
Chromosome analysis of *Quercus castaneifolia*

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Abstract The results showed that all studied cells of each population, the basic chromosome number was $x=12$ and all of them were diploid. Karyotype analysis of each population was conducted separately and several indices (TL: Total Length, LA: Long Arm, SA: Short Arm, CI: Centromer Index, AR: Arm Ratio, R- value, DRL%: Difference of Relative Length and TF%: Total Form) were determined. Karyotype formula was $12m$ in all studied populations. The length of chromosomes in all populations was estimated as 1.55-2.68 micrometers. The longest chromosome was observed in chromosome number 1 from population 4 (Gorghan) which was 2.68 micrometers and the shortest one was related to the chromosome number 12 from population 5 (Zanoos) which was 1.55 micrometers. Considering of chromosomal classification, all the studied populations were placed in class 1A of Stebbins which showed that there is a symmetry in the studied karyotypes. The other estimated indices also showed that in chromosomes are relatively symmetric in all populations that indicated this species is primitive and undeveloped.

Keywords: Cytogenetic, Iran, Karyotype, Oak, *Quercus castanifolia*

Introduction

Breeding can be raised the potential yield under environmental constraints to improve adaptiveness of paramount importance (Mohammadi *et al.*, 2014). Cytogenetic concerns a branch of genetics related to the construction of chromosomes. Cytogenetic is regarded as the primitive and basic achievements in breeding to determine the number of chromosomes and ploidy levels which necessary in selection of proper breeding method (Javadi *et al.*, 2006). Cytological methods is facilitated to determine the chromosomal structure and recognize specific chromosomes. Moreover, karyotype analysis is related to analysis of the appearance, number and construction of chromosomes in terms of size, location of centromere and other chromosomal information. The analyses of chromosomal characteristics and cytogenetic information are provided the recognition of plant species karyotype structure and diversity among different populations in a species. The genome of individuals is

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contained genetic information and gene expression which is appeared in phenotypic traits so, any changes in chromosome construction and size resulted in different phenotypic traits appearances. Karyotypic concerns the species populations which are important to each different population showing a fspecific genomic adaptation for the environmental conditions (Tabandeh Saravi *et al.*, 2013). Variation in karyotype structure e.g. number, type and size and chromosome behavior during cell division can be explained the genetic differences (Sheydaie *et al.*, 1997). However, the cytological studies is provided the valuable information for available gene pool of the country which could be using in gene bank (Hesamzadeh *et al.*, 2009). Therefore, the local germplasm in breeding programs is important to new traits in the genetic pool (Ozdemir Eroglu *et al.*, 2016). Seed and chromosome morphologies are considered to be useful to solve the systematic and evolution (Kocyigit and Alp, 2018). *Quercus* L. is a genus including 300 to 600 species (Johnson *et al.*, 2002) and the number of base chromosome is $n=12$, the most species are diploid (Demerico *et al.*, 1995) and polyploidy that rarely occurs in this genus (Tabandeh Saravi *et al.*, 2012). This genus includes different species of evergreen, deciduous trees and shrubs which are expanded from cold climates to tropical forests in Asia and America. The main growing habitats of *Quercus* species are central and Eastern Taurus Mountains and Amanos, Anatolia in Turkey, Northeastern of Iraq, Northwestern of Syria and western parts of Iran (Browicz 1994). Northern Zagros forests start from Shahoo ridge on the border of Kermanshah provinces and continue to the north of Piranshahr in West Azerbaijan. The area of the Northern Zagros forests is about 449000 hectares which *Quercus* species covers 106316 hectares (i.e. 24%) with pure or mixed populations (Fattahi, 1998). *Quercus* species in the world, the *Quercus castanifolia* C. A. Meyer is one of the most industrially important with ecofriendly values of Northern Zagros in Iran (Gorji Bahri, 1987) which distributed in Northern forests of Iran (from Astara to Golestan and Bojnurd) and karkaz in high altitudes (Sabeti, 2002). Unfortunately, in recent years for various reasons, the size of this species is decreased in natural habitats. Moreover, there is a few information in genetic potential of different species populations that scattered over a relatively large area of the northern forests. Therefore, the study aimed to determine the best recipe for cytogenetic studies by optimizing the method to prepare chromosomes and to determine its chromosomal karyotype and morphology of *Quercus castanifolia* by analyzing different chromosome populations to present the best for cytogenetic studies in the north of the country.

Materials and methods

The five accessions of *Quercus castaneifolia* from several northern forests in Iran during the years 2018-2019 were studied. Karyological observations were based on the material collected from natural populations. The accessions were used for somatic karyotype are Population 1: Astara, Population 2: Babolsar, Population 3: Chenaran, Population 4: Ghorghan, and Population 5: Zanoos.

They were brought to Urmia University in early growing season. Seedlings were transferred to plastic pots containing a mixture of garden soil, peat moss and perlite. In order to facilitate the preparation of root samples without any damage, the pots were divided to two parts. The underside surface inner pots were totally removed and placed on second pots which the one third of their volume was filled with sand, the fresh roots of inner pots could grow into the sand of the second pots. Sampling was conducted daily from the root meristem (root tip meristem which divides mitotic continuously). Roots with the length of 0.5-1 cm were collected during different times of the day which in the case of *Quercus*, the best time of sampling in Urmia condition was 08:00 - 09:00 O'clock in the morning. The fresh roots of ten seedlings from each population were used for chromosome analysis. Then, the following levels including pre-treatment, fixation, storing, hydrolysing, staining, observation, imaging of the cells and analysing of the chromosomes were done. For cytological studies the root tips squash techniques were used by early workers (Agayev, 1996; Sikdar and De, 1967; Shrivastava and Joshi, 1972; Reddy, 1973) and more developed by Pillari *et al.* (1981) with minor modification to find the suitable for the present study. Pre-treatment was done using 0.5% saturated alpha-bromo naphthalene solution in water for 6 hours, followed by washing with water 3 times. After pre-treatment, the roots were washed with distilled water and placed in fixing solution. These solutions made the chromatin to precipitate and also killed the cells quickly. The main goal of fixation was to keep the cell structure as well as preserving all forms of cell divisions using Lewitsky solution. After fixation, it is necessary to keep the samples for relatively long time at 4 °C, since it was not possible to hydrolyze all samples together, then the samples were placed in ethanol 70% and followed by fixation and washing. Hydrolysis degrades intracellular walls and helps distribution of chromosomes and cells. In this study, hydrolysis fixed samples was done using NaOH 1N for 20 min at 60 °C. Staining of root apical meristem is necessary for definition of chromosomes and their better visibility followed by hydrolysis. Staining ability of chromosomes is related to the chromophores which contain molecules called Aksuchrome with ability to keep the color. The

staining of root meristem was done using %2 Aceto Orcein for 48 hours. Root meristems after treated with cytase enzyme for removing cell walls followed by staining (Agayev, 1996). Root tip samples were squashed and microscopic slides were prepared by above mentioned procedures. The samples were studied using light microscope and the cells in metaphase with the best distributed and stained chromosomes were selected and photographed. The analyses of images were carried out using Micromeasure 3.3 as well as SPSS (21) softwares. Standard karyotype was prepared using selective metaphase and chromosome parameters including the length of long arm (L), the length of short arm(S), the total length of chromosomes (TL), arm ratio (AR), r-value(S/L), centromer index (CI) and Relative length (RL) as calculated for chromosomes. The volume of each chromosome was measured assuming that it is made of two cylinders corresponding to two sister chromatids. The chromosomes were assored in to different categories on the basis of arms ratio following Levan *et al.* (1964) (M=1.0, m=1.0-1.7, sm=1.7-3.0, st=3.0-7.0) (Figure 1). The classification of karyotypes in relation to their degree of asymmetry were done according to Stebbins (1971) (Table 1). Also, the ideogram of each populations was based on the length of short and long arms using Excel software. The arrangement of chromosomes in ideogram was considered from left to right as well as from the largest to the smallest total chromosomes length (TL).

Table 1. The classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971)

Ratio	Proportion of chromosomes with arm ratio >2:1			
	1.00(1)	0.99-0.51(2)	0.50-0.01(3)	0.00(4)
Largest/smallest	1A	2A	3A	4A
<2:1 (A)	1A	2A	3A	4A
2:1-4:1(B)	1B	2B	3B	4B
>4:1(C)	1C	2C	3C	4C

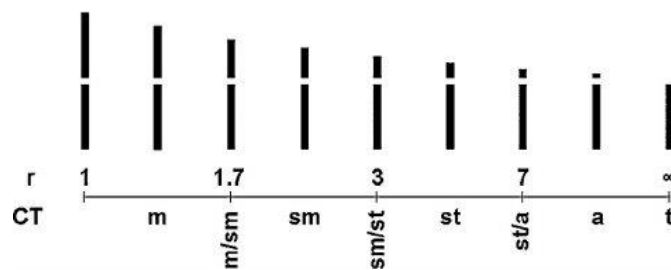


Figure 1. Chromosomes categories on the basis of arms ratio, Levan *et al.* (1964)

Results

Results of the mitotic metaphase plates in studied populations as well as the karyotype images and populations evolutionary status showed that all examined cells in each population, the basic chromosome number was $x=12$ and all were diploid. Karyotype analysis was conducted separately for each population and indices including the length of chromosomes, the length of long arm, the length of short arm, Arm Ratio, r-value, types of chromosomes and centromeric index were determined. The karyotype formula was $12m$ in all studied populations (Tables and Figures 2-6). The chromosomes type in all populations was metacentric without any satellite. The length of chromosomes in all populations was calculated as 1.55-2.68 μm . The longest chromosome was observed in chromosome number 1 from population 4 (Gorghan) which was 2.68 micrometers and the shortest one was related to the chromosome number 12 from population 5 (Zanoos) which was 1.55 micrometers. In population 1 which belongs to northern parts of Astara city, total chromosomes length varied from 1.63 to 2,52 μm . The longest arm was 1.29 μm and the shortest arm was 0.76 μm . Centromere of all chromosomes are at the median region, all of the chromosomes in this population is metacentric. No satellites were observed in the karyotypic of this population. Arm Ratio (L/S) varied from 1.02 to 1.21 and r-value (S/L) varied from 0.83 to 0.98. The karyotype of this population consisted of 12 pairs of median region (m) chromosomes (Table 2). Also, somatic metaphase, karyogram and haploid ideogram in population 1 of *Q. castaneifolia* (Astara) was shown in Figure 2.

Table 2. Chromosome characteristics in *Q. castaneifolia* (Population 1: Astara)

Pair	TL	LA	SA	SAT	AR	TYPE	r-value	CI	%L	%S	RL
1	2.52	1.29	1.23	–	1.05	m	0.95	48.81	5.00	4.77	9.78
2	2.48	1.25	1.23	–	1.02	m	0.98	49.59	4.85	4.77	9.62
3	2.42	1.26	1.16	–	1.09	m	0.92	47.93	4.89	4.50	9.39
4	2.41	1.32	1.09	–	1.21	m	0.83	45.42	5.12	4.23	9.35
5	2.39	1.22	1.17	–	1.04	m	0.96	48.95	4.73	4.54	9.27
6	2.38	1.20	1.18	–	1.02	m	0.98	49.58	4.65	4.58	9.23
7	2.36	1.19	1.17	–	1.02	m	0.98	49.58	4.62	4.54	9.15
8	1.92	0.99	0.93	–	1.06	m	0.94	48.44	3.84	3.61	7.45
9	1.90	0.97	0.93	–	1.04	m	0.96	48.95	3.76	3.61	7.37
10	1.70	0.88	0.82	–	1.07	m	0.93	48.24	3.41	3.18	6.59
11	1.67	0.91	0.76	–	1.20	m	0.84	45.51	3.53	2.95	6.48
12	1.63	0.85	0.78	–	1.09	m	0.92	47.85	3.30	3.03	6.32
Total	25.78										

*: metacentric

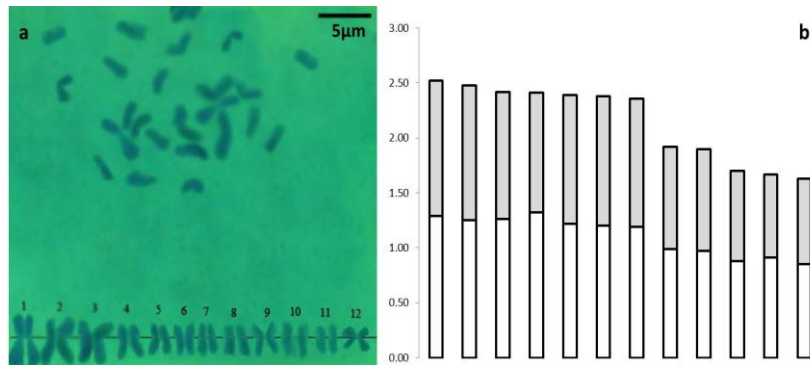


Figure 2. Somatic metaphase, karyotype (a) and haploid idiogram (b) in *Q. castaneifolia* (Population 1: Astara)

In population 2 which belongs to eastern parts of Babolsar, total chromosomes length varied from 1.65 to 2,59 µm. The longest arm was 1.32 µm and the shortest arm was 0.81 µm. No satellites were observed in the karyotypic of this population. Arm Ratio (L/S) varied from 1.02 to 1.15 and r-value (S/L) varied from 0.87 to 0.98. The karyotype of this population consisted of 12 pairs of median rignon (m) chromosomes (Table 3). Also, somatic metaphase, karyogram and haploid ideogram in population 2 of *Q. castaneifolia* (Babolsar) was shown in Figure 3.

Table3. Chromosome characteristics in *Q. castaneifolia* (Population 2: Babolsar)

Pair	TL	LA	SA	SAT	AR	TYPE	r-value	CI	%L	%S	RL
1	2.59	1.32	1.27	-	1.04	m	0.96	49.03	5.30	5.10	10.41
2	2.47	1.30	1.17	-	1.11	m	0.90	47.37	5.22	4.70	9.92
3	2.39	1.22	1.17	-	1.04	m	0.96	48.95	4.90	4.70	9.60
4	2.38	1.20	1.18	-	1.02	m	0.98	49.58	4.82	4.74	9.56
5	2.25	1.16	1.09	-	1.06	m	0.94	48.44	4.66	4.38	9.04
6	2.00	1.07	0.93	-	1.15	m	0.87	46.50	4.30	3.74	8.04
7	1.93	1.00	0.93	-	1.08	m	0.93	48.19	4.02	3.74	7.75
8	1.90	0.96	0.94	-	1.02	m	0.98	49.47	3.86	3.78	7.63
9	1.88	0.98	0.90	-	1.09	m	0.92	47.87	3.94	3.62	7.55
10	1.76	0.89	0.87	-	1.02	m	0.98	49.43	3.58	3.50	7.07
11	1.69	0.86	0.83	-	1.04	m	0.97	49.11	3.46	3.33	6.79
12	1.65	0.84	0.81	-	1.04	m	0.96	49.09	3.37	3.25	6.63
Total	24.89										

*: metacentric

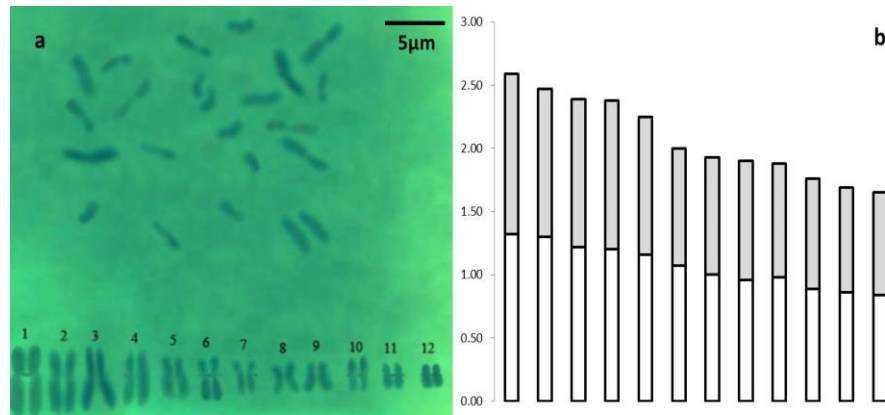


Figure 3. Somatic metaphase, karyotype (a) and haploid idiogram (b) in *Q. castaneifolia* (Population 2: Babolsar)

In population 3 which belongs to central parts of Chenaran rigion, total chromosomes length varied from 1.60 to 2.44 µm. The longest arm was 1.39 µm and the shortest arm was 0.78 µm. The satellites were not observed in the karyotypic of this population. Arm Ratio (L/S) varied from 1.01 to 1.32 and r-value (S/L) varied from 0.76 to 0.99. The karyotype of this population consisted of 12 pairs of median rigion (m) chromosomes (Table 4). Also, somatic metaphase, karyogram and haploid ideogram in population 3 of *Q. castaneifolia* (Chenaran) was shown in Figure 4.

Table 4. Chromosome characteristics in *Q. castaneifolia* (population 3: Chenaran)

Pair	TL	LA	SA	SAT	AR	TYPE	r-value	CI	%L	%S	RL
1	2.44	1.39	1.05	-	1.32	m	0.76	43.03	5.46	4.12	9.58
2	2.40	1.35	1.05	-	1.29	m	0.78	43.75	5.30	4.12	9.42
3	2.39	1.22	1.17	-	1.04	m	0.96	48.95	4.79	4.59	9.38
4	2.36	1.20	1.16	-	1.03	m	0.97	49.15	4.71	4.55	9.27
5	2.35	1.18	1.17	-	1.01	m	0.99	49.79	4.63	4.59	9.23
6	2.21	1.15	1.06	-	1.08	m	0.92	47.96	4.52	4.16	8.68
7	2.19	1.12	1.07	-	1.05	m	0.96	48.86	4.40	4.20	8.60
8	2.18	1.11	1.07	-	1.04	m	0.96	49.08	4.36	4.20	8.56
9	1.99	1.06	0.93	-	1.14	m	0.88	46.73	4.16	3.65	7.81
10	1.69	0.89	0.80	-	1.11	m	0.90	47.34	3.49	3.14	6.64
11	1.67	0.86	0.81	-	1.06	m	0.94	48.50	3.38	3.18	6.56
12	1.60	0.82	0.78	-	1.05	m	0.95	48.75	3.22	3.06	6.28
Total	25.47										

*: metacentric

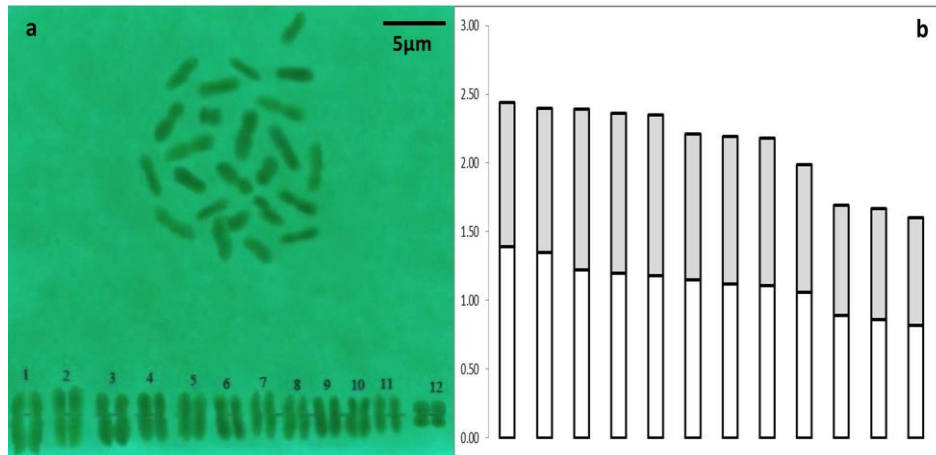


Figure 4. Somatic metaphase, karyotype (a) and haploid idiogram (b) in *Q. castaneifolia* (Population 3: Chenaran)

In population 4 which belongs to western parts of Ghorghan rigion, total chromosomes length varied from 1.75 to 2.68 μm . The longest arm was 1.44 μm and the shortest arm was 0.87 μm . The satellites were not observed in the karyotypic of this population. Arm Ratio (L/S) varied from 1.01 to 1.22 and r-value (S/L) varied from 0.82 to 0.99. The karyotype of this population consisted of 12 pairs of median rigion (m) chromosomes (Table 5). The somatic metaphase, karyogram and haploid ideogram in population 4 of *Q. castaneifolia* (Ghorghan) was shown in Figure 5.

Table 5. Chromosome characteristics in *Q. castaneifolia* (population 4: Ghorghan)

Pair	TL	LA	SA	SAT	AR	TYPE	r-value	CI	%L	%S	RL
1	2.68	1.44	1.24	–	1.16	m	0.86	46.27	5.44	4.68	10.12
2	2.63	1.39	1.24	–	1.12	m	0.89	47.15	5.25	4.68	9.93
3	2.59	1.32	1.27	–	1.04	m	0.96	49.03	4.98	4.80	9.78
4	2.46	1.28	1.18	–	1.08	m	0.92	47.97	4.83	4.46	9.29
5	2.39	1.24	1.15	–	1.08	m	0.93	48.12	4.68	4.34	9.03
6	2.23	1.20	1.03	–	1.17	m	0.86	46.19	4.53	3.89	8.42
7	2.11	1.16	0.95	–	1.22	m	0.82	45.02	4.38	3.59	7.97
8	2.04	1.03	1.01	–	1.02	m	0.98	49.51	3.89	3.81	7.70
9	1.95	0.99	0.96	–	1.03	m	0.97	49.23	3.74	3.63	7.36
10	1.86	0.96	0.90	–	1.07	m	0.94	48.39	3.63	3.40	7.02
11	1.79	0.91	0.88	–	1.03	m	0.97	49.16	3.44	3.32	6.76
12	1.75	0.88	0.87	–	1.01	m	0.99	49.71	3.32	3.29	6.61
Total	26.48										

*: metacentric

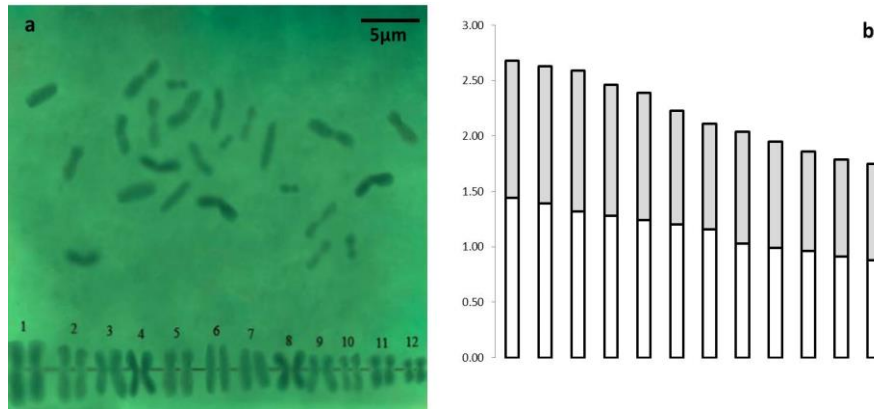


Figure 5. Somatic metaphase, karyotype (a) and haploid idiogram (b) in *Q. castaneifolia* (Population 4: Gorgan)

In population 5 which belongs to Zanoos rigion, total chromosomes length varied from 1.55 to 2.48 µm. The longest arm was 1.36 µm and the shortest arm was 0.73 µm. The satellites were not observed in the karyotypic of this population. Arm Ratio (L/S) varied from 1.01 to 1.21 and r-value (S/L) varied from 0.82 to 0.99. The karyotype of this population consisted of 12 pairs of median rigion (m) chromosomes (Table 6). The somatic metaphase, karyogram and haploid ideogram in population 5 of *Q. castaneifolia* (Zanoos) was shown in Figure 6.

Table 6. Chromosome characteristics in *Q. castaneifolia* (population 5: Zanoos)

pair	TL	LA	SA	SAT	AR	TYPE	r-value	CI	%L	%S	RL
1	2.48	1.36	1.12	-	1.21	m	0.82	45.16	5.60	4.61	10.21
2	2.42	1.29	1.13	-	1.14	m	0.88	46.69	5.31	4.65	9.97
3	2.38	1.25	1.13	-	1.11	m	0.90	47.48	5.15	4.65	9.80
4	2.35	1.19	1.16	-	1.03	m	0.97	49.36	4.90	4.78	9.68
5	2.27	1.14	1.13	-	1.01	m	0.99	49.78	4.70	4.65	9.35
6	2.19	1.12	1.07	-	1.05	m	0.96	48.86	4.61	4.41	9.02
7	1.13	1.07	1.06	-	1.01	m	0.99	49.77	4.41	4.37	8.77
8	2.08	1.06	1.02	-	1.04	m	0.96	49.04	4.37	4.20	8.57
9	1.94	0.99	0.95	-	1.04	m	0.96	48.97	4.08	3.91	7.99
10	1.80	0.93	0.87	-	1.07	m	0.94	48.33	3.83	3.58	7.41
11	1.69	0.89	0.80	-	1.11	m	0.90	47.34	3.67	3.29	6.96
12	1.55	0.82	0.73	-	1.12	m	0.89	47.10	3.38	3.01	6.38
Total	24.28										

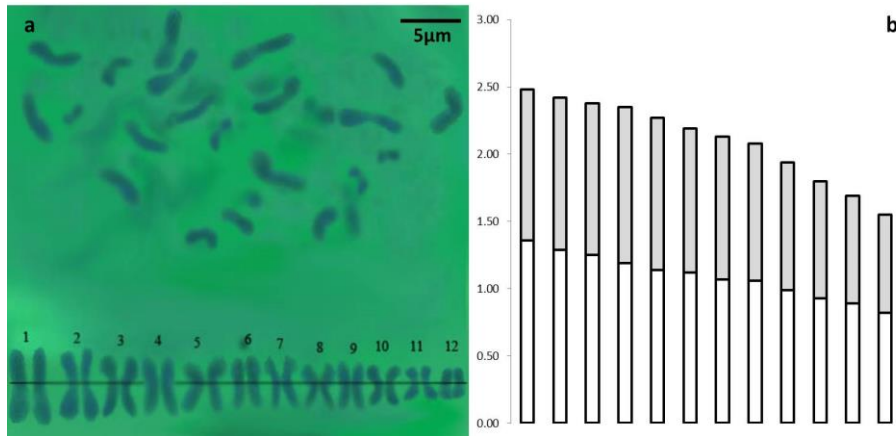


Figure 6. Somatic metaphase, karyotype (a) and haploid idiogram (b) in *Q. castaneifolia* (Population 5: Zanoos)

The mean of chromosome characteristics in five accessions of *Quercus castaneifolia* belongs to Northern parts of Iran that was shown in Table 7 and Figure 7. Mean of total chromosomes length varied from 1.64 ± 0.07 to $2.54 \pm 0.09 \mu\text{m}$. The longest arm was $1.36 \pm 0.06 \mu\text{m}$ and the shortest arm was $0.79 \pm 0.05 \mu\text{m}$. The satellites were not observed in the karyotype of these populations. Arm Ratio (L/S) varied from 1.04 ± 0.02 to 1.16 ± 0.12 and r-value (S/L) varied from 0.87 ± 0.09 to 0.97 ± 0.02 . The karyotype of this species consisted of 12 pairs of median region (m) chromosomes (Table 6). The haploid ideogram of *Q. castaneifolia* was shown in Figure 7.

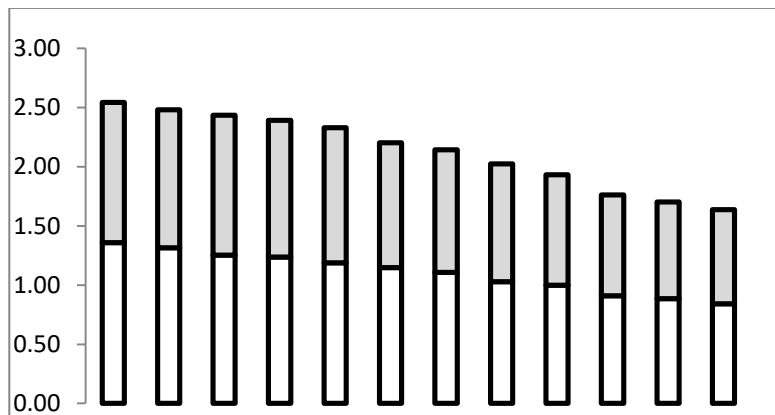


Figure 7. Haploid ideogram in *Q. castaneifolia* populations

Table 7. Mean Chromosome characteristics in *Q. castaneifolia*

Pai r	TL	LA	SA	S A T	AR	TY PE	r-vlue	CI	% L	% S	RL	% TF	%D RL
1	2.54± 0.09	1.36± 0.06	1.18± 0.09	—	1.16± 0.12	m	0.87± 0.09	46.46± 2.53	5. 36	4. 66	10. 02	48. 47	3.57
2	2.48± 0.09	1.32± 0.05	1.16± 0.08	—	1.14± 0.10	m	0.89± 0.07	46.91± 2.09	5. 19	4. 59	9.7 7		
3	2.43± 0.09	1.25± 0.04	1.18± 0.05	—	1.06± 0.03	m	0.94± 0.03	48.47± 0.72	4. 94	4. 65	9.5 9		
4	2.39± 0.04	1.24± 0.06	1.15± 0.04	—	1.07± 0.08	m	0.93± 0.07	48.30± 1.72	4. 88	4. 55	9.4 2		
5	2.33± 0.07	1.19± 0.04	1.14± 0.03	—	1.04± 0.03	m	0.96± 0.03	49.02± 0.76	4. 68	4. 50	9.1 8		
6	2.20± 0.14	1.15± 0.06	1.05± 0.09	—	1.09± 0.06	m	0.92± 0.05	47.82± 1.47	4. 52	4. 15	8.6 8		
7	1.94± 0.48	1.11± 0.08	1.04± 0.10	—	1.07± 0.09	m	0.94± 0.07	48.28± 1.93	4. 37	4. 08	8.4 5		
8	2.02± 0.12	1.03± 0.06	0.99± 0.06	—	1.04± 0.02	m	0.97± 0.02	49.11± 0.43	4. 06	3. 92	7.9 7		
9	1.93± 0.04	1.00± 0.04	0.93± 0.02	—	1.07± 0.05	m	0.94± 0.04	48.35± 1.05	3. 93	3. 68	7.6 1		
10	1.76± 0.07	0.91± 0.03	0.85± 0.04	—	1.07± 0.03	m	0.94± 0.03	48.35± 0.74	3. 59	3. 36	6.9 4		
11	1.70± 0.05	0.89± 0.03	0.82± 0.04	—	1.09± 0.07	m	0.92± 0.06	47.92± 1.54	3. 49	3. 22	6.7 1		
12	1.64± 0.07	0.84± 0.02	0.79± 0.05	—	1.06± 0.04	m	0.94± 0.04	48.50± 1.03	3. 32	3. 13	6.4 5		
To tal	25.38	13.28	12.30										

Discussion

All the accessions possessed a chromosome number of $2x=2n=24$ are supported the earlier studies by Tabande Saravi (2012). The karyotype analysis revealed that there was no secondary constriction and sub-terminal chromosome in any of the five accessions of *Q. castaneifolia*. When the karyotype asymmetry is taken into consideration the asymmetrical karyotypes are supposed to be more advanced than the symmetrical ones (Stebbins, 1950). Among the different accessions of *Q. castaneifolia* in the present study all the accessions having maximum number of metacentric chromosomes may be considered as the most primitive. But none of them showed sub-terminal chromosome, which is the characteristic of advanced forms. However, on the basis of karyotype analysis in the present study along with the number of

metacentric chromosomes and lack of sub-terminal chromosomes was observed in all the accessions of *Q. castaneifolia* which may be considered them as primitive type. It has been suggested that asymmetrical karyotypes that has more advanced than symmetrical ones (Stebbins, 1950) and the changes in symmetry are usually associated with chromatin loss. The concept of symmetry vs asymmetry has been proposed on the basis of predominance of metacentric and submetacentric chromosomes of approximately same size. Whereas increasing asymmetry has been related to shift of centromeric position from median/submedian to terminal/subterminal or through and differences in the relative size between the chromosome of the complement, thus making karyotype more heterogeneous. Since, the classification of Stebbins (1971) has been frequently used as qualitative method for assessing karyotype asymmetry and describing the typical relationships between different taxa, which recently followed by Seijo and Fernandez 2003 in *Lathyrus* and He *et al.* (2004) in between *Davidia involucrata* and *Camptotheca acuminata*. The investigations based on seven methods revealed the separation of different karyotype asymmetry and parameters of the broad intervals used by Stebbins, explaining only one quantitative parameter (Paszko, 2006).

The research finding is other estimated indices expressing that chromosomes are relatively symmetric in all populations to be indicated this species is primitive and undeveloped.

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