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Effective Method to Resolve the Chromosome Numbers in *Pistacia* Species (Anacardiaceae)

Mohannad Al-Saghir[1*], Shadi Abu Baker[2], Robert Pusok[1]

[1]Department of Environmental and Plant Biology, Ohio University Zanesville, Zanesville, OH, USA
[2]Department of Chemistry, Ohio University Zanesville, Zanesville, OH, USA
Email: [*]al-saghi@ohio.edu

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Abstract

The genus *Pistacia* L. belongs to the Anacardiaceae family and includes at least eleven species. Cytogenetic studies addressing the genus *Pistacia* are rather few. Chromosome numbers of the different *Pistacia* species, revealed by these studies, are questionable due to the fact that poor chromosome counting protocols were used, and these protocols are hampered by the extremely small-sized chromosomes of *Pistacia* species. The aim of this study was to develop a more effective method to resolve the chromosome numbers in *Pistacia* species using a fluorescent microscope. The method described here is modified from the Sigma Plant Protoplast Digest/Wash Solution protocol. The method used here is highly effective for karyotyping analysis and studying population genetics of *Pistacia* species. Moreover, it is easy and can be reproduced for other species that have smaller chromosomes. This method can be used for plant herbarium specimens or field plants. This study provides valuable chromosomal data for cytogeneticists and plant breeders who are working on this genus. It provides additional insight into understanding the taxonomic and phylogenetic relationships among *Pistacia* species. The chromosomes described here are also suitable for gene and genome mapping.

Keywords

***Pistacia*, Cytogenetics, Chromosome Numbers, Fluorescent Microscopy**

[*]Corresponding author.

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1. Introduction

The genus *Pistacia* L. belongs to the Anacardiaceae family and includes at least eleven species [1]. The genus includes *Pistacia vera* L., the cultured pistachio, which has edible nuts and considerable commercial importance [1]. The other species grow in the wild and their seeds are used as a rootstock seed source and sometimes are used for fruit consumption, oil extraction, or soap production [1].

The pistachio is native to the arid zones of Central Asia; it has been cultivated for 3000 - 4000 years in Iran and was introduced into Mediterranean Europe by Romans at the beginning of the Christian Era [2]. Pistachio cultivation extended westward from its center of origin to Italy, Spain, and other Mediterranean regions of Southern Europe, North Africa, and the Middle East, as well as to China, and more recently to the United States and Australia [3]. Pistachios are adapted to a variety of soils and are probably more tolerant of alkaline and saline soil than most tree crops [4].

Chromosomal data have been valuable resource for cytogeneticists and plant breeders. They often provide more insight into taxonomic and phylogenetic relationships [5] [6].

Cytogenetic studies addressing the genus *Pistacia* are rather few. Previous studies showed that all *Pistacia* species are diploid with chromosome numbers 2n = 24, 28, and 30. 2n = 28 was reported for *P. atlantica* or its subspecies by several researchers [7]-[9]. However, a recent study by Ila *et al.* [10] reported the chromosome number for the first time as 2n = 30 for the same species.

Chromosome number of *P. chinensis* was reported as 2n = 24 [11] [12]. Chromosome number of *P. eurycarpa* was reported as 2n = 30 [10] for the first time. Chromosome number of *P. intergimma* was reported as 2n = 30 [13]-[16]. Chromosome number of *P. khinjuk* was reported as 2n = 24 [9] and as 2n = 30 [8]. 2n = 24 was reported for *P. lentiscus* [7] [9] [17] and 2n = 30 [18]. Chromosome number for *P. terebinthus* was reported as 2n = 30 [8] [10] [17]. Chromosome number for *P. vera* was 2n = 30 [7] [8] [19]-[21].

Chromosome numbers of the different *Pistacia* species are questionable and controversial due to the fact that poor chromosome counting protocols were used [10]; these protocols are hampered by the extremely small-sized chromosomes of *Pistacia* species and a few cell divisions were visible in a single root tip [21].

The aim of this study was to develop a more effective method to resolve the chromosome numbers in *Pistacia* species using a fluorescent microscope and provide more insight into understanding the cytogenetics and phylogeny of the genus *Pistacia*. The method described here is modified from the Sigma Plant Protoplast Digest/Wash Solution protocol (Sigma, St. Louis, MO, USA).

2. Materials and Methods

2.1. Plant Materials

Leaves from the herbarium specimens of *Pistacia atlantica* Desf., *P. khinjuk* Stocks., *P. lentiscus* L. and *P. terebinthus* L. were used. The specimens were obtained from the Massey Herbarium, Department of Biological Sciences, Virginia Polytechnic Institute and State University Tech, Blacksburg, Virginia. All plants were collected by the first author in Jordan in 2004.

2.2. Chromosome Preparation

In this study, the Sigma Plant Protoplast Digest/Wash Solution protocol (Sigma, St. Louis, MO, USA) was modified and can be reproduced specifically for *Pistacia* species as the following:

1 g of leaf tissue was gathered from each specimen and sliced into 1 mm strips with a sharp blade. The tissue strips were placed in 50 mL conical vials, each filled with 20 mL of Plant Protoplast Digest/Wash Solution (Sigma, St. Louis, MO, USA). After being mixed via inversion for 5 min, the Digest/Wash Solution was removed leaving only the leaf tissue. To the leaf tissue 10 mL of the digestion enzyme solution was added and mixed via inversion for 2 min. The mixture was then gently agitated on the platform shaker for one hour.

After 1 h, 50 µL of each mixture was diluted into 4 micro centrifuge tubes each containing 450 µL of Digest/Wash Solution. The mixtures were spun at 100 × g-forces for 5 min; the supernatant was removed leaving the pellets intact.

20 mL of Digest/Wash Solution was added to the pellets and was mixed via inversion. The mixture was spun again at 100 × g-forces for 5 min. The supernatant was then removed and 10 µL of fixative was added to each pellet, the pellets were then re-suspended via inversion and chilled on ice for one hour. The chilled mixtures

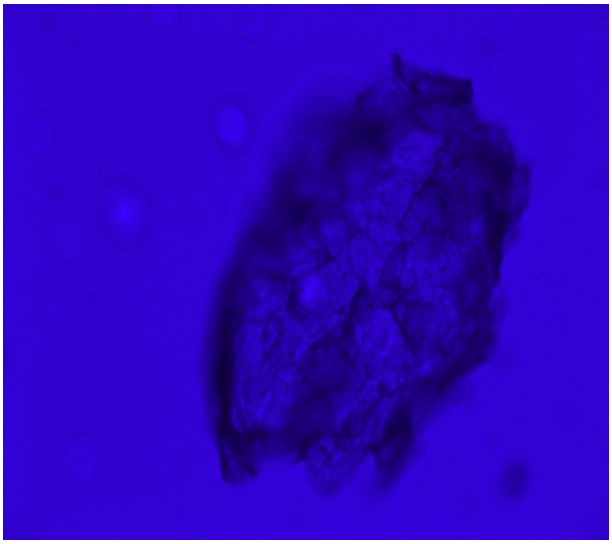

Figure 1. Cell with chromosomes (2n = 30) of *Pistacia atlantica* Desf.

were dropped onto room-temperature slides. The slides were stained with 4'-6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO, USA) and placed under fluorescent microscope (Leica, Wetzlar, Germany).

3. Results and Discussion

The results show that all used *Pistacia* species are diploid and they have the same chromosome number of 2n = 30 (**Figure 1**).

This study is the first to report the chromosome numbers using fluorescent microscope. The method was developed for *Pistacia* species because root-tips from the field are not possible to obtain and roots from seedlings are far too small. Moreover, the *Pistacia* species have very small chromosomes.

The method used here is highly effective for karyotyping analysis and studying population genetics of *Pistacia* species. Moreover, it is easy and can be reproduced for other species that have smaller chromosomes. This method can be used for plant herbarium or field plants.

This study provides valuable chromosomal data for cytogeneticists and plant breeders who are working on this genus. It provides additional insight into understanding the taxonomic and phylogenetic relationships among *Pistacia* species. The chromosomes described here are also suitable for gene and genome mapping.

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