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Original article

Variation of chromosomal complement in the Mexican plum *Spondias purpurea* L.: polyploid series in landraces cultivated by Mayan of Yucatan

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Summary

Polyploidy is a common phenomenon in domesticated plants, with polyploid plants containing more than two copies of the complete genome. Clonally propagated domesticated species can have uneven levels of polyploidy which would be unviable in nature. An evaluation was done of levels of polyploidy in eight Mexican plum *Spondias purpurea* landraces cultivated on the Yucatan Peninsula. Samples of the landraces from four locations were collected in the form of branch sections. These were grown in a greenhouse for three months, and root samples taken. Meristematic cells from the roots were processed and stained with Feulgen stain and aceto-orcein. Sections were viewed with a contrast microscope and chromosome counts done in metaphase cells to quantify the chromosomal complement. Diploid, triploid and tetraploid variations, in chromosomal complement, were observed. Presence of the polyploid series may have resulted from selection for fruit characteristics and landraces conservation through clonal propagation.

Keywords

Mexico, *Spondias purpurea* landraces, cytogenetic diversity, Anacardiaceae clonal propagation, home garden crop

Significance of this study

What is already known on this subject?

- Chromosome number for *Spondias purpurea* landraces is unknown, despite its fruit morphological variation that may be an effect of polyploidy.

What are the new findings?

- Variation in the chromosome number suggest a polyploid series with diploid, triploid and tetraploid forms of the species.

What is the expected impact on horticulture?

- Polyploidy may be associated with desirable traits for fruit production; this variation in ploidy levels is maintained by clonal propagation.

Introduction

Presence of more than two copies of the genome in the cell nucleus is known as polyploidy, which is a common phenomenon in flowering plants (Soltis *et al*., 2014a, b). A number of species exhibit intraspecific variation in levels of polyploidy in which individuals and/or populations with different levels of polyploidy coexist, sometimes sympatrically (Soltis *et al*., 2014c; Stebbins, 1940).

Maintenance of cultivated species with polyploid series is documented in 21 of 203 domesticated plants for food production, and is particularly important in tree species (Meyer *et al*., 2012). Polyploidy can cause gigantism in vegetative and reproductive structures, but gene overexpression can also lead to higher production levels of oils, sugars and other useful compounds. This can drive selection of polyploid forms for introduction into agriculture (Gepts, 2004; Meyer *et al*., 2012). Many polyploid forms are consequently found among crop species, especially those propagated by cloning (McKey *et al*., 2010; Meyer *et al*., 2012). This occurs because they reproduce vegetatively, avoiding the cost of sexual reproduction (Ramsey and Schemske, 2002; Zohary and Hopf, 2000). For example, some polyploid species are known to be sterile or genetically unstable (*e.g.*, triploidy, pentaploidy) in nature but are effectively grown as crops. Three vegetatively propagated crops with variation in polyploidy include potato (*Solanum* spp., Van Suchtelen, 1976); banana (*Musa* spp., Ortiz, 1995; Simmonds and Shepherd, 1955); and some cultivars of apple (*Malus* × *domestica* Brown, 1992; Einset and Pratt, 1963).

Spondias purpurea belongs to the family Anacardiaceae, which contains many species distributed in the tropics. This botanical family includes agriculturally important fruit tree species such as mango (*Mangifera indica* L.), cashew (*Anacardium occidentale* L.), pistachio (*Pistacia* spp.), pepper tree (*Schinus* spp.) and mombins (*Spondias* spp.). Within this family, spontaneous polyploidy has been documented in mango (Saúco *et al*., 2001), and it is quite possible that some cashew plants may be polyploid (Aliyu and Awopetu, 2007).

Known as Mexican plum (*ciruela* in Spanish), *S. purpurea* is widely grown in Mesoamerica. It has great potential as an agricultural product since it is accepted by consumers, can be propagated by cuttings, is highly drought resistant, and productive even in poor and shallow soils (Avitia-González *et al*., 2003). This species is widely distributed, and can be found in home gardens, orchards and natural populations in dry tropical forest along the Pacific coast from Sinaloa state in Mexico to Costa Rica, and along the Gulf of Mexico coast from Tamaulipas state to the Yucatan Peninsula in Mexico

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(Miller and Schaal, 2006). Cultivated landraces of *S. purpurea* on the Yucatan Peninsula are known as "*abal*", Mayan name that means "becoming limp" (Ruenes-Morales *et al.*, 2010). At least ten landraces have been recorded which are differentiated by their flowering-fruiting season and fruit characteristics (Ruenes-Morales *et al.*, 2010).

The chromosomal complement $2n = 32$ has been reported for *S. purpurea* accessions in Brazil (Almeida *et al.*, 2007). This coincides with reports for other species in this genus published to date in the Index to Plant Chromosome Numbers, the Chromosome Counts Database, as well as four species in Brazil (Almeida *et al.*, 2007). A number of studies have reported variation in *S. purpurea* fruit weight, size and sugar content across Mexico (Alia-Tejacal *et al.*, 2012; Guerrero *et al.*, 2011; Pérez-Arias *et al.*, 2008; Ramírez-Hernández *et al.*, 2008; Ruenes-Morales *et al.*, 2010; Vargas-Simón *et al.*, 2011). These three variables tend to increase in cultivated landraces, whereas in wild populations the fruit is small with low sugar content (Lins Neto *et al.*, 2014). Variation in fruit size has also been observed between landraces on the Yucatan Peninsula, with smaller fruit (2 cm long × 1.5 cm wide) in landraces such as *Abal ak* (wild plum) and larger ones (6 cm long × 3 cm wide) in landraces such as *Keken abal* (pig plum) (personal observation).

The variations in *S. purpurea* fruit size and sugar content reported in a number of studies may be associated with polyploid cultivated landraces. Confirming this hypothesis about a possible association between polyploidy and morphological and chemical variation in *S. purpurea* fruit requires systematic evaluations of the species' chromosomal complement in different landraces with contrasting fruit sizes. The present study objective was to generate a cytogenetic characterization, that is, an estimate of the mitotic index and chromosomal complement, for eight landraces of *S. purpurea* with different fruit sizes: small fruit (*Abal ak*, *Chi abal* and *Xuntura abal*); medium-sized fruit (*Campech abal*, *Xhuhi abal* and *Xhahal abal*); and large fruit (*Ek abal* and *Tuspana abal*).

Materials and methods

Species

Mexican plum *S. purpurea* is a deciduous to semideciduous tree, attaining heights of up to 15 m; most of its foliage is lost in the dry season. Its leaves are compound and imparipinnate, measuring from 10 to 20 cm long with 8 to 12 leaflets measuring from 1.9 to 4 cm long. Its external bark is grey to green in color, and rugose in texture with irregular fissuring. Flowers are pale rose to bright red in color, about 0.6 cm in diameter, have a calyx with five lobes and five petals grouped in panicles of three to five flowers (Vázquez-Yanes *et al.*, 1999; Ruenes-Morales *et al.*, 2010). Fruit are drupe and can range in color from yellow-green to orange, red and even dark purple. They weigh from 4 to 43 grams, have a polar diameter ranging from about 1.5 to 3.7 cm, and longitudinal diameter ranging from about 2.0 to 4.7 cm. Total soluble solids (sugars) content varies from 5° to 17° Brix. The endocarp is woody and yellow with five locules that can contain from zero to five seeds (Alia-Tejacal *et al.*, 2012; Guerrero *et al.*, 2011; Pérez-Arias *et al.*, 2008; Ramírez-Hernández *et al.*, 2008; Ruenes-Morales *et al.*, 2010; Vargas-Simón *et al.*, 2011). Seven of the studied landraces (*Abal ak*, *Chi abal*, *Campech abal*, *Xhuhi abal*, *Xhahal abal, Ek abal* and *Tuspana abal*) produce fruit in the dry season while *Xuntura abal* produces in the rainy season. All eight are among the ten landraces preferred by the Maya population of the Yucatan Peninsula (Ruenes-Morales *et al.*, 2010).

Study site and sampling

Material collection was done at four locations in the state of Yucatan, Mexico: 1) Hocabá, municipality of Hocabá (20°49'N; 89°15'W); 2) Dzityá, municipality of Mérida (21°02'N; 89°40'W); 3) Noc-ac, municipality of Mérida (21°07'N; 89°71'W); and 4) Maní, municipality of Maní (20°21'N; 89°19'W). At Hocabá, Dzityá and Noc-ac, one to two home gardens were identified containing *S. purpurea* landraces of interest for the study. Owners were consulted to confirm the landraces, and then permission was requested to detach two branches at least 5 cm in diameter and 90 cm long. At Maní, branches were collected from specimens growing in fields outside the town. Collected branches were cut into 30 cm long stakes for transport to the Biological, Agriculture and Livestock Sciences Campus of the Autonomous University of Yucatan (Universidad Autónoma de Yucatán – UADY), and a voucher of each one of the Hocabá's and Maní accessions were sent to MEXU herbarium, while the Noc-ac and Dzityá accessions were deposited at UADY herbarium (Table 1). The stakes were planted in pots containing an organically rich substrate (60% compost + 40% dead leaves), and the pots placed in a greenhouse. Four months after transplanting into the pots, once secondary and tertiary roots were present, roots were collected from each plant between 07:00 and 08:00 am. Collected roots were washed in

Table 1. Frequencies of cells observed with different chromosomic number on radicular meristem of eight folk variants of *Spondias purpurea* from backyards of Hocabá and Noc-ac and a wild population from Maní. In the last column voucher number and herbaria where the botanical examples were deposited are presented .

Variant	Locality	Chromosomic complement	Observed cells	Voucher number
Abal ak	Maní	16	8	R. Ruenes *et al.*, 1001 MEXU
Chi abal	Hocabá	16	8	R. Ruenes *et al.*, 1029 MEXU
	Noc-ac	32	8	J. Rivero, 9 UADY
Xuntura abal	Hocabá	16	10	R. Ruenes *et al.*, 1036 MEXU
Campech abal	Hocabá	24	7	R. Ruenes *et al.*, 1050 MEXU
Xhuhi abal	Noc-ac	16	10	J. Rivero, 2 UADY
Xhahal abal	Hocabá	16	7	R. Ruenes *et al.*, 1081 MEXU
Xhahal abal	Noc-ac	32	8	J. Rivero, 4 UADY
Ek abal	Hocabá	24	6	R. Ruenes *et al.*, 1007 MEXU
Ek abal	Dzityá	24	13	J. Rivero, 12 UADY
Tuspana abal	Hocabá	32	6	R. Ruenes *et al.*, 1004 MEXU

running water and treated with 0.02 M 8-hydroxyquinoline for 5 hours at 3 °C in darkness. Radicular apices were fixed in Farmer solution (3:1 absolute ethanol:glacial acetic acid), hydrolyzed in 1N HCl for 13 min, and washed with distilled water (Sharma and Sharma, 1980a). Meristematic cells were separated from the rest of the root by staining with Feulgen stain for 30 min in darkness, and then placing them on a slide with a drop of 1.8% aceto-orcein for immediate observation under a microscope (Sharma and Sharma, 1980b). Sections containing sufficient metaphase cells for a chromosome count were frozen and mounted in the middle of a DPX mount (Sigma-Aldrich 06522). Prepared cells were viewed using a Zeiss-PrimoStar microscope with phase contrast on a light field, and images for digital analysis taken with an Axiocam ARC5S camera.

Mitotic index

Estimation of the mitotic index (MI) was done as the proportion of dividing cells observed in a field at 400× final magnification, using the formula (Davidson, 1969):

$$MI = \frac{Number\ of\ dividing\ cells}{Number\ of\ observed\ cells} \times 100$$

Variation in the mitotic index was analyzed for seven of the studied landraces: *Abal ak*, *Chi abal*, *Campech abal*, *Xhuhi abal*, *Xhahal abal*, *Ek abal* and *Tuspana abal*. Estimates were generated for six randomly chosen fields per landrace. These were analyzed with an analysis of variance (ANOVA), and differences between average mitotic index values per landrace analyzed with a Tukey HSD test (Zar, 1999).

Chromosomal complement

Chromosome counts were done at 1,000× magnification in at least six cells in the late prophase and/or metaphase in the permanent preparations of each landrace from each collection location.

Results

Mitotic index values varied between landraces ($F = 5.27$; g.l. 4.38; $P = 0.0018$). The proportion of cells in mitosis was greater in *Xhuhi abal* (46.23% ± 5.77 S.E.) than in *Ek abal* (16.95% ± 7.81 S.E.) and *Abal ak* (8.73% ± 6.37 S.E.). Values were intermediate for the *Xhahal abal* (26.83% ± 5.22 S.E.) and *Tuspana abal* (23.57% ± 8.55 S.E.) landraces, which did not differ from either *Xhuhi abal*, or *Ek abal* and *Abal ak*.

Chromosome count ranged from 16 to 32 in the studied *S. purpurea* landraces (Table 1; Figure 1). Counts varied between the same landraces at different locations; at Hocabá the *Chi abal* and *Xhahal abal* landraces had 16 chromosomes whereas at Noc-ac they had 32 (Table 1; Figure 1).

Discussion

Both the mitotic index values and chromosome counts varied between the studied *S. purpurea* landraces. The mitotic index reflects the rate of cell division in a specific tissue. When values are compared among individuals growing under similar conditions, differences can be identified in growth and development in a tissue (Pereira *et al*., 2007). The *Xhahal abal* landraces had the highest number of cells in mitotic division in root tissues at the time of root collection, suggesting that the roots of this landrace grow more rapidly than those of the other studied landraces. *Spondias purpurea* propagates clonally, indicating that the speed of root development in the stakes of the *Xhahal abal* landrace would allow it to establish itself more rapidly. Confirmation of this possibility will require further research focusing in estimating both the biomass assigned to roots and root tissue growth rates.

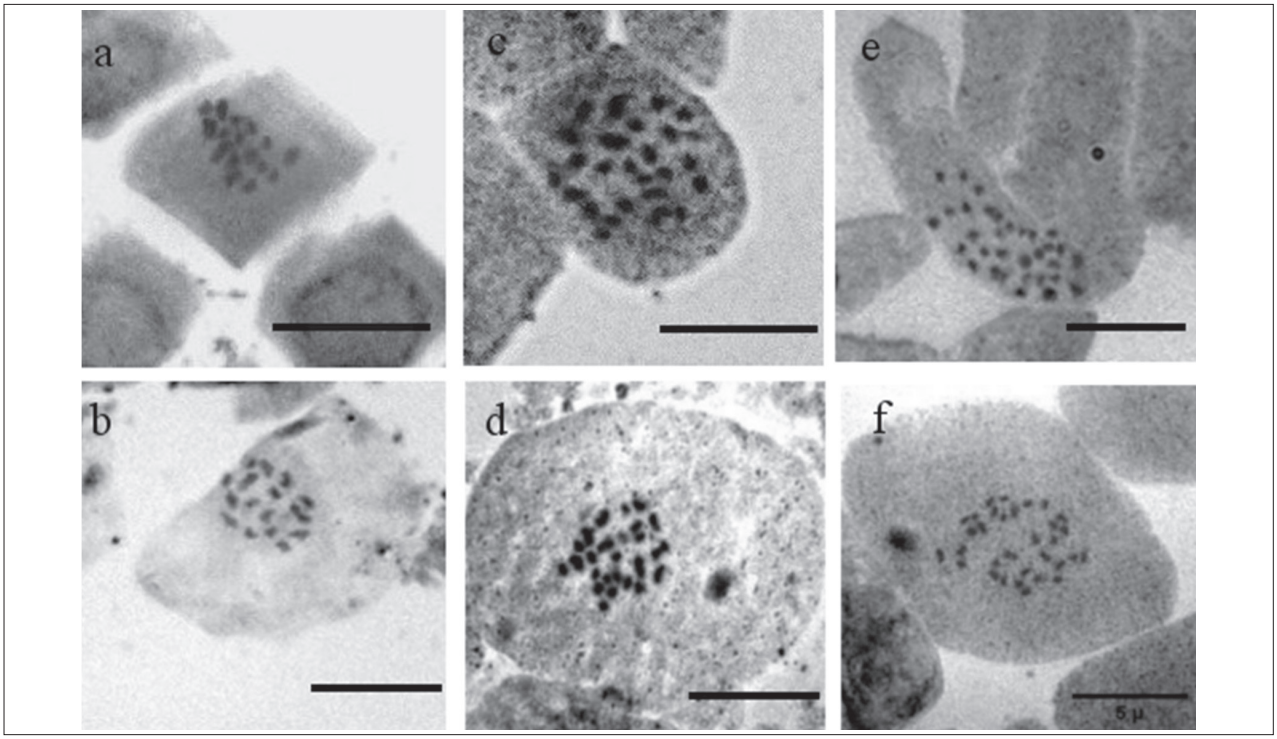

Figure 1. Microphotographs of chromosomes in radicular meristems cells of six landraces of *Spondias purpurea*: a) *Abal ak* from a wild population from Maní, $2n = 16$; b) *Xhuxhi abal,* $2n = 16$; c) *Ek abal*, $2n = 24$; d) *Campech abal*, $2n = 24$; e) *Tuxpana abal,* $2n = 32$ from Hocabá; and f) *Hahal abal*, $2n = 32$ from Noc-ac.

In this study, the chromosome complement of $2n=16$, and variation in the chromosome counts for a species of the genus *Spondias* were reported for the first time. The counts were multiples of eight (16, 24 and 32), implying duplication, triplication and quadruplication of the genome. In *S. purpurea*, the monoploid number (sensu Guerra, 2008) would therefore be $m=8$, because this is the common divisor for the 16, 24 and 32 chromosomic complement series. Based on the results, the basic number for this species would be $x=8$. This differs from the number reported for the *Spondias* genus ($x=16$) in the Index to Plant Numbers Chromosome database (Goldblatt and Johnson, 1979–2011), and in a study of six *Spondias* species, including *S. purpurea*, found in Brazil (Almeida *et al*., 2007). However, the probable basic number ($x=8$) derived from the present results does fall within the range of basic chromosome counts ($x=7$ to $x=16$) reported for Anacardiaceae genera (Mabberley, 1997). The discrepancies in basic numbers between the present results and those in the report on six cultivated *Spondias* species distributed in Brazil (Almeida *et al*., 2007) deserves further studies, that considered a comprehensive cytogenetic study of the genus as well the entire distribution of the widespread *S. purpurea* and its sympatric species *S. mombin* L. and *S. radlkoferi* Donn. Sm.

The tendency of certain angiosperm families to form polyploid complexes has facilitated their introduction into agriculture in environments where the wild ancestor would not grow (Meyer *et al*., 2012; Gepts, 2006). The presence of polyploid forms in cultivated *S. purpurea* may therefore be related to deliberate selection by Mesoamerican ethnic groups; in the present case, the Maya on the Yucatan Peninsula. The maintenance of polyploid lines in *S. purpurea* via vegetative propagation is likely due to selection by the Mayan. Selection of polyploid landraces and their introduction into agriculture is common when variations in polyploid levels are accompanied by larger fruit size and increased sugar content (Besnard *et al*., 2008; Gepts, 2004; Heslop-Harrison and Schwarzacher, 2012; Li *et al*., 2013; Meyer *et al*., 2012). The Mayan of Yucatan have a well-documented preference for large, sweet Mexican plum fruit (Ruenes-Morales *et al*., 2010). No data has yet been published directly supporting gigantism and/or higher sugar content in fruit in response to polyploidy in *S. purpurea*. However, the presence of triploid and tetraploid forms among cultivated landraces may be associated with selection for larger fruit (*Campech abal*, *Ek abal* and *Tuspana abal*) and/or sweet, or sweet and sour fruit (*Chi abal*, *Campech abal*, *Ek abal* and *Tuspana abal*). In contrast, diploid forms have smaller (*Abal ak*, *Chi abal*, *Xhuhi abal* and *Xhahal abal*) and/or more sour fruit (*Abal ak*, *Xhuhi abal* and *Xhahal abal*). Coexistence of diploid, triploid and tetraploid forms in cultivated *S. purpurea* in Yucatan has occurred in response to traditional management of this species in family home gardens, specifically vegetative propagation and the presence of multiple landraces in a single orchard. In this species, vegetative propagation has ensured that landraces with diploid, triploid and tetraploid chromosome complements are maintained. Coexistence of different levels of polyploidy in cultivated and clonally propagated species is common; in Mesoamerica it has also been documented in *Opuntia* spp. (Griffith, 2004; Segura *et al*., 2007) and *Agave tequilana* Weber (Palomino *et al*., 2003, 2008).

Spondias purpurea and its sister species *S. radlkoferi* and *S. mombin* L. clearly constitute an important research system for understanding how the evolution of polyploidy is associated with domestication. Further evaluations will be needed of the incipient domestication process of these species and how it relates to maintaining the polyploid complex, including flow cytometry to quantify cell nuclear DNA content (2CDNA) to corroborate changes in ploidy level. Comparative studies between wild and cultivated *Spondias* populations are needed that address three main areas: 1) the relationships between phenotype variation in fruit, floral and foliar morphometry and nutrient contents in accessions with different levels of polyploidy; 2) changes in cytotypes and cell nuclear DNA content associated with different levels of polyploidy; and 3) molecular level variation in nuclear and chloroplast markers. These will help to better understand the evolutionary history of *Spondias purpurea* and how it was affected by incipient domestication in Mayan home gardens in Yucatan, and other traditional agroecosystems.

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