

Viability of *Carica papaya* L. Seeds by the Tetrazolium Test

Sara M. C. Carvalho¹, Salvador B. Torres¹, Erivanessa C. Sousa¹, Danielle M. M. Sousa², Kleane T. O. Pereira¹,
Emanoela P. de Paiva¹, Janete R. Matias¹ & Brenna R. V. dos Santos¹

¹ Department of Plant Production, Federal Rural University of Semi-Arid, Mossoró, Brazil

² Department of Environmental and Technological Sciences, Federal Rural University of Semi-Arid, Mossoró, Brazil

Correspondence: Emanoela P. de Paiva, Federal Rural University of Semi-Arid, Rio Grande do Norte, Brazil. Tel: 55-084-99806-0517. E-mail: emanuelappaiva@hotmail.com

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Abstract

Germination test for papaya seeds demands thirty days to obtain the results, which is a long period for decision-making. Thus, rapid tests become essential tools to evaluate seed viability, and the tetrazolium test is an alternative for this purpose. Hence, this study aimed to establish the adequate procedure to apply this test, in order to reduce the time of evaluation of papaya seeds. For that, four tetrazolium solution concentrations (0.05, 0.1, 0.5 and 1%) and three staining times (3, 6 and 9 hours) were tested at temperatures of 35 and 40 °C. The experimental design was completely randomized in 4 × 3 + 1 factorial scheme (concentrations × staining times + one control = germination test). It was found that the tetrazolium test allows to evaluate the physiological quality of papaya seeds, thus reducing the time for decision-making. For the test, we recommend using the concentrations of 0.1% for 9 hours or 1% for 6 hours of staining, at temperature of 40 °C.

Keywords: Caricaceae, tropical fruits, rapid tests

1. Introduction

Papaya (*Carica papaya* L.) occupies a prominent place among the tropical fruits cultivated in Brazil and worldwide, due to the wide possibilities of utilization of the product and especially due to its composition (Jesus et al., 2016). Papaya production in Brazil in 2014 was equal to 1.58 million tons, equivalent to 16.67% of the global production (FAO, 2017), and the Bahia state is the largest national producer, followed by Espírito Santo and Minas Gerais, with productions of 718.7, 404.7 and 126.8 thousand tons, respectively, representing together approximately 80% of the Brazilian production in 2014 (IBGE, 2014).

The most cultivated papaya varieties in Brazil belong to the groups 'Solo' and 'Formosa'. 'Solo' group has fruits with reddish pulp and small size, weighing from 300 to 650 g, whereas fruits of the 'Formosa' group exhibit reddish orange pulp, mean weight of 1,000 to 1,300 g and are mostly formed by commercial hybrids (Dias et al., 2011).

Papaya can be propagated through cuttings, grafting and seeds. This latter method has been the most used in the economic exploitation of the crop, because the other two propagation methods do not have any advantage from the perspective of either vigor and yield or maintenance of the desirable features (Andrade & Jasper, 2013).

Seed quality evaluation through rapid tests that generate reproducible results has been a constant search of seed technologists and can help the decision-making regarding the use or disposal of lots, especially for species that require long periods for germination (Marcos-Filho, 2015). According to the Rules for Seed Evaluation, papaya requires 30 days to obtain this information through the standard germination test (Brasil, 2009). For Cunha and Gomes (2015), the delay to obtain results through the germination test, in some species, can be a limitation, especially when there is a demand for decision-making in the seed industry.

The tetrazolium test is based on the staining of living tissues in the presence of the solution of 2,3,5-triphenyltetrazolium chloride, which is reduced by the activity of dehydrogenase enzymes involved in the mitochondrial respiration activity. During respiration, hydrogen ions are released and react with the salt, which is colorless and soluble, forming triphenylformazan, a stable, non-diffusible, red-colored compound (Fogaça et al., 2011; Lazarotto et al., 2011). The efficiency of this test to evaluate seed viability depends on the development

and use of a method adapted to each species, such as defining adequate conditions for pre-conditioning, preparation, staining and evaluation of the seeds (Pinto et al., 2009).

Studies on the tetrazolium test with cultivated species have already been conducted to propose adequate methodology for its use, such as França Neto, Krzyzanowski, and Costa (1998) with soybean (*Glycine max* L. Merr.); Lima et al. (2007) with melon (*Cucumis melo* L.); Nery et al. (2007) with watermelon (*Citrullus lanatus* Thunb. Matsum.); Cervi and Mendonça (2009) with cotton (*Gossypium herbaceum* L.); Gaspar-Oliveira et al. (2010) with castor bean (*Ricinus communis* L.); Silva et al. (2013) with sunflower (*Helianthus annuus* L.) and Rodrigues et al. (2015) with cowpea (*Vigna unguiculata* L. Walp.).

Given the importance of papaya as a fruit species and considering that the evaluation of its seeds' quality through germination test is time-consuming, this study aimed to establish an adequate methodology to apply the tetrazolium test, in order to reduce the time necessary to evaluate the viability of seeds of this species.

2. Material and Methods

Papaya seeds of the 'Havaí' variety, 'Solo' group, were removed from ten ripe fruits. The sarcotesta was removed by friction on a sieve and the seeds were dried immediately after (Cavalcante et al., 2014). Water content was determined through the method of the oven at 105 ± 3 °C, for 24 hours (Brasil, 2009), and the seeds were stored in a cold chamber (17 °C and 60% relative air humidity) until the beginning of the experiment.

Based on preliminary tests, the seeds were pre-moistened in paper towel for 2 h and longitudinally cut using blades, but without separating their parts, to facilitate evaluation. Subsequently, the seeds were placed in 50-mL plastic cups and mixed with the 2,3,5-triphenyltetrazolium chloride solution at four concentrations (0.05, 0.1, 0.5 and 1%) for three staining times (3, 6 and 9 hours) at temperatures of 35 °C and 40 °C, in a Biochemical Oxygen Demand (B.O.D.) chamber in the absence of light. Four subsamples of 25 seeds were used in each combination. After each staining time, the tetrazolium solution was drained, the seeds were washed in running water, mixed with water and maintained in the refrigerator until the moment of evaluation.

Seeds were evaluated using a desktop magnifying glass and classified as viable or unviable, according to the color standard indicated by França-Neto et al. (1998): light pink (healthy tissue); intense red (deteriorating tissue) and without color (dead tissue). Not only the color, but also site, intensity and extension were considered. Results were expressed in percentage of viable seeds.

To obtain a standard for result comparison, a germination test was conducted simultaneously to the tetrazolium test, using four subsamples of 50 seeds arranged on paper towel moistened with distilled water at proportion of 2.5 times the weight of the dry paper at alternated temperature of 20-35 °C in a B.O.D. chamber. Seeds were evaluated 30 days after sowing and the results were expressed in percentage of normal seedlings (Brasil, 2009).

The experimental design was completely randomized in $4 \times 3 + 1$ factorial scheme (four solution concentrations \times three staining times + one control = germination test) at two temperatures (35 °C and 40 °C). The means of viable seeds obtained in the tetrazolium test were compared by Tukey test ($p \leq 0.05$), whereas the means of viable seeds obtained in the germination test (control) were compared by Dunnett test ($p \leq 0.05$), following the recommendations of Banzatto and Kronka (2006). Statistical analyses were performed using the program ASSISTAT 7.7, Beta version (Silva & Azevedo, 2002).

3. Results and Discussion

After drying, the seeds showed water content of 8.9% and the germination test resulted in 69% of normal seedlings.

The percentages of viable seeds obtained in the tetrazolium test in the different treatments (concentration \times exposure time) and germination test, at temperature of 35 and 40 °C are presented in Tables 1 and 2, respectively.

For the temperature of 35 °C (Table 1), the highest means of viability were found when seeds were exposed to the highest solution concentrations (0.5% and 1%). At the lowest concentrations (0.05% and 0.1%), viability was null, regardless of the seed exposure time. Although all treatments differed from the control at this temperature, the combination of highest concentration (1%) and longest time (9 hours) resulted in high percentage of viable seeds (84%).

Table 1. Means of viability of *Carica papaya* L. seeds by the tetrazolium test conducted at different concentrations and staining times, at temperature of 35 °C.

Periods (h)	Concentrations (%)			
	0.05*	0.1	0.5	1
3	0.0 aAy	0.0 aAy	2.0 cAy	1.0 cAy
6	0.0 aCy	0.0 aCy	42.0 aAy	30.0 bBy
9	0.0 aCy	0.0 aCy	32.0 bBy	86.0 aAy

Germination (%) 69 z				

Note. * Means followed by the same letter, uppercase in the row (A, B, C) and lowercase in the column (a, b, c), do not differ significantly by Tukey test at 0.05 probability level. Means followed by the same letter (z, y), between germination (control - germination test) and viability obtained in the tetrazolium test, do not differ significantly by Dunnett test at 0.05 probability level.

At temperature of 40 °C (Table 2), among the tested concentrations only the lowest one (0.05%) did not promote efficiency in any of the tested times of exposure. At 0.1 and 0.5% concentrations, the means of viability were statistically equal to those of the germination test when the seeds were exposed to 9 hours of staining. The 1% concentration, except for the 3-h time, was favorable to seed evaluation. In the 9-h staining time, it was possible to observe the highest means of viability, which did not differ from the control, except at the lowest concentration (0.05%). As occurred for the temperature of 35 °C, the 3-h time was not favorable to seed quality evaluation at any of the concentrations. However, the increase in the exposure time to 6 hours promoted favorable evaluation at the highest concentration (1%).

Table 2. Means of viability of *Carica papaya* L. seeds by the tetrazolium test conducted at different concentrations and staining times, at temperature of 40 °C.

Periods (h)	Concentrations (%)			
	0.05*	0.1	0.5	1
3	0.0 aBy	0.0 cBy	22.0 bAy	11.0 bABy
6	0.0 aBy	18.0 bBy	17.0 bBy	62.0 aAz
9	0.0 aCy	63.0 aBz	82.0 aAz	71.0 aABz

Germination (%) 69 z				

Note. * Means followed by the same letter, uppercase in the row (A, B, C) and lowercase in the column (a, b, c), do not differ significantly by Tukey test at 0.05 probability level. Means followed by the same letter (z, y), between germination (control - germination test) and viability obtained in the tetrazolium test, do not differ significantly by Dunnett test at 0.05 probability level.

Studies conducted with the tetrazolium test demonstrate highly variable results according to the studied species. While some are sensitive to low concentrations of the salt, papaya seeds in the present study required higher concentrations to develop a satisfactory color. The same could be observed for seeds of peanut (*Arachis hypogaea* L.) (Carvalho et al., 2009); black oat (*Avena strigosa* Sckreb.) and white oat (*Avena sativa* L.) (Souza et al., 2009, 2010) and barley (*Hordeum vulgare* L.) (Grzybowski et al., 2012). However, the concentration is also related to the time of seed exposure to the solution and they usually exhibit an inversely proportional relationship. This was clearly evident at temperature of 40 °C (Table 2), for which the 3-h time was inefficient at all concentrations, whereas the combinations of 6 hours at 1% and 9 hours at 0.1, 0.5 and 1% were statistically equal to the results of the germination test.

The temperature of 40 °C led to results statistically equal to those of the germination test, even at lower concentrations (0.1 and 0.5%), which did not occur for the temperature of 35 °C. This fact can be explained by the increase in the temperature of staining, corroborating AOSA (2009), which claims that seed color is more rapidly established at higher temperatures.

No studies were found on tetrazolium test for species of the Caricaceae family; thus, it was not possible to compare the behavior regarding the concentration, exposure time and color patterns of the seeds. Figure 1 shows some color patterns observed during the evaluation. In viable seeds, the tissues were light pink or bright red,

indicating living, firm and vigorous tissue. On the other hand, unviable seeds assumed a milky white color (partial or total), suggesting dead tissue, or intense red color in its entire extension, indicating an accelerated process of tissue deterioration.



Figure 1. *Carica papaya* L. seeds considered as viable by the tetrazolium test: seeds with light pink color (A); bright red color (B) and unviable: seeds completely uncolored (C); with discolored regions (D) and intense red color in the entire extension (E)

The temperature of 35 °C did not favor the evaluation of papaya seeds, because color development was difficult and many seeds did not stain, or such color was weak and not uniform. At this temperature, the values of viability were not consistent with that found for germination. In this case, what occurred was that many viable seeds were classified as unviable due to a failure in the staining process.

Although at temperature of 40 °C some treatments also made the evaluation difficult for not favoring an adequate color, the interpretation of the results was visually easier than at 35 °C, because the seeds showed stronger and more uniform color. Some studies with cultivated species have also demonstrated positive response for the staining at 40 °C, such as those using seeds of zucchini (*Curcubita pepo* L.) (Barros et al., 2005); cotton (*Gossypium hirsutum*) (Cervi et al., 2009); peanut (*Arachis hypogaea*) (Carvalho et al., 2009); jatropha (*Jatropha curcas* L.) (Pinto et al., 2009); black oat (*Avena strigosa*) and white oat (*Avena sativa*) (Souza et al., 2009, 2010); cucumber (*Cucumis sativus* L.) (Lima et al. 2010); wheat (*Triticum aestivum* L.) (Carvalho et al., 2013) and sorghum (*Sorghum bicolor* L. Moench.) (Carvalho et al., 2014).

Flores et al. (2015), working with rapeseed (*Brassica napus* L.) seeds, using concentrations of 0.5 and 1.0% at 30 °C/0.2, 0.5 and 1.0% at 40 °C, for 2, 4 and 6 h, found better results in the test at 0.5% concentration for 6 h at 40 °C, demonstrating a behavior very similar to that found in the present study, regarding the response of the seeds under high concentration, long exposure time and high temperature.

Although the data from the present study demonstrate the possibility of seed viability evaluation through four different treatments at 40 °C (Table 2), one should opt for the combinations of 1% for 6 h, which provides an evaluation in shorter time, or 0.1% for 9 h, which allows to use a lower quantity of reagent.

4. Conclusions

The time for viability evaluation of papaya seeds can be reduced by the tetrazolium test, which should be conducted at concentrations of 0.1% for 9 h or 1% for 6 h of staining, at 40 °C.

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