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### Spondias dulcis propagation by seeds and stem cuttings

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### ABSTRACT

**Objective**: To found simple and low-cost ways to increase the number of plants. In Chetumal, Quintana Roo, Mexico, a trial was established to evaluate two propagation forms from January to June 2023.

**Design/Methodology/Approach**: In soil enriched with bocashi, 22 seeds of seasoned fruits were sown. Also, four lots of stem cuttings were planted (five cuttings per lot) 30 cm in length, testing four average diameters (2.94, 2.34, 2.1 and 1.58, cm) that were also rooted in soil and bokashi, on the assumption that those with the largest diameter were also the oldest. The stem cuttings were obtained from 1.5- and 2.5-year-old trees. This latter experimental procedure was repeated, but stem cuttings were obtained from an 8-year-old tree, five lots of cuttings with the following average diameters (3.6, 1.92, 1.74, 1.7, and 1.16, cm) were evaluated; in addition, cuttings were treated with a stimulant to develop roots (Raizal<sup>®</sup> 400).

**Results**: The data analyzed in Excel<sup>™</sup> showed that germination is slow and irregular, 77% germination was reached after 2.5 months. Only the thickest cuttings from the 1.5-year-old tree developed abundant roots and vegetative shoots.

Findings/Conclusions: The propagation of *S. dulcis* is better done by seeds. Propagation by cuttings is possible, but with low success, only 8.8%.

Keywords: germination, Spondias dulcis, vegetative propagation.

### INTRODUCTION

In Mexico, the fruit of the species *Spondias dulcis* Parkinson, Anacardiaceae (Mitchel and Daly, 2015), is practically unknown because its origin is not native American. *S. dulcis* is native to the Indo-Malayan region of Tahiti (de Souza *et al.*, 2021). The tree was introduced in Jamaica in 1782 and is currently cultivated in several Caribbean countries. Fruit exportation is an important source of profit from foreign exchange in those countries (Bauer *et al.*, 1993; Mohammed *et al.*, 2017). It goes by several common names as Kedondong, Yellow plum, Polynesian plum, Belize plum, or June plum, among others (Bauer *et al.*, 1993) and ciruela-mango in

### Mexico.

In this study we decided to use the latter name; there are two genetic lines of *S*. *dulcis*, large and miniature trees. On large trees the fruits reach in average, 5-6 cm in diameter and 9-10 cm in length, with 200 g average weight.



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Whereas the fruits of miniature trees reach 4-5 cm in diameter and 5-6 cm in length, with 65 g average weight (Graham *et al.*, 2004; Mohammed *et al.*, 2017). The most widely cultivated genotype is that with the large fruit; the miniature type has received little attention (Graham *et al.*, 2024a), although it may have higher economical and nutritional value (Das *et al.*, 2019).

Both types of *S. dulcis* fruits can be consumed fresh or processed at home in various ways. In addition, they can be used harvested at different stages of development, either green, seasoned or ripe (Mohammed *et al.*, 2017). The fruits can even be processed into various added-value products and can be also valuable as raw material in the food and beverage industries. Likewise, the peel of ciruela-mango is a source of gelling agents for jam, candies, yogurt, and dairy drinks, among other products (Franquin *et al.*, 2005; Mohammed *et al.*, 2017). The fruit has nutraceutical properties; that is, it serves as food, and it helps to counteract symptoms of some diseases. For example, diabetes, indigestion, urinary tract infections, hypertension, and hemorrhoids (Islam *et al.*, 2013). Fruits are also used to treat heart and digestive problems; as well as for healing wounds, skin sores and burns (Das *et al.*, 2015).

The fruit is a drupe with a spiny-fibrous endocarp and is climacteric (Bauer *et al.*, 1993; Mohammed *et al.*, 2017). When the fruits are seasoned and ripe they are crunchy with pineapple scent and flavor; Graham (2004) and Bauer *et al.* (1993) described fruit flavor as sweet-and-sour. The miniature ciruela-mango has several ideal characteristics because fruits are available throughout the year, they are easily harvested due to the low height of the tree, which can be grown at high densities (Mohammed *et al.*, 2017). In addition, it is drought tolerant (Das *et al.*, 2015). Bauer *et al.* (1993) indicated that most *S. dulcis* plants are obtained by germination of seeds and few are obtained by cuttings. It is known that many seeds have certain difficulties in germinating. In fact, germination and dormancy are controlled by several genes that are affected by environmental and developmental conditions. In addition, the structures surrounding the seeds play a determining role in the inhibition and germination of the seed (Koornneef *et al.*, 2002).

For the large genotype of *S. dulcis*, 63% germination was reached at 60 days when combining mechanical scarification followed by imbibition in a solution of gibberellin plus cytokinin (350 mg  $L^{-1}$ ) for 12 hours. In contrast to the control, which consisted of imbibition with water and without scarification; in this treatment germination was three times lower (de Souza *et al.*, 2020). Precisely because of the scarcity of the ciruela-mango trees in Mexico, the aim was to find a simple and cheap way to increase the number of plants, in such a way that it can be achieved by any producer, without depending on expensive or difficult to obtain products.

### MATERIALS AND METHODS

This study was conducted from January to June 2023. The evaluated material was obtained from Spondias dulcis trees, an 8-year-old tree grown at the Autonomous University of the state of Quintana Roo, Campus "Bahía", located in the city of Chetumal. Also, from three other younger trees, two approximately 1.5 years-old and another 2.5 years old, these three trees are grown in a private home in Chetumal. The climate of Chetumal is warm

sub-humid, the average annual temperature in the state is 26 °C, the average rainfall in the state is around 1300 mm per year (Comisión Nacional del Agua, 2017).

Propagation of *S. dulcis* plants was made in two ways, using seeds (seed plus endocarp) and stem cuttings. The seeds came from one of the 1.5-year-old trees and consisted of a batch of 22 seeds taken from seasoned fruits, *i.e.* at physiological maturity (Figure 1).

Once the pulp of the fruit was removed, the seeds were immediately washed in tap water to remove as much as possible the remains of the pulp. Subsequently, seeds were exposed to the sun for 12 hours in order to dry them and at the same time disinfect them. Afterwards, seeds were germinated in a substrate made with a mixture of equal parts of red soil and bocashi (Restrepo, 2007); the latter component made from sheep manure. The substrate was poured into 2 kg nursery bags and moistened to field capacity. Then, each seed was sown at 3 cm depth. For five months, irrigation was applied every Monday, Wednesday and Friday, along with daily records of any seedlings emergence, from January 15 to June 15, 2023. Records were supplemented with the description of the morphology of seedlings in different weeks of development.

On the other hand, for vegetative propagation, a first block of stem cuttings derived from two young trees (1.5 and 2.5 years-old) was formed, obtaining 20 stem cuttings 30 cm in length (four average diameters 2.94, 2.34, 2.1 and 1.58, cm), which were grouped into four lots (five cuttings per lot) according to thickness and color. Those cuttings that were thicker and grayish brown were considered as older, while those greener and thinner were estimated to be younger. Before planting the cuttings, they were left to airing for a day; they were then planted in a substrate made with a mixture of 70% humus soil and 30% bocashi. This substrate was contained in nursery bags with a capacity of 2 kg where the cuttings were planted and received three irrigations per week.

Data recording was conducted from April to June 2023. The propagation by cuttings was repeated (in the second block of stem cuttings) in the same way as it was done with Block 1. But this time the stems were obtained from the 8-year-old tree and were grouped into five lots (average diameters 3.6, 1.92, 1.74, 1.7, and 1.16, cm), following the criteria already described. This time, stem cuttings were introduced in a solution (200 g in 20 L of water) of a commercial stimulant for roots emission (Raizal<sup>®</sup> 400) for 10 minutes. In addition, according to the specifications of the product, each nursery bag was irrigated



Figure 1. Spondias dulcis fruits (green, seasoned and ripe), and seeds (in front of fruits).

with 80 mL of the same solution. The data obtained from germination and vegetative propagation were organized, analyzed and graphed in Excel<sup>TM</sup>.

### **RESULTS AND DISCUSSION**

Seed germination was very irregular (Figure 2); it began at 36 days and after 76 days (2.5 months) 77% germination was achieved. Whereas, at 148 days (almost five months), 81.87% germination was reached. This result was very similar to that reported by de Souza *et al.* (2020) for the large genotype of *Spondias dulcis*, for which 63% germination was recorded in 60 days.

Although it is true that, in this study, there was only about 40% germination at 60 days, just 15 days later 77% germination had already been reached. This is a high percentage if we consider that seeds were not scarified or treated with any accelerator for germination, in contrast with de Souza *et al.* (2020) where mechanical scarification was combined with hormones.

The irregularity and slowness of germination appear to be a characteristic of the genus. Martins *et al.* (2019) noted that germination of *Spondias mombin* L. occurs between 6 and 24 months. In addition, they found that germination was different according to size, color, and the seed scarification point. In fact, medium-sized, brown, laterally scarified seeds reached 50% germination at 336 days, whereas in other treatments that percentage was reached up to 489 days.

The endocarp that covers the seeds of *S. dulcis* may be responsible for the germination slowness; however, in this species it would not be easy to remove the endocarp without damaging the seed. In order to optimize costs and time, three months is the maximum suggested time to get a good number of *S. dulcis* germinated seeds. A longer waiting would not be worth it because the subsequent germination rate is negligible.

The number of seedlings that emerged per seed ranged from one to five (Figure 3). At the same percentage (27.7%), seeds produced one, two, and three seedlings; fewer seeds yielded four or five seedlings. The time of emergence of seedlings from a single seed was

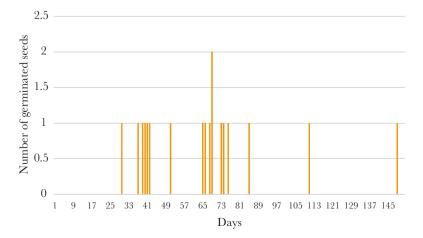


Figure 2. The irregular germination of *Spondias dulcis*.



Figure 3. Three Spondias dulcis seedlings which emerged from only one seed.

variable; some emerged up to 20 days after the first seedling emerged. The height and vigor of the plants originated from the same seed were also different.

According to the number of seedlings obtained from each seed, it was clear that within each one there may be between one and five embryos, perhaps more. Polyembryony is common in several genera of Anacardiaceae. Martínez-Ochoa (2010) reported 95 to 97.5% polyembryony rate in two cultivars of *Mangifera indica* L.; that author also found between 2 and 7 embryos per seed from zygotic and nucellar origin. Determination of the origin and number of embryos per seed will be one of the tasks to be solved for *S. dulcis*.

In *M. indica*, 2-5 seedlings were obtained when the seeds were germinated *in vitro* and only 2-3 when it was done *in vivo*. That same author stated that plants from nucellar embryos are believed to be more vigorous and are preferred for rootstocks. At five months, the survival of the plants was 100%, including those with up to five plants emerged from a single seed. Germination is hypogeal, the cotyledons are enclosed in the endocarp. The epicotyl lengthens and emerges curved just below the node of the two primordial leaves, both facing the ground.

Seedling growth is very fast; on the second day the seedling is fully erect, it has a length of about 4.5 cm, the single primordial leaves with smooth edges are about 2 cm long and are fully spread and opposite. While the first two nomophyl leaves (true typical leaves, pinnate) are compound and already exhibit the apical leaflet with serrated margin. The primordial leaves and the pinnate leaves are in a decussate arrangement, separated by very short internodes. On the third day the two pinnate leaves already show three leaflets, one at the tip and two laterals: the seedling measures approximately 5 cm (Figure 4).

Around the tenth day some primordial leaves turn yellowish and by the twentieth day they have already fallen off. As the development of the plant continues, the pinnate leaves increase in number of leaflets, all with serrated margins, increasing from three, to five, seven, nine, eleven, and thirteen leaflets. The latter was recorded in leaves of 35 cm



Figure 4. A three days-old *Spondias dulcis* seedling; two primordial smooth-edged leaves and two pinnate leaves, each containing three leaflets, are observed.

in length and 5 months-old plants, at this age the plants measured 89 cm in length, and they were still without lateral branches. However, it should be clarified that the plants continued in the same bags where germination occurred, that is, with some limitation for growth.

In relation to propagation by cuttings, those obtained from 1.5- and 2.5-year-old trees (Block 1) showed vegetative shoots after 10 days in four of the cuttings from lot 1. This lot consisted of the thickest stems obtained from a 1.5-year-old tree. Those same cuttings, after 22 days, all had emitted many shoots (between 5 and 7 per cutting); moreover, two cuttings already exhibited inflorescences in full development. Later, two months after planting, all four cuttings showed good vegetative growth (Table 1, Figure 5).

The well-developed leaves were about 35 cm long, each leaf with 11 or 13 leaflets. Likewise, in one cutting there were three clusters with fruits, nine fruits in total (Figure 6). Those fruits measured on average  $0.7 \text{ cm} \times 0.8 \text{ cm}$  in diameter and length, respectively. However, 10 days later the fruits were completely dry. In contrast, those cuttings with better-developed shoots also exhibited better roots, two months after planting (Figure 7).

			Lot 1					Lot 2			Lot 3			Lot 4						
Cutting Number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cutting Length (cm)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Cutting Diameter (cm)	3.3	2.8	2.9	2.8	2.9	2.4	2.3	2.4	2.3	2.3	2.2	2	2.1	2.3	2.1	1.5	1.4	1.6	1.5	1.9
Average Shoot Length (cm)	58.6	0	11.75	3.94	2.5	4.4	0	0	0	0	1.4	4	0	0	0	0	0	0	0	0
Number of leaves (Cutting)	55	0	11.25	6.4	7.2	2	0	0	0	0	7	12	0	0	0	0	0	0	0	0

Table 1. Average dimensions recorded in *Spondias dulcis* plants from Block 1, after 60 days.



Figure 5. Four out of five stem cuttings of Spondias dulcis exhibited good vegetative growth.



Figure 6. Spondias dulcis fruits developed in one of the cuttings.



Figure 7. *Spondias dulcis* stem cuttings with good vegetative growth which also formed abundant roots; in contrast to those who showed poor vegetative development.

In regard to the cuttings from the other lots in Block 1, only a few cuttings from lots 2 and 3 emitted shoots. But at two months the leaves of the shoots were small and dark green; likewise, these cuttings had not formed roots (Figure 7).

The cuttings of lot 4, thinner and younger stem cuttings, exhibited wilted tips at 10 days and continued to dry from tip to base. On the other hand, the cuttings obtained from the 8-year-old tree (block 2), treated with Raizal<sup>®</sup> 400 (Table 2), began bud break between 15 and 20 days. At 15 days, three cuttings in lot 1, the thickest and supposedly oldest cuttings, had buds smaller than 1 cm. The same condition was observed in two cuttings of lot 3 and in one of lot 4.

Although these same cuttings emitted more shoots later, they grew little and dried out in a short time, in such a way that after 45 days only three cuttings exhibited reduced shoots, with leaves also very small. The longest leaves only measured 6 cm on average and were dark green. Although at 45 days there were two shoots with inflorescences, these dried up a week later. None of the cuttings in Block 2 had developed roots by 45 days.

Lot 5, consisting of the thinnest and youngest stem cuttings, was already dry after 15 days. The high survival of seedlings, as found in this study, lowers the costs of seed propagation in ciruela-mango; quite opposite to what would happen if it were decided to multiply *S. dulcis* by stem cuttings. In fact, it was only 8.8% successful if you consider the total of the cuttings of both treatments. Moreover, it occurred when the cuttings did not receive rooting and were obtained from a 1.5-year-old tree (thick and greenish cuttings). This suggests that success depends on the vigor and perhaps the age of the parent plant.

In this study, the youngest and thinner cuttings with or without root stimulant died quickly. While the other stems with both treatments lived longer and emitted vegetative shoots. However, these shoots reached little development because they used the cutting reserves because they did not develop roots. More research remains to be done in this regard. Vega-Escobedo (2010) did not find an interaction among three doses of IBA (0, 3000 and 5000 ppm), two ages of the parent tree (1-5 and 7-16 years) and the location

			Lot 1			Lot 2					Lot 3							Lot 4	ł		Lot 5				
Cutting Number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Cutting Length (cm)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Cutting Diameter (cm)	3.7	3.5	3.4	3.5	3.9	2.2	1.9	1.9	1.9	1.7	2.7	1.7	1.5	1.4	1.4	1.8	1.8	1.9	1.5	1.5	0.9	1	1.4	1.2	1.3
Average Shoot Length (cm)	2.3	0	0	0	0	0	0	0	0	0	1.08	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Number of Leaves (Cutting)	8	0	0	0	0	0	0	0	0	0	3.25	0	0	0	0	0	0	0	0	3	0	0	0	0	0

**Table 2**. Average measurements obtained in *Spondias dulcis* plants in Block 2 after 45 days.

of the cutting site in the parent tree (basal, middle and apical). Tough, he did find 22% callus with 0% IBA in cuttings from the middle part of trees between 7 and 16 years old.

Jesus *et al.* (2004) also did not record root emission in cuttings of *S. dulcis* treated with different doses of IBA; while Rocha *et al.* (2020) concluded that vegetative propagation of *S. dulcis* was not feasible, but in *S. tuberosa* it achieved 11% success when cuttings were treated for 16 seconds in an IAA solution (10 g  $L^{-1}$ ).

### CONCLUSIONS

Germination of *Spondias dulcis* was relatively slow and irregular, with 77% germination in 2.5 months. Germination is hypogeal, the cotyledons remain within the endocarp. Seedlings develop two simple primordial leaves, while nomophiles (true leaves) are compound imparipinnates. Each seed germinated one and up to five seedlings, with a 100% survival rate at five months.

Vegetative multiplication of *S. dulcis* was successful without root stimulant, but it was only possible in the thickest and greenest cuttings, derived from a 1.5-year-old tree. In both treatments the younger, thinner cuttings died soon. Although all the other cuttings emitted vegetative shoots, most of these were completely dried by two months; the shoots that remained alive had very reduced growth because the cuttings had not emitted roots.

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