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STUDY OF GENETIC DIVERSITY OF APPLE TREE IN THE GUBIN REGION OF AZERBAIJAN

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Abstract. The work is devoted to the study of the genetic diversity of local apple varieties common in the Guba region of Azerbaijan. The purpose of this work was to study biomorphological characteristics, identify them based on molecular markers and determine the degree of genetic relationship between species and forms of apple varieties. Based on DNA analysis of apple tree varieties from the collection, patterns of distribution of genes that control the long-term preservation of fruits in apple tree varieties and forms were identified. Unique genotypes carrying rare alleles at microsatellite loci were identified.

Keywords: *Variety, genotype, genetic analysis, DNA markers.*

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1. Introduction

In Azerbaijan, as in most countries of the world, the most important fruit crop is the apple tree. She is a leader in increasing fruit production. Its wide distribution is due, first of all, to valuable dietary fruits, which are rich in vitamins, easily digestible amino acids, sugars, nitrogenous and other biologically active substances. In addition, apple fruits are valued for their preventive and therapeutic properties against many diseases, as well as the possibility of using them both fresh and processed all year round (Sadiqov, 2013; 2015). The long juvenile period of the apple tree, heterozygosity for many traits and self-incompatibility limit the possibilities of intensifying the breeding process and quickly obtaining new varieties. To evaluate hybrid forms for a number of morphological characteristics, a long period of time, measured in years, is required. This is especially true for assessing fruit parameters: shape, color, taste, chemical composition, etc. (Talibov & Bayramov, 2013).

The diversity of climatic conditions in our country requires the creation of a large assortment of varieties that are resistant to various types of stress and have high commercial and consumer qualities. This, in turn, poses certain challenges for breeders to create new high-quality apple varieties. Due to the need to intensify the selection process, it is advisable to use genetic methods based on DNA analysis. The use of molecular markers makes it possible to significantly speed up the identification of the

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starting material and analyze the results of crossing in a fairly short period of time. This makes it easier to select parental pairs for crossing, search for parental material in hybrid forms and analyze the introgression of useful traits from original forms to descendants. Marking apple tree varietal material will facilitate control over its uniformity when planting mother plantations, varietal cleaning of orchards and when selling planting material. DNA analysis, which directly characterizes the genome and not its phenotypic manifestation, can provide stable characteristics of the plant, neutral in relation to the environment and practically suitable for identifying genotypes, registering varieties and marking economically valuable genes and traits.

The purpose of this work was to study the genetic diversity of apple tree varieties and forms from the collection of the Research Institute of Fruit and Tea Growing of the Guba region of Azerbaijan using analysis of microsatellite genome sequences and to identify target alleles of genes for economically valuable traits in this collection using the method of molecular markers.

2. Materials and methods

The work was carried out by the Research Institute of Fruit and Tea Growing of the Ministry of Agriculture of the Republic of Azerbaijan, located on the territory of the Guba-Khachmaz economic zone, which accounts for 20% (Afunian *et al*., 2004) of all fruit plantings in the country and at the Institute of Genetic Resources MNOAR. The biological objects of the study were varieties and hybrid forms of apple trees from the collection of the Research Institute of Fruit and Tea Growing. A brief description of the starting material is given in Table 1.

To isolate DNA, young, healthy apple leaves were taken from the apical part of the shoot or buds beginning to bloom, one sample for each variety. DNA extraction was carried out according to the protocol proposed by Edwards et al. (1991) as modified by Forte (2002) using purification from polyphenolic compounds with lithium chloride. In addition, we used the Puchooa method (2004) adapted for working with plant material with a high content of phenolic compounds.

To analyze the collection of varieties and hybrid forms of apple trees, we searched for the most productive markers of the Vf gene in literary sources. To determine the presence of the Vf gene, the VfC marker was used, obtained from a study of the primary sequence of homologous members of the HcrVf family located in the Vf locus of the first chromosome (Afunian *et al*., 2004). To identify the Vf gene, PCR with primers VfC1 and VfC2 was used. Amplification was carried out in the following mode: 94^0C – 4 min, 30 cycles: 94^0C – 1 min, 58^0C – 1 min, 72^0C – 1 min; 72^0C – 7 min (Afunian *et al*., 2004). The gene involved in expansin synthesis in apple fruits was identified using the MD-Exp7SSR marker. The PCR reaction was carried out according to the following program: 94^0C – 120s, 35 cycles: 52^0C – 45s, 72^0C – 120s, 94^0C – 30s; 1 cycle 52^0C – 45s, 72^0C – 10 min. (Costa, 2008). Analysis of genetic polymorphism of apple varieties and hybrid forms was carried out using microsatellite primers (Table 2). The reaction was carried out according to the following program: 94^0C – 2 min 30 s; 4 cycles: 65^0C – 30s, 72^0C – 1 min, while the primer annealing temperature decreases by 1^0C with each cycle; 30 cycles: 94^0C – 30s, 60^0C – 1 min, 72^0C – 1 min; 72^0C – 5 min. (Gianfranceschi, 1998).

Table 1. Productivity and quality of fruits of new breeding varieties of apple trees

Variety	ripening	yield	Fruit weight (gr.)		Fruit volume	Fruit density
			average	maximum		
Fahima	Summer	163	137	143	140	0,97
Nigqar	Summer	150	138	140	141	0,97
Sulkh	autumn	183	176	184	279	0,63
Marfa	autumn	160	160	163	243	0,65
Khazar	winter	180	166	174	249	0,66
Ulvi	«------»	195	138	149	141	0,97
Vatan	«------»	170	145	155	220	0,65
Nubar	«------»	155	137	145	140	0,97
Chiriqtala	«------»	173	154	160	228	0,67
Davamli	«------»	145	154	160	228	0,67
Emil	«------»	185	153	155	226	0,67
Elvin	«------»	180	155	161	229	0,68
Gubinskoe autumn	«------»	155	164	170	247	0,66
Gubinskoe winter	«------»	154	160	179	247	0,64
Sevinqj	«------»	180	148	158	231	0,53
Shabran	«------»	175	137	142	140	0,97
Zumrud	«------»	165	155	166	230	0,67
Qizil tadj	«------»	180	159	168	234	0,68
Eldar	«------»	165	141	151	224	0,62
Maxmari	«------»	185	165	173	243	0,68
Nuran	«------»	153	145	153	220	0,66
Sarvan	«------»	161	154	166	228	0,67
Sadaf	«------»	185	215	225	314	0,68

Table 2. Characteristics of microsatellite loci used in the work

Name	Direct followerity (5′-3′)	Reverse sequenceity (5′-3′)	Fragment size	Number alleles	Marker type	Clutch group
CH01f03b	gag aag caa atg caa aac cc	ctc ccc ggc tcc tat tct ac	139–183	7	Mono-locus	9
CH03d11	acc cca cag aaa cct tct cc	caa ctg caa gaa tcg cag ag	115–181	6	Mono-locus	10
CH04e03	ttg aag atg ttt ggc tgt gc	tgc atg tct gtc tcc tcc at	179–222	11	Mono-locus	5
COL	agg aga aag gcg ttt acc tg	gac tca ttc ttc gtc gtc act	220–240	5	Mono-locus	10
CH02c02b	tgc atg cat gga aac gac	tgg aaa aag tca cac tgc tcc	78–126	5	Mono-locus	4
CH02g04	ttt tac ctt ttt acg tac ttg agc g	agg caa aac tct gca agt cc	132–197	7	Mono-locus	17
CH03d12	gcc cag aag caa taa gta aac c	att gct cca tgc ata aag gg	108–154	7	Mono-locus	6

CH03d07	caa atc aat gca aaa ctg tca	ggc ttc tgg cca tga ttt ta	186–226	8	Mono-locus	6
CH03d01	cgc acc aca aat cca act c	aga gtc aga agc aca gcc tc	95–115	7	Mono-locus	2
CH03d08	cat cag tct ctt gca ctg gaa a	tag ggc tag gga gag atg atg a	129–161	5	Mono-locus	14
Ch05g03	gct ttg aat gga tac agg aac c	cct gtc tca tgg cat tgt tg	135–192	6	Mono-locus	17
CH05g08	cca aga cca agg caa cat tt	ccc ttc acc tca ttc tca c	161–179	5	Mono-locus	1
CH04c07	ggc ctt cca tgt ctc aga ag	cct cat gcc ctc cac taa ca	98–135	8	Mono-locus	14
CH03a04	gac gca taa ctt ctc ttc cac c	tca agg tgt gct aga caa gga g	92–124	11	Mono-locus	5
MD-Exp7SSR	cat aga agg tgg cat gag ca	ttt ctc ctc aca ccc aaa cc	198-220	8	Mono-locus	6

Separation of amplification products with microsatellite primers was carried out by polyacrylamide gel electrophoresis (PAGE) in a vertical electrophoresis chamber Sequigen GT system (BIO-RAD). TBE was used as a buffer system. The gel was developed using the silver nitrate staining method (Benbouza *et al*., 2006). PCR products were previsualized on an agarose gel to confirm that amplification was successful. After this, the amplifier was separated by electrophoresis in a 6% polyacrylamide gel. This method made it possible to separate fragments that differ by 1 pair of nucleotides. To determine the length of the amplified fragments, a molecular weight marker 10bp DNA ladder (Invitrogen) (0.05 g/L) was used. After drying the gel, it was viewed on a light table, photographed and analyzed. To quantify polymorphism, the obtained data were presented in the form of a matrix of states of binary features, in which the presence or absence of amplicons of the same size in the electrophoretic spectra was considered as state 1 or 0, respectively. Dendrograms were constructed using the Past program. Calculation of genetic distances (GD) was performed using the Dyson coefficient of difference. ISSR is a fast, simple and easily reproducible method. ISSR markers typically exhibit high polymorphism and their greatest advantage is that no prior genome sequence information is required. When studying the genetic diversity of 71 apple genotypes, 68 points were synthesized on 8 ISSR primers characteristic of fruit plants, of which 47 (69%) were polymorphic and 21 (31%) were monomorphic. The number of amplified fragments per locus varied from 7 to 10.

3. Research results

SSR analysis was used to assess the variability of the apple genome. 15 microsatellite loci were analyzed on 71 plants of domestic and foreign varieties and forms of apple trees. The selected loci were characterized by a high level of information content. The number of alleles per locus varied from 10 (for the CH02c02b locus) to 20 (for the CH03d07 locus), which makes it possible to identify intervarietal polymorphism. The use of selected primers made it possible to obtain reproducible specific polymorphic electrophoretic spectra of SSR fragments for each apple sample under study. A total of 217 SSR fragments ranging in size from 76 to 250 bp were identified. A significant part of the detected alleles are rare, that is, they were found only once among the analyzed

genotypes. For example, in the CH02g04 locus, out of 17 detected alleles, 5 (with sizes of 185, 186, 189, 191 and 194 bp) can be classified as rare, since they were found in only one of the analyzed genotypes, while the allele with a length of 199 bp found in 18 samples.

Genotypes carrying target alleles of genes for long-term preservation of fruits have been identified. In particular, varieties have been isolated containing alleles of the Md-ACO1 and Md-ACS1 genes, which cause a reduced level of ethylene synthesis in fruits. Genotypes have been identified that carry combinations of Md-Exp7 gene alleles responsible for the long-term preservation of fruit hardness. The use of such varieties in breeding work as parent pairs makes it possible to create varieties with long-term resistance to adverse environmental factors. It has been established that analysis of microsatellite genome sequences in apple tree varieties and forms provides the breeder with additional information about the genetic similarities and differences of the breeding material, which will allow a more reasonable selection of pairs for crossing. Due to the identified high level of polymorphism of microsatellite loci, the possibility of using this method for assessing the hybrid stock and certification was established.

4. Conclusion

Based on the analysis of SSR spectra, genetic distances between samples were calculated. This involves calculating the Dyson similarity coefficient between pairs of samples. It was found that in the studied samples the values of similarity coefficients vary from 0 (between the Zumrud and Gobustan varieties) and 0.7 (between the Fakhima summer and Khazar winter varieties). At the same time, the similarity coefficient was high for varieties with a common ancestry.

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