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Karyological and Chromosome Analysis of *Quercus libani* in Iran

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Abstract: This study attempts to analyze the karyotype and chromosome structure in five populations of *Quercus libani* growing in Northern Zagros. Pre-treatment, fixation, hydrolysis and staining were conducted using root meristem and then microscopic samples were prepared and studied. The results showed that in 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 0.64-2.08 micrometers. The longest chromosome was observed in chromosome number 1 from population 1 and the shortest one was related to the chromosome number 12 from population 5. Considering of chromosomal classification, all the studied populations were placed in class B of Stebbins which showed that there is an average symmetry in the studied karyotypes. The other estimated indices also showed that in chromosomes are relatively symmetric in all populations that indicates this species is primitive and undeveloped.

Keywords: Chromosome, Cytogenetic, Karyotype, *Quercus libani*

İran'da Yetişen *Quercus libani'*nin Karyolojik ve Kromozom Analizi

Özet: Bu çalışmanın amacı Kuzey Zagros'ta yetişen beş *Quercus libani* popülasyonunda karyotip ve kromozom yapısını analiz etmektir. Ön muamele, fiksasyon, hidroliz ve boyama, kök meristem kullanılarak yapılmıştır ve daha sonra mikroskobik numuneler hazırlanıp, incelenmiştir. Sonuçlar, her popülasyonun çalışılan tüm hücrelerinde, temel kromozom sayısının x = 12 olduğunu ve hepsinin diploid olduğunu göstermiştir. Her popülasyonun karyotip analizi ayrı ayrı gerçekleştirilmiş ve birkaç indeks (TL: Toplam Uzunluk, LA: Uzun Kol, SA: Kısa Kol, SI: Sentromer İndeksi, KO: Kol Oranı, R-değeri, NUF%: Nispi Uzunluk Farklılığı ve TF %: Toplam Form) belirlenmiştir. Karyotip formülü çalışılan tüm popülasyonlarda 12m olarak belirlenmiştir. Tüm popülasyonlardaki kromozomların uzunluğu, 0.64-2.08 mikrometre olarak hesaplanmıştır. En uzun kromozom, popülasyon 1'den 1 numaralı kromozomda ve en kısa kromozom, popülasyon 1'den kromozom 12'de gözlenmiştir. Kromozomal sınıflandırma göz önüne alındığında, çalışılan tüm popülasyonlar, incelenen karyotiplerde bir ortalama simetri olduğunu gösteren Stebbins sınıf B'ye yerleştirilmiştir. Tahmin edilen diğer indeksler, ayrıca tüm popülasyonlarda kromozomların nispeten simetrik olduğunu; bu türün ilkel ve gelişmemiş olduğunu göstermiştir.

Anahtar kelimeler: Kromozom, Sitogenetik, Karyotip, *Quercus libani*

Introduction

Breeding to raise both yield potential and yield further under environmental constraints through improved adaptiveness will be of paramount importance (Mohammadi et al., 2014). Cytogenetic is a branch of genetics which studies the construction of chromosomes. Cytogenetic studies regarded as the primitive and basic achievements in breeding researches since determination number of chromosomes and ploidy levels are necessary in selection of proper breeding method (Javadi et al. 2008). Cytological methods facilitate and make it possible to determine the chromosomal structure and to recognize specific chromosomes. In other words, karyotype analysis includes the analysis of the appearance, the number and construction of the chromosomes in terms of size, the location of the centromere and the other chromosomal details. Also, the analyses of chromosomal characteristics as well as cytogenetic studies provide the recognition of plant

species karyotype structure as well as analyzing diversity among different populations of a species. The genome of individuals contains genetic information and the result of gene expression is appearing of phenotypic traits so, any changes in chromosome construction and size resulted in different phenotypic traits appearances. Karyotypic studies within populations of a species are important since each of different populations may show specific genomic adaptation with their environmental growing conditions (Tabandeh Saravi et al. 2013). Variation in karyotype structure (number, type and size) and chromosome behavior during cell division can explain the genetic differences (Sheydaie et al. 1997). Generally, cytological studies provide valuable information regarding available gene pool of the country which could be used in gene bank (Hesamzadeh et al. 2008). Therefore, using the local germplasm in breeding programs is very important to add new traits in the genetic pool (Ozdemir Eroglu et al., 2016). Also Seed and chromosome morphology have been considered useful for solving systematic and evolutionary obscures (Kocyigit and Alp, 2018). The *Quercus* L. is a genus with 300 to 600 species (Johnson et al. 2002) and its number of base chromosome is n=12 so the most of its species are diploid (Demerico et al. 1995) and polyploidy occurs rarely in this genus (Tabandeh Saravi et al. 2013). This genus includes different species of evergreen and deciduous trees and shrubs which have expanded from cold climates to tropical forests of Asia and America. *Quercus libani* G. Olivier is a certain species of Northern Zagros in Iran and its height can reach up to 10 meters with gray trunk like as old and grooved trees (Sabeti 1966). The main growing habitats of this species are central and Eastern Taurus Mountains and Amanos of 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 Kurdistan and Kermanshah provinces and continue to the north of Piranshahr in West Azerbaijan. The area of the Northern Zagros forests is about 449000 hectares which *Q. libani* covers 106316 hectares (i.e. 24%) as pure or mixed population (Fattahi 1998). This study aimed to define chromosome karyotype and morphology of *Q.libani* species by analyzing different populations in order to present the best instruction for cytogenetic studies in this species.

Materials and Methods

In this research, annual seedlings of the studied species were prepared from Research Institute of Forests and Rangelands (Botanical Garden of the country), Nursery of Natural Resources Offices. Five populations were used for this study.

Population 1: Urmia (Gasemlu)
Population 2: Piranshahr (East part of Piranshahr)
Population 3: Sardasht (North part of Sardasht)
Population 4: Baneh (kanisur)
Population 5: Bookan (central part of Bookan)

Then transferred 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 so 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 karyotype studies. Then the following levels including pre-treatment, fixation, storing, hydrolysing, staining. observation, imaging of the cells and analysing of the chromosomes were done.

1- Pre-treatment: this process was done using 0.5% saturated alpha-bromo naphthalene solution in water for 3 hours followed by washing with water.

2- Fixation: after pre-treatment, the roots were washed with distilled water and placed in fixing solution. These solutions make the chromatin to precipitate and also kill the cells quickly. The main goal of fixation is keeping the cell structure as well as preserving all forms of cell divisions using Lewitsky solution.

3- Storing: after fixation, it is necessary to keep the samples for relatively long time at 4ºC since it is not possible to hydrolyze all samples together sosamples were placed in ethanol 70% followed by fixation and washing.

4- Hydrolysis: 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 60ºC.

5- Staining: 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 Aceto Orcein. Root meristems after treated with cytase enzyme for removing cell walls followed by staining (Agayev 1996). Root tip samples were squashed and microscopic slides prepared followed 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, the length of short arm, the total length of chromosomes and centromer index (CI) were measured and recorded using Micromeasure software. Also, the ideogram of each populations was drawn 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).

Results and Discussion

Results of the mitotic metaphase plates in studied populations as well as the karyotype images and populations evolutionary status showed that in 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 and centromeric index were determined. The karyotype formula was 12m in all studied populations (tables and figures 1-5). The chromosomes type in all populations was metacentric without any satellite. The length of chromosomes in all populations was calculated 0.64-2.08 µm. The longest chromosome was observed in chromosome number 1 from population 1 belongs to Urmia (gasemlu) and the shortest one was related to the chromosome number 12 from population 5 which belongs to Bookan (central part of Bookan).

Table 1. Chromosome characteristics in *Q. libani* (Population 1: Urmia)

Chromosome Number	Centromer Index	Short Arm Length (µm)	Long Arm (µm)	Total Length (µm)	Chromosome Type
1	49.03	1.02± 0.17	1.06± 0.39	2.08±0.03	M*
2	45.21	0.85± 0.32	1.03± 0.47	1.88± 0.16	M
3	46.36	0.83± 0.06	0.96± 0.04	1.79± 0.08	M
4	43.31	0.68± 0.58	0.89± 0.52	1.57± 0.06	M
5	47.91	0.69± 0.12	0.75± 0.17	1.44± 0.05	M
6	47.69	0.62± 0.03	0.68± 0.10	1.3± 0.07	M
7	49.16	0.59± 0.29	0.61± 0.43	1.2± 0.13	M
8	48.62	0.53± 0.04	0.56± 0.04	1.09± 0.04	M
9	48.91	0.45± 0.16	0.47± 0.16	0.92± 0.04	M
10	50.00	0.43± 0.26	0.43± 0.31	0.86± 0.05	M
11	48.14	0.39± 0.29	0.42± 0.24	0.81± 0.05	M
12	45.33	0.34± 0.01	0.41± 0.01	0.75± 0.05	M

*: Metacentric

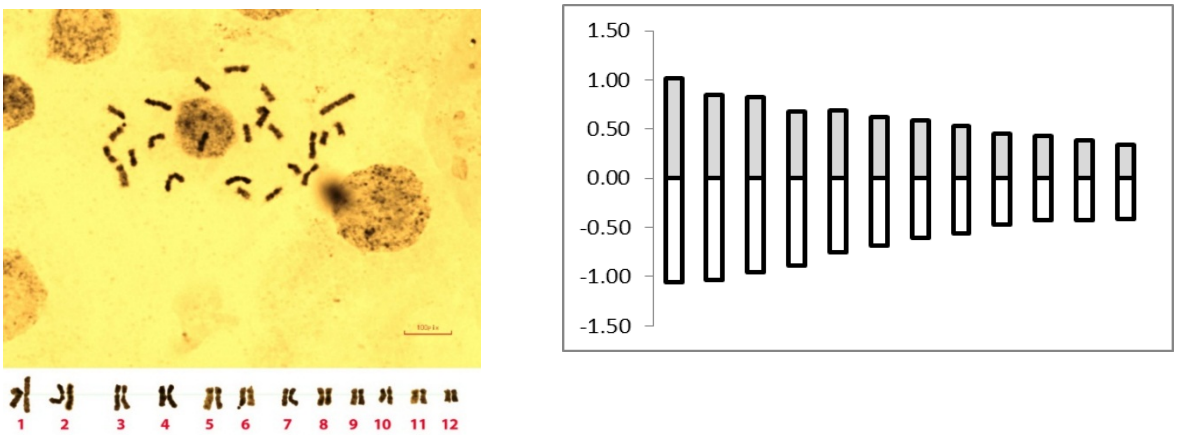

Figure1. Somatic metaphase, idiogram and karyotype in Q. *libani* (Population 1: Urmia).

Table 2. Chromosome characteristics in *Q*. libani (Population 2: Piranshahr)

Chromosome Number	Centromer Index	Short Arm Length (µm)	Long Arm (µm)	Total Length (µm)
1	49.01	1.00± 0.17	1.04± 0.39	2.04±0.01
2	46.19	0.85± 0.32	0.99± 0.47	1.84± 0.16
3	46.66	0.84± 0.06	0.96± 0.04	1.80± 0.08
4	45.66	0.79± 0.58	0.94± 0.52	1.73± 0.06
5	42.40	0.67± 0.12	0.91± 0.17	1.58± 0.05
6	42.75	0.59± 0.03	0.79± 0.10	1.38± 0.07
7	39.28	0.44± 0.29	0.68± 0.43	1.12± 0.13
8	40.20	0.39± 0.04	0.58± 0.04	0.97± 0.04
9	47.67	0.41± 0.16	0.45± 0.16	0.86± 0.04
10	49.41	0.42± 0.26	0.43± 0.31	0.85± 0.05
11	48.19	0.40± 0.29	0.43± 0.24	0.83± 0.05
12	48.75	0.39± 0.01	0.41± 0.01	0.80± 0.05

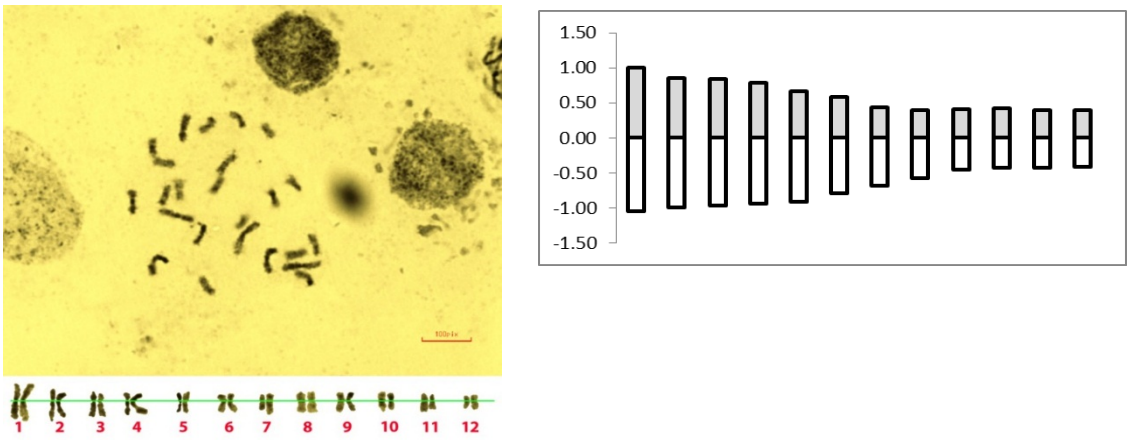

Figure 2- Somatic metaphase, idiogram and karyotype in *Q. libani* (population 2: Piranshahr).

Table 3. Chromosome characteristics in *Q. libani* (population 3: Sardasht)

Chromosome No.	Centromer Index	Short Arm Length (µm)	Long Arm (µm)	Total Length (µm)
1	46.73	0.86± 0.17	0.98± 0.39	1.84±0.06
2	46.62	0.83± 0.32	0.95± 0.47	1.78± 0.16
3	44.91	0.75± 0.06	0.92± 0.04	1.67± 0.08
4	43.42	0.66± 0.58	0.86± 0.52	1.52± 0.06
5	43.04	0.65± 0.12	0.86± 0.17	1.51± 0.05
6	43.83	0.64± 0.03	0.82± 0.10	1.46± 0.07
7	43.75	0.63± 0.29	0.81± 0.43	1.44± 0.13
8	43.57	0.61± 0.04	0.79± 0.04	1.40± 0.04
9	47.58	0.59± 0.16	0.65± 0.16	1.24± 0.04
10	49.53	0.53± 0.26	0.54± 0.31	1.07± 0.05
11	48.51	0.49± 0.29	0.52± 0.24	1.01± 0.05
12	41.66	0.35± 0.01	0.49± 0.01	0.84± 0.05

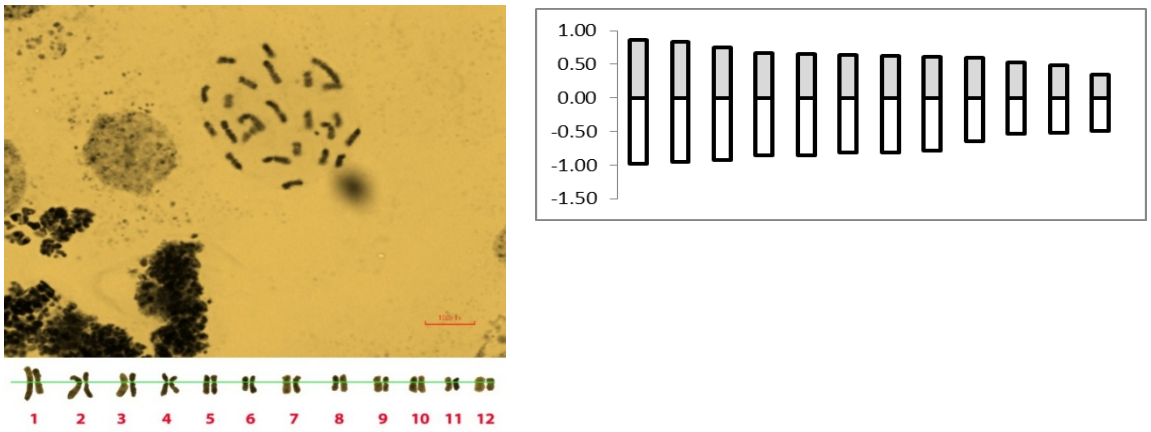

Figure 3- Somatic metaphase, idiogram and karyotype in *Q. libani* (population 3: Sardasht).

Table 4. Chromosome characteristics in *Q. libani* (population 4: Baneh)

Chromosome No.	Centromer Index	Short Arm Length (µm)	Long Arm (µm)	Total Length (µm)
1	49.75	1.00± 0.17	1.01± 0.39	2.01±0.03
2	46.77	0.87± 0.32	0.99± 0.47	1.86± 0.16
3	48.29	0.85± 0.06	0.91± 0.04	1.76± 0.08
4	44.37	0.71± 0.58	0.89± 0.52	1.60± 0.06
5	44.80	0.69± 0.12	0.85± 0.17	1.54± 0.05
6	43.62	0.65± 0.03	0.84± 0.10	1.49± 0.07
7	42.75	0.59± 0.29	0.79± 0.43	1.38± 0.13
8	39.02	0.48± 0.04	0.75± 0.04	1.23± 0.04
9	37.83	0.42± 0.16	0.69± 0.16	1.11± 0.04
10	49.54	0.54± 0.26	0.55± 0.31	1.09± 0.05
11	43.33	0.39± 0.29	0.51± 0.24	0.90± 0.05
12	43.50	0.38± 0.01	0.47± 0.01	0.85± 0.05

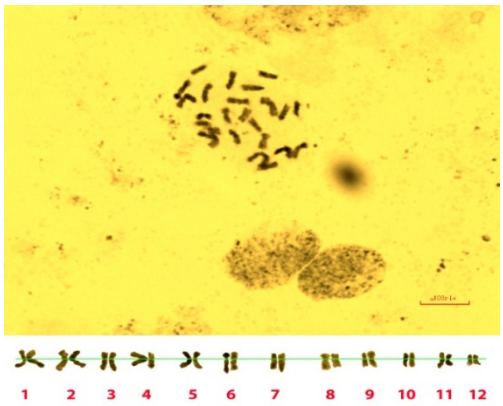

Figure 4- Somatic metaphase, idiogram and karyotype in *Q. libani* (population 4: Baneh).

Table 5. Chromosome characteristics in *Q. libani* (population 5: Bookan)

Chromosome No.	Centromer Index	Short Arm Length (µm)	Long Arm (µm)	Total Length (µm)
1	49.10	0.82± 0.17	0.85± 0.39	1.67±0.08
2	49.06	0.79± 0.32	0.82± 0.47	1.61± 0.16
3	46.76	0.65± 0.06	0.74± 0.04	1.39± 0.08
4	44.35	0.55± 0.58	0.69± 0.52	1.24± 0.06
5	37.86	0.39± 0.12	0.64± 0.17	1.03± 0.05
6	35.71	0.35± 0.03	0.63± 0.10	0.98± 0.07
7	38.29	0.36± 0.29	0.58± 0.43	0.94± 0.13
8	37.77	0.34± 0.04	0.56± 0.04	0.90± 0.04
9	37.34	0.31± 0.16	0.52± 0.16	0.83± 0.04
10	41.42	0.29± 0.26	0.41± 0.31	0.70± 0.05
11	41.79	0.28± 0.29	0.39± 0.24	0.67± 0.05
12	40.62	0.26± 0.01	0.38± 0.01	0.64± 0.05

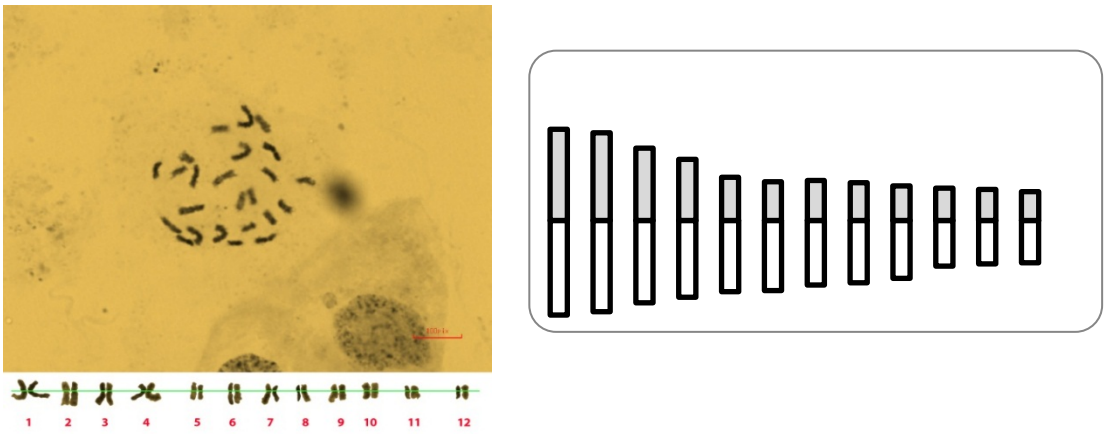

Figure 5- Somatic metaphase, idiogram and karyotype in *Q. libani* (population 5: Bookan).

The study of the karyotype of the various species is a preliminary step, but fundamental to cytogenetic research, which has been recognized since the beginning of the last century in order to classify the plants, to validate systematic data and to help solve the problems of classical taxonomy, so that Later, for the determination of kinship relations between species of a genus, not only the number of chromosomes, but also information such as size, morphology, location of the centromere, and the behavior of the chromosomes were also examined (Stebbins, 1971). Karyotype and chromosome components analysis in different species and populations of some gymnosperms species such aspine family are more or less easy due to their low number of chromosomes as well as large relative sizetorina and Mozgalina 2004). Results showed that the number of chromosomes in most economically important conifers was ranged from n=11 to n=13. On the other hand, chromosomal number in economically important broad-leaved trees such as *Alnus* sp., *Fagus* sp, *Magnolis* sp. and several species of *Prunus* ranged between n=7 to n=19 (Wright, 1976) which compatible with results obtained from *Q. libani* (n=12) in this study. Also The chromosome number of *Quercus libani* was given previously as mostly 2n=24 but also reported 2n= 22 (Fedorov 1974). The most important finding is that the result of chromosomes number analysis was compatible with findings reported in other *Quercus* species in Europe including *Q. frainetto* Ten, *Q. trojana* Webb, *Q. macrolepis* Kotschy, *Q. cerris* L, *Q. crenata* LAM, *Q. coccifera* L, *Q. virgiliana* Mill and *Q. dalechampii* Ten (Demerico et al. 1995; Aykut et al. 2011) which indicated that the fixed chromosome number of *Quercus* genus is 2n=24. It also shows that the basic chromosome number in this genus is x=12 so all species are diploid and generally polyploidy is more rare in this genus (Dzialuk et al. 2007). In the karyological study of oak species in Turkey (*Q. libani*, *Q. petraea*, *Q. coccifera* and *Q. infectoria*) the number of chromosomes in all species was 2n=24. Length of chromosomes in *Q. libani* was between 0.8 to 2.18 µm (Aykut et al. 2014), which was a little greater than present study (0.64-2.08 µm). Also *Q. libani* was reported as a different species among Turkish oak (Aykut et al. 2008), which it was the first karyotypic study on *Q. libani* in the world.

After that, in this study, karyotype of *Q. libani* have been done for the first time in Iran. So, this and other comparative studies can help supplement oak taxonomy.

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