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Full Length Research Paper

Investigation of genetic variation on different populations of *Silybum marianum* L. (Fabaceae) by using cytogenetic parameters

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This investigation was carried out on different ten diploid populations of *Silybum marianum* which was collected from different regions of Iran. The base number of chromosomes in total studied populations were equal to x = 17. On based of two ways Stebbin's table, all of the population located in the second class of this table, that indicated which total populations are similar as evolutionary. Analysis of variance showed significant differences for the total chromosome length, sum of long arms, coefficient of variation centromic index and asymmetric index ($p<0.01$), the largest and the smallest length of chromosome, sum of small arms, difference of range relative length and arm ratio ($p<0.05$). Factor analysis introduced three factors that justify nearly 96% from total variation among data. In the first factors, the total chromosome and sum of long arms had highly load factor and named as length chromosome factor. In the second factor asymmetric index had highly load factor and named as asymmetric factor. The third factor difference of range relative length had highly load factor. Cluster analysis grouped populations in 4 groups. The least Euclidean distance observed between Ahvaz and Sary population and the highest Euclidean distance was between Ahvaz and Rasht population. Analysis of variance on parameters among of clusters showed significant differences for all parameter except difference of range relative length, among groups. Ahvaz, Sary, Esfahan populations in the first group had maximum amount of total chromosome length, the largest and smallest length of chromosome, sum of long and small arms, difference of range relative length than other investigated populations. Kordestan population located in the fourth group from asymmetric index, coefficient of variation centromic index and arm ratio were better than other investigated groups.

Key words: *Silybum marianum* L., cytogenetic, chromosome.

INTRODUCTION

The milk thistle (*Silybum marianum* L.) is an annual or biannual herbaceous plant which is widespread in temperate American countries, Australia and Mediteranean climates (Cach et al., 1999; Hadolin et al., 2001). The seeds of this plant is including of the 3-oxyflavone silymarin, an isomeric mixture of three flavonolignans (silychristin, silydianin and silybin). Flavonolignans has the antihepatotoxic activity which can be used for medicinal purposes (Hikino and Kiso, 1984). *S. marianum* has been used in homoeopathic medicine in India, where its seeds are used in the control of the liver,related liver and gallstone conditions (Varma et al., 1980). Silybum belongs to the carduinae subtirbe of cardueae tribe in Asteraceae family. *S. marianum* is a diploid species with 2n = 2x = 34 chromosomes (Asghari-Zakaria et al., 2008; Ghaffari, 1989; Kamel, 2004; Mohamed, 1997; Wagner et al., 1974). They also reported six metacentric, ten submetacentric and one acrocentric chromosomes. One of the chromosomes (chromosome 1) had more or less large secondary constriction on centromeric region of its short arm. The SAT chromosome had minor variation in size among cells and populations, probably due to differences in chromosome contraction (Mohamed, 1997).

The main purpose of this survey is the investigation of genetic variation on different Iranian populations of

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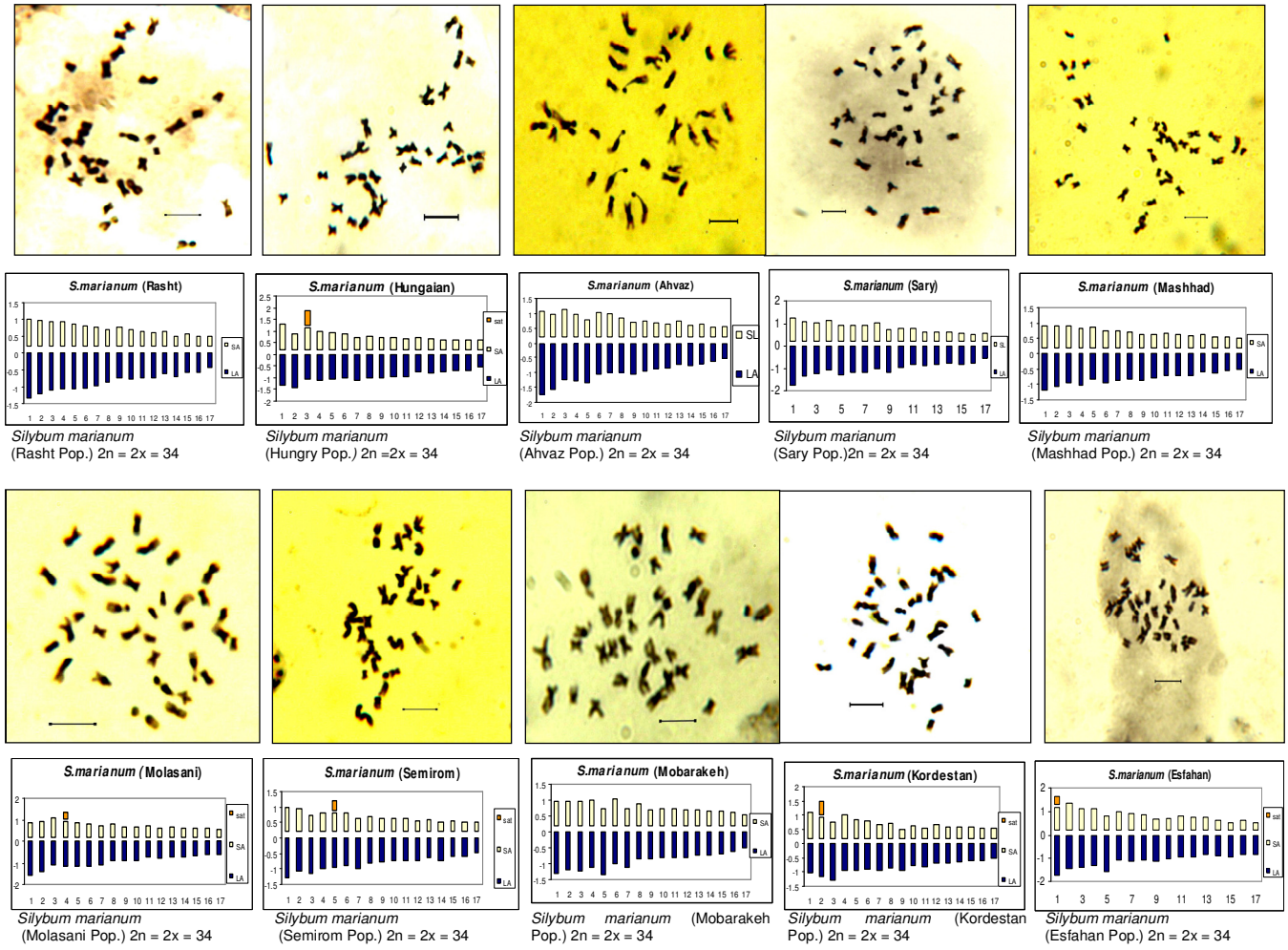

Figure 1. Somatic metaphase chromosomes and ideograms of *Silybum marianum* stained with aceto-iron-hematoxilin, scale bar 3 µm.

S. marianum L. by using cytogenetic parameters.

MATERIALS AND METHODS

Seeds of nine population of *S. marianum* collected from various geographical locations of Iran (kordestan, Semirom, Mobarakeh, Esfahan, Ahvaz, Molasani, Rasht, Sary and Mashhad) and one seed population belong to Hungry country. To observe metaphase plates, root-tip meristems were immersed in 0.01 M α-Boromo naphthalene at 4°C for 1 h and then fixed in cromic acid-formalin (2:1v/v) at 4°C for 34 h after which they were washed and preserved in ethanol at 4°C until used. To observe metaphase chromosomal, meristematic tissue were hydrolyzed with 1 N-NaOH for 18 min and stained with aceto iron hematoxylin at 25 to 30°C for 10 h. The roots were then gently squashed in a drop of 45% acetic acid. Images were captured with a BX50 olympus camera. For numerical karyotype analysis, chromosomes from three metaphases were measured for each population. Long and short arm of chromosome, chromosome length, total length of chromosome set, arm ratio and r-value were determined using micro measure software. Karyotype asymmetry was estimated using difference of relative length, coefficient of variation centromic index and asymmetric index. Chromosome type was determined according to Levan et al. (1964) and to study symmetric used from Stebbins and Romero-Zarco methods (Romero, 1986; Stebbins, 1971). For the share determination of parameters on variation between populations used from principal components analysis (PCA) and cluster analysis were performed.

Also, analysis of variance on parameters among of clusters was performed according to Duncan's method and Euclidean distance 7). Statistical analysis was performed by using SAS software.

RESULTS AND DISCUSSION

Mitotic chromosomes pictures and ideograms of the ten *S. marianum* population are shown in Figure 1. Results showed that *S. marianum* is a diploid species with 2n = 2x = 34 chromosomes. All of the populations except Kordestan population had chromosome of metacentric and submetacentric. Kordestan population was including 9 m^{sat} + 7 sm + 1 st karyotype formulae and had three type chromosome of metacentric, submetacentric and

Table 1. Results of karyotype parameters on different *Silybum marianum* populations. DRL%: difference of relative length, TF%: percentage of total from, A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index, AI: asymmetric index, AR: arm ratio, L%: relative length percentage of long arm, SC: set of chromosome.

Population	Scientific name	2 n	DRL%	TF%	A_1	A_2	AI	SC	Type	AR	L%
Ahvaz	*S. marianum*	34	6.30	37.39	0.38	0.31	3.55	2B	8 m + 9 Sm	1.77	62.56
Sary	*S. marianum*	34	6.96	37.63	0.39	0.29	3.52	2B	9 m + 8 Sm	1.80	62.37
Esfahan	*S. marianum*	34	5.10	36.40	0.49	0.28	2.81	2B	8 m + 9 Sm^{sat}	1.95	63.60
Rasht	*S. marianum*	34	5.90	38.22	0.36	0.30	2.47	2B	13 m + 4 Sm	1.72	61.78
Kordestan	*S. marianum*	34	4.84	36.96	0.39	0.26	4.25	2B	9 m^{sat} + 7 Sm + 1^{St}	2.78	63.04
Hungry	*S. marianum*	34	6.02	38.56	0.36	0.26	3.14	2B	7 m^{sat} + 10 Sm	1.71	61.44
Mashhad	*S. marianum*	34	4.93	38.34	0.37	0.25	1.91	2B	12 m + 5 Sm	1.69	61.66
Molasani	*S. marianum*	34	5.14	36.33	0.37	0.25	2.60	2B	6 m^{sat} + 11 Sm	1.82	63.59
Semirom	*S. marianum*	34	5.64	36.77	0.41	0.26	2.25	2B	9 m^{sat} + 8Sm	1.82	63.23
Mobarakeh	*S. marianum*	34	4.69	38.63	0.41	0.26	2.52	2B	14 m + 3 Sm	1.64	61.37

Table 2. Analysis of variance on karyotype parameters in different *Silybum marianum* populations. *, ** significant differences at probability levels of 0.01 and 0.05 respectively.

Source	DF	Square mean								
		TL	LC	SC	SSa	SLa	DRL	AI	CVci	AR
Population	9	**21.84	*0.31	*0.04	*2.86	**9.34	*1.63	**1.50	**18.56	*0.33
Error	20	5.86	0.09	0.01	1.03	2.68	0.60	0.08	0.30	0.12
Coefficient Variation	-	9.53	13.67	13.53	10.67	10.32	14.05	10.20	5.14	18.60

subtelocentric. The most number of metacentric chromosome belonged to Mobarakeh population with 14 m + 3 sm karyotype formulae. Esfahan, Hungry, kordestan, Molasani and Semirom populations had secondary constriction on centromeric region of its short arm. The most amount of total form percentage (TF%) belonged to Mobarakeh population and the least amount belonged to Esfahan and Molasani populations. The most amount intrachromosomal asymmetry index (A_1) and relative length percentage of long arm (L%) related to Esfahan population and the least amount related to Mobarakeh population (Table 1). Analysis of variance showed significant differences for all studied parameters ($p<0.05$). Mean comparison of studied parameters among populations showed that Esfahan population had the most amounts of TL, SC, SSa, SLa and the least amount of these parameters belonged to Mashhad population. Kordestan population was better of AI, CVci, and AR parameters than other populations, the least amount of DRL and AR belonged to Mobarakeh population. On based of TL parameter, studied populations located in 8 groups and according to AR, populations located in 2 groups (Tables 2 and 3). Factor analysis introduced three factors that justify nearly 96% from total variance among data. In the first factors, the total chromosome and sum of long arms had highly load factor and named as length chromosome factor. In the second factor asymmetric index had highly load factor and named as asymmetric factor. The third factor difference of relative length had highly load factor and named as range of chromosome (Table 4). Cluster analysis grouped populations in 4 groups (Figure 2). The least Euclidean distance was between Ahvaz and Sary population and the highest Euclidean distance observed between

Table 3. Mean comparison of karyoype parameters on different *Silybum marianum* populations. The same letter in each column indicates non significant differences.

Population	TL	LC	SC	SLa	SSa	DRL	AI	CVci	AR
Ahvaz	27.45 ^{abc}	2.63 ^{abc}	0.87^{b}	17.25^{abc}	10.18 ^{ab}	6.30 ^{ab}	3.54 ^{b}	11.38 ^{b}	1.76 ^{b}
Sary	28.46 ^{ab}	2.77 ^{a}	0.97 ^{ab}	17.73 ^{ab}	10.72 ^{a}	6.95^{a}	3.52 ^{b}	12.26 ^{b}	1.80 ^{b}
Esfahan	30.017 ^{a}	2.70 ^{ab}	1.17 ^{a}	19.08 ^{a}	10.93 ^{a}	5.10^{bc}	2.81 ^{cd}	10.14 ^{c}	1.94 ^{b}
Rasht	23.43 ^{cd}	2.14 ^{bcd}	0.75 ^{b}	14.45 ^{cd}	8.97 ^{ab}	5.90 ^{abc}	2.47 ^{de}	8.24 ^{d}	1.71^{b}
Kordestan	22.62 ^{d}	1.93^{d}	0.84 ^{b}	14.26 ^{cd}	8.35 ^{b}	4.83^{bc}	4.25 ^{a}	16.06 ^{a}	2.78 ^{a}
Hungry	26.38 ^{abcd}	2.39 ^{abcd}	0.94 ^{b}	16.21 ^{abcd}	10.17 ^{ab}	6.02^{abc}	3.14 ^{bc}	12.31 ^{b}	1.70 ^{b}
Mashhad	22.34 ^{c}	1.91 ^{d}	0.81 ^{b}	13.73 ^{d}	8.60 ^{b}	4.93^{cb}	1.90 ^{f}	7.66 ^{d}	1.69 ^{b}
Molasani	25.37 ^{bcd}	2.23 ^{abcd}	0.93 ^{b}	16.17 ^{abcd}	9.20 ^{ab}	5.13^{cb}	2.59 ^{de}	9.87 ^{c}	1.81^{b}
Semirom	22.44 ^{d}	2.06 ^{cd}	0.79 ^{b}	14.18 ^{cd}	8.26 ^{b}	5.64^{cab}	2.25 ^{ef}	8.47 ^{d}	1.82^{b}
Mobarakeh	25.46 ^{abcd}	2.05 ^{cd}	0.85 ^{b}	15.61 ^{bcd}	9.85 ^{ab}	4.69 ^{c}	2.52 ^{de}	10.20 ^{c}	1.64^{b}

Table 4. Principals components of karyotype parameters on different *Silybum marianum* populations.

Parameters	PCI	PCII	PCIII
TL	0.97	0.04	0.21
LC	0.85	0.06	0.49
SC	0.94	0.11	-0.20
SSa	0.92	-0.02	0.28
Sla	0.97	0.07	0.17
DRL	0.21	0.02	0.96
AI	0.19	0.93	0.26
CVci	0.13	0.96	0.06
AR	-0.17	0.88	-0.33
Total	4.49	2.61	1.54
Percent of variance	49.97	29.03	17.13
Cumulative percent	49.97	79	96.14

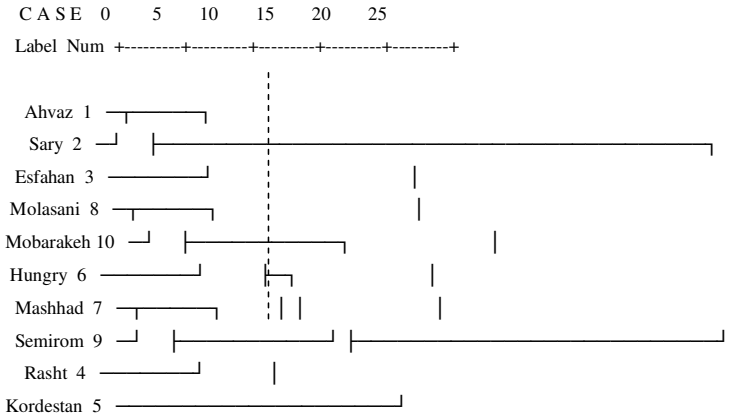

Figure 2. Dendrogram of cluster analysis using ward method on karyotype parameters in different *Silybum marianum* populations.

Ahvaz and Rasht population (Table 5). Analysis of variance on parameters among of clusters showed significant differences for all parameters except difference of range relative length, among groups. Ahvaz, Sary, Esfahan populations in the first group had maximum amount of total chromosome length, the largest and smallest length of chromosome, sum of long and small arms, difference of range relative length than other

Table 5. Euclidean distance between populations in cluster analysis on *Silybum marianum*.

Stage	Population	Population	Euclidean distance
1	1	2	0.97
2	8	10	2.1
3	7	9	3.29
4	1	3	5.63
5	6	8	8.09
6	4	7	10.59
7	4	6	15.38
8	4	5	20.69
9	1	4	31.59

Table 6. Analysis of variance on studied parameters among clusters in different *Silybum marianum* populations.

Parameters	Mean square	Mean within groups			
		Group 1	Group 2	Group 3	Group 4
TL	60.85 **	28.64 ^{a}	25.74 ^{b}	22.73 ^{c}	22.62 ^{c}
LC	0.83**	2.70 ^{a}	2.22 ^{b}	2.03 ^{b}	1.93 ^{b}
SC	0.07*	1.00 ^{a}	0.91 ^{ab}	0.78 ^{b}	0.84 ^{ab}
SSa	7.55**	10.61 ^{a}	9.74 ^{ab}	8.61 ^{bc}	8.35 ^{c}
Sla	25.74**	18.02 ^{a}	15.99 ^{b}	14.12 ^{b}	14.26 ^{b}
DRL	1.70	6.12 ^{a}	5.28 ^{ab}	5.49 ^{ab}	4.83 ^{b}
AI	3.78**	3.29 ^{b}	2.75 ^{c}	2.20 ^{d}	4.25 ^{a}
CVci	49.55**	11.26 ^{b}	10.79 ^{b}	8.12 ^{c}	16.06 ^{a}
AR	0.95**	1.83 ^{b}	1.72 ^{b}	1.74 ^{b}	2.78 ^{a}

**, * significant differences ($p<0.01$), ($p<0.05$). The same letter in each row indicates non significant differences.

investigated populations. Kordestan population in the fourth group was better from asymmetric index, coefficient of variation centromic index and arm ratio (Table 6). Therefore, karyotype of Esfahan and Kordestan investigated populations are asymmetric. Also, karyotypes of Mobarakeh and Mashhad investigated populations are symmetric. Kordestan and Esfahan Populations are more evolutional than other populations; Mashhad and Mobarakeh are elementrary than other populations. For generating of maximum variation in the plant society should be carried out sygosis between Kordestan and Esfahan with Mobarakeh and Mashhad populations. Our results that *S. marianum* is a diploid species with 2n = 2x = 34, is in agreement with the results of other research works concerning chromosomal study of Silybum (Asghari-Zakaria et al., 2000; Ghaffari, 1989; Kamel, 2004; Mohamed, 1997). In another study Van Loon (1974) and Asghari-Zakaria et al. (2000) showed that the chromosome number of this species was 2n = 34.

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