

Pregerminative treatments of yellow mombin (*Spondias mombin* L.) seeds

Tratamentos pré-germinativos em sementes de cajá (*Spondias mombin* L.)

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Abstract

The germination of *Spondias mombin* seeds is slow and irregular - it takes between 6 and 24 months for the process to complete. This causes serious difficulties to the plantlet producer since the plantlets are more expensive to produce and frequently are of different heights. The objective of this project was to establish techniques and strategies of yellow mombin (*Spondias mombin* L.) seed selection to promote and speed up germination. Having that in mind, two experiments were carried out. In the first, seeds (which are of the nucular type) of two colors (beige and brown) and four classes of size (small, medium-small, medium-large, and large) had their length, width, and weight determined. In the second experiment, the color (beige and brown) classified seeds were submitted or not to a fungicide treatment and to mechanical scarification of the endocarp (base, apex, lateral, and no scarification). The experiments were distributed according to a completely random design with four 20 seeds replications in the following factorial arrangements: 4x2 (size x color) and 4x2x2 (scarification x color x fungicide). Seedling emergence in sand, first count of germination, and mean time for germination were weekly determined up to 730 days; these evaluations were based on the determination of normal seedlings counting. Choosing brown and medium-large seeds is an efficient strategy to accelerate seedling emergence. Treating the seeds with fungicide was harmful to the germination process. The lateral scarification of brown seeds permitted a reduction in the period necessary for the completion of germination from 489 to 336 days.

Keywords: Dormancy; Mechanical scarification; Propagation

Resumo

As sementes de cajá apresentam germinação lenta e desuniforme, pois o processo normalmente tem duração de seis meses e dois anos. Tal característica constitui-se em entrave para os viveiristas, pois onera os custos de produção e resulta em mudas com tamanhos diversos. O objetivo deste trabalho foi estabelecer técnicas e estratégias de seleção de sementes de cajá (*Spondias mombin* L.) que promovam ou acelerem a germinação. Para tanto, foram realizados dois experimentos. No primeiro, foram avaliadas sementes (nuculânios) de duas cores (bege e marrom) e quatro classes de tamanho (pequena, média-pequena, média-grande e grande) caracterizadas por meio do comprimento, largura e massa. No segundo experimento, as sementes classificadas quanto à cor (bege e marrom) foram submetidas ou não à aplicação de fungicida e à escarificação mecânica do endocarpo (base, ápice, lateral e sem escarificação mecânica). Os experimentos foram instalados em delineamento inteiramente casualizado com quatro repetições de 20 sementes, nos seguintes arranjos fatoriais: 4 x 2 (tamanho x cor) e 4 x 2 x 2 (escarificação x cor x fungicida). Avaliou-se a emergência de plântulas em areia, primeira contagem e o tempo médio da emergência. Em ambos os estudos, a emergência foi avaliada semanalmente, até os 730 dias. Concluiu-se que a seleção de sementes marrons e média-grandes (31 x 18 cm e 2,16 g) constituiu-se em uma estratégia eficiente para aumentar e acelerar a emergência de plântulas. O tratamento das sementes com fungicida (metalaxil-M+fludioxonil) foi prejudicial ao processo germinativo. A utilização de sementes marrons e escarificadas na lateral possibilitou a aceleração da germinação (tempo médio de 489 para 336 dias).

Palavras-chave: Dormência; Escarificação mecânica; Propagação

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Introduction

Spondias mombin is a tree (Anacardiaceae) native to the tropical Americas. The species has been introduced in parts of Africa, India, Bangladesh, Sri Lanka and Indonesia (SACRAMENTO; SOUZA, 2009). Due to its ecological importance *Spondias mombin* is the most suitable species to restore the degraded areas in association with secondary forests species, that can be planted in the nearby areas of the Brazilian igapó forests (VALE; COSTA; MIRANDA, 2014). Additionally, yellow mombin fruits have an excellent flavor and aroma in addition to being a good producer of pulp, this being the reason of their use in preparation of refreshments, ice creams, jellies, and liquors (CARVALHO; ALVES, 2008).

One of the production problems to be solved for the commercial production of yellow mombin, is that it is related to the production of plantlets due to the slow and irregular germination of the seeds (AZEVEDO; MENDES; FIGUEIREDO, 2004). Yellow mombin seed germination starts 160 and ends 844 days after sowing (CARVALHO; NASCIMENTO; MÜLLER, 1998). Fungi development on the pulp remaining on the endocarp is pointed by farmers and researchers as an obstacle to the germination of some fruit species (VIEIRA; GUSMÃO, 2006; POLTRONIERI; AZEVEDO; SILVA, 2013) and this may be the case of yellow mombin. On the other hand, another explanation for the distribution of germination along a period of time would be seed dormancy (LOPES *et al.*, 2009; CARVALHO; NAKAGAWA, 2012). Mechanical scarification on the fruit distal region or near the embryo was sufficient to promote seed germination (FIRMINO; ALMEIDA; TORRES, 1997; LOPES *et al.*, 2009). This is a treatment that may be used to overcome seed dormancy (BRASIL, 2013).

The structure known as seed by the agronomists and used for the sexual production of yellow mombin, is a diaspore of the nuculanic type (CAVALCANTE *et al.*, 2009; CARVALHO; NAKAGAWA, 2012) from the botanical point of view. The nuculanium contains the true seeds surrounded by the woody endocarp which makes impossible their extraction without harming the embryo and thus reducing germination. The diaspores contain from zero to five seeds, although in 60% of the fruits only one true seed is found (AZEVEDO; MENDES; FIGUEIREDO, 2004).

Depending on its precedence, the nuculanium may be of different sizes with a length from 25.2 to 34.8 mm and a diameter between 13.2 and 21.2 mm (SOUZA *et al.*, 2000; AZEVEDO; MENDES; FIGUEIREDO, 2004). Nuculanium size may be related to the speed of germination; Azevedo, Mendes and Figueiredo (2004) reported that the largest diaspores bear seeds which primary roots are emitted more promptly than those of the smaller diaspores. It is supposed that the larger nuculanias were, during their formation, more well-nourished and thus have more vigorous seeds.

In addition to that, the larger nuculanias have a larger number of true seeds (AZEVEDO; MENDES; FIGUEIREDO, 2004) so that the sowing of the larger diaspores may enhance the chances of germination to occur and the development of at least one of those seeds and the production of plantlets.

Classifying fruit seeds as to size or weight is a strategy that may be adopted to make more uniform seedling emergence, seedling size and to get more developed plantlets. Larger or denser tamarinds (ALMEIDA *et al.*, 2010), Brazilian grape tree (WAGNER JÚNIOR *et al.*, 2011), and 'pitanga' (ANTUNES *et al.*, 2012) seeds were reported to be more vigorous than the smaller and lighter ones.

Fruit or seed color is also a simple criterion for field verification of stage of maturity viewing to establish the right moment for harvest, that is, when the seeds have reached physiological maturity and consequently maximum germination and vigor. (MARTINS *et al.*, 2004; SILVA *et al.*, 2009; CARVALHO; NAKAGAWA, 2012; LOPES; NÓBREGA; MATOS, 2014). Seeds should not be harvested before or after physiological maturity since, before, they have not reached maximum physiological quality and, after, because the deterioration process has already started (SILVA *et al.*, 2009; CARVALHO; NAKAGAWA, 2012). But, for the production of yellow mombin plantlets, the seeds are extracted from mature fruits picked right from the plants or those which have dropped to the ground, where they remain for variable periods of time (COSTA *et al.*, 2001). After the pulp is eliminated, the nuculanias are observed to display a clear or a dark surface - the dark surface results from the oxidation processes caused by the time they remained in contact with the fermenting pulp.

Taking in consideration the commercial potential of this fruit, the present research work viewed

the establishment of techniques and strategies for the selection of yellow mombin seeds capable of a germinating within shorter periods of time.

Material and Methods

Yellow mombin fruits were harvested during the months of April and May of 2012 from ten matrix plants in the College of Agrarian and Veterinarian Sciences in Jaboticabal, state of São Paulo, Brazil, or from small rural properties in the same state. The fruits were submitted to depulping by washing and friction on screens and running water after which they were dried for 24 hours in a shaded and ventilated place. The nuculanias containing the true seeds were named seeds, according to definition found in the Rules for Testing Seeds (BRASIL, 2013). These seeds were used to carry two experiments: in the first one, they were classified as to color - beige and brown and as to size: small, medium-small, medium-large, and large. Those classes were based on 100 seed samples (Table 1).

Table 1 – Yellow mombin seeds classification as to size and weight.

Tabela 1 – Classificação das sementes de cajá quanto ao tamanho e peso.

Size	Length (mm)	Width (mm)	Weight (g)
Small	25	14	1.093
Medium-small	29	16	1.623
Medium-large	31	18	2.159
Large	33	19	2.341

Immediately after the seeds were extracted from the fruits, they were submitted to drying on a table in the laboratory; the superficial water remaining in the seeds was removed with the help of paper towel and then the seed water content was determined by the oven method under a temperature of 105 ± 3 °C for 24 hours (BRASIL, 2009), two replications of 4 seeds each. Seed length and width were measured with the help of a digital caliper and their weight determined with the help of a 0.001 g precision scale.

In the second experiment, the seeds were classified as to color in beige and brown. These seeds were either treated or not with a fungicide and, with the help of an emery, submitted or not to mechanical scarification in the base, apex, and lateral. Immediately before sowing, the seeds were treated with the fungicide Maxim® XL (metalaxil-M+fludioxonil) solution at a concentration of 100 mL of the commercial product/100 kg of seeds.

In both experiments, the following seed attributes were evaluated:

Seedling emergence in sand - it was conducted with four 20 seed replications in sterilized sand which was moistened to 60% of its water holding capacity (BRASIL, 2013). The sand was placed in plastic boxes (30.2 x 20.8 x 6.3 cm) which were kept on a workbench for 730 days (temperature 26 ± 3 °C). Normal seedlings emerging from the substratum were weekly counted and removed. When two seedlings emerged from the same seed, they were counted as one for the calculation of the emergence percentage.

First count of emergence and mean time for emergence - both counts were made concomitantly with the seedling emergence in sand test. In the first count test, normal seedlings emerging at 180 days after sowing, a date established with basis on the moment when 50% of the final evaluation took place (NAKAGAWA, 1999).

The mean time for emergence (TME) - was calculated based on the seedling count at each week up to the final evaluation date. The data so obtained were used to calculate the TME by means of the formula proposed by Labouriau and Valadares (1976).

The means were compared by the Tukey's test at the level of 5% of probability. The replications

were distributed according to a completely random design the treatments arranged according to the following factorial combinations: 4 x 2 (size and color) in the first experiment and 4 x 2 x 2 (scarification x color x fungicide) in the second one. For the analysis of variances the data expressed in percentage were previously transformed in arc sen of the square root of $x + 0.05$. The means shown in the tables are non-transformed values.

Results and Discussion

The water content of recently harvested yellow mombin seeds, beige and brown, was not influenced by color or size - it was between 45.2 and 46.0%, respectively, independently of treatment. These moisture values are different from those reported by Gama *et al.* (2012), that is, 61.8%, probably because the seeds of this experiment were previously submitted to a 24-hour period of drying in a shaded room.

In the first experiment it was verified a significant interaction between color and size in the results of seedling emergence in sand. The speed of seedling emergence as evaluated by the first count and mean time for emergence tests were influenced only by the color of the seeds (Table 2).

Table 2 – Summary of the analysis of variance of yellow mombin seeds results as influenced by seed color and size.

Tabela 2 – Resumo da análise de variância em sementes de cajá classificadas quanto à cor e ao tamanho.

Source of variation	GL	Mean square		
		Emergence	First count	TEM
Color (C)	1	5.281*	8.000*	50.562*
Size (S)	3	8.531**	0.125 ^{ns}	8.123 ^{ns}
C x S	3	2.948*	3.000 ^{ns}	6.828 ^{ns}
Residue	24	0.865	1.479	3.946
CV (%)		14.80	25.96	26.94

Where in: MTE = mean time for emergence; CV = coefficient of variation; GL = degrees of freedom; ** = significant at the level of 1% of probability; * = significant at the level of 5% of probability; ^{ns} = non significant.

The brown seeds were verified to be superior to the beige ones (Table 3). The faster emergence of the brown seeds is probably due to their oxidation since, according to Sampaio *et al.* (2007), yellow mombin fruits undergo high oxidation soon after the fruits are harvested. Such a process may have caused a reduction in the level of seed dormancy and a softening of the endocarp mechanical barrier. This barrier is thought to be the cause of *Spondias mombin* seed dormancy (CARVALHO; ALVES, 2008).

Table 3 – Effects of seed color on the results of the first count (FC) and the mean time for seedling emergence (MTE) tests of yellow mombin seeds.

Tabela 3 – Primeira contagem (PC) e tempo médio de emergência (TME) de plântulas em areia de sementes de cajá (*Spondias mombin* L.), classificadas quanto à cor.

Tests	Color	
	Beige	Brown
FC (%)	18 B	28 A
MTE (days)	273 B	194 A

Means, in the same line, followed by the same large case letter, are not statistically different according the Tukey's test at the 5% level of probability.

It is important to emphasize these results of the mean time for seedling emergence test since this is a relevant characteristic for the production of commercial plantlets (COSTA *et al.*, 2001).

In Table 4, data of seedling emergence in sand as influenced by seed color and size are found. It is verified that medium-large seeds result in a larger number of seedlings independently of their color although, when the seeds were beige, the medium-large and the medium small resulted in statistically similar results. When the seeds were brown, the medium-large seeds did not differ from the large ones.

Table 4 – Effects of yellow mombin seed color and size on seedlings emergence in sand length x width; weight.

Tabela 4 – Emergência de plântulas em areia de cajá (*Spondias mombin* L.), classificadas quanto à cor (bege e marrom) e ao tamanho.

Size ¹	Color	
	Beige	Brown
Small (25x14 mm; 1,1 g)	39 bA	20 bB
Medium-small (29x16 mm; 1,6 g)	40 abA	26 bB
Medium-large (31x18 mm; 2,2 g)	68 aA	47 aA
Large (33x19 mm; 2,3 g)	37 bA	50 aA

Means followed by the same lower case letter in the column and capital in line, are not statistically different according the Tukey's test at the 5% level of probability.

The percentage value of seedling emergence displayed by medium-large seeds (between 47 and 68%) may be considered similar to the value of 60% reported by Firmino, Almeida and Torres (1997) also with yellow mombin seeds after treating them for dormancy breaking. These results suggest that seed selection with basis on visual characteristics may be enough to get seeds with improved germination performance.

According to Carvalho and Nakagawa (2012), larger seeds, usually, result from better nourished seeds during their maturation, which result in well-formed embryos with larger amounts of reserve substances. In the case of yellow mombin seeds, the higher seedling emergence could also be ascribed to the fact that the nuculanum has a larger number of true seeds (AZEVEDO; MENDES; FIGUEIREDO, 2004) which increases the chance that at least one of those seeds is successful in germinating. Brito Neto *et al.* (2009) also reported to have observed that medium and large size *Spondias tuberosa* Arr. Câm seeds displayed germination and vigor higher than the small ones.

In the second experiment, none of the examined factors had any significant influence on speed of emergence as determined by the first count test (Table 5). But, on the other hand, the mean time for seedling emergence was significantly affected by the interaction between color and scarification as well as fungicide and scarification. As to the percentage of emerged seedlings, it was reduced from 33 to 24% by the treatment of the seeds with the fungicide.

This negative effect of the fungicide may be due to toxicity resulting from the excess to which the product is absorbed and retained by the spongy fibers covering the nuculanum (seed) as shown by Azevedo, Mendes and Figueiredo (2004). These fibers form a tissue capable of absorbing water and this is the reason why the genus name is *spondias*, a Latin word meaning sponge. França Neto, Henning and Yorinori (2000) reported germination reduction and increment in abnormal seedlings in soybean seeds treated with Rhodiauram 500 SC.

When the effect of the interaction between scarification and color was examined, it was verified that the fastest germination (336 days) took place when brown seeds scarified on their lateral side were used (Table 6). This result was significantly higher than that of the check treatment seeds of the same color which mean germination time was of 489 days. In a similar way, the interaction between scarification x fungicide also resulted in the shortest mean time of emergence (392 days) for the seeds scarified in the lateral and without the application of fungicide, mainly when compared to the check treatment treated with

fungicide (472 days). On the other hand, the fungicide treated and scarified in the basal area of the seed also caused a reduction in the mean time for emergence. But, this treatment should not be used since, as shown in Table 5, the treatment of the seeds with the fungicide brought about a significant reduction in the final emergence percentage.

Table 5 – Summary of the analysis of variance of tests results of *Spondias mombin* seeds submitted or not to fungicide treatment and mechanical scarification.

Tabela 5 – Resumo da análise de variância em sementes de cajá (*Spondias mombin* L.), classificadas quanto à cor e submetidas ou não ao tratamento fungicida e escarificação mecânica.

Source of variation	GL	Mean square		
		Emergence	First count	TEM
Color (C)	1	1.562 ^{ns}	1.891 ^{ns}	54.347**
Fungicide (F)	1	10.562**	1.891 ^{ns}	5.568 ^{ns}
Scarification (S)	3	0.562 ^{ns}	1.016 ^{ns}	12.211*
C x F	1	0.062 ^{ns}	0.016 ^{ns}	3.122 ^{ns}
C x S	3	1.562 ^{ns}	0.682 ^{ns}	11.749*
F x S	3	0.562 ^{ns}	0.432 ^{ns}	14.771**
C x F x S	3	0.396 ^{ns}	0.057 ^{ns}	7.572 ^{ns}
Resíduo	48	0.750	0.693	3.475
CV (%)		16.02	52.74	13.72

Where in: MTE = mean time for emergence; CV = coefficient of variation; GL = degrees of freedom; ** = significant at the level of 1% of probability; * = significant at the level of 5% of probability; ^{ns} = non significant.

Table 6 – Mean time for the seedling emergence in sand of yellow mombin seeds classified as to color and submitted to mechanical scarification as well as when submitted or not to mechanical scarification and fungicide treatment.

Tabela 6 – Tempo médio de emergência de plântulas em areia de sementes de cajá (*Spondias mombin* L.), classificadas quanto à cor e submetidas à escarificação mecânica, bem como quando submetidas ou não ao tratamento fungicida e escarificação mecânica.

Scarification	Color		Fungicide	
	Beige	Brown	With	Without
Check treatment	460 bB	489 bB	472 bB	432 bB
Basis	432 bB	383 abA	366 aA	449 bB
Apex	471 bB	441 bB	427 bB	485 bB
Lateral	473 bB	336 aA	418 bB	392 aA

Means, in the same line, followed by the same large case letter, are not statistically different according the Tukey's test at the 5% level of probability.

Firmino, Almeida and Torres (1997) and Lopes *et al.* (2009) reported results that differed from those of this experiment - according to their data, seeds which had been mechanically scarified both at the base and the top of the seed germinated faster and to a higher proportion whereas our results indicated that scarifying the seeds as indicated by those authors did not result in faster germination (Tables 5 and 6).

The efficiency of the scarification procedure depends on ability and practice of the technician; if the scarification is too deep, it may result in damage to the embryonic axis or, if too superficial, it may be not enough to overcome the mechanical barriers that block seed germination (MARTINS *et al.*, 2012).

Conclusions

Choosing brown and medium-large seeds is an efficient strategy to accelerate seedling emergence. Treating the seeds with fungicide was harmful to the germination process.

The lateral scarification of brown seeds permitted a reduction in the period necessary for the completion of germination from 489 to 336 days.

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