



## Obtaining Java Plum Seedlings (*Syzygium cumini*) through Different Seed Extraction and Cleaning Methods

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### Authors' contributions

This work was performed in collaboration among all authors. Authors BAR and MSZ designed the study and performed the statistical analysis. Authors LEMB and DIB wrote the protocol and wrote the first draft of the manuscript. Authors HVN and BHNN managed the study analyzes. Finally, authors HRMA, PVGS and RAL managed the bibliographic searches. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/EJMP/2020/v31i630247

#### Editor(s):

(1) Dr. N. Karmegam, Government Arts College, India.

(2) Professor, Marcello Iriti, University of Milan, Italy.

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(3) Keith J. Stine, University of Missouri–St. Louis, USA.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55540>

Original Research Article

Received 06 February 2020

Accepted 12 April 2020

Published 20 April 2020

### ABSTRACT

Java plum (*Syzygium cumini*) belonging to the botanical family Myrtaceae, which also includes species of other tropical fruits widely consumed in Brazil, such as guava (*Psidium guajava* L.) and surinam cherry (*Eugenia uniflora* L.). The production of quality seedlings depends on several factors, including the method of extraction and cleaning the seed for planting. The experiment was conducted at the Federal Institute of Education, Science and Technology of Tocantins, Gurupi/TO city, from October 2018 to May 2019. The extraction methods used were: manual with friction on

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wire mesh; manual with the addition of sand and friction on wire mesh; manual with friction on a plastic sieve; fermentation at room temperature for three days; immersion in quicklime (CaO) solution for 24 and 48 hours; fermentation at room temperature with the addition of 5 pieces of sugar to 3 pieces of water, for 24, 48 and 72 hours; fermentation at room temperature, with the addition of 5 pieces of sugar to 1 of water, for 24, 48 and 72 hours. The following characteristics were evaluated: Root length, green mass of the aerial part, dry mass of the aerial part and root, first count of emergence and seedling emergence. All seeds were manually extracted and then submitted to different extraction methods. The best results were for the seeds that were applied to extraction by friction in plastic mesh, were soon immersed in quicklime solution for 24 hours and fermented at room temperature. The solutions that presented the best results were the solution with 5 proportions of water and 3 of sugar exposed during 24 hours (5:3) and also 5 proportions of water for only 1 sugar for 72 hours. These are the best treatments for the values of viability and vigor in Java plum seeds.

**Keywords:** *Java plum; seed viability; seedling quality; vigor; tropical fruits.*

## 1. INTRODUCTION

The java plum (*Syzygium cumini*) belonging to the botanical family Myrtaceae, which also includes species of other tropical fruits widely consumed in Brazil, such as guava (*Psidium guajava* L.) and surinam cherry (*Eugenia uniflora* L.). The Java plum can reach 10 meters high, straight trunk. It has simple leaves, opposite, coriaceous, glabrous, aromatic and 8 to 14 cm long, hermaphrodite flowers, white or yellowish, with long and numerous stamens, with almost whole calyx, 4 or 5 rounded petals in the shape of a hood, free stamens, undefined, binocular ovary with few eggs, its fruit is a drupe of 3 to 4 cm in length and has dark purple coloration and fleshy pulp that surrounds the brown seed, large [1].

The seeds have a length of about 14.79 to 18.46 mm, larger seed diameters ranged from 7.41 to 8.82 mm, diameters smaller from 7.71 to 9.22 mm and are related to fruit size and shape [2].

The tree is very ornamental, due to its beauty and edible fruits, however, it is not suitable for public spaces, as it has great potential to cause stains due to the presence of anthocyanins, hydrophilic antioxidant pigments as well fruits such as grapes (*Vitis* sp.) and bilberries (*Vaccinium* sp.).

In the literature, it is presented to us that java plum has phytochemical compounds of medicinal interest such as anti-inflammatory, antibacterial and antifungal. Its parts such as the bark of the stem have anti-inflammatory properties, the leaf has antioxidant properties such as flavonoids, saponins and tannins in which these can vary their concentrations during the seasons, with autumn being the largest concentration and the

fruit has antioxidant compounds and bioactive components such as anthocyanins and phenolics [3,4,5].

Java plum originates from Asia and has adapted very well to the soil and climate conditions of Brazil, becoming spontaneous in the Northeast region. Still, there is no large commercial production in the country, since information related to planting, plant management, post-harvest management, and fruit processing is limited and vague [6]. The fruits of Java plum are most often consumed naturally or processed. Because it presents important functional and medicinal characteristics, the production and consumption of Java plum should be encouraged, in the same way as studies in relation to their cultivation [7].

The propagation of java plum is made by seeds, presenting the characteristic of polyembryonic, but not always with desirable germination power, being influenced by moisture loss [8]. The extraction of seeds of fleshy fruits is usually done in a wet way due to the speed and efficiency of the process. Seeds of species that do not present mucilage involving the integument are practically ready for sowing after washing. However, the presence of mucilage closely adhered to the seeds requires subsequent processing operations for the elimination of this mucilage.

This is due to the fact that mucilage can impair seedling germination and development by favoring the development of microorganisms or containing germination-inhibiting substances [9]. The choice of the method of removal of the aryl is a function of the characteristics of the fruit, the way in which the seed is adhered to, of the

presence of mucilaginous envelope involving the seed, among others [10]. The objective is to obtain high-quality seeds; therefore, all efforts should be used to ensure the physiological quality of the same.

The production of quality seedlings depends on several factors, including the method of extraction and cleaning the seed for planting. Traditionally, seed extraction is done manually [11]. However, manually extracted seeds present many pulp residues and placental tissue adhered to the integument, in most cases impairing the viability and vigor of seeds and the need to test different methods of extraction of seeds.

This study aimed to evaluate the effect of different seed extraction methods on the physiological quality of Java plum seeds.

## 2. MATERIALS AND METHODS

The experiment was conducted at the Federal Institute of Education, Science, and Technology of Tocantins, Gurupi/TO city from October 2018 to May 2019. For this reason, Java plum seeds (*Syzygium cumini*) were directly removed from the fruits, which were collected in the same period. The harvested fruits were selected and submitted to pulping for the removal of seeds. The extraction methods used were: manual with friction over wire mesh; manual with addition of sand and friction over wire mesh; manual with friction over plastic sieve; fermentation at room temperature for three days; immersion in slaked quicklime (CaO) solution for 24 and 48 hours; fermentation at room temperature with added sugar in the proportion of 5:3 water and sugar, respectively, for 24, 48 and 72 hours; fermentation at room temperature with added sugar in the proportion of 5:1 of water and sugar, respectively, for 24, 48 and 72 hours.

The 12 trays used were composed of 25% Rice Straw (RS); 25% Bovine Cattle (BC) and 50% Black Soil (BS). The germination test contained 4 repetitions with 25 seeds per treatment, were submitted to two irrigations during the first 30 days and irrigated once a day in the remainder of the seedling formation period.

After the installation of the experiment, the data collection process began.

The following characteristics were evaluated:

- **Root Length (RL) and Shoot Length (SL):** Seedlings were removed from trays and with the aid of a ruler graduated in centimeters, measured from apical yolk to the end of the

apical root, and measured from the lap to the apex of the seedling. The results were expressed in centimeters, according to recommendations [12].

- **A Number of Leaves (NF):** After seedling removal, the number of leaves was counted. The results were expressed in a unit.
- **Dry Weight of Shoot (DWS) and root (DWR):** Seedlings after removal of substrates were properly cut and separated into roots and shoots, placed in paper bags, taken to a regulated greenhouse with forced air circulation at a temperature of 65°C, where they remained until they reached a constant weight. The results were expressed in grams by repetition, according to recommendations [12].
- **First emergency count (FE):** the first emergency count was performed at 20 days after sowing. The collected data were corresponding to the cumulative percentage of normal seedlings, with values recorded for each substrate.
- **Seedling Emergence (SE):** The count of the number of germinated seeds started from the first seedling emerged and extended to emergency stabilization in all substrates. The criterion used was that of normal seedlings that presented the perfect essential structures [13] and the results expressed as a percentage.

The data were submitted to variance analysis and the means compared by the Tukey test, using the statistical program Sisvar<sup>®</sup>.

## 3. RESULTS AND DISCUSSION

In general, the characteristics evaluated showed sensitivity when indicating differences in the form of extraction of the seeds of Java plum (Table 1), where the highest value of the root length was obtained with the seeds extracted manually through friction in mesh plastic (7.7 cm), immersion in quicklime solution for 24 and 48 hours (9.9 and 9.7 cm), respectively, fermentation at room temperature plus sugar (5:3) for 24 and 48 hours (9.6 and 9.9 cm), respectively, and fermentation at room temperature plus sugar (5:1) for 48 and 72 hours (9.2 and 9.4 cm), respectively and smaller in manual extraction through friction with steel mesh (5.3 cm), manual more sand through friction steel mesh (5.4 cm), fermentation at room temperature for 72 hours (5.6 cm), fermentation at room temperature plus sugar (5:3) for 72 hours and (5:1) for 24 hours (5.3 and 5.3 cm), respectively.

**Table 1. Root length (cm), shoot length (cm), number of leaves (n), dry root mass (g), shoot dry mass (g), first emergency count (%) and seedling emergence (%) of Java plum seeds, treated in different ways**

Treatments	RL	SL	NF	DWS	DWR	FE	SE
Manual + frictionsteelmesh	5.3 b	14.3 b	7.2ab	6.9ab	8.9ab	21.7 b	51.2 b
Manual + Sand + friction steel mesh	5.4 b	15.3ab	7.6ab	6.8ab	8.4ab	22.1 b	49.5 b
Manual + frictionplasticmesh	7.7ab	18.3a	8.5a	9.8a	11.6a	40.0a	95.6a
Fermentation at room T. 72 h	5.6 b	12.0 b	7.6ab	5.1 b	7.6 b	19.2 c	20.3 c
Immersion slaked quicklime(CaO)24 h	9.9a	19.6a	8.9a	9.2a	12.7a	39.6a	87.6ab
Immersion slaked quicklime(CaO)48 h	9.7a	19.4a	8.7a	9.9a	12.6a	41.1a	88.1ab
Ferm. at room T.+ sugar (5:3) 24 h	9.6a	19.1a	8.6a	9.6a	12.9a	39.4a	92.3a
Ferm. at room T. + sugar (5:3) 48 h	9.9a	18.6a	8.5a	9.6a	12.4a	39.1ab	91.2a
Ferm. at room T. + sugar (5:3) 72 h	5.3 b	12.3 b	7.2ab	6.4ab	7.7 b	24.5 b	55.7 b
Ferm. at room T. + sugar (5:1) 24 h	5.3 b	12.4 b	7.4ab	6.8ab	7.5 b	23.9 b	53.4 b
Ferm. at room T. + sugar (5:1) 48 h	9.2a	18.0a	7.1ab	9.4a	12.7a	41.7a	94.2a
Ferm. at room T. + sugar (5:1) 72 h	9.4a	18.5a	8.0a	9.1a	12.5a	40.6a	93.8a
CV (%)	4.9	7.5	1.3	4.8	5.5	13.4	13.7

CV = Coefficient of variation. Ferm = fermentation. T = temperature. + = with. The different letters mean differences between the means of the treatments in the column. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5%

Probably, the steel mesh damaged the seeds and fermentation at room temperature for 72 hours did not promote the complete removal of the pulp adhered to the seeds of Java plum. Silva [10] comments that the fermentation time of the seeds varies according to the species and period, also comments that fermentation has been used to degrade the gelatinous wrap that covers the seeds. Cardoso et al. [14], working with passion fruit seeds verified a positive effect on root length when they were subjected to the fermentation process.

It was also observed that the highest length value of the shoot was obtained in the manually extracted seeds through friction in plastic mesh (18.3 cm), immersion in quicklime solution for 24 and 48 hours (19.6 and 19.4 cm), respectively, fermentation at room temperature with sugar (5:3) for 24 and 48 hours (19.1 and 18.6 cm), respectively and fermentation at room

temperature with sugar (5:1) for 48 and 72 hours (18.0 and 18.5 cm), respectively, following the same trend of root length. Regarding the number of leaves, even with small variation (7.1 to 8.9 un), the seeds extracted in the plastic mesh, immersed in quicklime solution, fermented at room temperature with higher sugar concentration (5:3) for a shorter time and with lower sugar concentration (5:1) for longer stood out.

Regarding the highest values of the dry mass of the root and shoots (9.8 and 11.6 g) (Table 1), they were obtained with seeds extracted manually with the aid of friction in a plastic mesh. Immersed in quicklime solution for 24 hours (9.2 and 12.7 g) and 48 hours (9.9 and 12.6 g), respectively. For fermentation at room temperature in the ratio of 5 pieces of sugar to 3 pieces of water (5:3)/24 hours (9.6 and 12.9 g) and 48 hours (9.6 and 12.4 g), respectively,

fermentation at room temperature in the proportion of 5 parts of sugar to one of water (5:1) /48 hours (9.4 and 12.7 g) and 72 hours with (9.1 and 12.5), respectively for the same characteristics. Indicating that the immersion in quicklime solution for 24 hours is sufficient to complete removal of the pulp adhered to Java plum seeds, optimizing the time and quality of the seeds in the seedling production process.

The results obtained between the values of the first emergency count (Table 1) allowed to differentiate the methods of seed extraction at vigor levels, that is, they were influenced by seed extraction methods. The seeds were more vigorous when extracted manually through friction in plastic mesh (40%), immersed in quicklime solution for 24 and 48 hours (39.6 and 41.1%), respectively, fermented at room temperature with sugar (5:3) for 24 and 48 hours (39.4 and 39.1%) and concentration (5:1) for 48 and 72 hours (41.7 and 40.6%), respectively. Therefore, it is possible to obtain more vigorous seeds with shorter time by immersion in quicklime solution for 24 hours and fermented in higher sugar concentration (5:3) for 24 hours. Probably, these treatments promoted small risks in the tegument of seeds not causing injury to them and increasing the vigor.

It was also verified that fermented seeds at room temperature with sugar (5:3) for 72 hours and sugar (5:1) for 24 hours reduced vigor. Probably, excess fermentation has caused damage to the integument and cotyledons. Nascimento and co-workers [15], while working with cucumber and tomato seeds, found that vigor was affected as fermentation time and temperature were high. Similar results were found by Dos Santos [16] in cupuacu seeds when treatment involving fermentation in water which was used for period of 96 hours or more.

Regarding seedling emergence, once again the seeds extracted manually through friction in plastic mesh (95.6%), immersed in quicklime solution for 24 and 48 hours (87.6 and 88.1%), respectively, fermented at room temperature with sugar (5:3) for 24 and 48 hours (92.3 and 91.2%) and concentration (5:1) for 48 and 72 hours (94.2 and 93.8%). De Oliveira et al. [17], working with stored Java plum seeds, also found seedling emergence around 97%. Intermediate results were obtained in seeds extracted in the steel mesh, sand more steel mesh (51.2 and 49.5%), fermented at room temperature with

sugar (5:3 for 72 and 5:1 for 24 hours) (55.7 and 53.4%), respectively, and lower with fermentation at room temperature for 72 hours (20.3%).

#### 4. CONCLUSION

The seeds treated manually through friction in plastic mesh, immersed in slaked quicklime solution for 24 hours, fermented at room temperature with more sugar (5:3) for 24 hours and (5:1) for 72 hours provided the highest values of viability and vigor in java plum seeds.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:  
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