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Karyotyping of *Spondias* L. (Anacardiaceae) using fluorescent microscope

Abstract

The genus *Spondias* L. belongs to the Anacardiaceae family and includes 18species. 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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Mohannad G Al-Saghir
Department of Environmental and Plant Biology, Ohio University, Zanesville, USA

Correspondence: Mohannad G. Al-Saghir, Department of Environmental and Plant Biology, Ohio University, Zanesville, Ohio, USA, Email al-saghi@ohio.edu

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Introduction

The genus *Spondias* L. belongs to the Anacardiaceae family and includes 18species 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. tuberose*.[9] Recently, De Souza Alemdia et al.,[10] studied the karyotype differentiation among five *Spondias* species and the putative hybrid Umbu-cajá. 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* specieswere 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] 1g of dried leaf tissue was collected from each specimen and cut into 1mm sections using a sharp blade. The sections were placed in 50mL conical vials, each filled with 20mL of Plant Protoplast Digest/Wash Solution (Sigma, St. Louis, MO, USA).After being mixed via inversion for 5min, 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 2min. The mixture was gently agitated on the platform shaker for one hour. After 1h, 50µL of each mixture was diluted into 4micro centrifuge tubes each containing 450µL of Digest/Wash Solution.

The mixtures were spun at 100xg-forces for 5min; 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 100xg-forces for 5min. The supernatant was removed and 10µL 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 havevery 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
Spondias mombin L.	32	148031
Spondias x robe Vrbar	32	179743
Spondias dulcis Forst. F.	32	188070
Spondias purpurea L.	32	80867
Spondias radlkoferi J.D. Sm.	32	140725
Spondias laevis Griseb.	32	78925

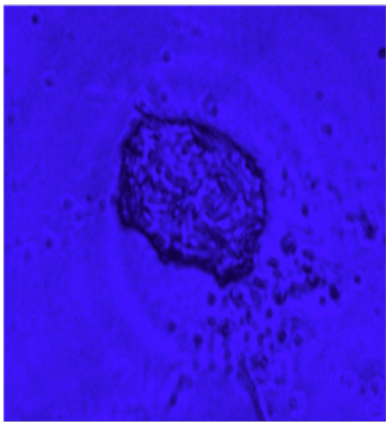

Figure 1 A typical cell with chromosomes (2n=32) of *Spondias mombin* L.

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Conflict of interest

The author declares no conflict of interest.

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