka F_1	Belorusskaya	Sev
Slava 1305	Slava	Sl13
Slava gribovskaya 231	Slava	Sl23
Stakhanovka 1513	Ditmarskaya rannyaya	Sta
Samples of *Brassica oleracea* var. *sabauda* L.		
Virtue 1340	Virtue	Ver
Moskovskaya kruzhevnitsa	Rosetochnaya	MosKr
Yubileinaya 2170	Ulmskaya	Ubi
Samples of *Brassica oleracea* var. *capitata* L. f. *rubra*		
Gako 741	Gako	Gak
Kamennaya golovka 447	Erfurtskaya	KamG
Rubin	Turnovskaya	Rub
Krasnokochannaya line I3	Erfurtskaya	RedL1

Note. Reproduction means that the sample is grown from the elite seeds.

Amplification of 21 microsatellite loci yielded 103 alleles, 4.9 per locus on average, of which 13 alleles were specific. The breeding samples Severyanka F_1, Belorusskaya 455, Podarok 2500, Amager 611, and Zimovka 1474 have the largest number of specific alleles (seven alleles). The amplification products vary in length from 130 to 410 bp. Sets of five alleles each were characteristic of the most informative SSR loci AF458409 and BZ523957 with the PIC values 0.90 and 0.86 for the primers, respectively (Fig. 1). However, less informative SSR loci with the PIC value 0.69 for the primers could contain a larger number of alleles, up to 13, like in CC969431, or 11, like in CC956699, CC969507, and AF113918. The lowest PIC value for the primers to AF180355 locus was 0.30, with only two alleles found.

2. Polymorphic microsatellite loci for marker-based estimation of the genetic relationships among headed cabbage (*Brassica oleracea* L.) breeding samples (Genetic collection of the Federal Scientific Vegetable Center)

Marker (GenBank NCBI)	Genes with polymorphic microsatellite sequences	Motif	Number of alleles	PIC
AF051772	Reproductive meristem gene 1 (REM1) mRNA	(gaa)5	3	0.81
AF051772(2)	B3 domain-containing protein REM1	(ct)6-1(ct)4-1(tcc)3	4	0.78
AF458409	Deoxycytidine deaminase (DCTD1), mRNA	(aga)6	5	0.90
AJ427337	mRNA for calmodulin 1 (cam1 gene)	(ga)5	3	0.61
BZ523957	Genomic clone BOKAH45	(ttg)6	5	0.86
CC956628	Brassica oleracea genomic clone BOIAA94, genomic survey sequence	(tc)5	2	0.50
CC956699	Brassica oleracea genomic clone BOIAB20, genomic survey sequence	(cac)9	11	0.69
CC969431	Genomic clone BOIAB19	(ga)6	13	0.61
CC969459	Genomic clone BOIAB94	(cgg)5	2	0.31
CC969497	Genomic clone BOIAA26	(tgc)5	2	0.83
CC969507	Genomic clone BOIAB15	(ct)5	11	0.74
X94979	mRNA for pollen coat protein	(atg)5	2	0.44

Continued Table 2

AF241115	Isolate HRI/CGN 5688 cauliflower gene	(at)5(ta)6	6	0.51
X92955	mRNA for pollen coat protein	(tttta)2(ata)7	3	0.57
AF180355	Isolate B265 ABI1 protein (ABI1) gene	(tc)16	2	0.30
AF113919	Phospholipase D2 (PLD2) gene	(at)6(gt)5	3	0.58
AF273844	Thioredoxin-h-like protein 1 (THL1) mRNA	(ctt)7	4	0.73
AF230693	Stearoyl-ACP desaturase (DELTA9-BO-1) gene	(ctt)3(ct)6(cttg)6	4	0.81
AF113918	Phospholipase D1 (PLD1) gene	(ct)7(at)7-1	11	0.60
U67451	Brassica oleracea homeotic protein boi1AP1 (Boi1AP1) mRNA	(at)9-1	6	0.81
AF241115(2)	Isolate HRI/CGN 5688 cauliflower gene	(ta)6-1	2	0.63

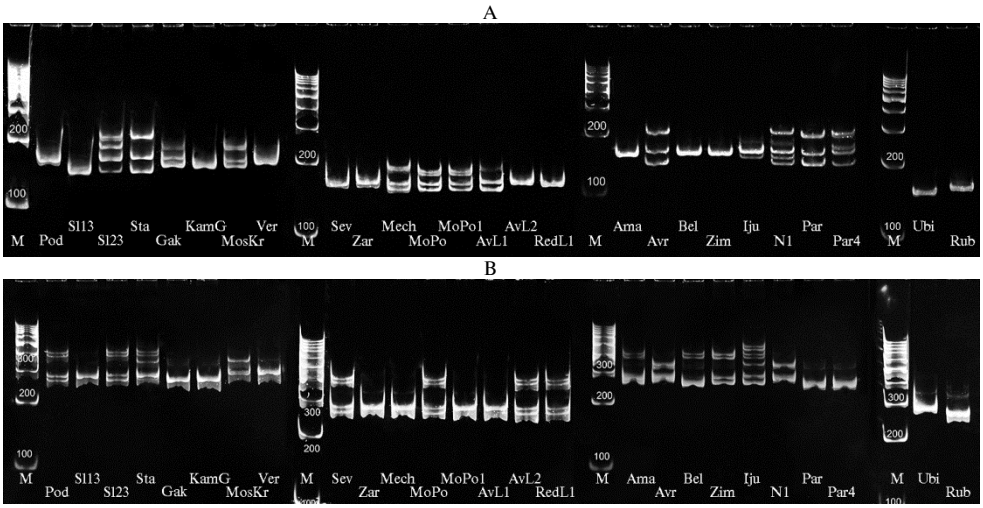

Fig. 1. Electophoregrams of microsatellite loci AF458409 (A) **and BZ523957** (B) **PCR amplification products in headed cabbage (*Brassica oleracea* L.) breeding samples** (Genetic collection of the Federal Research Center for Vegetable Growing, FRCVG). M — molecular weight marker (GeneRuler100 bp plus DNA ladder, Thermo Fisher Scientific, USA). For designations and description of samples, see Table 1.

In our study, the calculated PIC values were consistent with the values reported for the same markers by other researchers ($\chi^2 = 2.16$ at $p = 1.0$) [11, 12]. The markers for loci with a PIC value ≥ 0.5 turned out to be effective for genetic discrimination of related genotypes [25]. As a result, these markers allowed us to revealed 76% polymorphism among the studied breeding samples.

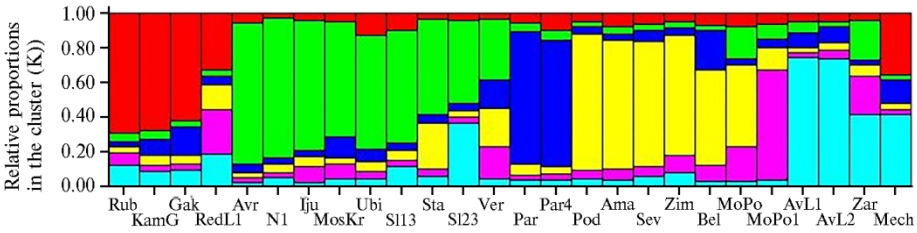

Fig. 2. Structure of the set of headed cabbage (*Brassica oleracea* L.) breeding samples (Genetic collection of the Federal Scientific Vegetable Center) **based on Bayesian analysis** (built with STRUCTURE software for six clusters, K = 6). For designations and description of samples, see Table 1.

It was shown that the tested breeding samples were best distributed among six clusters ($\Delta K = 2.05$) where they were grouped according to origin (Fig. 2). The calculated genetic distances varied from 0.060 to 0.186, with the greatest genetic distance between the Parus cultivar and the Mechta F_1 hybrid. Cabbage samples Severyanka F_1, Belorusskaya 455, Podarok 2500, Amager 611, and Zimovka 1474

of the Dutch group originating from North-Western Europe clustered together (Fig. 3). The high genetic similarity of cultivars Amager 611, Belorusskaya 455, and Podarok 2500 confirms that the latter was obtained through complex hybridization of the first two cultivars. The cultivars Belorusskaya 455, Zimovka 1474, and Podarok 2500 originated at the Gribovskaya Experimental Station were involved in production of a relatively new hybrid Severyanka F_1 advanced in quality and resistance to fusarium wilt, clubroot, and bacterioses. The cultivar Belorusskaya 455 obtained by selection of the earliest maturing forms in the Vitebsk region, has been zoned since 1943. The late-ripening cultivar Moskovskaya Pozdnaya 15, zoned in the same year and having a high productivity, formed a separate sub-cluster, closer to the samples of later ripeness, which explains local origin of the cultivar Moskovskaya Pozdnaya 15 from the Pyshkinskaya cultivar [26]. The genetic remoteness of this variety is also confirmed by its separate varietal type in the Central Russian group of varieties. The Parus cultivar with high quality parameters formed its own cluster, which proves the genetic remoteness of this cultivar from the general group of varieties and indicates a complex origin from various lines with economically valuable properties.

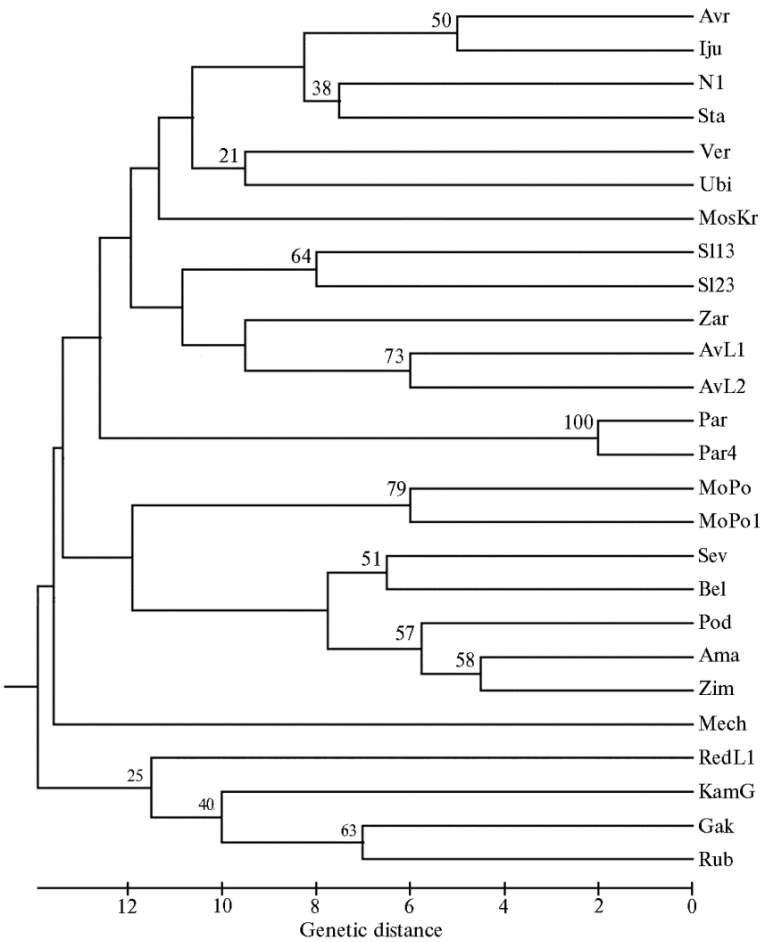

Fig. 3. UPGMA tree derived from data on microsatellite loci polymorphism of headed cabbage (*Brassica oleracea* L.) breeding samples (Genetic collection of the Federal Scientific Vegetable Center). Bootstrap replicates of 1000. For designations and description of samples, see Table 1.

Cultivars of the Ditmarskaya early type originating from Central Europe (Iyunskaya 3200, Stakhanovka 1513, Nomer pervyi gribovskii 147 and the early

ripening hybrid Avrora F_1 which has the Iyunskaya 3200 cultivar in the pedigree) formed a separate cluster. All these samples were early maturing. The cultivar Nomer pervyi gribovskii 147 zoned since 1943 was used to create cultivar Iyunskaya 3200, which is also consistent with its position in the dendrogram. The lines derived from the Avrora F_1 hybrid formed a separate subcluster. This confirms that the derived lines can be genetically quite distant from the hybrid. The traditional mid-season cultivars Slava Gribovskaya 231 and Slava 1305 of the Slava type, which were bred at the Gribovskaya Experimental Station and released in 1940, grouped in a separate subcluster.

The new mid-early hybrid Zarnitsa F_1 formed a separate branch of the dendrogram closer to the early ripening specimens, while the late-ripe hybrid Mechta F_1 formed a cluster at a 0.123 distance from the general group of cabbage specimens.

Savoy cabbage cultivars grouped into a separate subcluster within the white cabbage cluster. All of them belonged to different types of varieties, therefore, they were located genetically distantly from each other. The early-ripe cultivar Yubileynaya 2170 resulted from crossing the Savoy Viennese early cabbage and the white cabbage variety Nomer pervyi gribovskiy 147. The new mid-ripe cultivar Moskovskaya kruzhevnitsa with bubbly leaves turned out to be the most genetically distant from the other two cultivars of Savoy cabbage.

Traditional cultivars of red cabbage Kamennaya golovka 447 and Gako 741, bred at the Gribovskaya Experimental Station and released in 1943, belong to the Erfurt and Gako variety types, respectively, which is confirmed by their genetic remoteness. The Rubin variety and the I3 424-17 breeding line were grouped in the same cluster. Traditional varieties are well adapted to local growing conditions and can be donors of valuable genes conferring genetic variability and plasticity.

It should also be noted that the relatively recently bred cultivars Parus, Moskovskaya kruzhevnitsa, hybrids Zarnitsa F_1 and Mechta F_1 were quite distant from the main clusters consisting of traditional varieties. In addition, the breeding lines derived from promising F_1 hybrids significantly differed from the original form, which makes it possible to use DNA markers in inbreeding when obtaining hybrid-based lines.

In this study, a relatively small set of microsatellite markers successfully classified unique domestic head cabbages. El-Esawi et al. [27] used only twelve microsatellite loci to revealed a 75.7% polymorphism among twenty-five *Brassica oleracea* L. genotypes of which fifteen genotypes were cabbages, which is consistent with our findings.

Despite significant advances in the genetics of cabbage crops, the researchers mainly focus on the pathogen resistance [28-30], flowering time [31, head 32], morphology [33] and cracking [34, 35], with the lack of information on the genetic identification of cabbage varieties from different ripeness groups. Based on the genetic assessment of the studied breeding samples, we identified two main groups of white cabbage genotypes, with early maturation (the cluster of Avrora F_1, Iyunskaya 3200, Nomer pervyi gribovskiy 147, and Stakhanovka 1513) and late maturation (Moskovskaya pozdnyaya 15, Severyanka F_1, Belorusskaya 455, Amager 611, and Zimovka 1474). The Savoy cabbage genotypes in general were more precocious, which may explain their close genetic location to the group of more early maturing cultivars. Of these, the cultivar Virtue 1340 is the most late-ripening (the ripening period is about 130 days), while the white cabbage late-ripen genotypes need more than 140 days to reach ripeness.

Since environmental factors can influence varietal morphological traits and characteristics, cause phenotypic variability and hamper the assessment of varietal traits, DNA markers are the only tool for accurately identifying, maintaining and preserving genotypes for further selection [36]. DNA analysis with a set of known SSR markers allows reliable identification and selection of breeding samples from different ripeness groups and among valuable domestic cultivars, which are suitable for growing in all regions of Russia.

Thus, SSR markers were effective in detecting genetic variability among twenty-four genotypes of headed cabbage, including those similar in origin and belonging to the same cultivar. The results of DNA analysis confirm pedigrees of the traditional varieties and new hybrids. The genetic background of the genotypes can be decisive for design of crossing combinations in obtaining new breeding forms. The results of SSR analysis and genetic distance evaluation are "decision support information". This helps to select breeding material, control crossing combinations within groups of different ripeness, with preserving characters of the original cultivar types, and identify breeding lines in hybrid and/or varietal populations.

REFERENCES

1. Chepkemoi J. *The world leaders in cabbage production.* WorldAtlas, 2017. Available: worldatlas.com/articles/the-world-leaders-in-cabbage-production.html. Accessed: 15.03.2020.
2. Novotny C., Schulzova V., Krmela A., Hajslova J., Svobodova K., Koudela M. Ascorbic acid and glucosinolate levels in new czech cabbage cultivars: effect of production system and fungal infection. *Molecules*, 2018, 23(8): 1855 (doi: 10.3390/molecules23081855).
3. Lizgunova T.V. *Kul'turnaya flora SSSR. Tom 11. Kapusta* [Cultural flora of the USSR. V. 11. Cabbage]. Leningrad, 1984 (in Russ.).
4. Formisano G., Roig C., Esteras C., Ercolano M.R., Nuez F., Monforte A.J., Pico M.B. Genetic diversity of Spanish *Cucurbita pepo* landraces: an unexploited resource for summer squash breeding. *Genetic Resources and Crop Evolution*, 2012, 59(6): 1169-1184 (doi: 10.1007/s10722-011-9753-y).
5. Collard B.C.Y, Mackill D.J. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2008, 363(1491): 557-572 (doi: 10.1098/rstb.2007.2170).
6. Guichoux E., Lagache L., Wagner S., Chaumeil P., Léger P, Lepais O., Lepoittevin C., Malausa T., Revardel E., Salin F., Petit R.J. Current trends in microsatellite genotyping. *Molecular Ecology Resources*, 2011, 11(4): 591-611 (doi: 10.1111/j.1755-0998.2011.03014.x).
7. Lowe A.J., Moule C., Trick M., Edwards K.J. Efficient large-scale development of microsatellites for marker and mapping application in *Brassica* crop species. *Theoretical and Applied Genetics*, 2004, 108(6): 1103-1112 (doi: 10.1007/s00122-003-1522-7).
8. Márquez-Lema A., Velasco L., Pérez-Vich B. Transferability, amplification quality, and genome specificity of microsatellites in *Brassica carinata* and related species. *Journal of Applied Genetics*, 2010, 51(2): 123-131 (doi: 10.1007/BF03195720).
9. Thakur A.K., Singh K.H., Singh L., Nanjundanet J., Khan Y.J., Singh D. SSR marker variations in *Brassica* species provide insight into the origin and evolution of *Brassica* amphidiploids. *Hereditas*, 2018, 155(6): 1-11 (doi: 10.1186/s41065-017-0041-5).
10. Raza A., Mehmood S.S., Ashraf F., Khan R.S.A. Genetic diversity analysis of *Brassica* species using PCR-based SSR markers. *Gesunde Pflanzen*, 2019, 71(1): 1-7 (doi: 10.1007/s10343-018-0435-y).
11. Tonguc M., Griffiths P.D. Genetic relationships of *Brassica* vegetables determined using database derived sequence repeats. *Euphytica*, 2004, 137(2): 193-201 (doi: 10.1023/B:EUPH.0000041577.84388.43).
12. Louarn S., Torp A.M., Holme I.B., Andersen S.B., Jensen B.D. Database derived microsatellite markers (SSRs) for cultivar differentiation in *Brassica oleracea*. *Genetic Resources and Crop Evolution*, 2007, 54(8): 1717-1725 (doi: 10.1007/s10722-006-9181-6).
13. Saxena B., Kaur R., Bhardwaj S.V. Assessment of genetic diversity in cabbage cultivars using RAPD and SSR markers. *Journal of Crop Science and Biotechnology*, 2011, 14(3): 191-196 (doi: 10.1007/s12892-011-0018-2).
14. Zhu S., Zhang X., Liu Q., Luo T., Tang Z., Zhou Y. The genetic diversity and relationships of cauliflower (*Brassica oleracea* var. *botrytis*) inbred lines assessed by using SSR markers. *PLoS ONE*, 2018, 13(12): e0208551 (doi: 10.1371/journal.pone.0208551).
15. Artem'eva A.M., Kloke E., Chesnokov Yu.V. *Informatsionnyi vestnik VOGiS*, 2009, 13(4): 759-771 (in Russ.).

16. Izzah N.K., Lee J., Perumal S., Park J.Y., Ahn K., Fu D., Kim G.-B., Nam Y.-W., Yang T.-J. Microsatellite-based analysis of genetic diversity in 91 commercial *Brassica oleracea* L. cultivars belonging to six varietal groups. *Genetic Resources and Crop Evolution*, 2013, 60(7): 1967-1986 (doi: 10.1007/s10722-013-9966-3).
17. Shapturenko M., Khotyleva L., Vakula S., Jakimovich A.V., Zabara Yu.M., Khotyleva L.V. Informative EST-SSR markers for genotyping and intraspecific differentiation of *Brassica oleracea* var. *capitata* L. *Vavilov Journal of Genetics and Breeding*, 2016, 20(1): 51-56.
18. Chesnokov Yu.V., Artem'eva A.M. Evaluation of the measure of polymorphism information of genetic diversity. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2015, 50(5): 571-578 (doi: 10.15389/agrobiology.2015.5.571eng).
19. Pritchard J.K., Stephens M., Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*, 2000, 155(2): 945-959.
20. Earl D.A., vonHoldt B.M. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 2012, 4(2): 359-361 (doi: 10.1007/s12686-011-9548-7).
21. Peakall R., Smouse P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 2012, 28(19): 2537-2539 (doi: 10.1093/bioinformatics/bts460).
22. Nei M. Genetic distance between populations. *American Naturalist*, 1972, 106(949): 283-392 (doi: 10.1086/282771).
23. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 1978, 89(3): 583-590.
24. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. MEGA5: molecular evolutionary genetic analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 2011, 28(10): 2731-2739 (doi: 10.1093/molbev/msr121).
25. DeWoody J.A., Honeycutt R.L., Skow L.C. Microsatellite markers in white-tailed deer. *Journal of Heredity*, 1995, 86(4): 317-319 (doi: 10.1093/oxfordjournals.jhered.a111593).
26. *Elena Mikhailovna Popova. Uchenyi, selektsioner. Osnovopolozhnik nauchnoi otechestvennoi selektsii po kapustnym kul'turam. Nauchnoe nasledie vospominaniya, razvitie nauchnykh idei* /Pod redaktsiei V.F. Pivovarova [Elena Mikhailovna Popova. Scientist, breeder. The founder of domestic scientific selection of cabbage crops. Scientific heritage — memories, the development of scientific ideas. V.F. Pivovarov (ed.]. Moscow, 2007 (in Russ.).
27. El-Esawi M.A., Germaine K., Bourke P., Malone R. Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers. *Comptes Rendus Biologies*, 2016, 339(3-4): 130-140 (doi: 10.1016/j.crvi.2016.02.002).
28. Peng L., Zhou L., Li Q., Wei D., Ren X., Song H., Mei J., Si J. Qian W. Identification of quantitative trait loci for clubroot resistance in *Brassica oleracea* with the use of *Brassica* SNP microarray. *Frontiers in Plant Science*, 2018, 9(822): 1-8 (doi: 10.3389/fpls.2018.00822).
29. Kifuji Y., Hanzawa H., Terasawa Y., Ashutosh, Nishio T. QTL analysis of black rot resistance in cabbage using newly developed EST-SNP markers. *Euphytica*, 2013, 190(2): 289-295 (doi: 10.1007/s10681-012-0847-1).
30. Mei J.Q., Ding Y.G., Lu K., Wei D.Y., Liu Y., Disi J.O., Li J., Liu L., Liu S., McKay J., Qian W. Identification of genomic regions involved in resistance against *Sclerotinia sclerotiorum* from wild *Brassica oleracea*. *Theoretical and Applied Genetics*, 2013, 126(2): 549-556 (doi: 10.1007/s00122-012-2000-x).
31. Okazaki K., Sakamoto K., Kikuchi R., Saito A., Togashi E., Kuginuki Y., Matsumoto S., Hirai M. Mapping and characterization of FLC homologs and QTL analysis of flowering time in *Brassica oleracea*. *Theoretical and Applied Genetics*, 2007, 114(4): 595-608 (doi: 10.1007/s00122-006-0460-6).
32. Uptmoor R., Schrag T., Stützel H., Esch E. Crop model based QTL analysis across environments and QTL based estimation of time to floral induction and flowering in *Brassica oleracea*. *Molecular Breeding*, 2008, 21(2): 205-216 (doi: 10.1007/s11032-007-9121-y).
33. Lv H., Wang Q., Zhang Y., Yang L., Fang Z., Wang X., Liu Y., Zhuang M., Lin Y., Yu H., Liu B. Linkage map construction using InDel and SSR markers and QTL analysis of heading traits in cabbage. *Molecular Breeding*, 2014, 34(1): 87-98 (doi: 10.1007/s11032-014-0019-1).
34. Pang W., Li X., Choi S.R., Nguyen V.D., Dhandapani V., Kim Y.Y., Ramchiary N., Kim J.G., Edwards D., Batley J., Na J., Kim H.R., Lim Y.P. Mapping QTLs of resistance to head splitting in cabbage (*Brassica oleracea* L. var. *capitata* L.). *Molecular Breeding*, 2015, 35(5): 1-12 (doi: 10.1007/s11032-015-0318-1).
35. Su Y., Liu Y., Li Z., Fang Z., Yang L., Zhuang M., Zhang Y. QTL analysis of head splitting resistance in cabbage (*Brassica oleracea* L. var. *capitata*) using SSR and InDel makers based on whole-genome re-sequencing. *PLoS ONE*, 2015, 10(9): e0138073 (doi: 10.1371/journal.pone.0138073).
36. Korir N.K., Han J., Shangguan L., Wang C., Kayesh E., Zhang Y., Fang J. Plant variety and cultivar identification: advances and prospects. *Critical Reviews in Biotechnology*, 2013, 33(2): 111-125 (doi: 10.3109/07388551.2012.675314).