

# A COMPARATIVE CYTOLOGICAL STUDY IN THE D GENOME-BEARING SPECIES OF TRITICUM-AEGILOPS COMPLEX

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The wild D genome-bearing *Aegilops* L. species. are considered to be sources of useful alleles which can be used in bread wheat improvement. We examined the karyotype asymmetry of the D genome-bearing species and analyzed the data to look for their evolutionary correlations. A total number of eleven accessions belonging to seven D genome species and *Triticum aestivum* L. were used for this study. The observations of the analyzed factors showed that *Ae. vavilovii* (Zhuk.) Chen. posses the most symmetric karyotypes and *Ae. cylindrica* Host. the most asymmetric ones. Both species *Ae. crassa* Boiss. and *Ae. vavilovii* carrying a common genomic formula (XD) showed a similar karyotype asymmetry. The results of this study compared with the literature showed that the degree of karyotype asymmetry of the D genome has a correlation with the time of divergence from its ancestral progenitors. Our results suggest that the karyotype asymmetry analysis can be a useful tool to have an overall view over the genomic relationships and modifications and also a good measure to estimate the relative date of the origin of allopolyploidy of the D genome cluster.

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## Triticum-Aegilops

## D

از آنجا که گندم نان (*T. aestivum*) دارای ژنوم D می باشد، دارندگان ژنوم D از جنس *Aegilops* می تواند به عنوان منبع آلی مفید قابل انتقالی به گندم نان مورد استفاده قرار گیرد. در این تحقیق نحوه تقارن کاریوتایی دارندگان ژنوم D از جنس *Aegilops* و نیز گونه *T. aestivum* جهت بررسی ارتباطات تکاملی آنها مورد بررسی قرار گرفته است. در حدود ۱۱ جمعیت متعلق به هفت گونه دارنده ژنوم D برای این بررسی مورد استفاده قرار گرفت. بر اساس پارامترهای ارزیابی شده در این تحقیق، در میان کلیه گونه های مطالعه شده، گونه *Ae. cylindrica* نامتقارن ترین کاریوتایپ و در مقابل گونه *Ae. vavilovii* متقارن ترین کاریوتایپ را نشان می دهد. دو گونه *Ae. crassa* و *Ae. vavilovii* که دارای دو ژنوم مشترک (XD) هستند، سطح تقارن کاریوتایی مشابهی را نشان دادند. نتایج این بررسی با مقایسه با بررسی های پیشین نشان داد که درجه تقارن کاریوتایی ژنوم D با زمان انشقاق گونه از والد خود ارتباط مستقیم دارد. نتایج این بررسی پیشنهاد می کند که بررسی تقارن کاریوتایی می تواند ابزار مفیدی جهت شناخت ارتباطات و تغییرات ژنومی و نیز مقیاس مناسبی جهت تخمین تاریخ نسبی منشا آلویلی پلوئیدی در خوشه ژنوم D باشد.

## Introduction

Cytological and molecular studies in a group of related plant taxa allow biosystematists and plant breeders to understand the evolution and exploit the diversity of gene pools. As Kihara (1947) has noted, "the history of

the earth is recorded in the layers of its crust. The history of all organisms is inscribed in their chromosomes" understanding the characteristics of the plant cell nucleus is critical to learning about the genome, its behavior, modulation and generation of

biodiversity. This information is of value for both fundamental reasons and applications in plant breeding, Table 1. List of accessions of the D genome-bearing species of *Aegilops-Triticum* species used in this study and their locations.

Species	2n	Ploidy level	Genome type	Location
<i>Ae. tauschii</i>				
subsp. <i>tauschii</i>	14	2x	D	1, Iran, Azerbaijan, Khoy
subsp. <i>strangulata</i>	14	2x	D	406, Iran, Babolsar
<i>Ae. crassa</i>				
var. <i>crassa</i>	28	4x	XD	15700, Iran, Fasa
var. <i>crassa</i>	28	4x	XD	201, Iran, Yasooj
var. <i>macranthera</i>	28	4x	XD	15702, Iran, Shiraz
<i>Ae. cylindrica</i>				
var. <i>prokhanovii</i>	28	4x	DC	470, Iran, Yasooj
var. <i>cylindrica</i>	28	4x	DC	513, Iran, Jolfa
<i>Ae. ventricosa</i>	28	4x	DN	2270004, John Innes Centre
<i>Ae. vavilovii</i>	42	6x	XDS	2260002, John Innes Centre
<i>Ae. juvenalis</i>	42	6x	XDU	15649, Iran, Lordegan
<i>Triticum aestivum</i>	42	6x	ABD	90, Iran, Lorestan, Noorabad

ecology, phylogeography and genetics (Bennett, 1982 & 1984).

As Waines and Barnhart (1992) have pointed out the success of beard wheat (*Triticum aestivum* L., 2n = 6x = 42; AABBDD) and bread-making quality are largely affected by the D genome involved in its genome. The wild pool of the D genome distributed among *Aegilops* spp. are sources of disease resistance and other useful alleles that can be incorporated into the cultivated wheat (Waines & Barnhart, 1992). Thus, the cytological and molecular studies in this group are of brilliant importance.

The D genome-bearing species of the genus *Aegilops* have been classified at the sectional level into *Cylindropyrum* and *Vertebrata* sections, both possessing a D genome component derived from their diploid progenitor *Ae. tauschii* Coss. (DD) (McFadden and Sears, 1946; Wan et al., 2000). The above genomes are existed at three ploidy levels: diploid (*Ae. tauschii*), tetraploid (*Ae. cylindrica* Host., *Ae. ventricosa* Tausch. and the tetraploid cytotype of *Ae. crassa* Boiss.), and hexaploid (*Ae. vavilovii* (Zhuk.) Chen., *Ae. juvenalis* (Thell.) Eig. and the hexaploid cytotype of *Ae. crassa*) all founded on a basic chromosome number of seven (Slageren, 1994).

Although the D genome-bearing species have been the matter of intensive and long studies (Badaeva et al., 2002; Rayburn & Gill, 1987; Kimber & Zhao, 1983; Zhao & Kimber, 1984; Cunado et al., 1996; Saeidi et al., 2006, 2008; Sheidai et al., 2002), however a numerical and comparative karyotype analysis in this group seems to be still lacking.

This study is aimed to characterize the cytological and karyotypic details of *Aegilops* species-bearing the D genome and *T. aestivum* in order to construct a series of numerical parameters and a comparative cytological model for this group of species.

## Materials and methods

### Plant materials

The karyotype analysis was performed on ten accessions belonging to six *Aegilops* species of the D genome cluster and one from *T. aestivum*. Nine accessions belonging to *Ae. tauschii*, *Ae. crassa*, *Ae. cylindrica*, *Ae. juvenalis* and *T. aestivum* were collected from Iran and two accessions of *Ae. ventricosa* and *Ae. vavilovii* were received from John Innes Center (Norwich, England) (Table 1).

### Chromosome spread preparation

Somatic chromosomes of meristematic root tip cells were studied from germinating seeds using Agayev (1996) method. Briefly, pretreatment was performed in saturated solution of monobromonaphthalene, washed in distilled water for 10 min, fixed in chromic acid-formaldehyde mixture (1/1) at about 4 °C for 24 h, washed under tap water for 3 h. Then the materials were transferred into 70% ethanol solution and kept refrigerated till staining. For staining, the materials were transferred into distilled water for about 5-6 min and treated with 1N NaOH at 60 °C for 10 min, washed in distilled water thoroughly for 30 min then stained in aceto-iron-hematoxylin at 30 °C for 24 h, washed in distilled water for at least 30 min, macerated for 10-15 min in cellulase-pektinase enzyme solution at 37 °C.

Table 2. Parameters and their descriptions used for the measurements of this study.

	Parameters	Descriptions
1	Total haploid chromosome length (TCL)	Total Chromosome length of the haploid complement
2	Mean Chromosome Length (MCL)	Mean Chromosome length of the haploid complement
3	Total Form percent (TF%; Huziwara 1962)	Ratio between the shortest arms of the chromosomes and their total length; the TF% value is considered to be close to 50% in most symmetric karyotypes and less than 50% based on the degree of asymmetry
4	R (Siljak-Yakovlev 1986)	Ratio between the longest and the shortest arms of the chromosomes
5	Asymmetry index (AsI%; Arano & Saito 1980)	$100 \times \sum L / \sum TCL$ ; where $l$ is long arms in chromosome set and $TCL$ is total chromosome length in chromosome set
6	Karyotype formulae (Levan et al. 1964)	According to their arm ratios (long/short) designated by the position of the centromere: 1 (metacentric; M), 1-1.7 (metacentric; m), 1.7-3 (submetacentric; sm), 3-7 (subtelocentric; st), 7-39 (telocentric; t)].
7	intrachromosomal asymmetry index (A1; Romero Zarko 1986)	$= 1 - [\sum(b/B)/n]$ ; where $b$ and $B$ are the mean length of short and long arms of each pair of homologues respectively, $n$ is the number of homologues, The value of A1 is considered to be close to 0 if all chromosomes are metacentric and near to one if all chromosomes are telocentric.
8	interchromosomal asymmetry index (A2; Romero Zarko 1986)	$= s/x$ ; where $s$ and $x$ are standard deviation and the mean length of the chromosomes

Table 3. Karyotype features of the D genome-bearing species of *Aegilops-Triticum* ( $n$  = chromosome number, TL = Total haploid chromatin length, MCL = Mean Chromosome Length, SE = Standard Error, TF% = Total form percent, R = ratio between the longest and the shortest arms of the chromosomes, AsI% = Asymetry index, Karyotype formulae, A1 = intrachromosomal and A2 = interchromosomal asymmetry index).

Species	2n	TCL	MCL ± SE	TF%	R	AsI%	Karyotype formulae	A1	A2
<i>Ae. tauschii</i>	14	56.546	8.078 ± 1.330	40.17	1.51	58.29	5m + 2sm	0.302	0.164
<i>Ae. crassa</i>	28	110.392	7.885 ± 1.107	40.06	1.57	57.74	11m + 3sm	0.294	0.140
<i>Ae. cylindrica</i>	28	94.393	6.742 ± 0.817	31.58	1.48	67.62	1M + 6m + 3sm + 2st + 2t	0.500	0.121
<i>Ae. ventricosa</i>	28	99.528	7.109 ± 1.063	35.92	1.81	63.36	1M + 6m + 5sm + 2st	0.397	0.149
<i>Ae. vavilovii</i>	42	147.7	7.033 ± 1.462	41.81	2.07	57.24	2M + 13m + 6sm	0.260	0.207
<i>Ae. juvenalis</i>	42	165.883	7.899 ± 1.172	37.96	1.66	60.92	1M + 11m + 8sm + 1st	0.363	0.148
<i>T. aestivum</i>	42	207.584	9.884 ± 1.457	38.11	1.69	60.21	2M + 11m + 7sm + 1st	0.354	0.147

The roots were gently squashed in 45% acetic acid, on a slide glass and were observed and photographed under an Olympus AX-40 light microscope.

In order to characterize the karyotypic asymmetry 5-10 chromosome spreads from different individuals of each accession were examined. All chromosomal sizes were measured with computer-aided program Image Tool 3.0. The parameters measured in each metaphase chromosome spread are listed in Table 2.

### Results and Discussion

The morphological observations of our chromosomal studies are shown as karyotypes in Fig. 1. The resulted karyotype formulae and their analyzed parameters are shown in Tables 3 & 4.

The results showed that while sub-telocentric (st) together with telocentric (t) chromosome types appeared at low frequency (7.08%), the metacentric (m) and sub-metacentric (sm) types dominated the observed karyotypes with 92.92% frequency. The only two telocentric chromosomes observed, were exclusively indicators of *Ae. cylindrica* (see Table 3). Our results showed that satellites are restricted only to one chromosome in: *Ae. tauschii*, *Ae. ventricosa*, *Ae. cylindrica* and *Ae. vavilovii* and two chromosomes in *Ae. crassa*, *Ae. juvenalis* and *T. aestivum*. No B chromosome was observed among the materials studied.

Total chromosome length (TCL) was a variable parameter in this study; which can be caused by

Table 4. Average length of short and long arms and other characteristics regarding chromosomes of the D genome-bearing species of *Aegilops-Triticum* as observed in this study.

	<i>Ae. tauschii</i>			<i>Ae. crassa</i>			<i>Ae. cylindrica</i>			<i>Ae. ventricosa</i>			<i>Ae. varhovi</i>			<i>Ae. juvenalis</i>			<i>T. aestivum</i>		
	S	L	r	S	L	r	S	L	r	S	L	r	S	L	r	S	L	r	S	L	r
1	2.334 (±.69)	3.766 (±.07)	1.61 m	4.61 (±.08)	5.076 (±.10)	1.10 m	3.682 (±.12)	4.59 (±.09)	1.24 m	4.418 (±.12)	4.534 (±.12)	1.03 M	4.57 (±.17)	5.078 (±.23)	1.11 m	4.488 (±.07)	5.862 (±.19)	1.30 m	5.066 (±.04)	7.19 (±.09)	1.41 m
2	3.838 (±.12)	5.346 (±.06)	1.39 m	2.578 (±.06)	5.72 (±.11)	2.21 sm	3.198 (±.09)	4.578 (±.11)	1.43 m	2.514 (±.19)	5.29 (±.28)	2.10 sm	4.716 (±.17)	4.726 (±.28)	1.00 M	4.41 (±.18)	5.11 (±.13)	1.15 m	5.14 (±.10)	6.57 (±.06)	1.27 m
3	3.82 (±.12)	5.402 (±.17)	1.41 m	4.332 (±.21)	4.628 (±.16)	1.07 m	2.042 (±.08)	5.389 (±.17)	2.63 sm	3.452 (±.10)	4.348 (±.11)	1.25 m	3.646 (±.10)	4.756 (±.08)	1.30 m	3.806 (±.08)	5.614 (±.11)	1.47 m	4.69 (±.11)	6.604 (±.17)	1.40 m
4	2.332 (±.62)	4.344 (±.05)	1.84 sm	3.64 (±.11)	5.196 (±.05)	1.42 m	1.408 (±.04)	5.948 (±.08)	4.22 st	1.638 (±.04)	6.12 (±.17)	3.73 st	2.366 (±.14)	4.482 (±.09)	1.89 sm	2.71 (±.18)	6.146 (±.15)	2.26 sm	5.094 (±.08)	6.199 (±.09)	1.21 m
5	2.55 (±.09)	5.624 (±.06)	2.2 sm	3.616 (±.13)	5.138 (±.09)	1.42 m	2.792 (±.09)	4.56 (±.07)	1.63 m	2.11 (±.08)	5.546 (±.12)	2.62 sm	3.488 (±.14)	4.76 (±.11)	1.36 m	4.366 (±.19)	4.474 (±.12)	1.02 M	4.005 (±.05)	7.116 (±.03)	1.77 sm
6	3.546 *364	3.788 (±.04)	1.06 m	2.714 (±.06)	5.552 (±.16)	2.04 sm	2.6 (±.06)	4.378 (±.06)	1.68 m	1.712 (±.10)	5.758 (±.06)	3.36 st	3.534 (±.02)	4.122 (±.17)	1.15 m	3.858 (±.16)	4.914 (±.02)	1.27 m	5.476 (±.15)	5.61 (±.06)	1.02 M
7	4.276 (±.12)	4.696 (±.12)	1.09 m	2.66 (±.05)	5.17 (±.06)	1.94 sm	1.906 *744	4.134 (±.06)	2.16 sm	3.328 (±.17)	4.044 (±.16)	1.21 m	3.344 (±.14)	4.32 (±.12)	1.29 m	3.968 (±.09)	4.772 (±.17)	1.20 m	4.27 (±.15)	6.592 (±.11)	1.54 m
8				2.608 (±.04)	3.712 (±.16)	1.42 m	3.3 (±.16)	3.348 (±.09)	1.01 M	2.672 (±.09)	4.634 (±.15)	1.73 sm	3.076 (±.13)	4.648 (±.08)	1.51 m	2.716 (±.07)	5.93 (±.07)	2.18 sm	3.262 (±.22)	5.966 (±.07)	1.82 sm
9				3.056 (±.09)	4 (±.10)	1.30 m	2.5 (±.10)	3.744 (±.07)	1.49 m	2.098 (±.10)	4.58 (±.10)	2.18 sm	2.682 (±.04)	5.058 (±.26)	1.88 sm	3.916 (±.20)	4.548 (±.17)	1.16 m	2.508 *141	6.204 (±.29)	2.47 sm
10				3.292 (±.09)	3.952 (±.11)	1.20 m	2.374 (±.10)	3.732 (±.11)	1.57 m	1.714 (±.06)	4.726 (±.05)	2.75 sm	3.368 (±.13)	3.98 (±.12)	1.18 m	2.284 (±.11)	5.002 (±.14)	2.19 sm	3.308 (±.07)	6.986 (±.22)	2.11 sm
11				2.882 (±.10)	4.27 (±.10)	1.48 m	.774 (±.05)	5.328 (±.12)	6.88 st	2.586 (±.16)	3.876 (±.05)	1.49 m	3.248 (±.09)	3.964 (±.10)	1.22 m	3.25 *738	4.558 (±.10)	1.40 m	4.454 (±.08)	5.788 (±.10)	1.29 m
12				2.67 (±.12)	4.274 (±.14)	1.60 m	.654 (±.04)	5.45 (±.08)	8.33 t	2.962 (±.10)	3.162 (±.09)	1.06 m	2.662 (±.11)	4.584 (±.17)	1.72 sm	2.538 (±.19)	5.1057 (±.58)	2.18 sm	2.392 (±.06)	7.756 (±.10)	3.24 st
13				2.978 (±.09)	3.508 (±.12)	1.17 m	1.982 (±.07)	3.704 (±.06)	1.86 sm	2.438 (±.07)	3.592 (±.06)	1.47 m	3.156 (±.03)	3.686 (±.0517)	1.16 m	1.189 (±.03)	5.964 (±.10)	5.01 st	3.108 (±.09)	6.532 (±.06)	2.10 sm
14				2.602 (±.06)	3.544 (±.06)	1.36 m	.604 (±.02)	4.95 (±.10)	8.19 t	2.114 (±.005)	2.838 (±.10)	1.34 m	3.191 (±.05)	3.292 (±.09)	1.03 M	1.826 (±.10)	4.098 (±.16)	2.24 sm	3.872 (±.10)	5.53 (±.09)	1.42 m
15													2.428 (±.13)	3.978 (±.15)	1.63 m	3.068 (±.10)	3.89 (±.11)	1.26 m	3.382 (±.10)	5.38 (±.05)	1.59 m
16													2.734 (±.10)	3.494 (±.05)	1.27 m	2.38 (±.13)	4.593 (±.33)	1.92 sm	3.446 (±.06)	5.042 (±.10)	1.46 m
17													2.718 (±.03)	2.932 (±.14)	1.07 m	1.892 (±.07)	5.044 (±.14)	2.66 sm	3.668 (±.04)	4.816 (±.11)	1.31 m
18													2.402 (±.05)	3.25 (±.06)	1.35 m	3.014 (±.08)	3.662 (±.18)	1.21 m	2.93 (±.04)	5.368 (±.09)	1.83 sm
19													1.87 (±.10)	3.278 (±.08)	1.75 sm	2.64 (±.15)	4.095 (±.20)	1.55 m	3.008 (±.09)	4.882 (±.10)	1.62 m
20													1.868 (±.04)	2.966 (±.13)	1.58 sm	2.73 (±.10)	3.576 (±.08)	1.30 m	2.482 (±.09)	5.194 (±.10)	2.09 sm
21													1.434 (±.03)	3.19 (±.01)	2.21 sm	2.106 (±.06)	4.108 (±.18)	1.95 sm	3.57 (±.07)	3.674 (±.12)	1.02 M

\* Satellite. (Abbreviations: S: Short arm, L: Long arm, r: arm ratio, ±: standard error, Type: chromosome type according to LEVAN et al. 1964)

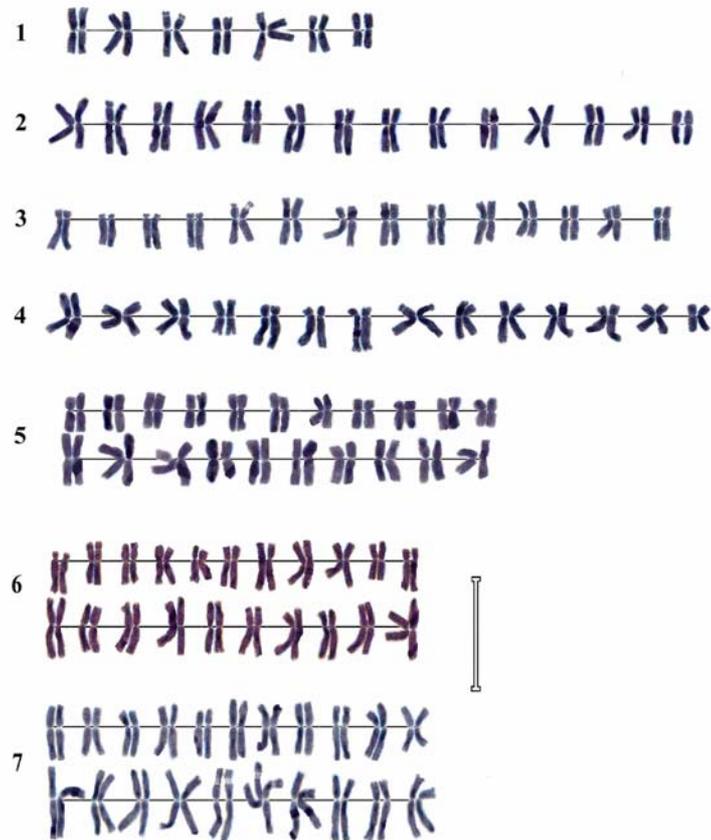


Fig. 1. Somatic chromosomes of the D genome-bearing species. 1. *Aegilops tauschii*, 2. *Ae. crassa*, 3. *Ae. cylindrica*, 4. *Ae. ventricosa*, 5. *Ae. vavilovii*, 6. *Ae. juvenalis*, 7. *T. aestivum*. Scale bar = 20µm.

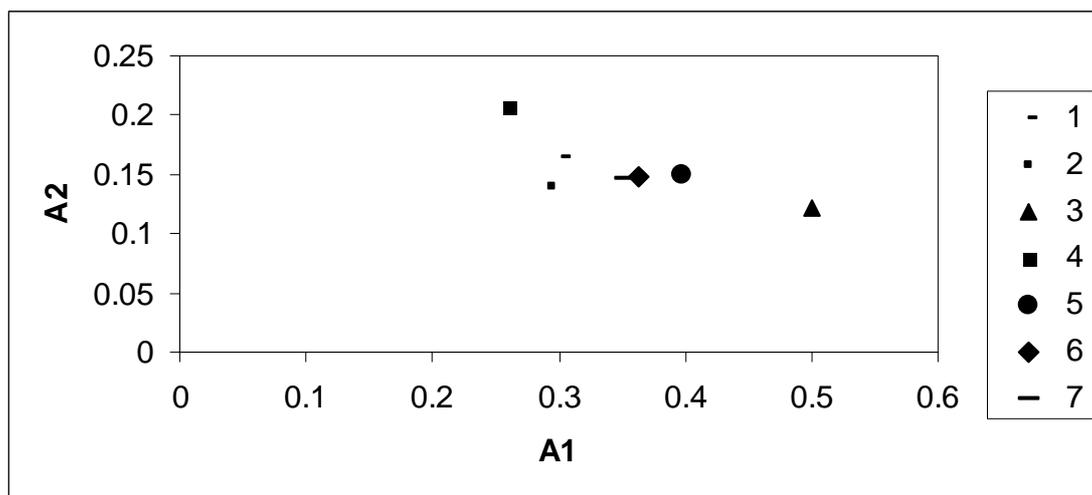


Fig. 2. Scatter diagram shows the relationships between the D genome-bearing species of *Aegilops-Triticum* based on the intrachromosomal (A1) and interchromosomal (A2) asymmetry indices. Values of A1 and A2 are summarized in Table 3. (1 = *Ae. tauschii*, 2 = *Ae. crassa*, 3 = *Ae. cylindrica*, 4 = *Ae. vavilovii*, 5 = *Ae. ventricosa*, 6 = *Ae. juvenalis*, 7 = *T. aestivum*).

different ploidy levels and presumably different genomic combinations (Table 3). As it was expected, the lowest (56.546  $\mu\text{m}$ ) and the highest (207.584  $\mu\text{m}$ ) TCL values were found in a diploid (*Ae. tauschii*) and a hexaploid species (*T. aestivum* with AABBDD formula) respectively; it must be mentioned that there is a big difference between the LT of *T. aestivum* (207.584  $\mu\text{m}$ ) and the other two hexaploids i.e., (*Ae. juvenalis*, TCL = 165.883 and *Ae. vavilovii*, TCL = 147.7). Based on these results it can be concluded that generally the chromosomal length in the genus *Aegilops* is shorter than that of *T. aestivum*. The highest chromosome length variation was found in *Ae. vavilovii* (XXDDSS) [SE (standard error) of MCL = 1.462  $\mu\text{m}$ ], and the lowest chromosome length variation was scored in *Ae. cylindrica* (SE of MCL = 0.817  $\mu\text{m}$ ) (Table 3). The ratio between the longest and the shortest arms (R) ranges from 1.48 in *Ae. cylindrica* to 2.07 in *Ae. vavilovii* (Table 3). Asymmetry Index (AsI%) ranged from the minimum 57.24 in *Ae. vavilovii* to the maximum 67.62 in *Ae. cylindrica* (Table 3).

Intra-specific length variation of short and long chromosome arms among the species- bearing the D genome showed that the coefficient of variability of short arms length was higher than that of the long arms length (Table 5); it can be interpreted that the TCL changes of the chromosomes is mainly influenced by the short arms rather than the long arms.

The degree of karyotype asymmetry as indicated by TF% values ranged between 31.58% (*Ae. cylindrica*) and 41.81% (*Ae. vavilovii*) (Table 3).

Three groups of the D genome-bearing species in the scatter diagram constructed based on A1 and A2 asymmetry indices (Fig. 2) were formed: (1) *Ae. cylindrica* with the most asymmetrical karyotype, (2) *Ae. vavilovii* with the most symmetrical karyotype and (3) a group of *Ae. tauschii*, *Ae. crassa*, *Ae. ventricosa*, *Ae. juvenalis* and *T. aestivum* with an intermediate place between the two latter groups. Inter- and intra-chromosomal asymmetry indices (A1 and A2 in Table 3) showed that *Ae. juvenalis* to be the most similar species to *T. aestivum*.

Based on the results of this study (the factors studied and the resulted asymmetry indices) *Ae. cylindrica* showed to be the most asymmetric karyotype (with the formulae of 1M + 6m + 3sm + 2st + 2t) among the species studied. Regarding the asymmetry indices observed in *Ae. cylindrica* it can be suggested that the karyotype asymmetry in this species is mainly affected by the place of the centromers rather than length of the chromosomes. *Ae. vavilovii* with the least chromosomal arm ratio variability, showed the most symmetric karyotype (with the formulae of 2M + 13m + 6sm)

Table 5. Coefficient of variability for intra-specific chromosome length variation of short and long arms for the D genome-bearing species of *Aegilops-Triticum* studied.

Chromosome arms	<i>Ae. tauschii</i>	<i>Ae. crassa</i>	<i>Ae. cylindrica</i>	<i>Ae. ventricosa</i>	<i>Ae. vavilovii</i>	<i>Ae. juvenalis</i>	<i>T. aestivum</i>
Short arm (in%)	10.27-18.82	5.88-10.42	2.02-12.34	4.58-12.35	2.32-7.39	1.93-7.25	3.02-6.92
Long arm (in%)	11.42-17.06	5.50-8.97	5.24-9.31	4.49-9.70	3.46-6.00	3.53-6.08	2.93-6.20

among the species studied. Regarding all the analyzed factors, a high similarity was found between *Ae. tauschii* (DD) and *Ae. crassa* (XXDD) (see Table 3). The high karyotype asymmetry similarity, as indicated by TF% and AsI% between *Ae. crassa* (XXDD; TF% = 40.06, AsI% = 57.74) and *Ae. vavilovii* (XXDDSS; TF% = 41.18, AsI% = 57.24), confirms the presence of two similar genomes (D and X) in common between these two species. The only other species possessing D and X genomes, *i.e.*, *Ae. juvenalis* (XXDDUU), showed no similarity regarding the degree of karyotype asymmetry with the above mentioned species.

As Romero Zarco (1986) has mentioned, karyotype asymmetry can be a good expression of the general morphology of karyotype in plants. According to the definition by Sharma (1990) symmetrical karyotypes are more primitive than asymmetrical ones; longer chromosomes than shorter ones; median centromeres with chromosome arms of equal length are more primitive than chromosomes with arms of unequal length; low basic numbers give rise to higher ones. Considering the above notions and the results of this study, it can be suggested that the asymmetric karyotypes observed within the D genome cluster species such as *Ae. cylindrica* are to be young. This suggestion is in accordance with the conclusion drawn by Badaeva, et al. (2002) who used C-banding and pAs1-FISH patterns, that *Ae. cylindrica* is a recently originated species. Based on the results of this study, *Ae. vavilovii* with the most symmetric karyotype, can be considered as the oldest relative to the hexaploid *Aegilops* species in the D genome cluster and also *T. aestivum*. This result is in accordance with Wang, et al. (1997) who based on the study of chloroplast and mitochondrial DNA variation suggested that *Ae. juvenalis* originated shortly before the other hexaploid species of the D genome cluster in *Aegilops*. However Badaeva, et al. (2002) believed that *Ae. juvenalis* is the oldest hexaploid among the respective species. In the same way, *Ae. crassa* showed a high symmetric karyotype (nearest species to the *Ae. vavilovii* in karyotype asymmetry; Table 3); suggesting a high chromosomal rearrangement event following the formation of this tetraploid species. Badaeva, et al. (2002) showed high genomic modifications in this species. Compare with its parental genomes, *Ae. ventricosa* with an intermediate karyotype asymmetry revealed some minor genomic modifications, that is in accordance with the observations of Dubcovsky, et al. (1994), Bardsley, et al. (1999) and Badaeva, et al. (2002) who noted minor differences between the *Ae. ventricosa* genomes and its progenitor species (*Ae. tauschii* and *Ae. uniaristata* Vis.).

Finally, the results of this study showed that the karyotype asymmetry analysis can be a good tool to reveal the genomic modifications and relative duration of allopolyploid formations in the D genome cluster. However, making any conclusion should be provided to considering the other genomic combinations involved.

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