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Genetic Diversity of Common Wild Wheat Species in Armenia Using ISSR Markers

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Abstract: Armenia is one of the primary genealogical centers of numerous crops, the wild relatives of many of which are still found in Armenia today. Population limits of three out of 4 species of wild relatives of endemic wheat in Armenia (*T. araraticum* Jakubz, *T. urartu* Tumanian ex Gandilyan, *T. boeoticum* Boiss.) have been reduced in recent decades as a result of various ecological and economic functions, they are facing the danger of extinction and are registered in the Red Book of Plants of the Republic of Armenia. In order to identify these species as selection raw material, genetic characterization of populations was carried out according to ISSR-PCR markers, as well as decoding genetic formulas and barcodes. As a result, according to the coefficients of the genetic pattern, the studied wild types of wheat are characterized as basic or typical gene pools with the least quantity and frequency of rare alleles. The populations are stable and in conditions of efficient number of isolates, the gene pool is capable of self-reproduction. The obtained results as genetic markers will provide an opportunity to further clarify the origin of the species, evolution, patterns of inheritance of useful economic traits, cell genetic characteristics of the species, natural resistance to adverse environmental factors and diseases, etc.

Keywords: Barcode, Ecosystem, Floristic Region, Locus, Population

Introduction

Genetic resources of flora and fauna are the wealth of each country. Being the output of natural evolution and human activities, they serve as a strategic material for ensuring food production and at the same time have a huge role in the process of maintaining the ecological balance. Genetic resources are a valuable starting material in the selection of cultivated plants and farm animals, contributing to the economic growth of each country and its overall activities, national autonomy, and food security (Eastwood *et al*., 2022).

The activities aimed at the conservation and sustainable use of genetic resources and ensuring food safety are closely related to the availability of genetic resources. The development issues in science and the latest technologies, economics and law, ethics, and international politics are closely intertwined with this question (Altukhova *et al*., 2004).

For many years, genetic resources have been considered the property of all mankind, which implied free and gratuitous access to genetic resources, regardless of their usage purpose (Begna, 2021). However, at the end of the 20th century, as a result of the reduction of the genetic diversity of flora and fauna on the one hand, and the development of biotechnology on the other hand, the value of genetic plasma as a starting selection material increased dramatically. From that perspective, the wild relatives of cultivated plants gained special value and demand in the world markets, which, being carriers of genes for drought, frost, diseases, and pest resistance, were of great interest for selection. Wild relatives of cultivated plants had no established value and were widely used as starting material in breeding programs (Avagyan, 2007; Yadav *et al*., 2022; Zhukovsky, 1971; Brezhnev and Korovina, 1981; Galluzzi *et al*., 2020).

According to the scientifically based theory of the distribution and centers of origin of agrodiversity, the

tropical and subtropical regions of the world, which are now mainly located in the areas of developing countries, are the main biodiversity centers of the planet. Due to insufficient material and technical base or highly qualified scientific potential, developing countries are not yet able to ensure reliable, guaranteed long-term conservation and efficient use of their genetic resources (Griffon and Hernandez, 2020; Panis *et al*., 2020).

At the end of the 20th century, due to the global change in the biosphere caused by human activity, the process of reducing global genetic diversity took on a threatening nature and became an important socio-economic, political, and ethnic problem. Currently, the preservation and diversity of the genetic pool play an extremely important role in the genetic homogeneity of the main agricultural crops, problems solution, genetic erosion prevention, economic growth of the country, food security of the population, as well as for the increase and sustainable development of health care system (Eastwood *et al*., 2022; Chandra and Idrisova, 2011).

Biodiversity is one of the main keys to the vital activity of the Armenian people and the socio-economic development of the country. The location and relief of Armenia have promoted the formation of a wide range of biodiversity, abundant endemism, and rich agro-biodiversity. On the small territory of Armenia (29.74 thousand km^2) there are about 3800 types of high-order flowering plants, and more than 17500 animals, about 500 of which are vertebrates (Biodiversity Assessment for Armenia, (2000); "5th national report of the Republic of Armenia to the convention on biological diversity" 2014; "6th national report to the convention on biological diversity of the Republic of Armenia" 2019). The number of lower plants and microorganisms exceeds several tens of thousands. Armenia takes up one of the leading places regarding plant density (more than 100 species per km^2). The number of endemic flora of Armenia is 144 species, which is about 3.8% of the total species diversity of its natural world ("5th national report of the Republic of Armenia to the Convention on Biological Diversity" 2014; "6th national report to the convention on biological diversity of the Republic of Armenia" 2019; Capacity Assessment for Biodiversity Conservation in Armenia, (2002).

Armenia is one of the most important centers of origin for a number of economically valuable plants and animals. Wild congeners of cereals and other crops, as well as those of domestic animals, have been so far introduced here. Armenia's biodiversity is rather rich in economically valuable plant species. About 2000 plant species are endowed with nutrients, fodder, medicine, cosmetics, essential oil, honey, and resin-producing properties (Zhukovsky, 1971; Medina Lozano and Díaz Bermúdez, 2021).

The majority of biodiversity has been utilized for centuries by the local people through traditional methods without considering the natural possibilities for ensuring the reproduction of bioresources. As a result of such a using approach, species degradation, and even extinction have gradually occurred, which has led to the overall impoverishment/ depauperization of biodiversity. This phenomenon has taken a more active course in the recent century related to the exacerbation of industrial, agricultural, transport, energy, and other types of environmental pollution, as well as due to intensive forests, pastures, and other ecosystem exploitation. The situation has become more acute in recent years due to the use of biological resources during the socio-economic restructurings. As a result, some habitats have been significantly degraded, and a number of species stood on the verge of extinction due to natural and anthropogenic impacts. Currently, almost half of the flora species of Armenia need protection to a greater or lesser extent. The red book of RA plants includes 386 species or 12% of the flora (Tamanyan *et al*., 2010). 61 of the mentioned species are included in the red book of the former USSR. 35 valuable plant species have completely disappeared from the territory of Armenia and now a number of plant species that are of great interest are on the verge of extinction. In addition, today there are no mechanisms in the republic for the availability of the country's genetic resources, which are based on preliminarily informed consent and signed mutually agreed contracts regarding the use of genetic material, as well as joint distribution of the results of their use. In the absence of a monitoring system, permits for the export and exploitation of genetic resources can also cause a reduction in their numbers (Salgotra and Chauhan, 2023).

Under the created conditions, it is necessary to develop a national strategy and carry out special coordinated actions in the field of access to national genetic resources, in order to preserve and sustainably use the country's biodiversity.

Moreover, above all these, the biodiversity known to date has been very randomly studied, especially concerning genetic and ecosystem methods.

Among the diversity of plants' genetic resources, wheat occupies a special place due to its scientific and practical significance. Wheat belongs to *the Triticum* genus of *the Poaceae* family. Three of the 4 species found in the world are widespread in Armenia (*T. araraticum* Jakubz, *T. urartu* Tumanian ex Gandilyan, *T. boeoticum* Boiss.), and are endemic to Armenia (Takhtajan, 2009a).

T. araraticum species grow at the altitude of 1200-1600 m above sea level at the middle mountainous zones, steppes, dry rocky slopes, sown areas, and field borders, as well as along the irrigation channels. It is

distinguished by high drought resistance and is also resistant to unfavorable environmental conditions. It is spread throughout floristic regions of Yerevan (neighborhood of Voghjaberd and Vedi villages) and Dareleges (neighborhood of Areni, Arpi, Getap, and Aghavnadzor villages) (Fig. 1) (Brezhnev and Korovina, 1981; Gandilyan, 2010; Tsvelev, 2006; Tamanyan *et al*., 2010; Takhtajan, 2009b).

It is registered in the Red Book of Flora of the Republic of Armenia and is a vulnerable species. The surface of the distribution area is less than 5000 km²; the surface of the residence area is less than 500 km². Since a significant part of the distribution area of the species is outside the borders of Armenia, the threat category has been reduced. It was included in the first edition of the Red Book of Armenia as a species under immediate threat of extinction (Tamanyan *et al*., 2010).

T. urartu species grows in the middle mountain zone, at altitudes of 1300-1500 m above sea level, in the steppes, on clay and rocky slopes, and in the coexistence of wild one-grained wheat and goatgrass species. It is used in the selection activities. Many amphidiploids were obtained by using this species in the "Scientific Center of Agro-Biotechnology" ANAU branch. It is spread in the floristic regions of Yerevan (neighborhood of Voghjaberd village, „Erebuni “State Reserve) (Fig. 1AB) (Tsvelev, 2006; Gandilyan, 2010; Shakaryan *et al*., 1988; 2003; Tamanyan *et al*., 2010; Takhtajan, 2009a). It is registered in the RA Red Book of Plants, is and endangered species. The surface of distribution and settlement areas is less than 500 km². The species is threatened by the reduction of distribution and habitat areas due to changes in habitat conditions related to the intensification of agricultural activities. It was included in the first edition of the Red Book of Plants of Armenia as a species under immediate threat of extinction (Tamanyan *et al*., 2010).

T. boeoticum species grows in the middle mountainous zones, at the altitude of 1250-1800 m high above sea level, in the dry clay slopes. It has many varieties (about 85). It is considered to be the predecessor of cultivated one-grained wheat (*Triticum monococcum* L.). It is used in the selection activities. Many amphidiploids have also been obtained using this species (Shakaryan *et al*., 1992; Gandilyan, 2010), and the mentioned variety is spread in the floristic regions of Yerevan and Dareleges (Fig. 1AB) (Brezhnev 1981; Gandilyan, 2010; Takhtajan, 2009b).

It is obvious that each species is composed of several different populations, which are formed due to the interaction of heredity, variability, and selection factors, have their specific gene pool, which is the sum of all isolates' genes constituting the given population which makes them different from other populations.

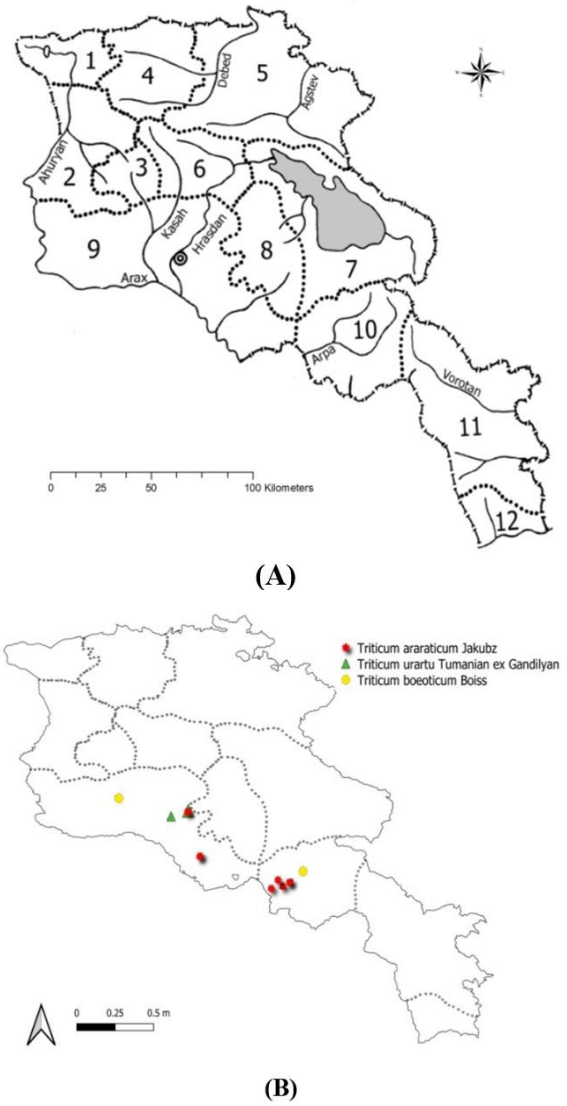


Fig. 1: (A) Map of the RA floristic regions: 1-Upper Akhuryan, 2-Shirak, 3-Aragats, 4-Lori, 5-Ijevan, 6-Aparan, 7-Sevan, 8-Geghama, 9-Yerevan, 10-Darelegis, 11-Zangezur, 12-Meghri; (B) and geographic distribution of the 3 wheat wild species in the RA

The genetic characteristics of the Armenian populations of wild wheat are very few or are absent at all in literature sources (Yesayan, 2010).

The current work presents the genetic characteristics of Armenian wild wheat populations according to ISSR-PCR markers.

Materials and Methods

Sampling

Three wild wheat species spread in Armenia have served as research objects: *Triticum araraticum* Jakubz.,

Triticum boeoticum Boiss., *and Triticum urartu* Tumanian ex Gandilyan. One population of the mentioned species is known in the Republic, the first two of which are found in Yerevan and Dareleges, and the third is found only in the floristic region of Yerevan (Tamanyan *et al*., 2010; Zhukovsky, 1971). For molecular genetic research, sampling in each floristic region was carried out by random selection from 33-35 plants, the distance between which was at least 50m. Certification of the Armenian wild wheat populations has been conducted in the ANAU laboratory of food quality control.

Molecular-Genetic Research

Molecular genetic research using the ISSR method (Vdovichenko, 2007) is carried out through the following stages:

- **Isolation of genomic DNA:** Total DNA isolation from seeds was carried out by the CTAB method (Padutov *et al*., 2007). 50 mg of sample tissue was used. The concentration of extracted DNA was determined using a NanoDrop One (Thermo Scientific, USA) spectrophotometer
- **PCR implementation (Primer Selection):** A 10µl reaction mixture for PCR had the following composition: 3.9µl water, 2µl buffer, 1µl dNTP, 0.4 µl primer, 0.1µl Taq-polymerase, 2µl (40 ng/µl) DNA, 0.6µl of $MgCl_2$. PCR was performed in the T-personal amplifier of the German company Biometra, in the following mode: DNA denaturation at 94°C for 4 min, then for 35 cycles of amplification: Denaturation at 94°C for 40 sec, annealing of primers at 52-64°C for 50 sec, elongation at 72°C for 40 sec, final elongation of chains at 72°C for 7 min. Marker DNA (100 bp +1.2+1.5+2+3 kb, 13 segments 100-3000 bp, "SolisBrodyne" Estonia) was used to determine the length of DNA segments
- **Electrophoresis Implementation:** Amplification results were subjected to electrophoresis on a 1.7% agarose gel using the Compact S phoresis apparatus of the German Biometric company. The gel was scanned through the gel-doc (bio-rad, usa) system. "quantity one" program was used in the Gel-Doc XR system to determine the length of DNA segments

Genetic Formulas and Barcoding

For the barcoding of DNA of three wild wheat varieties common in Armenia, the technology developed by Boronnikova (2013) has been used.

When writing the genera and species DNA fragments the two initials of the genus or species are indicated, for instance-TR_r750_{M27} (Sultangazina *et al*., 2020).

Ten-twelve bars are used to design a barcode, 4 of which determine the genera, 4-6 species, and 1-4 population. Fragments characteristic of the genera are highlighted in bold lines, species-in those of medium density, and population-in thin lines. The arrangement of the DNA fragments in the bars is made according to the length of the segments, in the order from the largest to the smallest (Boronnikova, 2009; Sultangazina *et al*., 2020).

Analysis of the Results

Amplification results are considered markers of the corresponding genomic DNA loci in the dominant inheritance pattern. The presence is considered as the homo- or heterozygous state of the dominant allele, and the absence-as the homozygous state of the recessive allele.

The following parameters were used to describe the genetic structure of the population:

1. The expected proportion of heterozygous genotypes in all populations as an index of total genetic diversity (H_T)
2. The expected number of heterozygous genotypes in subpopulations as an indicator of intrapopulation gene diversity (H_S)
3. The fraction of a population's genetic diversity in the total diversity, or an index of population segregation (G_{ST})

The index of genetic similarity of subpopulations was determined by Nei (1987) formula.

Population genetic analysis of wild wheat species distributed in different floristic regions was carried out using POPGENE 1.31 (Yeh and Boyle, 1999) and GENALEX6 software specialized for Microsoft Excel (Peakall and Smouse, 2006) determining the proportion of polymorphic loci (P_{95}) (Williams *et al*., 1990), expected heterozygosity (He), absolute (n_a) and effective (n_e) number of alleles at the locus, number of rare alleles (R) (Nei, 1987), Shannon's information Index (I) (Lewontin, 1972), mean number of morphs (µ) and fraction of rare morphs (h) (Zhivotovsky, 1980).

Statistical data processing was performed using SPSS and MS Excel programs, using standard methods of population-genetic research.

Results

Population-Genetic Analysis of Species

A molecular characterization is now a valuable tool for assessing variation in plant genetic resources. Polymorphism is also considered a useful selection tool in monitoring alien genome introgression in wheat breeding programs. In the present study, ISSR marker

systems were used to investigate the genetic variability of wheat genotypes.

Inter Simple Sequence Repeats (ISSR) markers or the analysis of inter-microsatellite segments is currently widely used for the genetic characterization of different crop varieties and wild species. It is used both in the activities of interspecies and intraspecies genetic variability, population genetic diversity, and species identification and in those of genome mapping and marking of useful economic traits (Alizadeh *et al*., 2017; Bylka *et al*., 2014). In this method, one or more primers with a length of 15-24 nucleotides consisting of 2-4 short tandem repeat and one selective nucleotide at the 3' end of the primer are used. Such primers enable to amplification of DNA segments that are located quite close to each other in the inter-microsatellite region. ISSR-PCR markers have a dominant inheritance pattern (Selezneva and Akhmedov, 2016; Bardukov *et al*., 2014; Kuluev *et al*., 2018).

Since an ISSR may be a conserved or non-conserved region, this technique is not useful for distinguishing individuals, but rather for phylogeographical analyses or maybe delimiting species, but still higher than that in actual gene sequences. The ISSR method does not allow determining the homo- and heterozygosity of organisms, which adds steps to the execution of the work but does not hinder the final result (Vdovichenko, 2007).

For the molecular genetic research of wild wheat species common in different floristic regions of Armenia, the selection of ISSR primers was made according to their effectiveness. All 15 primers designed for this purpose were individually tested on the genomic DNA of the studied species by PCR method. As a result, depending on the number of DNA segments (including polymorphic), according to the appropriate scale: 1-5 (1 is low, 5 is high), the effectiveness of the primers was determined (Boboshina and Boronnikova, 2012). As a result of evaluating the effectiveness of ISSR-PCR primers, it was found that on the species of *Triticum araraticum* Jakubz, 7 out of 15 primers were of very high (5), 3 of them high (4), 2-medium (3), 1-low (2) and 2 of very low efficacy. For the molecular-genetic research of the wheat wild species *T. araraticum* Jakubz. (Ta) in the floristic regions of Yerevan (Ta_1) and Dareleges (Ta_2), 7 ISSR primers were selected, 4 of which were dinucleotide: M_3 $(AC)_8CT$; M_{27} $(GA)_8C$; ISSR-4 $(TG)_8GC$; CR-215 $(CA)_6GT$, and three -trinucleotide: X_9 $(ACC)_6G$; X_{11} $(AGC)_6$; ISSR-9 $(ACG)_7G$ (Table 1). The average number of amplified DNA segments upon the use of the mentioned ISSR primers was 12 fragments, the maximum was 14 (X_9, X_{11}, CR-215), the minimum- 9 ($ISSR_4$). The average number of polymorphic DNA fragments was 8.7, the maximum was 11 (X_9, CR-215), the minimum-6 ($ISSR_4$). Overall, 61 or 73% of all 84 ISSR fragments identified were polymorphic Table 2. Besides, in the Ta_1 and Ta_2 floristic region of *T. araraticum* Jakubz, it has made $P_{95} = 0.718$ and $P_{95} = 0.734$, respectively.

Table 1: Efficacy of ISSR primers

			Primers efficacy *		
Primer's	Nucleotide sequence (5´-3´)	Annealing temperature, °C	*Triticum araraticum* Jakubz. (T_a)	*Triticum urartu* Gandilyan Tumanian ex (T_u)	*Triticum boeoticum* Boiss. (T_b)
M_1	$(AC)_8CG$	56	4	3	5
M_3	$(AC)_8CT$	54	5	5	5
M_{27}	$(CA)_8C$	52	5	5	4
X_1	$(CA)_6G$	61	1	2	2
X_9	$(ACC)_6G$	64	5	4	5
X_{10}	$(AGC)_6C$	64	3	5	1
X_{11}	$(AGC)_6$	64	5	5	5
ISSR-1	$(AC)_8T$	56	4	2	3
ISSR-3	$(TG)_8AA$	56	2	1	4
ISSR-4	$(TG)_8GC$	56	5	3	2
ISSR-8	$(GAG)_6G$	56	1	5	5
ISSR-9	$(ACG)_7G$	56	5	1	3
CR-212	$(CT)_8TG$	56	4	5	4
CR-215	$(CA)_6GT$	56	5	3	2
CR-216	$(GA)_6GG$	56	3	3	5

*5-very high, 4-high, 3-medium, 2-low, 1-very low

Table 2: DNA polymorphism of the wheat species *T.araraticum, T. urartu,* and *T. boeoticum* upon the use of ISSR primers

Species	Primers	Length of amplified fragments, kb	The amount of DNA fragment	Number of polymorphic DNA fragments, n	DNA polymorphism, %	Number of rare DNA rare DNA
Triticum	M_3	280-950	10	7	71	0
araraticum Jakubz	M_{27}	2500-1000	12	8	67	1
	X_9	380-1700	14	11	82	1
	X_{11}	200-1350	14	10	73	0
	ISSR-4	280-1300	9	6	62	0
	ISSR-9	300-1000	11	8	75	0
	CR-215	220-9500	14	11	78	0
	Total	-	84	61	73	2
Triticum urartu	M_3	200-8500	8	6	68	1
Tumanian ex	M_{27}	200-1000	12	9	77	1
Gandilyan	X_{10}	180-7500	10	6	59	0
	X_{11}	250-1250	14	9	66	0
	ISSR-3	300-1200	10	7	70	0
	CR-212	180-1150	14	11	76	1
	Total	-	68	48	71	3
Triticum boeoticum	M_1	180-1350	16	13	79	0
Boiss.	M_3	250-9000	18	13	73	0
	X_9	350-1750	11	8	69	2
	X_{10}	300-1100	14	11	76	0
	ISSR-8	350-1650	14	9	66	1
	CR-216	250-8500	12	9	78	0
	Total	-	85	63	74	3

There are two fragments with rare or below 5% occurrence frequency in the above-mentioned species. It has been registered in the Ta_1 floristic region. Such a low indicator of rare fragments proves the homogeneity and genetic isolation of the species (population). The most common criteria of genetic variability in populations are heterozygosity, which directly depends on the number of alleles.

The predicted heterozygosity in the loci of the selected group of the wheat species *T. araraticum* Jakubz. has made 0.282 fluctuating in the floristic regions within the range of 0.274 (Ta_1)- 0.292 (Ta_2). The effective number of alleles (ne) is a function related to polymorphic loci, the number, and frequency of alleles at a locus. It is considered to be an index for the genetic diversity of a population or species. The effective number of alleles estimates the inverse value of homozygosity and is the number of alleles with the same frequency, as a result of which the predicted heterozygosity in the population will be equal to the actual heterozygosity.

The absolute number of alleles at the locus (na) in the overall population of *T. araraticum* Jakubz. makes 1,852. The mentioned index acquired the following respective numerical values in the Ta_1 and Ta_2 floristic regions: 1.827 and 1.877. The effective number of alleles (ne) in the selected group was 1,466, recording the maximum value in the Ta_2 floristic region (ne = 1.476), and the minimum value in the Ta_1 floristic region (ne = 1.456).

Shannon's information index in the population of *T. araraticum* Jakubz. wheat species has made I = 0,440 amounting to Ta_1 (I = 0.447) and Ta_2 (I = 433) in the respective floristic regions.

The index of interpopulation diversity (μ) plays a key role in the characterization of genetic diversity. The initial value of this indicator is considered to be the frequency of Morph (as a result of our study, the frequency of ISSR markers). The more evenly the frequency of morphs is distributed, the closer the mentioned indicator to the maximum is. In the two floristic regions of the wild wheat variety *T. araraticum* Jakubz. Ta_1 (μ = 1,673) is distinguished by the highest homogeneity of allele frequency distribution and Ta_2 (μ = 1,737) floristic region by that of the lowest one Table 3.

The number of rare morphs (h) provides new information about the interpopulation diversity; in the case when μ gives an estimate of the degree of population diversity, the structure of the mentioned diversity is evaluated with the index of (h). In the case of dominant inheritance of ISSR markers, this indicator shows the number of null alleles. When h >0.3, the number of null alleles and homozygous recessive genotypes increases in the genetic structure of the population.

The value of h in two floristic regions of the wheat species *T. araraticum* Jakubz. was lower than 0.3, which points to the small number of null alleles in the structure of interpopulation; when characterizing the genetic structure of populations, the value of heterozygosity is used, which is otherwise called genetic diversity. Based on the above stated, the concept of total genetic diversity (H_T) was introduced, which is the heterozygosity of a selected group at all loci (H_S), it is an indicator of the average heterozygosity and segregation of populations (G_{ST}).

The total genetic diversity (H_T) of the selected group of *T. araraticum* wheat species makes 0.368. That is why the average heterozygosity gets lower (H_S = 0.277) (Table 4). The analysis of our research results reveals, that the lowest heterozygosity indices have been recorded in the case of X_{11} (G_{ST} = 0.093) and the highest ones in the case of $ISSR_4$ (G_{ST} = 201) primers. The differentiation of two floristic regions (Ta_1, Ta_2) of *T. araraticum* wheat species is weakly expressed, since the significant part of the considered genetic diversity is concentrated within the population (85.9 %), whereas the interpopulation variability is only 14.1% (Table 4) (p>0.95).

Six of all experimented 15 primers in the other studied wild wheat variety *Triticum urartu* Tumanian ex Gandilyan were of a very high (5), 1-of high (4), 4 medium (3), 2 of low (2) and 1 of very low efficiency. For molecular genetic research, 6 ISSR primers have been isolated, 3 of which were dinucleotide: M_9 $(AC)_8$ CT, M_{27} $(GA)_8C$, CR-212 $(CT)_8TG$, 3-trinucleotide: X_{10} $(AGC)_6C$, X_{11} $(AGC)_6$, ISSR-8 $(GAG)_6G$ (Table 2). Meanwhile, it should be noted that three of the selected primers, as high-efficiency primers (M_3, M_{27}, X_{10}) were used during the molecular genetic research of *T. araraticum* wheat species.

The average number of DNA segments amplified by the specified ISSR primers was 11.3 fragments, the maximum was 14 (X_{11}, CR-212), and the minimum was 8 (M_3). For comparison, it should be noted that X_{11} primer has also provided a synthesis of 14 DNA fragments in the case of wild wheat species *T. araraticum.*

On the whole, 48 or 71% of 68 amplified DNA fragments were polymorphic, the sizes of which fluctuated within 180-1280 bp. The maximum number of polymorphic loci in the selected group was 11 (CR-212), and the minimum was 6 (M_3, X_{10}). The portion of polymorphic loci (P_{95}) in the Yerevan floristic region of wild wheat variety *T. urartu* Tumanian ex Gandilyan has made P_{95} = 0.705 (Table 3). The number of rare fragments in the overall population is 4. The predicted heterozygosity (H_E) at all loci of the selected group was 0.208, the effective number of alleles (n_a) in the total population was 1,630, and the effective number of alleles (n_e) at the loci of the selected group was 1.362. Shannon Information Index in the selected group of *T. urartu* Tumanian ex Gandilyan wheat species makes 0.286. The low value of the interpopulation diversity index (μ = 1.629) points to more homogeneity of the allele frequency distribution (Table 3) (p>0.95).

The value of h in the selected group of wheat variety *T. urartu* Tumanian ex Gandilyan has made 0.156; it is smaller than 0.3, which is an indicator of the balanced structure of the population diversity and a small number of null alleles. Throughout the population genetic analysis of the wild wheat species *T. urartu*, it has been disclosed that the proportion of predicted Heterozygosity (H_T) in the selected group makes 0.284, while the expected (predicted) proportion (Hs) of heterozygous genotypes in the floristic regions has been equal to 0.211, which is lower than the index of H_T. The lowest value of Heterozygous genotypes (H_S) has been recorded in the case of M_{27} (H_S = 0,196), while the highest one-in case of ISSR-3 (H_S = 0.254) primers. Unlike the wild wheat variety *T. araraticum*, in this case, a considerable part of genetic diversity is concentrated within the population (G_{ST} = 76.3%), while the interpopulation variability makes 23, 7%.

Six of 15 ISSR primers tested for molecular-genetic investigations of wild wheat variety *Triticum boeoticum* were of very high (5), 3-high (4), 2of medium (3), 2-low (2), and -of a very low efficiency (Table 1). Three of 6 primers isolated for the population-genetic research of the mentioned wild wheat species were dinucleotide: M_1 $(AC)_8$ CG, M_3 $(AC)_8$CT, CR-216 $(GA)_6$GC, 3-trinucleotide: X_9 $(ACC)_6G$, X_{11} $(AGC)_6$, ISSR-8 $(GAG)_6C$ (Table 1).

For comparison, it should be mentioned that M_3 $(AC)_8$ CT and X_{11} $(AGC)_6$ primers were manifested as highly active primers during the molecular genetic research of the three wheat species. The total number of DNA fragments amplified during ISSR-PCR at two floristic regions (Tb_1, Tb_2) of wild wheat variety *T. boeoticum* is 85 with 180-1750 bp length. The average number is 10.5 fragments, maximum-18 (M_3), and the minimum-11 (X_9). 63 or 74% of amplified DNA fragments were polymorphous. The average number of polymorphic loci of the studied variety was 8.5, maximum-13 (M_1, M_3), and minimum-8 (X_9) (Table 2). Thus, the proportion of polymorphic locus (P_{95}) in the Tb_1 and Tb_2 floristic regions of wild wheat variety species *T. boeoticum* has made 0.714 and 0.768, respectively Table 2.

Predictable heterozygosity (H_E) in all loci of the selected group was 0.163, maximum-in Tb_2 (H_E = 0.168), and minimum-in Tb_1 (H_E = 0.157) floristic regions. The effective number of alleles (na) in the total population was 1.742, ranging from 1.715 to 1.768. The effective number of alleles (ne) in the loci of the selected group was 1.296, recording the following values in floristic regions: Tb_1 (ne = 1.287), Tb_2 (ne = 1.306). Shannon's information Index (I) indicates high diversity in the population: I = 0.384; 0.391 and 0.377 in Tb_1 and Tb_2 floristic regions, respectively. The lowest value in the number of rare alleles for all three wheat species was registered in the case of one-grained wheat (R = 1) with the maximum value in Tb_1 (R = 1) and minimum-in Tb_2 (R= 0) floristic regions.

The index of interpopulation diversity (μ) recorded a maximum value in Tb_1 (μ = 1.638) and a minimum in Tb_2 (μ = 1.623) floristic regions. The mentioned index is the lowest in the case of *T. boeoticum* wheat variety (μ = 1,634) among the studied three wild wheat species, which

testifies to the higher homogeneity of alleles frequency distribution. The value of the number of rare morphs (h) both in the overall population of wheat variety *T. boeoticum* (h = 0.156) and in Tb_1 (h = 0.153) and Tb_2 (h = 0.144) floritic regions is less than 0.3, which, as in case of the previous species, also points out the balanced structure of population diversity and a small number of null alleles (Table 3).

The predicted Heterozygosity (H_T) of the selected group of wild wheat species *T. boeoticum* (H_T) has made 0.259, while the expected proportion of heterozygous genotypes of the floristic regions (H_S) is 0.162, which is again lower than the H_T value for the other two cases (Table 4).

The predicted average heterozygosity and the very low number of rare fragments indicate the genetic homogeneity of the wheat species *T. boeoticum*. The lowest value of heterozygous genotypes (H_S) was recorded for M_3 (H_S = 0.144) and the highest value for M_1 (H_S = 0.182) primers.

The differentiation of Tb_1 and Tb_2 floristic regions is not strongly pronounced, since, as in two previously studied species, here again, the overwhelming part of genetic diversity (G_{ST} = 77.2%) is concentrated within the population, whereas the interpopulation variability makes 22.8% (Table 4) (p >0.95).

Table 3: The genetic diversity indicators in the floristic regions of three wild wheat species per ISSR markers

	Triticum araraticum Jakubz.			*Triticum urartu* Tumanian ex Gandilyan	*Triticum boeoticum* Boiss.		
Indicators	Ta_1	Ta_2	Total the population	Total in the population	Tb_1	Tb_2	Total in the population
H_E	0.274 (0,018)*	0,292 (0,017)	0,282 (0,019)	0,208 (0,011)	0,157 (0,014)	0,168 (0,012)	0,163 (0,012)
na	1,827 (0,381)	1,877 (0,332)	1,852 (0,349)	1,630 (0,309)	1,715 (0,492)	1,768 (0,475)	1,742 (0,468)
na	1,456 (0,336)	1,476 (0,311)	1,466 (0,303)	1,362 (0,331)	1,287 (0,311)	1,306 (0,296)	1,296 (0,334)
P_{95}	0,718	0,734	0,726	0,705	0,714	0,768	0,741
R	3	0	3	4	1	0	1
μ	1,673 (0,008)	1,737 (0,008)	1,705 (0,008)	1,629 (0,006)	1,638 (0,006)	1,629 (0,006)	1,634 (0,006)
h	0,165 (0,004)	0,154 (0,004)	0,160 (0,004)	0,156 (0,002)	0,153 (0,003)	0,144 (0,003)	0,155 (0,003)
I	0,447 (0,221)	0,433 (0,233)	0,440 (0,229)	0,286 (0,229)	0,391 (0,301)	0,377 (0,226)	0,384 (0,196)

* Numbers in brackets correspond to Standard Deviations (SD)

Table 4: The genetic structure and segregation of the floristic regions of three wild wheat species

Species	Primers	H_T	H_S	G_{ST}
Triticum araraticum Jakubz.	M_3	0,377 (0,011)	0,219 (0,031)	0,133
	M_{27}	0,322 (0,017)	0,281 (0,017)	0,175
	X_9	0,287 (0,013)	0,196 (0,011)	0,123
	X_{11}	0,336 (0,022)	0,281 (0,027)	0,093
	ISSR-4	0,262 (0,008)	0,224 (0,029)	0,201
	ISSR-9	0,377 (0,015)	0,288 (0,014)	0,116
	CR-215	0,384 (0,021)	0,268 (0,011)	0,148
	In the selected group	0,368 (0,029)	0,277 (0,014)	0,141
Triticum urartu Tumanian ex Gandilyan	M_3	0,260 (0,038)	0,202 (0,022)	0,236
	M_{27}	0,277 (0,021)	0,196 (0,019)	0,201
	X_{10}	0,301 (0,031)	0,218 (0,018)	0,254
	X_{11}	0,265 (0,034)	0,244 (0,026)	0,218
	ISSR-3	0,288 (0,031)	0,254 (0,022)	0,267
	CR-212	0,292 (0,028)	0,226 (0,017)	0,271
	In the selected group	0,281 (0,029)	0,211 (0,014)	0,237
Triticum boeoticum Boiss.	M_1	0,255 (0,022)	0,182 (0,009)	0,201
	M_3	0,198 (0,013)	0,144 (0,005)	0,195
	X_9	0,241 (0,025)	0,151 (0,012)	0,211
	X_{10}	0,263 (0,018)	0,177 (0,014)	0,266
	ISSR-8	0,301 (0,026)	0,148 (0,012)	0,258
	CR-216	0,233 (0,024)	0,171 (0,016)	0,234
	In the selected group	0,239 (0,019)	0,162 (0,014)	0,228

Table 5: Molecular genetic formulas of wild wheat

Species	The type of DNA fragments	Molecular-genetic formula
Triticum araraticum Jakubz.	Rod	$TR_2 1100_{X11}$; $TR_2 820_{M3}$; $TR_2 580_{M3}$; $TR_2 450_{X11}$
	Vid	$TA_V 970_{X9}$; $TA_V 800_{M27}$; $TA_V 600_{CR-215}$; $TA_V 550_{ISSR-4}$; $TA_V 320_{X11}$
	Polymorph	$TA_p 920_{ISSR-9}$; $TA_p 740_{X9}$; $TA_p 380_{X9}$
Triticum urartu Tumanian ex Gandilyan	Rod	$TR_2 1100_{X11}$; $TR_2 820_{M3}$; $TR_2 580_{M3}$; $TR_2 450_{X11}$
	Vid	$TU_V 850_{M27}$; $TU_V 700_{X10}$; $TU_V 470_{M27}$; $TU_V 410_{CR-212}$; $TU_V 340_{X10}$
	Polymorph	$TU_p 680_{ISSR-3}$; $TU_p 570_{M27}$; $TU_p 370_{X11}$
Triticum boeoticum Boiss.	Rod	$TR_2 1100_{X11}$; $TR_2 820_{M3}$; $TR_2 580_{M3}$; $TR_2 450_{X11}$
	Vid	$TB_V 1250_{M1}$; $TB_V 920_{X9}$; $TB_V 760_{ISSR-8}$; $TB_V 540_{CR-216}$; $TB_V 430_{M3}$
	Polymorph	$TB_p 1450_{X9}$; $TB_p 940_{ISSR-8}$; $TB_p 520_{X3}$

Genetic Formulae and Barcoding

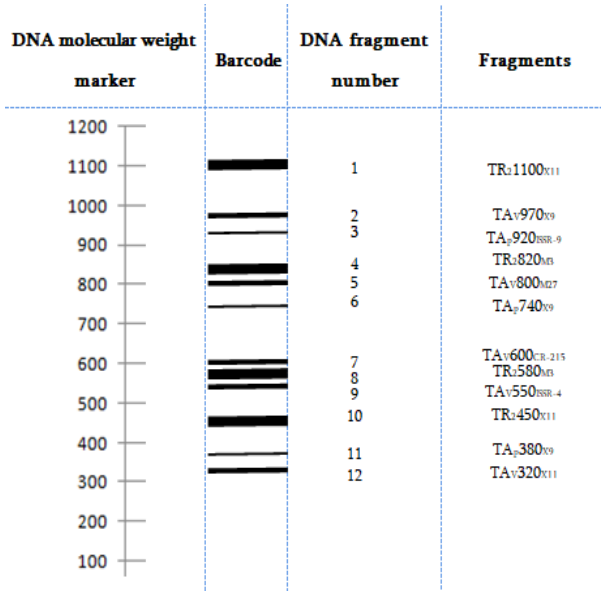
In general, all DNA fragments or ISSR markers are naturally divided into two groups: Monomorphic and polymorphic. Some of the monomorphic markers, which are characteristic of all three studied species of wild wheat and appear to have been formed before speciation, during the formation of the genus (*Triticum*), are called generic/racial and are denoted by the letter r as an index. The other group of DNA markers, which are unique to a given species, are called varietal and are denoted by the letter *v* as an index. The third group of DNA markers, which are polymorphic in nature and characteristic of different populations and floristic regions within the species, is called polymorphic and denoted by the letter p as an index. Such DNA fragments are called identifying markers (Caldwell and Kasarda 1978; Vdovichenko, 2007; Kuluev *et al*., 2018).

When drawing up molecular genetic formulae, 3 types of DNA fragments, the length of the fragment, and the primer as an index are indicated (Table 5).

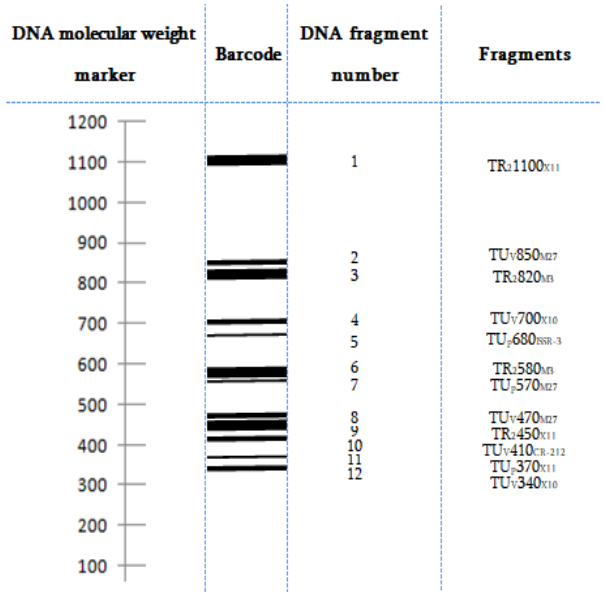
There are 4 DNA fragments selected as generic ones for passportization/certification of the Armenian populations of three wild wheat varieties, and for the creation of molecular-genetic formulae and barcodes, they are monomorphic in nature and common to all species. They have been most likely formed much earlier than the process of speciation and can be especially considered as markers identifying the genus *Triticum* (Kuluev *et al*., 2018).

There are 5 varietal DNA fragments in each version, which are characteristic of the given species. Polymorphic DNA fragments, being markers identifying different populations, floristic regions, or groups within species, are three for each version of our studies (Table 5).

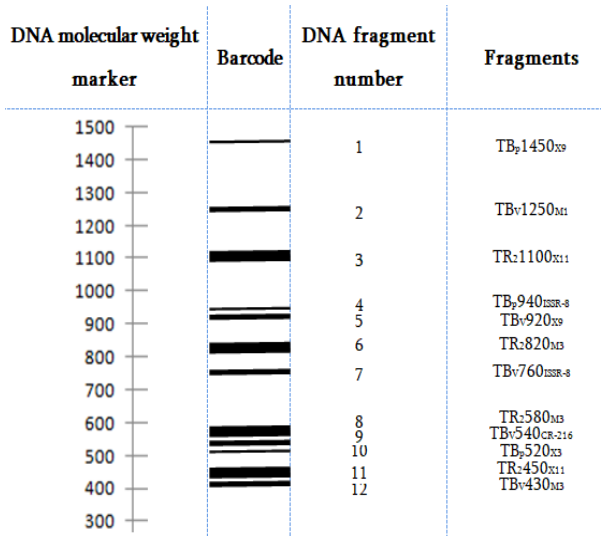
Molecular-genetic formulas and barcodes were compiled for the Armenian populations of three wild wheat varieties (Fig. 2 ABC).



(A)



(B)



(C)

Fig. 2: (A) Molecular-genetic barcodes of the Armenian populations of wild wheat varieties *Triticum araraticum* Jakubz; (B) *Triticum urartu* Tumanian ex Gandilyan; and (C) *Triticum boeoticum* Boiss

Discussion

Currently, the availability of genetic material and the diversity of the genetic pool play an extremely important role in solving the problems of genetic homogeneity of valuable agricultural crops, preventing genetic erosion, and promoting the country's economic growth and sustainable development. From this prospect, it is of paramount importance to have a clear insight into the genetic structure of various populations for the wild relatives of agricultural crops, e.g., allele funds and gene pool, degree of homozygosity, origin, genetic similarity, Quantitative Trait Loci (QTL), natural resistance to diseases, etc., using modern technologies and methods (Selyutina *et al*., 2014; Chokheli *et al*., 2018).

ISSR markers have been shown to be useful in genetic variation studies due to their excellent reproducibility and strength for polymorphism detection.

The identification of different varieties of agricultural crops and their wild relatives is carried out per a number of morphological features, which enables the assessment of the gene pool, genetic diversity, and variability of the population (Chokheli *et al*., 2018; Selezneva and Akhmedov, 2016).

Recently, a wide range of DNA markers have been used for genotype certification/passportization, population polymorphism assessment, genetic mapping, and phylogenetic research (Kuluev *et al*., 2018; Golovnina *et al*., 2007).

One of the methods of molecular identification of different crop varieties and wild relatives, as well as the biodiversity study is DNA barcoding. At the same time, it should be noted that to date no DNA site has been found in plants that can be used for DNA barcoding (Sultangazina *et al*., 2020).

The set of genotypes, the compiled barcodes, as molecular markers, could be used for the genetic passportization/certification and identification of the Armenian wheat populations, in future breeding programs to increase the genetic diversity among wheat genotypes. Increasing genetic diversity is very useful in Marker-Assisted Selection (MAS) for identifying genes controlling important traits in wheat.

The population-genetic indicators of studied wheat species prove that the populations are stable and the gene pool is capable of self-reproduction in case of an effective number of isolates. Passport data as genetic markers will allow further identification of the origin of the species, evolution, patterns of inheritance of useful economic traits, cell genetic characteristics of the species, and natural resistance to adverse environmental factors and diseases, which will serve as a basis for the implementation of marker selection.

Conclusion

Knowledge of the genetic diversity of wild relative species of wheat is crucial for their conservation as well as utilization in wheat breeding.

According to the coefficients of the genetic pattern (M, Me, P_{95}, H_E, ne, μ, I, H_T, H_S, G_{ST}, h), the studied wild wheat species are characterized as basic or typical gene pools, with the minimum number and frequency of rare alleles. The population-genetic indicators of all three species prove that the populations are stable and the gene pool is capable of self-reproduction in case of an effective number of isolates. Passport data as genetic markers will allow further identification of the origin of the species, evolution, patterns of inheritance of useful economic traits, cell genetic characteristics of the species, and natural resistance to adverse environmental factors and diseases, which will serve as a basis for the implementation of marker selection.

As a result, these genotypes and traits are deserving of more attention in future breeding programs aimed at improving wheat. The results of the population-genetic analysis may add helpful insights into the species' genetic diversity and provide essential knowledge for their selection as genetic resources in breeding new cultivars.

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Author's Contributions

Manvel Badalyan and Tatevik Aloyan: Conceptualization, methodology, software, validation, resources, data curation, written-original drafted preparation, written, reviewed and edited, visualization, supervision-project administration, funded acquisition.

Varya Dilanyan and Aghvan Sahakyan: conceptualization, methodology, resources, data curation, written reviewed and edited.

Satenik Kharatyan: Conceptualization software, validation, resources, data curation, written, reviewed and edited.

Andreas Melikyan: Conceptualization, software, data curation, written, reviewed and edited, supervision. Project administration, funded, acquisition.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved. There is no conflict of interest.

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