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ASSESSMENT OF GENETIC DIVERSITY OF *TRITICUM BOEOTICUM* AND *TRITICUM URARTU* POPULATIONS BY APPLICATION OF RAPD MARKERS

YESAYAN A.H., HOVHANNISYAN N.A.

Yerevan State University, Department of Ecology and Nature Protection,
Faculty of Biology,
E-mail: ayesayan@ysu.am, bionellibiotech@yahoo.com

18 RAPD markers where successfully amplified with *T. urartu* and *T. boeoticum* populations. The marker system proved to be effective in genetic assessment of 10 populations analysed. In the RAPD analysis a total of 114 bands were screened among which 55 were polymorphic across 10 populations of T. *boeoticum* and *T. urartu*. Dendrogram using UPGMA was originated from Nei's genetic distance. The RAPD dendrogram showed genetic differences among populations from the nearby geographical areas. Obtained results are showing importance of further investigations of genetic structure and genetic diversity within and between populations of studied plants with more valuable sampling to conclude more precisely the relationship between diversity and geographical origin.

Triticum boeoticum - Triticum urartu – PCR - RAPD markers - dendrogam

18 RAPD մարկերներ հաջողությամբ ամպլիֆիկացվել են *T.urartu* և *T. boeoticum* պոպուլյացիաների բույսերի ԴՆԹ-ներից: Ընտրված մարկերային համակարգը արդյունավետ է 10 ուսումնասիրվող պոպուլյացիաների համար: RAPD վերլուծության արդյունքում գնահատվել է 114 ամպլիֆիկացված հատվածներ, որոնցից 55 պոլիմորֆ էին: Նեի գենետիկական հեռավորության գնահատման արդյունքում կառուցվել է UPGMA դենդրոգրամ: Ըստ RAPD դենդրոգրամի տարբեր, մոտ աշխարհագրական պոպուլյացիաների միջև, բացահայտվել են գենետիկական տարբերություններ: Ստացված արդյունքները ցույց են տալիս ուսումնասիրվող բույսերի ներ- և միջպոպուլյացիոն գենետիկական կառուցվածքի ուսումնասիրությունների կարևորությունը գենետիկական բազմազանության և աշխարհագրական տարածվածության միջև փոխադարձ կապերի բացահայտման համար:

Triticum boeoticum (Triticum urartu (ՊՇՌ (RAPD մարկերներ (դենդրոգրամ

18 RAPD маркеров были успешно амплифицированы с ДНК популяций растений *T. urartu* и *T. boeoticum*. Выбранная система маркеров оказалась эффективной для оценки 10 исследуемых популяций. В результате RAPD анализа было оценено 114 амплифицированных фрагментов, из которых 55 оказались полиморфными. На основе оценки генетического расстояния Неи была построена UPGMA дендрограмма. По RAPD дендрограмме были выявлены генетические различия среди популяций, произрастающих в географически близко расположенных районах.

Полученные результаты показывают важность дальнейших исследований внутри- и межпопуляционной генетической структуры и генетического разнообразия исследуемых растений для более точного определения взаимосвязей между генетическим разнообразием и географическим распространением.

Triticum boeoticum - triticum urartu - ПЦР - RAPD маркеры - дендрограмма

Armenia is one of the main centers of distribution of wild wheat sites and domestication of common and emmer wheat [12, 3]. The genus *Triticum* comprises species of different ploidy levels from diploid (2n = 14) to hexaploid (2n = 42).

Triticum boeoticum Boiss. with the genome AbAb has been reported as a valuable source of desirable genes conferring protein quality, amino-acid content or resistance [8].

The habitats of wild wheat in Armenia are potentially the ideal areas to explore germplasm for suitable genes for introgression into cultivated wheat [11]. Therefore, it is supposed that the populations of *T.boeoticum* in this region contain high levels of genetic diversity and can be potential donors of useful genes for abiotic and biotic stress tolerance for breeding purposes.

Molecular markers provide a good estimate of genetic diversity since they are almost unlimited in number and are not influenced by the environment [10]. Different DNA markers have been developed in resent years such as RFLPs Restriction Fragment Length Polymorphisms), AFLPs (Amplified Fragment Length Polymorphisms), RAPDs (Random Amplified Polymorphic DNAs) and microsatellites SSRs (Simple Sequence Repeats) which can be used either separately or in combination, to assess genetic diversity. Molecular markers and their combinations were widely used for measuring genetic diversity in wheat cultivars and related wild species [1,4,5,6] but, our knowledge regarding molecular genetic variation in wild wheat relatives in Armenia is limited.

The main goal of this study was to evaluate genetic variability among populations of *T. boeoticum and T. urartu f*rom Armenia using RAPD.

Materials and Methodology. Accessions of wild wheat were obtained from seed banks of the Armenian State Agrarian University, ICARDA and IPK (Gatersleben, Germany) and from wild (Table 1)

Table 1. List of studied diploid wheat – *Triticum boeoticum* and *Triticum urartu*

N	**Species**	**Geographical region**	**Accession numbers Gatersleben (TRI), ICARDA (IG)**
1	*T. boeoticum*	Armenia (Erebuni Res.)	Erebuni Res.
2	*T. boeoticum*	Armenia (Yerevan)	IG126246
3	*T. boeoticum*	Armenia (Eghegnadzor)	-
4	*T. boeoticum*	Armenia (Kotayk)	IG 44938
5	*T.boeoticum*	Armenia (Ararat)	IG126286
6	*T. boeoticum*	Armenia (Vayots Dzor)	IG 137411
7	*T. urartu*	Iraq (Ninawa)	IG 109084
8	*T. urartu*	Iran (East Azerbaijan)	IG 45777
9	*T. urartu*	Turkey (Gaziandep)	IG 116203
10	*T.urartu*	Armenia	TRI6735

DNA isolation. DNA from young seedling was extracted by application two CTAB modified protocols [2]. DNA extracted by modified Doyle protocol with addition activated charcoal was used for RAPD analysis. DNA from young 7 – day-old seedlings were extracted by application a modified CTAB method [2].

PCR amplification and gel electrophoresis. For RAPD analyses 18 10 mer primers were used: OPA01, OPA02, OPA03, OPA04, OPA05, OPA11, OPA 13, OPA 18, OPE03, OPE04, OPE07, OPE11, OPE15, OPE20, OPC 02, OPC 05, OPC 11, OPC19 (Operon technologies).

Each sample contained 0.05 µl of Taq polymerase (Gatersleben, Genomzentrum), 2.5 µl PCR buffer, 10 µM of forward/reverse primers, 2.5 mM of each dNTP and about 50 ng of plant DNA. PCR conditions Biokom PCR amplifier the PCR programs were well workable on all machines. PCR amplification programs for RAPD markers are described in table 2.

Table 2. PCR amplification programs for different primer combinations used in 11 wild accessions of *Triticeae*

PCR programs for amplification RAPD markers	
OPA01, OPA02, OPA03, OPA04, OPA05, OPA11, OPA 13, OPA 18, OPE03, OPE04, OPE07, OPE11, OPE15, OPE20, OPC 02, OPC 05, OPC 11, OPC19	94^0C – 1.5 min, 40 cycles (94^0C – 30 sec., 36^0C – 1 min, 72^0C – 1 min), 72^0C – 10 min

PCR amplified RAPD DNA fragments were electrophoresed in 2% agarose gels at 110 V for about 2 hours, stained with ethidium bromide, visualized under UV light and photographed. Gel images were captured in TIF files and analyzed with the POPGENE32 software. The bands were scored in binary notation, with 1 and 0 for presence and absence of bands, respectively. Binary matrix was used to estimate the genetic similarities between pairs, by employing Nei index.

These coefficients were used to construct dendrogram using the unweighted pair group method with arithmetic averages (UPGMA), employing the Popgene 32 and treeview software.

Results and Discussion. 18 RAPD markers were amplified with 4 accessions of *T. urartu* and 6 of *T. boeoticum*. The marker system have proved highly effective in genetic assessment of 10 populations analysed. In the RAPD analysis by using 18 primers, a total of 114 bands were screened among which 55 were polymorphic (50%) across 10 populations of T. *boeoticum* and *T. urartu* (average of 6,2 bands per primer). The highest and the lowest number of polymorphic bands per assay unit were 2 and 15 respectively.

The observed number of allele, effective number of allele, Nei's gene diversity, Shannon's Information index and percentage of polymorphic were calculated and dendrograme was originated. The results obtained are presented in tab.3.

The amplified DNA fragments normally ranged from 100 to 2000 bp. An example of RAPD pattern, obtained with primer OPA02, is shown in Fig. 1.

The genetic similarity was estimated between pairs of populations (Tab.4). Estimates of genetic identity of RAPDs ranged from 0.11 to 0.98 with an average of 0.58.

The dendrogram using UPGMA as the clustering method, was originated from Nei's genetic distance (Fig. 2 and Tab. 4) based on the RAPD. Cluster analyses have separated 10 populations into two major groups based on their RAPD fragment similarities, and within group of *T. boeoticum* populations' three sub clusters was formed. The RAPD dendrogram showed the genetic differences among populations from the nearby geographical areas. These results are in good agreement with other analyses on genetic diversity carried out in different crop species and their wild relatives [6,7,9].

Table 3. Genetic diversity of *Triticum boeoticum* and *Triticum urartu* populations by RAPD markes

N	Population name	Observed number of alleles	Effective number of alleles	Nei's gene diversity	Shannon's Information index	The percentage of polymorphic loci %
1	T. boeoticum Yerevan	1.7500	1.3446	0.2069	0.3209	55.34
2	T. boeoticum Ararat	2.0000	1.6032	0.3497	0.5217	54.16
3	T. boeoticum Vayots Dzor	2.0000	1.6286	0.3673	0.5455	53.94
4	T. boeoticum Kotayk	1.9583	1.6481	0.3735	0.5493	50.00
5	T. boeoticum Yeghegnadzor	1.9583	1.6446	0.3735	0.5498	54.61
6	T. boeoticum Van	1.9583	1.6503	0.3754	0.5513	52.50
7	T. boeoticum Erebuni	1.9583	1.6192	0.3633	0.5391	54.17
8	T. urartu Armenia	2.0000	1.5881	0.3495	0.5267	55.68
9	T. urartu Erebuni	2.0000	1.6377	0.3799	0.5646	53.46
10	T. urartu Iraq	1.9583	1.6737	0.3899	0.5705	55.00
11	T. urartu Iran	2.0000	1.6097	0.3656	0.5462	51.67

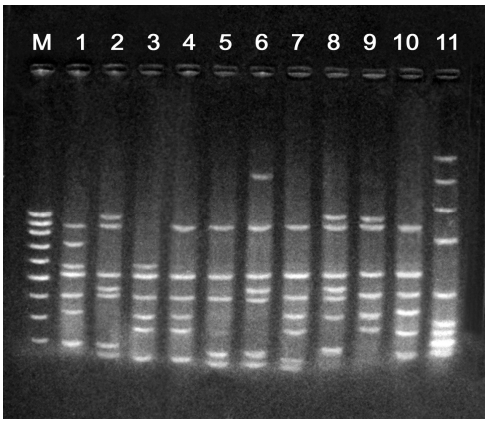

Fig. 1. RAPD patterns of 10 genotypes within the T. boeoticum (lines 1-7) and T. urartu (8-10) obtained with primer OPA02. M: DNA size markers (DNA Molecular Weight Marker, Fermentas, 100 bp ladder).

Although a limited number of accessions for each species was considered, there was a clear separation between and within accessions of *T .boeoticum* and *T. urartu* in the study. It is possible that this separation between populations may be due to predominance of self fertilization and geographic distribution.

Table 4. Nei's Original Measures of Genetic Identity and Genetic distance

pop ID	1	2	3	4	5	6	7	8	9	10	11
1	****	0.9684	0.9565	0.9231	0.7216	0.7737	0.9131	0.1113	0.1124	0,1885	0.1704
2	0.0321	****	0.9845	0.9683	0.7668	0.9415	0.9637	0.1538	0.1548	0.1444	0.1386
3	0.0445	0.0156	****	0.9832	0.9824	0.9637	0.9747	0.1453	0.1416	0.1346	0.1268
4	0.0800	0.0322	0.0170	****	0.9986	0.9807	0.9859	0.1368	0.1306	0.1346	0.1341
5	0.2817	0.0338	0.0177	0.0014	****	0.9842	0.9903	0.1413	0.1375	0.1376	0.1381
6	0.2350	0.0602	0.0370	0.0194	0.0159	****	0.9754	0.1097	0.1189	0.1286	0.1298
7	0.0909	0.0370	0.0256	0.0142	0.0097	0.0249	****	0.1533	0.1525	0.1563	0.1558
8	0.8867	0.0473	0.0563	0.0653	0.0605	0.0946	0.0478	****	0.9776	0.9516	0.9538
9	0.8917	0.0462	0.0602	0.0720	0.0645	0.0846	0.0486	0.0227	****	0.9779	0.9644
10	0.9182	0.0572	0.0676	0.0677	0.0644	0.0741	0.0447	0.0496	0.0224	****	0.9867
11	0.9388	0.0634	0.0760	0.0682	0.0639	0.0728	0.0452	0.0473	0.0362	0.0134	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

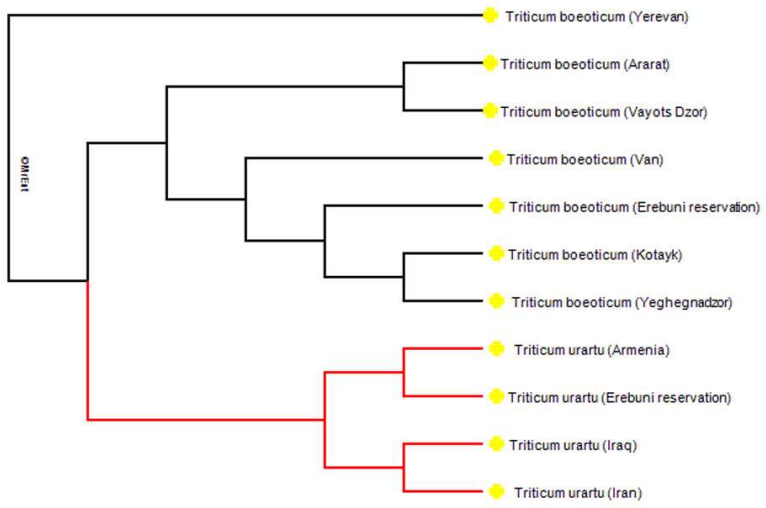

Fig. 2 Dendogram of 7 T.boeoticum and 4 T.urartu populations based on Nei genetic distance using UPGMA as the clustering method

According to our results the species *T.boeoticum* shows a high diversity comparable with the results obtained in West Iran [9]. From the results obtained, it becomes evident that geographically close regions could be ecologically quite different, which is the main driving factor in the high site-specific diversity in the close region.

Obtained results are showing importance of further investigations of genetic structure and genetic diversity within and between populations of studied plants with more valuable sampling to conclude more precisely the relationship between diversity and geographical origin.

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