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INVESTIGATING THE KARYOTYPE OF FIVE POPULATIONS OF "PLANTAGO MAJOR"SPECIES USING THE IMAGE ANALYSIS SYSTEM

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ABSTRACT

With the aim of investigating the Cytotaxonomy of a species of Plantago genus from the family of Plantaginaceae, collected from the habitats of the northern provinces of Iran, the karyotype of 5 populations belonging to P.major species is studied in the meristem cells of root tip using an image analysis system. The chromosomal dimensions such as the length of short arm and the length of the whole chromosome are measured and the AR and CI statistics are also calculated after preparation, staining, and microscopic investigation of samples and separation of chromosomes by Photoshop software through Micromeasure software in at least 3 appropriate cells of mitotic metaphase. The populations are measured based on the karyotype statistics including S%, %TF, A_1, DRL, A_2, VRC, Levan karyotype formula and grouping the Stebbins for studied populations. In this research, the studied populations of P.major had the chromosomal number of 2n=2x= 12 and the basic chromosome number was equal to x=6 in all populations and it was consistent with previous studies and this indicates the diploid species in Iran. Furthermore, according to the Levan Table, the chromosomes of studied populations were from the metacentric and submetacentric species.

Keywords: *Plantago major* , Cytogenetic Diversity, Chromosome, Karyotype, Northern Iran

INTRODUCTION

Plantago genus has about 483 species [21], 22 species of this genus have been reported from Iran in Flora Iranica (Mozaffarian, 1994). Most of its plants are have one or multiple years of age and there are a few perennial species for it. It has the dense and alternate Rosulates. The male and female flowers are inside the spikes until a narrow and extremely long cylinder with a few or a lot of flowers [11],The application of

cytogenetic information in systematic and phylogenetic nature of this genus has been considered from previous years. Rana (1996) has offered a new suggestion of classifying the Plantago genus based on the cytology knowledge and classified this genus into 6 subgenus including Coronopus Rahn, Albicans Rahn, Plantago major, Psyllium Juss, Littorella Rahn, and Bougueria Rahn, and has indicated that Plantago genus has 3 different chromosome bases including x= 4, x= 5, and x = 6.

The cultivation of this genus has been more taken into account due to the important components (they are more applied in pharmacy) in order to extract the active compounds [7],*Plantago major* has the biological active compounds like Polysaccharide, Glycoside, Lipid and Terpenoid, and Sorbitol [18], It has the astringent, anti-diarrhea, sweat-inducing, diuretic, and anti-rheumatism effects (Zargari, 1992). It is effective in curing the colic, jaundice, and liver diseases (Beara, 2000). Furthermore, it has the anti-inflammatory on the liver. (Turel et al, 2009) and it is especially applied in wound healing[2], *Plantago major* is spread in different parts of the Earth and grows in a large area of Europe, Asia, and North America [22],and [6],It is growing in almost all regions of Iran. It is essential to conduct the karyotype studies in the population of a species because different populations of a species show their unique genomic consistency with the environment wherein they grow, so that it is believed that the chromosomal analyses along with the genetic and morphological studies can be the reliable indices in assessing the relative relationship between the species of a genus [8],The investigation of difference in the number of chromosome, its morphology as well as the difference of ploidy level are utilized as the evidence for convergence between the species and somatic hybrids derived from the cell fusion [13],A lot of international studies are conducted by different researchers on determining the chromosome number and ploidy level of species and subspecies of a Plantago genus. McCullagh [14],has presented the first chromosome counting and morphology of chromosomes. Gorenf, Vasudevan and Favarger (1972) studied the polyploidy in this species. Santosh and Raghbir (2011) investigated the chromosome number of some species of Plantago genus in the northern India. Conducting the Cytotaxonomic study of this genus in the northern Iran, we will be able to provide the more accurate and detailed classification of this Taxon and provide the better and more effective key of identification by discovering the chromosomal status of plantago major

species populations in the north of country in addition to determining the level of karyotype symmetry.

MATERIALS AND METHODS

This study is conducted on five accessions from *P. major* species in different regions of northern Iran (Gorgan, Behshahr, Sari, Babolsar, and Babol). The studied seeds germinate after disinfection by bleach 15% for ten minutes and putting them on the wet filter paper inside the Petri dishes at 25 ℃, and the root tips are separated from the seeds when the root lengths reach 0.5-1 cm and are put as the pretreatment in solution of Alpha Bromonaphthalene 1% for 2 hours. The pretreatment refers to stopping the cell division at the metaphase stage. The next stages of this study apply the compounds such as the Farmer solution for 16-18 hours for stabilization of internal structure of cell and maintaining the original status as well as HCl 1N for hydrolysis for 5-10 minutes at 60°C and also ethyl alcohol 70% for storing the roots for a long time in addition to hematoxylin staining solution in order to investigate the shape and structure of chromosomes. Afterwards, the apical meristem of roots is separated and the chromosomal images are taken by adding acetic acid 45% and through the squash method (Agayev, 1996). The chromosomes of each cell are separated by Photoshop software and arranged on a page, and then the length of long arm, length of short arm, and total length of chromosome are measured in at least 3 appropriate mitotic metaphase cells through Micromeasure software.

Table 1: Characteristics of species and populations applied in this research

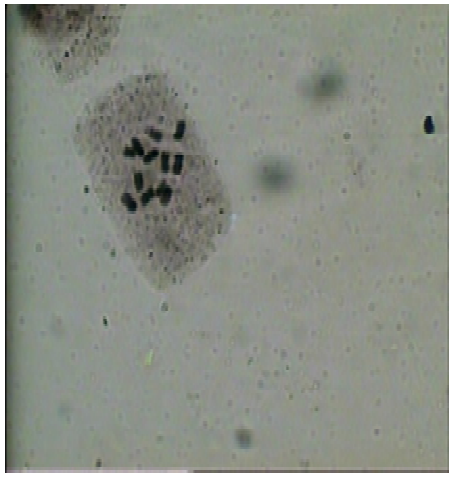
Species	Collection place	Height above sea level
P.major	**Mazandaran - Sari**	**1900**
P.major	**Mazandaran - Babol**	**2577**
P.major	**Mazandaran - Behshahr**	**400-660**
P.major	**Gorgan**	**3500**
P.major	**Mazandaran - Babolsar**	**-21**

The data is analyzed in the form of a completely randomized unbalanced method, and then the mean of each characteristic is compared by Duncan test at the level of 5%. The karyotype statistics S%, %TF (Huziwara, 1962), A1, DRL, A2 (Romrozarco, 1971), Levan karyotype formula (1962) and Symmetry Class (Stebbins, 1971) are measured for studied populations and the populations compared with each other. The mean chromosomal characteristics including the length of long and short arms are calculated for different populations through Excel software and the ideogram of each population drawn. The chromosomes are arranged based on the reduced length of chromosome and the centromeres aligned in drawing the ideogram.

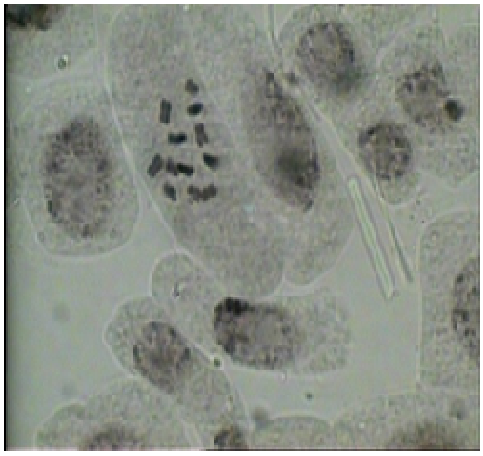
RESULTS

The mitotic metaphase images of studied species and populations are presented in

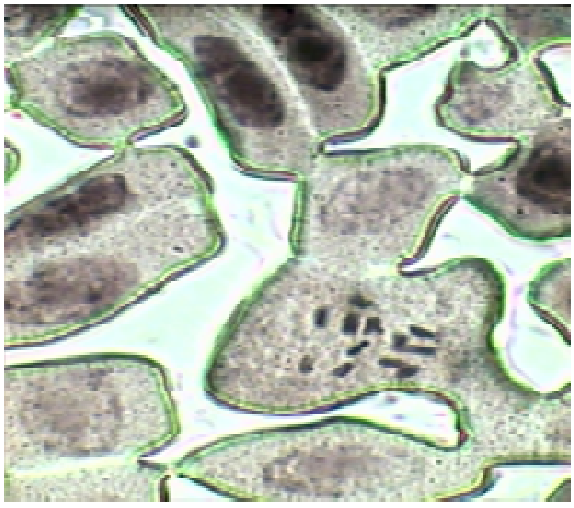
Figure 1. All of the chromosomes are not clearly seen in an image since they are multilevel. The mean chromosomal characteristics including the length of long and short arms are calculated through Excel software and the ideogram of each one drawn. (Figure 1)



Babol P.major



Sari P.major



Gorgan P.major

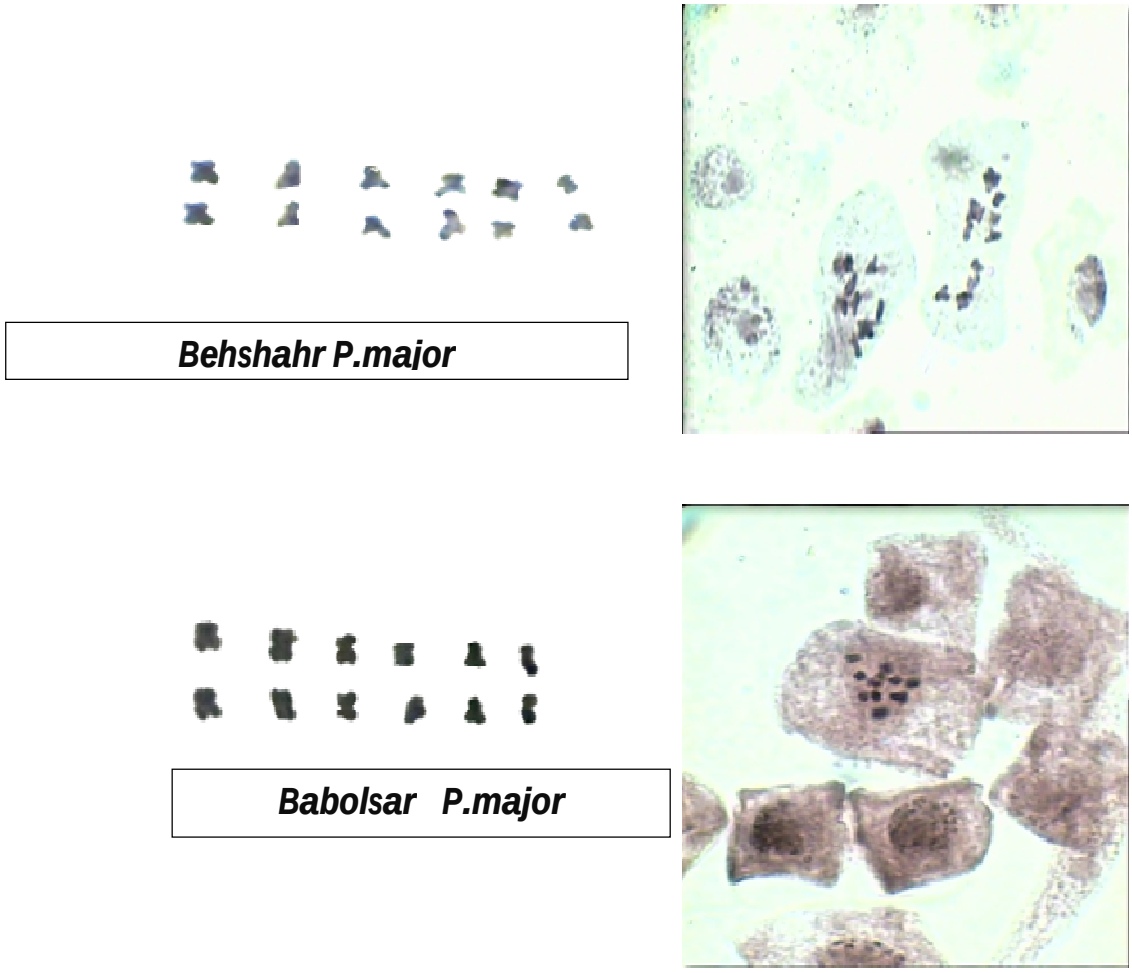


Figure 1: The mitosis metaphase image and karyogram of studied populations of *Plantago major* species

In drawing the ideogram of species, the chromosomes are arranged based on reducing the total chromosome length and the centromeres placed in the same direction. The parameters including the relative length of shortest chromosome (S%), the total form percentage (%TF), A1 (intra-chromosomal asymmetry index), the difference between the differences in the relative length (DRL) of chromosomes, Pearson dispersion coefficient (A2) as the inter-chromosomal asymmetry index, Value of Relative Chromatin (VRC), Levan karyotype formula and Stebbins grouping are measured for species and presented in Table 2.

Table 2: Measurement parameters of karyotype symmetry for five studied populations from *Plantago major* species

Population	%S	%TF	A1	DRL	A2	VCR	SC	Karyotype formula
Babol	14.21	38.40	0.36	4.78	0.10	4.05	1A	5m + 1sm
Sari	13.54	39.51	0.348	6.87	0.14	3.51	1A	6m
Behshahr	14.46	38.87	0.35	4.62	0.21	3.78	1A	5m + 1sm
Babolsar	12.63	38.31	0.38	7.56	0.20	3.32	1A	6m
Gorgan	13.27	36.46	0.42	5.94	0.13	4.25	1A	5m + 1sm

The results indicate that Babol proportion has 2n= x2x=12 chromosomes and its chromosomal base is x=6. The lengths of its longest and shortest chromosome are

equal to 4.61 and 3.46 respectively. In this species, the maximum and minimum lengths of long arm (L) are equal to 2.04 and 2.88, the lengths of short arm (S) equal to 1.40 and 1.73, the ratio of lengths of long to short arm of chromosome (AR) equal to 2.15 and 1.26, the centromeric indexes (CI) 0.31 and 0.44, and the percentages of relative chromosome length equal to 18.99 and 14.31. Furthermore, in this species, the length of a set of chromosomes (haploid) is equal to 24.31 µm, the total length of long arms 14.97 µm, and the total length of short arms 9.33 µm. This species with the karyotype formula of 5m+1sm is in class A1 in terms of karyotype symmetry and based on Stebbins table, and the average length of each chromosome is 4.05 µm. Its A1 is equal to 0.36, A2 0.12, S% equal to 14.24, %TF equal to 38.99 and DRL equal to 4.78 (Table 2). The ideogram of population for this species is presented in Figure 2.

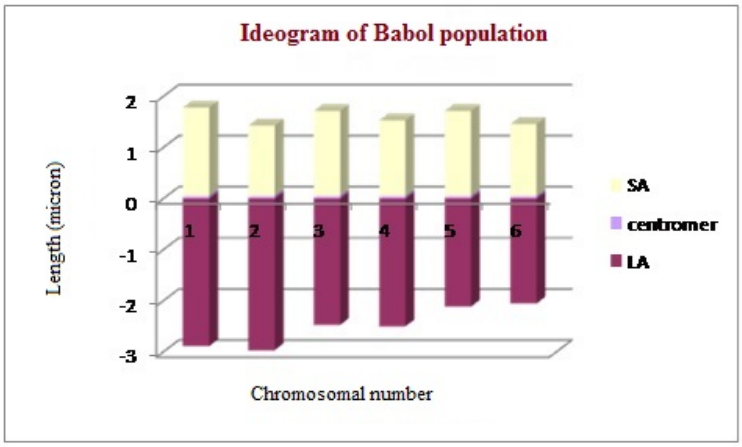


Figure 2: Ideogram of Babol population from *Plantago major* species

The study on Sari population indicates that the lengths of its longest and shortest chromosomes are equal to 4.30 and 2.85 respectively. In this species, the maximum and minimum lengths of long arm (L) are 2.43 and 1.68, the lengths of short arm (S) 1.87 and 0.161, the ratios of lengths of long to short arms of chromosome (AR) equal to 1.30 and 1.72, the centromeric indexes (CI) 0.36 and 0.43, and the relative lengths of chromosome are equal to 20.4 and 13.56. In this species, the length of a set of chromosomes (haploid) is equal to 21.09 µm, the total length of long arms 12.75 µm and the total length of short arms 8.33 µm. This species with the karyotype formula of 6m is in A1 Class based on Stebbins table in terms of karyotype symmetry, and the average length of each chromosome is equal to 3.54 µm. Its 1A is equal to 0.34, A2 equal to 0.14, S% 13.54, %TF 39.54 and DRL 4.62 and these are presented in Table 2. The ideogram of population for this species is presented in Figure 3.

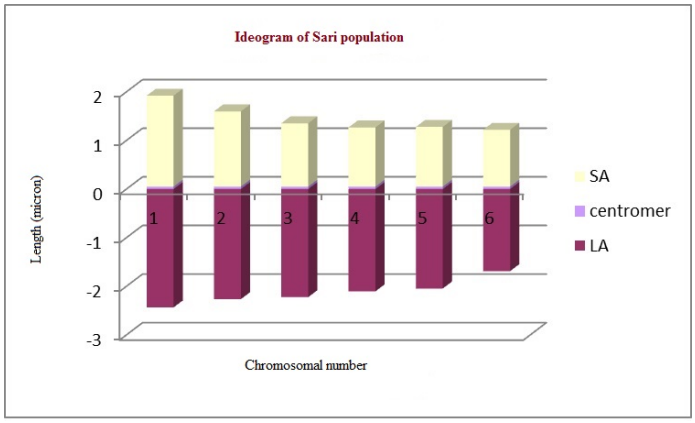


Figure 3: Ideogram of Sari population from *Plantago major* species

The study on Babolsar population indicates that the lengths of its longest and shortest chromosomes are equal to 4.02 and 2.52 respectively. In this species, the maximum and minimum lengths of long arm (L) are 238 and 1.60, the lengths of short arm (S) 1.65 and 0.92, the ratios of lengths of long to short arms of chromosome (AR) equal to 1.44 and 1.74, the centromeric indexes (CI) 0.36 and 0.41, and the relative lengths of chromosome are equal to 20.20 and 12.64. In this species, the length of a set of chromosomes (haploid) is equal to 19.92 µm, the total length of long arms 12.29 µm and the total length of short arms 7.63 µm. This species with the karyotype formula of 3m+3sm is in A1 Class based on Stebbins table in terms of karyotype symmetry, and the average length of each chromosome is equal to 3.32 µm. Its 1A is equal to 0.38, A2 equal to 0.16, S% 12.64, %TF 38.31 and DRL 7.56 and these are presented in Table 2. The ideogram of population for this species is presented in Figure 4.

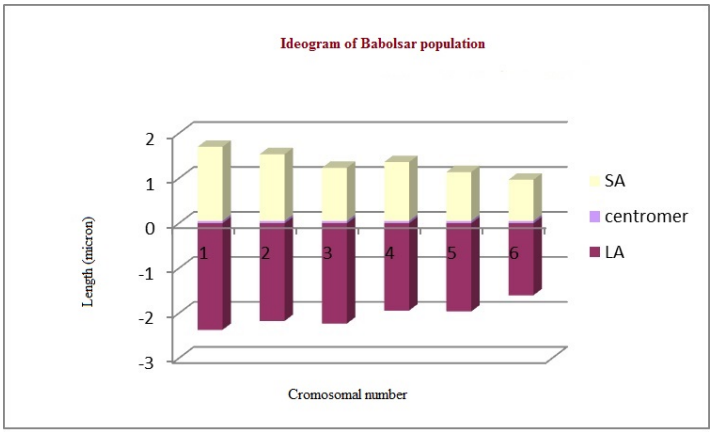


Figure 4: Ideogram of Babolsar population from *Plantago major* species

Plantago major species in Behshahr proportion has 2n= x2x=12 chromosomes and its chromosomal base is x=6. The lengths of its longest and shortest chromosome are equal to 4.32 and 3.28 respectively. In this species, the maximum and minimum lengths of long arm (L) are equal to 2.24 and 2.15, the lengths of short arm (S) equal to 1.01 and 1.88, the ratio of lengths of long to short arm of chromosome

(AR) equal to 2.25 and 1.30, the centromeric indexes (CI) 0.30 and 0.43, and the percentages of relative chromosome length equal to 19.22 and 14.60. Furthermore, in this species, the length of a set of chromosomes (haploid) is equal to 22.72 µm, the total length of long arms 13.67 µm, and the total length of short arms 8.83 µm. This species with the karyotype formula of 5m+1sm is in class A1 in terms of karyotype symmetry and based on Stebbins table, and the average length of each chromosome is 3.78 µm. Its A1 is equal to 0.35, A2 0.21, S% equal to 14.46, %TF equal to 38.87 and DRL equal to 4.62 (Table 2). The ideogram of population for this species is presented in Figure 5.

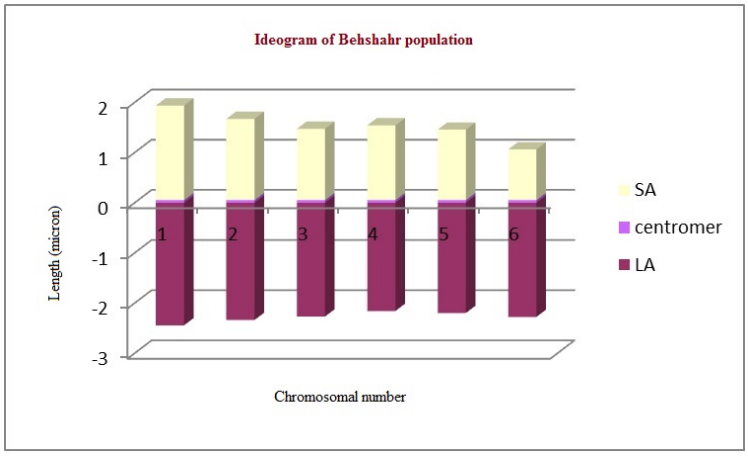


Figure 5: Ideogram of Behshahr population from *Plantago major* species

The studies on Gorgan population indicates that the *Plantago major* species in has 2n= x2x=12 chromosomes and its chromosomal base is x=6. The lengths of its longest and shortest chromosome are equal to 4.90 and 3.39 respectively. In this species, the maximum and minimum lengths of long arm (L) are equal to 3.34 and 2.23, the lengths of short arm (S) equal to 1.15 and 1.88, the ratio of lengths of long to short arm of chromosome (AR) equal to 2.13 and 1.43, the centromeric indexes (CI) 0.31 and 0.41, and the percentages of relative chromosome length equal to 19.21 and 13.27. Furthermore, in this species, the length of a set of chromosomes (haploid) is equal to 25.55 µm, the total length of long arms 16.23 µm, and the total length of short arms 9.31 µm. This species with the karyotype formula of 5m+1sm is in class A1 in terms of karyotype symmetry and based on Stebbins table, and the average length of each chromosome is 4.25 µm. Its A1 is equal to 0.42, A2 0.13, S% equal to 13.27, %TF equal to 36.46 and DRL equal to 5.94 and these are presented in Table 2. The ideogram of population for this species is presented in Figure 6.

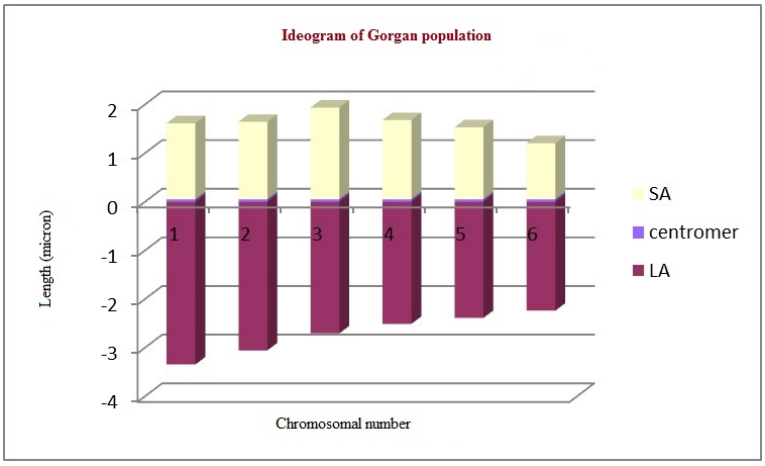


Figure 6: Ideogram of Gorgan population from *Plantago major* species

CONCLUSION

In this study, *Plantago major* species is diploid and has (2n=12) chromosomes. The same research is conducted by McCullagh [14],and Bocher (1955) and Rahan [16],on *P. major* species and the chromosomal number and ploidy level of this species equal to (2n= 2x= 12). Furthermore, Favarger and Vasudevan (1972) reported the chromosomal number of this species equal to 2n=6x= 36 in their studied in Himalayas. Moreover, Briggs [3],and Jain (1978) reported that this species is Hexaploid according to the chromosome counting in Italy. Santosh and Raghir (2011) reported the chromosomal number of *P. major* species equal to (2n=12.24) according to the cytological analysis of pollen meiosis in the northern India. According to the polymorphic study by Favarger and Vasudeva (1972) on this species, the studies indicate that the changes of quantitative survey reflect a change in the number of genes (phylogenetic). Badra and El-kholy [1],reported the chromosomal number and karyotype of Plantago genus equal to 2n=12 in Egypt. Ebadi et al [5],investigated the karyotype and karyomorphological changes in *Plantago ovata* Forsk in Iran and reported the chromosomal number of this species equal to 2n=2x=8. Mohsenzadeh et al (2008) reported the chromosomal number of *Plantago major* equal to 2x=12 in Iran.

The species and population with the shortest relative length of chromosome (S%) has more symmetric karyotype. In this study and among the population of studied species, Babolsar population with the value of 12.63 has the maximum value of this statistic and the most symmetric karyotype (Table 2). The more %TF is close to 50, the more it has the symmetrical karyotype. According to Table 2, the highest percentage equal to 39.54 belongs to Sari population. In other words and according to this statistics, P.major of Sari population has the most symmetric karyotype among the populations. Gorgan population with the value of 36.46 has the least amount of this statistics and the most asymmetric karyotype. The value of A1 as the index of intra-chromosomal asymmetry is lower among the metacentric chromosomes, so that if all chromosomes of a species are Meta (M), the value of A1 will be equal to zero, and thus it has the highest degree of karyotype symmetry. Based on this statistics, Gorgan population has the highest value and

most asymmetric karyotype. As shown in Table 2, two indexes, A1 and %TF, which show the intra-chromosomal symmetry or asymmetry, have inverse relationship with each other, so that the more %TF is increased, the more the value of As is reduced. In terms of DRL, since the lower values of this statistic indicate the higher symmetry of karyotype among the studied species, Sari population with the value of 4.62 has more symmetric karyotype than other populations. Babolsar population with DRL of 7.56 has the most asymmetric karyotype among the studied species (Table 2). The higher value of this statistic in a species or cultivar indicates that the karyotype is more asymmetric and that species or cultivar is in a higher degree of karyotype evolution. Pearson dispersion coefficient (A2), as the index of intra-chromosomal asymmetry, shows the karyotype asymmetry in terms of relationship between the sizes of different chromosomes. The more the value of A2, the more the difference between the length of chromosomes, the more asymmetric karyotype and thus the species more evolved. Accordingly, Behshahr species with statistic equal to 0.21 has the most asymmetric karyotype among the population of studied species and Babol population has the lowest value of this statistic equal to 0.10 and the most symmetric karyotype. Among the studied populations, Gorgan and Babolsar with the statistics of 4.25 and 3.32 microns have the highest and lowest Value of Relative Chromatin (VRC) respectively. On the basis of karyotype formula and based on the table presented by Levan, the studied chromosomes of Babolsar and Sari population have been from metacentric (m) type, and Babol, Behshahr, and Gorgan have metacentric and submetacentric types. According to Stebbins table, all populations of studied species have been in class A1. In general, according to karyotype statistics, namely, %S, %TF, A1, DRL and A2, Behshahr, Gorgan and Babol populations have the most asymmetric karyotype among the populations of species and the higher degree of karyotype evolution. Accordingly, Sari and Babol populations have the most symmetric karyotype among the studied populations (Table 2).

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