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Genetic Identification of Diploid and Tetraploid Wheat Species with RAPD Markers

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Abstract: The genetic diversity of 10 diploid and tetraploid wheat species was estimated using random amplified polymorphic DNA (RAPD) markers. Two species from diploid [*Triticum boeticum* (wild), *Triticum monococcum*] and five from tetraploid wheats [*Triticum dicoccoides* var. *arabicum* (wild), *Triticum dicoccum* var. *farrum*, *Triticum dicoccum* var. *atratum*, *Triticum durum* var. *hordeiforme* (Bereketli 95), *Triticum durum* var. *leucurum* (Sharq), *Triticum turgidum* var. *alboyadurum*, *Triticum turgidum* var. *salomonis* and *Triticum persicum*] were included for the analyses. Jacard's cluster analysis algorithm was used to determine genetic similarities.

There were two main classes in dendrogram: the varieties [*T. boeticum, T. dicoccoides* var. *arabicum, T. dicoccum* var. *farrum, T. dicoccum* var. *atratum, T .durum* var. *hordeiforme* (Bereketli 95), *T. durum* var. *leucurum* (Sharq)] assembled in one group and the species [*T. monococcum, T. turgidum* var. *alboyadurum, T. turgidum* var. *salomonis* and *T. persicum*] in another.

The same genotypes were also assessed in field conditions for structural analyses, which were carried out based on eight yield components. The dendrogram created was comparatively analyzed with the RAPD dendrogram. There were differences between genetic and phenotypic similarity of the studied accessions. Results indicated that some of genetically similar genotypes were different phenotypically.

Key Words: Plant genetic resources, biodiversity, wheat, RAPD, dendrogram, genetic relationships

Diploit ve Tetraploit Buğday Türlerinin RAPD Markerlar ile Genetik Tanımlanması

Özet: On farklı diploit ve tetraploit buğday türünün genetik farklılıkları RAPD markerlar ile tanımlanmıştır. Diploitlerden iki tür; *Triticum boeticum, Triticum monococcum* ve beş tetraploit buğday; *Triticum dicoccoides* var. *arabicum* (yabani), *Triticum dicoccum* var. *farrum*, *Triticum dicoccum* var. *atratum*, *Triticum durum* var. *hordeiforme* (Bereketli 95), *Triticum durum* var. *leucrum* (Sharq), *Triticum turgidum* var. *albayadurum*, *Triticum turgidum* var. *salomonis* ve *Triticum persicum* analizler için kullanılmıştır.

Dendrogramda türler iki grupta toplanmıştır. Varyete *T. boeticum, T. dicoccoides* var. *arabicum* (yabani), *T. dicoccum* var. *farrum* ve *T.m dicoccum* var. *atratum*, bir grupta toplanmıştır. *T. monococcum, T. turgidum* var. *albayadurum*, *T. turgidum* var. *salomonis* ve *T. persicum* türleri ise diğer grupta toplanmıştır.

Sekiz ürün için yapılan yapısal analizlerde aynı genotipler kullanılmış ve RAPD dendrogramı ile karşılaştırma yapılmıştır. Çalışılan aksesyonlarda genetik ve fenotipik benzerlikler arasında farklıklar bulunmuştur. Sonuçlara göre genetik olarak benzer genotiplerin fenotipik olarak farklı olabileceği söylenebilir.

Anahtar Sözcükler: Bitki Genetik Kaynakları, biyoçeşitlilik, buğday, RAPD, dendrogram, genetik ilişki

Introduction

The collection and conservation of plant genetic resources are important missions in the world and in Azerbaijan. The first "Genbank" for this purpose among the Caucasian Republics was created in Azerbaijan. In this Genbank, 8000 plant accessions are maintained, from which 1283 are wheat. The identification of accessions conserved in Genbank remains a problem. It is possible to reveal new gene origins using DNA markers, which can be used for selection in the future.

Wheat is the largest produced grain crop world-wide and has been extensively studied for a wide range of

agronomic traits located across the genome. Its large chromosomes and the capacity of the polyploidy genome to tolerate the addition or loss of chromosomes facilitated a fast progress in early wheat genetics using cytogenetic techniques. Identification of genetic diversity is one of the important tools in plant breeding. Morphological and cytogenetic traits used at present are not stable, are time-consuming and are affected by environmental conditions, but molecular markers are stable (1). Biochemical markers, such as isozymes and seed storage proteins, and DNA-based markers, such as restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD), have been used to evaluate the genetic diversity among wheat landraces (2-5). Other molecular markers, such as microsatellites and amplified fragment length polymorphisms (AFLPs), have also been used to study genetic relationships between wheat species. Among all these molecular markers, RAPD markers are simple, user- friendly, and cost- and time-effective. They have been used successfully for the evaluation of plant genetic resources in wheat genotypes (6-9).

The present study aimed to investigate DNA polymorphisms of different diploid and tetraploid wheat varieties of diverse origin.

Materials and Methods

From diploid wheats, *T. boeticum, T. monococcum,* and from tetraploid wheats, *T. dicoccoides var. arabicum, T. dicoccum var. farrum, T. dicoccum var. atratum, T. durum var. hordeiforme (Bereketli 95), T. durum var. leucurum (Sharq), T. turgidum var. alboyadurum, T. turgidum var. salomonis* and *T. persicum* species, mainly of Azerbaijan origin, were used in this study (Table 1).

Field experiments

During the 2005-2006 season, a yield trial (using the same 10 genotypes) was conducted in plots of 2 m length with between-row distance of 20 cm and plant distance of 5 cm. A randomized complete block design with three replications was used. At maturity, 10 plants were randomly selected from each plot for recording certain yield components. The studied characteristics were: plant height, number of productive boles, length of spike, weight of spike, number of spikelets, weight of grain, number of grains, and weight of 1000 grains. To examine interrelationships among the genotypes, a hierarchical cluster analysis was performed based on these traits.

Table 1. Diploid and tetraploid wheat species used in the study.

No.	Name	Origin	Genome	Number of chromosomes
1	*T. turgidum* var. *alboyadurum*	Azerbaijan	AABB	28
2	*T. durum* var. *leucurum*	Azerbaijan	AABB	28
3	*T. persicum*	-	AABB	28
4	*T. dicoccum* var. *atratum*	-	AABB	28
5	*T. durum* var. *hordeiforme*	Azerbaijan	AABB	28
6	*T. monococcum* L.	Azerbaijan	AA	14
7	*T. boeticum* Boiss.	Azerbaijan	AA	14
8	*T. turgidum* var. *salomonis*	Azerbaijan	AABB	28
9	*T. dicoccum* var. *farrum*	-	AABB	28
10	*T. dicoccoides* var. *arabicum*	Azerbaijan	AABB	28

DNA extraction

DNA was extracted from fresh leaf material collected from 10 different plants. After freezing by liquid nitrogen, leaves were grounded in a mortar with a pestle. 0.3 g from the powder was used for DNA extraction by appropriate modifications of the method described by Dellaporta et al. (10). DNA quality was tested using 1% agarose gel electrophoresis. For quantifying the amount of DNA, 20 µL of nucleic acid was added to 1980 µL of TE buffer and mixed and absorption (OD) read in a spectrophotometer at 260—280nm. The concentration of DNA was calculated according to the following formula:

DNA concentration (µg/µL) = [OD_{260} x 100 (dilution factor) x 50 µg/µL]/1000

PCR amplification

Polymerase chain reactions (PCRs) were carried out in a 25 µL volume, in a mixture containing 1 x PCR buffer (20 nm Tris-HCl pH 8.4, 50 nm KCl), 200 µm $MgCl_2$, 0.4 µm primer (random 10-mers), 1.0 units Taq DNA polymerase (Invitrogen, Carlsband, CA, USA) and 30ng genomic DNA. The amplification was performed in an MJ Research thermocycler (Model PTC 200, Waltham, MA). The temperature profile included 1 cycle of 3 min at 94°C, followed by 45 cycles of amplification. Each cycle of amplification had a denaturing step at 94°C for 1 min, an annealing step at 37°C for 1 min and an extension step at 72°C for 1 min. After the final cycle, the samples were held for 6 min at 72°C. Amplification products were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide in 1 x TBE buffer. The RAPD bands were visualized under UV light and photographed using a Polaroid DS-34 Direct Screen Instant Camera (Polaroid Corporation, Cambridge, MA, USA). The 100 bp DNA molecular marker (GIBCO-BRL) was used as a standard molecular weight marker.

RAPD primers

Fifteen RAPD primers used in the study were selected from among 87 primers by Mantzavinou et al. (11). The list of primers is shown in Table 2.

Data analysis

The RAPD bands were scored as 1 for the presence or 0 for absence of a particular DNA fragment of a similar length. Only reproducible and clear amplification bands were scored for the construction of the data matrix. The data matrix thus prepared was the input file for the calculation of Nei and Li's (12) coefficient between pair of cultivars using the appropriate procedures of the computer program NTSYS-pc v2.02i (13). The similarity matrices were used for the construction of dendrograms with unweighted pair-group method on arithmetic averages (UPGMA) (14).

Table 2. Nucleotide sequences of the 15 primers used in the study.

No.	Primer	Sequence (5'to 3')
1	OPA-07	GAAACGGGTG
2	OPA-08	GTGACGTAGG
3	OPB-08	GTCCACACGG
4	OPB-10	CTGCTGGGAC
5	OPB-20	GGACCCTTAC
6	OPE-02	GGTGCGGGAA
7	OPN-04	GACCGACCCA
8	OPN-05	ACTGAACGCC
9	OPN-08	ACCTCAGCTC
10	OPO-04	AAGTCCGCTC
11	OPO-05	CCCAGTCACT
12	OPO-06	CCACGGGAAG
13	OPO-12	CAGTGCTGTG
14	OPO-15	TGGCGTCCTT
15	OPAN-16	CAAGGTGGGT

Results

Electrophoresis of PCR products on 2% agarose gels containing ethidium bromide revealed different degrees of polymorphism for different primers (Table 3). For example, primer OPO-12 produced 13 bands, from which 10 were polymorphic. In OPE-02, 8 bands from 12 were noted as polymorphic bands, whereas in OPO-06, 10 from 16 bands were polymorphic. The highest percentage of polymorphism was observed using OPB-10 (90.9%) and OPA-08 (90%). Only 8 from 14 bands produced by primer OPN-04 were polymorphic, so the polymorphism percentage decreased to 57.1%. Total percentage of polymorphism for all primers used was 75%.

Results obtained from four primers (OPA-07, OPE-02, OPO–04, OPAN-16), photographed by a Polaroid DS-34 Direct Screen Instant Camera, are shown in Figure 1.

Genetic similarities

The genetic similarity for pairs of species was calculated using Nei and Li coefficients (Table 4) (12).

Grouping the accessions

Cluster analysis was carried out by the UPGMA method on the Nei and Li genetic similarity coefficients. The position of the genotypes in different clusters is presented in Figure 2. The dendrogram constructed with UPGMA based on Nei and Li's coefficient revealed that 10 genotypes fell into two distinct groups. The main cluster I included: *T. dicoccoides, T. dicoccum var. farrum, T. durum var. hordeiforme (Bereketli 95), T. dicoccum var. atratum, T. boeticum,* and *T. durum var. leucurum (Sharq). T. turgidum var. salomonis, T. turgidum var. alboyadurum, T. persicum* and *T. monococcum* formed cluster II. *T. dicoccides* and *T. dicoccum var. farrum* from cluster I are very similar genetically, and *T. durum var. leucurum* and *T. durum var. hordeiforme* located in cluster I showed high similarity. Cluster II, in its order, was divided into 2 subgroups (Figure 2). Two varieties of *T. turgidum: T. turgidum var. salomonis* and *T. turgidum var. alboyadurum* were assembled in one group. This could be because they are different varieties of the same species.

Table 3. The detected polymorphism of the 15 primers.

N	Primer	No. of amplified bands	No. of amplified bands	Degrees of polymorphism (%)
1	OPA-07	5	4	80.0
2	OPA-08	10	9	90.0
3	OPB-08	11	7	63.6
4	OPB-10	11	10	90.9
5	OPB-20	5	4	80.0
6	OPE-02	12	8	66.6
7	OPN-04	14	8	57.1
8	OPN-05	7	5	71.4
9	OPN-08	7	5	71.4
10	OPO-04	7	6	85.7
11	OPO-05	9	7	77.8
12	OPO-06	16	10	62.5
13	OPO-12	13	10	76.9
14	OPO-15	9	8	88.9
15	OPAN-16	5	4	80.0
Total		140	105	75.0

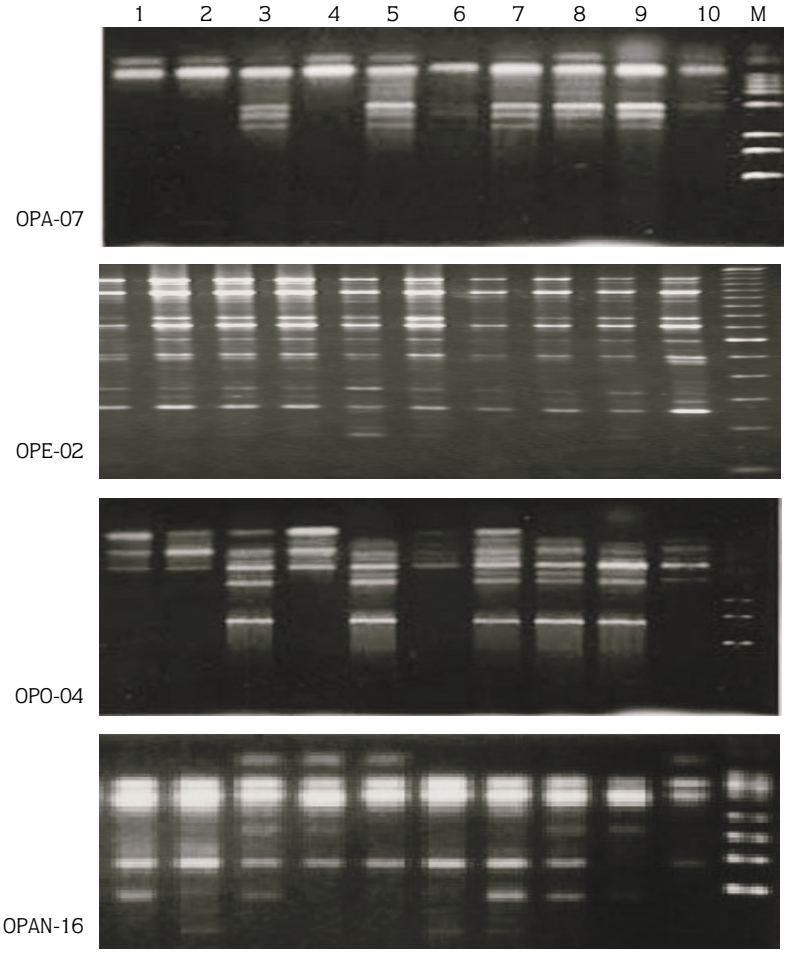

Figure 1. Representative RAPD pattern produced with primer OPA-07, OPE-02, OPO–04, OPAN-16 markers.
1. *T. turgidum var.alboyadurum*, 2. *T. durum var. leucurum (Sharq)*, 3. *T. persicum*, *4. T. dicoccum var. atratum*, 5. *T. durum var. hordeiforme (Bereketli 95*), 6. *T. monococcum*, 7. *T. boeticum*, 8. *T. turgidum var. salomonis*, 9. *T. dicoccum var. farrum*, 10. *T. dicoccoides var. arabicum*. M: Markers.

Table 4. Genetic similarity indexes of genotypes.

Genotype	1	2	3	4	5	6	7	8	9
T. dicoccoides arabicum	1								
T. dicoccum farrum	0.562	1							
T. turgidum salomonis	0.516	0.424	1						
T. boeticum	0.414	0.516	0.400	1					
T. monococcum	0.387	0.364	0.562	0.303	1				
Bereketli 95	0.533	0.625	0.322	0.414	0.387	1			
T. dicoccum atratum	0.452	0.545	0.312	0.466	0.437	0.581	1		
T. persicum	0.483	0.387	0.400	0.428	0.400	0.345	0.400	1	
Sharq	0.333	0.437	0.452	0.552	0.452	0.400	0.452	0.551	1
T. turgidum alboyadurum	0.529	0.529	0.588	0.485	0.514	0.529	0.514	0.545	0.470

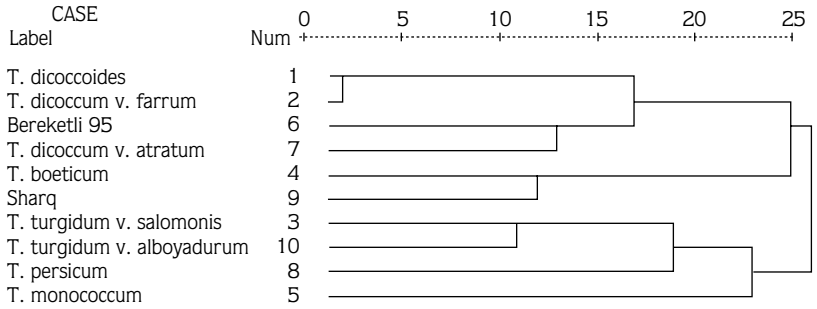

Figure 2. Dendrogram (UPGMA) showing the genetic relationships among 10 species of the genus *Triticum*.

In order to compare results obtained from genetic diversity with phenotypic diversity, the same accessions were also grown in the sown area of the Genetic Resources Institute of Azerbaijan National Academy of Sciences and were analyzed based on 8 yield components (Table 5). The dendrogram created for these yield components is shown in Figure 3.

The results were very interesting, and to a certain degree, were different from the genetic investigation. As in the first dendrogram, there were two main clusters. From the dendrogram it is seen that two different species - *T. boeticum* and *T. monococcum* - are close to each other based on yield components, but with RAPD markers, they were estimated as distant genotypes. In addition, *T. dicoccoides* and *T. persicum* were very similar for their phenotypic characteristics (yield components), whereas genotypically they were different for the primers used. The four accessions noted above formed cluster I. Cluster II consisted of 6 accessions, from which 3 united in one group and 2 in another. *T. durum var. hordeiforme (Bereketli 95*) constituted a separate group in cluster II. The varieties *T. turgidum var. alboyadurum* and *T. turgidum var. salomonis* showed similarity for both RAPD and structural analyses. The varieties belonging to *T. dicoccum* and *T. durum var. leucurum* (Sharq) showed similarity as well.

Discussion

The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials. Among them, the RAPD markers have been successfully used in wheat germplasm evaluation because of their many advantages.

Table 5. Structural analysis of diploid and tetraploid wheat species based on 8 yield components.

Name of varieties	Height of plant (cm)	Number of productive bole	Length of spike (cm)	Weight of spike (g)	Number of spikelets	Weight of grain (g)	Number of grain	Weight of 1000 grains (g)
T. boeticum	114±1.41	2.6±0.00	7.5±0.14	0.88±0.15	27.8±0.28	0.49±0.05	19.0±0.28	15.5±0.71
T. monococcum	104±1.41	4.8±0.28	6.6±0.14	0.86±0.32	29±1.41	0.52±0.23	26.7±1.83	18.0±0.42
T. dicoccoides var. arabicum	103±0.71	3.55±1.14	8.2±0.28	2.50±0.14	11.5±20.71	1.50±0.19	23.3±1.34	41.0±0.71
T. dicoccum var. farrum	131±2.12	4.3±0.14	6.1±0.14	1.95±0.40	16.0±0.00	1.45±0.25	33.2±1.13	40.6±0.85
T. dicoccum var. atratum	122±2.12	3.90±0.14	7.7±0.14	2.1±02.7	23.3±0.99	1.28±0.13	41.7±0.77	32.0±0.28
T. turgidum var. alboyadurum	114±0.71	2.4±0.00	6.45±0.82	4.36±0.69	20.1±0.14	3.35±0.61	54.8±0.28	56.9±0.00
T. turgidum var. salomonis	133±0.71	3.05±0.10	8.55±0.42	4.53±1.32	25.8±0.28	2.61±0.12	55.7±1.00	52.0±0.42
T. persicum	87.0±1.41	3.50±0.42	11.9±0.42	1.92±0.65	18.9±0.14	1.35±0.58	32.9±0.14	35.0±1.41
Bereketli 95	94.0±1.41	4.27±0.11	6.40±0.14	4.20±0.11	18.4±0.85	3.24±0.25	49.9±0.42	62.0±0.44
Sharq	132±4.24	3.5±0.13	7.40±0.14	3.47±0.18	19.6±0.92	2.39±0.36	41.6±0.92	52.0±0.00

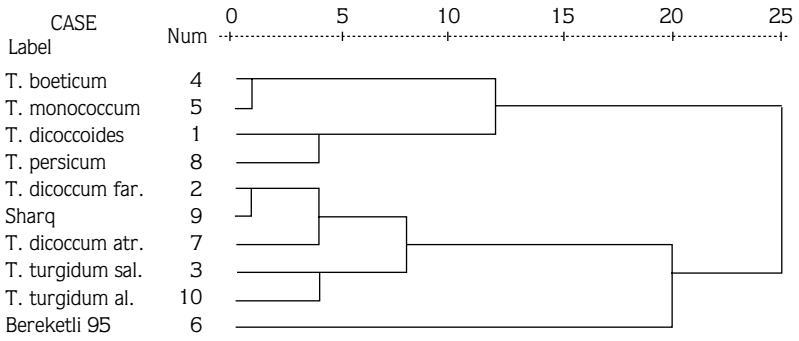

Figure.3. Dendrogram (UPGMA) showing the relationship among 10 species of *Triticum* based on structural analysis. The scale is based on Nei and Li's similarity coefficient.

However, some doubts have been expressed regarding the reproducibility of the RAPD technique and the suitability of the RAPD markers for genetic diversity studies. The reproducibility of RAPDs can be achieved by using optimized PCR conditions and by scoring only reproducible bands.

The suitability of the RAPD technique for genetic diversity studies and germplasm evaluations has been shown in many studies (5,6,8,15,16). The data on genetic relationships among wheat genotypes obtained from the RAPDs are in agreement with the data obtained using other markers. Castagna et al. (17) found that genetic similarity values calculated from RAPD markers were very similar to those calculated with RFLP markers for intraspecific comparisons of 49 diploid wheat accessions. In the study of 6 wheat species, Nagaoka and Ogihara (16) showed that genetic relationships estimated by ISSR (inter-simple sequence repeat) markers were identical with those from RFLP and RAPD markers. Recently, a weak correlation between the estimations of genetic diversity among wheat cultivars based on pedigree analysis and the RAPD method was reported (18,19). Low to moderate correlation coefficients have also been found between genetic diversity estimations based on RFLP and pedigree data in durum wheat (2), between the coefficient of parentage and AFLP data in hexaploid wheat (20), and between AFLP data and kinship in durum wheat (21). The discrepancy between the estimations of genetic diversity based on pedigree and the DNA data might be attributed to the different breeding objectives in the selection of these cultivars. Owing to the assumption of no genetic relationship between ancestors without a known pedigree, the calculations used in pedigree-based diversity measurements may result in an overestimation of the actual levels of genetic diversity. The genetic relationships inferred from molecular markers, such as RAPDs, should reflect a more reliable measure of genetic relationships.

The number of primers used in the RAPD method should be neither too small, because this could lead to a noninformative or biased analysis, nor too high, which could result in increased cost. Various numbers of primers have been used in the study of different species of the genus *Triticum* that revealed various degrees of polymorphism. Joshi and Nguyen (6) used 40 primers in studying wild and cultivated wheat and revealed 88% polymorphism among all accessions. With 26 UBC primers, Sun et al. (8) detected 62.5% polymorphism among 46 genotypes of *T. aestivum* and *T. spelta.* Pujar et al. (5) tested 81 Operon primers (kit A, F, J, V) and selected 21 primers that produced 3 to 13 polymorphic bands. A 78.2% polymorphism was detected among 64 genotypes of the species Triticum.

For our investigation, we used 15 primers as selected by Mantzavinou et al. (11) from among 87 primers for their polymorphism degree. The selected primers produced 4 to 10 polymorphic bands. Totally produced bands were 140, from which 104 were polymorphic. The polymorphism percentage among 10 different genotypes was 75%.

The genetic composition of a modern cultivar is usually homogeneous. The landraces are generally considered to be more heterogeneous than selected cultivars. In our studies, a high degree of genetic differences were observed among the genotypes belonging to *T. boeticum, T. monococcum*, *T. persicum* and *T. dicoccoides*. This may be because of usage of different species.

There were differences between genetic and phenotypic similarity in the studied accessions. For example, the RAPD primers used showed that *T. boeticum* and *T. monococcum* were genetically very different from each other, but in the dendrogram created based on yield components, these genotypes were grouped in one subcluster. The same objective laws were revealed for *T. dicoccoides* and *T. persicum*. For two varieties of *T. turgidum*, and two from *T. dicoccum*, the same degree of genetic and phenotypic similarity was noted.

Summarizing all results obtained from RAPD and structural analyses, we could conclude that phenotypically similar accessions are not always genotypically similar. However, different varieties of the same species mostly show phenotypic and genotypic similarity.

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