

DISTRIBUTION AND CHARACTERIZATION OF *AEGILOPS CYLINDRICA* HOST FROM IRAN

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Abstract

Jointed goatgrass (*Aegilops cylindrica* Host; $2n = 4x = 28$, C^cC^cD^dD^d) is a tetraploid remote relative of bread wheat (*Triticum aestivum* L; $2n=6x=42$, AABBDD) with two genomes and 28 chromosomes. The diversity center of this species is in the Fertile Crescent and in central Asia and could also be found in many places in Iran. In this experiment, 359 accessions provided by National Plant Gene Bank of Iran (NPGBI) were used. Based on the geographical distribution, the highest distribution of *A. cylindrica* is found in North, West and North-West regions of Iran. The data on the distribution of *A. cylindrica* showed that its distribution centers in Iran are more than those reported in the previous studies. Chromosome counting showed that all *A. cylindrica* accessions are tetraploid ($2n=4x=28$). Results of factor analysis for nine morphological chromosome traits showed that karyotypic variation within accessions are related to the length of chromosomes and there is difference between the accessions for their total chromosome length, but the karyotype of different accessions are almost the same for the symmetry. Low coefficient of variation in morphological traits as well as symmetric karyotypes of *A. cylindrica* species observed in this study could lead us to predict that *A. cylindrica* could be a recently evolved species among the remote relatives of bread wheat.

Introduction

Jointed goatgrass, originated from two species, is native to the Mediterranean, Middle East, Asia, and was also introduced to the Great Plains and the Pacific northwest of the United States (Kimber and Feldman, 1987; van Slageren, 1994). It is a winter annual grass weed that infests over three million ha of winter wheat in the Pacific Northwest and Great Plains regions of the USA (Dewey, 1996). It reduces winter wheat yields by interference and lowers harvested grain quality. Average yield loss due to *Aegilops cylindrica* infestations has been estimated to be 25% (Anderson, 1993; Donald and Ogg Jr, 1991). It has also been estimated that the economic cost of *A. cylindrica* to winter wheat producers in the western United States is \$145 million annually (Ogg, 1993). Jointed goat grass and winter wheat are closely related. Therefore, the development of selective herbicides to control this weed in winter wheat has been problematic.

A. cylindrica is a bushy type plant with 20-40 cm long culms. It is characterized by narrow, 4-5 cm long, and glabrous to sparsely hairy leaves, a narrow, lanceolate and 6-9 cm long spike, almost ended with two incomplete spikelets. Each spike consists of 6-11 spikelets and breaks off

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entirely or disintegrates into segments at maturity. Each spikelet holds one to three seeds that are reddish-brown in color and reach maturity in mid-summer, Fig. 1).

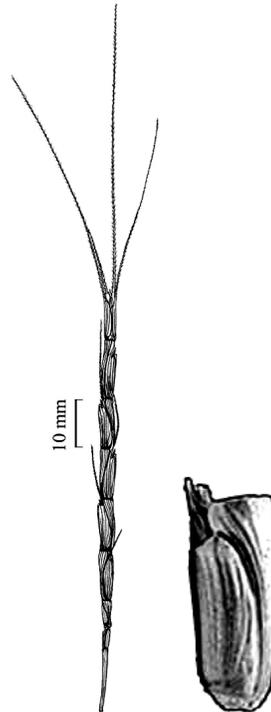


Fig. 1. Spike and spikelet of *A. cylindrica*.

The genomic constitution of *A. cylindrica* was determined by the analyses of chromosome pairing, storage proteins, isozymes, and differences in restriction length patterns of repeated nucleotide sequences (Linc *et al.*, 1999). Linc *et al.* (1999) identified the diploid species *A. caudata* L. ($2n=2x=14$, CC) and *A. tauschii* Coss. ($2n=2x=14$, DD) as the donor of the C and the D genome of *A. cylindrica*, respectively. Previously, the cytoplasm of *A. cylindrica* was shown to be contributed by *A. tauschii* (Maan, 1976; Tsunewaki, 1989) but, more recent analysis with chloroplast microsatellite markers has shown that both *A. tauschii* (D-type cytoplasm) and *A. markgrafii* (Greuter) K. Hammer. (C-type cytoplasm), now a synonym of *A. caudata*, have contributed their cytoplasm to *A. cylindrica* (Gandhi *et al.*, 2005). High genome homology shared between *A. cylindrica* and its progenitor species and low intra-species polymorphism in *A. cylindrica* indicated it as a new species with little chromosome changes. The D genome chromosomes of *A. cylindrica* species are more similar to *A. tauschii* biotypes and D^{cr2} genome of hexaploid cytotype of *A. crassa* Boiss. than D genome of bread wheat (Badaeva *et al.*, 2002; Caldwell *et al.*, 2004). These results indicate that there are different versions of D genomes for both *A. cylindrica* and *T. aestivum* L. species.

A. cylindrica has wide distribution from Western Europe to East Asia and even North America. The diversity center of this species is in the Fertile Crescent and in central Asia and could also be found in many places in Iran (van Slageren, 1994). *A. cylindrica* has spreaded westward to Greece, Bulgaria, Romania, Kosovo, Montenegro, Serbia, and Hungary. Northwards,

it is distributed in the Caucasus region and along the Black Sea coast. Though rare, this species is also present in the western arc of the Fertile Crescent involving Lebanon, Jordan, Syria, northern Iraq, and northwestern Iran (van Slageren, 1994).

The geographic distribution of *A. cylindrica* encompasses and extends beyond areas, where its diploid progenitors, *A. tauschii* and *A. markgrafii* can be found (Fig. 2).

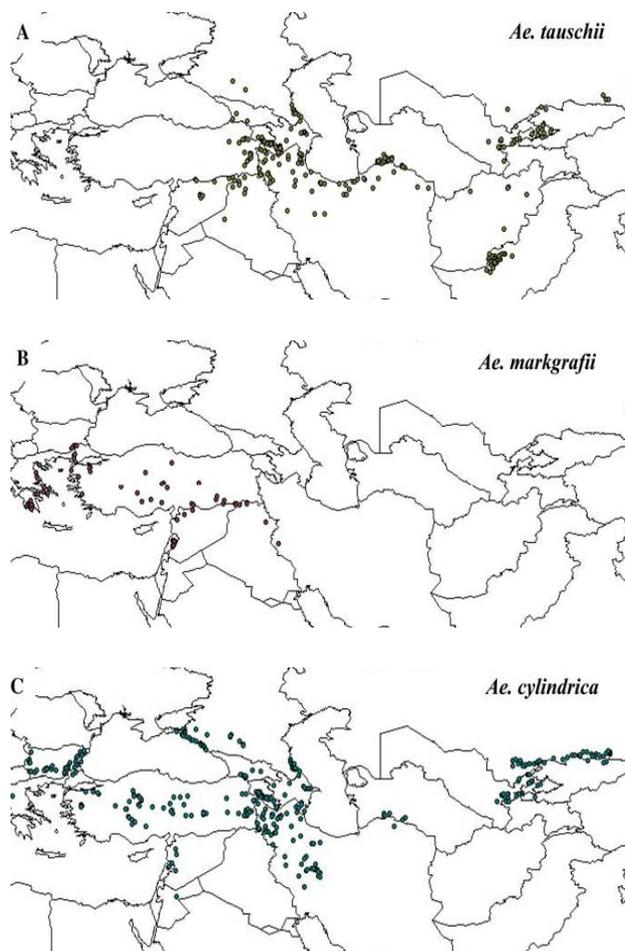


Fig. 2. Maps showing the distribution of collections of *A. tauschii* (A), *A. markgrafii* (B), and *A. cylindrica* (C). The geographic coordinates were obtained from the system-wide information network for genetic resources (SINGER; <http://singer.cgiar.org/>).

Throughout its range of distribution, *A. cylindrica* is considered as a weedy species, particularly in common wheat fields, where it chronically infests fields in the Mediterranean, the Middle East, Europe, and the United States of America (Dewey, 1996; Ogg and Seefeldt, 1999; van Slageren, 1994). Jointed goatgrass has also been suggested as a source of genetic variation for wheat improvement (Bouhssini *et al.*, 1998; Farooq *et al.*, 1992; Iriki *et al.*, 2001) because it is a close relative of common wheat and both species carry the D genome donated by *A. tauschii* (Kimber and Zhao, 1983; Riley and Law, 1965). In addition, natural hybridization between wheat

and jointed goatgrass suggests a potential for gene flow between these species under field conditions (Gandhi *et al.*, 2006; Zemetra *et al.*, 1998). Thus, there is considerable interest in understanding various aspects of the evolution of *A. cylindrica* for its better management and use. The D genome of hexaploid wheat has been shown to be more closely related to the D genome of *A. tauschii* subsp. *strangulata* (Eig) Tzvelev than to *A. tauschii* subsp. *tauschii* Coss. (Dvorak *et al.*, 1998; Lubbers *et al.*, 1991; Pestsova *et al.*, 2001), whereas the D-type plastome and the D genome of *A. cylindrica* are more closely related to *A. tauschii* subsp. *tauschii* than to *A. tauschii* subsp. *strangulata* (Gandhi *et al.*, 2005).

Although molecular genetic diversity and ploidy level of *A. tauschii* has been reported before (Bakhshi *et al.*, 2010; Levan *et al.*, 1964), no extensive study has been done to identify cytogenetic and morphologic characteristics of this species. We have collected many accessions from different regions of Iran that it could be remarkable to identify potential genetic diversity among these accessions. In this study, we analyzed chromosomes features of *A. cylindrica* as well as distribution and morphological characteristics of this species which is widely distributed in Iran. Since this species is a relative of bread wheat it is important to identify available genetic diversity in this species to be used in necessary condition in bread wheat breeding programs.

Materials and Methods

Plant material: 359 accessions were used in this experiment, which were provided from National Plant Gene Bank of Iran (NPGBI). These accessions were collected from sixteen provinces of Iran *viz.*, West Azarbaijan, East Azarbaijan, Ardebil, Zanjan, Qazvin, Kurdistan, Hamedan, Kermanshah, Ilam, Lorestan, Chaharmahal Bakhtiari, Mazandaran, Tehran, Esfahan, Semnan and Khorasan. A total of 23 traits were evaluated, 16 of which were qualitative and seven were quantitative. Evaluation of all traits was conducted using three replications of each accession. The mean and mode was calculated for quantitative and qualitative traits, respectively. Estimated statistical parameters traits were calculated for quantitative traits. Shannon and Weaver diversity index were calculated for measuring qualitative traits. Non-standard values of diversity index (Hc) and standard diversity index (SDI) was calculated as follows (Hennink and Zeven, 1990):

$$Hc = - \sum_{i=1}^n pi \log_e pi \quad SDI_e = Hc / \log_e n$$

In this formula, for certain traits, such as c, n, including the number of phenotypic classes and Pi is equal to the frequency of bushes.

Chromosome counting: Following the technique, developed at International Maize and Wheat Improvement Center (CIMMYT) institute (Mujeeb-Kazi and Miranda, 1985), root tips were collected between 9 AM to 10:30 AM, and then placed in a Petri dish, on a filter paper moistened with α -bromonaphthalene pre-treatment solution. The samples were pre-treated about 2.5 to 3.5 hours, but generally 3 hrs as pre-treatment time was used, that resulted satisfying chromosome contraction and high mitotic index. After pre-treatment, the root tips were transferred to vials, containing 0.2% aceto-orcein and refrigerated (4°C) until they were being used. Afterwards, the root tips were transferred to 2% aceto-orcein, in order to intensify the staining for 2 days before squashing. After staining the aceto-orcein was removed from the vial and 45% acetic acid was added to fill about a quarter of the vial. Vial was heated over a flame to bring the contents to a slow boiling. After boiling, the vial contents (45% acetic acid + root tip) were transferred into an evaporating dish.

A root tip was taken from it and placed over on filter paper to remove extra acetic acid. Apical root tip measuring 2-2.5mm was cut and placed on dry microscope slide. The root tip was squashed by an arrow-head needle, and a small drop of 45% acetic acid was quickly added to the squashed tissue. The slide was then slightly warmed and a cover glass was placed gently over the macerated cellular area. The cover glass slides were gently dabbed with coarse filter paper, the slide was heated slightly, placed between folded filter paper on a flat surface and thumb pressure applied directly to the cover glass. After squashing, the slide was suitable for observing chromosomes by microscope.

Karyotype preparation: In order to prepare karyotypes of *A. cylindrica*, 12 accessions of *A. cylindrica* were used (Table 1).

Table 1. Accessions of *A. cylindrica* used in karyotype study.

Province	City	Accession No.	Longitude		Latitude	
			Seconds	Degree	Seconds	Degree
West Azarbaijan	Naghadeh	50	45	22	36	57
Lorestan	Borujerd	96	57	20	37	28
Zanjan	Zanjan	363	48	29	36	40
Kermanshah	Songhor	286	47	34	36	47
East Azerbaijan	Maragheh	406	46	16	37	24
East Azerbaijan	Hashtrud	408	47	4	37	28
Ilam	Shirvan	312	46	34	33	46
Ardabil	Ardabil	332	48	17	38	15
Kermanshah	West Islamabad	379	46	32	34	7
East Azerbaijan	Urmia	45	45	2	37	32
Kermanshah	Javanrud	381	46	22	35	3
Hamadan	Hamadan	393	48	31	34	48

Study of karyotypes: Chromosomes were named according to the location of centromere in the chromosome (Levan *et al.*, 1964). In this study, comparison between karyotypes in different accessions of a species was performed by comparing their symmetry. The Stebbins method (Stebbins, 1971) has been used for determining the degree of symmetry. Additionally total form percentage index (TF) (Forni-Martins, 1994), the relative percentage of the longest chromosome to the shortest chromosomes (S) (Bennardello, 1994), coefficient chromosomal length variations (CV) (Sheidai *et al.*, 1996, the average ratio of long to short arm (R) (Bennardello, 1994) and range of chromosome length variation (V) (Datta and Agarwal, 1992) were calculated. Evaluation of karyotype evolution was calculated using Dispersion Index (DI) to show a few differences that were not visible in Stebbins indicators (Lavana and Srivastava, 1999). Factor analysis for morphological features of chromosomes based on principal component analysis and Varimax rotation was also conducted. For measuring different parts of the chromosomes and analyzing morphological data of chromosomes, Micromeasure software (Reeves, 2001) and the SPSS software were used, respectively.

Results and Discussion

Geographical distribution of *A. cylindrica* accessions in Iran: Investigation on collecting location and geographical distribution of *A. cylindrica* accessions reveals that this species predominantly grows in the range of 800 to 2000 meters altitude. Thus this species is adapted to mountainous ecosystems and not to low altitude ecosystems of Caspian shores, southern shore,

Khuzestan and Ilam. The results of the geographical distribution using ILWIS software also showed that the highest distribution of *A. cylindrica* was in north, west and north-west regions, including East Azarbaijan, West Azarbaijan and Kermanshah provinces, contrasting to that of the southern and southeast regions which showed the lowest distribution (Fig. 3). However, it may be found anywhere on the mountainous areas of Alborz and Zagros. Some populations could even be found on briny margins of Urmialake and Semnan, Northern Khorasan and around Qom. Whereas, *A. cylindrica* doesn't grow on salty deserts of central and southern Iran. While, it is abundant in central Iranian land, *A. cylindrica* is usually found in the more elevated northern strip of the central Iranian deserts and not in the more arid region of southern and central areas.

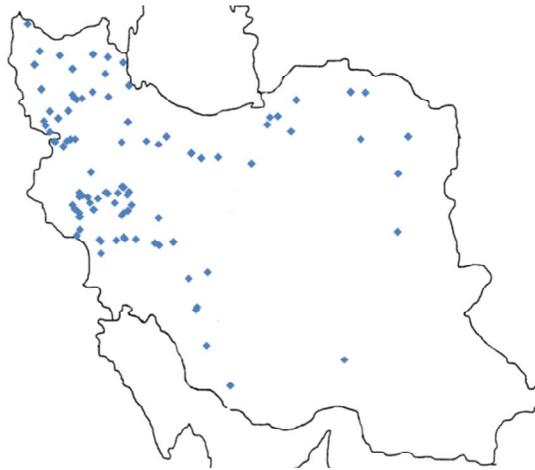


Fig. 3. Geographical distribution of *A. cylindrica* in Iran.

The present investigation on the geographical distribution of *A. cylindrica* showed that the distribution centers are more than that reported in a previous study conducted in Iran (Gandhi *et al.*, 2005). In the present study, in addition to north, west and northwest, northeast and southwest of Iran have also been identified as distribution centers for this species. The results also showed that this species is mostly present in mountain ecosystems and it is not found at low altitude ecosystems such as the margins and the southern coast ecosystems of Caspian Sea. Previous studies indicated that the evolution of bread wheat occurred in high altitudes of Caspian Sea ecosystems (Jaaska, 1981; Nakai, 1978). On the other hand, the results of this study showed that the *A. cylindrica* species has a wide distribution in this region. With this explanation, the possibility of challenging unproven hypothesis that *A. cylindrica* had a potential to be as a donor of hexaploid wheat D genome (Asghar *et al.*, 2001) might be more acceptable.

Statistical parameters for the traits of *A. cylindrica* species: Rachis width, spikelet seed number, plant height, kernel width and leaf number of rachis traits showed the highest phenotypic coefficient of variation, with 13.14, 11.33, 10.85, 10.43 and 10.11 percent, respectively. Most of these traits have been also observed among traits with high diversity in other collected accessions in Iran including *A. tauschii* and *A. crassa* (Aghaei *et al.*, 2008; Ranjbar *et al.*, 2007). Furthermore, maturity date, spikelet length and length of spikelet glume indicated the lowest phenotypic coefficient of variation with 4.80, 5.02, and 5.61 percent. Most standard deviations were also related to plant height, flowering date and maturity date and the lowest standard deviation were related to rachis width, spikelet width and width of spikelet glume (Table 2). White and flour form kernel, brown and glabrous glumes, moderate

fragility spikes, brown stamens and standing bushes were predominantly observed in the research field. Furthermore, growth habit showed the most variation using non-standard and standard diversity index of Shannon-Weaver (Peet, 1974). Thus, according to Shannon-Weaver Diversity index, growth habit could be introduced as the most effective qualitative trait to distinguish *A. cylindrica* populations (Table 3). Relatively low level of phenotypic variation coefficients for different traits were obtained in this study showing that this species is relatively new. However, high genetic diversity has been observed for *A. tauschii* in northern area of Iran (Aghaei *et al.*, 2008).

Table 2. Statistical parameters for quantitative traits evaluated in the collection of *A. cylindrica*.

	Mean	Mod	Middle	Minimum	Maximum	Range of variation	Standard deviation	Variance	Coefficient of variation
Plant height	59.67	59.00	60.33	38.33	89.67	51.33	6.47	41.88	10.85
Flowering date	65.12	67.00	66.00	58.00	93.00	35.00	4.34	18.82	6.66
Maturity date	89.23	87.00	88.00	58.00	98.00	40.00	4.28	18.32	4.80
Leaf number of rachis	3.46	3.33	3.33	2.67	4.33	1.67	0.35	0.12	10.11
Spikelet seed number	2.04	2.00	2.00	1.33	3.00	1.67	0.23	0.05	11.33
Spikelet number/spike	9.28	9.00	9.33	6.67	11.67	5.00	0.85	0.73	9.18
Node number of rachis	2.92	3.00	3.00	2.00	3.67	1.67	0.21	0.04	7.14
Kernel width	7.08	6.83	7.07	5.47	9.10	3.63	0.52	0.27	7.39
Spike length	8.65	9.00	8.67	6.00	10.33	4.33	0.74	0.54	8.53
Spikelet length	11.72	11.73	11.73	8.20	14.47	6.27	0.59	0.35	5.02
Length of spikelet glume	9.28	10.17	9.83	8.23	11.23	3.00	0.55	0.30	5.61
Kernel width	2.33	2.40	2.33	1.63	5.63	4.00	0.24	0.06	10.43
Spikelet width	2.69	2.67	2.67	2.27	3.63	1.37	0.17	0.03	6.51
Width of spikelet glume	2.88	2.87	2.87	2.40	4.27	1.87	0.19	0.04	6.70
Rachis width	1.27	1.17	1.27	0.83	1.90	1.07	0.17	0.03	13.14
Spike width	2.82	2.83	2.80	2.30	4.30	2.00	0.22	0.05	7.67

Table 3. Statistical parameters for qualitative traits evaluated in the collection of *A. cylindrica*.

	Mod	Range of variation	Minimum	Maximum	Standard diversity index	Non-standard diversity index
Kernel tissue	3	0	3	3	0	0
Stamen color	3	2	1	3	0.10	0.17
Kernel color	3	0	3	3	0	0
Glume color	2	0	2	2	0	0
Spike-axis fragility	3	2	3	5	0.04	0.07
Grows habit	1	4	1	5	0.58	0.92
Glume hairs	1	6	1	7	0.06	0.11

Karyotype analysis of accessions: Chromosome counting showed that most of *A. cylindrica* accessions are tetraploid ($2n = 4x = 28$). Furthermore, cytogenetic studies showed no aneuploid and B chromosome, but difference in chromosome length. Collecting place and accession numbers are

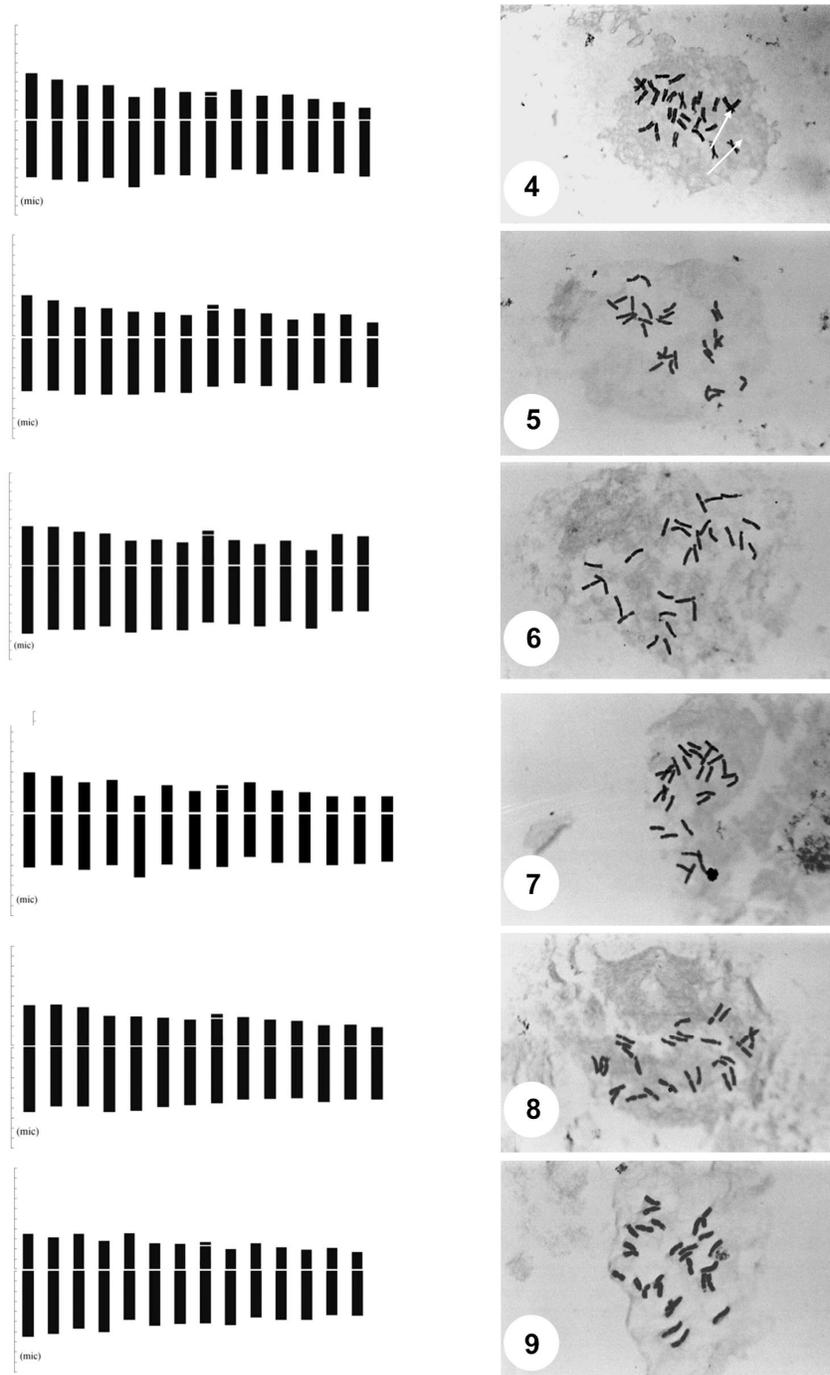
presented in Table 1 and chromosomes count, satellites count in karyotype and karyotypic formulae are shown in Table 5. The metaphase cell and ideogram of various accessions are presented in Figs. 4-15. All investigated accessions had a satellite in the short arm of chromosomes no. 8. The presence of satellite in the same pair of chromosomes is also reported (Karatagliis, 1989). Considerable variation was observed in total length of chromosomes (TLC) and the average length of chromosomes (C). High variation in length of chromosomes may be a sign of genome adaptation of this species to those places from where they have been collected. The largest chromosome (11.33 micrometer) was found in Zanjan accession and the smallest chromosome (4.87 micrometer) in Urmia accession and both of them were sub-metacentric. Maximum and minimum of long arm to short arm ratio was observed in Islam Abad Gharb (2.129) and in Shirvan (1.78) accessions, respectively. For total form percentage index (TF), the maximum (35.32) and minimum (31.18) TF was observed in Ardabil and Zanjan accessions, respectively. This data shows that karyotypes of Ardebil and Zanjan accessions have the highest and the lowest symmetry. The highest coefficient of variation (CV) was found in accessions from Naghadeh and the lowest in the Javanrud accessions. All of the accessions that were collected from the northwest and the west of Iran, belonged to A2 position of the Stebbins Table, indicating a relatively symmetrical karyotype for recently evolved species with short evolutionary history. Also, distribution index of chromosome (DI) showed that the Hashtrud accession had the highest DI (7.43) and Zanjan accession had the lowest DI (18.5). This observation indicated that the Hashtrud accession had the highest symmetry in contrast to Zanjan accession. DI could be more reliable than other indicators because three important karyotypic criteria, including variation in chromosomes length, centromere position and relative size of chromosomes, are involved in DI calculation (Table 6).

Table 5. Collecting place, chromosomes count, satellites count and karyotypic formula of *A. cylindrica*.

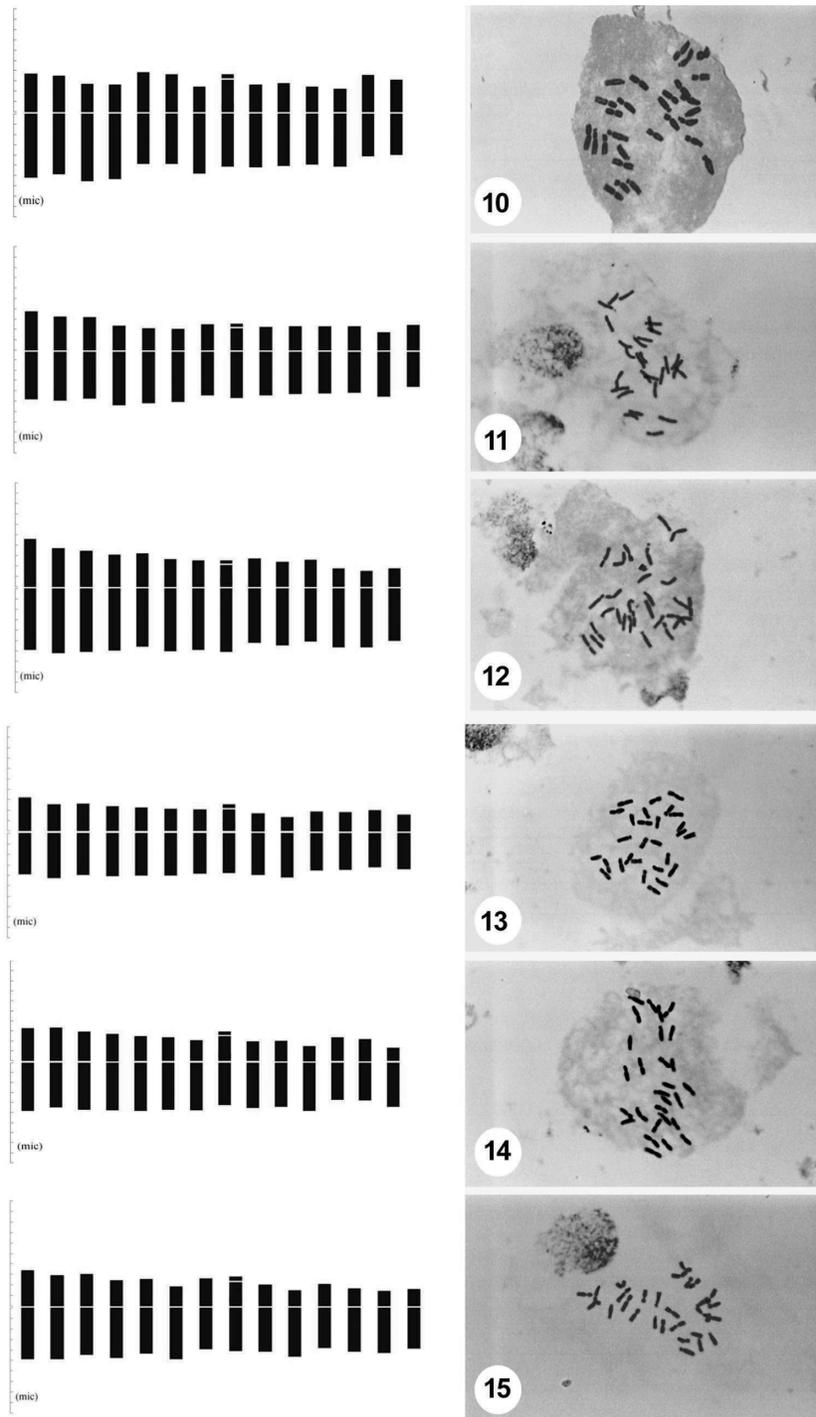
Collecting place	Chromosomes count	Satellites count	Karyotypic formula
Naghadeh	28	1	2m* + 6sm* + 6st*
Borujerd	28	1	1m + 8sm + 5st
Zanjan	28	1	2m + 6sm + 6st
Songhor	28	1	4m + 4sm + 6st
Maragheh	28	1	2m + 9sm + 3st
Hashtrud	28	1	1m + 9sm + 4st
Shirvan	28	1	5m + 8sm + 1st
Ardabil	28	1	3m + 9sm + 2st
West Islamabad	28	1	3m + 6sm + 5st
Urmia	28	1	1m + 12sm + 1st
Javanrud	28	1	1m + 10sm + 3st
Hamadan	28	1	2m + 7sm + 5st

* m; Metacentric chromosome, sm; Submetacentric chromosome and st; Subtelocentric chromosome.

Range of chromosome length variation, total length of chromosome, average of chromosome length, total length of chromosomes (TLC), centromere index, and standard deviation of chromosome length traits had the largest factor coefficients in the first factor. Because most of these traits depend on the chromosomes length, this factor is named length of chromosomes. This factor presents 65.83 percent of the total variance that shows there is great diversity for traits related to chromosome length among accessions. In the second factor, CV of chromosome length, minimum of short arm to long arm ratio percentage and distribution index



Figs 4-9: 4. Metaphase chromosomes picture and ideogram of Naghadeh population. *white flash shows satellites. 5. Metaphase chromosomes picture and Ideogram of Broujerd population. 6. Zanzan population. 7. Songhor population. 8. Maragheh population. 9. Hashtroud population.



Figs 10-15: 10. Metaphase chromosomes picture and ideogram of Shirvan population. 11. Ardebil population. 12. Islam Abad Gharb population. 13. Orumia population. 14. Javanroud population. 15. Hamedan population.

The results of factor analysis of 9 chromosomal morphological traits showed that the first two factors had Eigen values greater than one with 65.83 to 88.39 percent variance and these two factors were responsible for diversity in the accessions (Tables 7-8). traits had the largest

Table 6. Karyological characteristics of *A. cylindrica* accessions.

Collecting place	Stebins Index	CV of chromosome length	Distribution index of chromosome	Centromere index	Minimum of short arm to long arm ratio percentage	Total length of chromosomes (TLC)	Av. of short arm to long arm ratio	Av. of chromosome length	Total length of chromosome	Range of chromosome length variation
Naghadeh	2A	13.48	6.46	0.32	65.01	32.36	2.09	8.68	121.61	3.77
Borujerd	2A	12.09	5.84	0.32	66.51	32.54	2.07	7.57	105.97	3.12
Zanjan	2A	11.45	5.19	0.31	68.42	31.19	2.20	9.22	129.16	3.57
Songhor	2A	11.90	5.65	0.32	67.77	32.21	2.10	7.46	104.46	2.95
Maragheh	2A	12.84	6.41	0.33	67.13	33.31	2.00	8.43	118.12	3.41
Hashtrud	2A	15.06	7.44	0.33	60.76	33.07	2.02	7.70	107.88	3.87
Shirvan	2A	12.88	7.25	0.23	65.38	32.19	1.78	9.13	127.89	3.89
Ardabil	2A	11.19	6.12	0.35	69.74	35.33	1.83	6.76	94.64	2.49
West Islamabad	2A	12.99	6.10	0.31	64.19	31.96	2.13	8.27	115.87	3.71
Urmia	2A	11.15	5.93	0.34	69.07	34.73	1.88	5.85	81.94	2.18
Javanrud	2A	11.04	5.62	0.33	69.75	33.75	1.96	6.76	94.68	2.43
Hamadan	2A	12.98	6.61	0.33	65.82	33.71	1.97	6.45	90.38	2.77

Table 7. Eigen values, percentage of variance and cumulative variance factor.

Factors	Eigen values	Percentage of variance	Cumulative variance factor
1	5.93	65.83	65.83
2	2.03	22.56	88.39

Table 8. The first two factors derived from factor analysis for morphological traits of chromosomes.

Traits name	Factors	
	1	2
Range of chromosome length variation	0.822	0.554
Total length of chromosome	0.970	0.138
Average of chromosome length	0.970	0.138
Total length of chromosomes (TLC)	-0.906	-0.023
Minimum of short arm to long arm ratio percentage	-0.284	-0.910
Centromere index	-0.713	-0.187
Standard deviation	0.806	.0568
CV of chromosome length	0.244	0.947
Distribution index of chromosome	-0.007	0.923

factor coefficients. Thus, the second factor was named as karyotype symmetry due to all of these traits indicated karyotype symmetry of the accessions. The second factor accounted for 22.55 percent of the total variance, indicating that there was no great difference between accessions in terms of symmetry and confirming the placement of accessions at the 2A position of the Stebbins table. Therefore, the results of factor analysis show that karyotypic variation

within accessions is related to the length of chromosomes and there is difference between accessions for their total chromosome length; but the karyotype of different accessions are same for their symmetry and they are relatively symmetrical. As morphological studies were conducted, low coefficient of variation coupled with symmetric karyotype indicates *A. cylindrica* as a recently evolved species. *A. cylindrica* diversity centers are mostly located in the Northwest regions where the highest numbers of collection sites are distributed. We also observed that *A. cylindrica* accessions of Iran should be treated as a recently evolved species due to low diversity in morphological traits and symmetric karyotypes.

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