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Research Article

Investigation of chromosome variation in four *Aegilops* L. (Poaceae) species and populations in Iran

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Abstract

The genus *Aegilops* is one of the most important genera of the Poaceae family. It belongs to the tribe Triticeae with 12 species in Iran. The research karyological analyzed in 12 populations of 4 *Aegilops* species: *A. umbellulata* Zhuk., *A. tauschii* Coss., *A. columnaris* Zhuk. and, *A. triuncialis* L. were done. We used fresh grown root tips. Then α-bromonaphtaline, formaldehyde and chromium trioxide (1:1), 1 N NaOH and hematoxiline were used for pre- treatment, fixative, hydrolyser and chromosome staining agent, respectively. We used video Analysis system for each species with Micromeasure software. All populations in the secondary basic numbers were x=7. The ploidy levels were different. In *A. tauschii* and *A. umbellulata* were found 2n=14, and *A. columnaris* and *A. triuncialis* were found 2n=28. Detailed karyotype analysis allows us to group the different species and to postulate relationships among them.

Keywords: *Aegilops*, Chromosome, Karyology, Poaceae, Populations
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Introduction

The genus *Aegilops* L. (Poaceae) is one of the wheat relatives with a wide distribution in Iran, and is capable of making different complexes with each other and with *Triticum* L. genus. It has 12 species in Iran (Boissier 1844; Al- Mashhadani 1980). *Aegilops* is a western Asia-Mediterranean element found around the Mediterranean Sea and the western part of Asia (Bor 1968). The life form of Aegilops species is annual with a mainly outcrossing breeding system (Waines and Barnhart 1992). But some of the species such as *A. tauschii* is a self-pollinating (cleistogamic) goat grass species in the Triticeae tribe of the grass family. This genus is a pastoral plant that consists of only one donor species to the gene pool of *Triticum* L. (Lange and Jochemsen 1992a) and causes the evolution of hexaploid wheat (Waines and Barnhart 1992). *Aegilops tauschii* encompasses four morphological varieties, of which three, var. *typica*, var. *anathera*, and var. *meyeri*, are grouped taxonomically into *A. tauschii* ssp. *tauschii*, while the fourth is the monotypic *A. tauschii* ssp. *strangulata* (Eig 1929; Kihara and Tanaka 1958).

The ploidy levels of *Aegilops* species are diploid (2n = 2x = 14, x = 7), tetraploid (2n = 4x = 28, x = 7), and hexaploid (2n= 6x = 42, x = 7) (Bor 1968; Lange and Jochemsen 1992a, 1992b). All the diploid species have rather limited areas of distribution, while the tetraploid and hexaploid have a wider ecological adaptation (Hammer 1980). The *Aegilops* genus has played a major role in the constitution of durum and bread wheat genomes. It possesses wild species along with native races (Lange and Jochemsen 1992a,

1992b). Iran is one of the centers of distribution and variation of *Aegilops* in the world. Due to the importance of cultivated wheat, having a better knowledge about new genetic resources is necessary to improve wheat races.

Percival (1926) and Kihara (1937) studied the morphology and cytology of some hybrids of *Triticum* and *Aegilops*. Senyaninova (1932) recorded karyo-systematical investigation of *Aegilops*. Chennaveeraiah (1960) studied karyomorphologic and cytotaxonomic in *Aegilops*. Karataglis (1975) studied karyotype analysis on some diploid native Greek *Aegilops* species. Al-Mashhadani et al. (1980) recorded Karyotype analysis of five tetraploid *Aegilops* species native to Iraq. Badaeva et al. (1994) showed intraspecific karyotype divergence in *Triticum araraticum*. Ahmadabadi et al. (2005) recorded Intraspecific Karyotype Divergence of *Aegilops triuncialis* in the Northwest Regions of Iran. Results indicate the presence of high genetic variation among the populations of *A. triuncialis* species. This variation can be useful in breeding programs of polyploid wheat and to broaden the genetic variability of the gene pool. Karimzadeh et al. (2010) studied cytogenetic of some Iranian Wild Wheat Species (*Aegilops*) and OR-Banding.

Generally, taxa will cross more readily when brought to the same ploidy level. Exceptions, however, are encountered were cross ability at uneven levels is more successful. The 2n number alone, however, is of little use in determining species relationship. For that purpose, karyotype studies may provide more information. In addition, we need to examine chromosomal variation further due to morphological and physiological variation among the same *Aegilops* species collected in different places of the world (Kihara and Tanaka 1958).

Chromosome information is an important key for taxonomy, phylogeny, evolution, genetic and breeding in *Aegilops* plants. Here we present the report of the chromosome numbers, ploidy levels and comparison of karyotypic traits of some annual species and population of *Aegilops* genus in Iran. Our results are useful for a better understanding of its taxonomy and breeding purposes such as inter and intraspecific hybridization and genetic variation induction.

Materials and methods

The studied about 12 populations of 4 *Aegilops* species: *A. columnar*, *A. tauschii*, *A. triuncialis* and *A. umbellulata* with three populations for each species. The studied populations are listed in (Table 1). Vouchers deposited in the Herbarium of the natural research center of Fars province and in gene bank RIFR (Research Institute of Forest and Rangelands) from Iran. The mature seeds of four taxa were taken from the herbarium materials.

Table 1. The examined populations of *Aegilops* genus, Gene bank code, and location.

No.	Population	Gene bank code (RIFR)	Location
1.	*A. columnaris*	4134	Golestan: Minoodasht (1050m)
2.	*A. columnaris*	4135	Golestan: Minoodasht (1280m)
3.	*A. columnaris*	16500	Mazandaran: Larijan
4.	*A. tauschi*	13938	Golestan: Gonbad-kavoos
5.	*A. tauschi*	16379	Golestan: Tuska-stan
6.	*A. tauschi*	16426	Mazandaran: Alashet
7.	*A. triuncialis*	16418	Tehran: Abali
8.	*A. triuncialis*	16577	Fars: Fasa, Mianjangal
9.	*A. triuncialis*	15368	Fars: Shiraz, Hosain abad station
10.	*A.umbellulata*	15594	Fars: Fasa, Mianjangal
11.	*A. umbellulata*	8762	Fars: Shiraz, Hossein abad station
12.	*A. umbellulata*	3771	Fars: Darab, Layzagan

We used root tip meristems from seedling obtained by the germination of ripe seeds on wet filter paper in Petri dishes and left at 22°C temperature. Different pre-treatments were tested, and the best results were obtained for treating the root tips meristems with 0.5% saturated α-Bromo naphthalene at 4°C for 4-5 h, fixed in 10% formaldehyde and chromium trioxide (1:1 volume ratio) for 16 to 20 h at 4°C. Then the root tips were rinsed for 3 h in distilled water. Hydrolysis was carried out with 1 N NaOH at 60ºC for 20-25 min and used hematoxylin-iron for chromosome staining for 1-2 h at room temperature. Root tips were Squashed in a droplet of 45% acetic acid and lactic acid (10:1) (Wittmann 1965; Hesamzadeh Hejazi and Rasuli 2006). The preparations were observed with an optical microscope (BH2 Olympus supplemented Digital color video camera) at a magnification of about 2000x. Chromosomal recounts were done in at least five complete metaphases and used to prepare the karyotype by Adobe Photoshop 7.0 software and measured by Micro Measure 3.3 software for each genotype (Reeves and Tear 2000).

In each mitotic metaphase (at least 5 plates), cytogenetic parameters were calculated as: long arm (LA), short arm (SA), total length (TL), the percent of relative length of each chromosome (RL%), arm ratio (AR), centromeric index (CI), value of relative chromatin (VRC). Karyotype asymmetry was estimated by three different methods namely: total form percentage (TF%), a difference of relative length (DRL), intrachromosomal asymmetry index (A_1) and intrachromosomal asymmetry index (A_2).

Both indices A_1 and A_2 in (Romero-Zarco 1986) independent of chromosome number and size. Karyotype symmetry was determined according to Stebbins (SC) (Stebbins 1971). Chromosomes were identified according to Levan (Levan et al. 1964). For each species, karyograms and haploid idiograms were drawn based on the mean centromeric index and arranged in order of decreasing size. In order to determine the variation between species, one – way analysis of variance (ANOVA) was performed to compare the chromosomes pair in each population by Duncan's test. Factor analysis based on principal components analysis (PCA) was performed on standardized karyological data of populations. Cluster analysis using Ward's method was performed after calculation of Cophenetic correlation coefficient (*r*) to examine karyotype similarity among populations. Statistical analyses were performed using SAS ver. 6.12, 1996, JMP ver. 3.1.2, 1995 and Statisti XL ver 1.7, 2007 softwares.

Results

There was no different basis chromosome among the species (x=7). The somatic chromosome numbers (2n), karyotype formulae and parameters for the studied species are summarized in Table 2. Among 12 populations, 6 populations were diploid belong to *A. tauschii* and *A. umbellulata* and 6 populations were tetraploid belong to *A. columnaris*, *A. triuncialis* (Table 2). The pictures of the mitotic metaphases and their karyograms of the populations were presented in Fig. 1.

The mean value of chromosome's long arm was varied from 6.05 µm in *A. triuncialis* (16418) to 4.42 µm in *A. tauschii* (16379). Averages of chromosome's short arm were different from 3.84 µm in *A. tauschii* (16426) to 1.82 µm in *A. triuncialis* (15368). The total length of the chromosome was varied from 9.37 µm in *A. tauschii* (16426) to 6.72 µm in *A. triuncialis* (15368) and the mean value of chromosome's arm ratio was in the range from 2.75 in *A. triuncialis* (16418) to 1.38 in *A. tauschii* (13938) (Table 4).

In all of the population, the chromosomes were mainly of m (metacentric) type or sm (sub-meta centric) type. Satellites were observed in one chromosomes pair in *A. umbellulata* species and for two species *A. columnaris* and *A. triuncialis* which have two chromosomes pairs having satellites (Fig. 1).

Symmetry type of Stebbins (1971) and asymmetry indices of Romero-Zarco (1986) are given in (Table 2). According to of Stebbins category, *A. tauschii* species (13938 and 16379) populations were placed in 1A class, and *A. tauschii* species (16426) was placed in class 2A. *A. columnaris* species (4134, 4135 and 16500) populations were placed in 4A, 3B and 3A respectively. *A. triuncialis* species (16418, 16577 and 15368 populations) are classified as

4A, 3A and 4A category respectively. Finally, *A. umbellulata* species (15594, 8762 and 3771) populations are classified as 3A, 4A and 4A group respectively (Table 2).

Table 2. Karyotypic characters of different *Aegilops* taxa and populations

Taxon (population)	2n	A_1	A_2	%TF	DRL	VRC	SC	K.F.
A. *columnaris (4134)*	4x=28	0.623	0.114	26.72	2.62	7.92	4A	9sm+5st
A. *columnaris (4135)*	4x=28	0.554	0.194	30.01	5.09	7.63	3B	2m+9sm+3st
A. *columnaris (16500(*	4x=28	0.604	0.115	27.51	2.72	7.10	3A	12sm+2st
A. *tauschii (13938)*	2x=14	0.274	0.114	42.07	4.69	8.56	1A	7m
A. *tauschii (16379)*	2x=14	0.281	0.116	41.03	4.86	7.69	1A	7m
A. *tauschii (16426)*	2x=14	0.302	0.114	40.39	4.52	9.52	2A	6m+1sm
A. *triuncialis (16418)*	4x=28	0.635	0.127	26.30	2.93	8.44	4A	9sm+5st
A. *triuncialis (16577)*	4x=28	0.611	0.111	26.98	2.43	7.68	3A	11sm+3st
A. *triuncialis (15368)*	4x=28	0.620	0.088	26.65	1.97	7.01	4A	11sm+3st
A. *umbellulata (15594)*	2x=14	0.556	0.103	30.43	4.06	7.54	3A	5sm+2st
A. *umbellulata (8762)*	2x=14	0.584	0.105	28.89	4.63	8.40	4A	7sm
A. *umbellulata (3771)*	2x=14	0.596	0.084	27.83	3.45	7.33	4A	6sm+1st

2n: Diploid chromosome numbers A_1: intrachromosome asymmetry index, A_2: interchromosome asymmetry index, TF%: total form percentage, DRL: difference of relative length, VRC: value of relative chromatin, symmetry classes (SC) of Stebbins and karyotype formula (K.F.).

Figure 1. Representative mitotic plates of *Aegilops* – (a) *Aegilops tauschii* (13938), 2n=2x=14, (b) *Aegilops tauschii* (16426), 2n=2x=14, (c) *Aegilops tauschii* (16379) 2n=2x=14, (d) *A. umbellulata* (15594), 2n=2x=14, (e) *A. umbellulata* (8762), 2n=2x=14, (f) *A. umbellulata* (3771), 2n=2x=14, (g) *A. columnaris* (16500), 2n-4x=28, (h*) A. columnaris* (4134), 2n=4x=28, (i) *A. columnaris* (4135), 2n=4x=28, (j) *A. triuncialis* (16577). 2n=4x=28, (k) *A. triuncialis* (16418), 2n=4x=28, (l) *A. triuncialis* (15368) 2n=4x=28.

According to the Stebbins bilateral table, the studied populations were symmetrically classified. In the species with 1A class, *A. tauschii* (13938 and 16379) possesses the lowest A_1 and the highest TF% values. In the species with the B classes all populations have the highest values of A_2 and DRL; therefore, all of them have more asymmetric karyotypes (Table 2).

The highest VRC amongst all populations was obtained for *A. tauschii* (16426) and the lowest was obtained for *A. triuncialis* (15368). Based on intrachromosomal asymmetry, some populations had the most asymmetrical and evolutionary karyotype (are classified as 4A). According to interchromosomal asymmetry, *A. columnaris* (4135) had the most asymmetrical karyotype in all of the populations. A statistical comparison based on completely randomized design demonstrates that there are significant differences among the populations for all the measured traits (P<1%) (Table 3). The principal component analysis (PCA), of the karyotypic traits shows that the first two components account for 88.18% of total variation. The first component (67.52 %) is positively correlated to the total chromosome length, short arm length, arm ratio, DRL, A_1 and TF% which had the highest coefficients of eigen vectors, while the second component (20.66%) accentuates

long arm length and A2 parameters (Table 5). Grouping of the populations are studied based on their relative karyotypic as well as mitotic characteristics (Table 4, Fig. 3). Cluster analysis based on the Average method was used to group the species and populations. The results showed that there was the minimum distance between two populations of *A. columnaris* (4134) and *A. triuncialis* (16577) and the populations classified under three groups which certainly the first and the second components had the most significant role in separated classes (Fig. 3).

The diagram of the populations' dispersion, based on two first components showed the populations separated in three groups, which completely fits with the results obtained through the average grouping analysis (Fig. 2).

Table 3. The results of variance analysis for karyotypic data based on CRD design

		Mean of squares								
S.O.V	D.F	TL	LA	SA	AR	CI	DRL	TF	A_1	A_2
Populations	11	2.71**	1.03**	2.23**	1.41**	0.02**	5.30**	191.85**	0.100**	0.003**
Error	48	0.62	0.30	0.07	0.03	0.0003	0.58	2.95	0.002	0.0003
%C.V.		9.99	10.31	10.48	7.66	5.48	5.83	5.48	7.97	8.39

**: Significant at 1%.

Table 4. Mean of chromosomes analysis of *Aegilops* populations

Populations	TL	LA	SA	AR	CI	DRL	%TF	A1	A2
A. columnaris (4134)	7.76 bc	5.64 ab	2.12 cd	2.67 ab	0.27 d	2.99 cd	26.72 d	0.61 ab	0.13bc
A. columnaris (4135)	7.46 bc	5.16 abc	2.30 cd	2.27 c	0.30 bc	5.38 a	29.97 bc	0.55 ab	0.20a
A. columnaris (16500)	6.91 bc	4.96 bc	1.95 cd	2.54 abc	0.28 bcd	2.91 cd	27.97bcd	0.59 ab	0.12bc
A. tauschii (13938)	8.56 ab	4.96 bc	3.60 a	1.38 d	0.42 a	4.73 ab	42.12 a	0.26 c	0.12bc
A. tauschii (16379)	7.57 bc	4.42 c	3.15 b	1.41 d	0.41 a	4.62 ab	41.40 a	0.27 c	0.11bc
A. tauschii (16426)	9.37 a	5.53 ab	3.84 a	1.44 d	0.40 a	4.78 ab	40.41 a	0.30d c	0.12bc
A. triuncialis (16418)	8.27 abc	6.05 a	2.22 cd	2.75 a	0.26 d	3.16 cd	26.19 d	0.62 a	0.14b
A. triuncialis (16577)	7.65 bc	5.56 ab	2.09 cd	2.66 ab	0.27cd	2.57 d	26.85 cd	0.60 ab	0.11bc
A. triuncialis (15368)	6.72 c	4.90 bc	1.82 d	2.69 ab	0.26 d	2.37 d	26.43 d	0.61 ab	0.10c
A. umbellulata (15594)	7.54 bc	5.24 abc	2.29 cd	2.29 c	0.30 b	4.05 abc	30.42 b	0.54 b	0.11bc
A. umbellulata (8762)	8.24 abc	5.81 ab	2.43 c	2.41 bc	0.29 bcd	4.91 ab	29.02 bcd	0.56 ab	0.12bc
A. umbellulata (3771)	7.14 bc	5.10 abc	2.04 cd	2.50 abc	0.28 bcd	3.71 bcd	28.05 bcd	0.58 ab	0.10c

TL: total length of chromosome, LA: long arm, SA: short arm, AR: arm ratio, CI: centromeric index, DRL: difference of relative length, TF%: total form percentage, A_1: intra-chromosome asymmetry index, A_2: inter-chromosome asymmetry index.

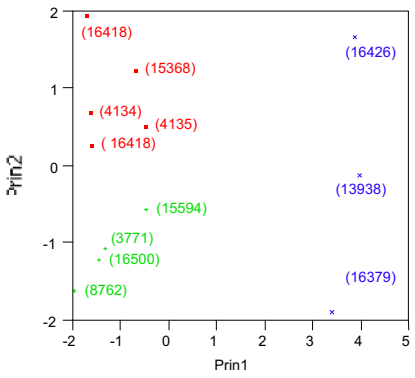

Figure 2. Scatter plot of 12 populations for the first two principals

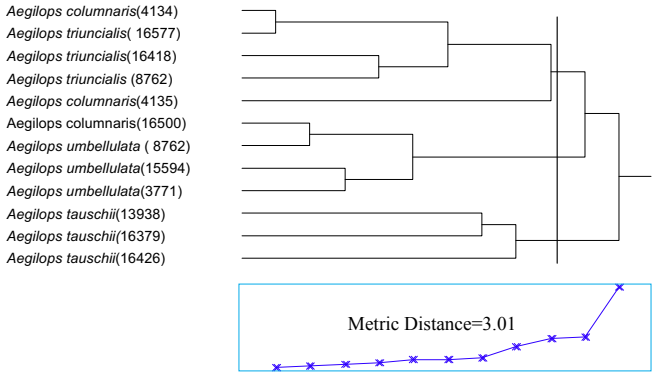

Figure 3. Dendrogram of 12 populations of *Aegilops* by analyzing nine karyotipic parameters using Ward 's cluster analysis method. Cophenetic correlation r=0.87

Table 5. Eigen vectors from the first two principal components for nine karyotype parameters to classify 12 populations of *Aegilops*

Parameters	First component	Second component
TL	0.25	**0.61**
LA	-0.17	**0.69**
SA	**0.41**	0.17
AR	**-0.43**	0.06
CI	**0.42**	-0.07
DRL	**0.34**	0.29
A1	**-0.43**	0.06
A2	-0.007	**0.32**
%TF	**0.42**	-0.07
Eigen Value	5.40	1.65
Percentage of Variance	67.52	20.66
Cum Percentage of variance	67.52	88.18

Discussion

The finding of this research reveals a detailed picture of the chromosome features in *Aegilops* species. Numerous reports, including those of (Bor 1968; Lange and Jochemsen 1992a, 1992b) have shown that the basis chromosome numbers for *Aegilops* genus no different basis chromosome among the different species (x=7) and ploidy levels are varied. In this study, twelve populations of four species of *Aegilops* possessed x=7 (2n=2x=14, 2n=4x=28).

Analysis of karyotype formulae showed that in *A. tauschii* species (2n=14) the number of "m" chromosomes is more than "sm" chromosomes, *A. umbellulata* species (2n=14) possessed the most "sm" type chromosomes. In tetraploid species, some species such as *A. columnaris* and *A. triuncialis* had the number of "sm" chromosomes more than "st" chromosomes.

At the interspecific level, quantitative and qualitative data allowed us the differentiation of several of the taxa studied. Among species, the most variable characters were the number of "m", "sm" and "st" chromosomes, as well as the number and position of satellites. (Fig. 1; Table 2). As a result, the species also could be differentiated by the number, type, and position of satellites.

Grouping of the populations based on taxonomic sections, confirmed the sections which introduced by Eig (1929) and Zhukovsky (1928).

The ratio of long arm /short arm chromosomes (AR) showed a high significant difference among some species belong to different sections, while other species are not clearly distinct (Table 4). Diploid species of *A. tauschii* (13938) for instance, had the lowest AR value (1.38), the highest TF% value (42.12) and the lowest A_1 value (0.26), exhibiting the most symmetrically karyotypes, while *A. triuncialis* (16418) with the highest AR value (2.75), the lowest TF% value (26.19) and the highest A1value (0.62) were introduced as the most asymmetrical karyotypes (Table 4). The pattern of variation of A_1 and A_2 values has been compared with the pattern of Stebbins' system in this study. In view of the fact that, fewer DRL value illustrated more symmetry of karyotype, *A. columnaris* (4135) and *A. triuncialis* (15368) respectively with DRL 5.38 and 2.37 values had the most symmetric and asymmetric karyotypes. Similarly, high DRL value leads to more changes in the construction of chromosomes.

The Duncan's test applied to the chromosome morphometric traits (LA, SA, TL, AR, DRL, TF%, A_1 and A_2) showed a highly significant difference among all examined populations belongs to different sections (Table 4).

The study revealed cytogenetic differences ($P<1$ %) in ANOVA for the karyological date as well as the ratio of long arms to short arms among diploid and tetraploid populations. So

these results indicate a significant quantitative change in the amount of chromatin in *Aegilops* species diversification (Table 2 and Table 3).

Considering the changes of interchromosomal asymmetry index (A_2) among diploid and tetraploid species, the lowest value exists in the diploid and some tetraploid species and the highest value only exists in the tetraploid species (*A. columnaris*) (Table 4).

Cluster analysis based on cytological data showed the populations with the lowest metric distance may lead us to use populations in crosses for inducing the highest genetic variations (Fig.3). However, the grouping of the *Aegilops* populations based on karyotypic data, partly agrees with either the taxonomic treatment of the genus *Aegilops* (Eig 1929 and Zhukovsky 1928) or phylogenetic analysis of the same species based on morphological characters.

Different populations of all species are classified as the same group except for *A. columnaris* (16500) majorly because of their different chromosome long arm and their DRL index. The present study shows the change in the chromosomal traits as one of the mechanism of inter and intraspecies diversification in the *Aegilops* genus as well as the earlier cytological reports. The differences in karyotype formulae and asymmetric indices found among the species suggest that structural changes of chromosomes may contribute to the diversification of the genus. These genomic differences could be used for breeding purposes. In general, cytological studies of the *Aegilops* species growing in Iran indicate the importance of polyploidy, chromosome structural changes, presumably quantitative changes in the amount of DNA and probably the role of growing sites in species diversification and suggest that such data may be used in the taxonomy and phylogenetic consideration of the genus.

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