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Cytogenetic Studies in Four Species of Flax (*Linum* spp.)

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Abstract: This research a karyological investigation which was carried out in three species of Flax, which is a multipurpose and valuable plant. Plants and achenes specimens were collected and identified from different ecological regions in West-Azerbaijan and Markazi Provinces, Iran. Root tip meristems obtained from germinated of achenes were pretreated with saturated solution of α -Bromonaphthalene and fixed in Levitsky solution and stained in Aceto-Iron-Hematoxylin. Stained slides were photographed by camera equipped microscopes and five appropriate metaphase plates were used for analyzing karyotype parameters. A number of statistical parameters were also estimated for all the species to investigate karyotype asymmetry. According to the results of this study *L. austeriacum* species is diploid that its karyotype consists of 18 chromosomes ($2n = 2x = 18$) whose size are between 2.246-3.44 micron and chromosome No. 1 includes satellite. All of the chromosomes were metacentric type. *L. nervosum* species, as this study explains, is diploid and its karyotype includes of 18 chromosomes ($2n = 2x = 18$). With size of 2.95-4.291 micron. Also according to the results of this research, *L. usitatissimum* species is diploid, with 30 chromosomes ($2n = 2x = 30$), that their size are between 1.292-2.968 micron. Type of all the chromosomes are metacentric except chromosome No. 4 that is submetacentric and its arms ratio is 1.8. The karyotype *Linum* spp. species consists 30 + 1 chromosome and is Aniploied. That the sizes of the chromosomes are between 1.224- 1.992 micron. Mostly chromosomes of this species are metacentric type except chromosomes No. 1 and 2. that are submetacentric. Based on investigating asymmetry, *L. austeriacum* is being estimated to be the most developed one among them. It needs more researches to be proved.

Key words: Chromosome, karyotype, *L. austeriacum*, *L. nervosum*, *L. usitatissimum*, satellite

INTRODUCTION

The genus of *Linum* of Linaceae comprises 200 distributed throughout the world, 15 of which occur in Iran (Sharifnia and Assad, 2001). Flax is making its mark in the world food supply as a functional food. Functional foods deliver a boost beyond what might be expected from their traditional nutrient content (Hasler, 2002).

Muravenko *et al.* (2001a), were studied C-banding patterns of the karyotypes of two closely related wild flax species, *Linum austeriacum* L. and *Linum grandiflorum* DESF. According results of this study the karyotype of both species were similar in the chromosome morphology and size. In each species, metacentric and acrocentric chromosomes (1.7-4.3 μ m) and satellite chromosome were observed (Muravenko *et al.*, 2001a).

Muravenko *et al.* (2004), fluorescence *in situ* hybridization was for the first time, used to study the chromosomal location of the 45S (18S-5. 8S-26S) and 5S ribosomal genes in the genomes of five flax species of the section *linum*. In *L. usitatissimum* L., *L. angustifolium*

Huds and *L. bienne* Mill, a major hybridization site of 45s rDNA was observed in the pericentric region of a large metacentric chromosome. Sites of 5s rDNA were colocalized with those of 45s rDNA, but direct correlation between signal intensities from the 45s and 5s rDNA sites was observed only in some cases (Murvenko *et al.*, 2004).

The C-banding technique was used to study flax chromosomes. Hetrochromatin was mainly locate in pericentromeric regions of chromosomes (Muravenko *et al.*, 2001b).

Muravenko *et al.* (2003), chromosome C-banding patterns were analyzed in three closely related flax species (*Linum usitatissimum* L., $2n = 30$; *L. angustifolium* Huds., $2n = 30$ and *L. bienne* Mill., $2n = 30$). In each case, the karyotype included metacentrics, submetacentrics and one or two satellite chromosomes. Chromosomes of the three flax species were similar in morphology, size (1-3 micron) and C-banding pattern and slightly differed in size of hetrochromatic regions (Muravenko *et al.*, 2003).

Karyotypic studies are used to compare the existing differences among individuals of a group, to clarify the

developmental process of changes in forming chromosomes of genome. And plays an important role in determining the affinities of species. It can be the first step in phylogenic analysis and the development of relative groups. One of the effects of the development process in plants is the changes that it makes in the absolute length of chromosomes, number and situation of satellites and chromosomal unit number. This information is used in plant classification too (Stebbins, 1971). Flax, for being autogamy, has a very low genetic diversity in the natural colonies. Therefore, syngamy will cause Flax to have got more genetic diversity and recombination virtue. The intra specific inoculations, performed between some of these species, are necessary to bring the desirable virtues in to a genotype; these species, karyotypic and chromosomal properties requires to be suitable. As a matter of fact in order to achieve a more and better recognition of flax, three species of this valuable plant will be karyologically studied in this research.

MATERIALS AND METHODS

Plant samples and seeds, were collected from the different eco-geographical regions of West-Azerbaijan and Markazi Provinces (Fig. 1). The experiments were

conducted in Karaj, institute of national plant Gene-Bank, Iran in 2005 year. In order cytogenetical studies seeds were sterilized in Sodium Hypochlorite solution for 30 min first. Then were germinated on damp filter paper in petri dish in 20-25°C and fresh root tips were collected for karyotypic study.

Different pretreatments were used and best result was obtained from saturated solution of α -Brumonaphtalene for four hours in 4°C (Agayev, 1998). And then were fixed in Levetsky solution for 48 h.

Next stage roots tip were hydrolyzed in normal one NaOH for 10 min, in 60°C. And were stained by Aceto-Iron-Hematoxilin for 16 h in 30-32 degree (Agayev, 1998). Finally in order to microscopic studies, Cytaze enzyme was used for destroy cell walls. Then were squashed in one drop of 45% Acetic acid and supplied microscopic samples and prepared samples were examined by camera equipped microscopes (Axiophot, Zeiss) with 10, 20, 40 degree binoculars and the best metaphase cells were selected. Then were re-examined by 100 degree binoculars and 5 metaphase plats were used for the analysis of karyotype parameters such as: (short arm length, long arm length, ratio of long arm length to short one, total length of chromosomes and relative length of

Fig. 1: Dispersion area the species of *Linum* in Iran

chromosomes and Centromeric index). Chromosomes were identified according to Levan method Levan *et al.* (1964) and karyogram of chromosomes was prepared after analyzing data and idiograms were designed using Excel software.

Parameters of symmetrical evaluation: Several parameters have been presented and applied for karyotypic symmetrical evolution; the following parameters were used in this research.

- TF%: Total Form Percentage; ratio of short arms of one taxa chromosomes/length of all chromosomes of that taxa
- DRL: The difference between minimum and maximum relative Length of chromosomes
- S%: Relative Length of the shortest chromosome
- TL: Total Length; Total length of a group of chromosomes by micron
- S/L: The ratio of the smallest chromosome to the longest one

RESULTS AND DISCUSSION

Cytogenetic evolution of *L. austeriacum* species:

According to the results of this study number of somatic chromosomes of *L. austeriacum* species is 18 chromosomes ($2n = 2x = 18$) and diploid, which is similar to the previous reports (Muravenko *et al.*, 2003).

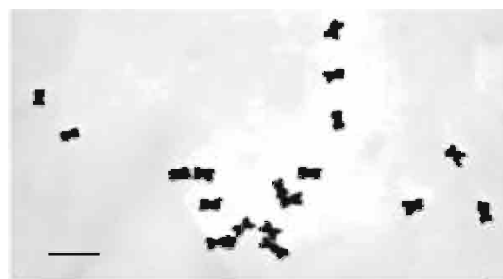
There is a pair of satellites on the short arm of one of the chromosomes. These satellites can be applied in other chromosomes as a chromosomal remark. Types of the chromosomes were determined by Levan method after the karyogram was prepared by five metaphase plates.

Results suggest that in this species the length of the longest chromosome (No. 1) and the shortest chromosome (No. 9) were (3.444 ± 0.103) with satellite and (2.246 ± 0.178) micron, respectively. All of the chromosomes were metacentric type. Average length of the chromosome was 2.56 ± 0.067 micron and the length of the total genome was 23.126 micron.

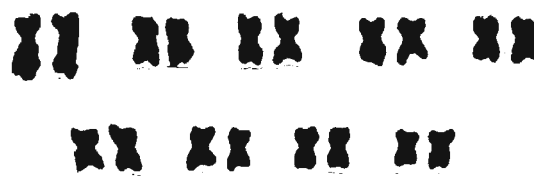
Karyotypic detail of this species are presented in Table 1 and Fig. 2A shows the metaphasic plate of this species and Fig. 2B shows karyogram prepared of this metaphase. Figure 7 shows the idiogram of this species with 9 haploid chromosomes. Average of ratio arms is 1.122 ± 0.020 is defined and chromosomal formula of this species is as link 1.

$$\text{Link 1} \rightarrow 2n = 2x = 18 = 12m + 2\text{sat} + 4M$$

Cytogenetic evolution of *L. nervosum* species: According to the results obtained of chromosomic images (Fig. 3),



A

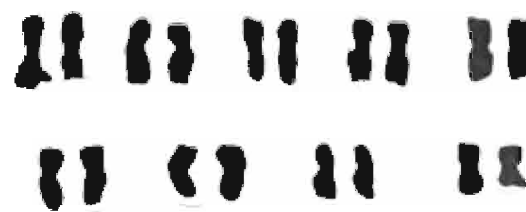


B

Fig. 2: A) Mitotic metaphase plate of *L. austeriacum*, B) Karyogram, constructed from chromosomes of this plate ($2n = 2x = 18$), Bar = 5 μ m



A



B

Fig. 3: A) Mitotic metaphase plate of *L. nervosum*, B) Karyogram, constructed from chromosomes of this plate ($2n = 2x = 18$), Bar = 5 μ m

the number of somatic chromosomes is 18 and diploid ($2n = 2x = 18$). Whilst widespread attempts have been done to find the resources of this species, yet no definite resource has been found. It seems that no study has been done in this case. The length of the longest chromosome

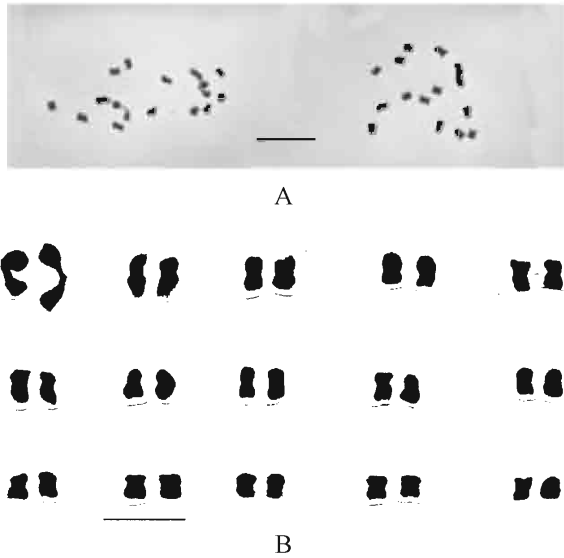


Fig. 4: A) Mitotic metaphase plate of *L. usitatissimum*, B) Karyogram, constructed from chromosomes of this plate ($2n = 2x = 30$), Bar = 5 μm

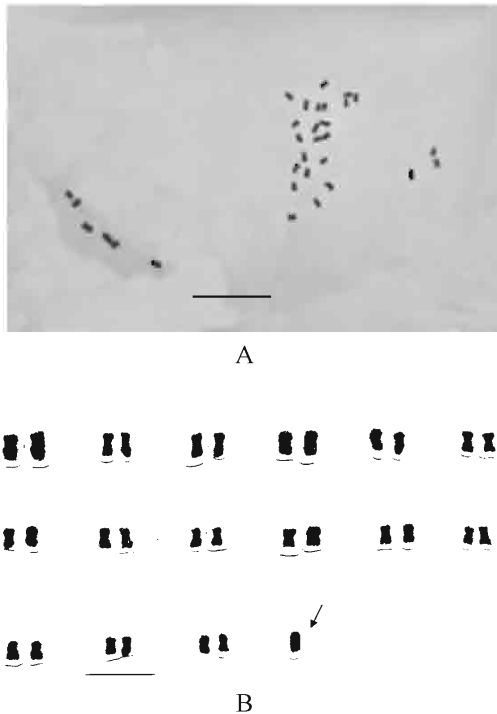


Fig. 5: A) Mitotic metaphase plate of *Linum* spp., B) Kayogram, constructed from chromosomes of this plate ($2n = 2x = 30+1$), Bar = 5 μm

(No. 1) and the shortest chromosome (No. 9) were (4.291 \pm 0.401) and (2.95 \pm 0.168) micron, respectively. All of

them were metacentric type. Average length of the chromosomes was 3.661 \pm 0.096 micron and the length of the total genome was 32.603 micron.

Karyotypic details of this species are shown in Table 2 and Fig. 3A shows the metaphase plate of this species and Fig. 3B shows karyogramme prepared of this metaphase.

Figure 8 shows the idiogram of this species with 9 haploid chromosomes. Average ratio of arms is 1.32 \pm 0.063 and its chromosomal formula is defined as link 2:

$$\text{Link 2} \rightarrow 2n = 2x = 18 = 16 m + 2M$$

Cytogenetic evolution of *L. usitatissimum* L. species:

Gained results of the studies based upon chromosomal images of the metaphases of this species (Fig. 3) show that the number of the somatic chromosomes is 18 and diploid ($2n = 2x = 18$) that is similar to Muravenko *et al.* (2003), reports in Russia. against to what these researchers said, there is no satellite on its chromosomes; this result which refers to the genotype of the plant, location of geographical plant and its growth place. In this species the length of the longest chromosome (No. 1) and the shortest chromosome (No. 15) were (2.968 \pm 0.133) and (1.292 \pm 0.110) micron, respectively and all of the chromosomes were metacentric type except number 4 which is a submetacentric chromosome and its arms ratio is 1.8. Average length of the chromosomes was 1.758 \pm 0.074 micron and total genome was 26.342 micron.

Karyotypic details of this species are shown in Table 3 and Fig. 4A shows the metaphase plate of this species and Fig. 4B shows karyogramme prepared of this metaphase.

Figure 9 shows the idiogramme of this species with 15 haploid chromosomes. Average ratio of arms is 1.323 \pm 0.049, the chromosomal formula of this species is defined as link 3:

$$\text{Link 3} \rightarrow 2n = 2x = 30 = 26 m + 2 sm + 2M$$

Cytogenetic evolution of *L. spp.* species:

According to the fact that this species has morphologic and karyotypic specialties different from cultivated species, such as that the petals are white and yellowish anthers and the peduncle are longer than those of cultivated ones and consists white seeds, it is probably a different species or is going to make new one. Having been studied, it is clarified that the number of somatic chromosomes is 30+1 chromosomes and aneuploid ($2n = 30 + 1$) (Fig. 5) for the reason that monosomic type is fatal case except in the some polyploids, then so aneuploidy of this plant is from trisomic type and gametes of this plant should be n+1 and n also additional chromosome should be

Table 1: Numerical data concerning the karyotype of *L. austeriacum* (N = 5)

No. Pair	Long arm (L)	Short arm (S)	Total (L+S)	Arm ratio (L/S)	CI S*100/(L+S)	Relative length	sat	Type	L (%)	S (%)
1	1.55±0.088	1.360±0.080	2.910±0.159	1.13±0.041	46.90±0.95	12.58±0.72	-	m	6.702	5.880
2	1.61±0.164	1.230±0.124	2.840±0.2881	1.30±0.012	43.30±0.20	12.28±1.303	-	m	6.961	5.318
3	1.50±0.127	1.290±0.061	2.800±0.135	1.16±0.128	46.15±2.602	12.08±0.611	-	m	6.486	5.578
4	1.37±0.065	1.310±0.100	2.680±0.165	1.04±0.040	48.46±0.947	11.58±0.745	-	M	5.924	5.664
5	1.32±0.117	1.210±0.086	2.530±0.183	1.11±0.082	47.82±1.77	10.94±0.828	-	m	5.707	5.232
6	1.28±0.115	1.220±0.070	2.500±0.185	1.04±0.037	48.80±0.88	10.81±0.835	-	M	5.534	5.275
7	1.28±0.103	1.140±0.075	2.430±0.172	1.12±0.047	47.10±1.09	10.49±0.777	-	m	5.534	4.929
8	1.17±0.089	1.076±0.097	2.240±0.178	1.08±0.058	47.90±3.11	9.71±0.804	-	m	5.059	4.652
9	1.16±0.080	1.037±0.072	2.197±0.103	1.11±0.030	47.20±0.66	9.50±0.678	1.25	m	5.015	4.484
Mean	1.36±0.039	1.208±0.0307	2.570±0.068	1.122±0.020						
Total	23.126								52.922	47.012

Chromosomal formula is $2n = 2x = 18 = 12m + 2m \text{ sat} + 4M$, L: Length of the long arm of the chromosome (µm), S: Length of the short arm of the chromosome (µm), CI: Centromer index, (m: Centromer in middle region of chromosome), (M: Centromer in center of chromosome), Centromere type according to Levan *et al.* (1964), sat: satellite, N = Number of mitotic metaphases used to determine the karyotype, L% and S%: Indices that express the contribution of each arm of each chromosome to the total length of karyotype

Table 2: Numerical data concerning the karyotype of *L. nervosum* (N = 5)

No. Pair	Long arm (L)	Short arm (S)	Total (L+S)	Arm ratio (L/S)	CI S*100/(L+S)	Relative length	sat	Type	L (%)	S (%)
1	2.437±0.177	1.854±0.187	4.291±0.401	1.314±0.037	43.20±0.707	13.130±1.171	-	m	7.451	5.668
2	2.350±0.100	1.570±0.159	3.936±0.258	1.480±0.057	40.290±0.94	12.040±0.701	-	m	7.185	4.849
3	2.082±0.145	1.709±0.089	3.791±0.233	1.218±0.024	45.080±0.52	11.600±0.635	-	m	6.365	5.225
4	1.907±0.093	1.845±0.020	3.752±0.101	1.033±0.007	49.173±0.221	11.472±0.370	-	M	5.830	5.641
5	2.237±0.062	1.498±0.066	3.725±0.038	1.486±0.101	40.214±1.48	11.398±0.109	-	m	6.809	4.580
6	2.107±0.045	1.530±0.055	3.637±0.091	1.377±0.067	42.070±1.228	11.130±0.268	-	m	6.442	4.678
7	1.857±0.096	1.504±0.049	3.361±0.121	1.234±0.063	44.750±1.18	10.284±0.354	-	m	5.678	4.598
8	1.942±0.093	1.320±0.150	3.262±0.195	1.471±0.145	40.470±3.05	9.981±0.529	-	m	5.937	4.036
9	1.649±0.109	1.301±0.041	2.950±0.168	1.267±0.059	44.101±1.09	9.026±0.492	-	m	5.042	3.977
Mean	2.062±0.064	1.57±0.0540	3.661±0.096	1.320±0.063						
Total	32.705									

Chromosomal formula is $2n = 2x = 18 = 16m + 2M$

Table 3: Numerical data concerning the karyotype of *L. usitatissimum* (N = 5)

No. Pair	Long arm (L)	Short arm (S)	Total (L+S)	Arm ratio (L/S)	CI S*100/(L+S)	Relative length	sat	Type	L (%)	S (%)
1	1.710±0.088	1.258±0.057	2.968±0.133	1.359±0.050	42.358±0.854	11.260±0.500	-	m	6.491	4.775
2	1.220±0.060	0.914±0.072	2.134±0.018	1.334±0.192	42.830±0.284	8.050±0.067	-	m	4.631	3.469
3	1.076±0.077	0.852±0.028	1.928±0.057	1.260±0.132	44.190±2.323	7.308±0.216	-	m	4.084	3.234
4	1.190±0.028	0.632±0.073	1.820±0.059	1.880±0.310	34.720±2.854	6.890±0.219	-	sm	4.517	2.399
5	0.992±0.016	0.764±0.043	1.756±0.053	1.298±0.072	43.500±1.322	6.700±0.200	-	m	3.765	2.900
6	0.988±0.037	0.734±0.027	1.722±0.042	1.346±0.072	42.620±1.414	6.527±0.164	-	m	3.750	2.786
7	0.958±0.035	0.792±0.076	1.738±0.080	1.209±0.123	45.560±2.506	6.589±0.297	-	m	3.636	3.006
8	0.986±0.054	0.742±0.090	1.740±0.088	1.328±0.208	42.640±3.697	6.595±0.331	-	m	3.743	2.816
9	0.972±0.020	0.690±0.072	1.664±0.081	1.411±0.117	41.460±2.270	6.308±0.304	-	m	3.689	2.619
10	0.900±0.014	0.716±0.033	1.616±0.047	1.256±0.041	44.300±0.875	6.125±0.177	-	m	3.416	2.718
11	0.846±0.013	0.726±0.023	1.572±0.020	1.165±0.052	46.180±1.144	5.959±0.075	-	m	3.211	2.756
12	0.860±0.026	0.640±0.047	1.500±0.062	1.343±0.079	42.660±1.638	5.686±0.234	-	m	3.264	2.429
13	0.870±0.028	0.634±0.048	1.500±0.064	1.365±0.081	42.260±1.682	5.686±0.238	-	m	3.302	2.406
14	0.768±0.040	0.624±0.006	1.392±0.032	1.230±0.078	45.014±1.587	5.284±0.124	-	m	2.915	2.368
15	0.650±0.058	0.642±0.051	1.292±0.110	1.012±0.006	44.820±0.216	4.904±0.412	-	M	2.467	2.437
Mean	1.001±0.051	0.764±0.028	1.758±0.075	1.323±0.0491						
Total			26.342						56.881	43.118

Chromosomal formula is $2n = 2x = 30 = 26 m + 2 sm + 2M$, sm: submetacentric

isochromosome. The longest chromosome (No. 1) and the shortest chromosome (No. 15) were (1.992±0.065) and (1.224±0.045) micron. Most of the chromosomes are of metacentric type except chromosomes number 1 and 2 which are of submetacentric type. Average ratio of arms in this species is 1.81 and 1.84. Average length of the chromosomes is 1.545±0.039 micron and the length of the total genome is 24.727 micron.

Karyotypic details of this species are shown in Table 4 and Fig. 6A shows the metaphase plate of this species and Fig. 6B shows karyogram prepared of this metaphase.

Figure 10 shows the idiogram of this species with 15 + 1 haploid chromosomes. Average ratio of arms is 1.262±0.033 and chromosomal formula of this species is described as link 4:

Table 4: Numerical data concerning the karyotype of *Linum* spp. (N = 5)

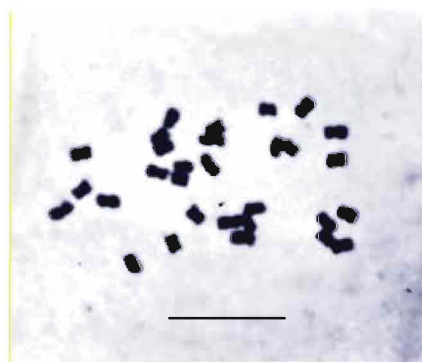
A No. Pair	Long arm (L)	Short arm (S)	Total (L+S)	Arm ratio (L/S)	CI S*100/(L+S)	Relative length	sat	Type	L (%)	S (%)
1	1.290±0.059	0.71±0.008	1.99±0.065	1.82±0.065	35.49±1.16	8.050±0.280	-	Sm	4.043	2.871
2	1.070±0.069	0.77±0.025	1.84±0.073	1.84±0.083	41.80±1.45	7.450±0.320	-	Sm	4.326	3.113
3	0.970±0.029	0.80±0.016	1.76±0.029	1.21±0.051	45.17±1.47	7.120±0.127	-	m	3.922	3.234
4	0.920±0.021	0.74±0.030	1.66±0.037	1.24±0.054	44.57±1.03	6.710±0.160	-	m	3.720	2.992
5	0.880±0.027	0.75±0.060	1.63±0.083	1.17±0.063	46.02±1.38	6.600±0.360	-	m	3.558	3.032
6	0.940±0.036	0.64±0.036	1.57±0.072	1.47±0.038	40.44±0.585	6.350±0.314	-	m	3.801	2.587
7	0.827±0.025	0.71±0.029	1.54±0.051	1.16±0.029	46.26±0.63	6.230±0.220	-	m	3.344	2.871
8	0.830±0.054	0.70±0.010	1.53±0.064	1.19±0.054	45.64±1.26	6.170±0.280	-	m	3.356	2.830
9	0.790±0.000	0.71±0.063	1.50±0.063	1.11±0.107	47.22±2.36	6.030±0.276	-	m	3.194	2.871
10	0.800±0.025	0.69±0.054	1.48±0.077	1.15±0.059	46.37±1.33	5.970±0.337	-	m	3.234	2.790
11	0.770±0.010	0.70±0.044	1.46±0.034	1.09±0.095	47.67±2.03	5.914±0.148	-	m	3.113	2.830
12	0.780±0.012	0.62±0.032	1.40±0.023	1.25±0.059	44.46±1.66	5.660±0.100	-	m	3.154	2.507
13	0.770±0.014	0.61±0.025	1.38±0.027	1.25±0.074	44.37±1.32	5.580±0.117	-	m	3.113	2.466
14	0.710±0.037	0.60±0.051	1.32±0.015	1.18±0.127	45.77±2.37	5.320±0.065	-	m	2.871	2.426
15	0.620±0.025	0.60±0.019	1.22±0.045	1.03±0.009	49.18±0.212	4.940±0.105	-	M	2.507	2.426
16	0.730	0.73	1.46	1.00	50.00	5.900	-	M	2.951	2.951
Mean	0.854±0.0281	0.69±0.015	1.55±0.039	1.26±0.033	45.026±0.574	6.28±0.172				

Chromosomal formula is $2n = 30+1 = 4 sm + 24 m + 3M$

Table 5: Karyotypic symmetrical parameters

species	2n	sat	S/L	TL	S (%)	DRL	TF (%)	Karyotypic formula
<i>L. austeriicum</i>	18	1	0.65	23.12	9.710	3.08	47.01	$2n = 12 m + 2 sat + 4 M$
<i>L. nervosum</i>	184.1	-	0.69	32.70	9.020	4.10	42.99	$2n = 16 m + 2 M$
<i>L. usitatissimum</i>	30	-	0.43	26.34	4.904	6.36	43.41	$2n = 26 m + 2 sm + 2 M$
<i>Linum</i> spp.	30+1	-	0.61	24.72	4.940	3.11	44.68	$2n = 4 sm + 24 m + 3 M$

TF%: Total form percentage, DRL: The difference between minimum and maximum, relative Length of chromosomes, S%: Relative length of the shortest chromosome, TL: Total Length; Total length of a group of chromosomes by micron, S/L: The ratio of the smallest chromosome to the longest one. sat: satellite



A



B

Fig. 6: A) Mitotic metaphase plate of *Linum* spp., B) Kayogram, constructed from chromosomes of this plate ($2n = 2x = 30$), Bar = 5 μ m

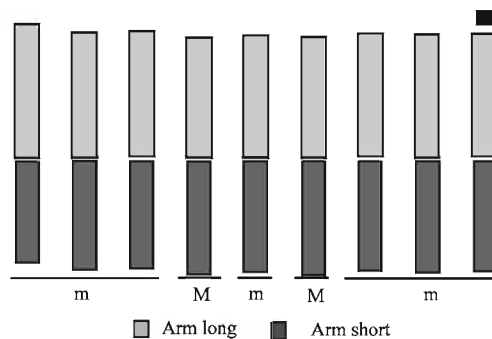


Fig. 7: Idiogram of haploid chromosomes of *L. austeriicum* L. $2n = 18$, Bar = 5 μ m

Link 4 $\rightarrow 2n = 30 + 1 = 4 sm + 24 m + 3 M$

Flax has different species but *L. usitatissimum* L. species from the point of commercial view is more valuable than the others as this species is of the oil seeds and fiber plants. Also medically and industrially it is valuable. Little cytological and limited chromosomal counting studies related to these species have been made all of which similar to our research (Muravenko *et al.*, 2001a; Muravenko *et al.*, 2003). The differences between absolute size of the chromosomes being manifested in species probably show the differences in one-by-one expansion of genes or the acceleration of

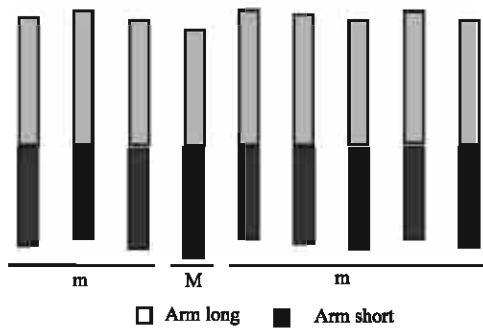


Fig. 8: Idiogram of haploid chromosomes of *L. nevosum* L. $2n = 18$, Bar = 5 μ m

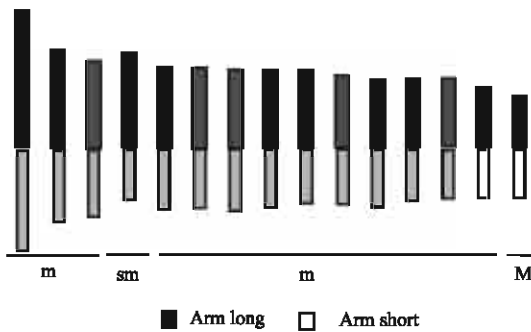


Fig. 9: Idiogram of haploid chromosomes of *L. usitatissimum* L. $2n = 30$, Bar = 5 μ m

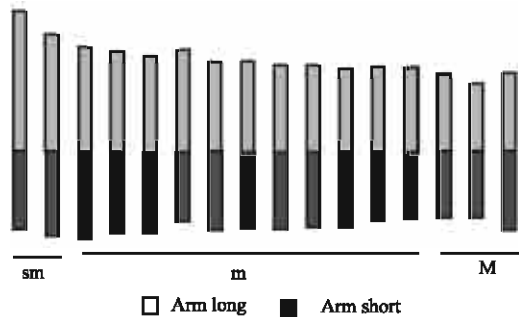


Fig. 10: Idiogram of haploid chromosomes of *Linum* spp., L. $2n = 30$, Bar = 5 μ m

polytenychromomemata. And the differences in number and position of satellite show the existing differences in place and size of nucleus areas. This phenomenon is followed by the differences in heterochromatination (Agayev, 1998). As observed from Table 1-4 absolute size of the chromosomes is different in these four species. It may be because of that the developmental process has effected the changes in absolute size of the chromosomes. Karyotype asymmetry parameters are presented in Table 5. According to these karyotypic parameters

symmetry is different in these three species as in TF% view the most is for *L. austeriacum* species and the least for *L. nevosum* species. It should be mentioned that TF% parameter has an opposite relation with karyotype symmetry. So *L. nevosum* has the most symmetrical karyotype and *L. austeriacum* has the non-symmetrical karyotype. And from DRL parametrical view the less amount shows the less symmetry. In vegetative series, symmetrical karyotypes are primary and the tension is from symmetrical to non-symmetrical side. So based on this statement, it seems that *L. austeriacum* type has the most non-symmetric karyotype ratio to the others among which have been studied. And probably, it is more developed than two other types that proving this these needs more examinations to be performed. There are spreaded karyotypical diversity in ploid level, the number of the base chromosomes and karyotypical asymmetry parameters within (our) four studied cases. Which could not only make genetical diversity, but also prevent of performing successful intra specific inosculation that this research was being performed in order to show the possibility of this.

For doing intra-interspecific inosculation, choosing of base parents should be performed on the basis of high similarity at karyotypes of course in respect of these parameters, could prevent of successfully doing intra specific inosculation; subsequently lead to inabequancy in reproduction and also producing fules seeds as results. In other words, it is possible the results of this inosculation to be somewhat barren. High autogamy and lack of karyotypical asymmetry in flax species have made the act of breeding so difficult. As previously mentioned, Mouravenko *et al.* (2001a), conducted the study on the (*L. austeriacum* L. and *L. grandiflorum*), so the results of this study are not similar to our results both in size and morphology of chromosomes.

There are two reasons of those dissimilarities: first, the material and methods and the facilities of lab used, by them, were different by those that we-applied. Socond, these species are dispersed in different climates throughout the world, so that the diversity of karyotype and in the number of base chromosomes have been brought into different populations of the species.

Finally, the most important result taken of this study is *Linum* spp. species/population that has shown the different morphologic and karyotypic diversity in comparison with other species. There are several interesting cases about this species that we would make to point to some items:

- This species/population was wild plant and collected from Markazi province's regions.

- This species/population was not showing similarity with any present species in Iran.
- The study performed on this species/population showed (that), it had high seed yield in comparison with cultivated genotypes.

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