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CERRADO PITANGA SEED STORAGE

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ABSTRACT

Problems are observed in the storage of seeds of some fruit species of the Cerrado, due to their low longevity and tolerance to desiccation. Thus, the present study aimed to verify the behavior of the pitanga seeds from the Cerrado in relation to their storage and implications in the emergency. The work was conducted at Goiano Federal Institute - Ceres Campus. The experimental design adopted was completely randomized, arranged in subdivided plots, with the plots represented by *Eugenia Calycina* seeds and the subplots by the factorial $2 \times 3 \times 4 + 1$, consisting of two temperatures, 25 °C and 7 °C, stored in glass bottles, bags plastics and aluminum foil and four storage periods: 30, 60, 90 and 120 days after harvest, and the seeds sown immediately after harvest (control). The control showed higher percentage of emergence and speed index of emergence than the contrasts, indicating that the storage of pitanga seeds from the Cerrado influences the emergence of seedlings. Fresh pitanga seeds provide faster emergence and greater uniformity and should be sown immediately after harvest. *Eugenia calycina* seeds show intermediate behavior in relation to storage and desiccation.

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INTRODUCTION

Due to its geographical location, climate and soil conditions, Brazil has a natural abundance of fruits, however, only a few of them are being exploited commercially, perhaps due to limited amount of information available on their properties (Donado-Pestana et al., 2015). Studies can contribute to the development of new strategies to promote agribusiness, creating social and economic benefits, besides adding scientific value to these native fruits and contributing to the conservation of biodiversity where these species occur (Sardi et al., 2017). Among the varieties of species, only a few edible fruits are consumed by the local population and are commercially exploited, such as pequi (*Caryocar brasiliense*), buriti (*Mauritia flexuosa*), cagaita (*Eugenia dysenterica* DC) and mangaba (*Hancornia speciosa*) (Donado-Pestana et al., 2015). *Eugenia calycina* Cambess, popularly known as pitanga, belongs to the family Myrtaceae, and is an endemic species in Brazil, found in Cerrado region (Sousa et al., 2015). Species of the genus *Eugenia* have great economic and pharmacological potential in the commercial exploitation of edible fruits, wood, essential oils and ornamental plants (Sardi et al., 2017).

This species can be used for landscaping, in the manufacture of hedges, borders, rinks, in addition to being attractive to birds. Studies demonstrated that fruits of the genus *Eugenia* have beneficial properties that can be explored in the nutritional industry (Migues et al., 2018), being useful in the prevention of some human diseases (Sousa et al., 2015). These fruits are rich in phenolic compounds, flavonoids and ellagitannins with antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, antifungal, antiproliferative, antibacterial and anticholinesterase properties (Borges et al., 2014; Sardi et al., 2017). The main method of propagation of *Eugenia calycina* is via seed. Thus, information on seed storage is an important attribute to assess the reproductive potential of forest species, with the behavior of seed storage being based on the response to dehydration (Galindo-Rodriguez & Roa-Fuentes, 2017). This study aimed to verify the behavior of pitanga seeds from the Cerrado (*Eugenia calycina*) in relation to their storage and implications in the emergency.

MATERIALS AND METHODS

The experiment was carried out from October/2016 to May/2017, in the experimental area of the Goiano Federal

Institute, Ceres Campus, Brazil, located at road GO 154, Km 3 (5°21'0.84 "S and 49°35'55.40 "W). Seed storage was performed in Seed Analysis Laboratory (SAL) and germination tests were conducted in a greenhouse. The climate of the place, according to the Koppen classification, is Aw type (hot and semi-humid with a well-defined season, from May to September), with an annual average temperature of 25.4 °C, with minimum and maximum averages of 19.30 and 31.5 °C, respectively. Pitanga fruits were collected in different plants (matrices), in October, this being the period of greatest availability of fruits per plant. The collection was carried out in naturally occurring areas, in the city of Rianópolis, GO (15°26'01.4 "S, 49°32'41.2" W). After collecting the fruits, the seeds were manually pulped and washed in running water until complete removal of the mucilage, then they were stored on paper towels inside plastic trays until the excess water was removed. Subsequently, the seeds were homogenized to compose a single batch and treated with an insecticide IMIDACLOPRID NORTOX® (0.3 L 100 kg⁻¹ of seeds). The emergency tests were carried out in polystyrene trays with 128 cells. The tray was filled with sand, one seed per cell was placed and then covered with a thin layer of sand. The experimental design adopted was completely randomized, with four replications and 15 seeds per plot. The treatments were arranged in subdivided plots, these being represented by *E. Calycina* seeds and the subplots by the factorial 2x3x4 + 1, with two temperatures (25 °C - stored in Biochemical Oxygen Demand; and 7 °C - stored in refrigerator), three containers storage (glass bottles, plastic bags and aluminum foil), four storage periods (30, 60, 90 and 120 days after harvest) and the control (T). The control sowing was carried out right after the harvest, and the plot was composed of four replications, with 15 seeds. The emergency of percentage (EP) and the emergency speed index (ESI) were evaluated, calculated using the formula proposed by Maguire (1962), (Equation 1). The plants that presented the cotyledons totally free and open were considered as emerged. The evaluations were carried out weekly.

$$ESI = E1/N1 + E2/N2 + \dots + En/Nn \quad (1)$$

Considering,

ESI = emergency speed index (plant day⁻¹);

E1, E2, ... En = number of normal seedlings computed in the first, second and last count (plant);

N1, N2, ... Nn = number of days from sowing to first, second and last count (day).

The data were subjected to analysis of variance, using the contrast test, and the averages obtained were compared using the *Tukey test*, with the aid of the Sisvar version 5.7 software.

9 contrasts were made to compare with the control, the seeds being stored in glass jars (contrast 1), plastic bags (contrast 2), aluminum foil (contrast 3), 7 °C (contrast 4), 25 °C (contrast 5); and sowing time: 30 days (contrast 6), 60 days (contrast 7), 90 days (contrast 8) and 120 days (contrast 9) after harvest. The choice was to perform a contrast for each factor, so that it was possible to compare each storage factor with the control.

RESULTS AND DISCUSSION

The emergence of *E. calycina* seedlings began 45 days after sowing (DAS). This result differs from that found by Bulow *et al.* (1994), who observed an emergency time of about 10 to 16

days after sowing. These authors also state that this difference may be related to the type of substrate used. Another possible cause for this discrepancy between the emergency values would be the genetic variability presented by the species, since for the present study only sand was used for seedling emergence, and according to (Sousa *et al.*, 2014) it does not offer physical impediment to seedling emergence. There is a significant difference between treatments for both the percentage of emergence (EP) and the emergency speed index (ESI). For the EP variable, only the contrasts 4, 6 and 7 were not significant and for the variable ESI, all the contrasts were significant (Table 1).

Table 1. Analysis of variance for emergency percentage (EP) and emergency speed index (ESI)

VS	MS (EP)	MS (ESI)
Treatment	2894.635**	0.005**
VC (%)	30.630	45.100
Contrast 1	844.982*	0.013**
Contrast 2	1,305.988**	0.014**
Contrast 3	938.853*	0.011**
Contrast 4	642.759 ^{NS}	0.014**
Contrast 5	1,581.098**	0.012**
Contrast 6	152.419 ^{NS}	0.002*
Contrast 7	324.093 ^{NS}	0.004**
Contrast 8	6,437.600**	0.028**
Contrast 9	5,716.550**	0.027**
Error	166.074	0.001

** : significant in 1% of probability; * : significant in 5% of probability; ^{NS} : non-significant.

VS: Variation Source; MS: Medium square; VC: Variation Coefficient.

Table 2. Comparison of adequate averages to additionally test the proposed contrasts vs. the Control for emergency percentage (EP) and emergency speed index (ESI)

Contrasts	EP	ESI
C1 vs T	42.92 vs 58.33	0.047 vs 0.107
C2 vs T	39.17 vs 58.33	0.045 vs 0.107
C3 vs T	42.08 vs 58.33	0.051 vs 0.107
C4 vs T	45.14 vs 58.33	0.045 vs 0.107
C5 vs T	37.64 vs 58.33	0.051 vs 0.107
C6 vs T	65.00 vs 58.33	0.082 vs 0.107
C7 vs T	68.05 vs 58.33	0.074 vs 0.107
C8 vs T	15.00 vs 58.33	0.017 vs 0.107
C9 vs T	17.50 vs 58.33	0.018 vs 0.107

The results of the present study demonstrated that temperature (contrast 4 and 5), packaging (contrast 1, 2 and 3) and storage time (contrast 6, 7, 8 and 9) directly influence the emergence process of seedlings of Cerrado pitanga. According to Jeromini *et al.* (2015), the constitution of the packaging, the temperature and the storage environment are important factors for maintaining the physiological quality of the seeds, however, the ideal storage condition depends on the species. Thus, in the construction of knowledge about seed storage, Galindo-Rodríguez & Roa-Fuentes (2017) highlight the importance of understanding the following terms: orthodox and recalcitrant. The term orthodox refers to seeds that can be dried at low humidity (approximately 5%) and at low temperatures without damage, and recalcitrants refer to seeds that cannot survive low humidity levels in long term (Dresch *et al.*, 2014). In addition to these two terms, Ellis *et al.* (1990) proposed a third classification, which designates seeds that exhibit a storage behavior between orthodox and recalcitrant, called intermediate behavior, which does not tolerate low temperatures for prolonged periods. It was observed that the newly collected seeds had higher EP and ESI than the contrasts (Table 2), indicating that when newly collected and sown,

seedlings emerge faster, with greater uniformity and a higher percentage of emerged seedlings. Regarding storage, there was a significant drop in both EP and ESI, but the emergency process continued, even at low rates, demonstrating that *E. calycina* shows an intermediate behavior regarding storage (Table 2). These results corroborate with Cardoso & Lomônaco (2003), who reported that *E. calycina* seeds quickly reduce the viability for germination, after being harvested, a fact that hinders the perpetuation of these individuals. Delgado & Barbedo (2007) describe that the seeds of *E. uniflora*, *E. brasiliensis* and *E. involucrata* are also in an intermediate position regarding the sensitivity to desiccation. Studying *Eugenia uniflora*, Comin *et al.* (2014) observed that the different seed storage periods influenced the percentage of normal and abnormal seedlings whose values were reduced with the increase in the storage periods and, consequently, the percentage of non-germinated seeds increased. The authors associate this behavior with the loss of moisture in the seeds, which negatively interferes with their physiological processes. Evaluating the desiccation tolerance of seeds of six native fruit species of the genus *Eugenia*, Delgado & Barbedo (2007) observed that, among the studied species, those that presented less sensitivity to desiccation were the seeds of non-domesticated species. Thus, the fact that *E. calycina* still presents an emergency, even if stored for long periods, may be linked to its high intrapopulation genetic variability, seen in non-domesticated species, being, therefore, a strategy to guarantee the survival of its descendants in unfavorable conditions. The results also confront the performance of phenotypic plasticity as a mechanism that generates phenotypic variability and point out its importance in adaptive and evolutionary processes (Cardoso & Lomônaco, 2003). In working with *E. uniflora* seeds, a domesticated species and very common in orchards and gardens, Comin *et al.* (2014) observed that the seeds sown soon after harvest expressed 55% of emergence and when stored gradually lose vigor, not tolerating storage for more than 20 days, in the refrigerator. These results corroborate the studies by Delgado & Barbedo (2007), who observed that domesticated species have less tolerance to storage. Bulow *et al.* (1994) confirm that the maximum emergence speed in *E. calycina* is obtained from freshly harvested seeds. The same authors reported that, if storage is necessary, the seeds should be packed in plastic bags, inside the fruits, in a refrigerator, until, approximately, 35 days after the harvest, because this way their conservation is prolonged. Justo *et al.* (2007), studying *Eugenia pyriformis* seeds, observed that drying during storage promotes significant ultrastructural damage, therefore, drying and storing for prolonged time should be avoided, considering that it causes gradual cellular disruption. According to Kohoma *et al.* (2006), the reduction of metabolism, by decreasing the temperature, is an important technique of seed conservation during storage for species intolerant to desiccation. With this technique, the aforementioned authors were able to maintain the emergency percentage of 60% in *Eugenia brasiliensis*, stored for six months at a temperature of 7 °C. Scalón *et al.* (2012) reinforce that this information about storage is important for conservation of seeds of native fruit species, and from this knowledge it is possible to adopt correct procedures for harvesting, transportation and processing of seeds.

Conclusions: *E. calycina* seeds present intermediate behavior in relation to storage and desiccation. Pitanga seeds from the Cerrado should be sown soon after harvest.

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