

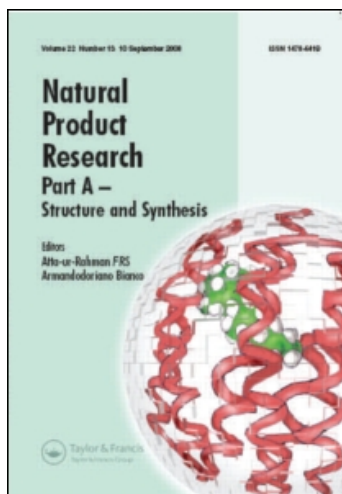
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Emine Ayaz^a; Süreyya Namli^a

^a Faculty of Science and Art, Department of Biology, The University of Dicle, Diyarbakir, Turkey

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The karyotype analysis of *Pistacia vera* L. from Turkey

Emine Ayaz* and Süreyya Namli

Faculty of Science and Art, Department of Biology, The University of Dicle, Diyarbakir, Turkey

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A detailed karyotype analysis was developed for *Pistacia vera* L. grown in Turkey. *In vitro* roots obtained from mature seeds were used as plant material. The chromosome number of *P. vera* L. was found to be $2n = 30$ at *c*-metaphase of mitosis cell investigated for all of the materials. Centromere type of all chromosomes were determined as median, submedian, subtelocentric, telocentric and total lengths of chromosome pairs were found between 35.4 and 5.97 μm . An idiogram was constructed from the average chromosome length, arm ratio and centomere type for each of the chromosome pairs.

Keywords: *Pistacia vera* L; karyotype analysis; chromosome

1. Introduction

The genus *Pistacia* is a member of Anacardiaceae family, and *Pistacia vera* L. is one of the 11 species of this genus (Stuessy, 1990; Yaltirik, 1967c). *Pistacia vera* L. produces edible nuts, large enough to be commercially acceptable and cultivated widely in Iran, California and the Mediterranean regions of Europe (Harandi & Ghaffari, 2000). The complete summary and taxonomic descriptions for *Pistacia* are provided by Zohary. Zohary described 11 *Pistacia* species, divided into four sections: *Lentscella*, *Lentiscus*, *Butmela* and *Terebinthus*, containing *P. vera* L. (Zohary, 1952). There are six *Pistacia* species in Turkey (Yaltirik, 1967a), five of which (*P. vera*, *P. khinjuk*, *P. atlantica*, *P. terebinthus* and *P. lentiscus*) were described by Zohary and one (*P. eurocarpa*) by Yaltirik (Yaltirik, 1967b, 1967c).

For taxonomic identification and classification of *Pistacia*, diagnostic traits such as leaves, flowers, fruits and growing habit of the trees have been used. However, leaf characteristics (size, shape, number of leaflets) are used for the primary characters among these diagnostic traits (Parfitt & Badenes, 1997). Floral characters have been used less for identification in *Pistacia* but have been used above the genus level (Wannan & Quinn, 1991). This is surprising because there is considerable variability for inflorescence structure and nut morphology. Due to these distinctions, identifying species is difficult.

*Corresponding author. Email: eayaz@dicle.edu.tr

There is little cytological information for *Pistacia* species in the literature. The first research on the chromosome number of *P. vera* L. ($2n=30$) was reported by Zohary. Chromosome counts were performed on three *Pistacia* species: *P. lentiscus* with $2n=24$, *P. atlantica* with $2n=28$ and *P. vera* with $2n=30$. The chromosome counts were also evaluated on *Pistacia* species by Harandi, Behboodi, Abd-mishani, and Ghaffari (1996) and Harandi and Behboodi (1997): *P. vera* as $2n=30$, *P. khinjuk* as $2n=24$ and the other subspecies of *P. atlantica* as $2n=28$ (Harandi et al., 1996; Harandi & Behboodi 1997). Since then, other researchers have also reported the same chromosome numbers for *P. vera* L. (Maggs, 1973; Ozbek, 1978). However, the diploid chromosome number for *P. vera* L. has been reported both as $2n=30$ and as $2n=32$ by Janick (2002) and Janick, Barghchi and Alderson (1989).

The development of karyotype analysis is difficult on pistachio because its chromosomes were extremely small, frequently having only a few cell divisions visible in a single root tip. Chromosome studies are often useful in suggesting taxonomic and phylogenetic relationships (Raven, 1975; Stuessy, 1990). The aim of this study is to determine chromosome numbers, karyotype analysis and idiogram of *Pistacia* species in root tip cells which are obtained *in vitro*. This study provides for further information on pistachio cultivars for future cytogenetic research.

2. Materials and methods

Pistachio (*Pistacia vera* L.) seeds were collected from the Gaziantep province of south-east Turkey. Chromosome study carried out in meristematic cells of root tips obtained from seeds germinated in *in vitro* conditions. For this study, mature seeds of *P. vera* L. used as material were first washed with tap water for 15 min, then they were kept in 20% sodium hypochlorite (NaOCl) for 30 min. Later they were cleaned from NaOCl by rising them in sterilised water five times each time for 5 minutes. The seeds isolated were transferred separately into 1/1 MS (Murashige & Skoog, 1962) media containing 3% sucrose (w/v) and agar (5.5% w/v). Media were adjusted to pH 5.7 prior to autoclaving (120° for 20 min.). The mature seeds were left to grow under 3000 lux at 16/8 photoperiod and at 25 + 2°C.

Mature seeds of *P. vera* L. were germinated in 10 days under *in vitro* condition. When meristematic root tips reached 1–1.5 cm, they were cut off and pretreated with paradichlorobenzene for 4 h, fixed with acetic alcohol (1:3) for 24 h and stored in 70% alcohol at 4°C. Stored root tips were washed and hydrolysed in 1N HCl for 5–12 min at 60°C and stained in feulgen for 2 h (Elçi, 1982). To confirm staining, quality root tips were kept in water for 15 min and squashed for preparations. Measurements of maximum and minimum chromosome sizes were based on drawings of five metaphase plates in this species, compared with a micrometric scale. The karyotype analysis was designed according to the nomenclature system advocated by Levan, Fredga and Sandberg (1964).

3. Results

The present study demonstrated that *P. vera* L. is a diploid species with $2n=30$ chromosome complement (Figure 1). *Pistacia vera* had a karyotypic formula of $8sm + 4m + 2st + 1t$. No satellite has been observed for this species. The length of chromosomes ranged from 35.4 to 5.97. Arm ratios are between 1.19 and 4.57. The relative

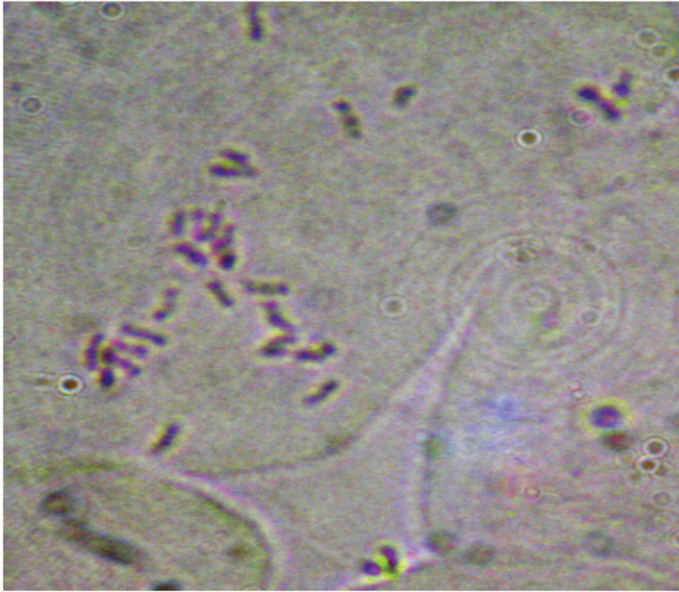


Figure 1. Somatic metaphase chromosomes of *P. vera* L. ($2n=30$).

Table 1. Measurements (μm) of somatic metaphase chromosomes of *P. vera* L.

Chromosome pair no.	Total length	Long arm length (L)	Short arm length (s)	Satellite length	Arm ratio ($L s^{-1}$)	Relative length (%)	Centromere type
I	35.4	19.3	16.1	—	1.19	12.27	m
II	25.8	17.2	8.6	—	2.0	8.94	Sm
III	23.6	15.0	8.6	—	1.74	8.18	Sm
IV	23.6	15.0	8.6	—	1.74	8.18	Sm
V	22.5	16.1	6.4	—	2.51	7.80	Sm
VI	22.5	13.9	8.6	—	1.61	7.80	Sm
VII	22.5	12.9	9.6	—	1.34	7.80	m
VIII	21.4	15.0	6.4	—	2.34	7.42	Sm
IX	19.3	15.0	4.3	—	3.48	6.69	St
X	16.1	11.8	4.3	—	2.74	5.58	Sm
XI	15.0	10.7	4.3	—	2.48	5.20	Sm
XII	15.0	8.6	6.4	—	1.34	5.20	m
XIII	10.7	6.4	4.3	—	1.48	3.71	m
XIV	8.97	7.9	1.07	—	7.38	3.11	t
XV	5.97	4.9	1.07	—	4.57	2.07	St

Note: Total length of haploid complements: $288.34 \mu\text{m}$.

lengths of chromosomes varied between 12.27 and 2.07 and the total length of the haploid complement is $288.34 \mu\text{m}$ (Table 1). According to the schematic representation of this karyotype, the largest chromosome was median. Of the remaining chromosomes, eight were submetacentric, three were metacentric, two were subtelocentric and one was telocentric (Figure 2).

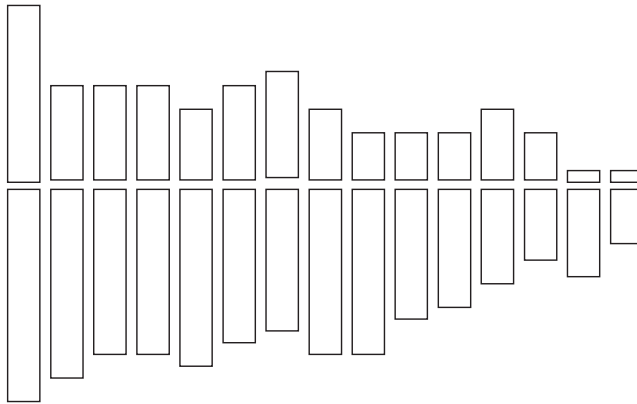


Figure 2. Idiogram of *P. vera* L.

4. Discussion

The limited chromosome reports in this species (*P. vera* L.) have so far been made by a few workers (Yaltirik, 1967c; Murashige & Skoog, 1962; Janick, 2002; Barghchi, & Alderson, 1989; Harandi et al., 1996; Bochantseva, 1972). The chromosome number observed for *P. vera* L. ($2n=30$) confirms previous reports by Zohary (1952), Bochantseva (1972) and Ghaffari and Harandi (2002) and differs from the number $2n=30$ and $2n=32$ reported by Janick (2002). Barghchi and Alderson (1989) and Ghaffari and Harandi (2002) investigated some *Pistacia* species from Iran and reported that the chromosome complement of *P. vera* was $2n=30$, with four metacentric pairs, eight submetacentric pairs and three acrocentric pairs. One pair of metacentric chromosomes was heterochromatic in nature (Ghaffari & Harandi, 2002). However, we found that eight were submetacentric, four were metacentric, two were subtelocentric and one was telocentric. Despite the similarities in chromosome number, the length of three chromosome pairs seem to be different with observed contrasting morphology and these might be important in the understanding of species differentiation. This is the first report including measurements (μm) of somatic metaphase chromosomes of *P. vera* from the Turkey region. In our analysis, the chromosome number $2n=30$ was the most common, followed by $2n=32$. This divergence may be due to dysploid changes between populations. This species has minute chromosomes that sometimes appear associated, resulting in miscounts.

Our results have shown that further karyological approaches, especially including chromosome morphology and karyotype analysis, could be valuable in future taxonomic and evolutionary studies.

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