

Karyotyping of *Spondias* L. (Anacardiaceae) Using Fluorescent Microscope

Abstract

The genus *Spondias* L. belongs to the Anacardiaceae family and includes 18 species. Cytogenetic studies addressing the genus *Spondias* are rather few. Previous studies did not include all *Spondias* species and the chromosome number is questionable due to the fact that poor chromosome counting protocols were used. In this paper, the karyotypes of five *Spondias* species were studied using fluorescent microscope to provide in-depth insights to understanding the cytogenetics and phylogeny of the genus *Spondias*. The results show that all studied *Spondias* species have the same chromosome number which is $2n=32$ based on all previous morphological and molecular studies, which clearly suggest a very close genetic relationship among *Spondias* species, and the chromosome numbers reported by various cytogenetic studies.

Keywords: *Spondias*; Cytogenetics; Chromosome numbers; Fluorescent microscopy

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Introduction

The genus *Spondias* L. belongs to the Anacardiaceae family and includes 18 species with at least nine Neotropical species [1]. *Spondias* is native to tropical America and Asia, and Madagascar. *Spondias* has been used as far as 6500 B.C., in the Tehuacán Valley of Mexico [2]. *S. mombin* L. (Cajá), *S. tuberosa* Arruda Câmara (Umbu) and *S. purpurea* (Serigüela) are the most economically important species in the genus for agro-industrial applications. Chromosomal data are a valuable resource for cytogeneticists and breeders as they provide better insights into taxonomic and phylogenetic relationships among the species [3,4]. Cytogenetic studies addressing the genus *Spondias* are rather limited. Previous studies showed that studied *Spondias* species were diploids with somatic chromosome number of $2n=32$. The only cytogenetic data available are the chromosome numbers ($2n=32$) of three species: *S. pinnata* [5-7], *S. mombin* [8], and *S. tuberosa* [9]. Recently, De Souza Alemdia et al. [10] studied the karyotype differentiation among five *Spondias* species and the putative hybrid Umbucajá. All the studied species and hybrid presented the same chromosome number ($2n=32$) and morphology. Previous studies did not include all *Spondias* species and chromosome numbers of the different *Spondias* species are questionable because inefficient chromosome counting protocols were employed [11]. These protocols are hampered by the small sized chromosomes of *Spondias* species and a few cell divisions were visible in a single root tip [11]. The aim of this study was to develop more effective method to resolve the chromosome numbers in *Spondias* species using fluorescent microscope and provide more insight into understanding the cytogenetic and phylogeny of the genus *Spondias*.

Materials and Methods

Plant materials

Leaves from the herbarium specimens of six *Spondias* species were used. The species studied are listed in (Table 1). The

specimens were obtained from University of Florida Herbarium (FLAS), Gainesville, Florida. For this study, the Sigma Plant Protoplast Digest/Wash Solution protocol (Sigma, St. Louis, MO, USA) was modified as the following [12]: 1 g of dried leaf tissue was collected from each specimen and cut into 1mm sections using a sharp blade. The sections 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. Ten mL of the digestion enzyme solution was added and mixed via inversion for 2 min. The mixture was gently agitated on the platform shaker for one hour. After 1 h, 50 μ L of each mixture was diluted into 4 micro centrifuge tubes each containing 450 μ L of Digest/Wash Solution.

The mixtures were spun at 100 x g-forces for 5 min; the supernatant was removed leaving the pellets intact. Twenty mL of Digest/Wash Solution was added to the pellets and was mixed. The mixture was spun at 100x g-forces for 5 min. The supernatant was removed and 10 μ L of fixative was added to each pellet; the pellets were re-suspended via inversion and chilled on ice for one hour. Then, the mixtures 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).

Results and Discussion

The results show that *Spondias* species are diploid and they have the same chromosome number of $2n=32$ (Table 1 & Figure 1). This study is the first to report the chromosome numbers using fluorescent microscope and the first to report the chromosome number for all studied species except *S. mombin*. The method was developed initially for *Pistacia* L. species because root-tips from the field are not possible to obtain and cuttings do not make roots easily. Roots from seedlings are rather small. Moreover, the *Pistacia* species have very small chromosomes [12]. This study shows that the same method can be used successfully to

study the chromosome number among *Spondias* species. Somatic chromosome number of $2n=32$ for *S. mombin* has earlier been reported [8,10]. These findings were supported by the current study (Figure 1). Chromosome number of *S. purpurea* was reported as $2n=32$ [10]. The current study is in agreement with these findings. Moreover, De Souza Alemdia et al. [10] found that all the analyzed *Spondias* species exhibited similar karyotypes and small chromosomes. This study provides valuable chromosomal data for potential use by the cytogeneticists and plant breeders. This study also provides additional insight into understanding the taxonomic and phylogenetic relationships among *Spondias* species.

Table 1: List of *Spondias* species used; Their chromosome number and plant identification numbers.

| Species | Chromosome Count (2n) | Plant ID |
|-------------------------------------|-----------------------|----------|
| <i>Spondias mombin</i> L. | 32 | 148031 |
| <i>Spondias x robe</i> Vrbar | 32 | 179743 |
| <i>Spondias dulcis</i> Forst. F. | 32 | 188070 |
| <i>Spondias purpurea</i> L. | 32 | 80867 |
| <i>Spondias radlkoferi</i> J.D. Sm. | 32 | 140725 |
| <i>Spondias laevis</i> Griseb. | 32 | 78925 |

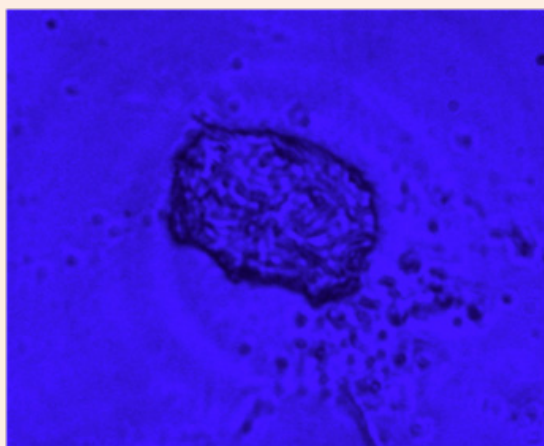


Figure 1: A typical cell with chromosomes ($2n=32$) of *Spondias mombin* L.

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Conflict of Interest

None.

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