Physical and physiological indicators of the quality of soursop seeds (*Annona muricata* L.)

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ABSTRACT

Objective: The present study aimed to carry out the analysis of the physical and physiological quality of soursop seeds, since there is very little information on the subject.

Design/Methodology/Approach: The material was collected at physiological maturity. The seeds were extracted from fruits in commercial maturity. They were subjected to a physical and physiological quality analysis: physical purity, humidity content of the seed, weight of 1000 seeds, integrity test of the seed with the X-ray equipment, evaluation of germination and the evaluation of viability by the tetrazolium method. A completely randomized experimental design was used in all the physical quality variables and tetrazolium tests. Other hand, a completely randomized factorial design (3×7) was used in the germination evaluation.

Results: The viability results obtained by the tetrazolium method showed over 59% viable seeds, while in the germination test with the germinative pretreatments only 11.33% germination was obtained in the seeds from which the cover was removed.

Findings/ Conclusions: Therefore, it was concluded that the moment of obtaining the plant material is important for its germination.

Keywords: fruit trees, germination, viability, humidity content.

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INTRODUCTION

In Mexico, the soursop (*Annona muricata* L.), is considered the most important species of the Anonaceae family, due to its commercial value (Reyes *et al.*, 2018); since this is one of the fruits with the greatest commercial acceptance in the world due to its creamy pulp, sweetness and excellent flavor (Márquez *et al.*, 2013). These fruits are harvested at physiological maturity, but if they are harvested before this maturity, they do not ripen well and the pulp can acquire a bitter taste (Jiménez *et al.*, 2017).

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Moreover, the collection of good quality seed allows its use for its conservation (Elizalde *et al.*, 2017). Two types of tests can be found, those that measure the proportion of viable seeds (germination tests) and those that evaluate the state of viability, among them are the viability test by the tetrazolium method, which allows classifying the seeds as viable or not viable (Masetto *et al.*, 2007). This test is based on the activity of respiration dehydrogenases, which chemically reduce the colorless tetrazolium solution to formazan (Elizalde *et al.*, 2017), giving a red color to living tissue, pink tones to partially damaged tissue, and white to tissue not viable (ISTA, 2016).

The present work was proposed because there is little information related to this species. Therefore, the objective is to obtain information on the analysis of the physical and physiological quality of soursop seeds.

MATERIALS AND METHODS

This study was conducted at the National Genetic Resources Center, National Institute of Forestry, Agriculture and Livestock Research (CNRG-INIFAP) located in Tepatitlan de Morelos, Jalisco. Four samples were used, which were kept stored in a cool and dry place at room temperature.

Variables evaluated in the physical quality seeds analysis

Physical purity. The total sample was weighed and, later, the components of pure seed, inert material and other seeds were separated. Once each of the components were separated was weighed, expressing their value as a percentage (ISTA, 2016).

Weight of 1000 seeds. Eight replicates of 100 seeds were randomly taken; each was weighed on an analytical balance. Then the average of the eight replicates was obtained, and the variance, standard deviation, and coefficient of variation were also calculated with these values. If the coefficient of variation did not exceed 4% (for not chaffy seeds), the result was taken as valid. The result of the determination was obtained by multiplying by 10 the average weight obtained from the eight repetitions of 100 seeds (ISTA, 2016).

Humidity content. This was done using an electronic humidity meter (termobalance method). One gram of seed was weighed was crushed. Subsequently, the ground seed was placed in a thermobalance (AND-MS-70), three repetitions per sample were performed (Rao *et al.*, 2007).

X-rays. Four repetitions of 25 seeds were counted, which were used to be radiographed with X-ray equipment (Faxitron X-Ray MX-20®), with this test the physical integrity of the seed was evaluated, which the percentage of seeds full, empty, damaged by insects and mechanically damaged were evaluated.

Variables evaluated in the physiological quality seeds analysis

Viability. This analysis was carried out by the tetrazolium method (ISTA, 2016). The seeds were removed from the cover and soaked in distilled water for 24 h, after which a ventral longitudinal cut was made. For this, 20 seeds were used, with four repetitions. The seeds were incubated in a 1.0% tetrazolium solution for 24 h, at 30 °C, in the dark, and the staining obtained in the embryo was later evaluated.

The seed disinfection protocol consisted of scarifying the seeds, then they were left to soak for 24 h in distilled water, washed three times with soap (Axion Tricloro®) for ten minutes each. They were placed in a Captan® solution (3 $g \cdot L^{-1}$) for one hour and the excess was removed. Inside the laminar flow hood, they were subjected to a 70% ethanol solution for two minutes and later to a 1% commercial chlorine solution for ten minutes, the relevant rinses were performed to remove the excess. Subsequently, they were placed in a solution of citric and ascorbic acid at a concentration of 1 $mg \cdot L^{-1}$ each and were incubated for one hour. This was done in order to know if this protocol affected the viability of the seed.

Germination. Seeds were placed in transparent acrylic germinating boxes. A mixture of agrolite substrate and Canadian peat (peat most®) (2:1) was used. The seeds underwent different pre-germination treatments (Table 1).

15 seeds per box were placed and four repetitions per treatment were evaluated, this was done for sample 2, 3 and 4. The germination boxes were placed in a germination room at a temperature of 25±3 °C, with a photoperiod of 16 h light and 8 h dark. After sowing, they were irrigated with Captan® (3 $g \cdot L^{-1}$), to avoid the appearance of fungus. The germination percentage was evaluated in a period of 30 days.

A completely randomized experimental design was used for the physical quality variables and for the seed viability test; in the case of germination, a completely randomized factorial experimental design (3×7) was carried out, an analysis of variance was carried out and the comparisons of means (Tukey, $\alpha=0.05$) were carried out using the statistical software SAS version 9.3. The values expressed in percentage were transformed with the arcsine function $\left(\sqrt{X/100}\right)$.

RESULTS AND DISCUSSION

There are very few studies related to this subject, so the results were compared with other works that were carried out on similar seeds.

Variables evaluated in the physical quality seeds analysis

The characteristics of the seed samples showed a variability. Table 2 shows the analysis of the physical purity of the seeds, where an average initial weight of 612.685 g was obtained, with an average of 1,379 seeds and 95% pure seed.

Table 1. Pre-germination treatments applied to soursop seeds.

Number	Treatment
1	Complete seed
2	Complete seed with imbibition in water for 24 h
3	Seed without cover
4	Seed without cover with imbibition in water for 24 h
5	Seed with a small cut
6	Sulfuric acid application for five minutes
7	Application of gibberellins 100 ppm for 24 h

Table 2. Analysis of physical purity of soursop seeds.

Sample	Starting weight (g)	Inert matter weight (g)	Purity (%)	Number of total seeds
1	973.21	12.73	98.69	1,888
2	511.82	35.23	93.11	1,230
3	485.95	35.23	92.74	1,198
4	479.77	6.91	98.55	1,195
Average	612.685	22.53	95.77	1,379.25

Table 3 shows the weight of 1000 seeds, where sample 1 presented the highest weight with 541.42 g and samples 2 and 3 presented the lowest weight. Viveros *et al.*, (2015), in *Enterolobium cyclocarpum* (Parota) seeds found a weight of 1000 seeds of 836.4 g, a result higher than that obtained by Meza and Bautista (2007), in soursop seeds that was 336 g, data similar to that obtained in the present investigation in samples 2 and 3. Regarding the humidity content, sample 4 presented the highest value (36.46%) but samples 2 and 3 presented the lowest humidity content. Authors such as Viveros *et al.* (2015), in parota seeds found an average humidity content of 9.7% in whole seed and 5.3% in crushed seed, data below those obtained in the present work in soursop seeds, since in crushed seed it was obtained a humidity content greater than 27.8%, in a bibliographic review carried out by Magnitskiy and Plaza (2007), reported that the humidity content at the time of dissemination of recalcitrant seeds of tropical trees varies between 23% in *Pourouma cecropiifolia* Mart., 25% in *Bertholletia excelsa* Humb. Bonpl, 46-51% in *Euterpe espiritosantensis* Fernand palm, and 47-53% in *Eugenia dysenterica* D.C.

Regarding the X-ray analysis, samples 2 and 3 had 100% and 99% full seeds respectively, the damage caused by insects is minimal since the percentages obtained for this cause are less than 20% in samples 1 and 4. Viveros *et al.*, (2015), found in seeds of parota that 98% showed a developed embryo and 93% of the seeds with cotyledons in this condition.

Variables evaluated in the physiological quality seeds analysis

The results of the disinfection protocols did not show statistical differences. As observed in Figure 1, the seeds without disinfection protocol in sample 1 showed 86% viable seeds and in samples 2 and 3 they presented 61% viability. On the other hand, to the seeds that the disinfection protocol was applied to be introduced into *in vitro* culture, sample

Table 3. Comparison of Tukey means of the physical quality analyzes of four soursop seed samples.

Sample	Thousand seed weight (g)	Humidity content (%)	X-Rays analysis	
			Filled seeds (%)	Insect damaged seeds (%)
1	541.41 a	32.84 ab	81.00 b	19.00 a
2	386.30 c	28.34 b	100.00 a	0.00 b
3	383.74 c	27.82 b	99.00 a	1.00 b
4	410.72 b	36.46 a	82.00 b	18.00 a

Values with the same letter are statistically similar (Tukey, α=0.05).

1 exhibited 81% viable seeds and samples 2 and 3 presented 59 and 60% viable seeds, respectively. Lobo *et al.* (2007), found a percentage of viable soursop seeds of 69% and custard apple seeds of 79.5%. In the case of parota seeds, they found an average of 75.5% viability (Viveros *et al.*, 2015). In this investigation it was found that the viability percentage was higher than 58.75% and 61.2%, with disinfection protocol and without disinfection respectively, which means that they maintain a good percentage of viability, similar to that reported by Lobo *et al.* (2007).

Pre-germination treatments were carried out; however, germination did not increase.

As observed in Table 4, the percentages for all treatments were low. However, the cover removal treatment registered the highest percentage of germination with 11.33%, followed by the complete seed treatment with 7.50%. The seeds of tropical trees have inherent dormancy, which results in delayed and non-uniform germination (Joseph, 2014), such as soursop, which is why heterogeneous germination can be observed.

Joseph (2014) found that the earliest germination in soursop seeds soaked for 72 h in water was 22 days after sowing, with a percentage of 13%, and 25 days after sowing in seeds soaked in cold water for 96 h had 40% germination. Seeds were extracted from ripe fruits, which were pulped and washed, air-dried for 48 hours, and stored for 38 days. While Meza and Bautista (2004) found that soaking in water for 24 h and the control (without soaking)

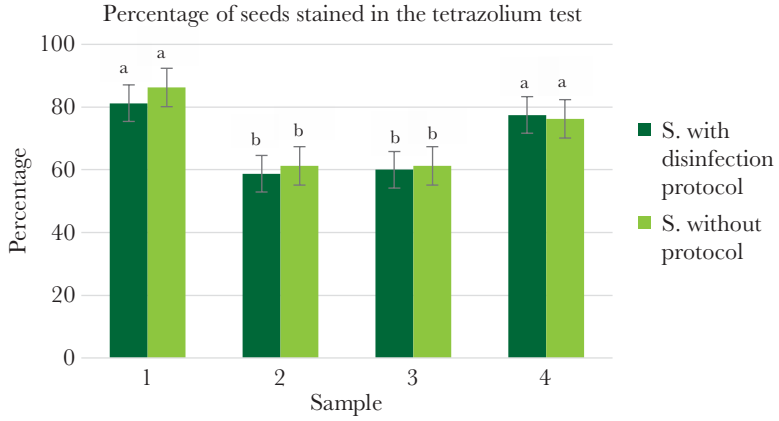

Figure 1. Seeds stained in the tetrazolium test. Values with the same letter are statistically similar (Tukey, α=0.05)

Table 4. Comparison of Tukey means of pre-germination treatments in soursop seeds.

	Treatment	Germination (%)
1	Complete seed	7.50 ab
2	Complete seed with imbibition in water for 24 h	5.53 ab
3	Seed without cover	11.33 a
4	Seed without with imbibition in water for 24 h	0.00 b
5	Seed with a small cut	2.50 b
6	Sulfuric acid application for five minutes	5.24 ab
7	Application of gibberellins 100 ppm for 24 h	3.03 b

Values with the same letter are statistically similar (Tukey, α=0.05).

simultaneously started germination at 17.66 days, but the treatment with scarification in sulfuric acid for 2 min was at 19.33 days.

Therefore, it is possible that, although a part of germination is determined by the conditions required for the embryo to emerge, it could be said that there is an interaction between the growth potential of the embryo and the restrictions imposed by the tissue that surrounds it (Lobo *et al.*, 2007). In the same way, it is important to mention that Meza and Bautista (2004) and Lobo *et al.* (2007), used seeds of fully ripe fruits and soft to the touch, for which they obtained a germination percentage higher than that was obtained in the present investigation since despite the fact that the fruits were physiologically mature, they did not feel soft.

CONCLUSIONS

The viability percentage of soursop seeds can be maintained above 60% in seeds stored at room temperature for three weeks after extraction. This viability percentage is not affected by the application of the seed disinfection protocol to be introduced to *in vitro* culture. And that the moment of harvesting the fruits for the extraction of the seed is vital for the seeds to germinate.

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