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Cytological study of chromosome and genome composition of Iranian Lettuce (*Lactuca sativa* L.) accessions

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ABSTRACT

In this study, 15 different types of Iranian lettuce accessions that were collected from different regions were investigated. Using root apical meristem, the number and size of the chromosomes were measured and the karyotypic formula was determined. The basic chromosome number was 9 (n=9) in all accessions and their chromosomal types were metacentric, submetacentric and subtelocentric. Analysis of variance showed significant differences between chromosomal characteristics. The maximum and minimum length of the genome was belonged to Qom (62.45 µM) and Babol (19.94 µM) accessions, respectively. Symmetrical and asymmetrical karyotypes were observed between the accessions. Formula in Ahwaz accession was 12 m + 4 sm + 2 t, which had the maximum centromic index (40.93%), while Fasa accession with 4 m + 10 sm + 4 st karyotypic formula had the minimum value (28.73). There were differences in having satellites on chromosomes between the accessions, some of them had 2 satellites, some 1 and the others had not any satellite. In principle component analysis, two components had more than 82.75% of the data variations. Based on the first and second components, the accessions separated into three groups. Cluster analysis of karyotypic traits based on UPGMA method separated accessions in three groups. The results of this study are useful for accession's classification.

Keywords: Cytogenetic, Karyotype, Lettuce, Satellite, Symmetry.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a dicotyledonous plant which belongs to Asteraceae family and Chicorideae subfamily. This plant is one of the important leafy vegetables which are used for salad and fresh marketing, also some types of them is used in baked type [7]. This genus has more than 100 species and 6 types. Different types of lettuce include; Romaine (cos), Crisp head (Iceberg), Butter head, Stem (Asparagus), Leaf (Cutting) and Oilseed lettuce [16]. In modern lettuce breeding, the genetic resources of lettuce species that conserved in the world's gene bank are very important [10]. The role of wild species, such as *L. serriola*, *L. aculeate*, *L. saligna* and *L. virosa* has been intensively studied by lettuce breeders [2]. Lettuce species have been shown to be diverse by morphological analysis and molecular and biochemical markers [9]. Chromosomal studies have played an important role in accelerating crop improvement [3]. On the other hand, cytogenetic studies are also related in the study of plant evolution and diversification [18]. For concluding developmental trends within particular plant groups and analyzing traits, karyotype characteristics has provided valuable data, such as changes in chromosome numbers [15], also, in chromosome length, and in karyotype symmetry [19]. Lebeda et al. [9] reported that there are 98 species of lettuce (*Lactuca* spp.) in the world, which included from 17 European, 15 African, 12 American, 3 Australian and 51 Asian species. In these 51 species, the greatest species richness is distinguished in Iran, India and Pakistan (15, 18 and 23 species, respectively). Lebeda [8] divided the number of lettuce chromosomes into three groups; a) n=8 in European and Himalayan species, b) n=9 in Indian, Mediterranean, African, Asian and some European species, and c) n=17 in Northern American from Canada to Florida. The origin of Asian species *L. aculeata* and *L. scarioloides* is 2000-3000 m altitude of Afghanistan, Iranian Azerbaijan and Iraq Kurdistan [8]. There are many studies about the chromosome number of this genus [5, 15, 17]. In these studies, evolution and phylogenic relationship of inter-specific in lettuce were evaluated. Karyotypic analysis and chromosome structure in domestic lettuce (*L. sativa*) and wild lettuce (*L. virosa, L. serriola, L. saligna*) were studied by FISH method [13]. The results showed that n=9 in all species, but the size of chromosome in *L. saligna* is shorter than the other three species. On the other hand, *L. virosa* had the longest chromosomes between the other three species, also had different karyotypic shape between them. *L. virosa* has a different pair of satellite chromosome. The maximum karyotipic similarity was between *L. sativa* and *L. seriola* in comparison with *L. sativa* and *L. virosa* [4]. Although only one satellite pair of chromosomes has been reported in *L. virosa* and two pairs of satellite in *L. sativa, L. serriola* and *L. saligna* [4] but the results of Matoba et al. [13] studies showed only one pair satellite in *L. saligna* and two pairs of that in *L. serriola*, *L. saligna* and *L. virosa*. In some species, which have different names in production areas using of cytogenetically markers is a useful method for naming and identifying of them [2]. The use of inter-specific crossing is a useful technique to increase environmental stress tolerance, diseases, yield, increasing quality and to overcome agricultural deficiency [8]. There is a high diversity in Iranian lettuce accessions and these accessions were considered as a valuable resource for breeding programs. Because there is no any cytological study about Iranian lettuce, in this study, we counted and measured chromosomes in selected accessions at metaphase of mitosis. Also, the karyotype of accessions was determined to identify chromosome number. Current study evaluated the shape and size of the chromosomes in all accessions to identify the possibility of inter-specific crossing by using multivariate statistics, too.

MATERIALS AND METHODS

In this study, 15 accessions of Iranian lettuce were investigated by chromosome specifications. The seeds were collected from Iranian research centers (Table 1). The seeds were sterilized by 5% bleach water for 15 min and after washing were placed in Petri dishes on wet filter paper in germinator at 20°C. After 72 h, the rootlets length was 1.5-2 cm; the apical meristem was used for cytogenetically studies and was placed in pretreatment for 4 h in 0.002 M 8-hydroxyquinoline solution at room temperature. After washing by double distilled water, the material was fixed in Acetic acid-ethanol (3:1) and stored at 4°C in refrigerator. After 24 h, three times washing of roots, samples stored in 70% ethanol. For cytogenetically studies in mitotic phase, the samples were hydrolyzed in 1% hydrochloric acid for 5 min in 60°C water, and then stained with 2% aceto-orcein in 30°C for 1 h. After squashing, five mitotic metaphase spreads were studied for each accession.

In all accessions, for the numerical characterization of the karyotypes, the following parameters were calculated; total chromosome length, long arm length, short arm length, arm ratio, centromic index was measured by Micro measure software. Also, other karyotypic parameters such as total form percentage, differences of relative length and relative length of shortest chromosome were measured. To determine karyotypic symmetry in the accessions used two-sided Stebbins table. Ideograms were constructed by organizing the chromosomes into grouped according to their centromeric index, ordering them by decreasing length within each category, and finally numbering them consecutively using the same scheme. Parameter means were compared by one-way ANOVA after Bartlett test of homogeneity. Distribution diagram was achieved based on the first and second components (PCoA). Analysis of variance accomplished for chromosomal traits analyzing. The comparison of traits mean was multi-range Duncan in 1% probability. To determine the share of each trait in diversion between the accessions, principle component analysis was accomplished. Classification of accessions clustering was performed using the unweighted pair-group method (UPGMA). For statistical analysis of data, SPSS ver.16 was used.

RESULTS AND DISCUSSION

Qom accession was stem lettuce and has anthocyanine, but Neishaboor was leaf lettuce. The other accessions were similar morphologically and all of them were romaine type. Figs. 1 and 2 show the metaphase mitotic chromosomes and ideogram of accessions. The basic genome chromosome number was 9 (n=9) and all accessions were diploid (2n=2x=18). Previous studies confirmed these results [13]. Karaj, Neishaboor, Gorgan, Babol, Varamin, Shiraz, Zirehi, Hamadan, Jahrom and Parsabad accessions have not satellite. Abtavil, Ahvaz and Fasa accessions have 1 pair of satellite; also Borazjan and Qom have two pairs of satellites. Other studies showed that lettuce species have one or two pairs of satellites [4]. Matoba et al. [13] reported that *L. sativa* has 2 pairs of satellites. The studies of

Matoba [14] demonstrated that there was not relationship between the number of satellites and other karyotypic traits. Although the number of basic chromosome is the same in all of the accessions, but there is diversity in size of chromosomes. These results have conformity with Matoba et al. [13] in karyotypic analysis and chromosomal structure. All of the accessions have little chromosomes; therefore, these accessions have a lower chromosome length mean in comparison with other species. The comparison of genome length showed that the maximum and minimum genome length belong to Qom (62.45 µM) and Babol (19.94 µM). Karyotypic formula of the accessions determined based on Levan et al. [11] by arm ratio. Table 2 shows karyotypic characteristics. The results showed that the accessions have metacentric, submetacentric and subtelocentric, which is confirmed by other studies on *L. sativa* [6, 13]. Evident from Tables 3 and 4, the analysis of variance and mean comparison of chromosomal traits showed significant differences between total length of chromosome, length of long arm, length of short arm, relative length and centromeric index in 1% probabilities. These significant differences show karyotypic variety between the accessions. Because of these results, study about chromosomes to determine the evolution and relativity between the accessions is necessary. There were a high level of diversity between the accessions in short and long arm length (Table 2). The results showed Ahwaz with 12 m + 4 sm + 2 st karyotypic formula had the highest total polymorphic percentage (40.93), while Fasa with 4 m + 10 sm + 4 st karyotypic formula had the lowest total polymorphic percentage (28.73). Jahrom had the highest (56.78%) and Fasa had the lowest (19.61%) relative length of the shortest chromosome (S %). Varamin had the highest differences of relative length (DRL) (14.05%) and Jahrom had the lowest (6.22 %).

Eukaryotic chromosomes can vary in shape, number and size. These traits are subject to evolutionary changes and may vary between and even within individual organisms [20]. The size of the chromosome is also a trait subject to evolutionary change. This variation could originate by reciprocal translocation, sequence amplification loss of dispensable part and insertion [20]. The instructing of chromosome evolution could be for an increase [1] and decrease in chromosome size [12]. The size of the chromosomes varied in lettuce species [13]. In current study, these characteristics were between 9.41 µM in Qom and 0.93 µM in Parsabad, respectively. In lettuce species, the short chromosomes should be a more ancestral character than longer ones [9] and this suggestion is identical with the proposal that species with a lower DNA content are more initial [6, 13]. Karyotypic formula in the lettuce species are moderately symmetrical and consist often exclusively of m and sm chromosomes, also st chromosomes observed in this genus. *L. sativa* and *L. serriola* have only one pair and *L. saligna* has two pairs and *L. virosa* has three pair's st chromosomes [13]. Present study also showed 9 types of different karyotypic formula in the accessions (Table 2). Representative karyotype of present accessions show that, Abtavil, Ahvaz, Babol and Shiraz accessions have only one pair st and Karaj, Hamadan and Fasa accessions have two pairs st and others accessions without any st chromosomes.

Table 1. Code, origin, type and geographical location of different Iranian lettuce accessions.

			Geographic location				
			Longitude		Latitude		Altitude (m)
Code	Origin	Type	Minute	Degree	Minute	Degree	
1	Abtavil	Romaine	27	50	46	28	0
2	Borazjan	Romaine	38	51	21	29	0
3	Ahvaz	Romaine	17	48	25	31	30
4	Karaj	Romaine	27	51	48	35	1360
5	Qom	Stem lettuce	53	50	38	34	930
6	Neishaboor	Leaf lettuce	47	58	12	36	1210
7	Gorgan	Romaine	17	53	28	36	45
8	Babol	Romaine	12	53	34	36	45
9	Varamin	Romaine	39	51	19	35	915
10	Shiraz	Romaine	22	52	37	29	1540
11	Kazeroon	Romaine	24	51	25	29	985
12	Hamadan	Romaine	31	48	48	34	1851
13	Parsabad	Romaine	38	48	28	38	1280
14	Jahrom	Romaine	33	53	30	28	1050
15	Fasa	Romaine	39	53	56	28	1370

Chromosomal symmetry is one of the karyotypic parameters, that most used to explain evolution in plants. This trait is widely accepted to be more preparatory than asymmetrical ones, when the comparison is between the same groups of vascular plants [20]. Neishaboor, Jahrom, Qom and Babol accessions failed in “2A” and “3A” category had the most symmetrical karyotype. While Zirehii, Parsabad and Fasa accessions failed in “3C” category had asymmetrical karyotype, which shows evolutions. Other accessions failed in “3B” category had asymmetrical karyotype. In lettuce, it was not detected a relationship between asymmetry and advanced taxa. We found an association between asymmetrical karyotype and shorter total chromosome length (Table 2). The possible evolutionary direction in lettuce would be from accessions with smallest chromosomes and more asymmetrical karyotypes in regions considered primaries, to accessions with longer chromosomes and more asymmetrical karyotypes. Therefore, increases in chromosome length are attached in karyotype symmetry. Probably, increase amount of DNA was unequally spread in the chromosome arms of the complement, giving rise to accessions with larger chromosomes.
Principle component analysis (Table 5) was done to determine the role of each studied traits. The results showed that traits classified into two components which had 82.75% of the variety between the accessions. The first component showed chromosome length, long arm length and short arm length had the most effect. Therefore, this component affected by the chromosomal traits. The most effective trait in the second component was arm ratio. To classify the accessions, cluster analysis was done based on UPGMA and accessions in 20% of Euclidean distance classified in three separated. Centromeric index (11.11), short arm length and arm ratio are the clear characteristics of this group. The second groups had two subgroups. The first group consisted of Abtavil and Borazjan accessions. The similarities of centromer index had two subgroups which consist of; 1) Neishaboor, Gorgan, Babol, Varamin, Shiraz, Zirehii, Hamadan, Parsabad and Jahrom accessions, and 2) Ahwaz, Karaj and Qom accessions. Based on the mean comparison, Qom accession had 6.94 µM total length, 4.64 µM long arm length and 2.30 µM short arm length. Also, this accession had the most number of submetacentric (16 sm) and the lowest number of metacentric chromosome, and the highest average chromosome length. Based on morphological studies, this accession belongs to stem lettuce. Other accessions in the second group had the same arm ratio, and centromeric index. These accessions had the highest number of metacentric chromosomes. Based on Table 2, Fasa accession was the only accession in the third group, which had the lowest arm ratio (0.41) and the lowest average of centromeric index (28.73). Also, it had the lowest relative length of shortest chromosome (19.61 S %). The most similarity was between Abtavil and Borazjan accessions, and the least was between Fasa with Abtavil and Borazjan accessions. Based on the results, although these accessions in morphological traits are similar, but they had different chromosomal traits. Also, these accessions in karyotypic formula, Stebbins symmetry class and the average length of the genome were different. Totally, Babol accession had the lowest total length of the genome (19.94 µM) and Qom had the highest total length (62.45 µM). Karyotypic studies about these accessions were for the first time.

Distribution diagram (PCoA) based on the first and second components separated the accessions into three groups. The greater importance of such characteristics was revealed in cluster and PCoA (Fig. 3). Qom (5) accession it is distinguishable and stood far apart from all genotypes in the study due to, the highest total length, long arm and short arm (6.94, 4.64, 2.30 µM), respectively. In Babol (8) accession, it is distinguishable and stood far apart from the all genotypes due to presence the lowest total length, long arm and short arm (2.22, 1.34, 0.87 µM, respectively). Also Abtavil (1) with the lowest centromer index (11.11%) and Fasa (15) with the highest arm ratio (2.80 µM) were distinguished and stood far apart from the other accessions. Cluster and principal component analysis on characters revealed the existence of variability among the investigated accessions. The greater importance of such characteristics was revealed in cluster and PCoA. Total length, long arm and short arm had positive values in PC1. At the same time, arm ratio had positive values in PC2.

Table 2. Chromosome and karyotypic characteristics of different Iranian lettuce accessions.

Accession	TL	SA	LA	r-value	AR	d-value	Longest chromosome	Shortest chromosome	CI	S%	DRL%	SAT	Category	Karyotype
Abtavil	47.05	17.02	30.03	1.72	0.57	13.01	7.11	3.11	36.17	43.74	8.49	2	3B	8M+6m+2sm+2st
Borazjan	44.95	16.59	28.36	1.71	0.58	11.77	7.14	3.05	36.91	42.72	9.1	4	3B	10m+8sm
Ahvaz	40.92	16.75	24.17	1.44	0.69	7.42	6.83	2.18	40.93	31.92	11.38	2	3B	12m+4sm+2st
Karaj	51.1	18.56	32.54	1.75	0.57	13.96	7.97	3.34	36.32	41.91	8.46	-	3B	10m+4sm+4st
Qom	62.45	20.68	41.75	2.01	0.49	21.07	9.41	5.16	33.11	54.84	6.81	4	3A	2m+16sm
Neishaboor	42.92	16.04	26.89	1.67	0.59	10.85	6.28	3.5	37.37	55.73	6.48	-	2A	8m+10sm
Gorgan	33.57	12.75	20.83	1.63	0.61	8.08	5.73	2.27	37.98	39.62	10.29	-	3B	10m+8sm
Babol	19.94	7.85	12.09	1.54	0.65	4.24	3.01	1.51	39.37	50.17	7.62	-	3A	12m+4sm+2st
Varamin	25.34	9.5	15.84	1.67	0.59	6.34	4.81	1.25	37.49	25.99	14.05	-	3B	10m+8sm
Shiraz	35.63	12.9	22.74	1.76	0.57	9.84	5.11	1.87	36.21	36.59	9.11	-	3B	10m+6sm+2st
Zirehii	38.95	14.8	24.15	1.64	0.61	9.35	6.14	1.45	38.00	23.62	12.04	-	3C	12m+6sm
Hamadan	29.09	10.31	18.79	1.82	0.55	8.48	4.44	2.11	35.44	47.52	7.69	-	3B	10m+4sm+4st
Parsabad	24.85	9.15	15.71	1.72	0.59	6.56	4.14	0.93	36.82	22.46	12.92	-	3C	10m+8sm
Jahrom	43.56	15.22	28.34	1.86	0.54	13.12	6.27	3.56	34.94	56.78	6.22	-	2A	8m+10sm
Fasa	36.69	10.54	26.16	2.48	0.41	15.62	6.22	1.22	28.73	19.61	13.64	2	2C	4m+10sm+4st

Total length of chromosome (TL) in µM, sum of short arms (SA) in µM, sum of long arms (LA) in µM, relative length (RL), arm ratio (AR), centromic index (CI), ratio of long arm to short arm (r-value), long chromosome-short chromosome (d-value) in µM, difference of relative length (DRL), relative length of shortest chromosome (S%), satellite number (SAT).

Table 3. Analysis of variance for the karyotypic characteristic of different Iranian lettuce accessions.

		MS					
Source of variation	df	TL	RL	LA	SA	AR	CI
Accession (A)	14	39.57**	0.000011**	18.42**	4.49**	0.11**	0.017**
Chromosome (C)	8	42.28**	0.0033**	21.81**	3.78**	0.12**	0.19**
A × C	112	2.09**	0.0001**	1.22**	0.43**	0.078**	0.13**
Error	270	0.007	0.000004	0.007	0.014	0.006	0.0009
CV (%)	-	2.05	5.36	3.19	7.64	13.39	8.21

***: Significant at 1% probability levels, TL: total length of chromosome, RL: relative length, LA: long arm length, SA: short arm length, AR: arm ratio, CI: centromic index*

Table 4. Mean comparison of different Chromosome and karyotypic traits in Iranian lettuce accessions.

Accession code	TL	LA	SA	AR	CI
1	5.23c	3.34c	1.89bc	1.44b	11.11c
2	4.99cd	3.15cd	1.84bc	1.76b	11.22c
3	4.55ef	2.69ef	1.86bc	1.64b	39.78a
4	5.67b	3.62b	2.06ab	1.78b	37.33a
5	6.94a	4.64a	2.30a	2.11ab	33.44ab
6	4.77de	2.99cde	1.78bc	1.70b	38.33a
7	3.73h	2.31fg	1.42de	1.62b	38.89a
8	2.22k	1.34i	0.87f	1.80b	39.22a
9	2.82j	1.76h	1.06f	1.69b	38.11a
10	3.96gh	2.52f	1.43de	1.87b	37.11a
11	4.33fg	2.68ef	1.64cd	1.64b	38.78a
12	3.23i	2.09gh	1.15ef	2.10ab	34.89ab
13	2.7j	1.75h	1.02f	1.83b	36.44a
14	4.84cde	3.15cd	1.69cd	1.89b	35.56ab
15	4.08gh	2.91de	1.17ef	2.80a	29.44b

Table 5. Principal component analysis of karyotypic traits in different Iranian lettuce accessions.

Parameter	First component	Second component
TL	0.989	0.072
LA	0.974	0.193
SA	0.962	-0.168
AR	-0.050	0.995
CI	-0.461	0.102
Eigen value	3.07	1.07
Relative variance (%)	61.32	21.40
Cumulative variance (%)	61.32	82.75

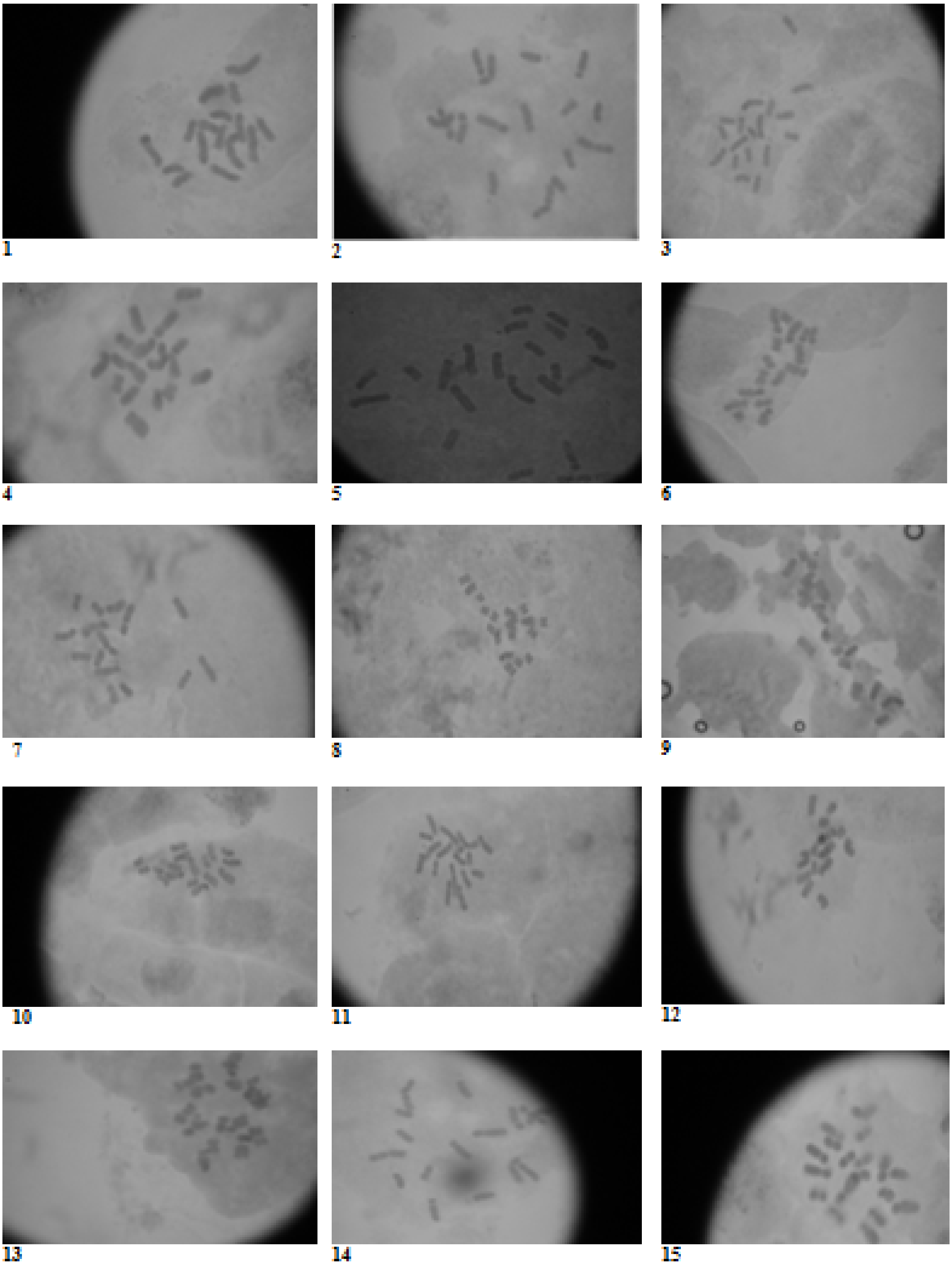

Fig. 1. Metaphase mitosis of different Iranian lettuce accessions (numbers 1 to 15, refer to Table 1).

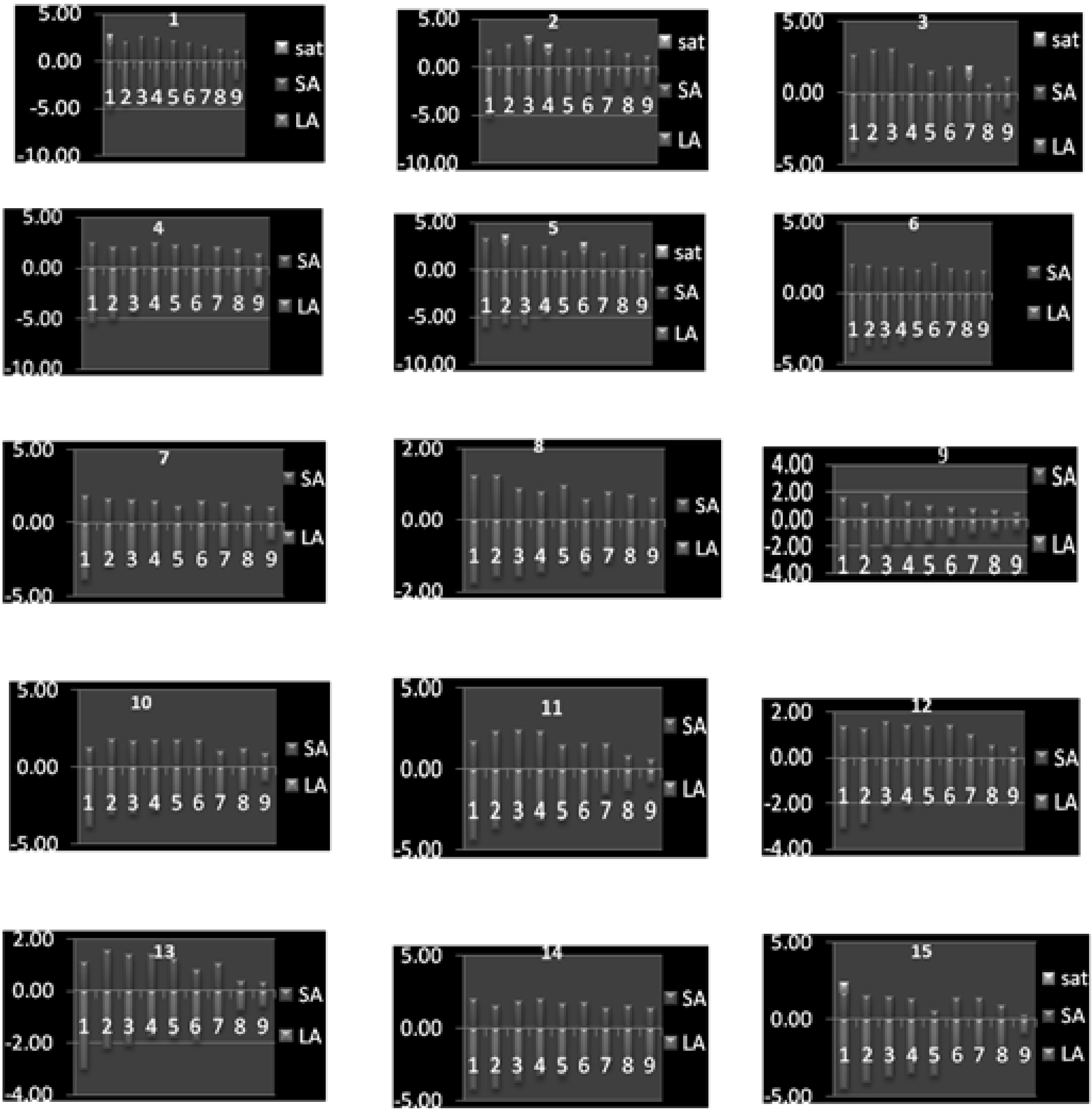

Fig. 2. Ideogram of different Iranian lettuce accessions (numbers 1 to 15, refers to Table 1).

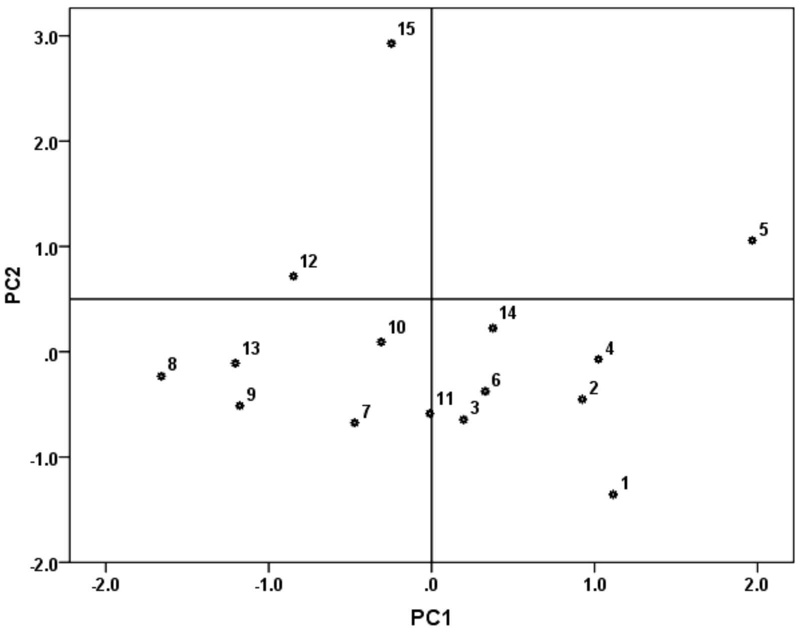

Fig. 3. PCoA based on chromosomal characters (Code 1 to 15, refers to Table 1).

CONCLUSION

In conclusion, diversity and heteromorphism between chromosomes in Iranian lettuce accessions could be possibility due to their origination from different regions or their being evolved through repatterning after transfer to another region (s). The results showed that chromosome counting, measuring and determining of different characteristics such as chromosome length, karyotypic formula and chromosomal symmetry are suitable tools to find effective crossings and periodic diversification of lettuce accessions. Also, our results are effective to classification and selection of Iranian accessions. In general, further studies are needed to genetic constitution and chromosome structures using genetic mapping and genomic *in situ* hybridization techniques.

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REFERENCES

[1] P.E. Brandham and M.J. Doherty, *Ann. Bot.*, **1998**. 82, 67-73.
[2] I. Dolezalova, I., A. Lebeda, J. Janecek, A.J. Cihalikov, A.E. Kristkov and O. Vranova, *Gen. Res. Crop Evol.*, **2002**. 49, 383-395.
[3] P.P. Jauhar, *Crop Sci.*, **2006**. 46, 1841-1859.
[4] W.J.M. Koopman and J.H. DeJong, *Acta Bot. Neerl*, **1996**. 45, 211-222.
[5] W.J.M. Koopman, J.H. DeJong and I.M. De Vries, *Plant Syst. Evol.*, **1993**. 185, 249-257.
[6] W.J.M. Koopman, E. Guetta, C.C.M. Vande Wiel, V. Vosman and R.G. Enberg, *Amer. J. Bot.*, **1996**. 85, 1517-1530.
[7] A. Lebeda, E.J. Ryder, R. Grube, I. Dolezalova and E. Kristkova, *Vegetable Crops. Boca Raton, CRC Press, Tailor and Francis Group*, **2007**. 377-472.
[8] A. Lebeda, *Report on Research Programme OECD Biological Resource Management for Sustainable Agricultural Systems, Wellesbourne*, **1998**. *U.K., HRI.*
[9] A. Lebeda, I. Dolezalova and D. Astly, *Gen. Res. Crop Evol.*, **2004**. 51, 167-174.
[10] A. Lebeda, I. Dolezalova, E. Krıstkova, M. Kitner, I. Petrzelova, B. Mieslerova and A. Novotna, *Euphytica*, **2009**. 170, 15-34.
[11] A. Levan, K. Fredga and A. Sandberg, *Hereditas*, **1964**. 52, 201-220.
[12] E. Martel, V. Poncet, F. Lamy, S. Siljak-Yakovlev, B. Lejeune and A. Sarr, *Plant Syst. Evol.*, **2000**. 249, 139-149.
[13] H. Matoba, T. Mizutani, K. Nagano, Y. Hoshi and H. Uchiyama, *Hereditas*, **2007**. 144, 235-243.
[14] H. Matoba, H. Uchiyama and T. Koyama, *Chromosome Sci.*, **2001**. 5, 73-77.

[15] J.A. Mejias, *Botanica Helv*., **1993**. 103, 113-130.
[16] P. Mercado-Ruaro and A. Delgado-Salinas, *J. Bot.*, **1998**. 85, 1-9.
[17] M.A. Mikel, *Hort. Sci.*, **2007**. 42, 489-493.
[18] J. Ortega, B. Navarro and B. Estudios, *Botanica Macaron*, **1997**. 3, 73-78.
[19] M.R. Pedro and D.S. Alfonso, *Gen. Mol. Biol.*, **2000**. 23 (4), 985-987.
[20] L. Poggio, G. Gonzalez and C.A. Naranjo, *Botanica J. Lin. Soc.*, **2007**. 155, 171-178.
[21] I. Schubert, *Current Opin Plant Biol.*, **2007**. 10, 109-115.