2026 International Nuclear Atlantic Conference – INAC 2026
Escola de Guerra Naval, Rio de Janeiro, RJ, August 24 - 28, 2026

Radiosensitivity of Eugenia uniflora seeds to gamma irradiation for germination and conservation purposes

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1. Introduction

Eugenia uniflora L., belonging to the Myrtaceae family and popularly known as pitanga, is a fruit species of wide occurrence and significant socioeconomic and environmental importance. Its adaptability to diverse climates allows its global dispersion, not being restricted to Brazil. The fruit, a berry of 1.5 to 3 cm with eight longitudinal grooves, exhibits coloring ranging from orange to dark purple. The flowers, white, hermaphrodite, and melliferous, are abundant in pollen, while its oval and shiny leaves, in the initial stage, present a wine hue. The pitanga tree can reach up to 8 meters in height. In addition to food value, studies point to antioxidant and healing properties, as well as the presence of vitamin C, giving it relevance in the pharmaceutical and cosmetic areas.

Pitanga seeds are important for the perpetuation of the species. Their classification as recalcitrant seeds or those with intermediate sensitivity to desiccation represents a challenge for long-term conservation in traditional seed banks. The short longevity of the pitanga seed is an important factor, a shared characteristic of recalcitrant seeds. Even under ideal humidity conditions, the germination capacity can be lost rapidly in a few months. According to Carvalho and Nakagawa (2012). Unlike orthodox seeds, which tolerate water loss and low metabolic activity to enter cryptobiosis, recalcitrant seeds do not possess the complex mechanisms to withstand desiccation, making prolonged storage by conventional methods unfeasible. This particularity emphasizes the need to keep seeds hydrated after collection and highlights the importance of alternative ex situ and in situ conservation strategies, as well as a deep understanding of their germinative behavior for reforestation and biodiversity programs.

Seed germination is an essential biological process, physiologically defined as the events that begin with water imbibition and culminate with radicle protrusion. This process comprises three phases: (1) imbibition (rapid weight gain by water absorption), (2)

metabolic activation (protein synthesis, enzymatic activation, respiration), and (3) growth and protrusion (new weight gain and embryo emergence).

Ionizing irradiation is a technology employed in the treatment of food and propagation materials, using sources such as Gamma rays (Cobalt-60), X-rays (below 5 MeV), or electrons (below 10 MeV). Its application is justified by technological requirements and/or health benefits to the consumer, and should not replace good hygiene or agricultural practices. Applied doses must be proportional to the desired purposes, respecting limits to ensure product safety, wholesomeness, and integrity. (Levy et al., 2018).

Studies indicate that the viability of its seeds decreases drastically when water content is reduced. For example, research such as that of Figueiredo et al. (2012) in "Revista Brasileira de Fruticultura" shows that dehydration negatively impacts the germination and vigor of *Eugenia uniflora* seedlings. This suggests that, for success in germination, seeds must be kept hydrated from collection.

Considering the gaps regarding the use of irradiation in seeds of species with sensitivity to desiccation, this study aimed to monitor the effects of gamma radiation doses on the germination viability and extension of the desiccation rate of pitanga seeds for conservation purposes.

2. Methodology

The experiment was conducted at the LADAI Laboratory, Center for Radiation Technology (CETER) of the Energy and Nuclear Research Institute (IPEN), São Paulo, SP, between June and December 2024.Seeds were manually extracted, selected for homogeneity of size and appearance, and prepared in batches of 20 seeds for each treatment, with four replicates per batch. The sanitization process involved washing in running water, immersion for 5 minutes in sodium hypochlorite, and subsequent washing in distilled water. The seeds were then dried at room temperature in a controlled environment.

Irradiation was performed at CETER/IPEN, using a Cobalt-60 source (Gammacell 220). The assays took place at room temperature and in the presence of air, with applied doses of 0 Gy (control), 50 Gy, and 100 Gy and a dose rate of 307.51 kGy/h in June 2024. After irradiation, the seeds were kept in a desiccator at 25 °C.

Prior to germination, each batch of seeds was subjected to a 2-hour imbibition in demineralized water. Germination assays were started at different times post-irradiation (days 0, 20, 30, 50, and 60).

Germination tests were performed on a vermiculite substrate, previously moistened and arranged in a 3 cm thick layer. The experiment was conducted for 120 days at 25 °C, following the Rules for Seed Analysis (RAS 2009). Substrate hydration was maintained on alternate days with sterile distilled water using a spray bottle. Controlled environmental conditions included a temperature of 25 ± 2 °C and a photoperiod of 12 hours light/12 hours dark.

Radicle protrusion was the criterion for determining germination, with counts performed on days 10, 20, 30, 50, and 60 after irradiation. Data, including seed identification, observation date, radicle protrusion status (yes/no), approximate radicle length (with a millimeter ruler), and additional observations (presence of fungi, abnormalities), were recorded in a spreadsheet. Data analysis included exploratory analysis for normality and outlier detection, and the Hartley test for homogeneity of variances.

3. Results and Discussion

Preliminary analyses indicated that the ideal phase for irradiation, aiming for shelf life extension, is when seeds are as close as possible to their natural dispersal moisture content, i.e., before any attempt at desiccation. Additionally, it was observed that germination decreased progressively over the storage period in all treatments.

The counts of germinated seeds per replicate (20 seeds/batch) and the calculated average for each treatment were as follows:

Non-irradiated seeds (Control - 0 Gy): The counts of germinated seeds in the four replicates were: 17, 12, 12, 9. The average germination for the control group was 12.5 seeds per batch, corresponding to an average germination rate of 62.5%. The observed variability (between 9 and 17 seeds) can be attributed to the intrinsic biological heterogeneity of the seeds or small controlled environmental fluctuations.

Seeds irradiated with 50 Gy: The counts of germinated seeds in the four replicates were: 11, 14, 10, 8. The average germination for this dose was 10.75 seeds per batch, totaling an average germination rate of 53.75%. Although the average is slightly lower than that of the control, the occurrence of one replicate with 14 germinated seeds suggests that this dose does not have a consistently severe inhibitory effect, indicating a resilient germinative capacity in some seeds.

Seeds irradiated with 100 Gy: The counts of germinated seeds in the four replicates were: 5, 3, 6, 2. The average germination for this dose was only 4.0 seeds per batch, corresponding to an average germination rate of 20.0%. This dose consistently demonstrated the lowest germination rates in all replicates, confirming a sharp inhibitory effect on seed viability.

The control group (0 Gy) exhibited the highest germination average, establishing a reference point for seed viability under the experimental conditions. The 50 Gy dose resulted in a reduced germination average compared to the control, but with some variability between replicates, which suggests that, although there is a negative impact, some seeds may possess greater resilience or

that this dose may induce complex effects that deserve further investigation. Interestingly, it was observed that, regarding storage time, the 50 Gy doses were able to maintain higher seed germinability compared to the control throughout the experimental period.

4. Conclusions

The results of this study, reinforced by the inclusion of four replicates per batch, robustly demonstrate an inversely proportional and dose-dependent relationship between gamma irradiation and the germinative capacity of *Eugenia uniflora* seeds. The 100 Gy dose manifested a drastic and consistent inhibitory effect on radicle protrusion, significantly making the seeds unviable. In contrast, the 50 Gy dose, while presenting a lower germination average than the control, showed greater variability among replicates, indicating a possible heterogeneous response of the seeds or the ability of certain seeds to resist this dose. Based on the results obtained and literature results, we can conclude that doses lower than 50Gy may, in certain plant species, maintain higher germinability than the control over storage time. We suggest that lower doses be tested in other studies. The process of hormesis using ionizing radiation is a finding of particular interest and suggests promising potential for the application of irradiation in managing the longevity of these recalcitrant seeds.

This work contributes to the understanding of the radiosensitivity of *Eugenia uniflora*, a species whose seeds are difficult to conserve by conventional methods. The final considerations point out that the use of ionizing radiation can be a sustainable tool to face the challenges imposed by tropical recalcitrant seeds. To optimize the application of this technology, balancing disinfection with the maintenance of viability and shelf life extension, future research with doses of 10; 15; 25 Gy and 50 Gy will be essential to determine the thresholds and precise biological effects.

Acknowledgements

The authors would like to thank IPEN-CNEN/SP, CNPq, and CNEN for financial support.

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