

## Phylogenetic comparison of the A genome using karyotype analysis in some *Triticum* species

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### Abstract

The Karyotype analysis was performed on 46 wheat accessions belonging to five species (*Triticum monococcum*, *T. urartu*, *T. durum*, *T. turgidum* and *T. aestivum*) and two subspecies (*T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum*) carrying A genome. All chromosomal sizes were measured with computer-aided program Micro Measure 3.3. Software. RL, TCL, MCL, arm ratio, centromeric index, TF%, mean of long and short arms, AsI%, S%, DRL, A1, A2 and karyotype formula were calculated for each chromosome. All the accessions were placed in 1A category of stebbines asymmetry categories. The scatter diagram based on A1 and A2 constructed three groups of karyotype asymmetry in the accessions studied: 1- *T. aestivum* with the highest asymmetrical karyotype, 2- *T. monococcum*, *T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum* with the lowest asymmetrical karyotype and 3- *T. urartu*, *T. turgidum* and *T. durum* being with an intermediate between the two previous groups. *T. monococcum* based on the A1 and A2 index (asymmetric index) had the oldest and the most primitive karyotype among diploid species. According to the results, it might be suggested that *T. durum* is more primitive than *T. turgidum* and *T. monococcum* could be considered as a donor of A genome to *T. durum* and *T. aestivum*.

**Key words:** A genome, Karyotype analysis, Phylogeny, *Triticum*

### Introduction

Plant nuclear genomes are enormously variable. Chromosome number, the degree of gene clustering, and chromosome size can all differ considerably, even between closely related species (Kellogg and Bennetzen, 2004).

The earliest work on wheat chromosomes relied on reconstructions made from microtomal serial sectioning of root tips. The development of squash technique reduced the possibilities of misinterpretations. Studies of the morphology of chromosomes in *Triticum* species have been made by many workers, including Schulz-Schaeffer and Haun (1961), Khan (1963), Kimber (1971), Johnson and Dhaliwal (1978), Kerby and Kuspira (1987), Miller (1987) and Khan (2005).

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Because of different workers had to do without standardization of techniques in different laboratories, their reports showed inconsistencies in the standard karyotype analysis based on chromosome length and arm ratio still holds its merit (Jahan and Vahidy, 1989). The analysis of plant genomes has provided insight into how these evolutionary events have occurred and the rate at which evolution could have taken place. Wheat has played a major role in the development of the world civilization. The domestication of wheat was a major event in the world civilization because it allowed humans to change from nomadic hunter gathers to permanent residents of specific locations. Many useful genes from wild species are available to be used in breeding Programmers as source of genes for disease resistance applying different chromosomal engineering techniques involving backcrosses, "bridges" crosses and selection of cytological as well as desired agronomic characteristics (Stalker, 1980; Dhalival *et al.*, 1986; Gale and Miller, 1987). By 1951, the work of Lilienfeld and colleagues had established many of the genomic relationships of the diploid and polyploid *Triticum* L. and *Aegilops* L. species on the basis of chromosome pairing in hybrids (Lilienfeld and Kihara, 1951). These studies have continued to the present and have accurately classified chromosome translocations (Naranjo, 1995; Naranjo *et al.*, 1988; Maestra and Naranjo, 1999; Jauhar *et al.*, 1991). Archaeological evidence has shown that *Triticum turgidum* L. (AABB) has been grown in both Mesopotamia (Tigris and Euphrates River Valley) and in the Nile River Valley 10,000 years ago. Because wild *T. tauschii* (Coss.) Schmalh. is found only in the mountain region of south Russia, west Iran and north Iraq, it is thought that the hybridization that produced *T. aestivum* L. have occurred in these regions. It has been suggested that this occurred as recently as 8,000 years ago which coincides with the development of collective settlements by man (Wazuddin and Driscollt, 1986).

The goal of the present study was to find relationships between *Triticum* species using karyotype analysis and molecular markers.

## Materials and Methods

### Plant materials

A total of 46 wheat accessions were collected for this study. The materials were taxonomically identified based on Rahiminejad and Kharrazyan (2005). The Karyotype analysis was performed on the accessions belonging to five species and two subspecies carrying A genome (*Triticum monococcum* L., *T. boeoticum* Boiss. subsp. *thaodar* (Reut.) Schieman, *T. boeoticum* subsp. *boeoticum*, *T. urartu* Tumanian ex Gandilyan, *T. durum* Desf., *T. turgidum* and *T. aestivum*) (Table 1).

### Chromosome spread preparation

*Triticum* seeds were germinated at 25°C on moist filter paper in Petri dishes. Actively-growing root tips, 1 cm in length, were excised from the germinating seeds and pretreated with  $\alpha$ -bromonaphthalene for 4-6 hours at 4°C in refrigerator and fixed in chromic acid-formaldehyde mixture (1:1 of 1% chromic acid + 10% formaldehyde) at about 4°C for 24 hours. The root tips were transferred into 70% ethanol solution and kept refrigerated till staining. Subsequently, the root tips were hydrolyzed in 1 N NaOH at 60°C for 10 minutes, stained for 24 hours in hematoxylin stain at 30°C, and squashed in 45% glacial acetic acid. Before squashing, the root tips were treated with cellulase-pectinase enzyme solutions at 37°C for 10-15 minutes. The selected cells were photographed under an Olympus AX-40 light microscope. Karyotypes were obtained from well-spread metaphase plates.

All chromosomal sizes were measured with computer-aided program Micro Measure 3.3. Software (Reeves, 2001). Relative length in proportion to total genome length (RL), total chromosome length (TCL), mean chromosome length (MCL), arm ratio (short arm length/long arm length) with their respective standard errors and centromeric index (length of

the short arm/total length of the chromosome X 100) (Arano and Sattio, 1980), total form percentage (TF%) (Huziwara, 1962), centromeric index mean of long and short arms (CI), asymmetry index (AsI%) (Arano and Sattio, 1980), S% (The shortest chromosome length/the longest chromosome length), DRL (difference of range relative length), intrachromosomal asymmetry index (A1) (Romero-Zarko, 1986), interchromosomal asymmetry index (A2) (Romero-Zarko, 1986), Stebbin's classification (Stebbins, 1971) and karyotype formula (Levan *et al.*, 1964) were calculated (Table 2). The data analyzed with Excel, SAS (version 14) and SPSS (version 16) statistical softwares.

Table1. List of scientific name, accessions codes\*, ploidy level, kind of genomes and collecting localities used for comparative cytological study of Iranian *Triticum* species

Species	Accession	2n	Ploidy level	Genome	Locality and Altitude(m)
<i>T. monococcum</i>	T. mono-30	14	2x	A	Kermanshah: Gardaneh Reno(1480)
	T. mono -10	14	2x	A	Kurdistan: 3 km to Saghez (1620)
	T. mono -41	14	2x	A	Isfahan: Semirom to Yasouj (2100)
	T. mono -39	14	2x	A	Arak toward Malayer (2020)
	T. mono -40	14	2x	A	Tehran: Taleghan valley (1850)
<i>T. boeoticum</i> subsp. <i>thaodar</i>	T.b.t.-37	14	2x	A	Kurdistan: 5 km after Jenan to Saqez (1770)
	T.b.t.-8	14	2x	A	Chaharmahal va Bakhtiari: Shahr-e-Kord, Shapoorabad to Jooneghan (2090)
	T.b.t.-34	14	2x	A	Arak: 15 km to Malayer (1840)
<i>T. boeoticum</i> subsp. <i>boeoticum</i>	T.b.b.-19	14	2x	A	Ilam toward Kermanshah: Gardaneh Reno (1370)
	T.b.b.-5	14	2x	A	Lorestan: 35 km to Khorram Abad from Malavi (1100)
	T.b.b.-20	14	2x	A	Kermanshah: 10 km to Harsin (1330)
	T.b.b.-86	14	2x	A	Kermanshah toward Kamyaran (1340)
	T.b.b.-3	14	2x	A	Kohgiluyeh va Boyer-Ahmad near Yasouj
<i>T. urartu</i>	T.ura-156	14	2x	A	West Azarbaijan: Maku (1580)
	T.ura-84	14	2x	A	Kermashah: 10 km to Saqez from Asadabad (1320)
	T.ura-2	14	2x	A	Ardabil: 10 km to Kaghazkanan (1349)
	T.ura-8	14	2x	A	Chaharmahal va Bakhtiari: between Gandoman and Lordegan (2080)
	T.ura-59	14	2x	A	Kurdistan: Saqez (1770)
<i>T. durum</i>	T.duru-86	28	4x	AB	Kermanshah: Kamyaran (1440)
	T.duru-24	28	4x	AB	Lorestan: Malavi toward Khorram Abad (1200)
	T.duru-166	28	4x	AB	Chaharmahal va Bakhtiari: DoAb Samsami (2000)
	T.duru-1	28	4x	AB	Kohgiluyeh va Boyer-Ahmad (990)
	T.duru-165	28	4x	AB	Chaharmahal va Bakhtiari: near Chaghakhor lake (2190)
	T.duru-109	28	4x	AB	West Azarbaijan: Sardasht to Baneh (1050)
	T.duru-15	28	4x	AB	Khuzistan: Haftgel to Masjed Soleiman (550)
	T.duru-126	28	4x	AB	Kurdistan: 6 Km to Alamoot (1660)
	T.duru-7	28	4x	AB	Chaharmahal and Bakhtiari: Borojen to Izeh (2190)
	T.duru-109	28	4x	AB	West Azarbaijan: Sardasht to Baneh (1050)
<i>T. turgidum</i>	T.turgi-211	28	4x	AB	West Azarbaijan: Khoy (1110)
	T.turgi- 45	28	4x	AB	Chaharmahal va Bakhtiari: Bazoft (2190)
	T.turgi- 2	28	4x	AB	Kohgiluyeh va Boyer-Ahmad: Yasouj (2880)

Species	Accession	2n	Ploidy level	Genome	Locality and Altitude(m)
<i>T. aestivum</i>	T.turgi- 43	28	4x	AB	Chaharmahal and Bakhtiari: Bazoft Morez valley (2000)
	T.turgi- 8	28	4x	AB	Chaharmahal and Bakhtiari: Borojen to Izeh (2190)
	T.turgi- 10	28	4x	AB	Khuzistan: Izeh (900)
	T.turgi- 194	28	4x	AB	Kurdistan: between Sanandaj and Saghez (1595)
	T.turgi- 80	28	4x	AB	Kermanshah: Mahi Dasht (1290)
	T.turgi- 25	28	4x	AB	Lorestan: Malavi toward Khorram Abad (1200)
	T.turgi-120	28	4x	AB	East Azarbaijan: Ahar (1320)
	T.aest-47	42	6x	ABD	Chaharmahal and Bakhtiari (2000)
	T.aest-74	42	6x	ABD	Ilam: Do Rahe (1410)
	T.aest-129	42	6x	ABD	Booshehr: Bandargah to Deilam (17)
	T.aest-73	42	6x	ABD	Khuzistan: Karkheh (13)
	T.aest-97	42	6x	ABD	Malayer 50 km to Arak (2010)
	T.aest-96	42	6x	ABD	Tehran: Firooz Kuh (1700)
	T.aest-107	42	6x	ABD	West Azarbaijan: Boukan to Mahabad (1290)
	T.aest-49	42	6x	ABD	Isfahan: Daran (2190)
T.aest-82	42	6x	ABD	Kermanshah: Mahi Dasht (1290)	
Chinese spring	C.S.	42	6x	ABD	Provided by the Institute of Plant Biology, University of Zurich, Switzerland

\*All samples are kept in the herbarium of Isfahan University

Table 2. The means of karyotypic characters of forty-six mitotic chromosomes in diplo, tetra and hexaploids species of the genus *Triticum* used in this study (n = Chromosome number, TCL = Total haploid chromatid length, MCL = Mean of chromosomes length, TF% = Total form percent, Cent.Index = Centromeric Index (= S/(L+S)), Lon.Ch. = Longest chromosome length, Sho.Ch. = Shortest chromosome length, ML = Mean of large arms, MS = Mean of short arms, r = Arms ratio, AsI% = Asymmetry index, S% = Ratio between the shortest and longest of the chromosomes percent, DRL = Difference of range relative length, A1 = Intrachromosomal asymmetry, A2 = Interchromosomal asymmetry (C.V.) and RL = Relative length of chromosomes)

Species	2n	TCL	MCL±SE	TF%	Cent.Index	Lon. Ch.	Sho.Ch.	M.L. arm
<i>T. monococcum</i>	14	59.610	8.515 (±1.02)	43.29	0.431	9.775	7.349	4.816
<i>T. urartu</i>	14	48.316	7.606 (±0.36)	42.35	0.423	10.161	7.518	4.433
<i>T. boeoticum</i> subsp. <i>thaodar</i>	14	70.277	8.482 (±0.13)	41.60	0.415	11.457	8.589	5.871
<i>T. boeoticum</i> subsp. <i>boeoticum</i>	14	74.758	10.679 (±1.55)	42.72	0.427	12.233	9.186	6.105
<i>T. turgidum</i>	28	130.133	9.294 (±1.94)	40.66	0.404	13.007	7.81	5.475
<i>T. durum</i>	28	156.210	11.157 (±1.54)	41.437	0.41	13.35	8.151	6.545
<i>T. aestivum</i>	42	241.002	11.475 (±0.88)	40.831	0.406	14.834	7.528	6.777

Species	M.S. arm	r	AsI%	SI%	DRL	A1	A2	RL
<i>T. monococcum</i>	3.698	1.27	56.69	74.55	4.201	0.232	0.114	14.28
<i>T. urartu</i>	3.256	1.36	57.67	74.16	3.767	0.264	0.146	14.28
<i>T. boeoticum</i> subsp. <i>thaodar</i>	4.168	1.41	58.39	75.41	4.002	0.283	0.114	14.28
<i>T. boeoticum</i> subsp. <i>boeoticum</i>	4.554	1.33	57.11	71.25	3.715	0.244	0.102	14.28
<i>T. turgidum</i>	3.819	1.47	59.35	62.425	3.409	0.299	0.147	7.14
<i>T. durum</i>	4.615	1.43	58.63	60.277	3.707	0.276	0.147	7.14
<i>T. aestivum</i>	4.698	1.45	59.085	50.418	3.099	0.305	0.166	4.76

## Results and Discussion

The results showed that about 33% of diploid accessions had sub-metacentric (sm) chromosomes in their Karyotype formula and other ones had only metacentric chromosomes. Tetraploid accessions had 2-4 sub-metacentrics (sm) and hexaploid accessions possessed 2-5

sub-metacentric (sm) chromosomes in their Karyotype formulae (Table 1).

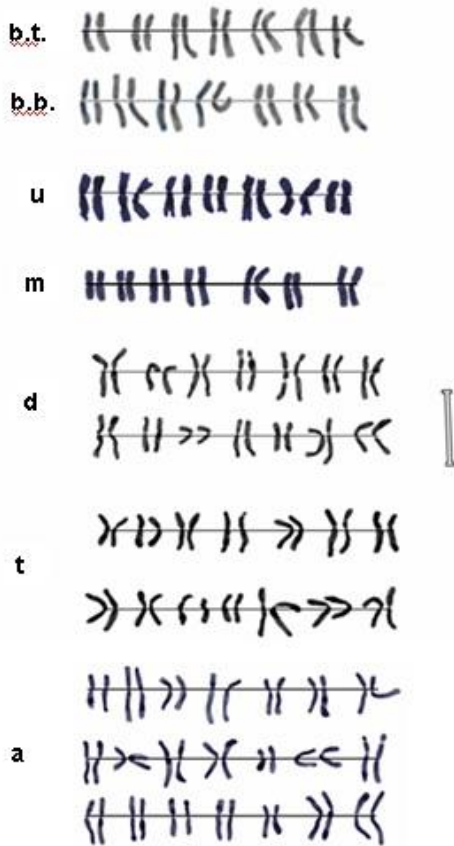


Figure 1. Mitotic metaphase in *Triticum* species studied. b.t. *T. boeoticum* subsp. *thaodar*; b.b. *T. boeoticum* subsp. *boeoticum*; u. *T. urartu*; m. *T. monococcum*; d. *T. durum*; t. *T. turgidum*; a. *T. aestivum*. Scale Bar = 20  $\mu$ m. Arrow shows the satellite chromosome

All the accessions without T.duru-1, T.aest-73 and T.aest-47 (2A category), were placed in 1A category of Stebbins (1971) asymmetry categories (not showed in these Tables). Total chromosome length depended on the ploidy levels and chromosome numbers and consequently this parameter was too variable (Table 2).

TCL values varied between genotypes (Table 2). The TCL value ranged from 48.316  $\mu$ m (*T. urartu*) with MCL of  $7.606 \pm 0.63$   $\mu$ m to (*T. aestivum*) with MCL of  $11.475 \pm 0.88$   $\mu$ m. The most variable chromosomes in length SE of MCL were found in *T. turgidum* (SE of MCL = 1.94  $\mu$ m) whereas the most similar chromosomes were scored in *T. boeoticum* subsp. *thaodar* (SE of MCL = 0.13  $\mu$ m) (Table 2). The TF% value ranged from 43.29 (*T. monococcum*) to 40.66 (*T. turgidum*) (Table 2). The CI ranged between 0.431 (*T. monococcum*) and 0.404 (*T. turgidum*). The longest and the shortest chromosome length were found in *T. aestivum*. The highest mean length arm was in *T. aestivum*, and the lowest mean was in *T. urartu*. The arm ratio ranged from 1.27 in *T. monococcum* to 1.47 in *T. turgidum*. The ASI% varied from 56.69 (*T. monococcum*) to 59.35 (*T. turgidum*). The SI% ranged from 75.41 (*T. boeoticum* subsp. *thaodar*) to 50.418 (*T. aestivum*). DRL in *T. monococcum* was the highest (4.201) and in *T. aestivum* was the lowest (3.099). In general, A1 and A2 values showed the high degree of karyotype symmetry in the majority of the genotype studied (Razik Kamel, 2006). A1 ranged from 0.305 (*T. aestivum*) to 0.232 (*T. monococcum*) and A2 ranged from 0.102 (*T. boeoticum* subsp. *boeoticum*) to 0.166 (*T. aestivum*) (Table 2). Among the diploid species, the TCL value varied from 74.758  $\mu$ m (*T. boeoticum* subsp. *boeoticum*) to 48.316  $\mu$ m (*T. urartu*). Between tetraploid accessions the

TCL value ranged between 156.210  $\mu\text{m}$  (*T. durum*) and 130.133 (*T. turgidum*). Based on the result of this study, it could be concluded that generally, the chromosomal length in the *T. urartu* was shorter than other species of the genus *Triticum*. In this species, minimum value of TF%, CI and A1 belonged to *T. boeoticum* subsp. *thaodar* and maximum showed in *T. monococcum*. Also among this group (diploids) arm ratio (r) and AsI% in *T. monococcum* were the lowest and in *T. boeoticum* subsp. *thaodar* was the highest. Results showed that coefficient of variability for intra-specific chromosome length variation of short and long arms (Table 3) was higher for short arms and that it can be concluded that short arms were more effective in TCL than long arms. Karyotype studies were principally based on the idea that symmetrical karyotypes were more primitive than asymmetrical ones; and this holds true for longer chromosomes compared to shorter ones; median centromeres with arms of equal length are more primitive than chromosomes with arms of unequal length; low basic numbers give rise to higher ones. These features are based on the comparison between karyotypes of known relative antiquity, as determined through classical taxonomy (Sharma, 1990).

Table 3. Coefficient of variability for intra-specific chromosome length variation of short and long arms for *Triticum* species used in this study

Chromosome arms	<i>T. monococcum</i>	<i>T. urartu</i>	<i>T. boeoticum</i> subsp. <i>thaodar</i>	<i>T. boeoticum</i> subsp. <i>boeoticum</i>	<i>T. turgidum</i>	<i>T. durum</i>	<i>T. aestivum</i>
Short arm (in%)	9.88 – 18.64	9.52 – 19.09	13.57 – 16.93	7.85 – 15.75	17.25 – 21.82	9.46 – 24.51	17.38 – 24.77
Long arm (in%)	7.60 – 13.46	8.88 – 15.48	10.70 – 13.01	11.64 – 14.06	11.63 – 16.82	8.19 – 18.32	12.93 – 19.09

The scatter diagram based on A1 and A2 (Romero-Zarco, 1986) constructed three groups of karyotype asymmetry in the accessions studied: 1- *T. aestivum* (a) with the highest asymmetrical karyotype, 2- *T. monococcum*, *T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum* with the lowest asymmetrical karyotype and 3- *T. urartu*, *T. turgidum* and *T. durum* with an intermediate between two previous groups (Figure 2) (Jalilian and Rahiminejad, 2011).

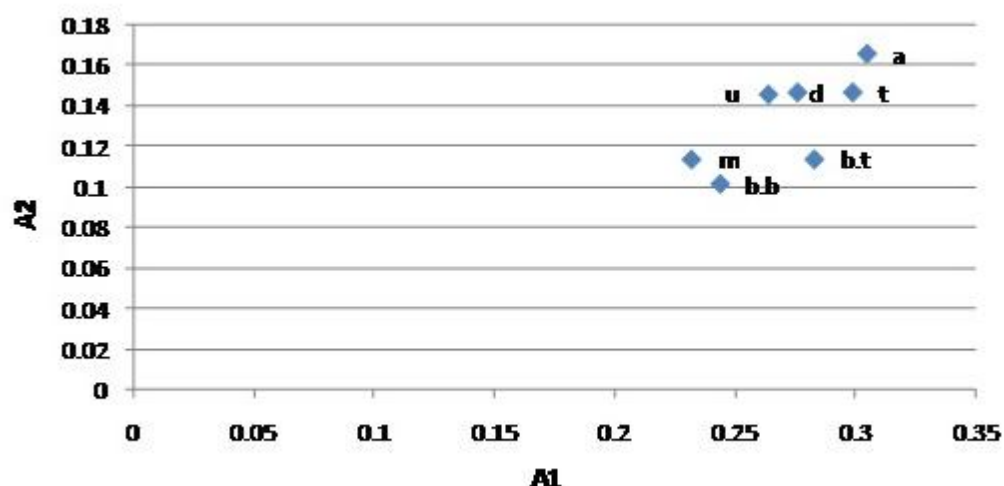


Figure 2. Scatter diagram show the relationships between the *Triticum* species based on the intrachromosomal (A1) and interchromosomal (A2) asymmetry indices. Values of A1 and A2 are summarized in Table 2. (m. *T. monococcum*; u. *T. urartu*; b.b. *T. boeoticum* subsp. *boeoticum*; b.t. *T. boeoticum* subsp. *thaodar*; d. *T. durum*; t. *T. turgidum*; a. *T. aestivum*)

In the UPGMA dendrogram (Figure 3), the five *Triticum* species studied were divided into three groups: (1) A: (*T. monococcum* and *T. urartu*), (2) B: (*T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum*), and (3) C: (*T. durum*, *T. turgidum* and *T. aestivum*) (Figure 3) (Akhavan and Saeidi, 2010; Jalilian and Rahiminejad, 2011).

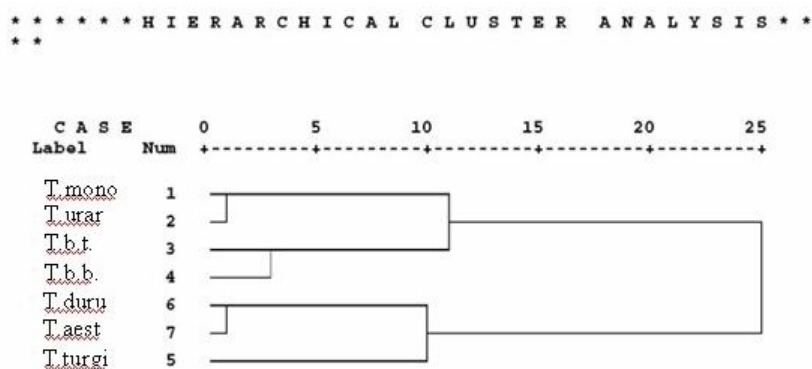


Figure 3. The UPGMA dendrogram (used Euclidian Distance) shows relationships between *Triticum* species based on chromosomal characters (see Table 2). T.mono. *T. monococcum*; T.urar. *T. urartu*; T.b.t. *T. boeoticum* subsp. *thaodar*; T.b.b. *T. boeoticum* subsp. *boeoticum*; T.duru *T. durum*; T.turgi. *T. turgidum*; T.aest. *T. aestivum*.

According to the results, it might be suggested that *T. durum* was more primitive than *T. turgidum* and *T. monococcum* could be considered as a donor of A genome to *T. durum* and *T. aestivum*. This hypothesis verified SSR analysis (Ehtemam *et al.*, 2010; Keshavarzi *et al.*, 2012).

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## مقایسه فیلوژنتیک ژنوم A در برخی از گونه‌های جنس *Triticum* با روش تحلیل کاریوتیپ

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### چکیده

کاریوتیپ ۴۸ جمعیت از پنج گونه (*T. urartu* و *T. turgidum*، *T. monococcum*، *T. durum*، *Triticum aestivum*) و دو زیرگونه *T. boeoticum* subsp. *boeoticum* و *T. boeoticum* subsp. *thaodar* دارای ژنوم A از جنس *Triticum* تحلیل شد. کروموزوم‌ها با نرم‌افزار Micro Measure 3.3 اندازه‌گیری شد. ویژگی‌های کاریوتیپی شامل: تعداد کروموزوم، طول کلی کروموزوم، میانگین طول کروموزوم‌ها، درصد شکل کلی کاریوتیپ، اندیس سانترومری، طول بلندترین کروموزوم، طول کوتاه‌ترین کروموزوم، میانگین طول بازوهای بلند، میانگین طول بازوهای کوتاه، نسبت بازو، اندیس عدم تقارن کاریوتیپی، درصد نسبت بین کوتاه‌ترین و بلندترین کروموزوم‌ها، تفاوت دامنه طول نسبی کروموزوم‌ها، اندیس تقارن درون کروموزومی، اندیس تقارن میان کروموزومی، طول نسبی کروموزوم‌ها و فرمول کاریوتیپی اندازه‌گیری و محاسبه گردید. تمام جمعیت‌ها در گروه A1 طبقه‌بندی Stebbins قرار گرفتند. تحلیل خوشه‌ای بر پایه ضرایب A1 و A2 سه گروه کاریوتیپی را در جمعیت‌های مطالعه شده ایجاد کرد: گروه نخست شامل *T. aestivum* با بیشترین عدم تقارن کاریوتیپی، گروه دوم شامل *T. boeoticum* subsp. *boeoticum*، *T. boeoticum* و *T. turgidum* subsp. *thaodar* و با کمترین عدم تقارن کاریوتیپی و گروه سوم شامل *T. durum*، *T. durum* و *T. urartu* و با جایگاهی مابین دو گروه قبل. گونه‌های دیپلوئید *T. monococcum* بر اساس ضرایب A1 و A2 قدیمی‌ترین و ابتدایی‌ترین کاریوتیپ را داشت. با توجه به نتایج به نظر می‌رسد *T. durum* ابتدایی‌تر از *T. turgidum* باشد و احتمالاً *T. monococcum* می‌تواند به عنوان دهنده ژنوم A به *T. durum* و *T. aestivum* به حساب آید.

**واژه‌های کلیدی:** ژنوم A، تحلیل کاریوتیپ، فیلوژنی، *Triticum*