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Environment and storage period for conservation of surinam cherry seeds (*Eugenia uniflora L.*)

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Abstract

The Surinam cherry tree is a native Brazilian fruit tree with potential for use in several segments. Normally, it is propagated by seeds, but its biggest obstacle is the fact that it is recalcitrant, making its germination capacity unviable if stored in an unsuitable environment. The objective of this work was to evaluate the viability of Surinam cherry seeds stored under natural environmental conditions and freezing and cooling temperatures, in three different packages (ziplock, kraft paper and vacuum) during nine periods (0, 5, 10, 15, 20, 25, 30, 60 and 90 days). The study was carried out at UTFPR - Campus Dois Vizinhos. The experimental design used was completely randomized, adopting analysis for each type of packaging in a 3 x 9 factorial scheme (storage temperature x period), with four replications of 50 seeds per experimental unit. The germination, germination speed index and average germination time were analyzed. According to the results, the Surinam cherry seeds lost their viability in Kraft paper, vacuum and ziplock packaging from the thirtieth day onwards when kept in natural and cooling conditions. The seeds did not tolerate the use of freezing temperatures, regardless of the maintenance period. Under natural conditions, using Kraft paper packaging, germination remained above 40% up to five days, different from other environments, in which vacuum and ziplock remained up to 15 days in natural conditions and, from 25 to 30 days under cooling, respectively.

Keywords: germination, Myrtaceae, native fruit, propagation

Introduction

The Brazilian flora is rich in plant, animal and microorganism species, which constitute the diversity of Brazilian biomes. Among the plant species, there are those that produce fruits, playing a fundamental role in feeding the fauna and when used for human consumption, they bring numerous health benefits (antidiarrheal, antipyretic, diuretic and hypotensive)(Auricchio et al., 2003).

In this same context, among the native fruit trees, the Surinam cherry tree stands out for being widely known, since it is found from north to south of the country and for presenting suitability in the use of its fruits for the food, pharmaceutical and cosmetics industries, its plant for ornamentation of residences or internal and external public spaces or for insertion in areas of permanent preservation or agroforestry (Oliveira, 2022).

To take full advantage of its potential, it is important to first obtain quality seedlings. One of the biggest obstacles to propagating Surinam cherry trees using seeds is that they are used quickly after being extracted from the fruit, since they are recalcitrant and do not tolerate desiccation, losing their germination capacity.

Due to this recalcitrant behavior, it is necessary to adopt seed conservation techniques, aiming to produce seedlings throughout the year, not only during production periods.

Among the techniques adopted for seed conservation, there are those that aim to reduce their metabolic activity, keeping them in low temperature conditions, in some cases negative, which can be an alternative for testing recalcitrant seeds, since it allows them to be kept with a high moisture content without germination occurring during storage.

However, when storing *Campomanesia xanthocarpa* seeds at low temperatures, Melchior et al.

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(2006) observed a loss of germination capacity, which could not withstand storage under these conditions or desiccation, a fact that may be linked to the conditions in which the seeds were stored, making it necessary to adopt packaging. Studies showed that storing seeds in ziplock-type polyethylene packaging was effective in preserving their viability (Borba and Perez, 2009; Antunes et al., 2012; Medeiros, 2000; Carlesso et al., 2008). The same effect was observed with the use of vacuum packaging (Danner et al., 2011).

Therefore, the need arose to carry out the present study, seeking to elucidate whether there is an influence of packaging in preserving the viability of Surinam cherry seeds under natural temperature, cooling and freezing conditions for up to 90 days of storage.

Material and Methods

The experiment was carried out at the Plant Physiology Laboratory of the Federal Technological University of Paraná - Dois Vizinhos Campus, located in the municipality of Dois Vizinhos, southwest of Paraná. Seeds from ripe fruits extracted from 10 mother plants belonging to the collection of the institution. The altitude of the site is 520 m, with latitude of 25º, 42', 52'' S and longitude of 53º, 03', 94'' W. According to the international Köppen classification, the local climate is Cfa (subtropical, humid). Annual precipitation varies from 2000 to 2500 mm (IAPAR, 2024). The soil is classified as Dystroferric Red Nitosol with clayey texture, characterizing deep, porous and well permeable soils (Bhering et al., 2008).

After the fruits were collected, they were manually pulped using a fine-mesh sieve, resulting in the seeds, which were then washed five times in running water to remove any remaining pulp residue. After extraction, the seeds were divided into batches and placed in different types of packaging (zip lock plastic bags, Kraft paper bags and vacuum-sealed plastic packaging using a Sulpack svb 500 vacuum sealer).

After placing the seeds in their respective packaging, they were stored under different temperature conditions [freezing (-18ºC), cooling (5ºC) and natural (25ºC±5ºC)]. The seeds were stored for 0, 5, 10, 15, 20, 25, 30, 60 and 90 days.

The experimental design used was completely randomized, adopting a 3 x 9 factorial scheme (storage temperature x storage period) for each type of packaging, with four replicates of 50 seeds per experimental unit.

After each storage period, the seeds were placed on Germtest® paper moistened with twice the weight of the paper, inside a gerbox-type box (sanitized with 70% alcohol) with a lid and kept in a germination chamber at a temperature of 25ºC, with no photoperiod (CREMASCO et al., 2017).

After 40 days, germination (%), germination speed index (GSI) (Maguire, 1962) and average germination time (days) (AGT) (Labouriau, 1983) were evaluated.

The data obtained were submitted to the Lilliefors normality test, and the germination data were transformed using arcsine square root of x/100, and the IVG and TMG data were transformed using square root of x+1. The data were then submitted to analysis of variance (ANOVA), followed by Duncan's comparative mean test (α = 0.05) for the qualitative factor and interaction of factors.

Results and Discuss

According to the results analyzed when Kraft paper packaging was used, there was a significant effect of the storage temperature x period interaction for germination, GSI and AGT (**Table 1**).

It was observed that the seeds kept in Kraft paper had the highest germination in a natural environment among the periods of zero, five and ten days, of cooling of zero and five days and of freezing on day zero. However,

Table 1. Germination (%), Germination Speed Index (GSI) and Average Germination Time (AGT) of Surinam cherry (*Eugenia uniflora*) seeds stored in Kraft paper bags kept in three temperature conditions (natural, refrigerator and freezer) for 90 days

Storage period (days)	Natural			Refrigerator			Freezer		
	Germination (%)	GSI	AGT	Germination (%)	GSI	AGT	Germination (%)	GSI	AGT
0	47.00 aA *	6.34 aB	6.34 a	47.00 aA	6.34 aB	6.34 aB	47.00 aA	6.34 aA	6.34 aA
5	41.5 aA	13.5 aA	13.49 aA	40.74 aA	10.8 aAB	10.8 aAB	0.0 bB	0.0 bB	0.0 bB
10	35.33 aA	15.7 aA	15.69 aA	30.70 aB	9.2 bAB	9.16 bAB	0.0 bB	0.0 bB	0.0 cB
15	7.31 bB	4.69 bB	4.69 bB	22.65 aB	12.9 aA	12.88 aA	0.0 cB	0.0 cB	0.0 cB
20	7.98 bB	7.38 aB	7.38 aB	12.70 aC	9.8 aAB	9.77 aAB	0.0 cB	0.0 bB	0.0 bB
25	4.99 aB	4.68 aB	4.68 aB	2.36 bD	2.4 bC	2.29 bC	0.0 cB	0.0 cB	0.0 cB
30	0.0 aC	0.0 aC	0.0 aC	0.0 aE	0.0 aD	0.0 aD	0.0 aB	0.0 aB	0.0 aB
60	0.0 aC	0.0 aC	0.0 aC	0.0 aE	0.0 aD	0.0 aD	0.0 aB	0.0 aB	0.0 aB
90	0.0 aC	0.0 aC	0.0 aC	0.0 aE	0.0 aD	0.0 aD	0.0 aB	0.0 aB	0.0 aB
VC (%)				20.91	17.82	9.49			

*Means followed by distinct uppercase letters in the same column and lowercase letters in the same row differ from each other by Duncan's test (α = 0.05) it is important to emphasize that the Surinam cherry seeds germinated up to 25 days in the natural and cooling conditions, with a variation in their averages occurring at 10 and 25 days in the natural environment and for 15 and 20 days with cooling. These results were maintained with the GSI (Table 1).

In the freezing temperature condition, there was a total loss of germination capacity from storage in the freezer after five days. This occurred after 30 days for the natural or cooling conditions (Table 1).

Possibly, the loss of germination viability of the Surinam cherry seeds when exposed to freezing temperature is linked to the formation of ice crystals between the intercellular spaces or in the protoplast region, consequently affecting the functionality of the cell wall or the plasma membrane, respectively.

When the plant is exposed to a slow freezing environment, as in the freezer used, the intracellular water crystallizes, forming ice crystals inside the cells. This process leads to perforation of the protoplasm, making germination unfeasible. Similar results were obtained by Scalon et al. (2012), in *Eugenia pyriformis* L. when stored in a freezer. Regarding GSI, in natural conditions, the highest averages occurred when the seeds were kept for five and 10 days; when cooled for five, 10, 15 and 20 days; and when frozen only on day zero (Table 1).

The results obtained with the cooling of the seeds, which extended the superiority up to 20 days, may be linked to the reduction of metabolic activities, allowing for lower sugar consumption and greater availability later to the embryo.

The AGT presented the shortest time in days in the condition in which the seeds were not stored, characterizing time zero of the three temperature conditions. The longest times, that is, the greatest delay occurred at five, 10, 15, 20 and 25 days in the natural environment condition, which together with days 15 and 25 did not differ from the cooling condition (Table 1).

When it is desired to store the seed, the first objective is to preserve its germination capacity efficiently and preferably without altering its initial vigor. The use of Kraft paper together with the natural environment condition kept the seeds viable for up to 25 days, as well as in cooling (Table 1), assuming that in the ambient temperature conditions, there was a greater consumption of sugars during storage and consequently required more time for embryo activation to occur, allowing its germination.

In an attempt to evaluate the desiccation resistance of Surinam cherry seeds, Langer et al. (2016) observed that germination viability was maintained for up to 10 days in paper bags. In addition, the authors observed that seeds stored in glass packaging presented the highest germination averages. In vacuum plastic packaging, a significant interaction was also obtained between the factors storage temperature x storage period in the germination, GSI and AGT variables (**Table 2**).

The highest germination rates occurred in seeds kept in natural conditions at times zero, five, 10 and 15 days, differently when controlled temperatures were used, in which cooling was maintained with superiority at times zero, five, 15 and 20 days and, in freezing only when not stored, that is, at time zero (Table 2).

It is important to emphasize that despite being recalcitrant, the Surinam cherry seeds remained viable for up to 30 days in both natural and refrigerated environments (Table 2), proving, to a certain extent, the efficiency of using vacuum packaging in maintaining greater moisture in the seed, which allowed its germination capacity to be maintained.

In addition, it was found that, at five, 10 and 15 days, the highest germination averages occurred in the natural and refrigerated environments, with the latter

Table 2. Germination (%), Germination Speed Index (GSI) and Average Germination Time (AGT) of Surinam cherry (*Eugenia uniflora*) seeds stored in vacuum packaging, kept in three temperature conditions (natural, refrigerator and freezer) for 90 days.

	Natural			Refrigerator			Freezer		
Storage period (days)	Germination (%)	GSI	AGT	Germination (%)	GSI	AGT	Germination (%)	GSI	AGT
0	43.74 aA*	7.21 aA*	9.98 aA	43.74 aA	7.21 aA	9.84 aA	43.74 aA	7.21 aA	9.84 aA
5	43.74 aA	6.76 aB	7.55 aC	44.99 aA	8.83 aA	10.60 aA	0.0 bB	0.0 bB	0.0 bB
10	43.44 aA	10.33 aA	12.00 aB	43.99 aB	8.38 aA	8.58 aA	0.0 bB	0.0 bB	0.0 bB
15	44.74 aA	9.98 a	10.30 aB	47.78 aA	9.11 aA	8.77 aA	0.0 bB	0.0 bB	0.0 bB
20	20.62 bB	12.82 aA	25.67 aA	51.50 aA	10.26 aA	8.17 bA	0.0 cB	0.0 bB	0.0 bB
25	12.33 bC	10.37 aA	31.07 aA	40.49 aB	11.77 aA	10.46 bA	0.0 cB	0.0 bB	0.0 bB
30	9.97 bC	1.94 bD	13.35 aB	30.24 aC	10.39 aA	8.89 bA	0.0 cB	0.0 cB	0.0 bB
60	0.0 aD	0.0 aE	0.0 aE	0.0 aD	0.0 aC	0.0 aB	0.0 aB	0.0 aB	0.0 bB
90	0.0 aD	0.0 aE	0.0 aE	0.0 aD	0.0 aC	0.0 aB	0.0 aB	0.0 aB	0.0 bB
VC (%)				21.08	17.41	15.66			

*Means followed by distinct uppercase letters in the same column and lowercase letters in the same row differ from each other using Duncan's test (α = 0.05).

Table 3. Germination (%), Germination Speed Index (GSI) and Average Germination Time (AGT) of Surinam cherry (*Eugenia uniflora*) seeds stored in ziplock plastic bags kept in three temperature conditions (natural, refrigerator and freezer) for 90 days.

Storage period (days	Natural			Refrigerator			Freezer		
	Germination (%)	GSI	AGT	Germination (%)	GSI	AGT	Germination (%)	GSI	AGT
0	49.77 aA*	6.37 aC	8.35 aA	49.77 aA	6.37 aC	8.35 aA	49.77 aA	6.37 aC	8.35 aA
5	40.23 aAB	5.87 bA	7.91 aA	44.48 aB	8.72 aB	10.67 aA	0.31 bB	0.0 cB	0.0 bB
10	29.26 bB	3.06 bB	4.68 bB	52.53 aB	10.96 aB	10.42 aA	0.29 cB	0.0 cB	0.0 bB
15	48.73 aA	7.20 aA	6.80 aA	46.72 aB	9.00 aB	8.85 aA	0.0 bB	0.0 bB	0.0 bB
20	36.23 bAB	5.05 bA	5.73 aA	46.74 aB	9.15 aB	8.03 aA	0.0 cB	0.0 cB	0.0 bB
25	32.88 aB	4.74 bA	5.42 bA	40.99 aB	11.94 aB	10.47 aA	0.0 bB	0.0 cB	0.0 cB
30	3.30 bC	1.40 bC	11.05 aA	65.60 aA	23.68 aA	9.87 aA	0.0 cB	0.0 cB	0.0 bB
60	0.0 aD	0.0 aD	0.0 aC	0.0 aC	0.0 aC	0.0 aB	0.0 aB	0.0 aB	0.0 aB
90	0.0 aD	0.0 aD	0.0 aC	0.3 aC	0.0 aC	0.0 aB	0.0 aB	0.0 aB	0.0 aB
VC (%)				17.45	14.67	23.99			

*Médias seguidas por letras distintas maiúscula na mesma coluna e minúscula na mesma linha, diferem entre si pelo teste de Duncan (α = 0,05)

remaining at 20, 25 and 30 days (Table 2), which allowed us to observe the advantage of using low temperatures and vacuum packaging, both of which have the function of reducing seed metabolism, which is necessary for recalcitrant seeds, since keeping them moist can activate the germination process during storage and, to prevent this from happening, attempts are made to control the processes linked to catabolism in the seed.

In an attempt to increase the viability of recalcitrant jabuticaba seeds (*Plinia* sp.), Danner et al. (2011) observed that vacuum packaging with phosphate buffer reasonably maintained germination viability for up to 65 days of storage. This is because jabuticaba seeds stored under vacuum allowed the seed moisture to be maintained.

Regarding GSI, the highest averages in the natural environment occurred at 10, 15, 20 and 25 days, which, together with 5 and 30 days, maintained superiority with the cooling environment. Comparing the environments on each day, the superiority for GSI occurred almost entirely in natural and cooling conditions, except for 30 days, in which only cooling presented the highest average (Table 2), thus demonstrating the advantage of keeping the seeds at low temperatures in order to conserve metabolic activities more slowly, providing less consumption of reserves.

For AGT, the shortest germination time occurred on day zero, in all environmental conditions. Within five, 10 and 15 days, the shortest times were in the natural and cooling conditions, and at 20, 25 and 30 days only in the natural environment (Table 2), showing that the storage condition at a lower temperature affects the metabolism of the seed in a way that not only favors its conservation, but also slows down the resumption of embryo growth for germination.

The germination in ziplock packages showed the highest averages in the natural environments when stored at zero, 5, 15 and 20 days, cooling at zero and 30 days and freezing only at zero days, this time allowing the only viability of the seeds. Similar superiority results were obtained with GSI, with the inclusion of only 25 days in the natural environment (**Table 3**).

The results obtained with freezing associated with ziplock allow us to point out that the Surinam cherry seeds did not tolerate negative storage temperatures, affecting the viability of their embryos, as early as the fifth day.

The cooling environments had the highest averages at all times in the range from zero to 30 days, but did not differ statistically from the natural condition at times five, 15 and 25 days, this behavior being maintained with AGT. The results obtained regarding AGT had the shortest time in the natural environment with 10 days, at the cooling temperature of zero to 30 days and, in the freezing only with zero, this time already presented as the only one to present germination (Table 3).

Conclusion

Surinam cherry seeds are sensitive to storage conditions, losing viability under freezing temperatures regardless of packaging. Refrigeration, especially with Ziplock packaging, best preserved viability for up to 30 days. Under natural conditions, Kraft paper was effective only for short-term storage of up to five days.

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