Karyological studies in *Triticum monococcum* subsp. *aegilopoides* and *Aegilops cylindrica* species grown wild pairwise in west Iran

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Abstract

The wild-relative gene pools of wheat are a rich source of genetic variation for wheat improvement. Karyotypes of eight genotypes of *Triticum monococcum* subsp. *aegilopoides* and eight genotypes of *Aegilops cylindrica*, which grown wild next to each other in different regions in the west of Iran were studied. Root tips were treated in α-bromonaphthalene solution, fixed in chromic acid-formaldehyde fluid, hydrolyzed in 1 N NaOH and staining with hematoxylin. Variation observed in karyotypic formulas of *A. cylindrica* showing some structural changes of the chromosomes. *Aegilops cylindrica* classified in 3A class and *T. m.* subsp. *aegilopoides* classified in 1A class of Stebbin's classification. *Triticum monococcum.* subsp. *aegilopoides* has been suggested to be a relatively old origin because possessing metacentric chromosomes while submetacentric and subtelocentric chromosomes in *A. cylindrica* suggest a relatively recent origin of the species. The karyotypic symmetry indices used in this study pointed to a much more asymmetric karyotype of *A. cylindrica* than *T. m.* subsp. *aegilopoides.*

Keywords: Chromosome, karyotype, mitosis, wheat, wild relative species

بررسی کاریولوژی گونه‌های *Triticum monococcum* subsp. *aegilopoides* و *Aegilops cylindrica* رشد یافته مجاورتی وحشی در غرب ایران*

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خلاصه

خویشاوندان وحشی گندم منبع غنی از تنوع ژنتیکی برای اصلاح گندم می‌باشند. کاریوتیپ هشت ژنوتیپ اینکورن وحشی و هشت ژنوتیپ *Aegilops cylindrica* که به صورت طبیعی در مجاورت یکدیگر در نواحی مختلف غرب ایران می‌رویند، مورد مطالعه قرار گرفت. نوک ریشه‌ها در محلول آلفابرومونفتالین پیش تیمار، در محلول فرمالدهید- کرومیک اسید تثبیت، در سدیم هیدروکسید یک نرمال هیدرولیز و در هماتوکسیلین رنگ‌آمیزی شدند. در فرمول‌های کاریوتیپی *A. cylindrica* تنوع مشاهده شد که نشان‌دهنده تغییرات ساختاری کروموزوم‌ها است. *Aegilops cylindrica* در کلاس 3A و اینکورن وحشی در کلاس 1A استبینز طبقه‌بندی شدند. از لحاظ تکاملی، گونه *Triticum monococcum.* subsp. *aegilopoides* به دلیل داشتن کروموزوم‌های یکنواخت متاسنتریک جزو گونه‌های متقارن و ابتدایی و گونه *A. cylindrica* با داشتن کروموزوم‌های ساب متاسنتریک و ساب تلوسنتریک یک گونه پیشرفته به شمار می‌رود. شاخص‌های تقارن کاریوتیپی مورد بررسی در این مطالعه نشان‌دهنده نامتقارنی کاریوتیپی بیشتر *A. cylindrica* در مقایسه با اینکورن وحشی بود.

واژه‌های کلیدی: خویشاوندان وحشی، کاریوتیپ، کروموزوم، گندم، میتوز

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Introduction

Wild wheat and wild relatives of wheat (*Triticum aestivum* L.) are valuable sources of genes for desirable agronomic traits, including resistance to biotic and abiotic stresses (Feldman and Sears 1981). Wide hybridization has been used in wheat improvement to introgress agronomically important traits such as disease and pest resistance as well as abiotic stress resistance from wild relatives to cultivated species. *Aegilops* species played an important role in the evolution of wheat as being the closest related taxa and progenitors of two wheat genomes B and D. Some of *Aegilops* species are member of secondary gene pool of wheat, which have at least one homologous genome with wheat genome (Schneider *et al.* 2008). *Aegilops cylindrica* is an allotetraploid species (2n=4x=28 DDCC genome) which grows in northern, western and central regions of Iran, and also widespread in west Asia, west, north and east Europe (Schoenenberger *et al.* 2006). It has already been demonstrated as being an indispensable source of salinity tolerance (Farooq *et al.* 1992, Arzani A., unpublished data), cold tolerance (Iriki *et al.* 2001) and Hessian fly resistance (El Bouhssini *et al.* 1998).

Triticum monococcum subsp. *aegilopoides* or wild einkorn is a diploid species with AA genome (2n=2x=14). It grows in northern and western regions of Fertile Crescent (Hopf & Zohary 2000, Heun *et al.* 1997). The domesticated einkorn cultivates in marginal regions and possessing desirable traits such as resistance to diseases and pests as well as nutrition quality (Heun *et al.* 1997, Konvalina *et al.* 2010).

Cytogenetic techniques have been used in *Triticeae* species to manipulate genes in different genomes for wheat breeding. In addition, cytogenetic information is valuable to dissect natural evolution and speciation (Gill & Fribe 2002). Due to easy chromosome manipulations, wheat has been considered as a model in polyploid cytogenetic studies. Karyotype studies describe number, size and morphology of the chromosome sets in related species (Singh 2003) and has been of great worth in understanding interrelationships and delimitation of the taxa.

Introduction of desirable genes from wild relatives into a crop plant either with classical plant breeding or modern plant breeding with the aid of biotechnology tools requires the detailed karyotypic information. Hence, karyotype analysis is still an important tool for taxonomy, phylogeny and diversity studies. Arzani *et al.* (2000) evaluated the immature pollen-mitosis for characterization of 14 single chromosomes (haploid set) of *Aegilops cylindrica* collected from west of Iran and reported six submetacentric and one acrocentric chromosomes belonging to genome C as well as six submetacentric and one metacentric chromosomes belonging to genome D. They also reported that, from two satellited chromosomes C_A and 5D, only the NOR of chromosome 5D produced nucleolus. Bordbar *et al.* (2009) examined the karyotype asymmetry of the D genome-bearing species and found that, *A. vavilovii* (Zhuk.) Chen. and *A. cylindrica* Host. possessed the most symmetric and asymmetric karyotypes, respectively. They identified *A. cylindrica* as a recently originated species. Karimzadeh *et al.* (2010) evaluated the karyotypes of 15 *Aegilops* populations including three diploid populations and 12 tetraploid populations. Bakhshi *et al.* (2010) reported that, there were differences in chromosome lengths of Iranian
A. cylindrica accessions and lacked both B chromosomes and aneuploids. They pointed out that, the variation of DNA content and chromosome length was probably due to the gain or loss of DNA content during the evaluation of these species and cytotypes in different environments. Arabbeigi *et al.* (2011) investigated the karyotypic features of 21 wheat genotypes including diploid, tetraploid and hexaploid species and belonging to wild, cultivated and synthetic groups. Synthetic hexaploid wheats had the greatest karyotype asymmetry indices, which is explained based on their many different stages of selection and introgression in the breeding program. Ehtemam *et al.* (2014) analyzed the karyotype of 46 wheat accessions belonging to five species (*Triticum monococcum*, *T. urartu*, *T. durum*, *T. turgidum* and

T. aestivum) and two subspecies (*T. boeticum* subsp. *thaodar* and *T. boeticum* subsp. *boeticum*) and based on asymmetry indices divided them into three groups: 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 an intermediate between the other groups. They also reported that, *T. monococcum* had the oldest and the most primitive karyotype among diploid species.

Although, several studies have examined the karyotypic features of *A. cylindrica*, there are no studies that have assessed: 1. karyotype analysis of *Triticum monococcum* subsp. *Aegilopoides* and 2. karyotype comparison between the genotypes of *Triticum monococcum* subsp. *aegilopoides* and *A. cylindrica* which naturally grow side-by-side in different areas in west of Iran. Hence, the purpose of this study was to investigate the karyotypic characteristics of eight genotypes of *Aegilops cylindrica* and eight genotypes of *Triticum monococcum* subsp. *aegilopoides*, which grow wild pairwise in west of Iran.

Materials and Methods

Eight genotypes of *T. monococcum* subsp. *aegilopoides* and eight genotypes of *A. cylindrica* grown wild in west of Iran were collected and used in this study. Details of genotypes and collection sites are given in Table 1. Seeds were germinated in Petri dishes and roots with a length of 3–4 cm were dissected and subjected to the α- bromonaphthalene pretreatment at 4° C for 6 h. Then, samples were fixed in chromic-acid formaldehyde fluid (1:1 of 1% chromic acid + 10% formaldehyde) at 4° C for 36 h. Root tips were hydrolyzed in 1 N NaOH at 60° C for 10 min and finally stained with hematoxylin (4%) for 2 h at room temperature. Stained root tips were squashed in 45% acetic acid. Chromosome images were taken under a Nikon Eclipse E600 light microscope using the Photograb 300Z software (Fuji Photo Film Co. Ltd).

Five samples were utilized per genotype and ten appropriate images were selected and measured by Dn-2 Micro-image process software. Karyotypic features such as total length of chromosome (TLC), total long arm (TLA), total short arm (TSA), symmetry index (SI), total form percentage (TFP), difference of relative length (DRL), intra chromosomal asymmetry index (A_1), inter chromosomal asymmetry index (A_2) (Romero Zarco 1989) were estimated and Stebbin’s classification was done. Ideogram of every genotype was drawn based on mean chromosome length (μm) and arranged in descending order of size.

Results and Discussion

Results of karyotypic analysis in eight genotypes belonging to each *T. monococcum* subsp. *aegilopoides* and *A. cylindrica* species are given in Table 2. Moreover, the representative photos of mitotic chromosomes for all of the examined genotypes are given in figures 1–3, show chromosome ideogram of *A. cylindrica* and *T. monococcum* subsp. *aegilopoides* genotypes, respectively.

Triticum monococcum subsp. *aegilopoides*

Triticum monococcum subsp. *aegilopoides* genotypes characterized to as 1A class of Stebbin's classification. The highest and the lowest values of TFP% belonged to Tm3 (43.23) and Tm7 (40.99) genotypes, respectively. The highest and the lowest symmetry indices belonged to Tm8 (72%) and Tm3 (38.46%) genotypes. Tm7 (0.3) and Tm3 (0.21) possessed the highest and the lowest interchromosomal asymmetry index. The highest and the lowest intrachromosomal asymmetry indices belonged to Tm3, Tm4 (0.16) and Tm8 (0.11), respectively. Only one pair of satellited chromosomes was observed in the karyotype of *T. m.* subsp. *aegilopoides* genotypes. Although, Camara (1943), Coucoli & Skorda (1966), Giorgi & Bozzini (1969), Kerby & Kuspira (1988), Arabbeigi *et al.* (2011), and Ehtemam *et al.* (2014) analyzed the karytotype of *T. monococcum*, no report is available on the karyotypic characteristics of *T. m.* subsp. *aegilopoides.*

Aegilops cylindrica Host

All of the *Aegilops cylindrica* Host genotypes classified in 3A class of Stebbin's classification. The most frequent type of chromosomes of this species on the basis of Levan *et al.* (REF) categories were submetacentric and subtelocentric while Ac2, Ac4, Ac5 and Ac8 genotypes categorized as having metacentric chromosomes. Results of the current study are broadly in agreement with those of Arzani *et al.* (2000), Bordbar *et al.* (2009) and Karimzadeh *et al.* (2010), who also found either metacentric or submetacentric as the most frequent type of chromosomes of this species.

Total form percentages varied in the genotypes with the highest value belonging to Ac8 genotype (35.4%). The highest and the least values of symmetry index belonged to Ac2 and Ac6 genotypes, respectively. The highest and the least interchromosomal asymmetry index belonged to Ac4 (0.46) and Ac2 (0.41) genotypes, respectively. On the other hand, Ac7 (0.17) and Ac8 (0.14) genotypes possessed the highest and the least intrachromosomal asymmetry index. Two pairs of satellited chromosomes were observed in the karyotype of the *A. cylindrica* genotypes, which is consistent with that of Arzani *et al.* (2000). The results of the current study are consistent with those of Bakhshi *et al.* (2009) and Arabbeigi *et al.* (2011) who reported variation for the symmetry index among the genotypes.

- Comparisons between *Triticum monococcum* subsp. *aegilopoides* and *Aegilops cylindrica*

Asymmetry index, implying variation in the length of chromosomes, was estimated 63.8% for *T. m.* subsp. *aegilopoides* and 58.4% for *A. cylindrica*, with the lower the value the higher the asymmetry in karyotype. Mean of total form percentage, demonstrating variation in the centromere positions of the meiotic chromosomes, was estimated 40.9 for *T. m.* subsp. *aegilopoides* and 34.1% for *A. cylindrica*. The lower is the amount of total form percentage the higher will be the asymmetry in karyotype. Mean of interchromosomal asymmetry index, indicating variety in position of centromeres, varied from 0.26 in *T. m.* subsp. *aegilopoides* to 0.44 in *A. cylindrica*. Mean of intrachromosomal asymmetry index, revealing which variety in the length of chromosomes, slightly differed between *T. m.* subsp. *aegilopoides* (0.15) and *A. cylindrica* (0.16). The higher is the value of either interchromosomal or intrachromosomal the greater will be both the asymmetry index and the asymmetry of karyotype.

Some differences were observed among eight genotypes of each species for example length of chromosomes. These could be explained by two possibilities: sampling biases the chromosome preparation/observation at different mitotic stages as well as the structural changes of chromosomes (Sybenga 1992). These results are consistent with those of Sheidai *et al.* 1996 and Karimzadeh *et al.* 2010, who claimed that, the changes in chromosomes type from metacentric to submetacentric and telocentric is due to the evolutionary changes within species.

Existence of submetacentric and subtelocentric chromosomes showed evolutionary changes in *A. cylindrica*. Sheidai *et al.* (2000) reported that, different *Aegilops* populations belonged to 2A, 4A, 3B and 4B class of the Stebbin's classification, while Karimzadeh *et al.* (2010) classified *Aegilops* populations into 1A and 1B classes. The shifts of the centromere from median to subterminal or terminal position may attribute to the changing in size of chromosomes as the evolutionary forces which led to a greater asymmetry (Yousefzadeh *et al.* 2010).

Two pairs of satellite chromosomes were observed in the karyotype of the *A. cylindrica* genotypes. It is likely that, each of the satellited chromosome pair belongs to one of the C or D genomes. Likewise, Arzani *et al.* (2000) reported that, *A. cylindrica* collected from west Iran possesses a chromosome number of 2n=28 and carries 2 satellited chromosomes of which one belongs to each of C and D genomes. However, our results differed from those reported either by Arabbeigi *et al.* (2011) who observed three chromosome pairs possessing NOR or Bordbar *et al.* (2009) who reported one satellited chromosome. Karimzadeh *et al.* (2010) reported that, the number of satellited chromosomes ranged from 0 to 2 among the studied *A. cylindrica* accessions. The observation of Badaeva *et al.* (2002) explained in part these inconsistencies, by detecting two major NOR loci and two additional minor NOR loci in *A. cylindrica* using FISH technique.

All of the cytological features have pointed to the higher symmetric and primitive karyotype of *T. m.* subsp. *aegilopoides* than *A. cylindrica*. These observations are in agreement with those of Gill & Friebe (2002), Arabbeigi *et al.* (2011) and Ehtemam *et al.* (2014), who reported that, *T. monococcum* was more primitive than other wheat species. The asymmetrical karyotypes are more advanced than symmetrical ones (Stebbins 1971).

The presence of gametocidal genes (*Gc*) genes in *A. cylindrica* may be considered as one of the reasons of the structural changes observed in this species. Likewise, Link *et al.* (1999) have observed frequent chromosomal rearrangements in *A. cylindrica* and other *Aegilops* species possessing *Gc* genes.

Table 1. Plant materials (*Aegilops cylindrical* and *Triticum monococcum* subsp. *aegilopoides*) and characteristics collection regions

Genotype*	Locality	Longitude	Latitude	Altitude (m)
Ac1, Tm1	Paa-ghaleh village, Kamyaran	46º 53' 475" E	34º 43' 336" N	1408
Ac2, Tm2	Gelkan village, Sanandaj-Marivan Rd.	46º 55' 268" E	35º 24' 33" N	1675
Ac3, Tm3	Km 55, Kermanshah-Ravansar Rd.	46º 42' 022" E	35º 39' 089"N	1397
Ac4, Tm4	Km 55, Kermanshah-Ravansar Rd.	46º 42' 022" E	34º 39' 089" N	1397
Ac5, Tm5	Km 55, Kermanshah-Ravansar Rd.	46º 42' 022" E	34º 39' 089" N	1397
Ac6, Tm6	Km 44, Kermanshah-Ravansar Rd.	46º 46' 602" E	34º 33' 457" N	1335
Ac7, Tm7	Km 44, Kermanshah-Ravansar Rd.	46º 46' 602" E	34º 33' 457" N	1335
Ac8, Tm8	Km 44, Kermanshah-Ravansar Rd.	46º 46' 602" E	34º 33' 457" N	1335

* A = *A. cylindrica*, T = *T. monococcum* subsp. *aegilopoides*, m = Meter

Table 2. Karyotypic characteristics of *Aegilops cylindrica* and *Triticum monococcum* subsp. *aegilopoides* genotypes

Genotype	TLC	TLA	TSA	SI	TFP	DRL	A1	A2	KF
Ac1	112.4	74.4	38	60.1	34	3.7	0.45	0.15	12sm+2st
Ac2	112.8	72.8	39.9	61.9	35.4	3.4	0.41	0.15	3m+3sm+8sm
Ac3	113.2	74.7	38.5	58.5	34	3.7	0.44	0.16	3sm+11st
Ac4	112.3	75.1	37.2	57.9	33.3	4	0.46	0.15	1m+6sm+7st
Ac5	105.2	69.2	36	57.3	34.3	3.8	0.45	0.16	1m+3st+10sm
Ac6	103.4	67.9	35.5	56.8	34.4	3.9	0.44	0.15	2st+12sm
Ac7	127.5	81.6	42.9	58.3	33.6	3.9	0.45	0.17	4st+10sm
Ac8	111.4	74	37.4	57.8	33.5	3.3	0.45	0.14	1m+5st+8sm
Tm1	74.6	42.6	32	38.5	42.9	6.8	0.21	0.16	7m
Tm2	74.2	42.9	31.3	50.7	42.2	5.8	0.27	0.13	7m
Tm3	61.7	35.2	26.5	69.9	43.2	5	0.24	0.13	7m
Tm4	76.4	44.8	31.6	42.3	41.7	6.9	0.29	0.16	7m
Tm5	74.6	43.2	31.4	64.4	42.1	6.3	0.26	0.14	7m
Tm6	82.4	49.2	34.1	49.4	41.4	5.9	0.28	0.13	7m
Tm7	76.2	44.8	31.4	48.7	41	6.1	0.3	0.14	7m
Tm8	60.2	34.3	25.4	72	42.2	4.6	0.27	0.11	7m

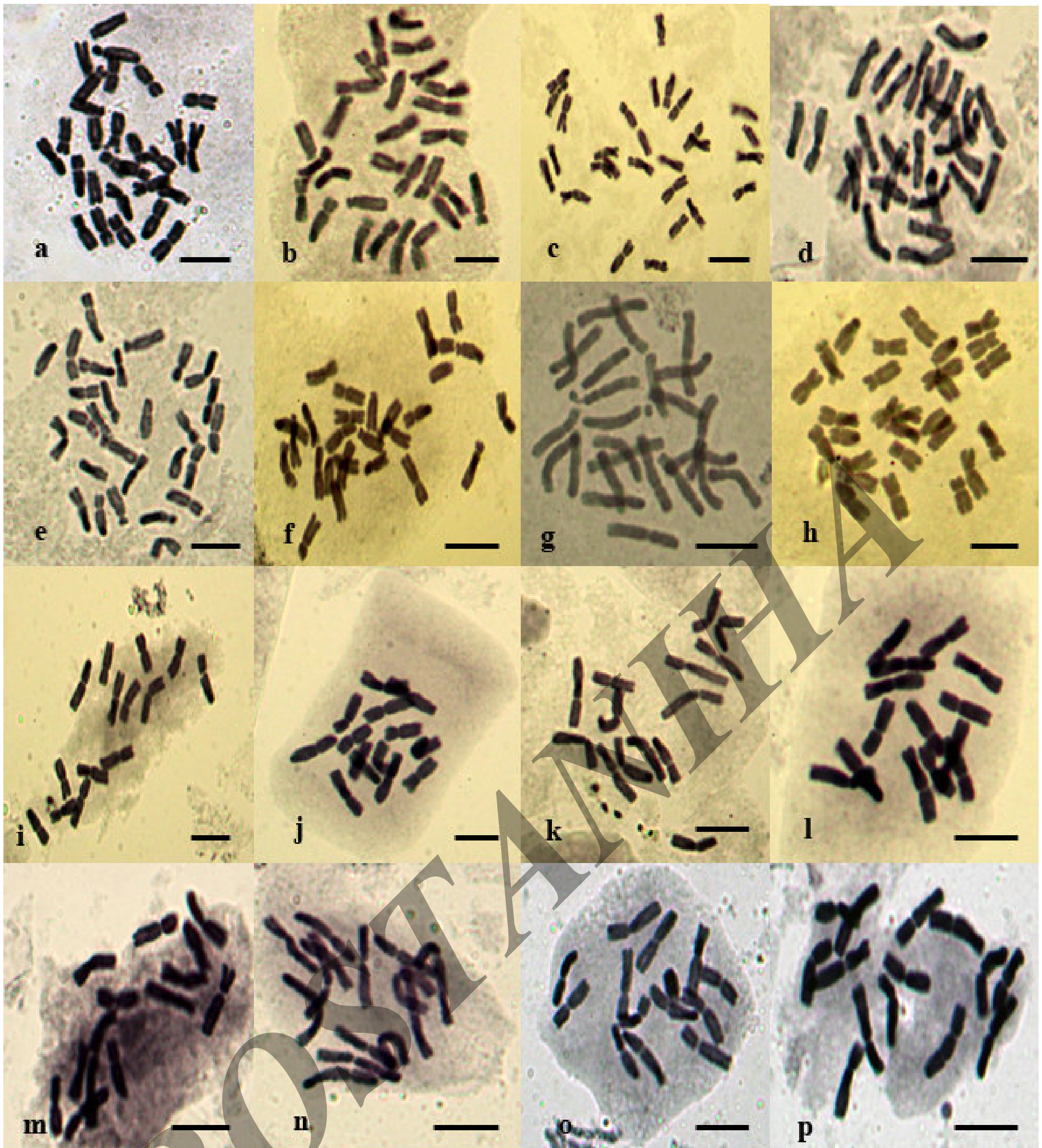

Fig. 1. Mitotic chromosomes of genotypes of *Aegilops cylindrica* and *Triticum monococcum* subsp. *aegilopoides*: a. Ac1, b. Ac2, c. Ac3, d. Ac4, e. Ac5, f. Ac6, g. Ac7, h. Ac8, i. Tm1, j. Tm2, k. Tm3, l. Tm4, m. Tm5, n. Tm6, o. Tm7, p. Tm8 (Bar = 10 µm).

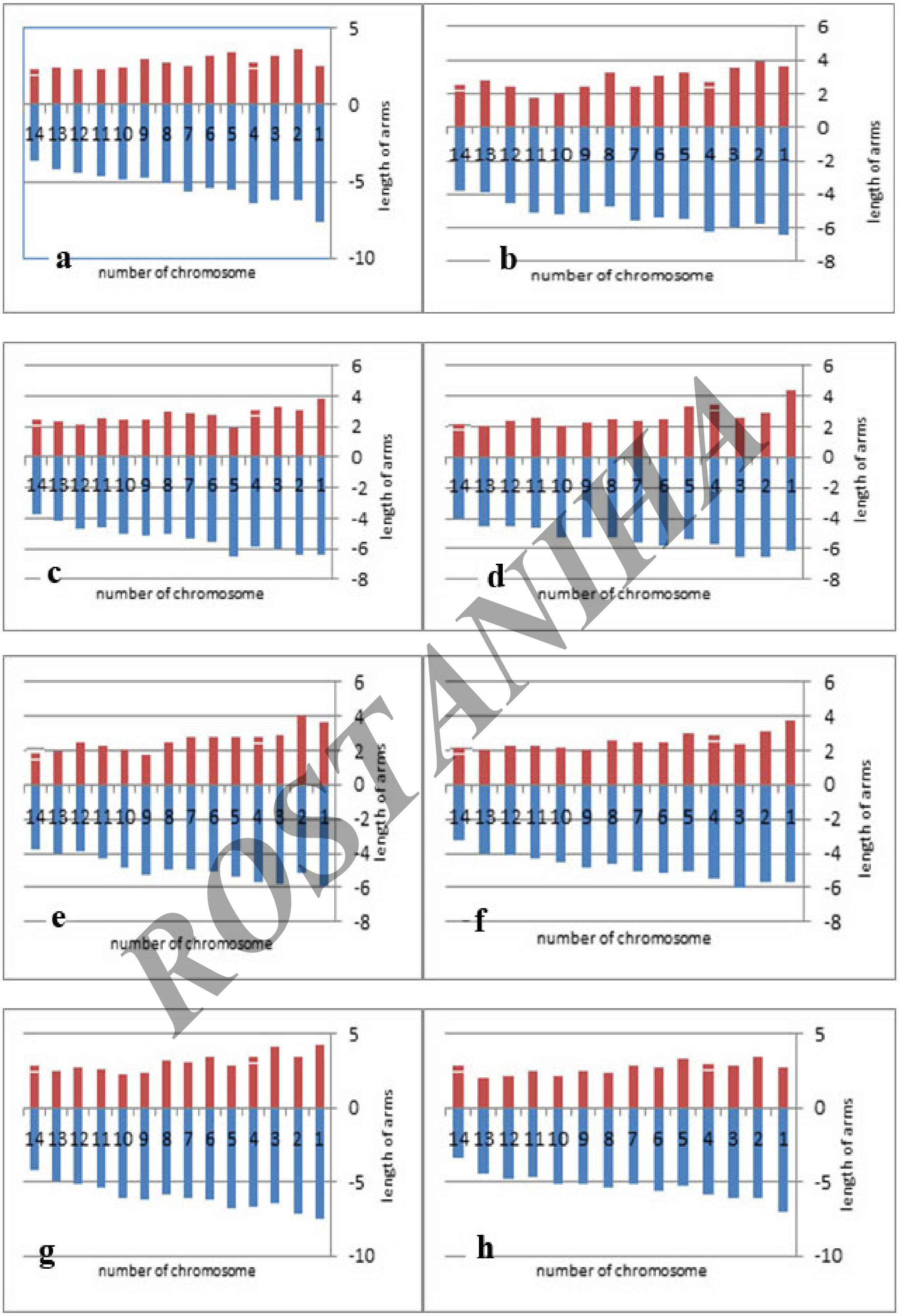

Fig. 2. Ideogram of genotypes of *Aegilops cylindrica*: a. Ac1, b. Ac2, c. Ac3, d. Ac4, e. Ac5, f. Ac6, g. Ac7, h. Ac8 (length of the arms represented based on μm).

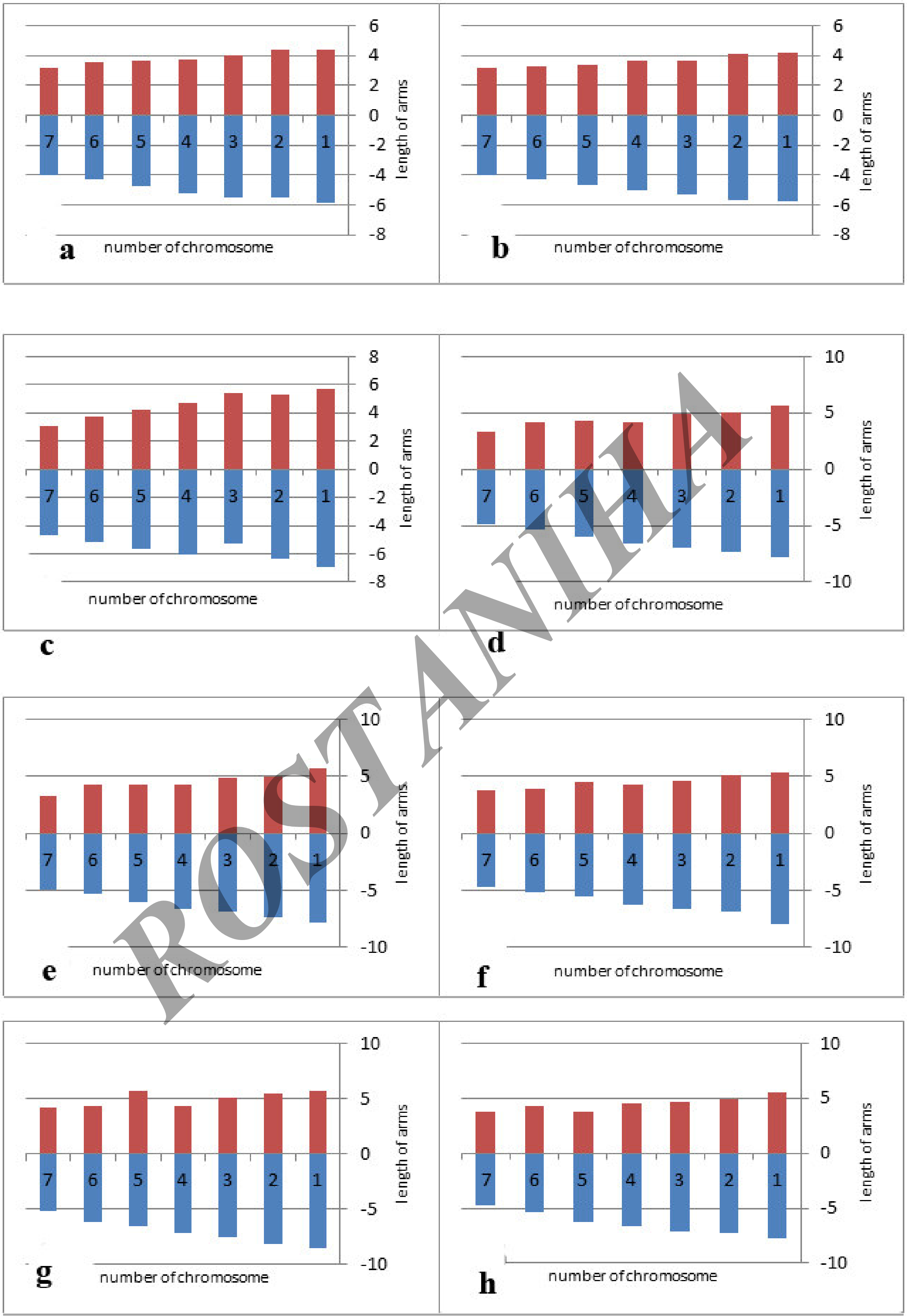

Fig. 3. Ideogram of genotypes of *Triticum monococcum* subsp. *aegilopoides*: a. Tm1, b. Tm2, c. Tm3, d. Tm4, e. Tm5, f. Tm6, g. Tm7, h. Tm8 (length of the arms represented based on µm).

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References

Arabbeigi, M., Arzani, A. & Saeidi, G.H. 2011. Study of karyotype and nucleolar organizer regions (NORs) in wild, synthetic and cultivated wheats. Emirates Journal of Food and Agriculture 23: 196–203.

Arzani, A., Poursiahbidi, M. & Mortazavi, S.E. 2000. An acetocarmine staining procedure for chromosome banding studies of immature pollen in *Triticeae*. Journal of Agricultural Science and Technology 2: 167–175.

Badaeva, E.D., Amosova, A.V., Muravenko, O.V., Samatadze, T.E., Chikida, N.N., Zelenin, A.V., Friebe, B. & Gill, B.S. 2002. Genome differentiation in *Aegilops*. 3. Evolution of the D-genome cluster. Plant Systematics and Evolution 231: 163–190.

Bakhshi, B., Aghaei, M.J., Bihamta, M.R., Darvish, F. & Zarifi, E. 2010. Ploidy determination of *Aegilops cylindrica* host accessions of Iran by using flow cytometry and chromosome counting. Iranian Journal of Botany 16: 258–266.

Bordbar, F., Saeidi, H. & Rahiminejad, M.R. 2009. A comparative cytological study in the D genome-bearing species of *Triticum-Aegilops* complex. Iranian Journal of Botany 15: 240–247.

Camara, A. 1943. Estuo, comparative do cariotipos no genero *Triticum*. Agronomia Lusitana 5: 95–117.

Coucoli, H.D. & Skorda, E.A. 1966. Further evidence on the karyotype of *Triticum monococcum* L. and *Triticum durum* DESF. Canadian Journal of Genetics and Cytology 8: 102–110.

Ehtemam, M.H., Rahiminejad, M.R., Saeidi, H. & Ebrahim, F. 2014. Phylogenetic comparison of the A genome using karyotype analysis in some *Triticum* species. Taxonomy and Biosystematics 21: 11–20.

El Bouhssini, M., Benlhabib, O., Nachit, M.M., Houari, A., Bentika, A., Nsrellah, N. & Lhaloui, S. 1998. Identification *Aegilops* species of resistant sources to Hessian fly (Diptera: *Cecidomyiidae*) in Morocco. Genetic Resources and Crop Evolution 45: 343–345.

Farooq, S., Iqbal, N., Asghar, M. & Shah, T.M. 1992. Intergeneric hybridization for wheat improvement VI. Production of salt tolerant germplasm through crossing wheat (*Triticum aestivum*) with *Aegilops cylindrica* and its significance in practical agriculture. Journal of Genetics and Breeding 46: 125–132.

Feldman, M. & Sears, E.R. 1981. The wild genetic resources of wheat. Scientific American 244: 102–112.

Gill, B.S. & Fribe, B. 2002. Cytogenetics, phylogeny and evolution of cultivated wheat. FAO Plant Production and Protection Series 30: 71–88.

Giorgi, B. & Bozzini, A. 1969. Karyotype analysis in *Triticum*: I. Analysis of *T. turgidum* (L.) Thell. and some related tetraploid wheats. Cytologia 22: 249–258.

Heun, M., Schafer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B. & Salamini, F. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278: 1312–1314.

Hopf, M. & Zohary, D. 2000. Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe, and the Nile valley (3rd ed.). Oxford University Press. p. 38.

Iriki, N., Kawakami, A., Takata, K., Kuwabara, T. & Ban, T. 2001. Screening relatives of wheat for snow mold resistance and freezing tolerance. Euphytica 122: 335–341.

Karimzadeh, G.H., Ashkani, S., Ahmadian Tehrani, P., Davoudi, D. & Mirzaghaderi, G.H. 2010. Cytogenetic studies of some Iranian wild wheat species (*Aegilops*) and OR banding. Faculty of Agriculture, Iranian Journal of Field Crop Science 41: 305–313 (In Persian with English summary).

Kerby, K. & Kuspira, J. 1988. Cytological evidence bearing on the origin of the B genome in polyploid wheats. Genome 30: 36–43.

Konvalina, P., Capouchova, I., Stehno, Z. & Moudry, J. 2010. Agronomic characteristics of the spring forms of the wheat landraces (einkorn, emmer, spelt, intermediate bread wheat) grown in organic farming. Journal of Agrobiology 27: 9–17.

Levan, A., Fredga, K. & Sandbeg, A. 1964. Nomenclature for centromeric position on chromosome. Hereditas 52: 201–220.

Link, G., Friebe, B.R., Kynast, G.R., Molnar-lang, M., Koszegi, B., Sutka, J. & Gill, B.S. 1999. Molecular cytogenetic analysis of *Aegilops cylindrica* Host. Genome 42: 497–503.

Ohta, S. 1990. Genome analysis of *Aegilops mutica* Boiss. based on the chromosome pairing in interspecific and intergeneric hybrids (PhD Thesis).

Romer-Zarco, C. 1989. A new method for estimating karyotype asymmetry. Taxon 35: 526–530.

Schneider, A., Molnar, L. & Molnar-Lang, A. 2008. Utilisation of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica 163: 1–19.

Schoenenberger, N., Guadagnuolo, R., Savova-Bianchi, D., Kupfer, P.H. & Felber, F. 2006. Molecular analysis, cytogenetics and fertility of introgression lines from transgenic wheat to *Aegilops cylindrica* Host. Genetics 174: 2061–2070.

Sheidai, M., Vojdani, P. & Alishah, O. 1996. Karyotype studies in *Gossypium herbaceum* cultivars of Iran. Cytologia 61: 365–374.

Sheidai, M., Arman, M., Mohamadi, S. & Zehzad, B. 2000. Notes on cytology and seed protein characteristics of *Aegilops* species in Iran. Nucleus 43: 118–128.

Singh, R.J. 2003. Plant Cytogenetics. 2nd ed. CRC Press, Boca Raton Florida.

Stebbins, G.L. 1971. Chromosomal Evaluation in Higher Plant. Edward Arnold. London.

Sybenga, J. 1992. Cytogenetics in Plant Breeding. Springer-Verlag, Berlin, Heidelberg.

Yousefzadeh, K., Houshmand, S., Madani, B. & Gomez, P.M. 2010. Karyotypic studies in Iranian wild almond species. Caryologia 2: 117–123.