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RESEARCH NOTE

Sodium hypochlorite for removal of the sarcotesta from newly extracted and stored papaya seeds[1]

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ABSTRACT – The objective of this study was to evaluate the effect of sodium hypochlorite on removal of the sarcotesta and the germination process of newly harvested papaya seeds before and after storage for 44 days. Nine treatments were evaluated using a completely randomized design with six treatments in a split plot arrangement and three additional treatments (control), each treatment with eight replications. The plots consisted of methods for breaking dormancy: M1 – sieve method + drying to 7%, M2 – sieve method + drying to 7% + NaOCl, and M3 – NaOCl + drying to 7%. The split plots consisted of different storage periods (0 and 44 days). The three additional treatments consisted of A - NaOCl at 6% for 12 hours, B – sieve method, and C – intact seeds in running water for 24 hours. Germination and the first count of germination were evaluated. Results showed that sodium hypochlorite is effective in removal of the sarcotesta, but there is a negative effect on germination. Storage for 44 days also had a negative effect on germination. Removal of the sarcotesta by the sieve method followed by drying to 7% moisture content was effective in promoting germination of papaya seeds.

Index terms: *Carica papaya* L.*,* active chlorine, germination.

Hipoclorito de sódio na remoção da sarcotesta de sementes de mamão recém-extraídas e armazenadas

RESUMO – Objetivou-se avaliar o efeito do hipoclorito de sódio na retirada da sarcotesta e no processo germinativo de sementes de mamão recém-extraídas, previamente armazenadas, antes e após armazenamento por 44 dias. Foram avaliados nove tratamentos segundo delineamento inteiramente casualizado, sendo seis tratamentos no esquema de parcelas subdivididas e três tratamentos adicionais (controles). As parcelas foram constituídas pelos métodos para quebra de dormência: M1 – Método da Peneira + Secagem a 7%; M2 – Método da Peneira + Secagem a 7% + NaOCl; e M3 – NaOCl + Secagem a 7%. As subparcelas pelo tempo de armazenamento (zero e 44 dias). Os três tratamentos adicionais consistiram em: A – NaOCl a 6% por 12 horas; B – Método da Peneira e C – Sementes intactas em 24 horas em água corrente. Avaliou-se o teste de germinação e a primeira contagem do teste de germinação. O uso do hipoclorito de sódio é eficiente para retirar a sarcotesta, porém afeta negativamente a germinação. O armazenamento por 44 dias é prejudicial à germinação. A remoção da sarcotesta pelo método da peneira, seguida da secagem até 7% de teor de água foi eficiente para propiciar a germinação das sementes de mamão.

Termos para indexação: *Carica papaya* L.*,* cloro ativo, germinação.

Introduction

Papaya (*Carica papaya* L.) consumption has grown continuously, which may be due to the considerable benefits it has for health, in addition to its flavor, which is pleasing to most, making papaya stand out among the most important tropical fruits grown in Brazil and in the world.

Although there is a trend toward vegetative propagation,

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papaya propagation for commercial purposes basically occurs through seeds (Carlesso et al., 2009). Although production companies have their protocols already defined for production, an optimized process for seed germination is not known. The lack of knowledge of the species is directly reflected in production, leading to high seed cost and, consequently, high production cost.

Papaya seeds have slow and irregular germination, and the entire process may take from 4 to 8 weeks (Tokuhisa et al., 2007a). Dormancy in papaya seeds has been reported in the literature as a cause of reduction in the process of fruit and seed production (Wagner, 1985; Schmildt et al., 1993; Tokuhisa et al., 2008). However, there is no consensus on the intensity of dormancy and its true cause.

Various studies have indicated the sarcotesta as one of the factors that negatively affects both germination speed and germination percentage (Wagner, 1985; Schmildt et al., 1993; Cavalcante et al., 2014; Jesus et al., 2016) due to the presence of inhibitors (Melo and Seleguini, 2013).

Among the main methods used for removing the sarcotesta in papaya seeds are removal by friction with autoclaved sand, removal with a blender, and removal through pressure in a sieve (Schmildt et al., 1993; Cavalcante et al., 2014).

According to Dias et al. (2015), even with removal of the sarcotesta, dormancy may continue, due to the presence of phenolic compounds that inhibit germination that are in other seed structures. Tokuhisa et al. (2007b) found that dormant papaya seeds contain phenolic compounds, with a greater concentration in the sclerotesta, followed by the sarcotesta, though practically absent from the embryo and endosperm.

For Dias et al. (2015), the time of harvest of the fruit also affects the concentration of phenolic compounds in the sarcotesta and sclerotesta of papaya seeds, explaining the variation in intensity of dormancy found by Tokuhisa et al. (2008), which is stronger in fruit harvested in the period of mild temperatures. In contrast, seeds of fruit harvested in periods of high temperatures do not exhibit dormancy (Viggiano et al., 2000). However, for Aroucha et al. (2005), seeds of fruit harvested at higher temperatures have dormancy to a lower degree compared to seeds extracted from fruit harvested in other periods of the year.

In addition to removal of the sarcotesta, Leonel et al. (1998) emphasize the need for use of gibberellic acid in newly harvested seeds so as to increase germination; results of germination without it were practically null. However, because of its high cost, its use on a large scale is unviable. Nevertheless, Martins et al. (2005) found high germination values in seeds before storage, with an increase at three months, followed by a decrease at six months of storage, reaching values below the initial values.

Companies of the papaya sector need seeds to maintain production throughout the year. Considering the extended time and dependence on labor in preparing seeds for removal of the sarcotesta, in addition to the high cost of gibberellic acid recommended for increasing germination in papaya seeds, there is a need for efficient and economical solutions to resolve the problems currently faced by the sector. In light of the above, the aim of this study was to analyze the effect of sodium hypochlorite on removal of the sarcotesta and on the germination process of newly extracted papaya seeds, just as on previously stored seeds and on papaya seeds to be stored.

Material and Methods

Papaya fruit of the cv. Golden, “Solo” group was used, which came from the company Caliman Agrícola S/A, in Linhares, ES, Brazil. The study was carried out in the Seed Research Laboratory of the Department of Plant Science on the campus of the Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Fruits were harvested at stage one of maturity, up to 15% of the peel with yellow coloring. They were stored for three days, at which time their entire peel had turned yellow. The fruit was cut lengthwise and the seeds were extracted manually. The seeds were homogenized and selected in regard to integrity, size, and predominant color. After that, the moisture content of the seeds was evaluated and the treatments began.

Moisture content was determined using four replications of 100 seeds by the oven method at 105 °C for 24 hours (Brasil, 2009), and the results were expressed in percentage.

Nine treatments were evaluated (Table 1) in a completely randomized experimental design, with six treatments in the split plot arrangement and three additional treatments. Eight replications of 50 seeds were tested, and each replication was composed of the mean of two determinations. The plots were constituted by the methods of breaking dormancy, M1 – sieve method + drying to 7%, M2 – sieve method + drying to 7% + NaOCl, and M3 – NaOCl + drying to 7%, while the split plots were constituted by storage time, 0 and 44 days. Three additional treatments (controls) were also used, which were directly applied to newly extracted seeds, i.e., intact seeds: A – NaOCl at 6% for 12 hours; B – sieve method (fermentation in a box with 10 mL of distilled water for 24 hours, followed by removal of the sarcotesta from the seeds in a sieve under water); and C – intact seeds washed in running water for 24 hours. The additional treatment A (6% of active chlorine for 12 hours) was chosen based on results of pre-tests carried out prior to this study, whose preliminary results indicated effective removal of the sarcotesta.

Table 1. Description of the treatments used in the experiment.

Method	Treatment	
Additional	A	Newly extracted seeds imbibed in NaOCl, 6% active chlorine, for 12 hours.
Additional	B	Sieve method (fermentation of newly extracted seeds in a box with 10 mL of distilled water for 24 hours, followed by removal of the seed sarcotesta in a sieve under water).
Additional	C	Intact seed (newly extracted) washed in running water for 24 hours.
Method 1	D	Sieve method + drying to 7% moisture content + no storage.
Method 1	E	Sieve method + drying to 7% moisture content + storage for 44 days.
Method 2	F	Sieve method + drying to 7% moisture content + no storage + 6% NaOCl for 12 hours.
Method 2	G	Sieve method + drying to 7% moisture content + storage for 44 days + 6% NaOCl for 12 hours.
Method 3	H	6% NaOCl for 12 hours + drying to 7% moisture content + no storage.
Method 3	I	6% NaOCl for 12 hours + drying to 7% moisture content + storage for 44 days.

The proportion of seeds (number) per volume (mL) of the sodium hypochlorite solution for imbibition was 380:127, which is equivalent to 0.334 mL of solution per seed.

The sieve method was based on removal of the seeds from the fruit and, after that, storage in a PVC box (containing water) for 24 hours so that fermentation begins. After this procedure, the seeds were placed in a sieve under a water jet, and light pressure was manually applied on the seed over the sieve for removal of the sarcotesta.

Initially, the drying process occurred in the shade. After that, the seeds were dried in a complementary manner in a greenhouse during the day, and the temperature of the mass of seeds was monitored so as not to go beyond 40 °C; at night, the seeds were placed in the laboratory and remained under forced air circulation. The complementary drying process continued until the seeds reached a moisture content of 7.0% at five days.

Seeds were stored in semipermeable plastic bags (plastic with a thickness of 0.10 mm) and kept in a refrigerator at 10 °C for a period of 44 days.

For the germination test, the recommendations contained in the Rules for Seed Testing (Brasil, 2009) were followed, but with four replications of 50 seeds. The experiment was carried out in a B.O.D. chamber with alternating temperatures of 20 and 30 °C, with 16 hours in the dark and eight hours of exposure to the light, respectively. Seeds were sown in rolled paper toweling, wetted with a volume of water 2.5 times the weight of the dry paper. The rolls were kept within plastic bags for the purpose of maintaining moisture. At 30 days, the number of normal seedlings (germination), abnormal seedlings, dormant seeds, and dead seeds was calculated. Together with the germination test, the first count of germination test was carried out, obtaining the percentage of normal seedlings at 15 days after setting up the test.

Germination was determined by the total percentage of normal seedlings at 30 days after setting up the experiment. The treatments composed of the methods of breaking dormancy and storage time were compared to the additional treatments in regard to the number of normal seedlings, abnormal seedlings, dormant seeds, dead seeds, and first count of germination.

The data obtained in the experiments were subjected to normality and homogeneity of variance tests, followed by analysis of variance at 5% probability. The effect of the factors, methods for breaking dormancy, and storage time on the response variables was evaluated by the Tukey test at 5% probability. The treatments, composed of the factors of breaking dormancy and storage time, were compared to the additional treatments (A, B, and C controls), applying the Dunnett test at 5% probability, whereas the additional treatments were compared among themselves by the Tukey test at 5% probability. All the analyses were carried out using the R computational program (R Core Team, 2013).

Results and Discussion

The average moisture content of the seeds determined before the beginning of the experiments was 74.4%. Among the factors that affect the germination and vigor of papaya seeds, moisture content is noteworthy (Viggiano et al., 2000).

In the result of analysis of variance for evaluation of the effect of the factors, methods for breaking dormancy, and storage time, in regard to germination, it was observed that the interaction among the method and storage factors was significant at 5% probability.

Analysis of variance in reference to the deployment of the methods of breaking dormancy within each storage time showed that in the absence of storage, there was no difference between the M1 and M2 methods (M1 – sieve method + drying to 7%, treatment D; and M2 – sieve method + drying to 7% + NaOCl, treatment F), whereas both differed from method M3 (M3 – NaOCl + drying to 7%, treatment H) (Figure 1).

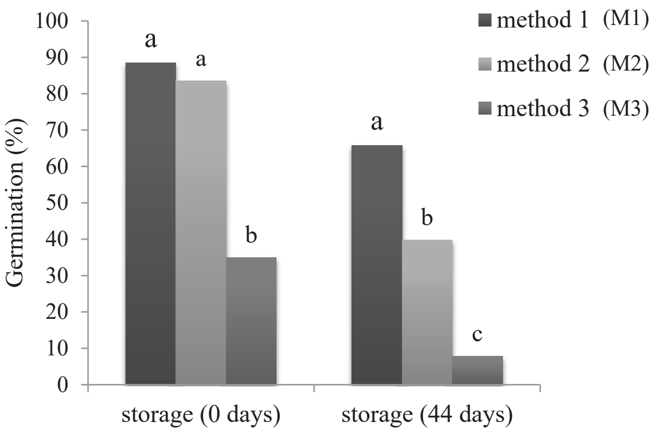

Figure 1. Germination of papaya seeds at 30 days for the different methods within each period of storage.

According to the mean values of methods M1, M2, and M3 in the absence of storage (Figure 1), sodium hypochlorite was detrimental when applied before drying, exhibiting a mean value of 35.0% germination (M3 – treatment H) under these conditions, a result significantly lower than the mean values of 88.5% and 83.5% obtained for the M1 (treatment D) and M2 (treatment F) methods, respectively.

After 44 days of storage, the three methods differed significantly among themselves at 5% probability. The M1 method (M1 – sieve method + drying to 7%, treatment E) was superior to the others, revealing the detrimental effect of sodium hypochlorite on the stored seeds (Figure 1).

In Figure 1, it can be seen that the sieve method, followed by drying of seeds to 7%, both in unstored and stored seeds (treatments D and E, respectively), had a mean value greater than the others. In unstored seeds, the M1 method did not differ significantly from M2 (treatments D and F, respectively). However, the treatment in regard to M1 is more advantageous since it has one step less, reducing costs in seed preparation. After the 44 days of storage, the seeds undergoing methods M2 and M3 (treatments G and I, respectively), both with use of sodium hypochlorite, did not have the ability to recover biological functions for a high percentage of germination. Results with stored seeds, both with use of sodium hypochlorite before drying (M3 – treatment I), and after drying and storage (M2 – treatment G), showed lower germination potential, that is, they were negatively affected by the use of sodium hypochlorite compared to M1 (treatment E), a treatment without the use of sodium hypochlorite.

Probably, the seeds absorbed the sodium hypochlorite and, as of the beginning of the drying process (treatments H and I) and after drying (treatments F and G), the sodium hypochlorite may have been absorbed in a drastic manner by various layers of the seeds, seriously harming structures essential for germination. According to Venturini et al. (2012), drying is a process of elimination of liquids from the surface and from the inside of a material, which occurs through evaporation and transfer of heat and weight. According to the authors, drying is used to impede the decomposition of biological materials, and to facilitate transport and storage. The negative effect may have occurred through an exchange of water among the seed layers, causing the hypochlorite to reach regions such as the embryo, affecting germination.

The result for analysis of variance in regard to the deployment of storage within each method showed that storage of 44 days was detrimental to germination at 30 days for all the seeds that received the methods for breaking dormancy (treatments E, G, and I) (Figure 2). These results contrast with those found by Berbert et al. (2008), in which the percentage of germination after removal of the sarcotesta by the sieve method and drying to 8% moisture content was 75.0%, increasing to 87.0% after four months of storage. Nevertheless, after eight months of storage, the authors observed a reduction in germination to 66.0%.

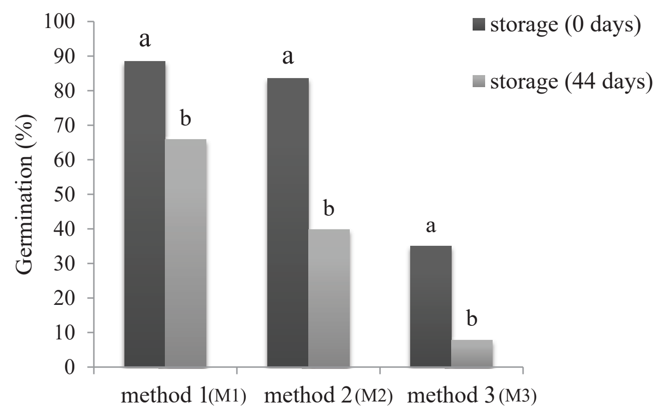

Figure 2. Germination of papaya seeds at 30 days for different times of storage within each method of breaking dormancy.

In this study, germination values of 88.5% were found in the initial period (treatment D), while after storage, there was a reduction to 65.8% (treatment E) for method M1. For method M2, without storage, germination was 83.5% (treatment F) decreasing to 39.8% (treatment G) after storage. For M3, although germination was low before storage, there was a further fall after storage, passing from 35.0% (treatment H) to 7.8% (treatment I) (Figure 2).

There was a detrimental effect from the use of sodium hypochlorite before drying (Method 3 – treatments H and I), which was accentuated when the seeds were stored for 44 days (treatment I). Storage of seeds for 44 days, in general, was a prejudicial factor through considerably reducing the germination percentage in method 1 (E), method 2 (G), and method 3 (I) (Figure 2).

The analyses of variance for the mean values of normal seedlings (germination), abnormal seedlings, dormant seeds, and first count of germination for the treatments in relation to each control (additional treatments A, B, and C) and among the controls were significant at the level of 5% probability. Nevertheless, at the level of 5% probability, significant differences of the treatments were not observed for the number of dead seeds in relation to the controls and for the controls among themselves.

Summarized results for the different treatments in relation to the controls and the controls among themselves are shown in Table 2 and 3, respectively, which were evaluated based on the mean values of normal seedlings, abnormal seedlings, dormant seeds, and first count of germination. The best results for normal seedlings were treatments D and F, with mean germination of 88.5% and 83.5%, respectively, which were better than controls A (37.5%), B (56.5%), and C (22.0%). The highest mean value for normal seedlings in treatment D (sieve method + drying to 7% moisture content + no storage) dispenses with the use of sodium hypochlorite (6% active chlorine for 12 hours), thus eliminating a step and reducing costs. Comparing treatments B and D, it can be observed that drying to 7% after removal of the sarcotesta was beneficial for increasing the percentage of normal seedlings.

Table 2. Effect of treatments on germination/normal seedlings (NS), abnormal seedlings (AS), dormant seeds (DS), and normal seedlings in first count of germination (FC).

Treat	NS				AS				DS				FC			
		A	B	C		A	B	C		A	B	C		A	B	C
		37.5	56.5	22.0		37.5	56.5	22.0		37.5	56.5	22.0		37.5	56.5	22.0
D	88.5	+	+	+	0.0	ns	ns	ns	11.5	-	-	-	81.5	+	+	+
E	65.8	+	ns	+	4.5	+	+	+	28.3	-	-	-	62.3	+	+	+
F	83.5	+	+	+	0.0	ns	ns	ns	16.3	-	-	-	63.5	+	+	+
G	39.8	ns	-	+	8.3	+	+	+	51.3	-	ns	-	29.3	+	ns	+
H	35.0	ns	-	+	0.0	ns	ns	ns	65.0	ns	+	-	11.3	ns	-	ns
I	7.8	-	-	-	7.5	+	+	+	84.5	+	+	ns	3.5	ns	-	ns

+ Significant and superior in relation to the control in the column by the Dunnett test at 5% probability.
- Significant and inferior in relation to the control in the column.
ns Not significant in relation to the control in the column.

The treatments with highest mean values for normal seedlings (treatments D and F) are among those that had the lowest values of abnormal seedlings, not differing from the controls, and also exhibiting the lowest rates of dormant seeds, which were significantly worse than the controls (Table 2).

Treatment D was notable in seedling performance in the first count of germination test, with the highest mean value (Table 2), superior to the mean values of the controls.

The controls did not differ statistically among themselves for number of abnormal seedlings. However, the impediment to germination brought about by the presence of the sarcotesta could be observed in treatment C, which exhibited a low value of normal seedlings and a high number of dormant seeds (Table 3); this has been confirmed by authors (Dias et al., 2012). In contrast, soon after removal of the sarcotesta (treatments A and B), there was a considerable increase in germination. However, this increase was not sufficient for there to be a high germination percentage of papaya seeds. Visually, the use of 6% sodium hypochlorite for 12 hours in intact seeds (treatment A) was effective in removal of the sarcotesta; however, it hurt seed germination, probably because of affecting some structure essential for germination (Table 3).

Table 3. Effect of the controls on normal seedlings (NS), abnormal seedlings (AS), dormant seeds (DS), and normal seedlings in the first count of germination (FC).

Treat	NS	AS	DS	FC
A	37.5 B	0.0 A	62.5 B	6.0 B
B	56.5 A	0.0 A	43.5 C	31.5 A
C	22.0 C	0.0 A	78.0 A	3.3 B

Mean values followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Although it had a low mean value, the sieve method (treatment B) stood out among the controls, exhibiting higher values of normal seedlings and first count of germination and lower values of dormant seeds (Table 3). Such results show the need for removal of the sarcotesta in the germination process, corroborating various authors (Wagner, 1985; Schmildt et al., 1993; Tokuhisa et al., 2008), although such a procedure in itself is not sufficient for the best germination performance of the seeds.

Although sodium hypochlorite removed the sarcotesta, the concentration, time of imbibition, and proportion used in the present study probably had a toxic effect on the embryo. New studies with sodium hypochlorite are suggested, varying the concentration, time, and proportion. Another factor for analysis is other storage times, all with a view toward improving seed quality.

Conclusions

The use of sodium hypochlorite at 6% active chlorine for 12 hours of imbibition at the proportion of 380 seeds per 127 mL of solution (0.334 mL of solution per seed) was effective for removing the sarcotesta; however, it was prejudicial to germination.

Storage of seeds for 44 days was detrimental to germination.

Removal of the sarcotesta by the sieve method followed by drying of seeds to a moisture content of 7% is effective for increasing the germination rate.

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