RESEARCH NOTE

Ratio of seeds and sodium hypochlorite solution on the germination process of papaya seeds[1]

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ABSTRACT – Sarcotesta, a mucilaginous layer found in papaya seeds, negatively affects the germination of papaya seeds and its removal has been manually performed. The use of sodium hypochlorite can be an alternative to manual removal of sarcotesta in papaya seeds. The objective of this study was to evaluate the effect of different active chlorine concentrations (0, 2, 4, 6 and 8%) during 24 hours of immersion, in different proportions (10:200, 50:200, 100:200, 200:200, 300:200, 400:200, 500:200 and 600:200) of seeds number and solution volume (mL) to identify the best combination for an effective remotion of sarcotesta to improve the germination process. Germination test and first count were performed. A completely randomized design with four replications was used. Regression analysis was performed for quantitative variables with significant effect by F-test at 5% probability. Papaya seeds immersion in the ratio of 10 seeds per 200 mL of NaOCl solution (10:200, i.e. 20 mL of solution per seed), at a 2% sodium hypochlorite concentration during 24 hours of imbibition, can be used as an alternative for sarcotesta remotion for freshly harvested papaya seeds, because besides the effective remotion of sarcotesta, this technique improves the germination process.

Index terms: *Carica papaya*, seed technology, NaOCl.

Proporção de sementes e solução de hipoclorito de sódio no processo germinativo de sementes de mamão

RESUMO – A sarcotesta, camada mucilaginosa presente nas sementes de mamão, afeta negativamente o seu processo germinativo e sua retirada tem sido realizada manualmente. O uso do hipoclorito de sódio pode ser uma alternativa à remoção manual da sarcotesta. Objetivou-se avaliar o efeito de diferentes concentrações de cloro ativo (0, 2, 4, 6 e 8%), durante 24 horas de imersão, em diferentes proporções (10:200, 50:200, 100:200, 200:200, 300:200, 400:200, 500:200 e 600:200) entre sementes (número) e volume (mL) da solução de hipoclorito de sódio respectivamente, visando identificar a melhor combinação para a retirada efetiva da sarcotesta de maneira a contribuir no processo germinativo. Foram avaliados o teste de germinação e a primeira contagem. Empregou-se o delineamento estatístico inteiramente casualizado com quatro repetições. Para as variáveis quantitativas, que apresentaram efeito significativo pelo teste F a 5% de probabilidade, foi realizada a análise de regressão. A imersão de sementes de mamão na proporção de 10 sementes por 200 mL de solução de NaOCl (10:200, ou seja, 20 mL de solução por semente), na concentração de 2% de cloro ativo por 24 horas, pode ser uma alternativa para a retirada da sarcotesta em sementes recém-colhidas, pois, além da retirada efetiva da sarcotesta, favorece a germinação.

Termos para indexação: *Carica papaya*, tecnologia de sementes, NaOCl.

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Introduction

Papaya occupies a prominent place among the most important tropical fruits grown in Brazil and worldwide. This is due to the great possibilities of using the product, mainly due to its composition. However, the lack of knowledge about the species has a direct impact on its production, resulting in high cost of the seed and therefore high production costs, which affects the final product.

Studies have shown that the presence of exotesta, also called sarcotesta, negatively affects the germination of papaya seeds due to the presence of phenolic compounds (Tokuhisa et al., 2007; 2008; Melo and Seleguini, 2013). Commonly, the removal of sarcotesta has been performed manually, using seeds friction in sieves under running water (Santos et al., 2009; Melo and Seleguini, 2013), friction with coarse sand (Melo and Seleguini, 2013), or even scrubbing with plastic bristles brushes (Melo and Seleguini, 2013). Manual procedures often employed in large companies in the papaya industry, for lack of an alternative, besides being time consuming, are not feasible for a large number of seeds.

The use of sodium hypochlorite may be an alternative to manual removal of sarcotesta in papaya seeds. For coffee seeds, sodium hypochlorite was as efficient as manual removal of parchment to increase and accelerate the emergence of seedlings (Lima et al., 2012). In papaya, besides the withdrawal of sarcotesta, sodium hypochlorite can act in the sclerotesta or part of it, favoring the germination of freshly harvested seeds. Additionally, sodium hypochlorite has a beneficial effect on the disinfection of seeds (Picolotto et al., 2007; Brasil, 2009).

Due to lack of information on the use of sodium hypochlorite in papaya seeds, pretests were conducted and preliminary results to this study, using sodium hypochlorite, were efficient in the removal of sarcotesta in papaya seeds with high germination in freshly harvested seeds, being a potential product to replace the manual removal of sarcotesta.

Although there are several reports on the benefit of sodium hypochlorite solution on seeds of various species, it is important to highlight that knowledge about the ratio of sodium hypochlorite solution for a given amount of seeds, as well as active chlorine solution concentration and soaking time, are of utmost importance to characterize the methodology to be employed.

The aim of the present study was to evaluate the effect of different ratios of seeds and sodium hypochlorite solution with different concentrations of active chlorine to identify the best combination for an effective removal of sarcotesta in papaya seeds in order to contribute to the germination process.

Material and Methods

The study was conducted at the Seed Analysis Laboratory of the Plant Science Department at the Federal University of Viçosa (UFV) in Brazil.

Papaya seeds of the "Solo" group and Golden variety were employed. The fruits were harvested at maturity stage one, up to 15% yellow skin coloration, and stored for three days, at which time all bark presented the yellow color. The fruits were longitudinally cut and seeds were manually extracted with a spoon, and the seeds of all fruits were homogenized and selected for integrity, size and color. Then the water content of the seeds was evaluated and the treatments started.

The determination of water content was performed using four replicates of 100 seeds by the greenhouse method at 105 ± 3 °C for 24 hours (Brasil, 2009) and the results were expressed as a percentage.

The seeds were immersed in sodium hypochlorite (NaOCl) solution at concentrations of 0, 2, 4, 6 and 8% of active chlorine for 24 hours in the ratios of 10:200; 50:200; 100:200; 200:200; 300:200; 400:200; 500:200 and 600:200, which is equivalent to 20; 4; 2; 1; 0.7; 0.5; 0.4 and 0.3 mL of solution per seed.

The active chlorine concentrations were obtained by dilution of commercial sodium hypochlorite in distilled water. Immersion of seeds was conducted in clear plastic boxes for germination under screens (to ensure full contact of the seed with the liquid), using different ratios. The boxes were maintained in a room at constant temperature of 18 °C. At baseline and at four-hour intervals the solution temperature was measured in different ratios and also the 0:200 ratio, i.e., in the absence of seeds for reference purposes of their effect on the changes in the solution temperature. To obtain the temperatures, an Incoterm® thermometer was used, with a scale of 10 – 60 °C and a 1 °C division. Subsequently, the seeds were washed in running water for 60 seconds to eliminate the sodium hypochlorite residue in the seeds.

After the treatments, the seeds physiological quality was evaluated by germination test and germination first count following the recommendations by the Rules for Seed Testing (Brasil, 2009), conducted in a B.O.D. (Biochemical Oxygen Demand) type growth chamber at alternating temperatures of 20 and 30 °C, being 16 hours in the dark and eight hours of light exposure, respectively. Paper rolls moistened with a water volume equivalent to 2.5 times the mass of the dry paper were used. The rolls were kept inside plastic bags in order to maintain moisture. The percentages of normal seedlings were calculated at 15 and 30 days after testing. Seedlings that reached total length of 2.5 cm and showed all essential structures complete and intact were considered normal.

The experiment was a completely randomized design, with each treatment consisting of four replications, where each replication consisted of the average of two determinations, i.e., two subsamples. For the germination study the treatments were arranged in a 5 x 8 factorial arrangement, being five active chlorine concentrations (0, 2, 4, 6 and 8%) and eight different proportions, considering the number of seeds per volume (mL) of solution (10:200, 50:200, 100:200, 200:200, 300:200, 400:200, 500:200 and 600:200).

The data were subjected to tests of normality and homogeneity of variance, and then analysis of variance. The F-test was used at 5% probability. For the quantitative variables, which had a significant effect by the F-test, regression analysis was performed. All analyzes were performed using the R computer program.

Results and Discussion

The initial moisture content of the seeds, determined soon after its extraction, was 78.1%. The temperature was obtained directly with the sodium hypochlorite solution, considering the different proportions (number of seeds per 200 mL of solution), concentration (active chlorine, %) and soaking time (hours). The temperature varied from 20 to 22 °C.

The solution temperature tends to increase with increased proportion (the number of seeds is greater for the same volume of solution), which may be explained by the acceleration in the oxidation process, increasing the solution temperature. Furthermore, the solution temperature tends to increase with higher concentrations of active chlorine. High concentrations of chlorine contribute to accelerate the oxidation process, resulting in an increased solution temperature (Donini et al., 2005).

The solution temperature tends to be higher at the beginning of the immersion time, and as time passes the reaction occurs, showing a chlorine consumption, resulting in the drop in temperature, and reaching a variation of 2 °C. It is also possible that, over time, there has been a reduction in active chlorine content due to natural degradation by exposure to light, contact with organic material and temperature, among other things, slowing down the reactions which triggered an increase in the solution temperature. The temperature rise could be a factor that affects the germination of seeds. However, there was virtually no temperature variation, and the highest temperature recorded in this study has not exceeded 22 °C. Therefore it has not affected seed germination. According to Carlesso et al. (2009), papaya seeds with initial moisture content of 53% had their vigor hindered when submitted to the temperature of 58 °C, but the germination was not affected.

Equation 1 represents the model selected to evaluate the effect of factors such as proportion (number of seeds per 200 mL of solution) and concentration (active chlorine, %) on germination, from the regression analysis. The model was chosen taking into account the coefficient of determination (R^2) and the significance of the regression coefficients.

$$G = 28.75 + 5.15C_{Cl} - 1.11 \cdot 10^{-2} P_o - 0.95C_{Cl}^2 \quad R^2 = 0.36 \qquad (1)$$

Where:

G = germination, %;

C_{cl} = active chlorine concentration, %;

P_0 = ratio, number of seeds per 200 mL of solution.

Figure 1 presents the germination values due to proportion and concentration factors. It was found that maximum germination tends to occur at a ratio of 10:200 (20 mL of solution per seed) at a concentration of about 2.7% of active chlorine.

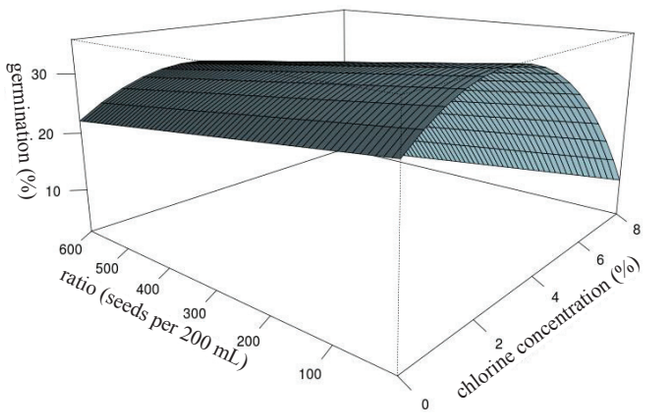

Figure 1. Response surface for germination at 30 days of papaya seeds due to the proportion and concentration of active chlorine.

Figure 2 shows cuts on the response surface for better viewing the influence of proportion and concentration factors on germination. From the analysis of Figure 2 it was observed that germination tends to increase with lower aspect ratio, i.e., greater volume of solution per seed. Furthermore, it tends to be higher at lower concentrations.

Although, according to the model, germination has not exceeded 40%, it is worth noting that in proportions of 10:200 and 50:200, which correspond to 20 and 4 mL of solution per seed, for the concentration of 2% of active chlorine average germination was of 80 and 76%, respectively. The results obtained in these treatments can be considered satisfactory for freshly harvested papaya seeds.

A larger proportion tends to accelerate the oxidation reaction, so that this one is more intense when compared with minor proportions; this also occurs with concentration. However,

it is possible that, over time, there has been a reduction in active chlorine content due to natural degradation by contact with large amounts of organic material, causing that more active chlorine proportions were not enough to sarcotesta withdrawal and not favoring germination. However, in smaller proportions, with active chlorine concentrations of 2 to 4%, germination tends to be greater (Figure 2), probably due to an amount sufficient to remove sarcotesta in all the seeds without compromising other seed structures and thus favoring germination. In the same proportions, concentrations of 6 and 8% were probably harmful to the embryo, reducing germination.

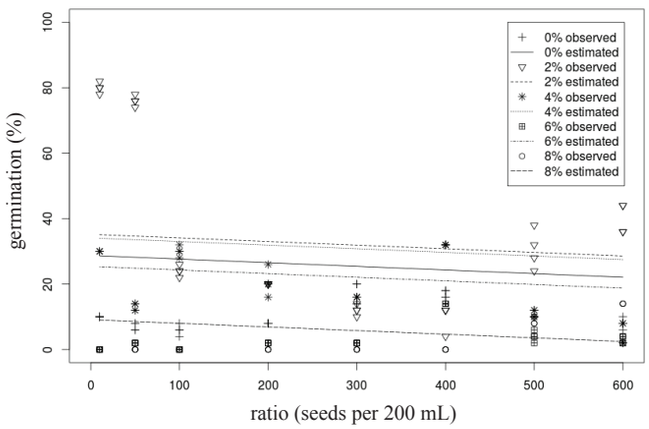

Figure 2. Cuts on the response surface for germination at 30 days of papaya seeds due to the proportion and concentration of chlorine.

As the oxidation reaction occurs, there is a consumption of chlorine and organic matter, which can lead to the elimination of sarcotesta. This elimination also occurs during another type of reaction: fermentation. According to Alves et al. (2009), fermentation is a process commonly used in seeds in order to eliminate mucilage in several species. However, a high oxidative process, due to the increased concentration of active chlorine for a period of 24 hours, may damage essential structures of the seed, thus jeopardizing germination.

In general, as the ratio increases, that is, the smaller the volume of solution per seed, the least sarcotesta is removed and the lower the germination. Chlorine concentrations of 2 to 4%, regardless of the proportion, probably have not affected the embryo, at least not drastically, since germination remained higher than for the control. On the other hand, in concentrations of 6 and 8%, regardless of the proportion, there was probably damage to the embryo, resulting in germination below the one for the control.

Equation 2 represents the model selected to evaluate the effect of factors such as proportion (number of seeds per 200 mL of solution) and concentration (active chlorine, %) on counting, from the regression analysis. The model was chosen taking into account the coefficient of determination (R^2) and the significance of the regression coefficients.

$$G = 29.36 + 1.25C_{Cl} - 3.66 \cdot 10^{-2} P_o - 3.12 \cdot 10^{-1} C^2_{Cl} \quad R^2 = 0.32 \quad (2)$$

Where:

G = germination, %;

C_{cl} = active chlorine concentration, %;

P_0 = ratio, number of seeds per 200 mL of solution.

Figure 3 shows the response surface for the first count due to proportion and concentration factors. It was found that maximum germination tends to occur at a ratio of 10:200 at a concentration of about 1.3% of active chlorine.

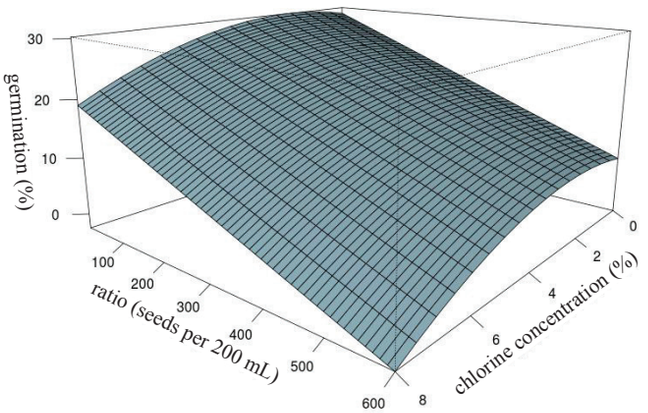

Figure 3. Response surface for the first count at 15 days of the papaya seed germination test due to the proportion and concentration of chlorine.

Figure 4 shows cuts on the response surface for better viewing the influence of proportion and concentration factors on germination for different levels of the factors. From the analysis of Figure 4, it was observed that germination in the first counting tends to increase with lower proportion. Furthermore, it is greater at lower concentrations, being similar to the behavior obtained in germination at 30 days.

According to the model, germination has not exceeded 40%. However, it is important to note that in the proportion of 10:200, which corresponds to 20 mL of solution per seed for the concentration of 2% of active chlorine, average germination was 60%. The result obtained in this treatment can be considered satisfactory for freshly harvested papaya seeds.

Although larger proportion and concentration can accelerate the oxidation reaction, as discussed earlier, with reduced chlorine content, due to natural degradation, this was not enough to remove sarcotesta, leading to low seed vigor by the first count test. While at lower ratios, even with

low concentration of active chlorine (2%), germination at first count test tends to be higher (Figure 4), probably due to an amount sufficient to remove sarcotesta in all seeds without compromising other seed structures, thus promoting germination at 15 days.

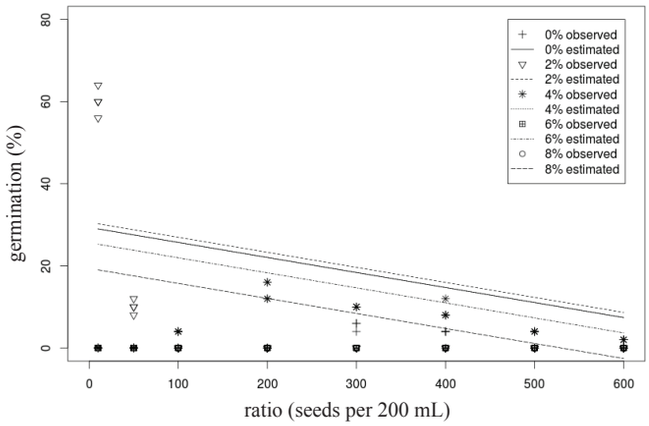

Figure 4. Cuts on the response surface for the first count at 15 days of the papaya seed germination test due to the proportion and concentration of chlorine.

It was observed that the use of active chlorine can be an alternative to manual removal of sarcotesta in freshly cut papaya seeds, because in addition to sarcotesta withdrawal it can favor the germination process. Under the conditions of this study, active chlorine was favorable in both the first count test at 15 days, as in the total germination test at 30 days after its installation, with germination of 60 and 80%, respectively.

Conclusions

The use of 20 mL of sodium hypochlorite solution per seed at a concentration of 2% of active chlorine for 24 hours is an alternative for the manual removal of sarcotesta in papaya seeds.

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