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Full Length Research Paper

Assessment of cytological and morphological variation among Iranian native *Iris* species

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In order to study the morphological and cytological variation among Iranian native *Irises*, first, morphological traits were analyzed in order to clarify taxonomic relationships among taxa and validity of diagnostic characters. Floral and vegetative characters were measured in 54 plant samples belonging to five species during peak of the flowering season in 2008. Analysis of variance showed significant differences. Result of analysis shows that the most variation coefficient belongs to leaf width. Correlation of coefficients defined between the date of the first blooming of the flowers and the date of the least blooming of the flowers, flowers surface and diameter had the positive and significant correlation. Factor analysis showed that only three factors define almost near to 92% variance among characters. Secondly, in cytological variation each species showed different karyotypic formula such as 2n = 2sm+ 14st+4t for *Iris meda*, 2n = 8m + 16sm + 20st with one pair of terminal satellite chromosomes for *Iris caspica*, 2n = 26m + 18sm for *Iris spuria*, 2n= 14m + 12sm + 8st with one pair of terminal satellite chromosomes for *Iris pseudacorous* and 2n= 8m + 20sm + 20st for *Iris germanica*. This is the first karyotypic report in *I. caspica*, *I. spuria* and *I. meda* species in Iran. The results seemed to provide enough genetic evidence to identify each species and useful data to clarify the interspecific relationships among Iranian native *Iris* species.

Key words: *Iris*, karyotype, Iranian native, chromosome analysis, genome.

INTRODUCTION

About 80 genera and 1500 *Iris* species of the family Iridaceae are found worldwide (Park et al., 2006). Because of the beautiful shape of the flowers and the elegance of the elongated leaves, these plants are commonly found in gardens and widely used in floral arrangement. Because of their popularity, new varieties of these species are worth developing by the horticulture industry. Wendelbo (1977) reported the occurrence of twenty wild *Iris* species in Iran which is, highly debated amongst scientists due to the ambiguity in the *Iris* classification and identification. Therefore, more cytological and molecular research is needed for this purpose.

Cytogenetic researches based on chromosome studies have been conducted as a useful tool in providing data with regard to the chromosome numbers and composition (Martinez et al., 2005). These data are used to identify species and to track down interspecific relationships (Martinez et al., 2005). Recently, chromosome researches were conducted in native *Iris* species of Iran with regard to its horticultural importance. Several reports on chromosome research in the genus *Iris* have been made. Azizian et al. (1993) investigated karyotypes of two species of the genus *Iris* native to Iran: *Iris germanica*, 2n = 32; *Iris pseudacorus*, 2n = 38.

MATERIALS AND METHODS

The species were collected from different parts of Iran and planted

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Table 1. Description of morphological characters recorded in Iranian *Iris* populations.

Number	Character	Description	Descriptor
1	Leaf height	From ground to the highest point (could be the peak of the curve) (in cm)	LH
2	Leaf width	In the point of deviation from stem (in cm)	LW
3	Leaf arch	Categorical character, coded by 1 = erect, 2 = semi curved and 3 = curved	LA
4	Stem height	From ground to fall bottom (in cm)	STH
5	Stem gap	The ratio of the gap between leaves and flower, and stem height. (stem height – leaf height)/stem height	SG
6	Flower height	From fall bottom to standard top (in cm)	FH
7	Flower diameter	At the height of the pollination tunnel (in cm)	FD
8	Flower/stem height	Ratio determines the size of the flower compared with stem height	FH/STH
9	Flower surface	Flower diameter * flower height (in cm2)	FSU
10	Flower shape	Ratio determines the flower shape (Feinbrun-Dothan, 1986)	FS
11	Flower age	date From blooming to withered	FA
12	Fall width	In its broadest place (in cm)	FAW
13	Fall height	From start to fall bottom (in cm)	FAH
14	First blooming date	Date of the first flowering	FB
15	Last blooming date	Date of the last flowering	LB
16	Beard condition	0= no Beard exist, 1= Beard exist	RC
17	Flower colour	Categorical character coded by 1 to 6	FC

in the research field Abouraihan campus, Pakdasht is located in southeastern Tehran characterized by 200 mm average annual precipitation. The regions where these plants were collected are as follow: *Iris meda*: Karaj, Kordan, 1800 m, 5.05.2006; *I. germanica:* Chalus, Koshke bala, 1800 m, 11.05.2006' *Iris caspica:* Mazndaran, Caspian sea coast, 400 m, 1.08.2006; *Iris spuria:* Kerman, Jiroft, Farash village, 600 m, 14.02.2007; *I. pseudacorus:* Mazandaran, rice farm area, 100 m, 15.05.2006.

Fifty-four wild populations belonging to five *Iris* species recorded in the different parts of Iran were investigated in this study. Morphological measurements were taken during the peak of flowering season (mid-February to early April) in 2007 to 2008. The populations were scored for 17 morphological characters (Table 1). Ten characters are descriptors of floral morphology, while three describe shape and size of leaves (one leaf, the second from the centre of the leaf-fan, was measured in each individual). The remaining three characters are descriptors of stem structure. All the characters chosen have previously been considered diagnostic for the taxonomy of *Oncocyclus Irises* (Feinbrun-Dothan, 1986). The flower colour and raceme condition were used as diagnostic characters in the past (Dinsmore, 1934; Feinbrun-Dothan, 1986).

Pearson correlation was performed to determine the interrelationships between traits. Principal component analysis (PCA) was utilized to show the patterns of co variation of quantitative variables among accessions. Statistical analyses were made using the SPSS package. For cytological study, fresh root tips were collected between 10.00 a.m. and 12.00 noon. The roots with 2 to 3 cm length were used for aceto-iron-hematoxilin staining method following Aghayev (1998). The roots were stored in 8-hydroxyl-quinalin (0.002 Mol) for 3 h as pretreatment and fixed in Lewitscky solution (acid cromic 1% and formalin 10% with ratio of 1:1 W/V) for 24 h in 4°C. Then were washed in water for 3 h and stored in ethanol 70% in 4°C for long time storage. The roots with 1 cm length were hydrolyzed in 1 N NaOH for 10 min at 60°C and then washed with distilled water for 30 min. The hydrolyzed roots were stained with aceto-iron-hematoxilin (4%) for 17 h in 30°C and then washed with distilled water for 3 min. One millimeter of root tips was cut and treated in cytase enzyme for 1 h at 25°C. Treated root tips were squashed using the finger in one drop of acetic acid (5%) on a lame using a standard squash method (Aghayev, 1998) magnifications and photographs were taken for each species. Total length of each chromosome, length of short and long arms, arm ratio and satellites were measured by micro measure (version 3.3; Colorado State University, Fort Collins, CO) and mean and variance of each trait was calculated and studied in each species.

RESULTS AND DISCUSSION

Morphological study

Table 2 summarizes the results of quantitative characters. The results show the existence of high variability in flower and stem traits, in a similar range of variation to that in a collection included the known geographical distribution of Iranian native *iris*. The populations evaluated in this study showed a lower range of variability for phenological data than those evaluated in a collection of Iranian native *Iris* (Azizian et al., 1993). The lesser variation for these traits can be as a consequence of the more restricted geographical range of the *Iris* collection in this study. Table 3 shows the percentage of each class for flower colour. The coloured flower category includes completely blue flowers and white ones that have some blue or pink pigmentation on the standard petal, lateral petal or both. The great percentage of accessions had colored flowers. Data of hereditability of flower color and

Table 2. Mean, standard deviation, maximum and minimum for the quantitative evaluated traits (see text for the explanation of descriptor codes).

Descriptor	Mean	Standard deviation	Maximum	Minimum
LH	35.52	12.95	55.00	14.00
LW	2.14	1.41	4.70	0.28
STH	35.70	13.3	60.00	8.23
SG	0.09	0.35	0.48	-0.09
FH	9.14	3.60	13.00	3.76
FD	3.14	1.73	6.69	0.70
FH/STH	0.27	0.09	0.46	0.11
FSU	33.49	26.02	84.96	2.95
FS	0.32	0.13	0.54	0.14
FA	4.22	1.44	7.00	1.00
FAW	2.21	1.73	0.41	0.41
FAH	4.80	2.57	1.71	1.71
FB	22.98	10.81	41.00	7.00
LB	28.09	11.17	46.00	11.00

Table 3. Class frequency of Iranian native *Iris* non metric characters.

Descriptor	Class	Frequency (%)
Flower colour	White	33.4
	Coloured	50
	Mixture	16.6
Beard condition	No beard	50
	Exist beard	50
Leaf arch	Erect	66.8
	Semi curved	16.6
	Curved	16.6

Table 4. Correlation coefficient between quantitative traits (see text for the explanation of codes).

Descriptor	LH	LW	STH	FH	FD	FS	FSU	FH/STH	SG	FA	FB	LB	FAH
FAW	0.22^{ns}	0.89**	0.31^{ns}	0.66**	0.63**	0.18^{ns}	0.63**	0.32^{ns}	0.24^{ns}	0.61**	0.07^{ns}	0.15^{ns}	0.87**
FAH	0.42^{ns}	0.9**	0.58*	0.81**	0.81**	0.16^{ns}	0.85**	0.09^{ns}	0.42^{ns}	0.48*	0.24^{ns}	0.29^{ns}	
LB	0.82**	0.19^{ns}	0.46^{ns}	0.65**	0.72**	0.37^{ns}	0.71**	0.2^{ns}	-0.23^{ns}	0.69**	0.99**		
FB	0.82**	0.13^{ns}	0.45^{ns}	0.61**	0.71**	0.4^{ns}	0.69**	0.19^{ns}	-0.25^{ns}	-0.62**			
FA	0.6**	0.51*	0.32^{ns}	0.67^{ns}	0.61**	0.1^{ns}	0.59**	0.38^{ns}	0.13^{ns}				
SG	0.23^{ns}	0.53*	0.72**	0.41^{ns}	0.01ns	-0.73**	0.18^{ns}	-0.72**					
FH/STH	0.26^{ns}	0.11^{ns}	-0.55*	0.03^{ns}	0.28ns	0.75**	0.13^{ns}						
FSU	0.74^{ns}	0.69**	0.66**	0.58**	0.98**	0.37^{ns}							
FS	-.03^{ns}	0.02^{ns}	-0.36^{ns}	0.02^{ns}	0.53*								
FD	0.67**	0.62*	0.53*	0.82**									
FH	0.75**	0.83**	0.79**										
STH		0.81**		0.58*									
LW		0.33^{ns}											

Ns, * and **; non significant, significant at P ≤ 0.05 and P ≤ 0.01, respectively.

Table 5. Correlation of the analyzed traits with the three first principal axes F1, F2 and F3 (see text for the explanation of codes).

Descriptor	F1	F2	F3
LH	0.74	0.32	-0.55
LW	0.77	0.30	0.47
STH	0.61	0.74	-0.23
FH	0.91	0.29	-0.05
FD	0.97	-.07	0.008
FS	0.49	-0.76	0.11
FSU	0.9	-0.21	0.01
FH/STH	0.26	-0.85	0.24
SG	0.07	-0.96	0.21
FA	0.77	-0.37	-0.11
FB	0.72	-0.11	-0.67
LB	0.68	0.22	-0.69
FAH	0.87	0.22	-0.39
FAW	0.80	-0.03	0.54
% Variation explained	53.26	22.66	15.84
% Variation accumulated	53.26	75.92	91.76

isozymes heterozygosity indicated a variable level of out crossing depending of environmental conditions (Tuba and Dogan, 2008). In this study, 16.6% of accessions have a curved leaf. This might be a response to the increase of radiation towards the desert (Wanli, 1996; Wanli and Zhangcheng, 1998). Correlations between metric characters were calculated (Table 4), many of them were as expected. The strongest positive correlations were between first blooming date and last blooming date, flower diameter and flower surface, fall height and leaf width. Significant negative correlations ($P < 0.01$) were found between stem gap, flower shape and flower/stem height, flower age and first blooming date. Correlations between some combinations of traits, for example, between flower diameter and flower surface, were large ($r^2 = 0.98$), whereas correlations between other pairings, such as between flower diameter and stem gap were small ($r^2 = 0.01$). This result cannot be attributed to the peculiar properties of the individual plants such as genetic variations, but may be explained by the genetic and the developmental relationships between some combinations of traits (Wanli and Zhangcheng, 1998).

Result of PCA is summarized in Table 5 which shows the correlation of each character with the three principal components, the percentage of variation explained by these components, the variability explained and accumulated by the 3 PC and F1 explained by 53.26% of variability. In this axis, the traits with the most important contribution were related to flower architecture (flower height, flower diameter, fall height and fall width). F2 (22.66% of variation) was mainly due to traits related to stem size. F3 (15.84% of variability) was positively correlated with the phenological characters, first, flower appearance date and last, blooming date. The total amount of variability accounted for the three principal components was 91.76%; this high percentage indicates that the traits showed a strong association.

Cytological study

I. caspica had 2n = 22 chromosome number: 4 pairs of the chromosomes are m-type, 8 pair sm-type and 10 pair st-type. The pair of chromosome 5 of this species had satellites (Figures 1a and 2). Total form percentage (TF %) in this species was 30.93%. In studied metaphasecells in this species, arms ratio of the chromosomes ranged between 1.10±0.04 and 5.30±0.88 (Table 6) and all of the chromosomes with arm ratio lower than 1.7 were metacentric (Levan et al., 1964). The mitotic metaphase cells of *I. spuria* consisted of 22 pairs of chromosomes; including 13 pairs of m-type and 9 pairs of sm-types without satellites (Figures 1b and 3). The TF percentage in this species was 39.18%. In studied cells in this species, arm ratio of the chromosomes ranged between 1.18±0.03 and 2.94±0.7 (Table 7). The somatic chromosome complement of the mitotic metaphase cells of *I. meda* consisted of 10 pairs of chromosomes; including 1 pair of sm-type, 7 pairs of St-type and 2 pairs of t-type without satellites (Figures 1c and 4). The TF percentage in this species was 16.85% and arm ratio of the chromosomes ranged between 5.08±0.22 and 7.23±0.04 (Table 8). The mitotic metaphase cells of *I.*

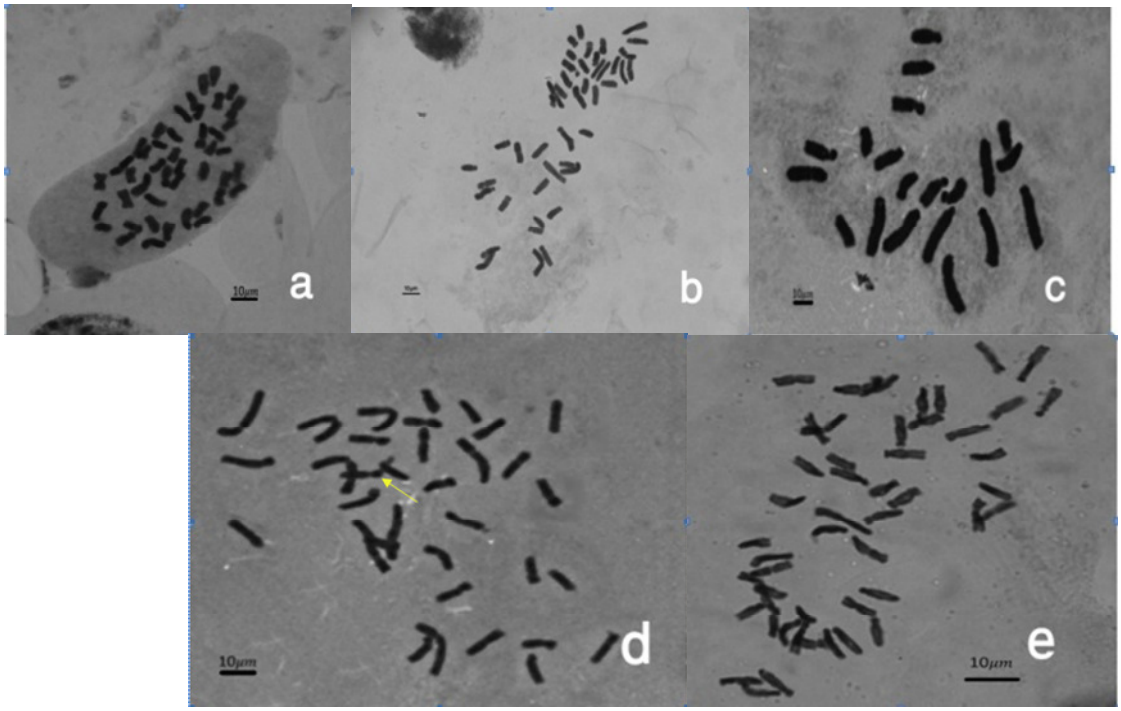

Figure 1. Somatic chromosomes in root-tip cells of three species of Iranian native *Iris* a: *I. caspica* (2n = 44); b: *I. spuria* (2n = 44); c: *I. meda* (2n=20); d: *I. pseudacorus* (2n = 34); arrow shows location of satellite, and e: *I. germanica* (2n = 48).

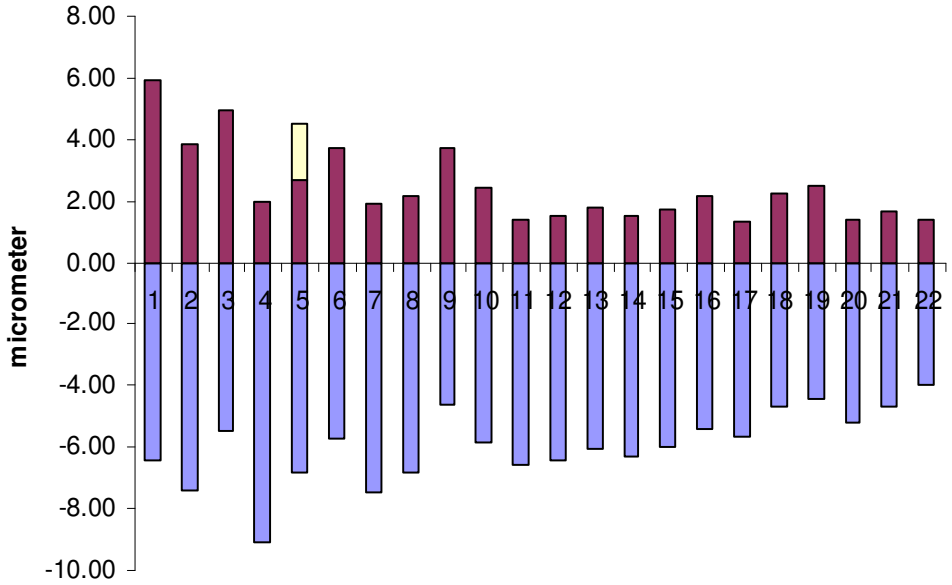

Figure 2. Idiogram of *I. caspica.*

pseudacorus consisted of 17 pairs of chromosomes; including 14 pairs of m-type and 12 pairs of sm-types and 8 pairs of St-type with one pairs of terminal satellite chromosomes (Figures 1d and 5). The TF percentage in this species was 35.30% and arm ratio of the chromosomes ranged between 1.03±0.01 and 3.85±0.16 (Table 9). In *I. germanica* the somatic chromosome complement of the mitotic metaphase cells consisted of 24 pairs of chromosomes; including 8 pair of m-type, 20 pairs of sm-type and 20 pairs of st-type (Figure 1e and 6)

Table 6. Length and relative length of chromosome and arm ratio index in *I. caspica.*

Chromosome number	Chromosome arm		Total length	Arm ratio	Relative length	Chromosome type
	Long arm	Short arm				
1	6.45±0.07	5.92±0.20	12.37±0.20	1.10±.04	47.67	m
2	7.43±0.89	3.87±0.65	11.30±0.49	2.65±0.9	34.83	sm
3	5.44±0.25	4.95±0.28	10.40±0.52	1.11±0.02	47.67	m
4	9.11±0.53	1.96±0.29	11.08±0.28	5.30±0.88	18.00	st
5	6.83±0.40	2.70±0.15	9.53±0.33	2.61±0.30	28.33	sm
6	5.74±0.21	3.73±0.38	9.46±0.20	1.67±0.26	39.00	m
7	7.50±0.29	1.91±0.21	9.41±.09	4.20±0.51	20.17	st
8	6.80±0.08	2.20±0.21	9.00±0.21	3.26±0.35	24.50	st
9	4.61±0.20	3.71±0.10	8.32±0.18	1.25±0.07	44.50	m
10	5.88±0.48	2.42±0.46	8.30±0.04	3.00±0.6	29.50	sm
11	5.39±0.56	2.18±0.33	7.56±0.44	2.84±0.50	29.33	sm
12	6.29±0.13	1.51±0.08	7.80±0.10	4.24±0.32	19.17	st
13	4.68±0.18	2.24±0.14	6.92±0.24	2.14±0.17	32.33	sm
14	4.45±0.42	2.47±0.32	6.91±0.22	2.17±0.56	36.00	sm
15	5.18±0.08	1.38±0.16	6.56±0.19	3.98±0.4	20.83	st
16	4.66±0.30	1.66±0.03	6.32±0.32	2.80±0.14	26.50	sm
17	3.97±0.45	1.41±0.03	5.39±0.44	2.84±0.36	27.00	sm
18	6.60±0.59	1.40±0.16	7.99±0.46	5.15±0.82	18.17	st
19	6.02±0.52	1.74±0.11	7.75±0.44	3.59±0.42	23.17	st
20	5.68±0.30	1.31±0.08	6.99±0.29	4.43±0.40	19.17	st
21	6.03±0.74	1.81±0.03	7.84±0.75	3.32±0.40	24.17	st
22	6.43±1.09	1.52±0.17	7.95±0.93	4.81±1.07	22.50	st
Total	5.96±0.14	45.2±0.12	4.28±0.17	11.3±0.15	75.28	

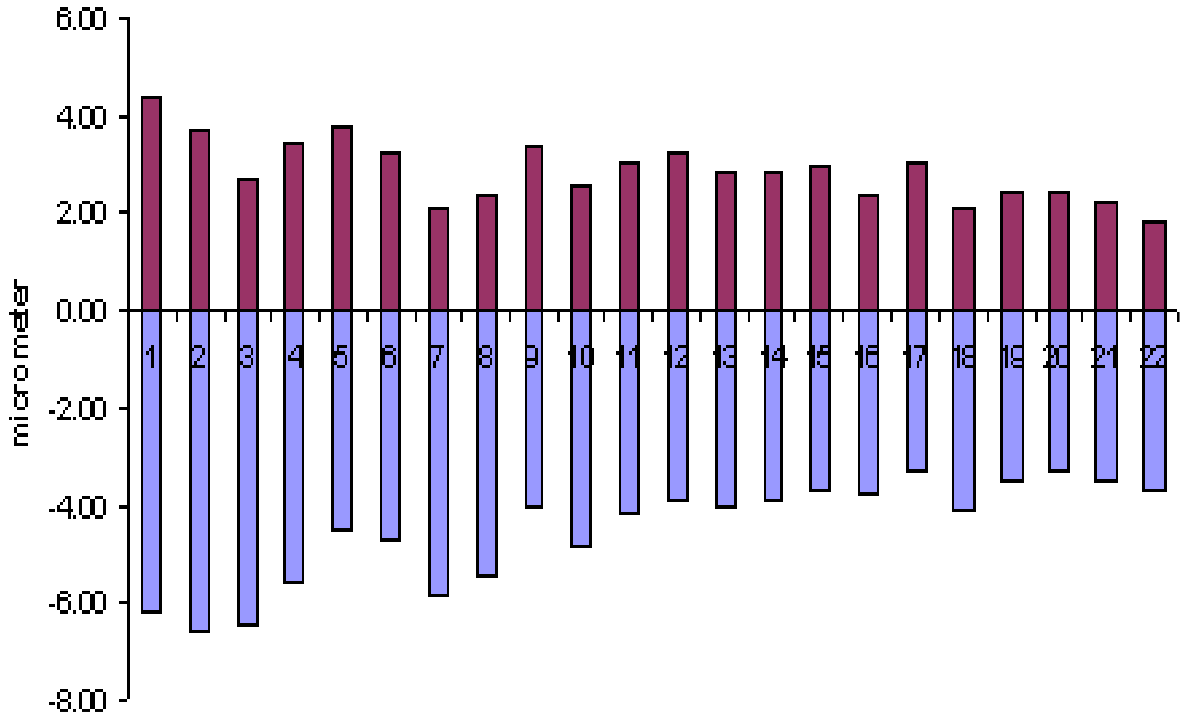

Figure 3. Idiogram of *I. spuria.*

Table 7. Length and relative length of chromosome and arm ratio index in *I. spuria.*

Chromosome number	Chromosome arm		Total length	Arm ratio	Relative length	Chromosome type
	Long arm	Short arm				
1	6.18±0.34	4.41±0.06	10.59±0.43	1.41±0.08	41.83	m
2	6.57±0.36	3.72±0.75	10.28±0.68	2.65±0.92	34.67	sm
3	6.44±0.35	2.75±0.17	9.02±0.21	2.42±0.25	30.67	sm
4	5.60±0.23	3.46±0.30	9.06±0.45	1.67±0.13	38.00	m
5	4.53±0.19	3.82±0.20	8.36±0.37	1.19±0.03	45.83	m
6	4.69±0.29	3.27±0.37	7.96±0.63	1.50±0.12	40.50	m
7	5.83±0.31	2.12±0.08	7.95±0.36	2.75±0.13	26.83	sm
8	5.41±0.17	2.35±0.43	7.76±0.53	2.94±0.70	28.83	sm
9	4.03±0.23	3.40±0.14	7.44±0.37	1.18±0.03	45.83	m
10	4.81±0.40	2.56±0.12	7.37±0.35	1.92±0.23	35.33	sm
11	4.18±0.18	3.10±0.15	7.27±0.32	1.35±0.02	42.33	m
12	3.89±0.22	3.22±0.12	7.11±0.34	1.21±0.03	45.33	m
13	4.03±0.30	2.87±0.08	6.90±0.38	1.40±0.07	41.67	m
14	3.90±0.33	2.87±0.07	6.77±0.40	1.35±0.09	42.83	m
15	3.66±0.20	3.00±0.22	6.66±0.40	1.24±0.05	44.67	m
16	3.79±0.19	2.40±0.29	6.18±0.40	1.71±0.24	38.17	sm
17	3.32±0.22	3.08±0.23	6.40±0.44	1.09±0.02	48.17	m
18	4.09±0.46	2.12±0.04	6.20±0.46	1.94±0.23	35.33	sm
19	3.49±0.06	2.47±0.52	5.96±0.51	2.15±0.70	39.00	sm
20	3.26±0.11	2.48±0.29	5.75±0.39	1.40±0.15	42.50	m
21	3.47±0.14	2.22±0.28	5.69±0.4	1.69±0.20	38.00	m
22	3.67±0.50	1.89±0.07	5.55±0.56	1.92±0.22	35.50	sm
Total	4.49±0.11	2.89±0.08	7.37±0.15	1.73±0.08	39.17	

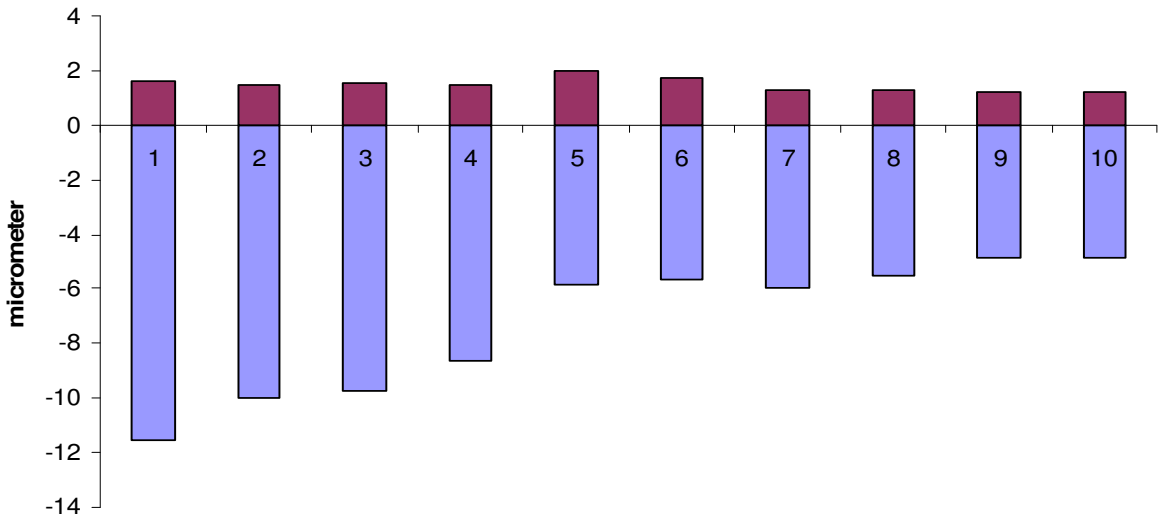

Figure 4. Idiogram of *I. meda*.

The TF percentage in this species was 31.41% and armratio of the chromosomes ranged between 1.16±0.46 and 4.43±0.95 (Table 10). We counted 2n = 44 as the chromosome number of *I. caspica.* This is the first report on this species. This study could not show the basic set of chromosomes, because the ideogram does not show significant differences among them. Therefore, this species need to advanced cytotaxonomical methods for diagnosing basic set of chromosomes. The chromosome counting of *Iris spuria* showed 2n = 2x = 44. Roy et al.

Table 8. Length and relative length of chromosome and arm ratio index in *I. meda*.

Chromosome number	Chromosome arm		Total length	Arm ratio	Relative length	Chromosome type
	Long arm	short arm				
1	11.55±0.51	1.61±0.08	13.16±0.55	7.23±0.33	12.17	t
2	10.01±0.23	1.45±0.13	11.46±0.16	7.21±0.68	12.83	t
3	9.74±0.27	1.55±0.7	11.28±0.28	6.38±0.39	13.50	st
4	8.61±0.3	1.45±0.05	10.06±0.30	5.98±0.29	14.50	st
5	5.87±0.18	2.00±0.10	7.86±0.11	3.00±0.23	25.50	sm
6	5.62±0.11	1.74±0.06	7.36±0.14	3.25±0.10	23.67	sm
7	5.95±0.11	1.29±0.03	7.24±0.12	4.65±0.12	17.83	st
8	5.50±0.21	1.26±0.12	6.75±0.14	4.59±0.50	18.50	st
9	4.88±0.11	1.19±0.10	6.06±0.05	4.29±0.39	19.50	st
10	4.90±0.18	1.19±0.10	6.09±0.28	4.21±0.23	19.50	st
Total	7.26±0.31	1.47±0.04	8.73±0.32	5.08±0.22	17.75	

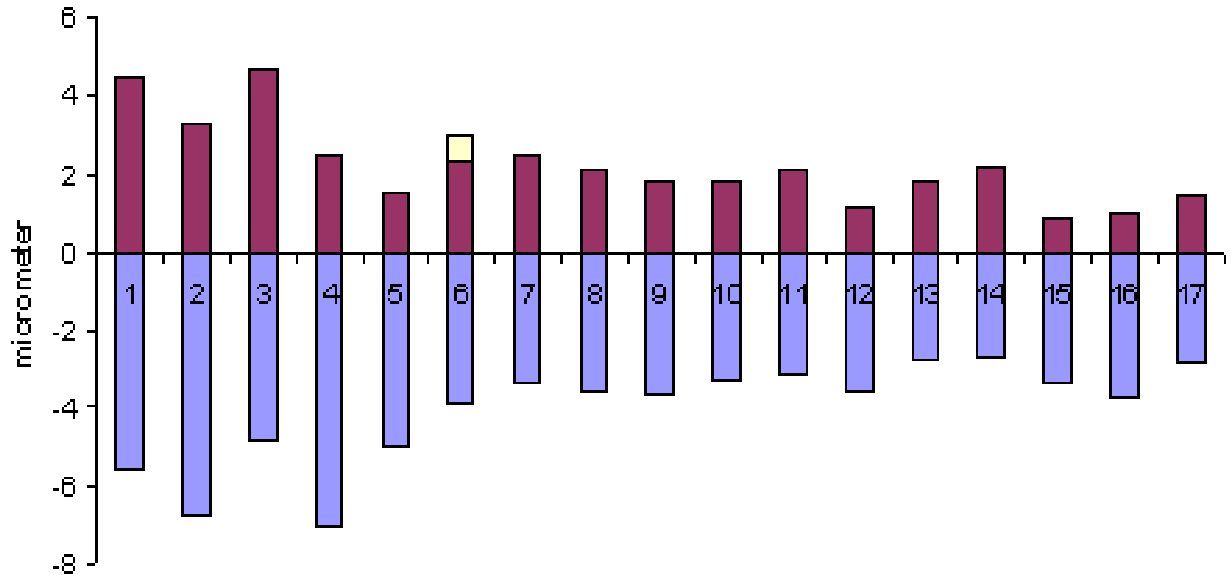

Figure 5. Idiogram of *I. pseudacorus*.

(1988) reported that this species have 2n = 2x = 40 chromosomes. Also, Goldblatt and Takei (1997) reported that the basic set of chromosomes in the majority of *Iris* species are more than 10. The ideogram could not show any differences among the chromosomes, so it seems that the basic set of chromosomes are 11. The chromosome counting of *Iris meda* showed 2n = 2x = 20. This observation was in accordance with Avishai and Zohary (1977). This species had the biggest chromosomes among the studied species and the lowest TF% among other species studied. This result indicates that media species has ten basic set of chromosome. Also, TF percentage was more exposed to chromosomal changes than other species. The chromosome counting of *I. pseudacorus* showed 2n = 2x = 34. This observation, was in accordance with Valdes (1980), Strid and Franzen (1981) and Laublin and Cappadocia (1992). According to Azizian et al. (1993) research, karyotypes species showed 2n = 38. In their research, the length of the biggest chromosome was 5.2 µm and the length of the smallest chromosome was 1.5 µm. The chromosome counting of *I. germanica* showed 2n = 48. This observation was in accordance with Pandita (1979).

According to Azizian et al. (1993) this species has 2n = 32 and with increase in the ploidy level, the karyotype asymmetry also increases in this species. This high level of polyploidy seen in Iranian native irises is common in the Iridaceae. Goldblatt and Takei (1997) also reported in the Iridaceae family, is there a polyploidy. Many genera, including *Iris*, *Moraea*, *Crocus* and *Gladiolus*, have extensive polyploid series (Goldblatt and Takei, 1997).

Table 9. Length and relative length of chromosome and arm ratio index in *I. pseudacorus.*

Chromosome number	Chromosome arm		Total length	Arm ratio	Relative length	Chromosome type
	Long arm	Short arm				
1	5.55±006	4.50±036	10.06±0.09	1.28±0.13	44..50	m
2	6.76±0.06	3.32±0.04	10.06±0.09	2.04±0.01	32.67	sm
3	4.8±0.1	4.68±0.084	9.48±0.18	1.03±0.01	49.33	m
4	7.09±0.04	2.46±0.02	9.55±0.06	2.88±0.02	28.30	sm
5	4.96±0.13	1.52±0.04	6.48±0.09	3.28±0.03	23.17	st
6	3.86±0.15	2.38±0.04	6.24±0.13	1.63±0.08	38.17	m
7	3.38±0.05	2.5±0.02	5.89±0.07	1.36±0.02	42.33	m
8	3.52±0.1	2.1±0.11	5.62±0.04	1.70±0. 12	37.17	sm
9	3.66±0.16	1.81±0.05	5.47±0.13	2.04±0.12	33.33	sm
10	3.25±0.04	1.80±0.07	5.06±0.08	1.81±0.07	36.00	sm
11	3.18±0.15	2.1±0.09	5.27±0.17	1.53±0.1	39.67	m
12	2.72±0.02	2.17±0.02	4.89±0.03	1.2±0.0	39.67	m
13	3.56±0.18	1.17±0.1	4.47±0.1	3.2±0.36	25.00	st
14	3. 7±0.04	0.97±0.04	4.67±0.07	3.85±0.16	20.67	st
15	2.76±0.14	1.81±0.04	4.58±0.1	1.54±0.11	39.67	m
16	2.82±0.05	1.45±0.05	4.26±0.07	1.96±0.08	34.00	sm
17	3.31±0.03	0.89±0.03	4.21±0.17	3.71±0.05	21.50	st
Total	4.05±0.11	2.21±0.11	6.27±0.21	2.12±0.09	34.56	

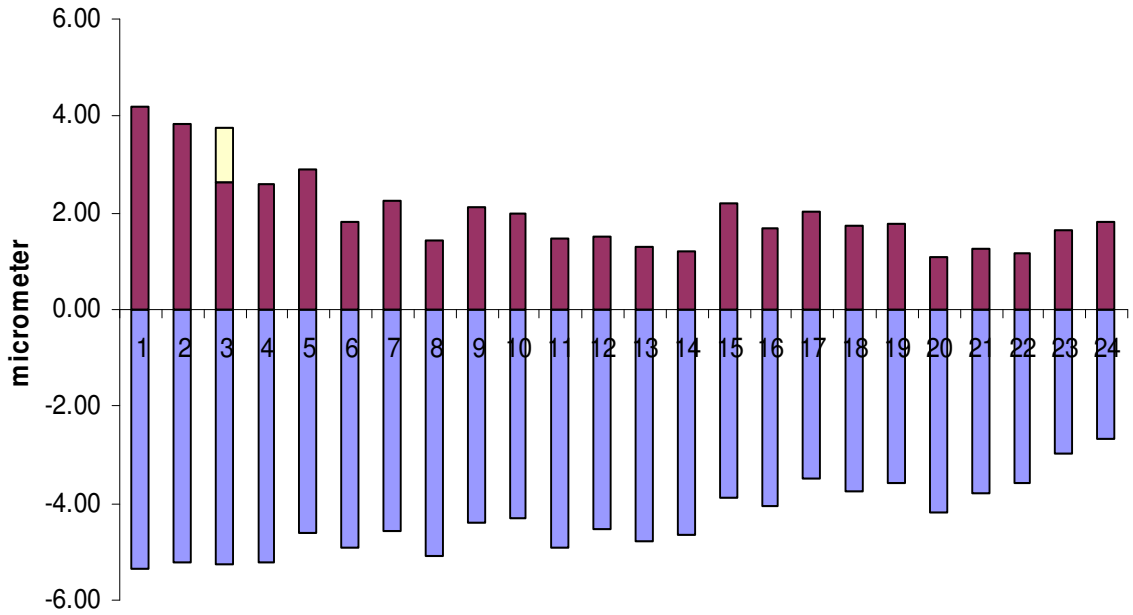

Figure 6. Idiogram of *I. germanica.*

Goldblatt and Takei (1997) suggested that in the early evolution of the Iridaceae, there was a burst of polyploidy followed by descending dysploidy in many genera. They considered ascending dyploidy to be uncommon in plant evolution. In studied species, B chromosome was not showed. This observation was in accordance with Johnson and Guner (2002) who reported that B chromosome is not common among *Irises*. Roy et al. (1988) reported that the chromosome number of *I. spuria* is 2n = 2x = 40. Our results show that the chromosome number are 44 in *I. spuria.* That indicated aneoploidy in this species. Regarding to karyotype formula of five *Iris* species, the polyploidy and chromosomal structure rearrangement has an important role in *Iris* speciation

Table 10. Length and relative length of chromosome and arm ratio index in *I. germanica.*

Chromosome number	Chromosome arm		Total length	Arm ratio	Relative length	Chromosome type
	Long arm	Short arm				
1	5.35±0.31	4.19±0.12	9.54±0.28	1.29±0.10	44.00	m
2	5.22±0.47	3.85±0.19	9.08±0.48	1.37±0.15	42.83	m
3	5.29±0.09	2.64±0.55	7.93±0.46	2.46±0.44	31.83	sm
4	5.24±0.28	2.57±0.52	7.81±0.28	3.18±1.16	36.83	st
5	4.61±0.63	2.89±0.19	7.50±0.46	1.71±0.31	39.67	sm
6	5.04±0.4	1.66±0.43	6.72±0.23	4.43±0.95	24.43	st
7	5.35±0.31	2.24±0.24	6.83±0.19	2.20±0.34	33.17	sm
8	5.11±0.140	1.44±0.05	6.55±0.16	3.57±0.11	22.00	st
9	4.39±0.37	2.81±0.34	6.51±0.09	2.79±0.49	32.67	sm
10	4.3±0.31	1.99±0.09	6.29±0.16	2.21±0.20	31.83	sm
11	4.94±012	1.46±0.09	6.29±0.11	3.47±0.24	22.50	st
12	4.55±0.28	1.52±0.26	6.07±0.12	3.65±0.80	25.70	st
13	4.81±0.17	1.30±0.11	6.11±0.08	3.91±0.48	21.67	st
14	4.68±0.29	1.22±0.09	5.90±0.21	4.6±0.54	21.00	st
15	3.88±0.29	2.18±0.27	5.88±0.09	2.02±0.41	37.00	sm
16	4.08±0.13	1.66±0.15	5.74±0.1	2.59±0.30	29.00	sm
17	3.51±0.30	2.30±0.15	5.54±0.08	2.25±0.73	36.50	st
18	3.78±0.30	1.74±0.36	5.52±0.08	3.29±1.11	31.33	st
19	3.58±0.26	1.77±0.31	5.35±0.06	2.65±0.72	32.83	sm
20	4.18±0.07	1.08±0.03	5.26±0.08	3.91±0.12	20.33	st
21	3.79±0.79	1.26±0.04	5.05±0.1	3.02±0.05	24.67	st
22	3.58±0.13	1.16±0.12	4.74±0.12	1.16±0.46	24.50	m
23	2.97±0.10	1.64±0.16	4.61±0.12	1.64±0.28	35.32	m
24	2.69±0.16	1.82±0.18	4.45±0.14	1.82±0.27	40.67	sm
Total	4.34±0.08	1.91±0.08	6.31±0.11	1.91±0.11	30.79	

procedure. Also, the existence of many different chromosomes in individual species may imply the adaptation of this species with ecological circumstance. There is a relation between the DNA content and acclimation with colder climates (Benet, 1976; Price et al., 1981). This study showed that *I. meda* species that was collected from mountainous zones had bigger chromosome as well as bigger genome than other species collected from warmer climate.

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